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Epidemiological trends of knee injuries in Al-Karak, Jordan: MRI based cross-sectional study

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Abstract

Knee injuries, particularly ligament and meniscus injuries, are among the most prevalent musculoskeletal injuries, particularly in the active population. However, limited data are available on the epidemiology of and associated risk factors in the Jordanian population. To determine the prevalence and patterns of ligament and meniscus knee injuries and evaluate their association with demographic and anthropometric variables in patients presenting with knee pain at Al-Karak Governmental Hospital, Jordan. This retrospective cross-sectional study included 175 patients diagnosed with ligament or meniscal knee injuries via MRI between September 2018 and January 2022. Sociodemographic and clinical data were collected, including age, sex, body mass index (BMI), occupation, injury type, and side. Descriptive statistics, Pearson's chi-square tests, and multivariable logistic regression were performed to identify significant predictors and associations between variables. This study analyzed 175 participants, predominantly aged 18–50 years (68%), with a small female majority (53%). Most participants had a BMI > 25 (63%) and injuries were more common on the right side (58%). ACL injuries were the most frequent ligament injuries (58.3%), while meniscus injuries were highly prevalent (83.4%), with 64% of patients experiencing concomitant ligament and meniscus injuries (CLMI). Participants with a BMI > 25 had significantly higher odds of ligament (OR = 4.35, $p < 0.001$), meniscus (OR = 3.5, $p < 0.01$), and concomitant injuries (OR = 6.06, $p < 0.001$). Left-sided injuries were associated with higher odds of ligament injuries (OR = 2.99, $p < 0.05$). While age and occupation showed no significant association with most injuries, students had increased odds of ACL injuries (OR = 4.18, $p < 0.05$). Ligament and meniscus knee injuries are highly prevalent among Jordanian patients, particularly among overweight individuals. BMI has emerged as a significant risk factor across injury types, underscoring the need for targeted preventive strategies among high-risk populations. These findings emphasize the importance of early diagnosis and risk stratification in populations vulnerable to traumatic knee injuries.

Keywords: knee injury, ligament injury, meniscus injury, Jordan, obesity

1. Introduction

The knee is the largest and most biomechanically complex joint in the human body, and is among the most frequently injured musculoskeletal structures (1). With a mean incidence of 2.3 per 1,000 individuals, knee injuries impose significant socioeconomic burdens and contribute to substantial disability (2). Ligament injuries account for approximately 40% of knee trauma, patellar injuries for approximately 25%, meniscal tears for approximately 10%, and other types of injuries for the remaining 25% (1). A 2019 study reported an ACL tears incidence of 68.8 per 100,000 individuals (3). Posterior cruciate ligament (PCL) injuries represent 0.65%–3% of all sports-related knee injuries (4). Meniscal injuries are also prevalent, with an incidence of 61 cases per 100,000 persons and a prevalence ranging between 12% and 14% (5).

Most knee injuries occur through non-contact mechanisms, particularly involving the anterior cruciate ligament (ACL), which is a critical stabilizer of the joint and is often injured alongside other structures such as the meniscus (6,7). In contrast, multiple ligament knee injuries (MLKI) are typically associated with knee dislocations resulting from high-energy motor vehicle accidents or low-velocity sports injuries (8).

Regarding risk factors for developing such knee injuries, several risk factors have been identified for anterior cruciate ligament (ACL) injuries, including increased body mass index (BMI), greater joint laxity, and reduced femoral notch width (9). Posterior cruciate ligament (PCL) injuries are most commonly associated with trauma or direct impact on the knee (4). Additionally, studies have shown that male sex, increased BMI, advancing age, and occupational activities involving repetitive knee strainsuch as kneeling, squatting, or climbing stairsare significant risk factors for developing meniscal tears (10).

Despite the global significance of these injuries, only a few

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studies have focused on meniscal and ligament injuries in Jordan. This study aimed to assess the prevalence and types of knee injuries among patients presenting with knee pain in Al-Karak, Jordan between September 2018 and January 2022. Additionally, it sought to explore the associations between knee injuries and demographic risk factors, including age, sex, and body mass index (BMI).

2. Materials and Methods

2.1. Study setting

This study was conducted at the Al Karak Governmental Public Hospital in September 2018 and concluded by the end of January 2022.

2.2. Study sample

A retrospective cross-sectional study of the medical records of 175 patients was performed. Eligible participants were patients with ligament or meniscal knee injuries living in Al-Karak Province, Southwest Amman, Jordan. Patients with osteoarthritis, fractures, or bone cancer were excluded from this study.

2.3. Data collection

Knee injury was diagnosed using MRI and confirmed by an attending radiologist. Data collection tools designed for the study included variables such as type of injury (Anterior Cruciate Ligament (ACL), Posterior Cruciate Ligament (PCL), Anterior Horn of Medial Meniscus (AHMM), Posterior Horn of Medial Meniscus (PHMM), Anterior Horn of Lateral Meniscus [AHLM], posterior horn of medial meniscus [(, anterior horn of lateral meniscus [AHLM), Posterior Horn of Lateral Meniscus (PHLM), Medial Collateral Ligament (MCL), Lateral Collateral Ligament (LCL), ligament injury (LI), meniscus injury (MI), multi-meniscus injury (MMI), concomitant ligament and meniscus injuries (CLMI), and multi-ligament injury(MLI)), side of injury (right, left), age (less than 40 years, 40 to less than 60 years, 60+years), sex (male, female), weight, and height to estimate the Body Mass Index (BMI) categories (25 >,25 <), and occupation. See supplementary files for different MRI images of knee injuries. All knee MRI studies were interpreted by board-certified musculoskeletal radiologists at the Al-Karak Governmental Hospital, following international diagnostic standards.

Meniscus injuries were classified according to the Stoller grading system (grades 1–3, based on signal intensity and meniscus morphology) (11). Ligament injuries, including ACL, PCL, MCL, and LCL injuries, were evaluated based on morphological disruption, abnormal signal intensities on T2-weighted images, and fiber discontinuity following standardized musculoskeletal radiology guidelines (12). The radiologists underwent uniform institutional training and used a predefined institutional protocol to ensure consistency in reporting. All MRI interpretations were independently finalized. In cases of complex or ambiguous findings, a consensus diagnosis was reached through a double reading. Radiologists were blinded to the patients' clinical histories to minimize diagnostic bias.

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 28 for Windows. Descriptive statistics including frequencies and percentages were computed to present the variables. Alpha was set at 5%, and chi-squared tests were performed to assess the association and relationships between BMI and other variables. Logistic regression analysis was conducted for ACL, PCL, MI, LI, and CLMI, using personal predictors. The results of the logistic regression are presented in tables showing the odds ratio (OR) for each predictor.

3. Results

In total, 175 participants were included in this study. The majority were aged between 18-50 years (68%), followed by participants over 50 years (30.3%), and those under 18 years (1.7%). More females (53%) participated than males (47%). Most participants had BMI greater than 25 (63%). Regarding the side of injury, the right side was more frequently affected (58%) than the left (42%). Occupation-wise, the largest groups were housewives and teachers (24.7% each), followed by students (14%), industrial workers (13%) and retired individuals (15%). Among the reported injuries, anterior cruciate ligament (ACL) injuries were the most prevalent (58.3%), followed by meniscus injuries (83.4%) and multi-ligament injuries (52.9%). Posterior cruciate ligament (PCL) injuries were less frequent (15.4%), as were lateral collateral ligament (LCL) (8.6 %) and medial collateral ligament (MCL) (9.7 %) injuries (Table 1). Meniscus injuries were highly prevalent, affecting 83.4% of the participants, and 64% of the participants had concomitant ligament and meniscus injuries (CLMI), (Fig. 1).

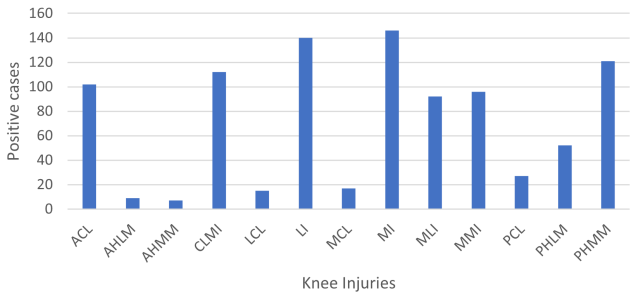

Fig. 1. Presents a bar plot illustrating the distribution of the injuries

Males and females with a BMI > 25 were more prone to ACL and meniscus injuries than those with a BMI < 25. The highest frequency of ACL injuries was observed among females with BMI > 25 (20%), followed by males with BMI > 25 (18.3%). Similarly, posterior horn medial meniscus (PHMM) injuries were predominantly reported among females with a BMI > 25 (26%), while males in the same BMI group showed slightly lower occurrences (20.6%). Posterior cruciate ligament (PCL) injuries and other less common injuries (e.g., AHLM and AHMM) demonstrated a consistent pattern of higher prevalence among individuals with higher BMI, regardless of sex (Table 2).

Table 1 Sociodemographic characteristics of participants at Al-Karak governmental hospital in 2022

Characteristics	N (%)	Characteristics	N (%)	Characteristics	N (%)
Age		**Multi-ligament**			
18>	3 (1.7)	**Injury**		**PCL**	
18-50	119 (68)	Yes	92 (52.9)	Yes	27 (15.4)
50<	53 (30.3)	No	83 (47.4)	No	148 (84.6)
Sex		**CLMI**		**LCL**	
Male	82 (47)	Yes	112 (64)	Yes	15 (8.6)
Female	93 (53)	No	63 (36)	No	160 (91.4)
BMI		**Meniscus Injury**		**AHMM**	
25>	64 (37)	Yes	146 (83.4)	Yes	7 (4)
25<	111 (63)	No	29 (16.6)	No	167 (96)
Side of Injury		**Multi-meniscus Injury**		**PHMM**	
Right	102 (58)	Yes	96 (55)	Yes	121 (69.1)
Left	73 (42)	No	79 (45)	No	54 (30.9)
Occupation		**ACL**		**PHLM**	
Housewife	43 (24.7)	Yes	102 (58.3)	Yes	52 (29.7)
Nurse	5 (2.9)	No	73 (41.7)	No	123 (70.3)
Soldier	6 (3.4)				
Teacher	43 (24.7)				
Student	25 (14)				
Industrial workers	22 (13)				
Unemployed	4 (2.3)				
Retired	27 (15)				
Ligament Injury		**MCL**		**AHLM**	
Yes	140 (80)	Yes	17 (9.7)	Yes	9 (5.1)
No	35 (20)	No	158 (90.3)	No	166 (94.9)

Table 2 Injuries across gender and BMI categories

Injuries	Gender and BMI								
	Male		Female			Male		Female	
	BMI <25	BMI >25	BMI <25	BMI >25		BMI <25	BMI >25	BMI <25	BMI >25
ACL					**PHLM**				
Yes	21 (12%)	32 (18.3%)	14 (8%)	35 (20%)	Yes	25 (14.3%)	28 (16%)	9 (5.1%)	14 (8%)
No	13 (7.4%)	16 (9.1%)	16 (9.1%)	28 (16%)	No	9 (5.1%)	20 (11.4%)	9 (5.1%)	14 (8%)
PCL					**AHLM**				
Yes	3 (1.7%)	12 (6.9%)	3 (1.7%)	9 (5.1%)	Yes	1 (0.6%)	4 (2.3%)	0 (0%)	4 (2.3%)
No	31 (17.7%)	36 (20.6%)	27 (15.4%)	54 (30.9%)	No	33 (18.9%)	44 (25.1%)	30 (17.1%)	59 (33.7%)
MCL					**LI**				
Yes	0 (0%)	5 (2.9%)	1 (0.6%)	11 (6%)	Yes	25 (14.3%)	42 (24%)	20 (11.4%)	53 (30.3%)
No	34 (19.4%)	43 (24.6%)	29 (16.6%)	52 (30%)	No	9 (5.1%)	6 (3.4%)	10 (5.7%)	10 (5.7%)
LCL					**MLI**				
Yes	5 (2.9%)	5 (2.9%)	2 (1.1%)	3 (2%)	Yes	11 (6.3%)	32 (18.3%)	10 (5.7%)	39 (22.3%)
No	29 (16.6%)	43 (24.6%)	28 (16%)	60 (34%)	No	23 (13.1%)	16 (9.1%)	20 (11.4%)	24 (13.7%)
AHMM					**MI**				
Yes	1 (0.6%)	4 (2.3%)	1 (0.6%)	1 (0.6%)	Yes	23 (13.1%)	45 (25.7%)	22 (12.6%)	56 (32%)
No	33 (19%)	44 (25.3%)	28 (16.1%)	62 (35.6%)	No	11 (6.3%)	3 (1.7%)	8 (4.6%)	7 (4%)
PHMM					**MMI**				
Yes	20 (11.4%)	36 (20.6%)	19 (11%)	46 (26%)	Yes	12 (6.9%)	36 (20.6%)	12 (6.9%)	36 (20.6%)
No	14 (8%)	12 (6.9%)	11 (6.3%)	17 (10%)	No	22 (12.6%)	12 (6.9%)	18 (10.3%)	27 (15.4%)

Regarding ACL and PHHM injuries, none of the predictors, including age, BMI, side of injury, or occupation, were significantly associated with ACL injury ($p > 0.05$). However, students had higher odds of ACL injury (OR = 4.18, $p < 0.05$). Demographic factors showed similar non-significant associations, Table 3).

Table 3. Logistic regression analysis of ACL and PHMM in relation to demographic predictors

	ACL			PHMM			
	OR	**CI**	**p-value**	**OR**	**CI**	**p-value**	**Reference**
Age 18>	1.52e +6	0 – inf	> 0.05	2.7	0.2 - 32.7	> 0.05	18-50
50<	1.24	0.47-3.25	> 0.05	1.23	0.4 - 4	> 0.05	
BMI	1.82	0.87 - 3.76	> 0.05	1.35	0.64 - 2.86	> 0.05	BMI <25
Side	1.24	0.64 - 2.38	> 0.05	1	0.5-2.1	> 0.05	Right
Occupation Housewife	0.5	0.17 - 1.45	> 0.05	2.34	0.7 - 7.6	> 0.05	Teacher
Nurse	1.56	0.23 - 10.7	> 0.05	3.2	0.3-34.7	> 0.05	
Soldier	1.03	0.27 - 10.11	> 0.05	3.64	0.35 - 31.24	> 0.05	
Student	**4.18**	**1.17 - 14.9**	**< 0.05**	0.57	0.20 – 1.65	> 0.05	
Industrial worker	0.31	0.28 - 2.36	> 0.05	1.04	0.35 -3.09	> 0.05	
Retired	1.03	0.27-3.77	> 0.05	3	0.62 - 14.26	> 0.05	
Unemployed	0.81	0.28 - 2.36	> 0.05	0.72	0.09-.5.65	> 0.05	

For ligament injuries, menisci, and concomitant meniscus and ligament injuries, BMI was significantly associated with all injury types. Participants with BMI > 25 had a significantly higher risk of ligament injuries (OR = 4.35, $p < 0.001$), meniscus injuries (OR = 3.5, $p < 0.01$), and concomitant injuries (OR = 6.06, $p < 0.001$). Additionally, the side of injury was a significant predictor of ligament injuries, with left-sided injuries associated with higher odds (OR = 2.99, $p < 0.05$) but was statistically insignificant for meniscal injuries and CMLI. Age was a statistically insignificant predictor of all the injuries (Table 4).

Table 4 Logistic regression outcomes for LI, MI, and CLMI utilizing demographic predictors

	Ligament Injury			Meniscus Injury			Concomitant Meniscus and Ligament Injury			
	OR	**CI**	**p-value**	**OR**	**CI**	**p-value**	**OR**	**CI**	**p-value**	**Reference**
Age 18>	2.18e+6.40	0 – inf	> 0.05	0.9	0.07 – 10.8	> 0.05	2.99	0.25 - 35.3	> 0.05	18-50
50<		0.10–1.53	> 0.05	2.9	0.82 – 10.9	> 0.05	0.63	0.29 - 1.38	> 0.05	
BMI	**4.35**	**1.72 - 11**	**< 0.001**	**3.5**	**1.43-8.43**	**< 0.01**	**6.06**	**2.9 - 12.7**	**< 0.001**	BMI<25
Side	**2.99**	**1.21-7.42**	**< 0.05**	0.67	0.29 - 1.55	> 0.05	1.5	0.75 - 2.94	> 0.05	Right

4. Discussion

This study offers valuable insights into the demographic distribution, prevalence, and associated risk factors for various knee injuries, particularly ligament and meniscal injuries. Our findings showed that anterior cruciate ligament (ACL) and meniscus injuries were the most commonly reported, with a substantial proportion of participants (64%) presenting with concomitant ligament and meniscus injuries (CLMI). The high prevalence of ACL (58.3%) and meniscus injuries (83.4%) aligns with prior research, including findings by Joseph et al., who reported that ACL injuries occurred in nearly half of all knee injuries (13). Similarly, Adams et al. identified meniscal injuries as the second most frequent type of knee injuries (14).

The considerable proportion of CLMI (64%) observed in our study is consistent with previous findings indicating that approximately 79% of patients with ACL injuries are found to have a concomitant meniscal tear during arthroscopic evaluation and that they are present in nearly half of the ACL injury cases (15). This is clinically significant, as CLMI can lead to greater biomechanical alterations, increased joint instability, and a higher risk of developing osteoarthritis (OA) than isolated ligament injuries (16).

Our results also indicate that higher body mass index (BMI) is a significant risk factor for knee injuries. Participants with a BMI > 25 had significantly higher odds of ligament injuries (OR = 4.35), meniscus injuries (OR = 3.5), and CLMI (OR = 6.06). These findings support the hypothesis that increased body weight imposes greater biomechanical stress on the knee joint, thereby increasing the vulnerability to injury (17). Biomechanically, a higher BMI results in greater axial compressive forces, which may accelerate meniscal intrasubstance degeneration, leading to meniscal tears or

maceration (18). This is supported by Ford et al., who found a significant association between elevated BMI and the need for meniscal surgery in both males and females (19). Furthermore, Tomihara et al. and Ang et al. also found that a higher BMI was associated with concomitant meniscal injury and irreparable meniscal tears during primary anterior cruciate ligament reconstruction in young patients (20,21).

Despite the strong association between BMI and overall knee injuries, our subgroup analysis found no statistically significant association between age, BMI, side of injury, or occupation and the odds of specifically developing ACL or posterior horn medial meniscus (PHMM) injuries. However, students had significantly higher odds of ACL injuries (OR = 4.18, $p < 0.05$), possibly reflecting their greater involvement in physically demanding activities or sports. This finding is consistent with Etzel et al., who reported an increased risk of ACL injury in young adults and adolescents, as well as with Evans et al., who found higher ACL injury rates among athletes involved in football, soccer, and basketball (22,23). However, previous literature suggests that certain occupations, such as military service, are associated with a higher incidence of ACL injuries than civilian populations, which is contraindicated in our results (24).

Regarding the side of injury, right-sided injuries were more frequent overall. However, left-sided injuries were significantly associated with the risk of ligament injuries (OR = 2.99, $p < 0.05$). These findings are comparable to those of Rahmadian et al., who reported a predominance of right knee injuries (56.9%) in Indonesian patients, potentially due to right-sided dominance and more frequent use in daily activities (25).

This study has several limitations that should be acknowledged to contextualize its findings. First, its retrospective cross-sectional design inherently limits causal inferences. While associations between demographic variables (such as BMI and occupation) and injury type were identified, the directionality and temporality of these relationships could not be established. Longitudinal or prospective cohort studies are better suited to assess the causality and temporal patterns of knee injuries. Second, the study was conducted in a single governmental hospital in Al-Karak, Jordan, which may limit the generalizability of the findings to other regions of the country or to different healthcare settings. The sample may not be fully representative of the broader Jordanian population, particularly individuals who seek care in private clinics or do not undergo MRI due to financial or accessibility barriers.

Third, selection bias was possible, as only patients who underwent MRI and were diagnosed with ligament or meniscus injuries were included. This excluded individuals with mild injuries managed conservatively or those misdiagnosed clinically without MRI confirmation, potentially underestimating the true burden of knee injuries in the region. Fourth, the study did not evaluate detailed activity levels, sports participation history, or previous injury history, which are factors known to influence the risk of knee injuries. The use of "occupation" as a proxy for activity level may not accurately capture the biomechanical demands placed on the knee joint in daily life or during sports activities.

Fifth, although MRI interpretations were performed by experienced musculoskeletal radiologists following standardized protocols, inter-rater reliability was not assessed. Lastly, anthropometric data were derived from patient records, and inaccuracies in height or weight documentation could have led to the misclassification of BMI categories. Despite these limitations, this study provides meaningful insights into the epidemiology and risk factors of knee injuries in a Jordanian population and serves as a foundation for future larger-scale multicenter research efforts.

This study highlights the multifactorial nature of knee injuries, emphasizing the critical role of BMI and activity level on injury risk. While demographic variables such as age and sex were not statistically significant predictors on their own, BMI and occupation demonstrated strong associations, reinforcing the importance of weight management and activity modification in injury prevention strategies.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: M.H.K., J.H.J., I.M.A., E.E., Y.H., Z.S., A.A.A., Design: M.H.K., J.H.J., I.M.A., E.E., Y.H., Z.S., A.A.A., Data Collection or Processing: M.H.K., J.H.J., I.M.A., E.E., Y.H., Z.S., A.A.A., Analysis or Interpretation: M.H.K., J.H.J., I.M.A., E.E., Y.H., Z.S., A.A.A., Literature Search: M.H.K., J.H.J., I.M.A., E.E., Y.H., Z.S., A.A.A., Writing: M.H.K., J.H.J., I.M.A., E.E., Y.H., Z.S., A.A.A.

Ethical Statement

This study was approved by the Ethics Committee of the Faculty of Medicine, Mutah University, Al-Karak, Jordan (reference number: 222022).

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Research Article

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The histological examination of vena saphena magna as a graft in coronary artery bypass surgery

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Abstract

Coronary artery bypass surgery (CABG) is one of the most frequently performed surgical procedures worldwide, owing to the increasing incidence of coronary artery disease, which remains a leading cause of death globally. Following CABG, the development of occlusion in vein grafts is one of the significant indicators of poor prognosis, resulting in an increased risk of recurrent ischemic events and repeat revascularization. In the early and late postoperative periods, the use of healthy grafts with intact endothelium is critical to reduce mortality and morbidity rates. In this study, the histopathological findings of the vena saphena magna grafts in 12 patients who underwent CABG were evaluated to identify any preoperative degenerative changes in the grafts. The findings of the study showed that preoperative morphological degeneration was minimal in most grafts. However, in one sample, intimal fibrosis narrowed the lumen, while another sample exhibited mild medial sclerosis. In contrast, all other samples showed minimal or no degeneration. Consequently, assessing the histopathological condition of vessels before the operation is crucial to avoid using unsuitable grafts and to direct the surgeon to use different grafts if necessary. This study highlights the importance of examining the histology of vena saphena magna grafts to ensure their suitability as a bypass conduit in CABG surgery.

Keywords: coronary artery bypass surgery, vena saphena magna, graft, histopathology

1. Introduction

Today, in coronary surgery, a healthy blood vessel (graft) is taken from the chest or leg area, and this blood vessel is connected beneath the blocked heart artery. This new pathway improves blood flow to the heart muscle. The grafts used contain healthy veins or arteries harvested from a separate area; the number of grafts needed will depend on how severe the coronary heart disease is and how many coronary blood vessels are narrowed. Mostly used grafts are arterial grafts, which are biologically divergent conductance arteries used in coronary artery bypass surgery, including the internal mammary artery, vena saphena magna, radial artery, and right internal mammary artery. Due to its ease of access and because its wall contains a higher percentage of elastic and muscular fibers compared to other superficial body veins, the saphenous vein is used in coronary artery bypass surgery. The removal of the saphenous vein does not require a long incision; one or two incisions are made at the knee and a small incision in the thigh. This method minimizes scarring, allowing the patient to heal faster than conventional surgery. Despite many attempts to improve the results of using veins in coronary artery bypass grafting, they still tend to fail in the long term. There are many studies on this subject in the literature (1-3).

Studies have shown that 7–13% of grafts from the great saphenous vein used for coronary artery bypass develop occlusion within the first month after surgery, while 5–14% of venous grafts develop >50% luminal stenosis. Complete occlusion is observed in 15–26% of grafts after 6–8 months, with 5–10% having greater than 50% luminal stenosis (4). Comorbidities are repeatedly encountered in patients undergoing coronary artery bypass surgery; specifically, diabetes mellitus, hypertension, chronic obstructive pulmonary disease, and chronic kidney diseases are common in this patient group. Studies have shown that for diseases such as aging, diabetes mellitus, and chronic renal failure, structural changes in the vena saphena magna appear, increasing the chance of failure of vein grafts after surgery. Sun et al. showed differences in saphenous vein extracellular protein expression

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in diabetic patients, claiming that these contrasts may impact the long-term patency rate of saphenous veins. In another study, the negative effects of chronic renal failure on saphenous vein histology were reported (5). Patient-specific factors, such as age, diabetes, hypertension, and hyperlipidemia, are known to influence vascular health and may contribute to the development of degenerative changes in saphenous vein grafts. While our study did not specifically investigate the impact of these factors, future research should explore the relationship between patient comorbidities, graft histology, and long-term clinical outcomes (6). It is possible that the observed medial sclerosis is more prevalent in older patients or those with a history of hypertension. Larger studies are needed to determine the relative contribution of these factors to graft degeneration.

The importance of the histological study of vein grafts prior to their use in bypass surgery is vital for performing the preoperative evaluation of graft quality, which can provide the necessary knowledge to predict graft viability. The long saphenous vein is usually used as a graft for coronary artery bypass surgery. Histological changes observed after the implantation of the arterial system are well established in the literature, but little awareness has focused on the histological features of the long saphenous vein before grafting.

In a study by Hess et al., 1,828 patients who had undergone coronary bypass surgery were examined, and significant vascular insufficiency requiring re-intervention was detected in vein grafts in 42.8% of the patients 12–18 months after the operation (7). Harskamp et al. determined that disease (development of occlusion) in vein grafts after the coronary bypass is one of the indicators of poor postoperative prognosis (8).

This study aims to explore the histopathological characteristics of saphenous veins in patients who have undergone coronary artery bypass surgery. In particular, this investigation seeks to identify any preoperative degenerative changes in the grafts of saphenous veins and assess their potential implications for the patient's prognosis. The research aims to elucidate the relationship between the degenerative findings of saphenous veins and the overall health outcomes of patients following CABG. By investigating these relationships, this study will contribute to the broader understanding of the significance of saphenous vein histology in the context of CABG surgery. Additionally, this study intends to inform the development of evidence-based clinical practices related to saphenous vein grafting and potentially improve patient outcomes in the long term.

2. Materials and methods

This research employs a qualitative flexible experimental research design that aims to conduct a histological examination of vena saphena magna as a graft in coronary artery bypass surgery. This study includes 12 patients who underwent coronary artery bypass surgery due to coronary artery disease at Marmara University Faculty of Medicine, Department of Cardiovascular Surgery.

Inclusion criteria: Participants must have applied to the Marmara University Faculty of Medicine, Department of Cardiovascular Surgery due to coronary artery disease. Adult patients undergoing elective first-time CABG should be able to give informed consent and be aged >18 years.

Exclusion criteria: Patients from whom veins cannot be removed will be excluded from the study, as well as individuals who had prior CABG surgery within one month, pregnant or lactating women, those with a myocardial infarction <48 hours prior to surgery, and patients experiencing cardiac shock within 48 hours of surgery.

2.1. Histological examination (Light microscopy).

Vena saphena magna samples taken from three different regions (femoral, popliteal, and medial malleolus) were placed in 10% neutral buffered formaldehyde and sent to the pathology laboratory. Histological evaluation was performed by a pathologist blinded to patient data. Intimal fibrosis was scored according to the following criteria: 0 = normal intima, 1 = focal proliferation and minimal contraction, 2 = concentric proliferation and slight contraction, 3 = concentric proliferation and moderate narrowing (<50% narrowing of the lumen), 4 = significant narrowing (>50% narrowing of the lumen). Medial sclerosis was scored as follows: 0 = negative, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe (9).

3. Results

As a result of the examination, it was observed that the preoperative morphological degeneration was minimal in almost all grafts. Intimal fibrosis was observed in six of the 12 (50%) samples at varying degrees (Table 1, Fig. 1 and 2). In one sample (8.5% of all samples), intimal fibrosis was found to narrow the lumen (Fig. 3). Medial sclerosis was mild in three (25% of all samples) at varying degrees and negative in all other samples (Table 2, Fig. 4).

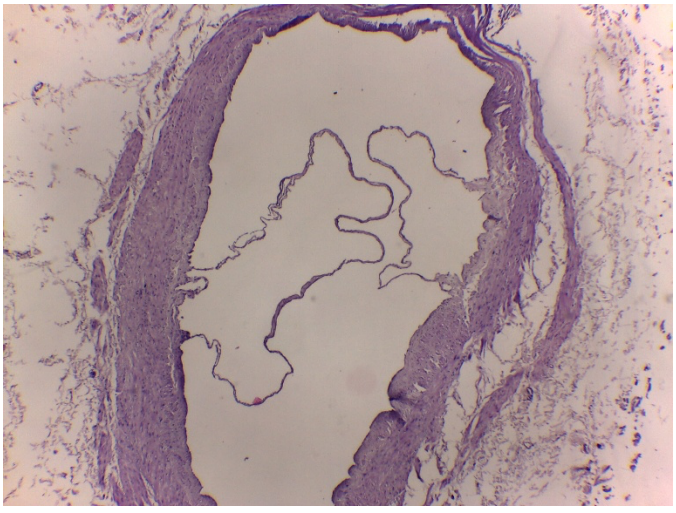

Fig. 1. A vessel with normal wall thickness with valves in the lumen (Hematoxylin and Eosin x40)

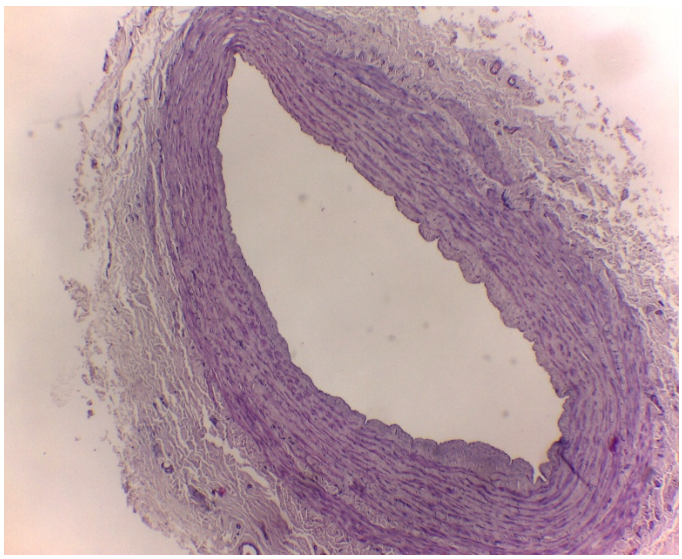

Fig. 2. The intima and media of the vein with a normal appearance (Hematoxylin and Eosin x40)

Table 1. The intimal fibrous thickening score by the number of patients

Patients no	Proximal	Medial	Distal
1	0	0	0
2	0	0	0
3	1	1	1
4	2	0	1
5	4	3	1
6	0	0	0
7	0	0	0
8	0	0	0
9	2	2	2
10	2	2	2
11	2	2	2
12	0	0	0

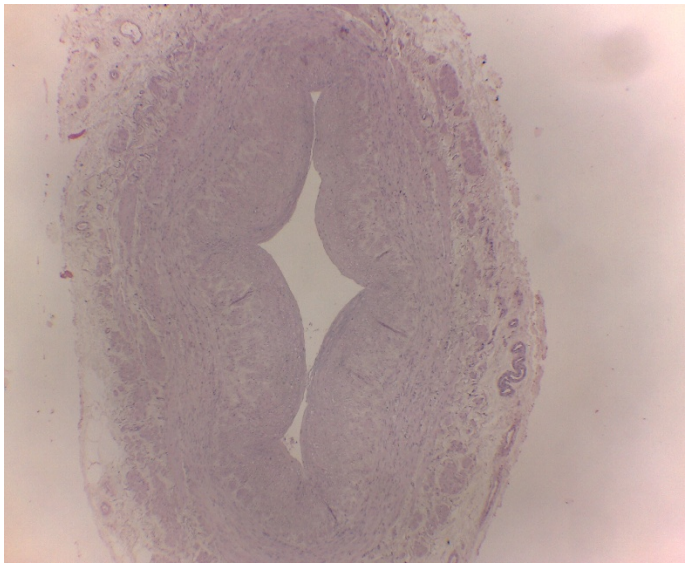

Fig. 3. Intimal and medial thickening causing severe narrowing of the lumen (Hematoxylin and Eosin x40)

Table 2. Medial fibrous thickening score by the number of patients

Patients no	Proximal	Medial	Distal
1	0	0	0
2	0	0	0
3	0	0	0
4	1	0	0
5	2	2	2
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	1	1	1
12	0	0	0

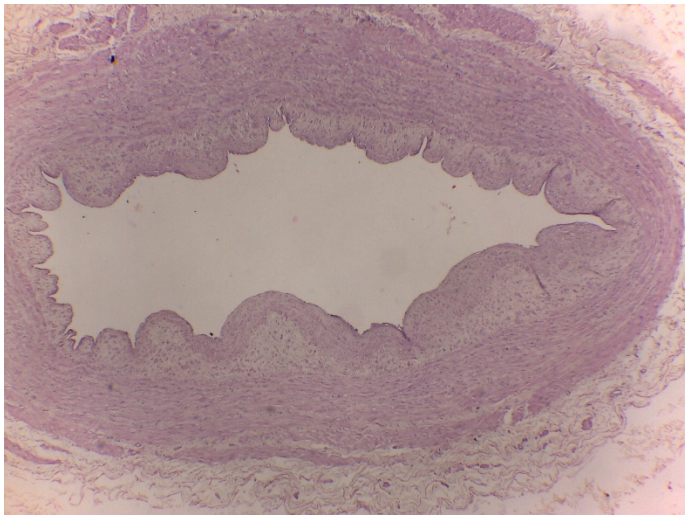

Fig. 4. Intimal and medial thickening causing narrowing of the lumen (Hematoxylin and Eosin x40)

4. Discussion

This study aimed to investigate the histology of vena saphena magna in the patient population who underwent coronary artery bypass surgery and select the degenerative findings of the grafts in these patients. Our study showed that although VSM grafts appear macroscopically normal, they may show some histopathological features such as intimal fibrosis and medial sclerosis. Intimal fibrosis was observed in six of the 12 (50%) samples included in our study, and medial sclerosis was identified in three samples.

The observed intimal fibrosis and medial sclerosis, even in minimal forms, may contribute to reduced graft compliance and increased resistance to blood flow. This, in turn, could accelerate the development of graft stenosis or occlusion, ultimately impacting long-term patency and increasing the risk of recurrent ischemic events.

Our findings align with previous research demonstrating that pre-existing structural abnormalities in saphenous vein grafts can negatively influence their long-term performance (10). However, our study provides a unique perspective by focusing on early histological changes in a general CABG population, allowing us to assess the baseline condition of the grafts before significant postoperative remodeling occurs.

Future studies should correlate these histological findings with angiographic follow-up data to determine the predictive value of preoperative graft histology for long-term clinical outcomes.

The research conducted by Lawrie et al. aimed to assess the morphological characteristics of grafts derived from the internal thoracic artery and the great saphenous vein before their utilization in aortocoronary bypass surgery. The primary objective was to determine the suitability and viability of these grafts for the surgical procedure. The findings of the study revealed significant necrosis of endothelial cells within the examined grafts, leading to the exposure of the underlying basement membrane, which became susceptible to interactions with blood cells. Notably, the observed chronic lesions primarily affected venous grafts and were characterized by distinct atheromatous plaques or thickening of the intima and media layers (9). These observations highlight the importance of preoperative assessment and careful consideration of the morphology of grafts in aortocoronary bypass surgery. Further research is warranted to investigate strategies to improve the quality and durability of grafts, particularly in the context of venous grafts.

In a separate investigation conducted by Perek et al., the histological characteristics of saphenous vein reconstruction were examined concerning aging and determining the optimal age limits for selecting grafts in clinical practice. Distal segments of venous grafts were collected from a cohort of 110 patients who underwent venous coronary artery bypass grafting (CABG). The results revealed a progressive and age-dependent thinning of the venous wall and tunica media, accompanied by elongation of smooth muscle cell (SMC) nuclei. These histological changes suggest potential impairment in the migration and proliferation rates of SMCs with advancing age. Interestingly, the results suggest that individuals aged 70 years and above may derive greater clinical benefits from venous CABG procedures compared to younger patients (10). The implications of these findings are significant, highlighting the need for considering chronological age when determining the most appropriate candidates for venous CABG procedures.

The aim of the study conducted at St. Mary's Hospital Medical School was to assess the preoperative quality of the long saphenous vein (LSV) wall using ultrasound and histology. A total of 40 limbs from 38 patients were included in the evaluation, and three segments of the LSV (ankle, knee, and mid-thigh) were examined preoperatively using ultrasound imaging. The results revealed significant insights into the histological characteristics of the LSV wall. Upon histological examination, only eight specimens exhibited a normal appearance, indicating the absence of notable abnormalities. In contrast, six specimens displayed severe fibrosis, suggesting a considerable degree of pathological tissue remodeling within the LSV wall. Importantly, varying grades of fibrosis were observed in different anatomical sites of the same vein, implying localized heterogeneity in the structural integrity of the LSV. These findings underscore the importance of comprehensive preoperative evaluation in assessing the quality and suitability of the LSV as a potential conduit in surgical interventions (11). By combining ultrasound imaging and histological analysis, a comprehensive understanding of the morphological characteristics of the LSV wall can be obtained, enabling surgeons to make informed decisions regarding its utilization in clinical practice.

The University of Copenhagen in Denmark conducted a study aiming to analyze the histopathological features of in situ vein bypass stenoses. In this study, a total of nineteen specimens of primary (n = 16) or recurrent (n = 3) vein graft stenosis were collected from seventeen patients during surgical revision. The median time interval between bypass surgery and stenosis excision was 5 months, with a range of 2 to 52 months. Upon analysis, the histopathological characteristics of the graft stenoses were identified and documented, showing moderate to severe intimal hyperplasia primarily composed of actin-positive but desmin-negative cells. The concurrent presence of fibrotic areas further contributed to the narrowing of the lumen (12). The findings emphasize the significant histological changes that occur within in situ vein bypass stenosis, showcasing the crucial role endothelial dysfunction plays in its pathogenesis.

A comprehensive investigation was conducted by the University of Padova Medical School to assess the condition of the venous wall prior to its utilization as an aortocoronary conduit. This study meticulously examined autologous saphenous veins used as bypass conduits in a consecutive series of 150 patients undergoing aortocoronary bypass procedures. To analyze potential age-related differences, the patients were stratified into four distinct groups based on their age. The histological examination yielded valuable insights into pathological features present within the venous structures. Notably, primary pathological findings encompassed a range of intimal fibrous thickening, sclerosis of the medial longitudinal muscular layer, and elastosis of the internal elastic lamina (8). These alterations underscore the necessity of assessing the pre-existing condition of the venous wall, which facilitates informed decision-making during bypass surgery and enhances our understanding of the processes underlying vein graft failure.

In a meticulous investigation by Hess et al., the primary objective was to gain a deeper understanding of the factors associated with vein graft failure (VGF) to enhance patient outcomes. The study involved an extensive examination of 1,828 participants enrolled in the Project of Ex Vivo Vein Graft Engineering via Transfection IV (PREVENT IV) trial. Angiographic assessments were performed between 12 to 18 months post-coronary artery bypass grafting (CABG) (6). Notably, the findings revealed that among the total cohort, 782 individuals exhibited VGF at the 12 to 18-month follow-up.

The detailed assessments highlighted associations between various demographic characteristics and comorbid conditions with VGF. The study underlines the complexity of VGF and the need for further investigation into its contributing factors to improve clinical outcomes.

The success of coronary artery bypass grafting (CABG) using the vena saphena magna (VSM) graft depends on the preoperative histopathological condition of the vessels. Therefore, preoperative examination is crucial in identifying suitable grafts and guiding the surgeon toward alternative options when necessary. In our study, the degree of preoperative morphological degeneration was evaluated in nearly all VSM grafts, with observations indicating that this degeneration was minimal. The presence of even mild intimal fibrosis and medial sclerosis could compromise the long-term patency of saphenous vein grafts, increasing the risk of thrombosis and accelerated atherosclerosis within the graft.

In the context of surgical coronary artery bypass procedures, the preoperative assessment of grafts is crucial in determining their viability and predicting long-term durability. Various methods have been proposed for preoperative screening of grafts, including angioscopy and intravascular ultrasound. Combining these methods allows for the detection of small lesions in the vessel wall, such as intimal hyperplasia, making the quality assessment of grafts more effective. However, it should be noted that ultrasound alone may not reliably identify these changes, which can be readily diagnosed using angioscopy or histological examination (13).

In this study, we employed light microscopy to examine biopsies of grafts taken from the great saphenous vein, which were remnants from surgical coronary artery bypass procedures. Our aim was to draw conclusions regarding the preoperative morphology of the grafts and correlate this with their future viability. Our results suggest that thorough preoperative assessment is indeed crucial, as even grafts that appeared macroscopically normal were found to carry histopathological findings of vascular abnormality such as intimal fibrosis and medial sclerosis. The use of a combination of screening methods, including light microscopic examination, angioscopy, and intravascular ultrasound, may aid in the detection of these lesions and improve the accuracy of preoperative quality assessment. Ultimately, these improvements could lead to better surgical outcomes and increased graft durability.

Preventing vein graft failure (VGF) is a critical objective in coronary artery bypass graft (CABG) surgery, and various preoperative measures have been proposed to enhance graft potency and durability. Intraoperative strategies aimed at minimizing graft trauma and optimizing handling techniques have proven effective in preventing early graft failure. These measures include avoiding graft distention, employing a no-touch technique, and minimizing graft manipulation during harvesting and implantation. However, their impact on late graft failure remains limited.

Pharmacological interventions have also been extensively investigated as a means to prevent VGF. Hypercholesterolemia has been identified as a risk factor for graft failure, and aggressive lipid-lowering therapy utilizing statins has demonstrated promising results in reducing neointimal hyperplasia—a common contributor to graft failure in CABG surgery. Antithrombotic therapy represents another avenue for preventing VGF by reducing the occurrence of thrombotic graft occlusion and potentially preventing distal embolization and associated myocardial ischemic injury. Aspirin, for instance, has exhibited efficacy in mitigating early graft failure through its antiplatelet properties; however, its long-term effect on graft patency is limited, and it does not inhibit hyperplasia development.

While these preoperative measures and pharmacological interventions have shown promise in mitigating VGF, further research is needed to optimize their effectiveness and identify additional strategies for preventing graft failure in the long term. A comprehensive understanding of the underlying mechanisms involved in VGF, coupled with advances in surgical techniques and adjunctive therapies, may pave the way for more targeted and effective approaches to enhance graft durability and improve clinical outcomes in CABG surgery.

Saphenous vein conduit selection is established based on lumen characteristics, so our study's limitations include:

1. Patients who required coronary bypass surgery and who would use vena saphena magna as a graft in this surgery were included in our study.

2. If the entire saphenous vein is used during the operation and there is no residual vein, these patients will be excluded from the study.

Our study is also limited by a relatively small sample size, which may affect the generalizability of our findings. Future studies with larger, multi-center cohorts are needed to validate these observations and to identify potential subgroups of patients who may be at higher risk for graft failure based on preoperative histology. Since this study was not a group comparison study and the sample size was small, statistical analysis was not performed, which also limits the robustness of our conclusions.

Ethical Statement

This study was approved by the Clinical Research Ethics Committee of Marmara University Faculty of Medicine (Protocol No: 09.2016.631; Date: 02.11.2016).

Conflict of interest

There is no conflict of interest.

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No to declare.

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None to declare.

Authors' contributions

Concept: A.H.M.A., K.A., A.M., Design: A.H.M.A., K.A., A.M., Data collection or Processing: A.T., C.C.U., M.E.D., S.T., Analysis or Interpretation: A.H.M.A., A.M., Literature Search: A.T., C.C.U., Writing: K.A., M.E.D., S.T.

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A study to determine the frequency of *van A* and *van B* genes in vancomycin-resistant *Enterococcus faecalis* strains obtained from clinical specimens in northwestern Iran

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Abstract

One of the significant global challenges today is the increasing prevalence of vancomycin-resistant enterococci (VRE). This study aims to understand the antibiotic resistance patterns, investigate the prevalence of VRE-causing genes in clinical samples, and evaluate the prevalence of enterococcal strains. For this study, 200 urine and blood samples were collected from patients visiting healthcare centers in Tabriz. Antibiotic susceptibility was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, and the minimum inhibitory concentration (MIC) for vancomycin was assessed using the E-Test method. Additionally, polymerase chain reaction (PCR) techniques were employed for precise bacterial identification and to examine the presence of vancomycin resistance genes *van A* and *van B*. Molecular analysis revealed that 100 out of 200 samples (50%) belonged to *Enterococcus faecalis* (*E. faecalis*). The isolates exhibited the highest resistance to penicillin (83%), tetracycline (43%), and ciprofloxacin (41%), while they showed the greatest sensitivity to linezolid (87%), imipenem (85%), and teicoplanin (70%). A total of 31 samples were identified as resistant to vancomycin, with 18 strains (58.06%) containing the vanA genotype, 8 strains (25.81%) containing the vanB genotype, and 5 strains (16.13%) harboring both *van A* and *van B* genes. Given the high prevalence of VRE strains, it is essential to evaluate these organisms in all clinical samples.

Keywords: *Enterococcus faecalis*, vancomycin, blood, urine, infection, vancomycin resistance

1. Introduction

Enterococci are Gram-positive, catalase-negative bacteria that naturally inhabit the gastrointestinal tract but can also cause serious hospital-acquired infections, such as UTIs, surgical wound infections, bacteremia, endocarditis, and meningitis. They are highly resilient, tolerating bile salts, high salt concentrations, and extreme pH (9.6), with optimal growth at 10–45°C and survival at 60°C for 30 minutes. Recent overuse of antibiotics has led to rising global resistance, notably in vancomycin-resistant (VRE) and linezolid-resistant (LRE) strains (1-6). The first reported case of VRE in animals dates back to 1933, influenced by avoparcin. Later, in 1988, the first human case of VRE was reported in the United Kingdom (7). In the 1980s, immunological, biochemical, and genetic studies (including DNA similarity, rRNA analysis, and 16S rRNA sequencing) revealed distinct differences between Enterococcal and non-Enterococcal group D streptococci. These findings led to the reclassification of enterococcal strains into a separate genus, Enterococcus (8). The genus Enterococcus includes over 50 species, with *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) being the most significant members (9). The *E. faecalis* species is the dominant strain in this group, accounting for 85 to 90 percent of clinical isolates, while *E. faecium* contributes to about 5 to 15 percent of this statistic (10). Enterococci possess surface components such as polysaccharide capsules, pili, and adhesive substances that allow them to form biofilms and ultimately establish persistent infections. Other factors that exacerbate bacterial infection within the host include

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hemolysin/cytolysin, serine protease, bacteriocin, gelatinase, and toxic oxygen metabolites (TOM) produced by the bacteria that lead to cellular damage (7). Studies have clearly established that vancomycin resistance in enterococci is mediated by *van* genes. To date, several genotypes within the *van* group have been identified including *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*. The *vanA* and *vanB* genotypes have been predominant worldwide (2, 11). *E. faecalis* can transfer resistance genes via conjugative transposons, pheromone-responsive plasmids, or broad-host-range plasmids (12). Among the glycopeptide resistance genes in enterococci, the *vanA* gene is the most prevalent. This genotype confers high-level resistance to vancomycin and teicoplanin and is often located on plasmids transferred through transposon Tn1546. The *vanB* genotype exhibits similar functionality to *vanA*; however, their regulation differs as the *vanB* operon can be located on either chromosomes or plasmids (13-16). With reports of various Enterococcal strains emerging globally and the ongoing trend of acquiring resistance to multiple antibiotics, a significant reduction in therapeutic options for controlling these infections has occurred (17, 18). Therefore, the objective of this research is to evaluate the prevalence of Enterococcal strains, antibiotic resistance patterns, and identify the key genes responsible for vancomycin resistance (*vanA* and *vanB*) in clinical samples isolated from hospitalized patients in Tabriz city.

2. Materials and methods

2.1. Sample Collection and Identification:

In this study, 200 clinical samples (including blood and urine) were randomly collected from patients visiting hospitals and healthcare centers in Tabriz between March 2024 and August 2024. Patient demographic data, such as gender, age, and hospitalization status (hospitalized or outpatient), were also recorded. Initially, all samples were cultured on nutrient agar (Merck, Germany) and incubated at 37 °C for 24 hours. Colonies were identified at the genus level using Gram staining, catalase testing, disk resistance determination, optochin sensitivity, bile esculin growth, growth in 6.5% sodium chloride, and L-pyrrolidonyl-β-naphthylamide (PYR) hydrolysis tests. Additionally, the standard strain *E. faecalis* ATCC 19433 was used for quality control of the culture media and diagnostic tests (19). For final species determination, molecular identification was performed using polymerase chain reaction (PCR) techniques. In this study, bacterial DNA extraction was conducted using an extraction kit (Invitek Stratec Business) manufactured in Canada, and two pairs of primers were utilized for genus and species identification (Table 1).

The PCR reaction was performed in a final volume of 25 µl, which included 1 µl of each primer, 1 µl of dNTPs, 1 µl of template DNA, 1.7 µl of MgCl2, 2.5 µl of buffer, and 14.8 µl of distilled water, along with 1 unit of Taq DNA Polymerase (all consumables were provided by SinaGen, Iran). The thermal cycler program (Eppendorf serial number 46752 5332) consisted of 35 cycles with the following temperature conditions: an initial denaturation at 94 °C for 10 seconds, annealing at 64 °C for 15 seconds, elongation at 72 °C for 15 seconds, and a final elongation at 72 °C for 5 minutes. After completing the electrophoresis duration, the gel containing the PCR products was placed in a tank with ethidium bromide solution (produced by SinaGen) for 15 to 20 minutes. The presence of the desired bands was observed under ultraviolet (UV) light using a Gel Document device from ATP (serial number 001-020508), and photographs were taken and printed. Following the analysis of the obtained samples to confirm whether the cocci bacteria were indeed *E. faecalis*, two samples of the PCR products with four different readings were sent for sequencing to Macrogen in South Korea through Fazapajouh Tehran.

Table 1. Primers Used in This Study

Primer type	Product size (bp)	Sequence (5´-3´)	Reference
E. faecalis	941	ATCAAGTACAGTTAGTCTTTATTAG ACGATTCAAAGCTAACTGAATCAGT	(20)

2.2. Antibiotic Sensitivity Testing:

To evaluate drug resistance, the standard Kirby-Bauer method was employed to assess the resistance of isolated strains against the following antibiotics: vancomycin (30 µg), ampicillin (10 µg), linezolid (30 µg), imipenem (10 µg), teicoplanin (30 µg), gentamicin (10 µg), penicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), nitrofurantoin (300 µg), and dalfopristin (30 µg). These tests were conducted on Mueller-Hinton agar (Merck, Germany). The standard strain *E. faecalis* ATCC 19433 was used as a control for the disks. Additionally, the minimum inhibitory concentration (MIC) was determined using the E-Test method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines from 2018. The results were interpreted based on CLSI recommendations, where an MIC of vancomycin greater than 32 µg/µl was considered resistant, an MIC of approximately 6-12 µg/µl was categorized as intermediate, and an MIC of less than 4 µg/µl was deemed sensitive.

2.3. Identification of the *van* gene:

In this study, two pairs of primers were utilized (Table 2), which were employed together in a multiplex PCR reaction (21). The PCR reaction was conducted with a final volume of 25 µl, comprising 0.4 µl of each primer, 0.5 µl of dNTPs, 0.5 µl of template DNA, 1.5 µl of MgCl2, 2.5 µl of buffer, 18.7 µl of distilled water, and 0.5 units of Taq DNA Polymerase (SinaGen, Iran). The thermal cycler program included 35 cycles with the following temperature conditions: an initial denaturation at 94 °C for 1 minute, annealing at 57 °C for 1

minute, elongation at 72 °C for 1 minute, and a final elongation at 72 °C for 10 minutes. Subsequently, the PCR product was evaluated on a 1% agarose gel using electrophoresis. After completing the electrophoresis duration, the gel containing the PCR products was placed in a tank with ethidium bromide for 15 to 20 minutes and then observed under UV light to visualize the bands. *E. faecalis* ATCC 51299 with the *vanA* gene and *E. faecium* ATCC 51599 with the *vanB* gene were used as positive controls, while *E. faecalis* ATCC 29212, which is sensitive to vancomycin, served as a negative control. For statistical analysis of the data, version 20 of SPSS software and the chi-square test were employed, with a significance threshold set at $p < 0.05$.

Table 2. Primers Used in This Study

Primer type	Sequence (5´-3´)	PCR product size (bp)	Reference
vanA	F: 5'AATACTGTTTGGGGGTTGCTC3'	734	(22)
	R: 5'CTTTTTCCGGCTCGACTTCCT3'		(22)
vanB	F: 5'GCGGGGAGGATGGTGCGA3'	420	(22)
	R: 5'GGAAGATACCGTGGCTCAAAC3'		(22)

3. Results

Out of the 200 collected samples, 169 samples (84.5%) were isolated from urine, while 31 samples (15.5%) were from blood. Among these, 120 samples (60%) were related to outpatient cases and 80 samples (40%) were from hospitalized patients. The average age of the patients was 37.4 ± 27 years, ranging from a minimum of 10 months to a maximum of 75 years. Of the obtained samples, 110 (55%) were from males and 90 (45%) were from females. After performing PCR, considering the expected size for genus and species, which is approximately 941 base pairs, the amplification resulting from the reaction with the specified primers confirmed a fragment of 941 base pairs. Out of the 200 Enterococcal samples tested, only 100 samples were identified as *E. faecalis* (Fig. 1). Among these, 62 samples were from males and 38 samples were from females, with 42 samples isolated from hospitalized patients and 58 samples from outpatients.

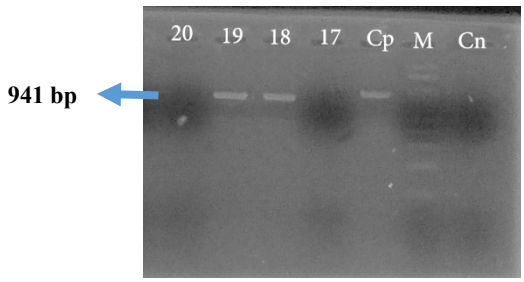

Fig. 1. Display of *E. faecalis* isolates (941 bp)
(M: 100 bp marker, Cn: negative control, Cp: positive control, 18 and 19: *E. faecalis* isolates)

Two samples, N70 and N32, were sent for sequencing to Macrogen, and both N70 and N32 were identified *as E. faecalis* using BLAST at the National Center for Biotechnology Information (NCBI). Based on the antibiotic sensitivity testing, *E. faecalis* exhibited the highest resistance to penicillin, tetracycline, and ciprofloxacin, while showing the greatest sensitivity to linezolid and imipenem (Table 3).

In this study, the determination of the MIC indicated that 31 samples were resistant to vancomycin. Among the resistant strains, 10 strains had an MIC of 48 µg/µl, 9 strains had an MIC of 64 µg/µl, and 12 strains had an MIC greater than 256 µg/µl (Figure 2). The vancomycin-resistant strains were isolated from urine samples with a frequency of 22 samples and from blood samples with a frequency of 9 samples. In this study, 18 vancomycin-resistant strains were isolated from outpatient cases and 13 strains were isolated from hospitalized patients.

Table 3. Percentage of Resistance and Sensitivity of Enterococcal Strains to Antibiotics (%)

Antibiotics	Sensitive	Intermediate	Resistant
Vancomycin	71	6	23
Ampicillin	65	3	32
Linezolid	87	4	9
Imipenem	85	1	14
Gentamicin	50	11	39
Teicoplanin	77	0	23
Penicillin	14	0	86
Ciprofloxacin	41	18	41
Tetracycline	54	3	43
Nitrofurantoin	70	18	12
Dalfopristin	59	11	31

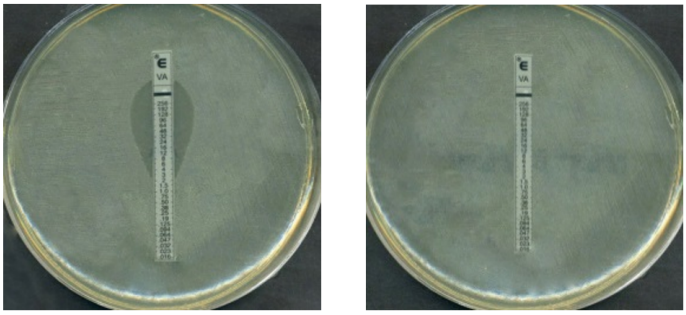

Fig. 2. Determination of Minimum Inhibitory Concentration (MIC) by E-Test

The results of the multiplex PCR indicated that 18 strains contained the *vanA* genotype, 8 strains contained the *vanB* genotype, and 5 strains contained both *vanA* and *vanB* genes (Figure 3). In this study, 88.89% of the *vanA* gene (16 strains) was observed in urine samples, while 11.2% (2 strains) was found in blood samples. The *vanB* gene was detected 100% in urine samples. Among the strains with the *vanA* gene, 61.12%

(11 strains) were from outpatient cases and 33.88% (7 strains) were from hospitalized patients. For the *vanB* gene, 75% (6 strains) were from outpatient cases and 25% (2 strains) were from hospitalized patients. Additionally, 40% (2 strains) of the strains carrying both *vanA* and *vanB* genes were isolated from outpatient cases, while 60% (3 strains) were isolated from hospitalized patients.

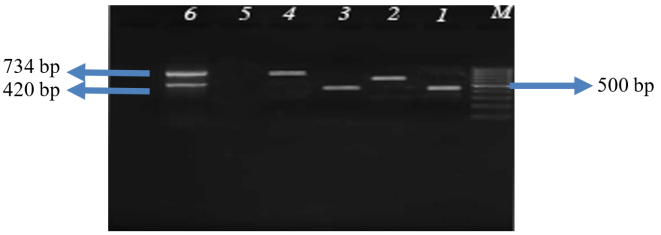

Fig. 3. Results of Amplification of Vancomycin-Resistant Enterococcus Genes by Multiplex PCR
M) indicates a 100 base pair marker, 1) *E. faecium* containing the *vanB* gene (positive control), 2) *E. faecalis* containing the *vanA* gene (positive control), 3) sample containing the *vanB* gene, 4) sample containing the *vanA* gene, 5) *E. faecalis* ATCC 29212, which is sensitive to vancomycin (negative control), 6) containing both *vanA* and *vanB* genes

4. Discussion

Enterococci are the second and third leading causes of urinary tract infections and bacteremia in humans (23). According to the present study, 50% of the 200 samples analyzed were identified as *E. faecalis*. The isolates exhibited the highest resistance to penicillin (83%), tetracycline (43%), and ciprofloxacin (41%), while they showed the greatest sensitivity to linezolid (87%), imipenem (85%), and teicoplanin (70%). Among the 31% of vancomycin-resistant samples, 58.06% contained the *vanA* genotype, 25.81% contained *vanB*, and 16.13% harbored both *vanA* and *vanB* genes. While our study identified vancomycin resistance in *E. faecalis* at significantly higher rates (23% by disk diffusion and 31% by Etest), Mohammadi et al. reported markedly lower resistance (9% by disk diffusion, with only 20% of these confirmed as resistant by Etest), highlighting both methodological differences in sensitivity and potential epidemiological variations in resistance prevalence across study populations and settings (24). Shahraki et al. reported an 18.6% vancomycin resistance rate using both Etest and antibiogram methods, which is lower than the findings of the current study (25). Our study's detection of significant VRE prevalence in outpatients (58.06%) contrasts with traditional hospital-associated epidemiology, suggesting potential community-acquired transmission through livestock exposure, asymptomatic colonization, or prior hospitalization. The outpatient VRE presence raises concerns about silent community spread and challenges empirical vancomycin use, necessitating enhanced outpatient surveillance, stricter antibiotic policies, and molecular typing to distinguish hospital versus community-associated clones for effective containment (26).

A study conducted by Sulaiman et al., found that 30% of all blood samples collected from children were *E. faecalis*, with 91.67% resistant to levofloxacin, 83.33% to amoxiclav, 66.67% to erythromycin, 58.33% to amikacin, 50% to ampicillin, 33.33% to cefotaxime and ceftriaxone, and finally, 25% resistant to vancomycin. Among the existing VRE samples, the *vanA* gene was identified in 89.88%, and the *vanB* gene in 77.78%, indicating that *vanA* is predominant, consistent with the findings of this study (27). According to research by Hosseini et al., which examined 48 enterococcal samples isolated from hospitalized and outpatient patients in southern Fars province, 27.09% of all samples belonged to *E. faecalis*. Overall, 43.75% of enterococcal isolates were resistant to vancomycin, with 40% carrying the *vanA* gene and 20% harboring the *vanB* gene (28). The statistical data from this research do not align with the results of the current study; however, similar to previous findings, the *vanA* gene is considered dominant. In a study by Malatrouni et al., conducted on 400 samples collected from patients in intensive care units (ICU) in Egypt, enterococcal isolates were identified in 12% of patients, with *E. faecalis* accounting for 66.7%. Among all enterococcal isolates, 41.7% were resistant to vancomycin, with the *vanA* gene observed in 85% of VRE samples, while the *vanB* gene was not found in any VRE isolates (29). Ultimately, the statistics and results obtained from this research exceed those of the current study and do not align; nevertheless, similar to findings from this study, the *vanA* gene remains predominant. According to data collected by Hammerum et al., between 2015 and 2022 in Denmark, among 4,862 clinical samples of VRE and Vancomycin-variable enterococci (VVE), approximately 60% were related to urine samples while the remainder were associated with other clinical specimens including blood and pus. About 2,504 samples (51%) contained *vanA E. faecium*, while 1,485 samples (30%) had *vanB E. faecium*; additionally, there were 62 cases (1.2%) with both *vanA* and *vanB* genes in *E. faecium*, 15 samples (0.3%) containing *vanA E. faecalis*, and 23 cases (0.4%) containing *vanB E. faecalis*. This comprehensive analysis highlights significant trends in antibiotic resistance among enterococci, particularly emphasizing the prevalence of *E. faecalis* as a major pathogen in urinary tract infections and its resistance patterns against various antibiotics across different studies and populations (30). According to a study conducted by Adimi et al., on 208 enterococcal isolates collected from clinical samples in three hospitals in Nigeria, 40.9% of the isolates were identified as VRE. In this study, *E. faecalis* constituted 28.2% of the VRE isolates. The *vanA* resistant phenotype was prevalent in 65.9% of the isolates (31). The high prevalence of the *vanA* gene in most studies may be attributed to the high transferability of transposons (28). According to a study by Dadashi et al., on human clinical samples worldwide, *E. faecalis* exhibited approximately 0.9% resistance to various antibiotics, with resistance to linezolid reported at 2.2%. The prevalence of linezolid-resistant *E. faecalis* was found to be 2.8% in Asia and 0.4% in Europe (32). In research conducted by Ghalavand et al., on 63 patients with catheter-associated urinary tract infections (CAUTI), 126 *E.*

faecalis isolates (63 urine samples and 63 fecal samples) were identified. Based on this study, *E. faecalis* isolated from urine and fecal samples showed resistance rates of 88.9% and 76.2%, respectively, against tetracycline, and 87.3% and 71.4%, respectively, against minocycline. According to the results obtained from this study, *E. faecium* showed no resistance to antibiotics such as linezolid, vancomycin, ampicillin, nitrofurantoin, and penicillin, which is contrary to the findings of the current study (33). Karna et al., reported a VRE prevalence of 25.3% among enterococcal samples in 2019, with the highest antimicrobial sensitivity recorded for linezolid (97.8%), teicoplanin (95.6%), and gentamicin (81.3%) (34), similar to the findings of this study where linezolid exhibited the highest sensitivity. In their study, Goudarzi et al. isolated 439 Enterococcus faecalis strains from 690 clinical samples, reporting 0% linezolid resistance and vanA/vanB gene prevalence rates of 72% and 22%, respectively. These findings are inconsistent with the results of our current study (35). The results from Moghimi Baghkhan et al. indicated that vancomycin resistance among enterococcal isolates in Iran was at 14% (36). The study reveals significant variations from reference data primarily due to geographic and population differences in patient demographics, sample sources, and regional antibiotic practices that shape resistance patterns, combined with methodological variations in study scale, techniques, and duration that influence data consistency, along with microbial genetic factors particularly the transposon driven *vanA* and *vanB* genotype showing location-dependent prevalence all of which collectively demonstrate antimicrobial resistance development through complex interactions between local microbiomes, clinical practices, and research methodologies, ultimately explaining apparent contradictions among similar studies.

The findings from this research and other studies indicate a high prevalence of VRE strains among hospitalized and outpatient patients. Variations in VRE prevalence across different studies may arise from factors such as sample size, duration of sample collection, age and gender of patients, as well as the diagnostic methods used for detecting VRE. Given that enterococci exhibit both intrinsic and acquired resistance to various antibiotics, conducting antibiotic susceptibility testing prior to drug administration is essential. Additionally, proper use of antimicrobial agents is recommended to prevent the emergence of more severe antibiotic resistance in bacteria. In summary, this research underscores the importance of continuous monitoring and identification of *E. faecalis* in clinical settings. Further investigations are necessary to clarify the underlying factors contributing to drug resistance and to develop more effective strategies for managing *E. faecalis* infections in clinical environments.

Ethical Statement

The study protocol was approved by the Clinical Research Ethics Committee of Islamic Azad University, Ahar Branch (Date: 2024, No: 22030507931003).

Conflict of interest

Authors declare that there is no conflict of interests.

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No to declare.

Authors' contributions

Concept: A.J.S., M.P., Design: A.J.S., Data collection or Processing: A.J.S., Analysis or Interpretation: S.V., M.F.S., G.K.I., F.G.I., K.S., K.H.K., Literature Search: S.V., M.F.S., G.K.I., F.G.I., K.S., K.H.K., Writing: S.V., M.F.S., G.K.I., F.G.I., K.S., K.H.K.

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Anticancer effect of ethanolic yellow hawthorn extract on chronic myeloid leukemia cells and acute myeloid leukemia cells

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Abstract

Cancer is a disease characterized by abnormal cell growth and invasion and metastasis of these cells to other tissues or organs of the body. Natural products have been used for centuries as drugs or in drug development, especially for the treatment of cancer. Besides, extracting natural products with several bioactive compounds has a promising effect on cancer treatment. In this study, we aimed to investigate the anticancer effect of the ethanolic extract of yellow hawthorn fruits on K562 (Chronic Myeloid Leukemia) and MOLM-13 (Acute Myeloid Leukemia) cell lines. The antiproliferative effect of the ethanolic extract of yellow hawthorn fruits was investigated in time- and dose-dependent manners. The Annexin-V/Propidium Iodide (PI) double staining was used to examine the apoptosis. Furthermore, cell cycle analysis is conducted by PI staining. The cell viability of K562 and MOLM-13 cell lines was significantly reduced by the ethanolic extract of yellow hawthorn fruits with IC50 values of 9144 µg/mL and 3515 µg/mL in 48-hour incubation time, respectively. Moreover, the results showed that the ethanolic extract of yellow hawthorn fruits caused an increased apoptosis by 12.7- and 8.87-fold changes in K562 and MOLM-13 cell lines compared to control groups, respectively. Ethanolic extract of yellow hawthorn fruit has reduced cell proliferation, induced apoptosis and arrested the cell cycle at G0/G1 phase by 71% in MOLM-13 and at G2/M phase by 80.3% and G0/G1 phase by 38.2 % in K562 cells. Further studies should be conducted to elucidate the mechanism of the effect of yellow hawthorn fruit on these cancer cells.

Keywords: yellow hawthorn, ethanolic extract/extraction, natural compounds, cancer, chronic myeloid leukemia, acute myeloid leukemia

1. Introduction

According to the World Health Organization's 2020 data, cancer is the second leading disease in the world, causing approximately 10 million deaths annually (1). Furthermore, it has been stated that the number of deaths it causes in the world will increase to approximately 16.2 million by 2040. Approximately 53,000 people are diagnosed with cancer every day, so it is important to develop novel treatment and diagnosis methods for cancer (2).

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm (MPN) resulting from the clonal proliferation of myeloid progenitor cells in the bone marrow (3). Chronic myeloid leukemia (CML) occurs in 1 or 2 out of 100,000 adults and results from the translocation of the Abelson murine leukemia viral oncogene from chromosome 9 to the BCR on chromosome 22, forming the BCR-ABL1 fusion oncogene. This translocation results in the shortened chromosome 22, known as the Philadelphia (Ph) chromosome (4). Furthermore, this translocation results in the expression of the BCR-ABL oncoprotein, which is a continuously active tyrosine kinase activating downstream signaling pathways such as PI3K/Akt, JAK/Stat5, and Ras/MAPK (5). Moreover, the BCR-ABL oncoprotein ensures the survival, development, inhibition of cell death and self-renewal of cancer cells (6, 7).

Acute myeloid leukemia (AML) is a hematological malignancy formed by genetic alterations in hematopoietic progenitor cells and results in the accumulation of altered cells as myeloid blasts (8, 9). In other words, AML is an aggressive type of cancer that occurs from the proliferation of clonal hematopoietic cells (10, 11). Acute myeloid leukemia (AML), although it can develop at any age, is most commonly diagnosed in individuals over the age of 55, with a median age at diagnosis of 68 years. (12). AML results from various mutations and chromosomal changes (13). In AML, mutations occur most notably in the FMS-like tyrosine kinase 3 (FLT3) (37%), NPM1 (29%), DNMT3A (23%) and N/KRAS (10%) genes (14). FLT3 encodes the receptor tyrosine kinase (RTK) in immature hematopoietic cells. When these immature hematopoietic cells become mature, the FLT3 gene expression is suppressed and tyrosine residues in the FLT3 undergo autophosphorylation and downstream signaling pathways such

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as PI3K/Akt and MAPK are activated and thus abnormal proliferation of cancer cells occurs (15).

Treatment of cancer with natural compounds is an effective method as natural compounds have anticancer effects which may arise from either whole extract of natural compounds or isolated phytochemicals such as, polyphenols, polysaccharides, terpenoids (16). Furthermore, natural products have lower toxicity compared to conventional chemotherapeutics and may decrease the side effects caused by chemotherapeutic drugs. Moreover, these compounds often demonstrate low cytotoxicity on healthy cells (17).

Hawthorn is a plant that grows on thorny shrubs and small trees belonging to the Rosaceae family of the Crataegus genus. It has approximately 280 species and grows in the temperate regions of Europe, East Asia, North America, and 21 species are found in Turkey (18, 19). It has been consumed worldwide for centuries as food, dietary supplements, as well as medicine in traditional treatment methods (20). Its berries, leaves, and flowers have been used for medical purposes for many years due to their antibacterial, anticancer, anti-inflammatory, and antioxidant properties and this plant has been used in the treatment of many diseases, such as cancer, due to its phytochemicals, remarkable effects, and safety profile (21). Hawthorn fruit is rich in various bioactive ingredients such as flavonoids, terpenoids, polyphenols, polysaccharides, and several organic acids (22). Phytochemicals are naturally occurring compounds in plants, fruits, etc. and can suppress the tumor growth, reduce the risk of cancer development by targeting the cellular mechanisms that are altered in cancer progression (23). It is reported that the phenolic extract of Hawthorn (Crataegus pubescens) fruit demonstrates hawthorn has high levels of carbohydrates, terpenes, phenols, and flavonoids (24). Furthermore, it is indicated that the fruits of hawthorn have major phytochemical content rather than its leaves (25). Studies have demonstrated that hawthorn fruit reduces cell proliferation, causes cell cycle arrest, particularly in the G2/M and S phases, and induces apoptotic cell death (26, 27).

Therefore, this research article aimed to investigate the anticancer effect of the extract of yellow hawthorn fruit on K562 and MOLM-13 cells, as no studies have been reported for these cell lines. In the present study, we report the anticancer activities of the ethanolic extract of yellow hawthorn fruit against K562 and MOLM-13 cell lines. Although there is a need for further investigation, our results suggest that the extract used in this study causes a decrease in cellular proliferation, induces apoptosis and causes cell cycle arrest.

2. Materials and methods

2.1. Materials

Soxhlet apparatus, condenser, and absolute ethanol were purchased from ISOLAB. The heating mantle was purchased from Weightlab. Cell lines were obtained from the German National Resource Center for Biological Material (DSMZ). RPMI 1640 was purchased from Serox. The stock solution of ethanolic yellow hawthorn extract is prepared as 250 mg of ethanolic extract and dissolved in 1 mL of RPMI medium for cell culture purposes and stored at -20°C.

2.2. Preparation of the ethanolic extract of yellow hawthorn fruit

Yellow hawthorn fruits were collected from Akarca Village of Nevşehir, Türkiye, in August 2023, soaked in pure water for 30 hours and dried in an oven at 55°C. After three days of drying in oven, they were cut into small pieces with a knife and their seeds were removed. 20 grams of the cut yellow hawthorn fruits were weighed for extraction and were placed on filter paper. The filter paper was closed with staples from both ends and placed in the Soxhlet extractor apparatus. 200 mL of absolute ethanol was added to the filter paper in a ratio of 1/10 to the amount of yellow hawthorn. The heating mantle was set to 80°C and the temperature was measured with a thermometer at certain intervals. The extract of yellow hawthorn fruits was taken in the Soxhlet extractor for a total of 2 cycles approximately for 6 hours. Then, excess solvent was removed under low pressure at 140 rpm at 35°C by suing rotary evaporator (19). In order to use further in vitro tests, the extract was lyophilized for 2 days at 0.05 mBar and -52°C.

2.3. Cell Culture and Maintenance

For cell culture experiments, K562 cells and MOLM-13 cells were grown in RPMI medium supplemented with 10% fetal bovine serum and 100 U/mL penicillin/streptomycin and incubated in a 5% CO_2 incubator at 37°C. When 80% cell density (approximately 2-3 days) was observed in the Petri, the medium of the cells was changed and passaged.

2.4. Cell Viability Assay

The antiproliferative effect of yellow hawthorn extract on K562 and MOLM-13 cells was investigated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) cell viability assay. The MTT assay is carried out with different concentrations (100 µg/mL, 250 µg/mL, 500 µg/mL, 1000 µg/mL, 2000 µg/mL, 3000 µg/mL, 4000 µg/mL, 5000 µg/mL, 10000 µg/mL). The cell viability assay for each cell line and for each incubation time was performed in triplicate. The cells were seeded in 96-well plates at $1x10^4$ density per well in 100 µL. The determined concentration of yellow hawthorn extract was added to each well and incubated for 24, 48 and 72 hours. Following the incubation, 10 µL of MTT dye was added to each well and incubated for 2 hours at 37°C and 5% CO_2. After incubation, 100 µL of dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan crystals and then incubated for 15 minutes on a waving shaker in the dark. Later, the absorbance was determined at 570 nm by using a Varioskan LUX microplate reader (Thermo Scientific) to measure the amount of formazan crystals, and a graph of the absorbance values obtained was created (28).

2.5. Cell Death Assay

The concentrations for cell death assays were selected as 9000

µg/mL and 15000 µg/mL for K562 cells, and 3000 µg/mL and 10000 µg/mL for MOLM-13 cells. The cells were seeded in 6-well plates as $5x10^5$ cells per well in 2 mL and incubated for 48 hours. After incubation, the cells were centrifuged at 300 × g for 5 minutes at 4°C and the supernatant was discarded after centrifugation. The resulting pellet was washed with 1 mL of PBS and then centrifuged at 300 x g for 5 minutes at 4°C. This step was repeated twice. After centrifugation, the supernatant was discarded, and the resulting pellet was solubilized with 200 µL of control and 100 µL of Annexin binding solution. After the pellet was homogenized, 2.5 µL Annexin V and 2.5 µL PI were added and incubated at room temperature in the dark for 15 minutes. After incubation, 400 µL Annexin binding solution was added to each tube. Finally, the cells were analyzed by BD LSRFortessa (Becton Dickinson) flow cytometry (28).

2.6. Cell Cycle Analysis

The same concentrations were used for the cell cycle experiment as in the apoptosis assay. The cells were seeded in 6-well plates as $1x10^6$ in 2 mL per well and incubated for 48 hours. The cells were centrifuged at 260 × g for 10 minutes at 4°C. The supernatant was discarded, and the pellet was homogenized by adding 1 mL of cold PBS and centrifuged again at 260 x g for 10 minutes at 4°C and this process was repeated twice. After centrifugation, 1 mL of cold PBS and 4 mL of cold ethanol were added to the cells and the cells were incubated at -20°C overnight for cell fixation. The next day, the cell suspension was centrifuged at 260 × g for 10 minutes at 4°C and the supernatant was discarded. The resulting pellet was dissolved with 1 mL of cold PBS and centrifuged again at 260 × g for 10 minutes at 4°C. Lastly, the supernatant was removed, and the pellet was homogenized by adding 1 mL of 0.1% Triton-X and 100 µL of RNAase (200 µg/mL) and incubated at 37°C for 30 minutes. After incubation, 25 µL of PI was added to the samples and incubated at room temperature for 15 minutes. Then, the samples were analyzed by BD Biosciences LSRFORTESS Cell Analyzer flow cytometry (29).

2.7. Statistical Analysis

The results are shown as mean±standard deviation. Statistical analysis was prepared using the GraphPad Prism 8.0.2 program. The statistical significance of the results was calculated using one-way analysis of variance (ANOVA). The value of $P \leq 0.05$ was considered statistically significant and the value of $P \leq 0.001$ was considered highly statistically significant.

3. Results

3.1. Yield of Ethanolic Yellow Hawthorn Fruit Extract

The yield is calculated by using general formula (30, 31).

$$yield = \frac{weight\ of\ dry\ extract}{weight\ of\ dry\ plant}\ X\ 100\%$$

The dried yellow hawthorn fruit weighed as 20 grams. After the extraction and powder production process in a freeze-dryer, the powder from of extract is weighed as 4 grams. According to these amounts, the yield was calculated as 20%.

$$yield = \frac{4\ grams\ of\ yellow\ hawthorn\ fruit\ extract}{20\ grams\ of\ dried\ yellow\ hawthorn\ fruit}\ X\ 100\% = 20\%$$

3.2. Ethanolic Yellow Hawthorn Fruit Extract Significantly Inhibited the Cell Proliferation of K562 and MOLM-13 cells

First, the effect of yellow hawthorn ethanolic extract on the proliferation of K562 and MOLM-13 cells was studied. Cells were treated with increasing doses of yellow hawthorn ethanolic extract for 24, 48 and 72 hours. As shown, yellow hawthorn ethanolic extract reduced the proliferation of K562 cells and MOLM-13 cells in a dose- and time-dependent manner (Fig. 1 and Fig. 2). From these dose-time curves, the IC50 values were calculated separately for 3 different treatment times. The IC50 value of K562 cells treated with yellow hawthorn extract for 24, 48 and 72 hours was calculated as 15948 µg/mL (Fig. 1, left). 9144 µg/mL (Fig. 1, middle) and 4858 µg/mL (Fig. 1, right), respectively. Among these results, IC50 value of 24 hours treatment was calculated as an approximate value, since cell viability was not observed under 50%. According to the results, the viability of K562 cells treated with yellow hawthorn extract decreased as the dose of yellow hawthorn extract increased. The IC50 value of K562 cells treated with 3 different periods decreased as well, with prolonged treatment. Thus, it was concluded that yellow hawthorn extract has a significant antiproliferative effect on K562 cells. On the other hand, MOLM-13 cells' viability was also significantly reduced in a dose and time-dependent manner. According to dose-time curves for MOLM-13 cells, IC50 values of treating MOLM-13 cells with yellow hawthorn ethanolic extract in 3 different time manners, which are 24, 48, and 72 hours, are 11772 µg/mL, 3515 µg/mL, and 1240 µg/mL, respectively (Fig. 2, left, middle, right, respectively). Similar to that of the K562 cell lines, IC50 value of 24 hours treatment was calculated as an approximate value, since cell viability was not observed under 50%. These results showed that yellow hawthorn ethanolic extract has an antiproliferative effect on MOLM-13 cells in a time- and dose-dependent manner. Our results demonstrate that ethanolic extract of yellow hawthorn shows better inhibitory effect with lower IC50 values on MOLM-13 cells compared to K562 cells for every incubation time periods.

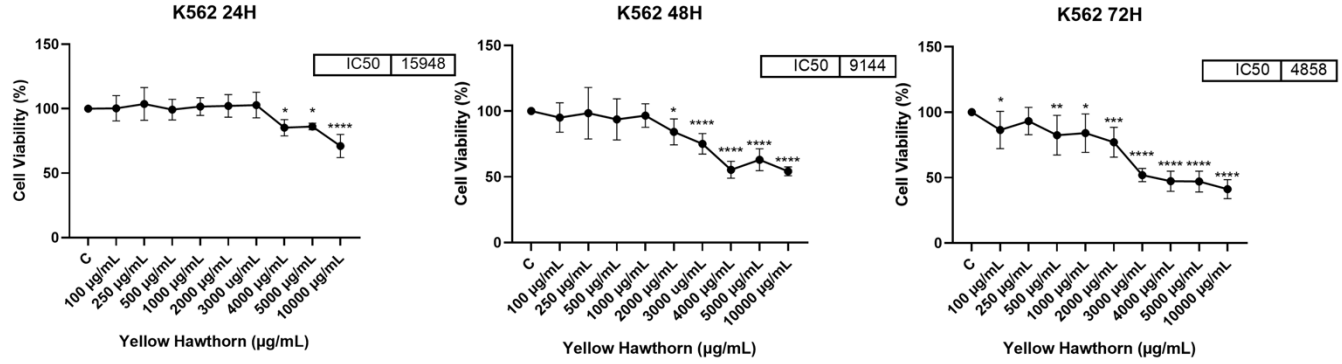

Fig. 1. Antiproliferative effect of ethanolic extract of yellow hawthorn fruit in K562 cell line at increasing doses for 24, 48, and 72 hours. Standard deviation was calculated according to the number of replicates and 3 independent replicates were made. All data are presented as mean ± S.D. (ns = P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001)

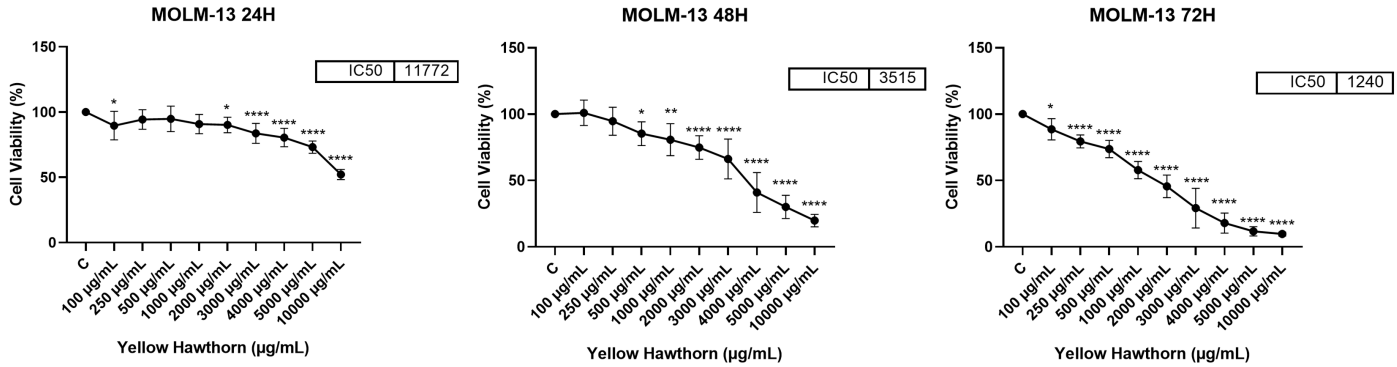

Fig. 2. Antiproliferative effect of ethanolic extract of yellow hawthorn fruit in MOLM-13 cell line at increasing doses for 24, 48, and 72 hours. Standard deviation was calculated according to the number of replicates and 3 independent replicates were made. All data are presented as mean ± S.D. (ns = P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001)

3.3. Yellow Hawthorn Ethanolic Extract Induced Apoptotic Cell death in CML and AML cells

K562 cells were treated with yellow hawthorn ethanolic extract for 48 hours at 9000 µg/mL and 15000 µg/mL, and MOLM-13 cells were treated with 3000 µg/mL and 10000 µg/mL. According to the results, it was shown that yellow hawthorn extract caused apoptotic cell death in K562 cells and MOLM-13 cells with increasing doses of yellow hawthorn extract. K562 cells treated with 15000 µg/mL yellow hawthorn extract increased the ethanolic apoptotic cell amount from 7.45% to 56.15% compared to the control (Fig.3, a). It is observed that ethanolic yellow hawthorn extract increased apoptosis by 4.4 times at the dose of 9000 µg/mL and by 12.7 times at the dose of 15000 µg/mL compared to the control (Fig. 3, b). On the other hand, late and early apoptotic cell population was determined and it was shown that yellow hawthorn extract caused a significant increase in late apoptotic cell population of K562 cells. According to the results, yellow hawthorn extract increased the amount of K562 cells undergoing late apoptosis from 2% to 45.2% at the dose of 15000 µg/mL compared to the control (Fig. 3, a). Furthermore, the viability of K562 cells decreased from 91.15% to 39.2% compared to the control. And also, as shown in the graph, the number of cells undergoing late apoptosis increased by 22.7 times compared to the control (Fig. 3, c). According to the apoptosis test results, it was determined that the ethanolic extract of yellow hawthorn fruit caused apoptosis in K562 cells. It was determined that increasing doses of yellow hawthorn extract significantly led to late apoptosis of K562 cells. On the other hand, it is indicated that at 10000 µg/mL, MOLM-13 cells undergo apoptotic cell death. It is indicated that with 10000 µg/mL dose of yellow hawthorn, ethanolic apoptotic (Q2+Q4) cell death is increased from 8.9% to 54.7% compared to the control. Moreover, the viability of MOLM-13 cells has decreased from 90.8% to 41.35% (Fig. 4, a). Furthermore, the total apoptotic cell death of MOLM-13 cells is increased by 8.87 times compared to the control (Fig. 4, b). It is shown that late apoptotic cell death is increased by 11.5 times with a dose of 10000 µg/mL as compared to the control. In addition to that, there is an increase in early apoptotic cell death, which is 7.8 times compared to the control group (Fig. 4, c). These results demonstrate that yellow hawthorn ethanolic extract causes apoptotic cell death in MOLM-13 cells and as the dose increases, the apoptotic cell death increases. Our data indicated that K562 cells are more sensitive and demonstrate a higher level of apoptosis compared to MOLM-13 cells.

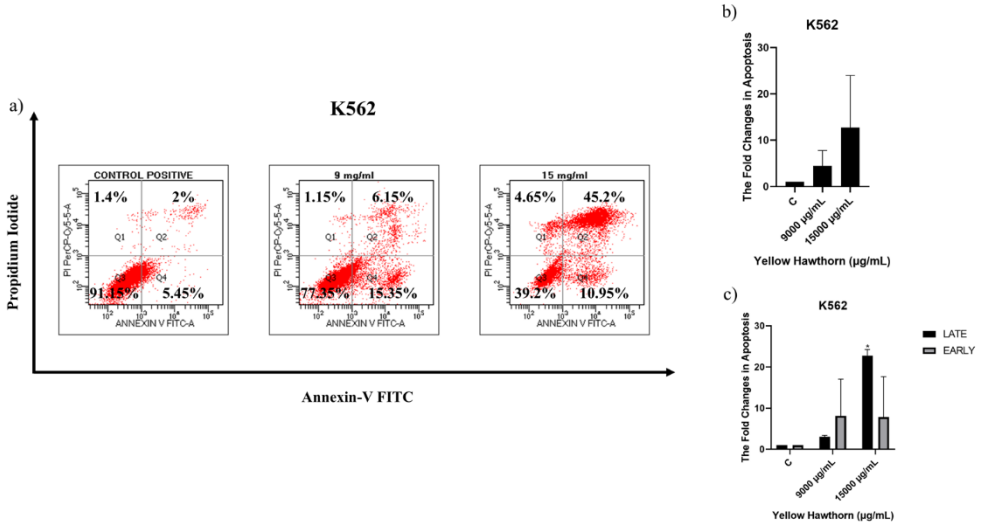

Fig. 3. a) Effect of yellow hawthorn ethanolic extract on apoptotic cell death **b)** Sample histogram of the effect of graphs on total apoptosis **c)** Sample histogram of the effect of graphs on late and early apoptosis. K562 cells were treated with yellow hawthorn ethanolic extract for 48 hours. Apoptosis analysis results and cell percentages are shown. Two independent repetitions were performed, and the results were combined and analyzed. The upper right part (Q2) of the graphs shown in Fig3 a) shows the number of cells that underwent late apoptosis, and the lower right part (Q4) shows the number of cells that underwent early apoptosis. The histogram shown in b) shows the number of cells that underwent total apoptosis (Q2+Q4). All data are presented as mean ± S.D. (ns = P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001)

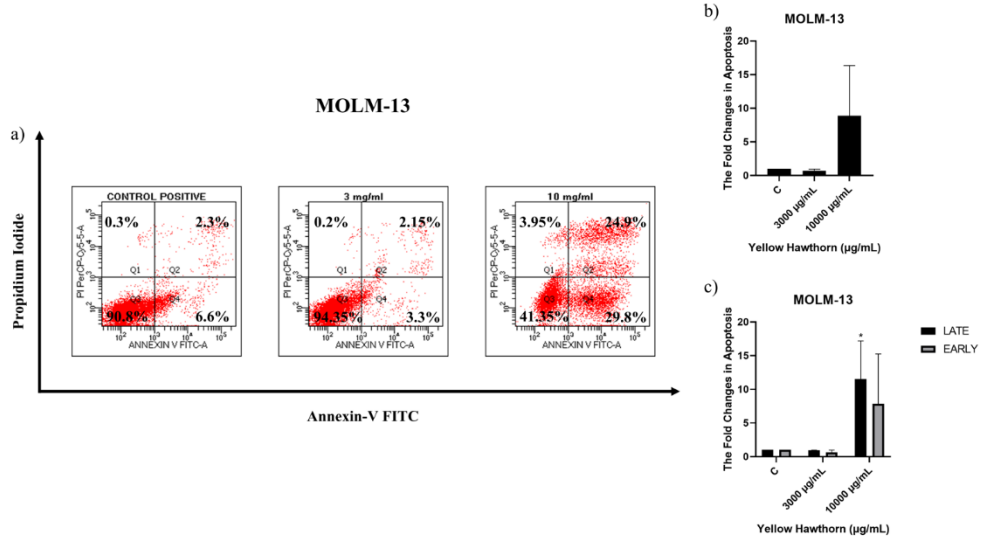

Fig. 4. a) Effect of yellow hawthorn ethanolic extract on apoptotic cell death b) Sample histogram of the effect of graphs on total apoptosis c) Sample histogram of the effect of graphs on late and early apoptosis. MOLM-13 cells were treated with yellow hawthorn ethanolic extract for 48 hours. Apoptosis analysis results and cell percentages are shown. Two independent repetitions were performed, and the results were combined and analyzed. The upper right part (Q2) of the graphs shown in Fig4 a) shows the number of cells that underwent late apoptosis, and the lower right part (Q4) shows the number of cells that underwent early apoptosis. The histogram shown in b) shows the number of cells that underwent total apoptosis (Q2+Q4). All data are presented as mean ± S.D. (ns = P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001)

3.4. Effect of Yellow Hawthorn Ethanolic Extract on Cell Cycle

Cell cycle experiments of K562 and MOLM-13 cells treated with yellow hawthorn extract were conducted with the same doses as indicated in the apoptosis assay. According to the given graph, the 9000 µg/mL concentration significantly arrested K562 cells treated with yellow hawthorn ethanolic extract in the G2/M phase and increased the cell percentage in this phase from 18.8% to 80.3% (Fig. 5, a and b). On the other hand, according to the histogram, yellow hawthorn extract caused an increase in the number of cells in the S phase of K562 cells from 15.35% to 30.8% with 15000 µg/mL (Fig. 5, a). Furthermore, the dose of 15000 µg/mL increased the number of cells in the G0/G1 phase from 4.35% to 38.2% compared to 9000 µg/mL (Fig. 5, a). On the other hand, MOLM-13 cells are arrested at the GO/G1 cell cycle phase with both 3000 µg/mL and 10000 µg/mL doses of yellow hawthorn ethanolic extract. Cell population in the G0/G1 phase increased by 53.1% to 70.6% for 3000 µg/mL, and 71% for 10000 µg/mL compared to the control (Fig. 6, a). The histogram shows that yellow hawthorn ethanolic extract has arrested the G0/G1 phase of the MOLM-13 cell line significantly, both with 3000 µg/mL and 10000 µg/mL (Fig. 6, b). Thus, it is indicated that yellow hawthorn ethanolic extract effects of cell cycle arrest on both K562 and MOLM-13 cells in different phases of the cell cycle.

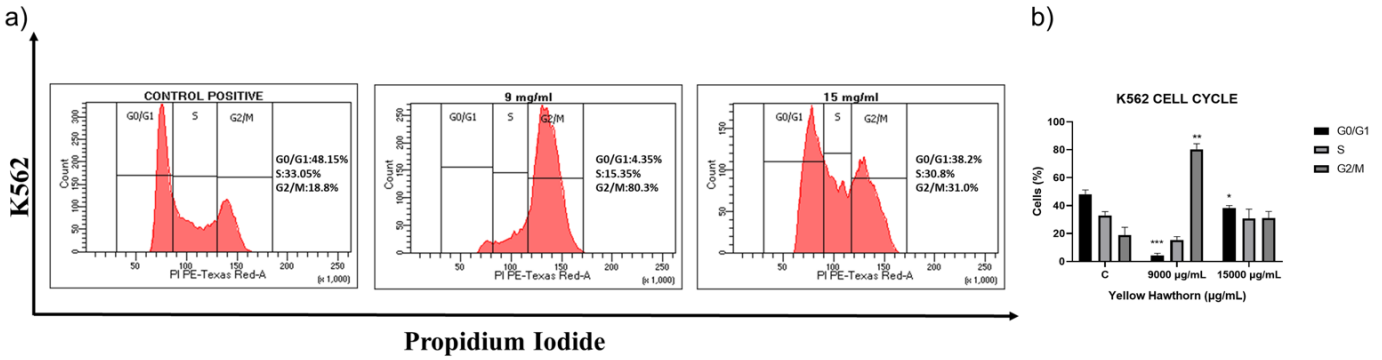

Fig. 5. a) Effect of yellow hawthorn ethanolic extract on cell cycle b) sample histogram of graphs. K562 cells were treated with yellow hawthorn ethanolic extract for 48 hours. Cell cycle analysis results are shown in Fig.5. Two independent replicates were performed and the results were combined and analyzed. All data are presented as mean ± S.D. (ns = P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001)

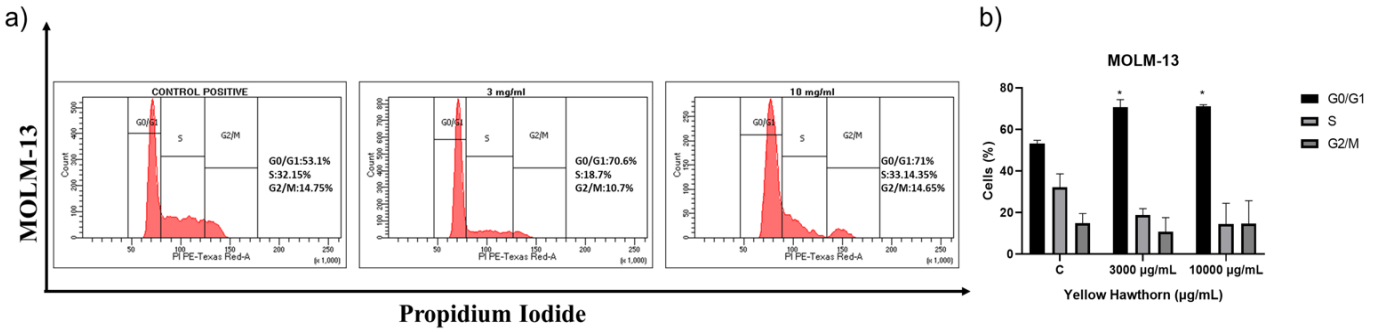

Fig. 6. a) Effect of yellow hawthorn ethanolic extract on cell cycle b) sample histogram of graphs. MOLM-13 cells were treated with yellow hawthorn ethanolic extract for 48 hours. Cell cycle analysis results are shown in Fig.5. Two independent replicates were performed and the results were combined and analyzed. All data are presented as mean ± S.D. (ns = P > 0.05, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001)

4. Discussion

Chronic myeloid leukemia (CML) is a myeloproliferative and cancer type that occurs as a result of the formation of the BCR-ABL1 oncoprotein (3). Acute myeloid leukemia (AML) is a type of hematological cancer which is characterized by the differentiation of hematopoietic stem cells into myeloid cells (8, 9).

Tyrosine kinase inhibitors are used in both CML and AML treatment. In CML, these inhibitors inhibit BCR-ABL1 and break the resistance caused by mutations caused by the BCR-ABL1 oncoprotein (32, 33). On the other hand, these inhibitors are used in AML to inhibit the FLT3 gene which is a tyrosine kinase mutation that activates downstream pathways (34, 35). However, patients with CML and AML develop resistance to tyrosine kinase inhibitors over time and regress treatment (36-38).

Traditional treatment methods have been used for centuries in countries and use natural products and their bioactive compounds (39). Natural products and isolated compounds from them are promising for drug discovery and development in cancer treatment (40). Paclitaxel is an example of an anticancer drug that is an isolated alkaloid (41). Especially, secondary metabolites found in natural products have anticancer effects as they regulate apoptosis, cell cycle, and proteins altered in pathways (42). Natural products are used in the treatment of different diseases and especially, in cancer treatment, as natural products have anti-inflammatory, antioxidant, and antitumor effects. (43). According to studies, it has been determined that compounds such as flavonoids, terpenes, alkaloids, etc. in natural products can also reverse multiple drug resistance (MDR) by regulating signaling pathways and related proteins (44). Ursolic acid which is a type of terpenes and widely found in hawthorn fruit, and it decreases the proliferation and invasion of human glioblastoma cells by downregulating the PI3K/Akt pathway (45, 46). On the other hand, flavonoids have anticancer ability by inhibiting the metabolic activation of cancer cells and downregulation of MAPK, FAK, and PI3K/Akt pathways (47). It is reported that the proliferation of MCF-7 cells which are breast cancer cells is suppressed, and apoptosis is induced by isoflavones (48).

This study examines the anticancer effect of the ethanolic extract of yellow hawthorn fruit on CML and AML cancer cell lines with respect to the induction of cell death and cell cycle arrest. In our study, ethanolic extract of yellow hawthorn significantly decreased the proliferation of MOLM-13 and K562 cell lines in a dose- and time-dependent manner, which is conducted for the first time. However, for the K562 cell line, the IC50 values for time periods are high compared to the MOLM-13 cell line's IC50 values. Similar to our results, it has been reported that the ethanolic extract of hawthorn fruits showed an anticancer effect by inhibiting the proliferation of DLD-1, a colorectal cancer cell line (19). Another study

demonstrated that the proliferation of HCT116 cells is inhibited with the treatment of polysaccharides extracted from hawthorn (26, 27).

As a further study, we continued analyzing the effect of ethanolic yellow extract on apoptosis. According to our data, K562 showed a better apoptotic response than MOLM-13 as their fold changes in apoptosis are 12.7- and 8.87-fold changes. Consistent with our data, Ma et al. reported that apoptosis is induced as apoptotic cell amounts are increased in a dose-dependent manner with the treatment of hawthorn extract in HCT116 cells with a lower dose range (125-1000 µg/mL) (26). Furthermore, the study by Zhou et al. revealed that in liver cancer cell lines, it has been investigated the proliferation of cells is suppressed and apoptotic protein levels are increased. Similarly, this study used the ethanolic extract of hawthorn fruits. Also, the anti-apoptotic protein BCL-2 level is decreased, and the apoptotic protein BAX level is increased, and apoptosis is induced by cleaving the caspase-9 and caspase-8 (21). On the other hand, it has been reported that methanolic hawthorn berry extract reduced the proliferation of glioblastoma cells with increasing PARP-1 levels, resulting in apoptosis induction (49, 50).

To further investigate, we examined the effect of the ethanolic extract on the cell cycle and our results demonstrated that the ethanolic extract induced cell cycle arrest at G2/M phase in K562 cells and the cell population is increased to 80.3% with treatment of 9000 µg/mL. In the same manner, in MOLM-13 cells, the G0/G1 phase is arrested with the treatment of ethanolic hawthorn extract and cells in this phase are increased to 70.6% (3000 µg/mL) and 71% (10000 µg/mL), respectively. The results of this study can be concluded that the ethanolic extract of yellow hawthorn fruit demonstrates the anticancer effect on CML and AML cell lines in terms of a decrease in cell viability, induction of apoptosis and cell cycle arrest. Similar to our data, it has been reported that hawthorn extract arrested cell cycle by a decrease in CDK and cyclin levels, also, p21 expression is increased and G1, S, and G2/M phases arrests are investigated (21). Similarly, Zhang et al. reported that hawthorn extract arrests the cell cycle in the S phase in colon cancer cells, and apoptosis is also increased in these cells. However, this hawthorn extract shows these biological responses with a lower dose (1000 µg/mL) compared to our doses (22). Taken together, we demonstrate that two cell lines with different genetic backgrounds could respond differently to the same yellow hawthorn extract in different biological assays. Two AML cells respond differently to the treatment because K562 and MOLM-13 cell lines belong to different types of hematological malignancies. These cells have different neoplastic transformations that drive their survival and unlimited division. Having different genetic backgrounds activates different signaling pathways, and thus different survival mechanisms would be induced. This could be a reason behind the different responses of these cell lines in terms of apoptosis and cell cycle arrest. In addition to genetic background differences, epigenetic regulation mechanisms could be different, which would have an impact on the response of cells to the same treatment. Besides, the metabolic activities of these cell lines could be variable due to their different FLT3 expression or Bcr-Abl expression profiles for MOLM-13 and K562, respectively. The different oncoproteins may lead to different escape mechanisms for these cell lines. We believe the merit of this study is to reveal the effect of the used natural product on different types of leukemia cells and although a consistent antiproliferative, antiapoptotic and cytostatic effect was observed for both cell lines, further studies should be conducted to reveal the differences between the cell lines.

In this study, the anticancer effect of yellow hawthorn fruit was investigated on chronic myeloid leukemia K562 and acute myeloid leukemia MOLM-13 cells. This study is novel as anticancer effect of yellow hawthorn fruit ethanolic extract has not been investigated before on K562 and MOLM-13 cell lines. Also, it is valuable to examine the effect of hawthorn fruit as an ethanolic extract in cancer treatment as it consists of many bioactive compounds and ethanolic extract gives efficient results compared to single isolated phytochemicals in cancer treatment.

In conclusion, ethanolic extract of yellow hawthorn fruit showed antiproliferative, apoptotic and cytostatic effects on MOLM-13 and K562 cell lines in a dose- and time-dependent manner. Furthermore, the ethanolic yellow hawthorn fruit extract induced apoptosis significantly and arrested the cell cycle in the G2/M phase in K562 and G0/G1 phase in MOLM-13 cells. As a future perspective, a mechanistic follow-up study should be conducted to reveal the underlying mechanism for apoptotic cell death. Some apoptotic and antiapoptotic marker proteins should be detected in response to the treatment of cell lines. In addition, the cell cycle regulatory mechanism should be detected by checking the expression levels of phase specific cyclin dependent kinases or cyclins. The G2/M cell cycle arrests may be due to microtubule disorganization and thus, a follow-up for our study would be further investigations on cytoskeletal organization upon extract treatment. Moreover, even though the motivation of our study was to use a natural product to have anticancer effect on cancer cells, aiming a minimal side effect for healthy cells, this should also be demonstrated using healthy controls for leukemia cells. Finally, the in vivo studies should be designed to show the anticancer effect in leukemia models. Lastly, although the results of the current study require further validation, we believe it will be a notable study for the demonstration of antiproliferative, apoptotic effects and cytostatic effects of the specific natural product on AML and CML cell lines. Further experiments are required for a better understanding of the effect and mechanism of yellow hawthorn ethanolic extract on these cell lines.

Ethical Statement

The authors declare that no ethics committee approval is required for this study.

Conflict of interest

The authors declare no conflict of interest.

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Authors' contributions

Concept: İ.A., Design: İ.A., Data Collection and Processing: A.N.A, Analysis or Interpretation: İ.A., A.N.A, Literature Research: İ.A., A.N.A, Writing: İ.A., A.N.A.

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Abdominal complications after geriatric hip fracture surgery: Epidemiological analysis and identification of risk factors

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Abstract

Abdominal complications, such as postoperative ileus or acute cholecystitis, though not commonly encountered, represent a significant challenge in the management of patients admitted to orthopaedic wards following surgical procedures. Our objective was to assess the prevalence of abdominal postoperative complications following geriatric hip fracture surgery and to identify potential risk factors. Elderly patients with a hip fracture, and operated on by the same surgical team in 2023 were evaluated. Demographic variables such as age and gender, fracture type, and surgical procedures (proximal femoral nail vs. partial prosthesis) were recorded. Abdominal complications that only developed during initial hospitalization were investigated. Of the 97 patients included in the study, the incidence of abdominal complications following geriatric hip fracture surgery was 6.1% (6 patients). The incidence for postoperative ileus, acute cholecystitis, spleen infarction, and rectal bleeding were calculated as 2.1%, 2.1%, 1%, and 1%, respectively. Further analyses demonstrated a weak correlation between the development of abdominal complications and operation type ($p=0.025$, $r=0.259$). No correlation was observed between age, gender and fracture type and the development of abdominal complications. Abdominal complications after geriatric hip fracture surgery are rare, and require a multidisciplinary approach to management. Surgeons should be aware of this potential complication and necessary assessments should be performed before discharge, especially in patients undergoing arthroplasty.

Keywords: arthroplasty, abdominal complication, acute cholecystitis, geriatric hip fracture, partial prosthesis, postoperative ileus

1. Introduction

As the world's elderly population grows, so does the incidence of geriatric health problems. Hip fractures are one of the most common and urgent orthopaedic pathologies in the elderly population (1,2). In contrast to their younger counterparts, hip fractures in the geriatric population typically occur subsequent to low-energy injuries. Female gender, decreased mobility, and low bone density represent risk factors for geriatric hip fractures (3,4). The type of treatment is planned according to the type of fracture, the activity level of the patient, and the accompanying comorbidities (5-7).

One of the most important problems in the treatment of geriatric hip fractures is early postoperative complications and their proper management. The complications that orthopedic surgeons most frequently cite as a cause for concern following a hip fracture include wound site problems, periimplantic fractures and deep vein thrombosis (7,8). Abdominal complications, such as postoperative ileus or acute cholecystitis, though not commonly encountered, represent a significant challenge in the management of patients admitted to orthopaedic wards following surgical procedures. These complications are usually associated with trauma and prolonged immobilization, and have the potential to impair both patient health and postoperative rehabilitation process (9,10).

The objective of this single-center study was to assess the prevalence of abdominal postoperative complications following geriatric hip fracture surgery and to identify potential risk factors.

2. Materials and Methods

Following approval from the ethics committee (decision no:

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E1-22-2916; date: October 5, 2022), all patients admitted to the study clinic with a hip fracture (intertrochanteric, subtrochanteric, or femoral neck) and operated on by the same surgical team in 2023 were evaluated. The study population comprised all patients aged 65 years or older who were able to mobilize (with or without support) before the fracture and who accepted surgical treatment. Patients who were followed conservatively, who were unable to mobilize prior to the fracture, who had a history of abdominal surgery, who had a pathological fracture, and who had a hip fracture as a result of polytrauma were excluded from the study. Furthermore, patients who were followed up in the postoperative intensive care units (ICU) more than 24-hours were also excluded from the study. These patients were excluded from the study on the grounds that ICU follow-up can be performed as a precautionary measure for the first 24 hours. However, the necessity for such follow-up beyond this period is both associated with a deterioration in the patient's general condition, and has a negative impact on the rehabilitation process. Therefore, these patients were excluded. In accordance with the established inclusion and exclusion criteria, a total of 97 patients who were followed-up prospectively were subjected to a retrospective evaluation.

All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

All patients underwent surgery within the initial 72-hour period following their admission to the hospital as an emergency case. Patients who were taking anticoagulants for any reason prior to surgery were discontinued after consultation with the Cardiology or Anesthesia clinics, and were switched to low-molecular-weight heparin (LMWH) prophylaxis. The administration of LMWH was withheld from patients on the night preceding the surgery. All patients received an enema the night before surgery.

All patients underwent surgery with the same surgical team, with arthroplasties performed in the supine position using an anterolateral approach with a modified Watson-Jones incision and internal fixation procedures conducted with a standard nail approach under a traction table.

After surgery, all patients are initially admitted to the post-anesthetic care units (PACU). Following this, they are either transferred to the orthopaedic ward or to the ICU, depending on whether follow-up in the latter is deemed appropriate.

In the postoperative period, LMWH prophylaxis and compression stockings (mechanical prophylaxis) were maintained in all patients. In accordance with the recommendations of the relevant clinic, patients who had used any anticoagulants prior to surgery were restarted on their medication in the postoperative period. No patient was restarted on their medicine without the postoperative recommendation of the relevant clinic and without completing a minimum of 72 hours of postoperative wound site follow-up. All patients were informed of and initiated into lower extremity isometric exercises on the first postoperative day, and all patients were mobilized to the extent they could tolerate on the first post-operative day.

The data set was analyzed in order to ascertain the correlation between abdominal complications and age, gender, and operation performed. In order to investigate the impact of advanced age and fragility, patients were grouped according to age, with those above and below 80 years of age. The surgical procedures performed were classified as internal fixation (proximal femoral nail-PFN) or arthroplasty (partial prosthesis-PP).

The complications were analyzed retrospectively by means of a review of the notes in the patient files, clinical archives and the hospital information record system. In order to ensure that only surgery-related complications were evaluated, complications that only developed during initial hospitalization after hip fracture (before discharge) were included in the evaluation. Surgical complications such as wound site problems, nerve damage and thromboembolism were not evaluated in detail, as they are not the focus of this study.

The IBM® SPSS® Statistics for Windows, version 26.0 (IBM SPSS Corp.; Armonk, NY, USA) software was used to perform the statistical analyses. The terms frequency and percentiles were used to describe the descriptive statistics of categorical data. The mean ± standard deviation and minimum-maximum values were used to define the parameter "age." Bivariate correlation analysis was conducted using Kendall's tau-b (for non-parametric variables) and Pearson (parametric variable) as correlation coefficients. At $P < .05$, statistical significance was taken into consideration.

3. Results

Of the 97 patients included in the study, 43 (44.3%) underwent internal fixation and 54 (55.7%) underwent partial prosthesis. In the present study, the incidence of abdominal complications following geriatric hip fracture surgery was 6.1% (6 patients). The incidence for postoperative ileus, acute cholecystitis, spleen infarction, and rectal bleeding were calculated as 2.1%, 2.1%, 1%, and 1%, respectively. Table 1 presents a detailed distribution of patient and surgical characteristics, and detailed incidences of separate abdominal complications.

Further analyses demonstrated a weak correlation between the development of abdominal complications and operation type ($p=0.025$, $r=0.259$). No correlation was observed between age, gender and fracture type and the development of abdominal complications ($p>0.05$ for each).

All patients who developed abdominal complications were referred to the General Surgery clinic in the early period, and their follow-up and treatment were undertaken by our team. Conservative treatment was preferred for all patients and no patient required secondary acute surgery.

Table 1. Detailed distribution of patient and surgery characteristics

		Geriatric Hip Fractures (n=97)	**Frequency – Percentage** (%)
Age (years)		81.86 ± 7.846 (Range: 65-99)	
Age	Under 80-years	33	34%
	Over 80-years	64	66%
Gender	Female	75	77.3%
	Male	22	22.7%
Operation	PFN	43	44.3%
	PP	54	55.7%
Abdominal Complication	None	91	93.8%
	Yes	6	6.2%
Abdominal Complication	None	91	93.8%
	Ileus	2	2.1%
	Acute cholecystitis	2	2.1%
	Spleen Infarct	1	1%
	Rectal Bleeding	1	1%

N: number of patients, PFN: proximal femoral nailing, PP: partial prosthesis

Table 2. Correlation analysis of variables related to the abdominal complications

		Patients With Abdominal Complications (n=6)	**Patients Without Abdominal Complications** (n=91)	**P**
Age		80.83 ± 5.879 (72-88)	81.92 ± 7.979 (65-99)	0.744
Age	Under 80-years	2 (6.1%)	31 (93.9%)	0.971
	Over 80-years	4 (6.3%)	60 (93.8%)	
Gender	Female	3 (4%)	72 (96%)	0.101
	Male	3 (13.6%)	19 (86.4%)	
Operation	PFN	0	43 (100%)	***0.025***
	PP	6 (11.1%)	48 (88.9%)	***r=0.259***

N: number of patients, P: statistical significance value, PFN: proximal femoral nailing, PP: partial prosthesis, r: correlation efficiency value

4. Discussion

Hip fractures represent a significant health concern among the elderly, being one of the most prevalent types of fractures in this demographic (8,11). The incidence of geriatric hip fractures is rising in parallel with the ageing of the population and the increased level of activity among elder patients in the daily routine. The annual incidence of geriatric hip fractures is estimated to be between 1-3%, with the prevalence in 2050 projected to reach 6.3 million cases per year (12). As with other geriatric patients, the management of geriatric hip fractures should be conducted in a multidisciplinary manner. The usual recommendations after hip surgery, such as mobilization as soon as possible, assume even greater importance in the geriatric population, given that these patients are at increased risk not only of complications that orthopaedic surgeons are accustomed to, but also of abdominal pathologies. The objective of this study was to ascertain the prevalence of abdominal complications following hip fracture surgery in the elderly and to identify any associated risk factors. The incidence of abdominal complications following geriatric hip fracture surgery was found to be 6.1% in our study. Further analysis revealed that only the type of surgery performed was found to be correlated to the abdominal complications.

Postoperative ileus (POI) is a feared postoperative complication, particularly following abdominal surgery. In particular, a lack of postoperative mobility and disturbances in magnesium and other electrolyte values are significant risk factors for the development of ileus. The occurrence of POI following major orthopaedic surgery is a topic of contention, largely due to the traumatic nature of the procedure and the subsequent limitations of mobilization. While the incidence of POI after all major operations is reported to be between 10-14% (13-15), the prevalence of POI following total joint arthroplasties has been documented to range between 0.3-4% (9,10,16). In the present study, the incidence of POI was calculated to be 2.1% after geriatric hip fracture surgery, in line with the literature.

In the existing literature, the incidence of acute cholecystitis in patients aged 70 years and above is reported to range between 13-50% (17-19). Conversely, the available literature on the incidence of acute cholecystitis in geriatric hip fractures is notably scarce. In 2023, Yuan et al. reported the incidence of acute cholecystitis following geriatric hip fracture surgery as 0.13% (17), based on a sample of 7,746 patients. In a meta-analysis of 15,210 geriatric hip fractures, the incidence of acute cholecystitis following geriatric hip fracture surgery was reported as 0.24% (20). In our study, the incidence of acute cholecystitis following a geriatric hip fracture was determined

to be 2.1%. The incidence rates observed in our study are considerably higher than those reported in the literature. The fact that our study was single-centered and the number of patients was relatively limited may have contributed to this discrepancy. It is important to consider that a number of factors, including gender distribution, body mass index and social predispositions, may also influence the development of acute cholecystitis.

The remaining two abdominal complications identified in our study, splenic infarction and rectal bleeding, are both potential complications that can be attributed to alterations and irregularities in anticoagulant utilization (21,22). In 2012, Boland et al. presented a case of rectal bleeding following total hip arthroplasty in a geriatric patient with preoperative oral anticoagulant use (22). Similarly, in the course of our study, we observed that the patient who had rectal bleeding had been taking an oral anticoagulant prior to the surgical procedure, had switched to LMWH prophylaxis following the fracture, and had resumed taking the oral anticoagulant in the postoperative period. Our study found the incidence of both complications to be 1%, and larger cohort studies may provide more objective incidences.

In accordance with the literature, a correlation was identified between the development of abdominal complications and arthroplasty procedures ($p=0.025$, $r=0.259$). On the other hand, age and gender were not found to be correlated with the development of abdominal complications ($p>0.05$). The existing literature on this subject contains a number of conflicting reports. Klasanet al. described the risk factors for POI after total joint arthroplasty as previous abdominal surgery, chronic renal failure, myocardial infarction, and hip arthroplasty, and they stated that age and gender were not found to be effective in the development of POI (9). Murphy et al., investigated the independent risk factors of POI and concluded that older age, male gender and open operative approach are important risk factors (13). Gender has been identified as a significant risk factor in the development of cholecystitis, with a higher incidence of the condition reported in women (23,24). Due to the limited sample size of our cohort, subgroup analyses based on each complication were not feasible. Consequently, risk factor analyses of abdominal complications were conducted as a unified whole. Despite the controversial results in the literature, the findings of our study indicate that age and gender are not associated with the development of abdominal complications in geriatric hip fractures. Statistical analysis revealed that only the type of surgery performed was found to be a significant predictor of the development of abdominal complications ($p=0.025$, $r=0.259$). When considering the underlying mechanisms, it is plausible that the supine position during arthroplasty, with assistants leaning on the patient's abdomen and the patient's fractured side's upper limb folded and placed on the abdomen, may contribute to this relationship.

It should be noted that our study is not without limitations. Our study was conducted at a single center with a relatively small number of patients. While the inclusion of operations performed by a single surgeon and the avoidance of surgical technique and time discrepancies as variables are advantageous, the limited number of patients precludes the possibility of subgroup analysis. Additionally, this retrospective study is limited by the lack of analyses of comorbidity, opioid use and length of hospitalization. The inclusion of data from multiple centers with a larger number of patients may provide more objective data and a more accurate representation of complication incidences.

Abdominal complications after geriatric hip fracture surgery are rare, and require a multidisciplinary approach to management. In this single-center study of geriatric hip fractures operated on by a single surgeon, the incidence of perioperative abdominal complications was calculated to be 6.1%. Surgeons should be aware of this potential complication and necessary assessments should be performed before discharge, especially in patients undergoing arthroplasty.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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Authors' contributions

Concept: E.N.G., B.G., S.E., Design: E.N.G., B.G., T.S., S.E., Data Collection or Processing: E.N.G., T.S., B.G., Analysis or Interpretation: E.N.G., B.G., T.S., S.E., Ö.D., Literature Search: E.N.G., B.G., Writing: E.N.G., T.S., B.G.

Ethical statement

This study was approved by the local ethics committee (decision no: E1-22-2916; date: October 5, 2022).

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Curcumin protects against ketoconazole-induced hepatotoxicity in Wistar rats

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Abstract

Ketoconazole (KZ) is a broad-spectrum antifungal drug that can cause hepatotoxicity. Curcumin (CM), an isolate of *Curcuma longa L.* is used in folk medicine to treat various ailments. This study assessed whether CM can protect against a rat model of KZ-induced hepatotoxicity. Thirty healthy adult Wistar rats (220-240g) were randomly grouped (n=5/group) and supplemented orally with CM (50, 100 and 200 mg/kg/day) prior to the oral administration of KZ (200 mg/kg/day) for 14 days. On day 15, the rats were weighed and anesthetized. Blood samples were collected, and sera were extracted for biochemical analyses. Liver samples were excised, weighed and processed for oxidative stress markers and histology. KZ significantly ($p < 0.01$) decreased body weight and significantly ($p< 0.001$) decreased liver glutathione, superoxide dismutase, catalase, and glutathione peroxidase levels when compared to control. KZ significantly ($p < 0.01$) increased liver weight and significantly ($p < 0.001$) increased serum gamma-glutamyl transferase, alkaline phosphatase, amino transferases, lactate dehydrogenase, total bilirubin and liver malondialdehyde levels when compared to control. KZ caused hepatocyte necrosis. However, CM supplementation significantly restored body and liver weights at 50 mg/kg ($p < 0.05$), 100 mg/kg ($p < 0.01$) and 200 mg/kg ($p < 0.01$) when compared to KZ. CM supplementation restored serum biochemical and liver oxidative stress markers at 50 mg/kg ($p < 0.05$), 100 mg/kg ($p < 0.01$) and 200 mg/kg ($p < 0.001$) when compared to KZ. CM restored liver histology. CM was effective against KZ-induced hepatotoxicity in a dose-related fashion.

Keywords: curcumin, ketoconazole, liver, prevention, toxicity

1. Introduction

Hepatotoxicity is a serious adverse drug reaction that occurs as a result of liver damage caused by toxic chemical substances or drugs leading to liver dysfunction, after ruling out other potential causes (1). Antifungals are commonly associated with hepatotoxicity. The risk of antifungal drug induced hepatotoxicity is complex and can be influenced by pre-existing liver diseases as well as various factors such as patient demographics, drug-drug interactions, comorbidities, environmental and genetic factors and chemical properties of the drug (2). Hepatotoxicity from antifungal drugs typically manifest as increased serum aminotransferase levels, but the clinical significance of these changes is not always clear. The incidence of hepatotoxicity from antifungal drugs varies widely with higher rates seen in patients taking azole antifungal drugs (2).

Ketoconazole (KZ) is a broad-spectrum azole-based antifungal drug. It works by inhibiting the biosynthesis of ergosterol in fungal cell which increases the permeability of mycetes, ultimately causing death (3). It is commonly used to treat patients with systemic fungal infections. Recently, clinical data from China has linked KZ with severe cases of hepatotoxicity (3). Additionally, based on clinical data, the United States issued safety information urging caution when using KZ due to the risk of potentially fatal hepatotoxicity. In humans. It has been associated with hepatitis, elevated transaminases, and hepatic necrosis (4). Literatures have shown that it may cause acute and severe liver failure, especially in individuals with pre-existing liver diseases. In addition, preclinical experiments have shown that KZ can altered liver structure (5).

Natural products serve as a repository for the identification and discovery of chemicals that can be processed and used for the treatment of ailments. Most drugs used clinically were sourced from natural products or their derivatives (6). Curcumin (CM) is a natural bioactive polyphenolic compound extracted from the rhizome of *Curcuma longa* Linn. It is an orange-red powder that is tasteless, and insoluble in water which has received significant recognition as a dietary supplement with potential therapeutic activities on a wide range of ailments (7, 8). These potential therapeutic activities

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include anticancer, anti-inflammatory, antiviral and antibacterial. Additionally, it has shown therapeutic potential in mental cognitive disorders, tumors, cerebrovascular and cardiovascular diseases (7). It has shown strong antioxidant activity through the scavenging of free radicals and the upregulation of antioxidant proteins. Its anti-inflammatory effect includes the suppression of proinflammatory cytokines (6,9). Aside from the aforementioned activities, it has shown promising liver protective activity in animal studies against ethanol and paracetamol-induce hepatotoxicity (10, 11). It also exhibited protective activity against liver oxidative stress and inflammation caused ochratoxin and carbon tetra chloride (9,12). In light of the aforementioned, this research novelty assessed the protective ability of CM against KZ-induced hepatotoxicity in Wistar rats.

2. Materials and Methods

2.1. Drugs, chemicals and animals

Thirty healthy adult Wistar rats of both sexes weighing 180-200g were randomly grouped into 6 groups of n=5 per group in metallic cages. The rats were purchased from the Animal handling units of the Faculty of Pharmacy, Madonna University, Nigeria. They were kept under standard temperature (25-30ºC) with 12-h light/dark cycle and had access to food and water freely. The rats were acclimated to laboratory conditions before the experiment began. The European Communities Council Directive of 24 November 1986 (86/609/EEC) for animal handling was followed (13). Ethical approval was obtained from the research ethics committee of the Department of Pharmacology/toxicology, Faculty of Pharmacy, Madonna University. Modified doses of KZ (200 mg/kg/day) (14) and CM (50-200 mg/kg/day) (15) were used. Piperine (20 mg/kg/day) was added to CM to increase bioavailability (15). KZ (2) and CM (15) were suspended in normal saline

2.2. Administration of drug and chemicals.

The rats were orally administered with the chemical agents daily for 14 days as follows; Groups I (Control) and II were administered with normal saline (0.2mL) and CM (200 mg/kg), respectively whereas group III was administered with KZ (200 mg/kg). Groups IV-VI were supplemented with CM; 50 mg/kg, CM; 100 mg/kg, and CM 200 mg/kg prior to the administration of KZ (200 mg/kg).

2.3. Animal sacrifice and sample collection

On day 15, the rats were euthanized with thiopental sodium (40 mg/kg). Blood samples were obtained from the heart in heparinized containers and evaluated for serum total bilirubin (TB), alanine amino transferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), conjugated bilirubin (CB) and alanine amino transferase (AST). Liver tissues were excised, rinsed in saline (0.9% NaCl) and preserved in neutral buffered formalin (10%) for histological analysis. Liver tissues were also processed to produce homogenates in ice-cold 20 mM tris hydroxymethyl aminomethane buffer. Thereafter, the homogenates were centrifuged (3000 X g for 30min at 4ºC), the supernatants obtained and evaluated for oxidative stress markers.

2.4. Evaluation of biochemical markers

Serum total bilirubin, AST, GGT, ALT, TB, ALP, LDH and CB were evaluated using an auto chemistry analyzer.

2.5. Assay of liver oxidative stress markers

Glutathione (GSH) and glutathione peroxidase (GPx) activities were analyzed as described by Rotruck *et al.,* 1973 (16) and Sedlak and Lindsay, 1968 (17), respectively. Superoxide dismutase (SOD) and catalase (CAT) activities were evaluated as documented by Sun and Zigman, 1978 (18) and Aebi, 1974 (19), respectively. Malondialdehyde (MDA) was analyzed as explained by Buege and Aust, 1978 (20).

2.6. Liver histology

Liver tissues were fixed for 24 h in neutral formalin (10%) solution and thereafter, dehydrated in ascending ethanol concentrations. The tissues were processed and fixed in paraffin wax. The processed tissues were sectioned (five-micrometer-thick), stained with hematoxylin and eosin and viewed with a Nikon Eclipse E200-LED microscope (Tokyo, Japan).

2.7. Data analysis

Results as mean ± standard error of mean (SEM) of five replicates. Two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test were used for data analysis with the aid of Graph Pad Prism (Version 5.0, Graph Pad Software Inc., La Jolla, California, U.S.A.). *P* values < 0.05, < 0.01 and < 0.00 were used to express significance.

3. Results

3.1. Protective effect of curcumin on the body and liver weights of ketoconazole- administered rats

CUM (200 mg/kg) had no significant ($p>0.05$) effects on the body and liver weights of rats when compared to the control. However, KZ (200 mg/kg) decreased body weight and increased liver weight significantly ($p<0.01$) in rats when compared to the control (Table 1). Interestingly, body and liver weights were restored significantly by CM; 50 mg/kg ($p<0.05$), CM; 100 mg/kg ($p<0.01$) and CM; 200 mg/kg ($p<0.01$) supplementation when compared to KZ (Table 1).

3.2. Protective effect of curcumin on the serum liver biochemical markers of ketoconazole- administered rats

Serum ALT, LDH, ALP, GGT, CB, AST and TB were normal ($p>0.05$) in CM (200 mg/kg) administered rats when compared to the control. The aforementioned biochemical markers were significantly ($p<0.001$) elevated in KZ (200 mg/kg) administered rats when compared to the control (Table 2). But the aforementioned serum biochemical markers were significantly restored in a dose-related fashion in CM; 50 mg/kg ($p<0.05$), CM; 100 mg/kg ($p<0.01$), and CM; 200 mg/kg ($p<0.001$) supplemented rats when compared to KZ (Table 2).

Table 1. Effect of curcumin on body and liver weights of ketoconazole- administered rat

Groups	Dose (mg/kg)	FBW (g)	ALW(g)	RLW (%)
Group I	NS	261.6±20.1	6.31±0.22	2.41±0.08
Group II	CUM 200	255.0±18.7	6.30±0.17	2.47±0.01
Group III	KZ 200	140.7±10.2*	14.24±0.45*	10.12±0.08*
Group IV	CUM 50 + KZ 200	200.9±19.3 [a]	11.51±0.43 [a]	5.73±0.25 [a]
Group V	CUM 100 + KZ 200	249.3±20.2 [b]	7.42±0.24 [b]	2.98±0.43 [b]
Group VI	CUM 200 + KZ 200	255.8±19.7 [b]	6.24 ±0.36 [b]	2.44±0.21 [b]

Data as mean ± SEM, n = 5. NS: Normal saline, KZ: Ketoconazole, CUM: Curcumin, *p < 0.01 Significant difference when compared to control, a p < 0.05; b p < 0.01 Significant difference when compared to KZ (ANOVA).

Table 2. Effect of curcumin on serum biochemical markers of ketoconazole- administered rats

Groups	Dose (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (g/dL)	CB (g/dL)	LDH (U/L)	GGT (U/L)
Group I	NS	40.11±2.00	42.51±6.00	35.83±4.32	5.72±0.21	3.20=±0.21	22.82±2.00	0.31±0.05
Group II	CUM 200	38.80±3.42	40.42±4.21	34.04±2.11	5.43±0.17	3.11±0.33	22.47±3.21	0.29±0.09
Group III	KZ 600	130.01±17.1*	135.70±15.0*	99.23±9.01*	17.74±1.00*	14.63±1.51*	87.16±10.5*	1.01±0.01*
Group IV	CUM50+KZ 200	97.62±10.2[a]	99.21±10.1[a]	77.15±7.16[a]	12.90±0.92[a]	11.25±0.62[a]	60.65±5.22[a]	0.76±0.02[a]
Group V	CUM 100+KZ200	65.91±8.66[b]	69.45±5.22[b]	55.96±3.21[b]	7.99±0.41[b]	7.66±0.77[b]	35.14±3.00[b]	0.56±0.01[b]
Group VI	CUM 200+KZ200	42.13±6.68[c]	47.24±6.71[c]	36.54±4.55[c]	5.97±0.66[c]	3.57±0.431[c]	23.09±2.11[c]	0.35±0.07[c]

Data as mean ± SEM, n = 5. NS: Normal saline, KZ: Ketoconazole, CUM: Curcumin, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferse, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, TB: Total bilirubin, ALP: Alkaline phosphatase. n=5, *p < 0.001 Significant difference when compared to control. a p < 0.05; b p < 0.01 and c p < 0.001 Significant difference when compared to KZ. (ANOVA).

3.3. Protective effect curcumin on liver oxidative stress markers of ketoconazole- administered rats

Liver SOD, GSH, MDA, CAT, and GPx levels did not change significantly (p>0.05) in CM (200 mg/kg) administered rats when compared to the control. KZ (200 mg/kg) significantly decreased liver SOD, GSH, CAT, and GPx and significantly elevated liver MDA levels at p<0.001 when compared to the control (Table 3). But liver SOD, GSH, MDA, CAT, and GPx levels were restored in a dose-related fashion by supplementation with CM; 50 mg/kg (p<0.05), CM;100 mg/kg (p<0.01), and CM; 200 mg/kg (p<0.001) when compared to KZ (Table 3).

3.4. Protective effect curcumin on the liver histology of ketoconazole- administered rats

The liver of rats in the control group (Fig.1.a) and CM (200 mg/kg/day) (Fig.1.b) administered group showed normal histology. In contrast, the liver of KZ (200 mg/kg) administered group showed severe hepatocytes necrosis and central vein congestion (Fig.1.c). Furthermore, the liver of rats in the group supplemented with CM; 50 mg/kg showed mild hepatocytes necrosis and inflammatory cells (Fig.1.d). The liver of rats in the group supplemented with CM; 100 mg/kg (Figure e), and CM; 200 mg/kg (Fig.1.f) showed congested central vein.

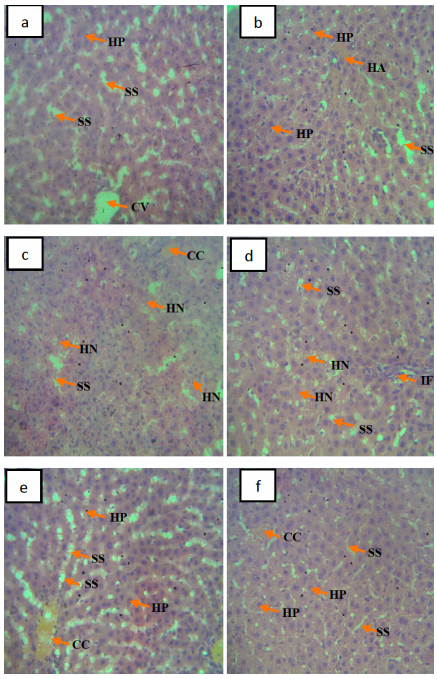

Fig.1. (a) and (b) are the liver of the control and CM (200 mg/kg) administered rats, respectively. (c) is the liver of KZ (200 mg/kg) administered rats. (d), (e) and (f) are the liver of CM; 50 mg/kg, 100 mg/kg, and 200 mg/kg supplemented rats, respectively. HP: Normal hepatocytes, CV: Normal central vein, HN: Hepatocytes necrosis, IF: Inflammatory cells, SS: Sinusoids, HA: Hepatic artery, CC: Congested central vein

Table 3. Effect of curcumin on liver oxidative stress markers of ketoconazole - administered rats

Group	Dose (mg/kg)	SOD (u/mg protein)	CAT (u/mg protein)	GSH (µg/mg protein)	GPX (u/mg protein)	MDA (nmol/mg protein)
Group I	NS (0.2mL)	43.20 ± 4.00	40.56± 4.01	23.62 ± 3.20	28.16 ± 3.00	0.13 ± 0.01
Group II	CUM 200	42.32 ± 3.45	40.72 ± 5.22	24.01 ± 3.35	29.19 ± 2.74	0.10 ± 0.05
Group III	KZ 200	17.65 ± 2.21*	18.32 ± 2.51*	7.73 ± 0.34*	9.69 ± 0.51*	0.78 ± 0.07*
Group IV	CUM 50+KZ 200	22.02 ± 2.00a	22.30 ± 2.37a	11.41 ± 1.62a	13.57 ± 1.22a	0.47 ± 0.01a
Group V	CUM 100+KZ 200	30.33 ± 3.71b	30.31 ± 3.56b	15.32 ± 1.44b	18.47 ± 2.31b	0.21 ± 0.06b
Group VI	CUM 200+KZ 200	39.21± 3.44c	38.55± 4.02c	21.22 ± 3.72c	27.23 ± 2.51c	0.15 ± 0.04c

Data mean ± SEM, n = 5. Normal saline, KZ: Ketoconazole, CUM: Curcumin, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde, GPX: Glutathione peroxidase, * $p < 0.001$ Significant difference when compared to control. a $p < 0.05$; b $p < 0.01$; c $p < 0.001$ Significant difference when compared to KZ. (ANOVA).

4. Discussion

The essential role of the liver in drug biotransformation makes it a primary target for toxicity (21). Metabolites, or parent drugs, can elicit different types of biochemical changes such as oxidative stress and covalent binding leading to signal transduction, endoplasmic reticulum and, mitochondrial stress which can cause cell death. Alternatively, these stress responses may indicate the occurrence of idiosyncratic drug associated hepatotoxicity (22). Oral KZ use has been linked to both acute and chronic hepatotoxicity (23). Studies have shown that CM is a naturally occurring substance with numerous biologic activities of medicinal importance (24). This study aims to explore the protective benefit of CM against KZ-induced hepatotoxicity in Wistar rats.

Body and organ weights are important factors in assessing test article-associated toxicities. Studies haves shown that changes in body and organ weights may be as a result of treatment-related effects (25). In this study the rats administered with KZ showed a decrease in body and an increase in liver weights. This aligns with decrease in body and increase in liver weights in rats administered with KZ (200 mg/kg/day) for 15 days (26). The increase in liver weight may indicate changes such as hepatocellular hypertrophy possibly due to peroxisome proliferation or enzyme induction (25, 26). On the other hand, the decrease in body weight could be due to decrease in body mass. Interestingly, supplementation with CM was able to restore both liver and body weights.

In clinical practice, the traditionally used biochemical markers to detect liver injury measures alterations in serum liver function indexes, and changes in liver tissues. The evaluation of liver function depends on serum biomarkers like total bilirubin, ALT, GGT AST, LDH and ALP (27, 28). In this study, KZ administration impaired liver function significantly by elevating serum ALT, GGT AST, ALP, LDH, TB and CB levels. This observation correlates with the elevated serum biochemical markers reported in rats administered with KZ (200 mg/kg/day) for 15 days (26). The elevation in TB level shows incapacitated liver activity, increased AST and ALT activities reflect hepatocellular necrosis, and increased ALP levels reflect damage to biliary epithelial cells or canalicular membrane (29, 30). Elevated TB may be a consequence of excess synthesis, poor liver absorption, altered metabolism, conjugation errors, or biliary excretion errors (31). The damage to the liver hepatic cell membrane is evidenced by increased AST and ALT circulation (32). However, this study observed that CM supplementation normalized liver function marked by restored serum liver biochemical markers in a dose-related fashion.

Exposure of the liver to reactive oxygen species (ROS) during metabolic functions and xenobiotics biotransformation can disrupt the redox balance causing oxidative stress. This can impair liver function, and modulate proinflammatory pathways contributing to diseases. Oxidative stress has been associated with the pathogenesis and progression of acute and chronic liver diseases (33). The liver possesses robust antioxidant defense mechanisms such as SOD, GPx, CAT and GSH which help maintain ROS at physiological levels. CAT and SOD protect the liver from superoxide radicals and hydrogen peroxide (34), while GSH, acts as a detoxifying agent removing toxic compounds and heavy metals (34). However excessive liver ROS activity can overwhelm these antioxidants leading to oxidative stress (35). In the current study, KZ caused significant liver oxidative stress characterized by low antioxidant levels and elevated MDA level, a primary marker of lipid peroxidation. This supports previous findings of rats administered with KZ (14). Similar results were reported with KZ (200 mg/kg/day) administration for 15 days (26). Furthermore, CM supplementation inhibited KZ-induced liver oxidative stress by stabilizing antioxidant and MDA levels in a dose-dependent manner. The observation may be attributed to CM's ability to scavenge or inhibits ROS, thereby preventing KZ-induced liver oxidative stress.

In addition to the elevated serum biochemical markers, this study observed altered liver histology marked by hepatocyte necrosis in rats administered with KZ. This is consistent with

the findings reported by Hamza *et al.,* in rats given 100 mg/kg/day of KZ for 5 days (14). It also agrees with similar observations reported in rats administered with KZ (100 mg/kg/day) for 15 days (26). The altered liver histology observed in this study, might be due to the induction of oxidative stress via ROS production by KZ causing damage to liver biomolecules (14). Nonetheless, supplementation with various doses of CM restored liver histology.

The mechanism by which KZ causes hepatotoxicity is yet to be established, but might be idiosyncratic or immune mediated as speculated by some studies (36). Additionally, studies have suggested a disruption in liver homeostasis through the generation of ROS leading to liver oxidative stress (14). Excessive ROS activity within the liver can damage proteins, lipids, and DNA, resulting in structural and functional liver impairment and potentially progressing to various liver diseases (37). The evident protective activity of CM observed in this study may due to its antioxidant effect through free radical scavenging. CM has the ability to scavenge free radicals, such as reactive oxygen and nitrogen species (7,38,39) and can enhance the activities of GSH, CAT, and SOD in neutralizing free radicals. Furthermore, it can inhibit ROS-producing enzymes like xanthine oxidase/hydrogenase and cyclooxygenase/lipoxygenase. As a chain-breaking antioxidant, CM is a lipophilic compound that efficiently scavenges peroxyl radicals and can also inhibit inflammation through various mechanisms (39, 40).

CM supplementation restored biochemical and liver histological changes-induced by KZ in a dose-related fashion. CM may have clinical benefit in KZ associated hepatotoxicity.

Conflict of interest

Authors declare that there was no conflict of interest.

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None to declare.

Authors' contributions

Concept: E.A., N.O.E., B.S.D., Design: E.A., N.O.E., B.S.D., Data Collection or Processing: E.A., N.O.E., B.S.D., Analysis or Interpretation: E.A., N.O.E., B.S.D., Literature Search: E.A., N.O.E., Writing: E.A., N.O.E., B.S.D.

Ethical Statement

Ethical approval was obtained from the research ethics committee of the Department of Pharmacology/toxicology, Faculty of Pharmacy, Madonna University.

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Is the risk of colorectal cancer increased after a diverticulitis attack? Is routine colonoscopy necessary? our clinical experience

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Abstract

Diverticular disease is common, particularly in Western societies and in older age groups. Diverticulitis, occurring in 10–25% of cases, is the most frequent complication of this condition. Computed tomography (CT) is the standard imaging modality for diagnosing diverticulitis. Previous studies have demonstrated an increased incidence of colorectal cancer (CRC) in colonoscopies performed after episodes of diverticulitis and its complications. This study aims to evaluate the necessity of colonoscopic assessment in differentiating colorectal cancers in patients diagnosed with diverticulitis and its complications via abdominal CT. Between January 2018 and December 2024, 290 cases diagnosed with diverticular disease and its complications were retrospectively analyzed in the General Surgery Department of Recep Tayyip Erdoğan University Training and Research Hospital. Twenty-one patients were excluded from the study due to mortality following perforation or lack of follow-up. Demographic and clinical data were obtained from hospital records. Among the 269 patients included in the study, malignancy was detected in 37 (13.8%) cases after elective colonoscopy. The mean age of patients diagnosed with colorectal malignancy was 70.3 ± 9.7 years, which was statistically significantly higher compared to patients without malignancy. CT evaluations of wall thickening at the diverticulitis site revealed malignancy in 22 of 105 patients (21%) with localized wall thickening. Additionally, tumors were identified in 12 of 13 patients (92.3%) with wall thickening in colonic segments outside the site of diverticular disease. The risk of malignancy in patients with diverticulitis increases with age. Many studies have recognized an increased incidence of colorectal cancer following diverticulitis episodes. Based on our findings, we recommend performing elective colonoscopy 6–8 weeks after the treatment process in patients presenting with diverticulitis due to the high prevalence of colorectal malignancies. While CT is considered the standard method for diagnosing diverticulitis, its limitations in detecting colorectal cancer should be acknowledged.

Keywords: diverticulosis, diverticulitis, colorectal cancer, colonoscopy

1. Introduction

Diverticular disease is commonly observed, particularly in Western societies and among older adults (1). While it is frequently identified asymptomatically during colonoscopies, it can also become symptomatic, leading to complications such as diverticulitis, hemorrhage, or perforation. Acute diverticulitis, observed in approximately 10–25% of patients, is the most common complication (2).

The imaging modality most frequently used for diagnosing acute diverticulitis and its complications is computed tomography (CT) (3). CT has a sensitivity and specificity of approximately 95% for the diagnosis of diverticulitis and its complications, making it the standard diagnostic method (4). However, due to imaging findings such as thickening of the bowel wall secondary to inflammation, there may be an overlap between diverticulitis and colorectal cancers (1,4). Several studies have reported an increased prevalence of colorectal cancer ranging from 0.5% to 11% in colonoscopies performed after an episode of diverticulitis (4–5). This rate is notably higher in cases of complicated diverticulitis compared to uncomplicated cases.

In this context, the American Gastroenterological Association (AGA) guidelines, as well as other similar guidelines, recommend performing colonoscopy following an episode of diverticulitis (6).

This study aims to evaluate whether colonoscopic assessment is necessary to differentiate colorectal cancers in patients diagnosed with diverticulitis and its complications based on abdominal CT findings. While some publications suggest that the risk of colorectal cancer in uncomplicated diverticulitis cases is similar to that observed in standard

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colonoscopic screenings, others support the notion that the cumulative risk increases when diverticulitis and its complications are evaluated collectively. Our objective is to determine the prevalence and diagnosis of colorectal cancer in cases of diverticulitis diagnosed via CT at our clinic and to provide concrete data on the necessity of colonoscopy in our region.

2. Materials and Methods

In our study, 290 cases diagnosed with diverticular disease and its complications between January 2018 and December 2024 at the General Surgery Department of Recep Tayyip Erdoğan University Training and Research Hospital were retrospectively analyzed. Among these, 21 cases were excluded due to mortality following perforation or lack of follow-up data.

The demographic data of the patients, clinical symptoms, and results of elective colonoscopies performed after treatment, along with any complications, were recorded retrospectively. This study was approved by the Non-Interventional Clinical Research Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (Approval Date: October 1, 2024; Approval Number: 2024/258).

2.1. Statistical Analysis

Data were analyzed using IBM SPSS Statistics (Version 29) for Windows (Armonk, NY, USA, IBM Corp.). Numerical and percentage values were presented for categorical variables, while mean and standard deviation values were used for the age variable. Relationships between categorical variables were assessed using the Chi-square test and Fisher's Exact Test. The Mann-Whitney U test was used to evaluate the relationship between age and tumor presence. A p-value of <0.05 was considered statistically significant for all analyses.

3. Results

A total of 290 patients with diverticular disease and its complications were retrospectively reviewed, 269 of which were included in the analysis after excluding 21 patients due to mortality following perforation or lack of follow-up.

The mean age of patients with a history of diverticulitis was 62.9 ± 12.2 years (range: 26–90). Among patients diagnosed with colorectal malignancy, the mean age was 70.3 ± 9.7, compared to 61.7 ± 12.2 in those without malignancy (Mann-Whitney U test, $p<0.001$). Colorectal malignancy was detected in 37 (13.8%) of the 269 patients who underwent elective colonoscopy (Table 1).

Table 1. Age and cumulative colorectal cancer data

	Colorectal Cancer Present	Colorectal Cancer Absent	p
The average age (± SD)	70.3 ± 9.7	61.7 ± 12.2	$p<0.001^*$
Associated Tumor (%)	37 (%13.8)	232(%86.2)	

*: Mann Whitney U is used. SD: Standard deviation

Among the patients who underwent colonoscopy following diverticulitis, 51.3% (n=131) were female, and malignancy was detected in 8.4% (n=11) of them. Of the 138 male patients, malignancy was identified in 18.8% (n=26). No statistically significant difference was found between genders (Fisher's Exact test, $p=0.013$) (Table 2).

Table 2. Gender and tumor localization data

		Colorectal Cancer Present		Colorectal Cancer Absent		Total group		*p*-value
		n	%	n	%	n	%	
Gender	Male	26	18.8	112	81.2	14	24,1	p=0.013*
	Female	11	8.4	120	91.6	44	75.9	
Same localization wall thickening on CT	Yes	22	21	83	79	105	39	p=0.006*
	No	15	9.1	149	90.9	164	61	
Different localization wall thickening on CT	Yes	12	92.3	1	7.7	13	4.8	P<0.001*

*: Fisher's Exact Test is used.

The most common initial diagnosis was diverticulitis (82.5%, n=222). The remaining cases included perforation (10.8%, n=29), abscess (4.5%, n=12), and bleeding secondary to diverticulosis (2.2%, n=6) (Table 3).

In terms of treatment, the majority of patients (84.8%, n=228) were treated medically without any intervention. Surgical interventions included Hartmann's procedure (10.8%, n=29) and diverticulitis excision or drainage combined with antibiotic therapy.

Table 3. Patient diagnoses

	Percentage (%) /Number (n)
Diverticulitis	82.5 (n=222)
Perforation	10.8 (n=29)
Abscess	4.5 (n=12)
Diverticulosis-related bleeding	2.2 (n=6)

3.1. Additional Results

When evaluating the localization of findings identified on CT by radiologists, the most common site was the descending colon/sigmoid colon in 92.2% (n=248) of cases. Colonoscopy findings showed that diverticula were most frequently observed in the descending and sigmoid colon, accounting for 74% (n=199) of the cases.

In the classification based on CT radiological findings, inflammation (43.9%, n=118) and wall thickening at the diverticulitis site (39%, n=118) were the most common observations.

When the wall thickening was localized to the same site as diverticulitis and its complications, malignancy was identified in 21% (n=22) of the 105 patients (Fisher's Exact test, p=0.006) (Table 2).

In contrast, in cases where wall thickening was present in a different colonic segment, 92.3% (n=12) of the 13 patients were found to have a tumor (Fisher's Exact test, p<0.001) (Table 2).

Among the 269 patients, only two cases (0.7%) presented with major complications such as perforation and bleeding, while no significant complications occurred in the remaining patients.

3.2. Discussion

The risk of malignancy in patients with diverticulitis increases with age. A meta-analysis by Koo et al. found an approximately 0.3% increase in risk per decade of life (4). Similarly, a study conducted in the United States reported a nearly twofold increase in colorectal cancer prevalence among patients over 65 years of age following diverticulitis (7). In our study, the mean age of patients diagnosed with colorectal malignancy was 70.3 ± 9.7 years, consistent with the literature (Table 1).

Multiple studies on the prevalence of colorectal cancer after a diverticulitis episode indicate malignancy rates of 0.5%–2% for uncomplicated diverticulitis and 5%–11% for complicated cases (4, 8, 9). When both categories are combined, cumulative malignancy risk ranges between 0.5% and 11%, as shown in numerous meta-analyses (8, 9). A community-based study by Jin-Dominguez et al. found that the prevalence of colorectal cancer within one year after an acute diverticulitis episode was nearly double compared to individuals without diverticulitis (10). In our study, colorectal malignancy was detected in 13.8% (n=37) of patients who underwent colonoscopy following a diverticulitis episode (Table 1).

Although a causal relationship between colorectal cancer and diverticulitis has not been established, both conditions share overlapping radiological features such as increased colonic wall thickening, soft tissue densities, and luminal narrowing (11, 14). Additionally, their shared localization in the sigmoid colon and factors such as prolonged colonic transit times and increased adenoma prevalence in these regions suggest similar risks. Moreover, studies suggest that mucosal inflammation may contribute to carcinogenesis (12, 13).

CT is the standard diagnostic modality for diverticulitis and its complications, with reported sensitivity and specificity levels of approximately 95% (3, 5). However, CT findings such as wall thickening and inflammation overlap with features of colorectal cancer (14). This overlap reinforces the recommendation for post-attack colonoscopic evaluation in many studies (3, 5, 8, 13).

In our study, wall thickening was identified on CT in 105 patients, 21% (n=22) of whom were diagnosed with colorectal cancer. Among 164 patients without significant wall thickening, 9.1% (n=15) were found to have colonic malignancies (p=0.006, Table 2). Furthermore, of the 13 patients with wall thickening in a different colonic segment, 92.3% (n=12) were diagnosed with malignancies (p<0.001, Table 2).

Recent studies suggest that colonoscopy may not be necessary for patients with uncomplicated diverticulitis due to the low malignancy risk, which is comparable to that of the general population (15). The World Society of Emergency Surgery (WSES) does not recommend routine colonoscopy after uncomplicated diverticular disease unless screening is indicated for other reasons (16). However, there is a lack of long-term follow-up studies on this issue. Conversely, the American Gastroenterological Association (AGA) and several meta-analyses advocate routine colonoscopy due to the increased risk of colorectal cancer (4-6, 8). For instance, Rottier et al. reported colorectal cancer prevalence of 0.4%–1.0% in screening colonoscopies compared to 2.1% after diverticulitis (8).

While some studies suggest that colonoscopy can be performed within six weeks after an attack (17), the consensus recommends waiting 6–8 weeks (18, 19). Although rare, colonoscopy-associated complications can occur, particularly after diverticulitis. Technical challenges during colonoscopy, such as luminal narrowing, angulations, and spasms, can complicate the procedure (20). In our study, one case of perforation and another of bleeding were observed following elective colonoscopy.

Based on our findings, we recommend performing an elective colonoscopy 6–8 weeks after the resolution of a diverticulitis episode to address the increased risk of colorectal malignancy. While CT is the standard imaging modality for diagnosing diverticulitis, its limitations in differentiating diverticulitis from colorectal cancer should be recognized.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions
Concept: A.Ö., O.B., Design: A.Ö., O.B., Data Collection or Processing: A.Ö., O.B., Analysis or Interpretation: A.Ö., O.B., Literature Search: A.Ö., O.B., Writing: A.Ö., O.B.

Ethical Statement
This study was approved by the Non-Interventional Clinical Research Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (Approval Date: October 1, 2024; Approval Number: 2024/258).

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Research Article

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Immunohistological examination of thyroid hormone receptor expressions in placenta in maternal hypothyroidism

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Abstract

Thyroid hormones are necessary for the development of a healthy fetus and placenta. In our study, we evaluated the thyroid hormone receptor expressions in the placentas of pregnant women with hypothyroidism. We evaluated term placentas in two groups: control (n=5) and hypothyroidism (n=5). THRα and THRß were expressed in the placenta, especially in syncytiotrophoblasts, and also in placental macrophages. THRα expression was decreased in the hypothyroid group compared to the control group, but it was statistically non-significant (p=0,850) . THRß expression was decreased in the hypothyroid group compared to the control group at a statistically significant level (p=0,004). The results showed that thyroid hormone receptors are associated with maternal hypothyroidism.

Keywords: maternal hypothyroidism, placenta, thyroid hormone receptors

1. Introduction

Thyroid hormones have a wide range of effects, specifically on metabolism, growth, development, and different organs. From a metabolic perspective, thyroid hormones are calorigenic and cause oxygen consumption and body heat generation. They increase protein catabolism, promote gluconeogenesis, increase glucose utilization, and regulate lipid metabolism (1–3). In some cases, thyroid hormones act by binding to proteins such as integrin αvβ3 on the plasma membrane, but most of their effects are usually achieved by binding intracellularly to thyroid nuclear receptors (THRs). T3 has a higher affinity for binding to THRs, while T4 is more effective at binding to integrin αvβ3. THRs act as ligand-dependent transcription factors by acting directly on thyroid hormone response elements (TREs) on gene promoters (canonical signaling). THRs can also act by non-canonical signaling by activating molecules such as phosphoinositide-3-phosphate kinase (PI3K), protein kinase B (AKT), and mitogen-activated protein kinases (MAPK). THRs are encoded by two different genes (α and β) located on chromosomes 17 and 3. THRα1, THRβ1, and THRβ2 are the major hormone-binding isoforms (4–6). Particularly T3 plays an important role in trophoblast differentiation and fetal neurodevelopment (5). THRα and ß are expressed in the nuclei of syncytiotrophoblasts, cytotrophoblasts, and extravillous cytotrophoblasts at increasing levels with advancing pregnancy (7).

Hypothyroidism is common during pregnancy and is seen between 3.5% and 18% (8–11). In general, the main cause of hypothyroidism is iodine deficiency. The American Thyroid Association (ATA) and the Turkish Endocrinology and Metabolism Association have determined TSH levels according to pregnancy periods according to Table 1 below (12). In the case of untreated hypothyroidism during pregnancy, the risk of miscarriage, placental rupture, premature birth, pre-eclampsia, and neonatal deaths may be observed (8,13,14).

Table 1. TSH Reference Ranges in The Evaluation of Thyroid Functions During Pregnancy in the First, Second, and Third Trimesters

Trimester	TSH Reference Range
First Trimester	0,1 – 2,5 mIU/ml
Second Trimester	0,2 – 3,0 mIU/ml
Third Trimester	0,3 – 3,0 mIU/ml

In our study, we aim to evaluate THR expressions in placentas with hypothyroidism during pregnancy from an immunohistological perspective.

2. Materials and Methods

2.1. Group design

Placentas that were discarded after delivery were taken from pregnant women who came to the Gynecology and Obstetrics Clinic of DEU Application and Research Hospital. While placentas taken from healthy pregnant women were used for the Control group (n=5), placentas from pregnant women diagnosed with hypothyroidism were taken for the Hypothyroidism group (n=5).

Patients in the hypothyroidism group were selected from pregnant women diagnosed with subclinical hypothyroidism based on TSH serum concentration and free T3 and T4 reference values (TSH>2.5 mIU/L in the first trimester and TSH>3.0 mIU/L in the second trimester)(15,16). Pregnant

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women were selected between the ages of 20-35. Consent forms were obtained from the patients before the study. Based on the patient's medical records and their responses to the health status questionnaire, patients with established risk factors such as congenital/acquired heart disease, obesity, multiple pregnancy, diabetes mellitus, hypertension, and smoking were excluded from the study (17,18).

2.2. Immunohistological examination

For immunohistochemical staining from placental samples, approximately 1 mm^3 tissue samples were placed in 10% formalin to fix the tissues and embedded in paraffin blocks. A microtome was used to cut the paraffin tissue blocks at 5 µm thickness on positive charged slides.

The slides were kept in an oven at 60ºC overnight. The next day, deparaffinization was carried out by passing through three series of xylene, the first of which was in the oven. The sections were passed through increasing alcohol series and brought to distilled water. Antigen retrieval was performed with citrate buffer (pH 6, 15-M103, Bio-Optica, Italy) to open antigenic epitopes that were closed during fixation. Slides were boiled in the microwave at 850W for 15 min and allowed to cool for 20 min. UltraVision Large Volume Detection System (TR-125-AL, Thermo Fischer, USA) was used for histochemical immunostaining. Slides were incubated with H_2O_2 for 15 min to block endogenous peroxidase activation and washed with PBS. Slides were incubated with Ultra V Block for 7 min. Slides were incubated with primary antibody [THRα (1:50, bs-6221R, Bioss, USA) and THRß (1:50, bs-11440, Bioss, USA)] overnight at +4°C in a humidified chamber. Slides were incubated with Streptavidin Alkaline Phosphatase for 10 min. The slides were washed with PBS. Slides were incubated with HRP Polymer for 15 min. Washed with PBS. Coloring of bound antibodies was achieved with a DAB chromogen (11718096001, Roche, Swiss) solution. The slides were washed with distilled water. Counterstaining was done with hematoxylin and covering was done with mounting medium (107960, Merck, Germany).

2.3. Positive area density measurement

After immunohistochemistry staining, 10 random fields were photographed from each section using a microscope (Euromex, IS.3153-PLFi/3) with a 40x objective. (1) The photos were opened in ImageJ and the photo was separated according to color channels with the color deconvolution plugin (2) Color channel windows were closed except for the brown color channel representing DAB staining (3) threshold was applied and brown stained areas were selected and (4) area measurement of the selected areas was performed.

2.4. Statistical analysis

All data were loaded into the GraphPad Prism 10 statistical program and the results are shown as mean ± standard error. After evaluating the normality and homogeneity of the data statistically, a nonparametric Mann-Whitney U analysis was performed to compare the two groups. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

In the control group, both THRα and THRß showed significant expression in syncytiotrophoblasts, while labeling was observed to be lower in the Hypothyroidism group. In addition, Hofbauer cells, which are placental macrophages, also gave a positive immunoreaction both THRα and THRß.

After ImageJ analysis, the THRα positive area density in the Control group was 3481584.50±260138.79, while in the Hypothyroidism group, it was 2921985.7±286587.20, and this decrease was found to be statistically non-significant ($p=0.416$). The THRß positive area density in the Control group was 3069027.90±404848.09, while in the Hypothyroidism group, it was 1817939.2±260711.31, and this decrease was found to be statistically significant ($p=0.013$).

4. Discussion

Thyroid hormone receptors show differences in hypothyroid placentas. In our study, THRα and THRβ showed different expressions in the control and hypothyroidism groups. While THRα and THRß gave strong immunoreactivity in the Control group, a weaker signal was observed in the Hypothyroid group.

In the study conducted with trophoblast cells, it was shown immunohistochemically that thyroid hormone receptors were expressed in cytotrophoblasts and syncytiotrophoblasts (19).

In a similar study on placentas with GDM, THRα expression was found to be at the strongest level in healthy placentas, while THRß expression was found to be weaker. THRα expression was also found to be lower in GDM placentas compared to healthy placentas. THRß expression showed similar expression in GDM placentas to healthy placentas (6).

In a study examining the relationship between IUGR and THR, similar to the GDM study, the strongest expression was for THRα. When they compared healthy and IUGR placentas, both THRα and THRß expression decreased significantly in IUGR placentas (7).

In a study conducted on spontaneous and recurrent miscarriages, THRα and THRß expressions in both placenta and decidua were significantly decreased compared to healthy pregnant women (20).

There is an important relationship between thyroid hormones and the immune system. Abnormal thyroid hormone secretion can affect immune functions. Hormones and endocrine transmitters bind to immune cells, leading to the production of factors that alter immune functions and regulate the intensity of the immune response. Studies have shown that THR is expressed in macrophages (21–23). In our study, we showed that THRα and THRß are expressed in Hofbauer cells.

As a result, it was observed that the effect of THRs changed in hypothyroidism as in different pregnancy complications. This change may pave the way for the development of

pregnancy complications caused by hypothyroidism.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: G.B., S.C.M.; Design: G.B., S.C.M.; Data Collection or Processing: G.B.; Analysis or Interpretation: G.B., S.C.M.; Literature Search: G.B., S.C.M.; Writing: G.B., S.C.M.

Ethical Statement

The study was approved by 'Non-Interventional Research Ethics Committee' of Dokuz Eylül University (2023/20-15). The investigation was carried out according to the Declaration of Helsinki. A consent form was taken from all volunteers.

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Evaluation of Infective Endocarditis Agents

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Abstract

Endocarditis is an inflammation or microbial infection of the heart valves and the endocardium of the heart. Bacteria that enter the blood due to various reasons can multiply in the endocardial layer of the heart and cause infection. In addition, these infections can be carried to different parts of the body through the bloodstream. This study aims to evaluate the distribution of microorganisms grown in blood cultures taken from patients diagnosed with endocarditis. The distribution of microorganisms grown in blood culture samples sent from patients diagnosed with endocarditis to our Ondokuz Mayıs University Medical Microbiology Laboratory between 2018-2021 was retrospectively examined. The distributions of the most frequently isolated bacteria from 63 strains obtained from blood samples are as follows; 61.90% Staphylococcus spp., 11.11% Streptococcus spp., 9.52% Enterococcus spp., 3.17% Escherichia coli, 1.58% Enterobacter cloacae, 6.34% Klebsiella pneumoniae, 1.58% Acinetobacter baumannii, 1.58% Stenotrophonomonas maltophilia, 1.58% Corynebacterium stratum, 1 1.58% Micrococcus spp. Oxacillin resistance was detected as 44.44% in S. aureus isolates. Carbapenem resistance was not detected in Enterobactarales bacteria. Vancomycin resistance was not detected in enterococcus isolates. Despite significant developments in the diagnosis and treatment of infective endocarditis, there has been no decrease in its incidence and mortality. Similar to many articles in the literature, it has been determined that the most frequently isolated pathogen is S. aureus. Knowing the distribution of infective endocarditis agents is important in guiding clinicians in both prophylactic and empirical treatment selection.

Keywords: infective endocarditis, diagnosis, therapy, prevention and control

1. Introduction

Infective endocarditis (IE) is defined as infection of the endocardial surface of the heart; it usually refers to infection of one or more heart valves or infection of an intracardiac device (1).

A variety of microorganisms can cause infective endocarditis (IE), The three most common causes of IE worldwide are staphylococci, streptococci, and enterococci. In the United States and most developed countries, *Staphylococcus aureus* is the most common cause of IE (2); Staphylococcal IE is a common cause of health care-associated IE (3); streptococcal IE is a common cause of community-acquired IE (4).

In a large cohort study involving 2,781 patients diagnosed with infective endocarditis (IE), the distribution of causative microorganisms was as follows: Staphylococcus aureus was the most frequently identified pathogen (31%), particularly associated with right-sided IE. This was followed by Viridans group streptococci (17%), enterococci (11%), coagulase-negative staphylococci (11%), and Streptococcus bovis (7%). Other streptococcal species, including nutritionally variant streptococci, accounted for 5% of cases. Non-HACEK (Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella) gram-negative bacilli and fungal pathogens were each detected in 2% of cases. The HACEK group, comprising fastidious gram-negative bacilli such as Haemophilus aphrophilus (currently classified as Aggregatibacter aphrophilus and A. paraphrophilus), Aggregatibacter actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, and Kingella kingae, was also responsible for 2% of cases. The remaining patients were diagnosed with culture-negative endocarditis (8%), polymicrobial infections (1%), or infections caused by various other rare pathogens (3%) (5, 6). Gram-negative bacterial species, such as *Escherichia coli* and *Klebsiella pneumoniae*, exhibit a lower affinity for adherence to cardiac valves when compared to gram-positive organisms (7). *Brucella* is an important cause of IE in endemic regions (8). Patients with ulcerative lesions of the colon due to carcinoma or inflammatory bowel disease have a predilection to develop IE due to *S. bovis* (9, 10). *Candida* spp. (11, 12) and *Aspergillus* spp. (13, 14) are the major causes of fungal IE.

Symptoms: The most common symptom of infective endocarditis (IE) is fever. Systemic symptoms such as fatigue, headache, muscle and joint pain, night sweats, abdominal pain and dyspnea are also common.

Diagnostic (modified Duke) criteria: The modified Duke criteria, which are widely used in the diagnosis, categorise patients into three groups: ‘definite IE’, ‘probable IE’ and ‘excluded IE’. These criteria were developed mainly for the evaluation of infections involving native left heart valves; however, their diagnostic sensitivity is lower in prosthetic

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valves, right heart involvement or pacemaker infections (15).

The diagnosis of infective endocarditis (IE) is based on the modified Duke criteria, which include major and minor clinical findings. Major clinical criteria consist of the identification of typical microorganisms associated with IE in two separate blood cultures, or persistently positive blood cultures. A single positive blood culture for Coxiella burnetii or a phase I immunoglobulin G (IgG) antibody titer greater than 1:800 is also considered a major criterion. Evidence of endocardial involvement demonstrated by echocardiography—such as vegetation, abscess, or partial dehiscence of a prosthetic valve—or the presence of new valvular regurgitation also fulfills the major criteria. (16)

Evidence of endocardial involvement includes at least one of the following major findings: a positive echocardiographic result indicating infective endocarditis, such as valvular vegetation, abscess formation, or partial detachment of a prosthetic valve; or the presence of newly developed valvular regurgitation. Minor criteria include the presence of risk factors (such as intravenous drug use or underlying structural heart disease), fever equal to or exceeding 38.0°C, vascular phenomena (e.g., arterial emboli or Janeway lesions), immunologic manifestations (e.g., glomerulonephritis or Osler nodes), and microbiological evidence that does not meet the major criterion threshold. The modified Duke criteria have been validated in several studies and remain a widely accepted tool for the clinical diagnosis of infective endocarditis (17, 18).

Blood cultures − At least three sets of blood cultures should be obtained from separate venous vessels prior to initiation of antibiotic therapy.

The diagnosis of infective endocarditis heavily relies on the importance of blood culture and the identification of grown microorganisms. Therefore, the objective of this article is to evaluate the distribution of microorganisms grown in blood cultures among patients diagnosed with endocarditis. As a summary in this comprehensive analysis of microorganism distribution in infective endocarditis, our goal is to gain a holistic understanding of the microbial landscape in the challenging condition. By integrating research findings and leveraging technological advancements, our aim is to enhance patient outcomes and alleviate the burden of this devastating infection. The aim of this study is to evaluate the distribution of microorganisms grown in blood cultures taken from patients diagnosed with endocarditis.

2. Materials and Methods

The distribution of microorganisms isolated from blood culture samples sent by patients diagnosed with endocarditis between the years 2018-2021 was retrospectively examined in our Ondokuz Mayis University medical microbiology laboratory.

The blood culture bottle samples sent to the laboratory were incubated in the fully automated Bact/ALERT 3D instrument (Biomerieux,France). The blood culture bottles showing growth signals were incubated onto 5% sheep blood agar and EMB agar media. The isolated bacteria was identified using the Vitex MS instrument (Biomerieux,France), and their antimicrobial sensitivity was determined using the Viteks2 compact automated system (Biomerieux,France).

3. Results

The distributions of the most frequently isolated bacteria from 63 strains obtained from blood samples are as follows; 61.90% (n=39) *Staphylococcus spp*., 11.11% (n=7) *Streptococcus spp*., 9.52% (n=6) *Enterococcus spp*., 6.34% (n=4) *Klebsiella pneumoniae*, 3.17% (n=2) *Escherichia coli*, 1.58% (n=1) *Enterobacter cloacae*, 1.58% (n=1) *Acinetobacter baumannii*, 1.58% (n=1) *Stenotrophonomonas maltophilia*, 1.58% (n=1) *Corynebacterium stratum*, 1.58% (n=1) *Micrococcus spp.* Among Staphylococcus aureus isolates, resistance to oxacillin, indicating methicillin resistance, was detected in 44.44% of the strains. In the Enterobacterales group, resistance to carbapenem antibiotics (e.g. imipenem, meropenem or ertapenem) was not observed. No vancomycin resistance was found among Enterococcus species isolates (Table 1). The gender distribution of the patients with isolated strains was 23.87% female and 76.11% male.

Table 1. Distribution of microorganisms grown in blood culture

Microorganism name	Total number of isolates	Antibiotic resistance
		Oxacillin (R)
Staphylococcus	**39**	**25**
S.aureus	18	8
S.hominis	8	6
S.epidermidis	16	11
S.capitis	2	1
S.haemolyticus	6	5
S.lugdunensis	1	0
Streptococcus	**7**	
S.sangius	2	
S.pneumoniae	1	
S.dysgalactiae	1	
S.mitis/oralis	1	
S.galiolyticus	1	
S.gordonii	1	
		Imipenem/ Meropenem (R)
Enterobacterales	**7**	**0**

Escherichia coli	2	0
Enterobacter cloacae	1	0
Klebsiella pneumoniae	4	0
		Vancomycin (R)
Enterococcus	**6**	0
***E**.faecalis*	3	0
***E**.faecium*	2	0
***E**.avium*	1	0
Acinetobacter baumannii	**1**	
Stenotrophonomonas maltophilia	**1**	
Cornybacterium stratum	**1**	
Micrococcus spp.	**1**	

The distribution of isolated microorganisms according to clinical departments is presented below. Among all departments, Staphylococcus species were the most frequently isolated microorganisms with a total of 39 isolates. These were most frequently detected in cardiology (n = 10), infection (n = 6) and coronary intensive care unit (n = 6) departments. Streptococcus species were isolated in 7 cases, the majority of which were in cardiology (n=3), infection (n=2) and coronary intensive care unit (n=2). Enterococcus species were isolated in 6 cases and were found in cardiology (n=3), infection (n=1), general surgery (n=1) and haematology (n=1). Among Gram-negative bacteria, Klebsiella spp. were isolated in four samples originating from the cardiology, nephrology, general surgery and coronary intensive care units (n=1 each). Escherichia coli was detected in two samples, both from the cardiology department. Enterobacter spp. was isolated from a single sample from the infection unit and Acinetobacter baumannii from a patient in the neurology department. Less frequently isolated organisms included Stenotrophomonas maltophilia (n=(n=1, coronary intensive care), Corynebacterium striatum (n=1, cardiology) and Micrococcus spp. (n=1, urology).

4. Discussion

The incidence of infective endocarditis (IE), which varies greatly from country to country, ranges between 3 and 10 per 100000 (19). The infection is usually associated with heart valves (native or prosthetic) or implanted cardiac devices (20). Despite all medical advances in the last 30 years, significant advances in the diagnosis and treatment of infective endocarditis, the incidence and mortality have not decreased (19; 21).

Blood cultures are the most important diagnostic method in IE. Patients have a low level of persistent bacteremia. Therefore, blood cultures taken at any time can show the etiologic agent. In patients who have not received antibiotics before, the chance of two blood cultures taken on admission being positive is around 90%. Therefore, 10 mL of venous blood from different veins at different times within the first 24 hours should be taken for three separate blood cultures. The most common microorganisms causing infective endocarditis are streptococci, staphylococci and enterococci (22; 23). It is estimated that staphylococci, streptococci, and enterococci collectively account for approximately 70–80% of all infective endocarditis cases. Among these, staphylococci—primarily Staphylococcus aureus—represent around 40–45%, viridans group streptococci contribute to 35–40%, and enterococci are responsible for roughly 10% (24; 25). Recent studies have shown that the frequency of staphylococci has increased in recent years (19; 26). In most cases of native-valve infective endocarditis, the causative agents are bacterial in origin. The most commonly isolated organisms include Staphylococcus aureus (accounting for approximately 35–40% of cases), followed by streptococcal species such as viridans group streptococci (~20%) and Streptococcus gallolyticus (formerly known as S. bovis, ~15%). Additionally, enterococci are identified in around 10% of patients (27).

Pehlivan et al. (1998); in their case reports, it was determined that infective endocarditis was detected in 7 (11.2%) of 62 patients who were hospitalized and followed up in their clinics between 1994-1997 and diagnosed with infective endocarditis using Duke criteria and the cases were examined prospectively. Four of these patients were female and three were male. *Staphylococcus aureus* was the causative agent in three of the patients, *Streptococcus viridans* in one, and *Staphylococcus epidermidis* in the other, while no growth was detected in two (28%) (28).

Çaylan et al. (2001), between 1997-2001, 32 endocarditis attacks in 30 cases were treated in our department. 22 (73.33%) of the patients were male and 8 (26.66%) were female. Blood cultures grew in 78.1% (25/32) of the attacks; *Staphylococcus spp.* (12), *Streptococcus spp.* (6), *Enterococcus spp.* (3), *Stenotrophomonas maltophilia* (2), *Pseudomonas aeruginosa* (1) and *Listeria spp.* (1) were the pathogens grown (29).

Şırlak et al. (2003): Patients operated on for infective valvular endocarditis in the Cardiovascular Surgery Clinic of Ankara University Medical Faculty between January 1990 and July 2001 were evaluated. During this period, 18 patients were operated for infective valve endocarditis. The ratio of patients operated on for infective valve endocarditis to the total number of patients operated on during this period was 0.231%. Eight of the patients were female (44.4%) and 10 were male (55.5%). Staphylococcus (22.2%), streptococcus (22.2%) and brucella (11.1%) were the causes of infective valve endocarditis in 4, 4 and 2 patients, respectively. However, in 8 patients (44.4%) the microorganism could not be detected (30).

Irdem et al. (2012), the microbiology and blood culture results of 36 patients diagnosed with definite and probable infective endocarditis were analyzed. The responsible agent was isolated in 14 (38.9%) of the cases by blood culture. The most frequently isolated agents were *S. viridans* 5 (13.88%), *S. aureus* 4 (11.11%), *S. epidermidis* 3 (8.33%) (21).

Lindberg et al. (2022), hospitalised adult patients with Gram-positive bacteraemia during 2017-2019 were evaluated retrospectively through medical records and the Swedish Death Registry. 480 patients with bacteraemia were included and definite endocarditis was diagnosed in 20 (7.5%), 10 (6.6 %), and 2 (3.2 %) patients with *S. aureus*, non-β-hemolytic streptococci and *E. faecalis*, respectively (31).

Despite the global data on infective endocarditis, comprehensive epidemiological studies focusing on Asian populations remain limited. In one investigation conducted within a Chinese cohort, Staphylococcus aureus was identified as the most common causative agent (23.4%), followed closely by streptococcal species (21.9%) (32).

Acet et al. (2024), blood cultures were positive in 75.5% of the cases (175 patients). The predominant causative organisms identified were: Streptococcus viridans (26.08%), Staphylococcus aureus (18.6%), Enterococcus faecalis (10.8%) (33).

Infective endocarditis was more frequently isolated from male study isolates in parallel with the general literature data on gender distribution (19; 34).

Blood culture-negative infective endocarditis is most frequently associated with prior antibiotic exposure, which can lead to sterilization of blood cultures. Retrospective analyses have reported that such cases may account for approximately 35% to 74% of all culture-negative endocarditis occurrences (35). Thanks to effective antibiotherapy and improved surgical techniques, IE is a disease with great advances in its treatment. The conventional management of infective endocarditis typically requires 4 to 6 weeks of intravenous antibiotic administration. Therefore, the overall treatment cost is significantly influenced by the prolonged duration of hospitalization and the expenses associated with intravenous therapy. However, despite all these advances, it remains a disease with high mortality and morbidity (36; 37; 38). Identification of the microorganisms causing IE is crucial for the diagnosis of the disease and determination of appropriate antimicrobial therapy (39).

IE remains a condition associated with considerable morbidity and mortality. To reduce adverse outcomes, more effective strategies and timely interventions are required. The incidence of healthcare-associated IE has been rising, with *Staphylococcus aureus* emerging as the predominant causative agent across various geographical regions. In this context, outpatient parenteral antimicrobial therapy and oral step-down approaches have gained attention as potential alternatives to prolonged hospitalization.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: Y.T.Ç., Design: Y.T.Ç., Data Collection or Processing: İ.B., M.C., Analysis or Interpretation: M.C., İ.B., Literature Search: Y.T.Ç., İ.B., M.C., Writing: Y.T.Ç., A.B., İ.B., M.C.

Ethical Statement

This study was approved by the Clinical Research Ethics Committee of Ondokuz Mayıs University (Date: 19.12.2024, Decision No: OMU KAEK 2024/462).

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Compliance with hepatocellular carcinoma screening and the effectiveness of screening in cirrhotic patients

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Abstract

Current guidelines recommend that patients with cirrhosis be screened for the development of HCC every 6 months. The aim of our study was to determine whether HCC screening is performed with appropriate methods and at appropriate intervals in cirrhotic patients, and to evaluate the outcomes of the screening. This research is a retrospective cohort study. The study included patients aged 18 and over, diagnosed with Child-Pugh class A or B cirrhosis, who applied to our clinic between 2010 and 2020. Patients were divided into two groups: those who underwent guideline-recommended surveillance (imaging with ultrasound, CT, or MRI and/or measurement of AFP every 4-8 months) and those who did not undergo recommended surveillance (insufficient screening or no screening). These groups were compared in terms of HCC development, curative treatment, and survival. A total of 641 cirrhotic patients were included in the study. Only 146 (22.7%) patients underwent guideline-recommended HCC screening. During the follow-up period, a total of 89 patients were diagnosed with HCC (42 patients (28.8%) in the surveillance group and 47 patients (9.5%) in the nonsurveillance group, $p<0.001$). In the surveillance group, the rate of early-stage HCC detection (83.3% vs. 40.4%), curative treatment rate (78.4% vs. 33.3%), and median survival time (74 vs. 21.7 months) were higher compared to the nonsurveillance group ($p<0.001$). HCC screening rates in cirrhotic patients are quite low. Guideline-recommended HCC screening in these patients results in earlier diagnosis and increases both the likelihood of receiving curative treatment and overall survival.

Keywords: surveillance, screening, hepatocellular carcinoma, cirrhosis

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor originating from hepatocytes. According to the World Health Organization's 2020 data, HCC ranks sixth in terms of incidence among all cancers and third among cancer-related causes of death (1) Prognosis for patients with advanced-stage HCC is generally poor, and treatment responses are limited. Clinical signs and symptoms related to the tumor often do not manifest until the early stages, making early diagnosis of HCC challenging in at-risk patients. Curative treatments for HCC, such as surgical resection, local ablation, and liver transplantation, can only be performed in early-stage cases. Hence, as in many other solid cancers, early diagnosis is crucial in HCC.

The majority of HCC patients have an underlying risk factor. Liver cirrhosis, viral hepatitis, and consumption of aflatoxin-contaminated foods are the most significant risk factors for HCC development. However, regardless of the cause, liver cirrhosis is recognized as the most significant risk factor for HCC (2). Approximately 90% of cases of HCC develop on a background of cirrhosis, with an annual HCC incidence rate of 1-8% reported in cirrhotic patients.[2] For this reason, many guidelines, including those from the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD), recommend screening for HCC in patients at risk for early diagnosis (3-5). However, there is no complete consensus among guidelines regarding the optimal screening protocol for HCC. Additionally, there is no clear consensus on the most appropriate method for diagnosis in these patients. Therefore, new studies are needed to assess the effectiveness of diagnostic methods and screening programs for HCC. The aim of our study was to determine whether HCC screening was performed in cirrhotic patients using appropriate methods and intervals and to evaluate the outcomes of the screening.

2. Materials and Methods

2.1. Patient selection and study design

A total of 1196 patients, including 752 males and 444 females,

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were screened between January 2010 and January 2020, who had presented to the Gastroenterology Clinic of Karadeniz Technical University Medical Faculty or were being followed up for liver cirrhosis in the gastroenterology department. Patients aged 18 and above with a diagnosis of cirrhosis were included in the study. Patients with malignancies other than HCC, those with Child-Pugh class C cirrhosis, those diagnosed with HCC at admission, and patients with missing medical records were excluded from the study. The patient selection flowchart is shown in Fig. 1. Patients were initially defined based on the International Classification of Diseases, Ninth Revision (ICD-9) codes for confirmed cirrhosis (456.0, 456.1, 456.20, 456.21, 567.2, 567.23, 571.2, 571.5, and 572.2). The diagnosis of cirrhosis was established based on typical cirrhosis findings in imaging methods (nodular appearance, heterogeneous echogenicity, decreased vascularity, caudate lobe hypertrophy, etc.), consistent laboratory findings (elevated serum bilirubin and INR, decreased serum albumin and platelets), and clinical signs related to cirrhosis (ascites, splenomegaly, esophageal varices, spider angiomas, palmar erythema, gynecomastia, hepatic encephalopathy, etc.). Demographic data of patients, date of cirrhosis and HCC diagnosis, number of lesions, largest lesion size, comorbidities, cirrhosis complications, and administered treatment methods were retrospectively retrieved from patient files and recorded.

The study was approved by the Karadeniz Technical University Health Application and Research Center Ethics Committee dated 14.12.2020 and numbered 48814514-501.07.01-E.14680.

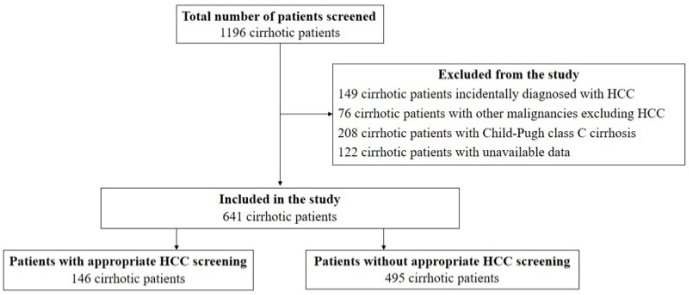

Fig. 1. The patient selection flowchart

2.2. HCC surveillance, detection, staging, and outcomes

All included patients were divided into two groups based on the follow-up interval: the surveillance group and the nonsurveillance group. Patients with an HCC diagnosis were categorized as the surveillance group if abdominal ultrasound, MRI, or CT had been performed for liver imaging within 4-8 months prior to diagnosis. Patients in the nonsurveillance group included those who had undergone liver imaging using any of the aforementioned methods within 8-24 months, or those who had not undergone any imaging at all. These groups were compared in terms of HCC development, curative treatment, and survival.

The diagnosis of HCC was made based on typical radiological findings according to the American Association for the Study of Liver Diseases (AASLD) criteria (4,5). In suspected cases, tumor biopsy was performed using imaging methods. Tumor characteristics, such as maximum diameter, number, and the presence of vascular invasion or distant metastasis, were determined. HCC staging was done using the Barcelona Clinic Liver Cancer (BCLC) system, and early HCC was defined as BCLC 0-A (6). HCC treatment was categorized as liver transplantation, surgical resection, local ablative treatment, transarterial chemoembolization (TACE) or transarterial radioembolization (TARE), systemic chemotherapy, or best supportive care. If HCC treatment included liver transplantation, surgical resection, or local ablative treatment, it was considered curative. Furthermore, using the Central Population Administration System - NVI (MERNİS), we defined overall mortality as any cause of death monitored until March 31, 2022.

2.3. Statistical Analysis

Categorical data were presented as counts (n) and percentages (%), while numerical data were presented as mean, standard deviation, median, and quartile values. The Mann-Whitney U test was used for the analysis of numerical data. The Chi-square or Fisher's exact test was employed for comparing categorical data. Survival rates of HCC patients were calculated using the Kaplan-Meier survival analysis. Differences in survival times were assessed using the Log-Rank test for treatment type, follow-up status, and HCC stage. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics according to follow-up status of all patients

Among the 641 patients in our study, 146 (22.7%) underwent HCC screening according to guidelines using any of the methods: USG, CT, or MRI (surveillance group), while it was found that 495 (77.3%) patients were not screened for HCC according to guidelines (non surveillance group). Among the 146 patients in the surveillance group, HCC developed in 42 (28.8%), and among the 495 patients in the nonsurveillance group, HCC developed in 47 (9.5%). The rate of HCC development was statistically higher in the surveillance group compared to the nonsurveillance group (28.8% vs. 9.5%, $p<0.001$). In Table 1, the relationship between patients with and without follow-up with multiple variables such as gender and etiology was evaluated and no significant difference was found (there is no difference in etiology and gender between follow-up/non-follow-up). Demographic characteristics, clinical findings, and laboratory results of the cirrhotic patients included in the study according to their follow-up status are shown in Table 1. Table 2 shows the etiology distribution of HCC, and no significant difference was observed between etiologic groups in terms of follow-up benefit ($p = 0,944$).

Table 1. Demographic characteristics according to patient follow-up status

Variable	Follow-Up Group	Non-Follow-Up Group	p-value
Total patients, n (%)	146 (22,7)	495 (77,3)	
Age, mean ± SD	61,38 ± (54-71)	60,24 ± (52-69)	*0,178*
Age group, n (%)			*0,226*
40 years and below	10 (6,8)	33 (6,7)	
41-55 years	44 (30,1)	112 (22,6)	
56-70 years	63 (43,2)	222 (44,8)	
71 years and over	29 (19,9)	128 (25,9)	
Gender, n (%)			***0,05***
Male	93 (63,7)	270 (54,5)	
Female	53 (36,3)	225 (45,5)	
Comorbid diseases, n (%)			
HT	63 (43,2)	223 (45,1)	*0,546 †*
DM	62 (42,5)	205 (41,4)	*0,548 †*
CKD	23 (15,8)	69 (13,9)	*0,745 †*
HF	18 (12,3)	71 (14,3)	*0,204 †*
HL	23 (15,8)	79 (16)	*0,349 †*
Etiology of Cirrhosis, n (%)			
Hepatitis B	42 (29)	102 (20,6)	
Hepatitis C	28 (19,3)	82 (16,6)	
Alcohol consumption	10 (6,9)	28 (5,7)	
NASH	9 (6,2)	49 (9,9)	
Biliary	8 (5,5)	28 (5,7)	
Cryptogenic	15 (10,3)	77 (15,6)	
Cardiac causes	6 (4,1)	16 (3,2)	
Vascular causes	4 (2,8)	10 (2)	
Metabolic causes	4 (2,8)	6 (1,2)	
Other	1 (0,7)	15 (3)	
Unknown	19 (12,4)	82 (16,6)	
Cirrhosis Complications, n (%)			
Variceal bleeding	23 (15,8)	74 (14,9)	*0,467 ‡*
Ascites	54 (37)	208 (42)	*0,277 ‡*
Peritonitis	13 (8,9)	25 (5,1)	*0,083 ‡*
Hepatic encephalopathy	30 (20,5)	73 (14,7)	*0,149 ‡*
Hepatorenal syndrome	0 (0)	7 (1,4)	
AFP, n (%)			*0,492*
10 ng/ml and below	116 (87,2)	361 (88)	
11-400 ng/ml	15 (11,3)	47 (11,5)	
400 ng/ml and above	2(1,5)	2 (0,5)	
Child-Pugh Score, n (%)			*0,934*
A	77 (52,7)	263 (53,1)	
B	69 (47,3)	232 (46,9)	
Fib-4, median (IQR)	4,44 (3,16-6,82)	4,74 (2,84-7,39)	*0,389*
APRI, median (IQR)	1,21 (0,62-2,09)	1,2 (0,7-2,2)	*0,437*
MELD-Na, median (IQR)	11 (9-15)	11 (9-15)	*0,190*
HCC status, n (%)			***<0,001***
HCC developed	42 (28,8)	47 (9,5)	
HCC not developed	104 (71,2)	448 (90,5)	

HT: Hypertension, DM: Diabetes mellitus, CKD: Chronic kidney disease, HF: Heart failure, HL: Hyperlipidemia, HCC: Hepatocellular Carcinoma, FIB-4: The Fibrosis-4, APRI: AST to platelet ratio index, MELD: Model for end stage liver disease † Comparison between patients with and without the respective comorbidities ‡ Comparison between patients with and without the respective complications **p*-value less than 0.05 was considered statistically significant

3.2. Demographic and clinical characteristics of patients diagnosed with HCC

Among the 641 patients in our study, 89 (13.9%) developed HCC during their follow-up. Among the patients diagnosed with HCC, 42 (47.2%) were in the surveillance group and 47 (52.8%) were in the nonsurveillance group. In the surveillance group, the rate of detecting early-stage (BCLC stage 0/A) HCC was higher compared to the nonsurveillance group (83.3% vs. 40.4%), the rate of detecting uni-nodular HCC lesions was higher (18.5% vs. 3.6%), and the rate of receiving curative

treatment was also higher (78.4% vs. 33.3%) (p<0.001). The mortality rate was lower in the surveillance group (38.1%) compared to the nonsurveillance group (72.3%) (p<0.001).

The demographic characteristics, laboratory findings, clinical features, and treatment outcomes of patients who developed HCC according to their follow-up status are presented in Table 2.

When Kaplan-Meier survival analysis was conducted based on the follow-up status of patients who developed HCC, the median survival of patients in the surveillance group was 74 months, while it was 21.7 months (95% CI, 12.7-30.7) in the nonsurveillance group (p<0.001) (Fig. 2).

Table 2. Demographic and clinical characteristics, laboratory findings, and treatment outcomes of patients who developed HCC according to follow-up status

Variable	All HCC Patients	Follow-up Group	Non-Follow-Up Group	p-value
Number of Patients n (%)	89 (100%)	42 (47,2%)	47(52,8%)	
Age, mean ± SD	67,27 ± 10,09	68,48 ± 9,22	66,19±10,8	*0,418*
Gender, n (%)				*0,084*
Male	62 (69,7)	33 (78,6)	29 (61,7)	
Female	27 (30,3)	9 (21,4)	18 (38,3)	
Etiology, n (%)				
Hepatitis B	41 (46,1)	18 (42,9)	23 (48,9)	
Hepatitis C	25 (28,1)	13 (31)	12 (25,5)	
Alcohol	5 (5,6)	3 (7,1)	2 (4,3)	
NASH	6 (6,7)	1 (2,4)	5 (10,6)	
Biliary	1 (1,1)	0	1 (2,1)	
Metabolic	1 (1,1)	0	1 (2,1)	
Cryptogenic	10 (11,2)	7 (16,6)	3 (6,4)	
Etiology Group, n (%)				*0,944*
Viral	66 (74,2)	31, (73,8)	35, (74,5)	
Nonviral	23 (25,8)	11, (26,2)	12, (25,5)	
HCC Diagnosis MELD-Na, n (%)				*0,222*
10 (%) and below	29 (34,9)	18 (43,9)	11 (26,2)	
11-18 (%)	48 (57,8)	20 (48,8)	28 (66,7)	
19 (%) and above	6 (7,2)	3 (7,3)	3 (7,1)	
MELD-Na Score, Median (IQR)	12(10-15)	11,5 (9-13)	12 (10-16)	*0,058*
AFP, median (IQR)	14,39(5,14-243,7)	12,12 (5,46-141,6)	38,87(5,14-258)	*0,322*
Largest Nodule Diameter, (%)				*0,068*
<2 cm	10 (11,2)	6 (14,3)	4 (8,5)	
2-3 cm	30 (33,7)	16 (38,1)	14 (29,8)	
>3 cm	49 (55,1)	20 (47,6)	29 (61,7)	
HCC Count				***<0,001***
Uninodular	45 (51,6)	27(18,5)	18 (3,6)	
Multinodular	44 (49,4)	15 (10,3)	29 (5,9)	
BCLC Stage, n (%)				***<0,001***
0/A	54 (60,7)	35 (83,3)	19 (40,4)	
B/C/D	35 (39,3)	7 (16,7)	28 (59,6)	
Diagnosis Method, n (%)				*0,279*
USG	9 (10,1)	2 (4,8)	7 (14,9)	
CT	41 (46,1)	20 (47,6)	21 (44,7)	
MR	39 (43,8)	20 (47,6)	19 (40,4)	
Treatment Method, n (%)				***<0,001***
Curative*	44 (53,7)	29 (78,4)	15 (33,3)	
Noncurative**	38 (46,3)	8 (21,6)	30 (66,7)	
Death Status n (%)				***<0,001***
Deceased	50 (56,2)	16 (38,1)	34 (72,3)	
Surviving	39 (43,8)	26 (61,9)	13 (27,7)	

HCC: Hepatocellular Carcinoma, MELD-Na: Model for End-Stage Liver Disease-Sodium, BCLC: Barcelona Clinic Liver Cancer, USG: Ultrasonography, CT: Computed Tomography, MR: Magnetic Resonance, AFP: Alpha feto protein,
*Curative treatment: liver transplantation, resection, RF, TACE (Transcatheter arterial chemoembolization);
** Noncurative treatment: systemic chemotherapy and best palliative treatment
p-value less than 0.05 is considered statistically significant.

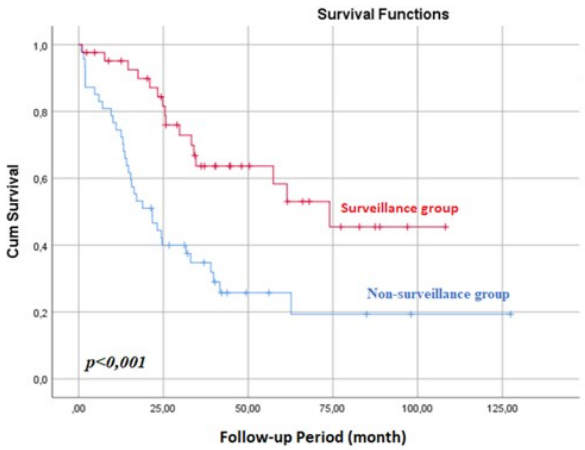

Fig.2. Survival analysis of patients diagnosed with hcc according to follow-up status

4. Discussion

Patients with cirrhosis are the highest-risk group for developing HCC, and the development of HCC in these patients is a significant cause of both mortality and morbidity (3,8). Therefore, guidelines such as AASLD and EASL recommend HCC screening every six months for cirrhotic patients with the aim of early detection and improved patient outcomes (3-5). However, despite guideline recommendations, low rates of HCC screening have been reported in these patients. In our study, the rate of cirrhotic patients undergoing HCC screening in accordance with guidelines was only 22.7%. Similar to our findings, several meta-analyses conducted between 2012 and 2021 reported HCC screening rates ranging from 18.4% to 24% (7-9). Our findings were also consistent with low adherence rates to HCC surveillance guidelines in various high-risk cohorts as reported in the literature (10-15). The markedly low HCC screening rates observed in cirrhotic patients can be attributed to several factors, including poor physician knowledge of screening guidelines, screening costs, and additional issues that may arise during contrast imaging, such as renal insufficiency, in these patients with accompanying comorbidities. Additionally, non-compliance of patients with physician recommendations could also have influenced adherence rates. To enhance screening rates in patients at risk, informing physicians about the recognition of chronic liver diseases, using nurse-patient reminder systems (such as phone calls, SMS, emails), and increasing patient-physician communication regarding HCC mortality could be beneficial. Furthermore, it is evident that there is a scarcity of studies assessing the effectiveness of HCC screening, highlighting the need for more research in this area.

In our study, the rates of HCC detection were statistically significantly higher in patients who underwent guideline-recommended HCC screening compared to those who did not (28.8% vs. 9.5%, *p<0.001*) (Table 1). Similar to our study, other cohort studies in cirrhotic populations have reported higher rates of HCC diagnosis in screened patients (11,13). These studies have demonstrated that early-stage, uninodular, or small-sized HCC lesion detection is associated with more frequent application of curative treatments and improved survival rates (10,13,16-19). Consequently, in light of our study's findings, we emphasize the need to include more cirrhotic patients who meet the criteria recommended by guidelines in screening programs.

HCC surveillance in cirrhotic patients is associated with a multitude of parameters prone to failure, including access to healthcare services in clinical practice, comorbid conditions, and cirrhosis-related complications. However, the absence of screening in these patients can lead to late-stage tumor detection (20). In our study, the rates of early-stage HCC (BCLC stage 0/A) detection were significantly higher in patients who underwent guideline-recommended HCC screening compared to those who did not (83.3% vs. 40.4%, p<0.001) (Table 2). Similar to our study, it has been demonstrated in cirrhotic patients that guideline-adherent HCC screening is associated with early-stage tumor detection (21-24). However, Singal et al.'s prospective study in 2021 involving 614 cirrhotic patients found that although a proportionally higher number of early-stage HCC lesions were detected in the surveillance group compared to the nonsurveillance group, no statistically significant difference was observed (62.5% vs. 50%, *p=0.69*) (25). Since only 26 of the 614 patients in this study developed HCC lesions during follow-up, the lack of statistically significant results may be explained by the small number of patients who developed HCC. Patients with very early and early stages of BCLC may be offered more effective survival-enhancing treatments than patients with intermediate and advanced stages (26). Therefore, it is an undeniable fact that including patients in the risk groups recommended by the guidelines in screening programs and detecting more early stage HCC will increase the number of patients reaching curative treatments.

As in all cancers, the goal for HCC patients should be to evaluate curative treatment options. Curative treatments depend on the stage of HCC disease and liver reserve. In early-stage HCC patients with preserved liver reserve, surgical resection and/or local ablative therapies are typically applied, whereas patients with a cirrhotic background should be evaluated for transplantation (3,15). Liver transplantation is one of the most frequent indications, especially in cases of HCC arising on a cirrhotic background. Liver transplantation for HCC treatment not only offers a curative approach for the tumor but also addresses the impaired liver function.[26] In our study, the rate of receiving curative treatment was significantly higher in the surveillance group compared to the nonsurveillance group (78.4% vs. 33.3%, p<0.001) (Table 2). Similar findings are supported by multicenter studies conducted in cirrhotic patients (16,21). In a retrospective study conducted by Singal et al. in 2017 involving 374 patients with HCC on a cirrhotic background, those diagnosed through surveillance had a higher rate of receiving curative treatment compared to nonsurveillance patients (30.6% vs. 13.0%,

p=0.02) (28). Unlike our study, this study included patients receiving incidental/symptomatic treatment and Child-Pugh class C patients. The inclusion of Child-Pugh class C patients in their study might explain the lower rate of curative treatments compared to our study (78.4%). It is evident that many early-stage HCC patients in the non surveillance group were deprived of curative treatments. Given the retrospective nature of our data collection, we were unable to determine the reasons for inadequate treatment utilization. A multicenter prospective study involving a larger number of patients is needed to identify limitations in treatment access. In light of these studies, interventions that facilitate access to curative treatment can improve the effectiveness of the HCC screening process.

In our study, the mortality rate in the surveillance group was lower (38.1%) compared to the nonsurveillance group (72.3%) ($p<0.001$). Survival analysis based on patients' surveillance status revealed that median survival of patients with surveillance was higher than that of patients without surveillance. Similarly, a meta-analysis conducted by Signal et al. in 2022, which included 59 studies and 145,396 patients, demonstrated an association between HCC surveillance and increased overall survival (18). In various cohorts of patients diagnosed with HCC between 2015 and 2018, as in our study, retrospective studies showed that patients who underwent guideline-adherent HCC screening had significantly longer median survival compared to those who did not undergo screening (17,26,29). Yang et al.'s retrospective study in 2020 involving 401 patients with HCC on a cirrhotic background found a proportionally higher median survival in the monitored group; however, unlike our study, no statistically significant difference was observed (14.5 months vs. 12 months, *p=0.375*) (2). In this study, the higher number of patients with severe liver disease in the monitored group compared to our study might have led to lower median survival rates in this cohort, and the impact of screening on survival might not have been significant. Similarly, Mancebo et al.'s prospective study in 2017 involving 770 cirrhotic patients found a proportionally higher median survival in the monitored patients; however, no statistically significant difference was observed (24.7 months vs. 14.2 months, *p=0.16*) (23). This could be explained by the limited number of non-monitored patients who developed HCC in this study. The aim of screening is to detect early-stage HCC and increase access to curative treatments that can improve survival. Although our study showed a higher median survival in the surveillance group compared to the non surveillance group, longer follow-up periods, larger prospective studies evaluating contrast agent-related complications, and the psychological effects of screening on patients are needed to confirm the benefits of HCC surveillance in current cohorts.

Our study is a substantial investigation encompassing a significant number of cirrhotic patients with an extended follow-up duration, emphasizing the significance of HCC surveillance in this patient group. However, there are certain limitations associated with our study primarily due to its retrospective nature. The foremost limitation is that our study is not a randomized controlled trial (RCT). Nevertheless, considering the results of a study where patients were surveyed about participating in an RCT (31) for HCC surveillance (99.5% declined randomization, and 88% opted for non-randomized surveillance), we observe that conducting randomized controlled trials may not be currently feasible. Additionally, being a single-center study, the generalizability of our findings may be limited. The data for our study were obtained from electronic records and patient files, leading to potential missing data as outcomes of excluded patients were not evaluated. Furthermore, it's possible that some patients continued their follow-up or received treatment at another facility after exiting our study. Another limiting factor is that deaths were due to non-HCC complications.

In conclusion, our study has revealed that HCC surveillance in cirrhotic patients falls short of the desired levels. Nonetheless, patients who underwent HCC surveillance exhibited higher rates of early-stage HCC detection, greater likelihood of receiving curative treatment, and higher median survival rates. To enhance HCC surveillance in cirrhotic patients, clinicians must understand the significance of adherence to screening and continue exploring options to enhance screening rates through system-based approaches and awareness campaigns. Furthermore, considering the substantial impact of adhering to the recommended time frame on overall survival, initiating patient-physician educational programs to achieve a 6-month screening policy in line with guidelines and improve compliance could be beneficial.

Conflict of interest

There is no conflict of interest in our study.

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Authors' contributions

Concept: Ü.Y.K., S.F., A.M.C., Design: Ü.Y.K., S.F., A.M.C., Data Collection or Processing: Ü.Y.K., S.F., C.K.S., S.N.K., M.E., A.M.C., Analysis or Interpretation: Ü.Y.K., S.F., C.K.S., S.N.K., M.E., A.M.C., Literature Search: Ü.Y.K., S.F., Writing: Ü.Y.K., S.F.

Ethical Statement

The study was approved by the Karadeniz Technical University Health Application and Research Center Ethics Committee dated 14.12.2020 and numbered 48814514-501.07.01-E.14680.

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In vitro evaluation of colistin and ceftazidime-avibactam activity against multi-drug resistant klebiella pneumoniae isolates

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Abstract

This study aimed to evaluate the in vitro effectiveness of colistin and ceftazidime-avibactam (CZA) antibiotics against multidrug-resistant (MDR) *Klebsiella pneumoniae* isolates. The study included 54 clinical isolates sent to the Medical Microbiology Laboratory of Kirsehir Education and Research Hospital between 2022 and 2023, which were resistant to multiple drugs in routine antibiotic susceptibility tests. Colistin susceptibility was evaluated using the microdilution method, while CZA susceptibility was assessed using the disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria. It was determined that 31.4% of the isolates were resistant to colistin, and 22.2% were resistant to CZA. No isolates were resistant to both antibiotics. Statistical analyses did not reveal a significant relationship between antibiotic susceptibility and gender, age, underlying disease, and sample type ($p>0.05$). However, colistin resistance has been found to be higher in intensive care units, whereas resistance to CZA has significantly increased in internal medicine units ($p =0.025$). The study's findings indicate that colistin and CZA may be important in treating multidrug-resistant *K. pneumoniae* infections. CZA provides an effective alternative for colistin-resistant isolates, while colistin is an effective alternative for CZA-resistant isolates. However, to enhance the efficacy of these agents, larger-scale studies and ongoing monitoring of resistance mechanisms are necessary.

Keywords: colistin, ceftazidime avibactam, klebsiella pneumoniae

1. Introduction

Klebsiella pneumoniae, although a component of the flora in the human body's gastrointestinal system and nasopharynx, is a Gram-negative bacillus that commonly acts as a pathogen in urinary tract infections, septicemia, pneumonia, nosocomial infections, surgical and catheter-related infections (1). *K. pneumoniae* is a significant nosocomial pathogen due to its rapidly increasing resistance to all currently available antibiotics, particularly carbapenems (2). The capsule structure, presence of siderophores, lipopolysaccharides, and fimbriae are essential virulence factors of these strains (1).

Resistance to at least one agent from three or more antimicrobial groups is defined as multidrug resistance (MDR); resistance to bacteria that are susceptible to two or fewer antimicrobial categories is termed extensive drug resistance (XDR); and resistance to all agents in all antimicrobial categories is known as pandrug resistance (PDR) (3). An infection caused by a multidrug-resistant microorganism can result in inadequate or delayed antimicrobial treatment and is linked to poorer patient outcomes. Among multidrug-resistant organisms, bacteria such as *K. pneumoniae* and *Acinetobacter spp.* can resist all currently available antimicrobial agents or may only be sensitive to older, potentially more toxic agents like polymyxins (3). The rising prevalence of multidrug-resistant Gram-negative bacterial pathogens worldwide is a primary global public health concern (4).

Antimicrobial resistance among Gram-negative pathogens, particularly resistance to β-lactam antimicrobials, often arises from the production of β-lactamases, which can significantly limit treatment options for serious bacterial infections. The rising prevalence of pathogens producing extended-spectrum β-lactamases (ESBLs) has resulted in increased use of carbapenems and growing dependence on them (4). Carbapenems are a class of beta-lactam antibiotics used to treat infections caused by *Enterobacterales* that produce ESBL and/or AmpC cephalosporinase. Although limited antibiotic options like colistin, tigecycline, and aminoglycosides are used to treat carbapenemase-producing pathogens, this has highlighted the urgent need for new antimicrobial agents (3,5). Antimicrobial resistance poses serious threats to modern medical practices. It is associated with the spread of resistance, treatment failures, and increased mortality rates. *K. pneumoniae* has emerged as a critical pathogen in this context. Strains that have developed resistance to last-resort antibiotics, such as carbapenems, have made treatment protocols much more complex (6,7). Colistin and ceftazidime-avibactam (CZA) are significant treatment options for these resistant infections. This study aims to evaluate the in vitro efficacy of these two agents against multidrug-resistant *K. pneumoniae* isolates.

2. Materials and Methods

This study included *K. pneumoniae* isolates from various

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clinics sent to the Medical Microbiology Laboratory of Kirsehir Education and Research Hospital between February 2022 and December 2023. These isolates were found to exhibit multidrug resistance in routine antibiotic susceptibility tests (Amikacin, amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, cefazolin, cefepime, ceftazidime, ceftolozane-tazobactam, ceftriaxone, cefuroxime, ciprofloxacin, levofloxacin, ertapenem, imipenem, meropenem, gentamicin, piperacillin-tazobactam, tigecycline, trimethoprim-sulfamethoxazole) and were stored as stock. Repeating isolates were not included in the study. The demographic data of the patients whose strains were included in the study (age, gender, sample type, clinical, and comorbidity status) were retrospectively obtained from the hospital automation system. Identifying isolates and antibiotic susceptibility tests in routine laboratory were determined using the VITEK2 compact system (bio-Merieux, France). Strains exhibiting multidrug resistance were stored at -20°C until the day of in vitro testing. On the day of the study, the preserved strains were revived by inoculating them onto blood agar and incubating them for 24 hours in an incubator. The colistin and CZA antibiotic susceptibility tests of the growing bacteria were conducted and evaluated as specified by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The colistin antibiotic susceptibility test was performed using the microdilution method. Antibiotic stock solutions were created based on the manufacturer's guidelines and stored at -80°C in a frozen state. For colistin, concentrations in the range of 64-0.25 µg/ml were prepared by performing serial two-fold dilutions in 96-well microplates containing freshly prepared cation-adjusted Mueller-Hinton liquid medium. The microplates were covered and incubated at 35 ± 2°C for 18-20 hours. At the end of the incubation, the wells with growth were visually evaluated. The CZA antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion method, and the CZA (10-4 µ) (Bioanalyse, Turkey) antibiotic disk was examined. Isolates with an inhibition zone diameter of ≥13 mm according to EUCAST criteria were considered CZA sensitive and were evaluated as sensitive. The data obtained were recorded in the study file and subjected to statistical analysis. The data analysis used the "IBM SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, USA) 30.0 for Windows" package. The results obtained from the SPSS software were interpreted based on variables such as gender, age, sample type, diagnosis, mortality, and type of service, as well as the effectiveness of these two agents. The variables in the descriptive statistics were shown as the number of cases, with the percentage (%) displayed. Pearson's chi-square and Fisher's exact tests were used in the data analysis, and a $p<0.05$ value was considered statistically significant.

3. Results

The colistin and CZA antibiotic susceptibility results of multidrug-resistant *K. pneumoniae* isolates obtained from clinical samples of 54 patients referred from various clinics were examined. Of the patients, 35 were male, 19 were female, and the average age was 72,22 (14,48). Among all the isolates, 17 (31.4%) were found to be resistant to colistin, while 12 (22.2%) exhibited resistance to CZA (Fig. 1). There are no isolates resistant to both antibiotics simultaneously. The susceptibility data for both antibiotics were analyzed using cross tables and the chi-square test, considering factors such as gender, age, underlying diseases, mortality, sample type, and the clinic from which the sample originated. According to the data results, no statistically significant relationship was found between the susceptibility of both antibiotics and gender, age, underlying disease, mortality, and sample type ($p > 0.05$). Although not statistically significant, the resistance rates for both antibiotics were higher in males, patients over 65 years old, patients diagnosed with pneumonia, respiratory samples for colistin, and blood-catheter samples for CZA. Statistically significant differences were found in the sensitivity rates to CZA among clinical units ($p = 0.025$), and the resistance rate was higher in patients from the internal medicine unit. Although not statistically significant, colistin resistance was observed to be higher in percentage among patients in intensive care units compared to those in other wards (Table 1).

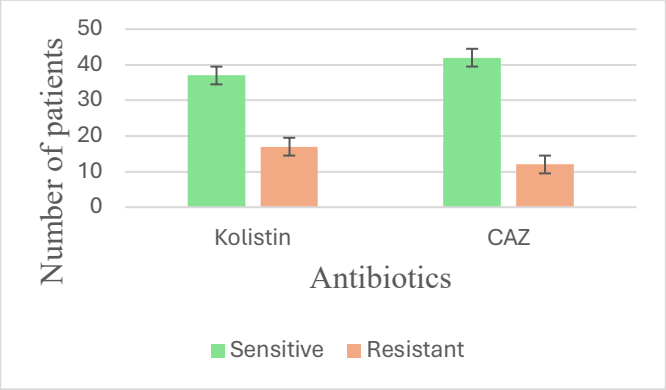

Fig. 1. Number of patients resistant and sensitive to colistin and ceftazidime avibactam

Table 1. Distribution of patients from whom colistin and ceftazidime-avibactam-sensitive and resistant strains were isolated according to demographic data [(%)]

	Colistin		p	CZA		p	Total
	Sensitive	*Resistant*		*Sensitive*	*Resistant*		
Gender			**0.547**			**0.879**	
Female	14 (%73.3)	5(%26.3)		15 (%78.9)	4 (%21.1)		19 (%100)
Male	23 (%65.7)	12 (%34.3)		27 (%77.1)	8 (%22.9)		35 (%100)
Age			**0.949**			**0.148**	
<65	9 (%69.2)	4 (%30.8)		12 (%92.3)	1 (%7.7)		13 (%100)
≥65	28 (%68.3)	13 (%31.7)		30 (%73.2)	11 (%26.8)		41 (%100)
Diagnosis			**0.378**			**0.368**	

UTI	7 (%70)	3(%30)		7 (%70)	3 (%30)		10 (%100)
Pneumonia	12 (%63.1)	7 (%36.9)		15 (%78.9)	4 (%21.1)		19 (%100)
Sepsis	5 (%100)	0 (%0)		2 (%40)	3 (%60)		5 (%100)
CVD	5 (%83.3)	1 (%19.7)		6 (%100)	0 (%0)		6 (%100)
AKI	1 (%33.3)	2 (%66.7)		3 (%100)	0 (%0)		3 (%100)
Wound Infection	2 (%50)	2 (%50)		3 (%75)	1 (%25)		3 (%100)
Others	5 (%71.4)	2 (%28.6)		6 (%85.7)	1 (%14.3)		7 (%100)
Mortality	15 (%65.2)	8 (%34.8)	**0.653**	16 (%69.6)	7 (%30.4)	**0.211**	23 (%100)
Sample Type			**0.741**			**0.386**	
Respiratory Track	14 (%60.9)	9 (%39.1)		20 (%87)	3 (%13)		23 (%100)
Urine	12 (%75)	4 (%25)		12(%75)	4(%25)		16 (%100)
Blood-Catheter	7(%70)	3 (%30)		6 (%60)	4 (%40)		10 (%100)
Wound	4(%80)	1 (%20)		4(%80)	1 (%20)		5 (%100)
Department			**0.138**			**0.025**	
ICU	26 (%61.9)	16 (%38.1)		35 (%83.3)	7(%16.7)		42 (%100)
Internal Medicine Service	8 (%88.9)	1 (%11.1)		4 (%44.4)	5 (%55.6)		9 (%100)
Surgery Service	3 (%100)	0 (%0)		3 (%100)	0 (%0)		3 (%100)
Total	**37 (%68.5)**	**17(%31.5)**		**42 (%77.8)**	**12(%22.2)**		**54 (%100)**

CZA: Ceftazidime-avibactam, UTI: Urinary tract infection, CVD: Cerebrovascular disease, AKI: Acute kidney injury, ICU: Intensive care unit, Other: malignancy, arrhythmia, cirrhosis, chronic kidney disease, hernia, etc

4. Discussion

In this study, we investigated the distribution of colistin and ceftazidime-avibactam (CZA) resistance among patients based on demographic factors, diagnosis, sample type, and hospital department. Our findings revealed no statistically significant association between gender and resistance to either colistin (p=0.547) or CZA (p=0.879). Similarly, age did not significantly affect resistance rates for colistin (p=0.949) or CZA (p=0.148). While most colistin-resistant strains were isolated from patients over 65 years old, the difference was not statistically significant. Regarding diagnosis, sepsis cases were exclusively linked to colistin-sensitive strains, while resistance was more common in pneumonia cases. Interestingly, acute kidney injury (AKI) showed the highest proportion of colistin resistance (66.7%), despite the limited number of cases. Regarding the department of hospitalization, resistance to colistin was more prevalent in patients from the intensive care unit (ICU) (38.1%) than in other departments. In comparison, the CZA resistance rate was significantly lower in ICU patients (16.7%) compared to those from internal medicine services (55.6%) (p=0.025). This finding highlights the importance of closely monitoring resistance patterns in ICUs, where antimicrobial resistance is typically higher. Furthermore, the distribution of resistant strains by sample type did not differ significantly, although respiratory tract samples showed a higher resistance rate to colistin (39.1%) than urine (25%). These findings suggest that while colistin resistance remains relatively consistent across demographic factors, ICU admission and specific diagnoses may increase the risk, warranting targeted antimicrobial stewardship interventions (Table 1).

It has been observed that various beta-lactamase genes (KPC, NDM, and OXA-48) are commonly found alongside the mcr-1 gene among the resistance mechanisms in MDR *K. pneumoniae* strains. This situation has brought treatment strategies to the forefront, particularly those involving broad-spectrum antibiotics (6). Colistin and CZA have been reported as critical agents for treating multidrug-resistant *K. pneumoniae* infections (8). CZA has been developed as a new β-lactam/β-lactamase inhibitor combination with in vitro and in vivo activity against *Enterobacterales* members that produce carbapenemase and OXA-48 (9). This drug has been found particularly effective in treating infections caused by multidrug-resistant Gram-negative bacteria (9,10). Colistin is an antibiotic from the polymyxin group, used as an effective agent against Gram-negative bacteria. However, serious side effects such as nephrotoxicity and neurotoxicity restrict its therapeutic use (8). The use of colistin has increased due to the rise in multidrug resistance among Gram-negative bacteria and the lack of new antibiotics to combat them. However, colistin resistance has also been frequently encountered in hospitalized patients (11,12).

According to data from a total of 10,998 Klebsiella isolates (9,098 *K. pneumoniae* and 1,900 *K. oxytoca* isolates) collected from 176 centers in 39 countries across Asia/Pacific, Europe, Latin America, and Africa/Middle East between 2012 and 2014, 2,821 isolates (25,7%) were found to be multidrug-resistant. The percentages of multidrug-resistant isolates varied among different countries; in some countries, relatively high rates of multidrug-resistant isolates were found (Brazil, Nigeria, Russia (>50%)), while in others, lower rates were observed (Australia, Denmark, Netherlands, Sweden, United Kingdom (<5%)). It was reported that 88.1% of meropenem-resistant multidrug-resistant isolates were sensitive to CZA (13). In a study by Van Duin et al., the first group of 38 patients with carbapenem-resistant *Enterobacteriaceae* infection was treated with CZA, while the second group of 99 patients was treated with colistin. At the end of 30 days, hospital mortality was reported to be significantly lower in the CZA group (9%) compared to the colistin group (32%), indicating that CZA could be a reasonable alternative to colistin in the treatment of carbapenemase-producing *K. pneumoniae* infections (14). Almangour et al. conducted a study comparing the efficacy of

CZA and colistin in treating carbapenem-resistant *Enterobacteriaceae* bacteremia, including a total of 230 patients; 149 patients received CZA and 81 patients received colistin-based treatment. *K. pneumoniae* was identified as the most prevalent isolated pathogen (n = 201; 87%). Of the isolates, 116 (78%) were sensitive to CZA, whereas 62 (76%) were sensitive to colistin. CZA proved superior for treating infections caused by carbapenem-resistant *Enterobacteriaceae*, showing higher clinical treatment rates, lower incidence of acute kidney injury, and reduced mortality rates compared to colistin-based regimens (15). In another study conducted in India, the effectiveness of colistin and CZA against multidrug-resistant *K. pneumoniae* was compared, with observations indicating that CZA had fewer side effects than colistin. However, continuous monitoring is necessary for resistance development (16). When the results of these studies were compared with our data, it was found that CZA (22.2%) had a lower resistance rate than colistin (31.5%), indicating similarity (Fig. 1).

The 2016 annual report from the Türkiye Public Health Institution, part of the Ministry of Health, indicated that the prevalence of multidrug-resistant *K. pneumoniae* in our country was 46.1%. In 961 of the 1394 multidrug-resistant *K. pneumoniae* isolates, colistin susceptibility was tested, and 271 (28.2%) were found colistin-resistant. Among 1229 carbapenem-resistant *K. pneumoniae* isolates, colistin resistance was 31.7% (17). In a study conducted in Ankara between September 2018 and December 2018, colistin resistance was determined to be 39.51% in 81 *K. pneumoniae* isolates with identified carbapenem resistance (18). In the study by Kaya et al., 95.7% of the multidrug-resistant *K. pneumoniae* were sensitive to CZA. In contrast, CZA resistance was not detected in the carbapenem and colistin-resistant *K. pneumoniae* isolates (19). In the study by Hosbul et al., 150 carbapenem-resistant *K. pneumoniae* isolates were examined between January 1, 2018, and February 1, 2021. Colistin resistance was 52% (n=78), while CZA resistance was 7.3% (n=11). Except for one of the 78 colistin-resistant isolates, all other colistin-resistant isolates were found to be sensitive to ceftazidime-avibactam. Colistin resistance was observed to be high in recent studies and in this study (31.4%) and it has been thought that colistin resistance in *K. pneumoniae* isolates in our country is increasingly becoming a dangerous issue. CZA resistance was higher in our data (22.2%) compared to other studies. This has been interpreted as using this newly developed drug to treat resistant isolates over time.

The study by Jayol et al. further suggests that ceftazidime/avibactam is an effective therapeutic option for treating infections caused by colistin-resistant and KPC- or OXA-48-producing *K. pneumoniae (20*). CZA therapy demonstrates lower mortality rates compared to colistin-based treatments in bloodstream infections caused by carbapenem-resistant *K. pneumoniae* producing OXA-48. In a multicenter study, the mortality rate was 14.3% when CZA was used as the initial therapy, whereas it was 37.7% when patients were switched from last-resort treatments such as colistin (p = 0.04). Additionally, initiating CZA on the same day the blood culture was obtained significantly reduced the mortality risk. These findings indicate that CZA is more effective than colistin in treating carbapenem-resistant *K. pneumoniae* infections, particularly in OXA-48 endemic regions (21). Like this study, the findings of our research, in which no *K. pneumoniae* isolate was found to be resistant to both colistin and CZA, showed that CZA can be used as an alternative in vitro for isolates resistant to colistin and that colistin can be used for isolates resistant to CZA. However, it should not be forgotten that more evidence is needed to prove this, and it must be supported by clinical studies. In a study evaluating the in vitro efficacy of the combination of CZA with various antimicrobial agents against carbapenem-resistant *K. pneumoniae*, the in vitro efficacy of the combination of CZA and COL shows irrelevant effects against the tested clinical isolates (22). Although ceftazidime/avibactam has emerged as a valuable therapeutic option against KPC-producing *K. pneumoniae*, evidence regarding its combination with colistin remains inconclusive and clinically problematic. In vitro time-kill assays conducted by Wang et al. demonstrated that the ceftazidime/avibactam-colistin combination exhibited only partial synergistic effects in a minority of isolates and failed to achieve superior bactericidal activity compared to monotherapy in most cases (23). Similarly, Borjan et al. reported a lack of synergy and no significant enhancement in survival outcomes in an in vivo *Galleria mellonella* model, further questioning the utility of this combination (24). Notably, the addition of colistin may not only lack synergistic benefit but also introduce substantial risk due to its well-documented nephrotoxicity (23,24). These findings collectively suggest that the ceftazidime/avibactam-colistin combination should be approached with caution and reserved only for cases where alternative regimens are either unavailable or ineffective, with careful consideration of potential toxicities and the absence of an additive antimicrobial effect.

There are some limitations in this study. One limitation is that the resistance test was conducted using different methods for the two types of antibiotics in the study's methodology. Colistin was examined with broth microdilution, while CZA was assessed using the disk diffusion method. Additionally, limitations include obtaining study data from a single center, a small number of patients, and the inability to determine resistance mechanisms in resistant strains.

The study suggests that colistin and CZA may be important agents in treating multidrug-resistant *K. pneumoniae* infections. However, antibiotic susceptibility varies according to patient characteristics. The study data indicate that the increasing resistance rates in patients over 65 years old

necessitate special attention when treating this group. The high resistance rates of CZA in blood-catheter samples may pose challenges for using this drug in treating invasive infections, and the high colistin resistance rates in intensive care units highlight the critical importance of antibiotic management in these settings (Table 1). Although CZA stands out as an effective alternative against carbapenemase-producing strains, the presence of resistant strains shows that this drug alone is not sufficient. Advanced studies involving larger patient populations, optimizing treatment strategies, understanding resistance mechanisms better, and ensuring continuous monitoring will help validate these results comprehensively.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: T.A.M., Design: T.A.M., Data Collection or Processing: T.A.M., Analysis or Interpretation: T.A.M., Literature Search: T.A.M., Writing: T.A.M.

Ethical Statement

Approval was obtained from Kirsehir Ahi Evran University Faculty of Medicine, Non-Interventional Clinical Research Ethics Committee with decision number 2024-4/15 dated 06/02/2024.

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Research Article

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Investigation of structural properties of transverse sinuses in neurosurgical cases by three dimensional volume rendering technique using magnetic resonance images

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Abstract

The detailed description of the neuroanatomical structure of the human brain's venous system, along with the examination of its neurophysiological, neurogenetic, neuropathological, and neurogeometric foundations, has not yet been sufficiently accomplished. The aim of this project is to provide accurate, useful, and up-to-date information on the structural characteristics and variations of the transverse sinuses key components of the brain's venous structures—based on neuroradiological studies. For this study, 44 patients (20 men and 24 women) aged between 20 and 79, who had undergone thin-slice magnetic resonance imaging (MRI) for various reasons at our neurosurgery clinic between 2016 and 2020, were randomly selected. The MRI images, obtained in DICOM format, were rendered into 3D using the volume rendering technique in the OsiriX program, enabling clear visualization of the transverse sinuses. Various angular, length, distance, width, and calibration measurements were then performed on these images. The resulting data were analyzed statistically using the SPSS v25 program. In the majority of patients, the right transverse sinus was dominant. No statistically significant correlation was found between dominance and gender or age ($p = 0.681$ and $p = 0.521$, respectively). The exit angle of the right transverse sinus from the torcular Herophili was found to be significantly greater than that of the left transverse sinus ($p = 0.013$). The diameters of both transverse sinuses were measured just before and just after the junction with the vein of Labbé, and the difference between the pre- and post-junction diameters was found to be statistically significant ($p < 0.05$). Average distances between the transverse sinuses and the corresponding mastoid processes were determined, but no statistically significant difference was found between the dominant and non-dominant sides ($p = 0.447$ and $p = 0.912$, respectively). The use of contrast-enhanced T1-weighted MRI combined with volume rendering to visualize the transverse sinuses in 3D by digitally removing the scalp and bone structures represents a significant advancement for the field of neurosurgery. This method highlights the importance of preoperative visualization of these veins in connection with surgical interventions. Measuring the distance of the transverse sinuses from defined anatomical landmarks and comparing these by age, gender, and dominance represents an innovative approach. Overall, this study will serve as a foundation for future research in brain venous anatomy. The numerical data and statistical findings presented here will be valuable for surgical planning and improving surgical success in centers lacking neuronavigation systems.The use of volume rendering technique with contrast-enhanced T1-weighted magnetic resonance imaging may contribute to the three-dimensional evaluation of the transverse sinuses for surgical planning. This method emphasizes the importance of preoperative visualization of venous structures. The findings of this study may provide a basis for future research on the venous anatomy of the brain and improve surgical success.

Keywords: transverse sinus, torcular herophilia, labbe vein, venous variations, magnetic resonance imaging, three dimensional volume rendering technique

1. Introduction

The venous vessels of the brain have been a less researched topic in the neurosurgical literature compared to the arterial system. Many anatomo-physiologic and cadaveric studies have focused primarily on the lateral surfaces of the brain, and detailed examination of the venous system has been relatively neglected. However, in recent years, with the development and more widespread use of surgical techniques in the midline and basal regions of the brain, interest in the venous system has increased markedly. The cerebral venous system is difficult to define as a standardized anatomical structure due to its large individual variations in size and connectivity (1, 2). The cerebral venous system plays a critical role in regulating cerebral blood flow and balancing intracranial pressure. This system, which lacks valve structures, exhibits a dynamic network of complex anastomotic connections (3). The interruption of the main structures of the venous system during surgical intervention or occlusion by mass effect may lead to venous infarction and cause severe neurologic deficits. However, the severity of the clinical picture varies depending on the extent of anastomotic connections in the venous system. In the presence of widespread anastomoses, obstruction may present with milder clinical symptoms, whereas in areas with limited anastomotic connections, obstruction may cause severe neurologic deficits. Especially in areas with insufficient anastomoses, damage to the venous system can lead to serious life-threatening clinical pictures such as hemiplegia, coma and death. (4, 5). In order to better understand the complex structure of the venous system of the brain, neuroradiological

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studies can provide accurate, up-to-date and localizing information, as in the arterial system. Such studies provide an important guide for surgical and interventional approaches by defining the anatomical variations of the venous system in more detail. The transverse sinuses are one of the main components of the cerebral venous system. It extends bilaterally in the groove located in the occipital bone, along the posterior margin of the tentorium cerebellum and drains into the internal jugular vein via the sigmoid sinus (6, 7). Like other cranial venous sinuses, it lies between the two leaves of the dura mater (8). The Labbe vein, also known as the inferior anastomotic vein, usually drains into the transverse sinus (9, 10). The Labbe vein is of great clinical importance because of its capacity to maintain venous drainage in the event of obstruction or damage to the transverse sinus. Therefore, preservation of the Labbe vein during surgical interventions is critical to maintain venous return and prevent serious neurologic complications. (11).

One of the main objectives of this study is to process contrast-enhanced T1 sequence magnetic resonance imaging (MRI) data of the transverse sinuses using the volume rendering technique and to make them visible in three dimensions by removing the scalp and bony structures. It is aimed that the data obtained as a result of this examination will provide guidance for the surgeon during surgical planning and operation and contribute to increasing surgical success, especially in centers where neuronavigation systems are not available.

Within the scope of the study, the diameters of the right and left transverse sinuses and the exit angles from the torcular Herophili will be evaluated and the effects of the involvement of the Labbe vein on sinus calibration will be examined. In addition, it is aimed to compare these anatomical parameters in terms of both sides and to determine whether they differ according to gender and age variables. In addition, the distances of the transverse sinuses to the anatomical reference points will be measured and analyzed according to various parameters.

2. Material and Methods

Between 2016 and 2020, 44 patients (20 males and 24 females) aged between 20 and 79 who underwent thin-slice MRI for various reasons at the Department of Neurosurgery were randomly selected. Patients under the age of 18, as well as those with a history of cranial hemorrhage, mass lesions, cranial surgery, or cranial radiotherapy, were excluded from the study.

Within the scope of the study, 20 male and 24 female patients aged between 20 and 79 years who applied to Ondokuz Mayıs University Department of Neurosurgery with various clinical indications between 2016 and 2020 and underwent thin section magnetic resonance imaging (MRI) were randomly selected.

After obtaining the patients' contrast-enhanced T1-sequence MRIs in DICOM format, the images were rendered into 3D using the volume rendering technique in the OsiriX software, making the transverse sinuses (TS) visible (Fig. 1). Subsequently, various measurements were performed on these images, including angles, lengths, distances, widths, and calibrations.

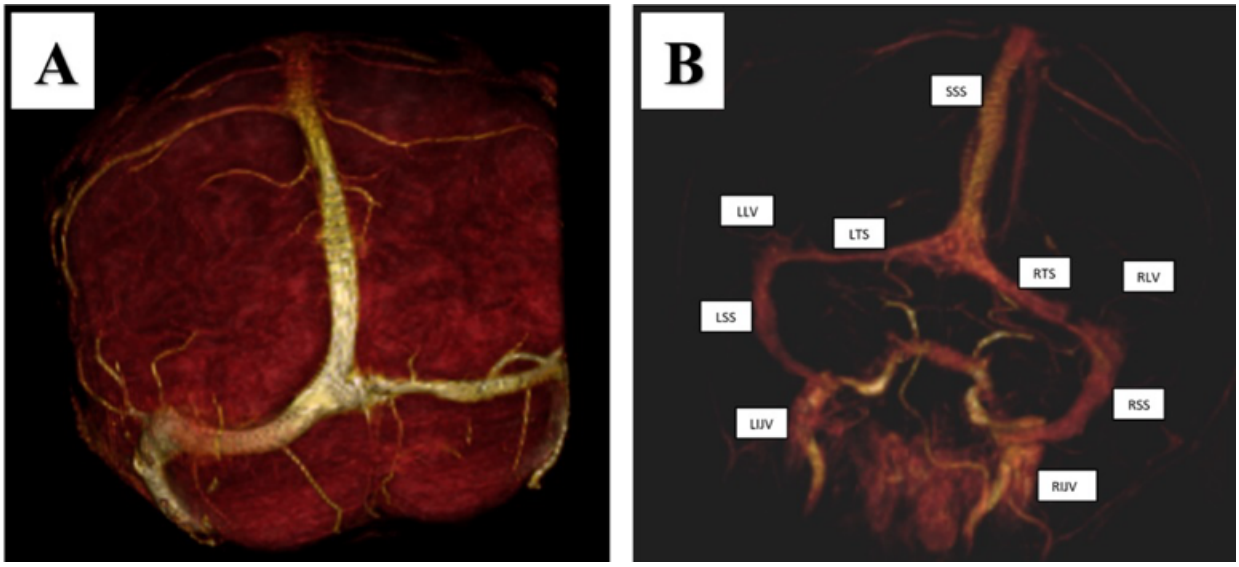

Fig. 1. Contrast enhanced T1 sequence MR images processed with 3D Volume Rendering Technique in OsiriX program. (SSS: superior sagittal sinus; LTS: left transverse sinus; LLV: left vein of labbe; LSS: left sigmoid sinus; LIJV: left internal jugular vein; RIJV: right internal jugular vein; RSS: right sigmoid sinus; RLV: right vein of labbe; RTS: right transverse sinus).
A: Skin, subcutaneous and bony structures removed.
B: Dose adjustment was made and venous system was visualized.

The data obtained were analyzed in IBM SPSS 25 (IBM Statistical Package for Social Sciences) program. Descriptive statistics were given as number, percentage, mean±standard deviation and median (minimum and maximum values). Since numerical variables were normally distributed, they were presented as mean±standard deviation. "Independent Samples T Test" was used for statistical significance between groups for variables that were found to comply with normal distribution

and homogeneity of variances of normal variables between groups was evaluated by 'Levene's test'. In the comparison of categorical variables, "Fisher Exact Test" was used by utilizing cross tabulations. In addition, the correlation between numerical variables was analyzed using the "Pearson Correlation Coefficient" since the variables were normally distributed. $p<0.05$ was considered statistically significant.

3. Results

Of the 44 patients who participated in the study, 24 (54.5%) were female and 20 (45.5%) were male. The ages of the patients ranged between 20-79 years with a mean age of 47.3±17.3 years. Female patients were aged between 20-79 years with a mean age of 46.5±19 years, while male patients were aged between 25-78 years with a mean age of 48.3±15.5 years, and no statistically significant difference was found between the mean ages according to gender (p=0.744) (Table 1).

Table 1. Demographic properties of the patients

	Female	Male	Total	p
	24 (54,5)	20 (45,5)	44	0.128**
Mean Age	46,5±19	48,3±15,5	47,3±17,3	0.744*

** Independent Samples T Test; ** Chi-square*

The dominant transverse sinus was right in 29 patients (65.9%), left in 11 patients (25%) and codominant in 4 patients (9.1%). In patients with dominant left transverse sinus, the proportion of women (54.5%) was higher than that of men (45.5%), whereas in patients with dominant right transverse sinus, the proportions of women (51.7%) and men (48.3%) were close to each other. In patients with codominance, the proportion of women (75%) was higher than that of men (25%). However, no statistically significant relationship was found between the gender of the patients and the dominant transverse sinus lateralization (p=0.681). Again, no statistically significant relationship was found between age and dominant sinus lateralization (p=0.521) (Table 2).

Table 2. The relationship between age-gender and dominant sinus lateralization

		Right Dominant TS	Left Dominant TS	Codominant TS	p
Gender	Female	15 (51,7)	6 (54,5)	3 (75)	0.681[a]
	Male	14 (48,3)	5 (45,5)	1 (25)	
Age		47,8 ± 18,8	43,8 ± 12,7	53,7 ± 18,8	0.521[b]

TS: Transverse Sinus
a: Fisher Exact Test, b: Independent Samples T Test

The exit angle of the left TS from the torcular herophilia was in the range of 70°-112° with a mean of 95.9±7.8; that of the right TS was in the range of 79°-116° with a mean of 100.1±7.7. The mean right TS angle was greater than the mean left TS angle and the difference between these means was statistically significant (p=0.013) (Table 3).

Table 3. Analysis of the exit angle of the transverse sinus from the torcular herophilia between the parties

	Left TS	Right TS	p
Exit Angle	95,9±7,8	100,1±7,7	0,013*

** Independent Samples T Test*

Both dominant and other TS diameter means were found to be higher after the addition of the labbe vein than before and this difference was statistically significant (p<0.05) (Table 4).

Table 4. Analysis of the change in TS diameter before and after the addition of labbe vein

	Pre-Labbe	Post-Labbe	p
Dominant TS diameter(mm)	6,9±1,7	9,1±1,3	<0.05*
Non-dominant TS diameter (mm)	4,7±1,4	6,9±1,4	<0,05*

** Independent Samples T Test*

When the relationship between the mean TS diameters measured and gender was analyzed, no statistically significant result was found (p>0.05) (Table 5).

Table 5. TS diameters of patients according to gender

	Female	Male	p
Pre-Labbe Dominant TS Diameter (mm)	7,2±1,8	6,7±1,6	0,323*
Post-Labbe Dominant TS diameter (mm)	9,2±1,3	9,0±1,4	0,572*
Pre-Labbe Non-dominant TS Diameter (mm)	4,6±1,4	4,7±1,3	0,826*
Post-Labbe Non-dominant TS Diameter (mm)	7±1,5	6,8±1,3	0,656*

** Independent Samples T Test*

The distance between the TS and the same side mastoid process was measured and compared according to gender. The measurement points were the point where the labbe vein joins the TS and the same side mastoid process, and the distance was calculated in centimeters. The mean distance between the dominant TS and the same side mastoid process was 2.1±0.1 in women and 2±0.1 in men. The mean distance between the nondominant TS and the same side mastoid process was 2.2±0.2 in women and 2.1±0.1 in men. There was no statistically significant difference between these measurements between male and female patients (p>0.05) (Table 6).

Table 6. Distance between the TS and the same side mastoid processes of the patients

	Female	Male	p
Distance of the dominant TS to MP	2,1±0,1	2±0,1	0,447*
Distance of the non-dominant TS to MP	2,2±0,2	2,1±0,1	0,912*

MP: Mastoid Process
** Independent Samples T Test*

The relationship between age and TS diameters was analyzed and no statistically significant correlation was found (p>0.05) (Table 7).

Table 7. The relationship between age and TS diameters

		Pre-Labbe Dominant TS Diameter	Post-Labbe Dominant TS Diameter	Pre-Labbe Non-dominant TS Diameter	Post-Labbe Non-dominant TS Diameter
Age	Pearson Correlation(r)	-0,156	-0,080	-0,059	0,069
	p	0,510	0,738	0,805	0,774

4. Discussion

No correlation was found between the gender and age of the patients included in the study and the lateralization of the dominant TS, and it was found that the right TS was dominant in the majority of the patients. Our results are similar to the literature (12, 13).

The exit angles of the transverse sinuses (TSs) from the torcular Herophili were analyzed, and it was observed that the exit angle of the right TS was statistically significantly greater than that of the left TS. This finding may be associated with the dominance of the right TS. No previous studies examining these angles were found in the literature.

As a result of the measurements, it was found that both dominant and nondominant TS diameter increased statistically significantly after the addition of the labbe vein. The reason for this may be thought to balance the pressure against the increased volume load with the loss of the labbe vein. If it were possible to measure blood flow velocities right here, it would be possible to determine whether the blood flow velocity, which should be low on the dominant side due to the laws of physics, is really low or high. There are some studies in which the diameter of the TS has been examined; however, it has been stated that no clear inference can be made due to morphologic variations (14).

It was observed that TS diameters on both sides were not gender-dependent, both left and right TS diameters increased in both sexes with the shedding of the labbe vein, but the amount of this increase did not change with gender. Recent studies have reported that TS thrombosis is more common in women (15). Although no anatomical dimensional or angular difference was found, it is likely that one of the reasons for this statistic in the clinic is the different functioning physiology between the two sexes.

The distance between the point where the labbe vein drains into the TS and the mastoid process was calculated in the patients and it was found that these measurements did not vary depending on gender. With these calculations, anatomical information was obtained to make the surgery safer and easier, including the skin incision and dissection phase. Although not similar to our measurements, there are studies showing the estimated localization of the sinuses and torcular herophilia by measuring the distances to the determined anatomical points (16).

It was found that TS diameters did not change with age. Many anatomical and physiological changes occur in the human body with aging. However, it is remarkable that the transverse sinus diameter remains constant. In studies, it has been reported that venous anatomy changes with age in pediatric patients and the sinuses reach maximum diameter at a certain age (17).

This study has revealed many important results, but it also has some deficiencies. Considering the diagnosis of the patients at the time of presentation to the clinic while making comparisons will show whether the results obtained change in patients presenting with different diagnoses and in which patient group they are more applicable. This is only possible by increasing the number of patients included in the study.

The venous system in the midline and basal regions of the brain, which has received more attention with the development of surgical techniques over time, often has variations in size and connections. This study has provided very important numerical and statistical data on the venous anatomy of the brain. We believe that these data will pioneer future studies on the cerebral venous system. It is a fact that as the number of studies and data increase, surgical planning can be made more accurately and surgical success will increase, especially in centers without neuronavigation systems.

In this study, the anatomical structure and variations of the transverse sinuses were analysed in detail and significant findings were obtained especially in terms of dominance orientation, exit angles and diameter variations associated with the Labbe vein. It was observed that the transverse sinuses were more dominant on the right side, but this dominance was not related to gender or age. Furthermore, there were significant differences between the exit angles of the sinuses from the torcular Herophilia and the involvement of the Labbe vein caused significant changes in sinus diameter. Distance measurements to the mastoid process were similar regardless of gender. The findings obtained may provide a better understanding of the anatomical position of the transverse sinuses in neurosurgical practice and may guide surgical planning. This study constitutes an important resource in terms of raising awareness of anatomical variations, especially in centres where neuronavigation is not available, and may form the basis for more comprehensive morphometric studies in the future.

Conflict of interest
The authors declared no conflict of interest.

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None to declare.

Authors' contributions
Concept: M.A., R.E., Design: M.A.E, Data Collection or Processing: M.A.E, Analysis or Interpretation: M.A.E, R.E., C.C., Literature Search: M.A.E, R.E., C.C., Writing: M.A.E., R.E., C.C.

Ethical Statement
This study was conducted with the approval of the Ondokuz Mayıs University Clinical Research Ethics Committee, based on our application numbered 2021000136-3 and approval decision number 2021/136.

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Review

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Age-related declines in stem cell function: Molecular insights and future therapies

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Abstract

Stem cells are fundamental units that support tissue homeostasis throughout life, thus, progressive loss of function with aging would result in defective maintenance and regeneration of tissues, which in turn increases vulnerability to age-associated diseases. This review summarizes the potential contributors such as telomere shortening, DNA damage accumulation, epigenetic changes and mitochondrial dysfunction to age-associated defects in stem cell function. We discuss the interactions of these intrinsic factors with changes in the stem cell niche, including blood-derived inflammatory signals and changes in the extracellular matrix that contribute to stem cell exhaustion. Despite this local accessibility and its known role in regeneration, our knowledge about the age-related loss of tissue-specific regenerative potential is limited largely to age-related changes in specific stem cell populations, including hematopoietic, mesenchymal, neural, muscle satellite and intestinal stem cells, and highlights the potential for tissue-specific regenerative impediments. Further, new therapeutic strategies against stem cell exhaustion are discussed, such as caloric restriction, genetic and epigenetic reprogramming, senolytics, stem cell transplantation, and mitochondrial-targeted therapies. It also discusses challenges in tumorigenesis, immune rejection, and long-term efficacy. The review then ends with a reminder of the importance of ongoing studies for generating applicable treatments to prolong healthy life and promote regenerative responses. The present extensive synthesis is intended to assist further regenerative medicine efforts by presenting the most promising therapies to counteract aging effects on stem cells.

Keywords: stem cells, cell aging, mitochondrial dysfunction, epigenetics

1. Introduction

1.1. Overview of stem cells

Stem cells are specialized cells found in most adult mammalian tissues that play a crucial role in maintaining tissue homeostasis and facilitating tissue repair and regeneration in response to damage (1). These cells are characterized by their ability to self-renew and differentiate into various cell types, although they make up only a small fraction of the total cells within any tissue (2). Their identification and study have been a challenge, but recent advances have led to the discovery of molecular markers that allow for the isolation of tissue-specific stem cells (3). This has opened new avenues for research into the molecular mechanisms governing stem cell behavior, including multipotentiality and self-renewal. Additionally, the development of stem cell–based therapies hold significant promise for regenerative medicine. Stem cells reside within specialized microenvironments, or niches, which regulate their activity (4). These niches are composed of various elements such as the extracellular matrix, neighboring cells, and locally secreted soluble factors. Importantly, these niches, and the systemic milieu that influences them, dynamically adapt to regulate stem cell function, especially with regard to aging(5). This review explores what is known about the aging process in several key stem cell populations, including hematopoietic stem cells (HSCs), embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), mesenchymal stem cells (MSCs), intestinal stem cells (ISCs), satellite cells (Muscle stem cells), EpSCs and neural stem cells (NSCs) (Fig. 1) (6). We examine the changes that occur in stem cell number and function with age, explore the factors that make stem cells susceptible or resistant to aging, and evaluate the extent to which stem cell dysfunction contributes to overall aging processes (7). Stem cell aging reveals age-related declines in stem cell populations. Studies on serial transplantations show that while stem cells can pass through multiple recipients, their regenerative capacity diminishes after a few passages (8). Donor stem cells become less competitive over time, and even in primary transplants, stem cells recover to only a fraction of their original numbers (9). This decline might be influenced by extrinsic factors such as the bone marrow environment, but still points to limitations in stem cell self-renewal with age (10).

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Age-related changes in stem cell populations significantly affect their function and regenerative capacity (11). HSCs, while capable of serial transplantation, exhibit limitations in longevity, typically sustaining only five passages (12). Over time, host stem cells gain a competitive advantage over transplanted donor cells, leading to diminished hematopoietic function (13). This limitation may arise from transplant-related manipulations, not reduced stem cell potency. Aged stem cells show higher apoptosis rates due to accumulated cellular damage, which hampers their self-renewal and differentiation (14). The efficiency of stem cells homing to their marrow environment also declines with age. Despite apoptosis being a crucial regulatory mechanism for stem cell populations, targeting it might improve aging outcomes (15). Studies involving genetically modified mice show that preventing apoptosis can increase stem cell numbers and enhance engraftment. Stem cell migration plays a role in normal hematopoiesis, as evidenced by cross-engraftment in conjoined mice (16).

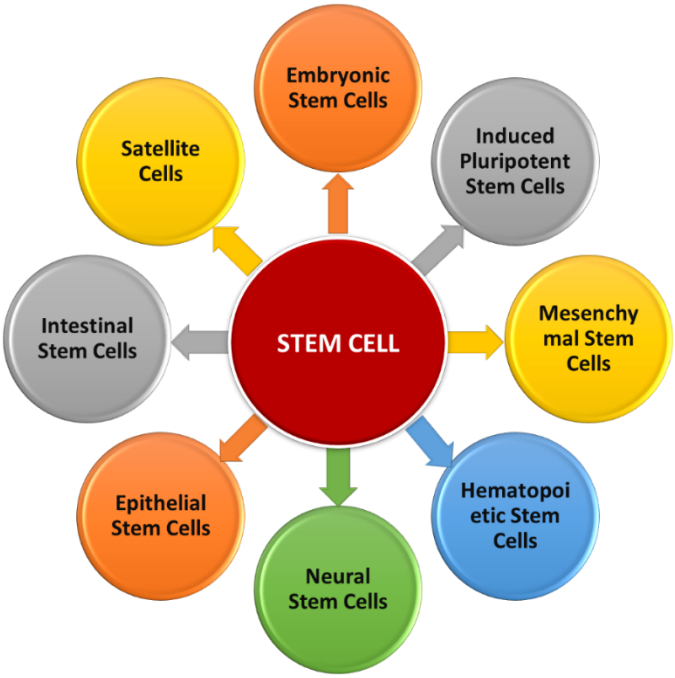

Fig. 1. Different types of stem cells

ESCs, derived from the inner cell mass of blastocysts, serve as a gold standard for pluripotency and self-renewal. While their direct therapeutic application remains limited due to ethical concerns and risks of teratoma formation, ESCs provide invaluable models for studying the molecular pathways governing cellular aging (17). Comparative studies between ESCs and aged somatic cells have revealed critical differences in DNA repair mechanisms, epigenetic regulation, and mitochondrial function that contribute to age-related cellular deterioration (18).

iPSCs have emerged as a revolutionary alternative, offering patient-specific models of aging and regeneration. By reprogramming somatic cells using Yamanaka factors (OCT4, SOX2, KLF4, c-MYC), iPSCs reset the aging clock, providing insights into cellular rejuvenation (19). However, recent studies show that iPSCs derived from aged donors may retain epigenetic memories of aging, limiting their functionality. Current research focuses on optimizing reprogramming techniques to generate "younger" iPSCs for autologous therapies in age-related diseases, such as Parkinson's and cardiovascular disorders (20).

MSCs found in bone marrow, adipose tissue, and umbilical cord, play a pivotal role in tissue maintenance and immune modulation. With advancing age, MSCs exhibit reduced proliferative capacity, increased senescence-associated secretory phenotype (SASP), and skewed differentiation potential—often favoring adipogenesis over osteogenesis (21). This shift contributes to age-related conditions like osteoporosis and sarcopenia. Strategies to rejuvenate aged MSCs, including mitochondrial transfer, senolytic drugs, and exosome-based therapies, are under investigation to restore their regenerative potential (22).

In the nervous system, NSCs residing in the subventricular zone and hippocampus sustain neurogenesis throughout life. Aging leads to a dramatic decline in NSC proliferation and differentiation, resulting in diminished cognitive plasticity and increased vulnerability to neurodegenerative diseases like Alzheimer's (23). Key factors implicated in NSC aging include accumulated DNA damage, dysregulated Wnt/β-catenin signaling, and chronic inflammation. Interventions such as exercise, caloric restriction, and pharmacological activation of neurogenic pathways show promise in mitigating these declines (24).

EpSCs are critical for maintaining the skin and other barrier tissues, undergo functional attrition with age. Reduced self-renewal capacity, coupled with impaired wound healing, leads to thinning epidermis, chronic ulcers, and increased susceptibility to infections (25). Similarly, ISCs located in the crypts of Lieberkühn experience age-related declines in regenerative activity, contributing to leaky gut syndrome, malabsorption, and systemic inflammation. The Wnt and Notch signaling pathways, essential for ISC maintenance, become dysregulated with age, offering potential therapeutic targets for preserving gut homeostasis in the elderly (26).

Satellite Cells (Muscle Stem Cells) are indispensable for skeletal muscle repair and regeneration. During aging, these cells enter a state of quiescence or senescence due to niche alterations, oxidative stress, and chronic inflammation, leading to sarcopenia—the progressive loss of muscle mass and strength. Emerging therapies, including stem cell transplantation, myostatin inhibition, and NAD+ boosters, aim to reactivate satellite cell function and restore muscle integrity in aged individuals (27).

1.2. The impact of aging on stem cells

Aging is a complex biological process characterized by the progressive decline in physiological function and increased

susceptibility to diseases, particularly those associated with impaired tissue repair and regeneration (28). One of the hallmarks of aging is the deterioration of stem cell function, a phenomenon commonly referred to as "stem cell aging." As organisms age, stem cells exhibit reduced regenerative capacity, increased senescence, and altered differentiation patterns (29). These changes compromise the ability of tissues to recover from injury and maintain homeostasis, contributing to the development of age-related disorders such as osteoporosis, neurodegenerative diseases, cardiovascular conditions, and sarcopenia (30). Several factors contribute to the decline in stem cell function with age, including genetic and epigenetic alterations, oxidative stress, mitochondrial dysfunction, telomere shortening, and changes in the stem cell microenvironment (niche) (31). The molecular pathways driving these changes are diverse, involving DNA damage accumulation, cellular senescence, disrupted signaling pathways, and a shift towards a pro-inflammatory systemic environment (32). Understanding the molecular mechanisms behind these processes is essential for developing therapeutic interventions aimed at rejuvenating aged stem cells and restoring regenerative potential (33).

1.3. Purpose of the review

This review aims to provide a comprehensive overview of the current understanding of stem cell aging, focusing on the molecular insights that drive the age-related decline in stem cell function. We will explore the general mechanisms underlying stem cell aging, such as telomere attrition, DNA damage accumulation, and mitochondrial dysfunction, as well as the specific effects on various stem cell populations, including HSCs, MSCs, NSCs, satellite cells, and ISCs. Additionally, we will discuss how changes in the stem cell niche and systemic factors influence stem cell aging. Finally, we will examine potential therapeutic strategies to counteract age-related declines in stem cell function, including caloric restriction, genetic and epigenetic reprogramming, senolytics, stem cell transplantation, and mitochondrial-targeted therapies. The review concludes with a discussion on the challenges and future directions for translating these insights into clinical applications aimed at improving healthy aging and regenerative outcomes. Fig.2 representing the stem cell aging and their mechanism.

2. Stem cell function and aging: general mechanisms

One of the hallmarks of aging is stem cell exhaustion, characterized by a gradual decline in the number and function of stem cells (34). Stem cell exhaustion manifests as a reduced ability to maintain tissue homeostasis, repair damaged tissues, and support the regenerative processes required for normal functioning (35). This decline is observed across various stem cell types, including HSCs, MSCs, NSCs, and muscle satellite cells, leading to age-related pathologies such as immune senescence, osteoporosis, neurodegeneration, and muscle wasting (36).

2.1. Factors causıng stem cell depletıon

Several intrinsic and extrinsic factors contribute to stem cell exhaustion (37). Intrinsic factors include DNA damage accumulation, telomere attrition, and metabolic changes, while extrinsic factors involve alterations in the stem cell niche, systemic inflammation, and age-related shifts in circulating factors (28). Stem cell exhaustion results in a reduced pool of functional stem cells, a decline in self-renewal potential, and aberrant differentiation patterns, which collectively contribute to impaired tissue regeneration and increased vulnerability to age-related diseases (38).

Telomere shortening and its role in stem cell aging

Telomeres are repetitive nucleotide sequences located at the ends of chromosomes, serving to protect genomic integrity during cell division (39). With each cell division, telomeres gradually shorten due to the "end-replication problem (40)." In stem cells, the enzyme telomerase counteracts this shortening by adding telomeric repeats to the ends of chromosomes, thus maintaining replicative capacity (41). However, as stem cells age, telomerase activity decreases, leading to progressive telomere shortening and an eventual loss of telomere integrity (42). Telomere attrition limits the replicative lifespan of stem cells, triggering cellular senescence or apoptosis when telomeres reach a critically short length (43). This phenomenon is particularly evident in tissues that require frequent cell turnover, such as the hematopoietic and intestinal systems (44). For example, in HSCs, telomere shortening impairs the ability to generate adequate immune cells, contributing to immune senescence (45). In 888NSCs, reduced telomerase activity and telomere shortening are associated with diminished neurogenesis and cognitive decline (46). The molecular mechanisms linking telomere shortening to stem cell dysfunction involve DNA damage response pathways (47). Critically short telomeres are recognized as DNA double-strand breaks, activating pathways such as p53, which induces cell cycle arrest and senescence. The accumulation of senescent stem cells further disrupts tissue function and propagates age-related degeneration (48).

DNA Damage Accumulation and Cellular Senescence

DNA damage is a key factor driving cellular aging, with stem cells being particularly susceptible due to their potential for long-term self-renewal (49). Accumulation of DNA damage occurs as a result of various factors, including endogenous metabolic processes, reactive oxygen species (ROS), and environmental insults (50). With age, the ability of stem cells to repair DNA damage diminishes, leading to the accumulation of genetic lesions and chromosomal instability (51).

One consequence of unresolved DNA damage in stem cells is the activation of cellular senescence, a state of permanent cell cycle arrest characterized by changes in gene expression, secretion of pro-inflammatory factors, and altered metabolic activity (52). Senescent stem cells contribute to the aging process by impairing tissue regeneration and creating a pro-inflammatory environment through the secretion of a distinct

set of factors known as the senescence-associated secretory, phenotype (SASP) (53). SASP factors include cytokines, chemokines, proteases, and growth factors that can disrupt the local stem cell niche and promote chronic inflammation (54).

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Oxidative stress is a significant contributor to DNA damage and cellular senescence in aging stem cells (55). The increased production of ROS, coupled with a decline in antioxidant defenses, leads to oxidative damage to DNA, proteins, and lipids (56). This is especially detrimental in stem cells, where maintaining genomic integrity is crucial for their regenerative function (57).

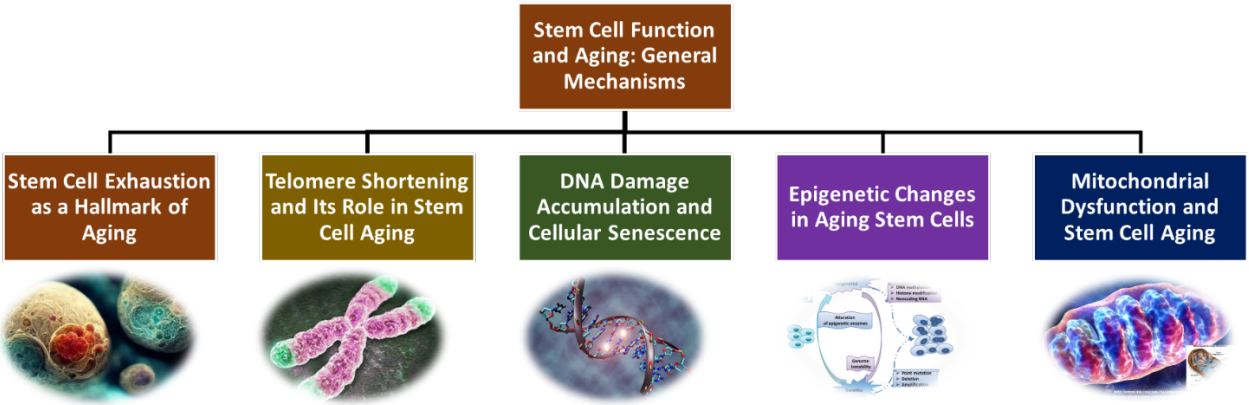

Fig. 2. Diagrammatic representation of stem cell aging and their mechanism

Epigenetic changes in aging stem cells

Epigenetic regulation, which includes DNA methylation, histone modifications, and non-coding RNA activity, plays a vital role in maintaining stem cell identity and function (58). However, aging leads to changes in the epigenetic landscape of stem cells, a phenomenon often referred to as "epigenetic drift (59)." This drift results in altered gene expression profiles, loss of stem cell identity, and compromised self-renewal and differentiation capacity (60). In aged stem cells, DNA methylation patterns become more heterogeneous, with regions of hypermethylation and hypomethylation (61). These changes can disrupt the expression of genes involved in stem cell maintenance, leading to impaired regenerative function (62). For example, hypermethylation of promoter regions associated with key self-renewal genes can reduce stem cell activity, while hypomethylation of repetitive elements may contribute to genomic instability (63). Histone modifications also change with age, affecting chromatin structure and gene accessibility (64). For instance, a reduction in histone acetylation may decrease the expression of genes essential for maintaining a stem cell's undifferentiated state, while increased histone methylation can contribute to the repression of genes involved in cell cycle regulation (65). Collectively, these epigenetic alterations limit the ability of aged stem cells to respond to regenerative signals effectively (66).

Mitochondrial dysfunction and stem cell aging

Mitochondria play a central role in cellular energy production, metabolism, and regulation of apoptosis (67). In stem cells, mitochondrial function is crucial for maintaining the balance between self-renewal and differentiation (68). However, mitochondrial dysfunction becomes more pronounced with age, leading to a decline in stem cell regenerative potential (69). The mechanisms underlying mitochondrial dysfunction in aging stem cells involve alterations in mitochondrial biogenesis, dynamics (fusion and fission), and mitophagy (selective autophagy of damaged mitochondria) (70). Age-related changes in these processes can lead to the accumulation of dysfunctional mitochondria, impaired energy production, and increased ROS generation (71). The resulting oxidative stress exacerbates DNA damage and cellular senescence, further compromising stem cell function (72). In addition to oxidative damage, shifts in stem cell metabolism from glycolysis to oxidative phosphorylation can contribute to aging-related declines (73). Young stem cells typically rely on glycolysis for energy production, a metabolic state that supports quiescence and reduces ROS generation. However, aged stem cells exhibit increased oxidative phosphorylation, which is associated with higher ROS levels and oxidative damage (74). This metabolic shift can also influence differentiation patterns, as certain lineage commitments may require specific metabolic states (75).

3. Age-related changes in specific stem cell populations

Aging impacts stem cell populations differently depending on the tissue type and functional requirements. The following subsections explore how specific stem cell types—hematopoietic, mesenchymal, neural, muscle satellite, and (ISCs)—experience age-related declines in function and the implications for tissue maintenance and repair.

3.1. Hematopoietic stem cells (HSCs)

HSCs are specialized cells found in the bone marrow that are essential for the continuous production of all blood cells throughout an individual's life (76). These cells are responsible for generating a diverse range of blood components, including oxygen-carrying red blood cells, infection-fighting white blood cells, and platelets, which are crucial for blood clotting and wound healing (77). Functionality of HSCs declines as individual age. Aging affects their regenerative capacity, making them less efficient at replenishing the blood system (78). Their self-renewable ability decreases over time, leading to a gradual depletion of the stem cell pool (79). One of the significant changes associated with aging is lineage skewing, where HSCs show a preference for producing specific types of blood cells, often favoring myeloid cells over lymphoid cells (80). This imbalance contributes to an increased risk of age-related hematopoietic disorders, such as anemia, reduced immune function, and an elevated likelihood of developing blood-related cancers (81).

Impact of aging on HSC Function

As individuals age, HSCs experience a significant shift in their blood cell production, moving from generating lymphoid cells, such as T cells and B cells, to favoring myeloid cell lineages like macrophages and granulocytes (82). This phenomenon, called "myeloid skewing," is a hallmark of aging and contributes to immune senescence—a decline in the immune system's efficiency (83). As a result, older adults face diminished adaptive immune responses, making them more vulnerable to infections and reducing the effectiveness of vaccines. Additionally, the decreased regenerative capacity of aged HSCs limits their ability to adequately replenish blood cells after injuries or physiological stress, increasing the risks of anemia, excessive bleeding, and other blood-related disorders (84).

Molecular mechanisms underlying HSC Aging

Several molecular mechanisms drive the age-related decline in HSC functions are mentioned below (Fig. 3):

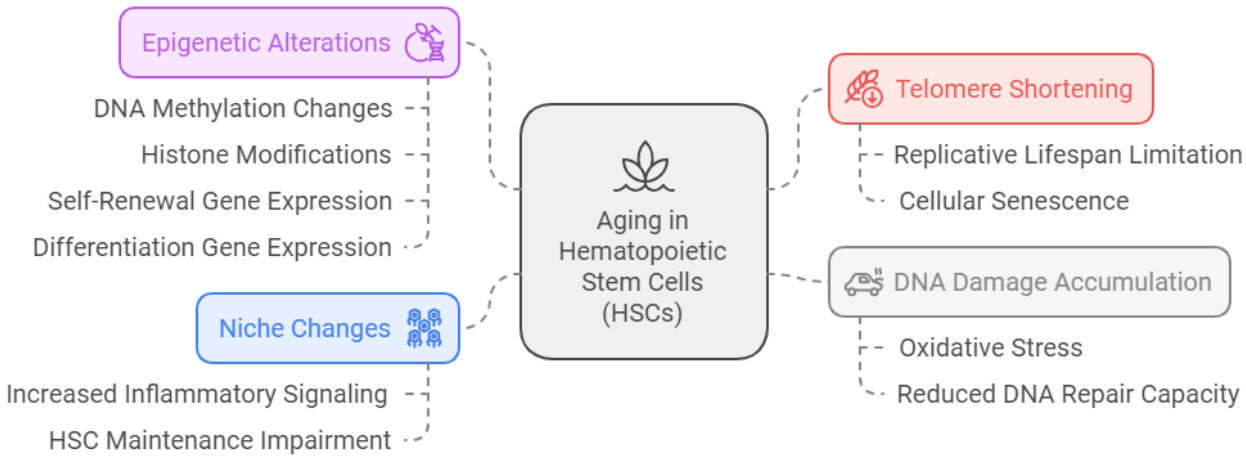

Fig. 3. Mechanism of age-related decline in hematopoietic stem cell

Telomere Shortening: HSCs undergo progressive telomere shortening as they age, which limits their ability to replicate over time (85). This gradual loss of telomere length leads to a reduction in the cells' regenerative capacity, ultimately contributing to cellular senescence (86). The resulting decline in HSC function is linked to aging and the reduced ability to maintain tissue homeostasis (87).

DNA damage accumulation: In aged HSCs, increased DNA damage occurs due to heightened oxidative stress and a diminished capacity for DNA repair (88). This accumulation of damage impairs the cells' normal functions, reducing their ability to regenerate and maintain healthy blood and immune systems, which contributes to age-related cellular dysfunction and disease (89).

Epigenetic alterations: Aging causes alterations in DNA methylation and histone modifications within HSCs, leading to changes in the regulation of genes essential for self-renewal and differentiation (90). These epigenetic changes disrupt the normal function of HSCs, impairing their ability to regenerate and differentiate, ultimately contributing to age-related functional decline (88).

Niche changes: As the bone marrow microenvironment, or niche, ages, it experiences increased inflammatory signaling and other alterations that impair its ability to support HSCs (91). These changes negatively affect HSC maintenance, reducing their regenerative capacity and function, which contributes to the overall decline in blood and immune system health with age (92).

3.2. Mesenchymal stem cells (MSCs)

MSCs are multipotent stem cells present in several tissues, including bone marrow, adipose tissue, and the umbilical cord (93). They possess the unique ability to differentiate into various cell types, such as osteoblasts (bone cells), chondrocytes (cartilage cells), and adipocytes (fat cells), making them essential for the repair and maintenance of bone,

cartilage, and fat tissues (94). As individuals age, MSCs experience a decline in their regenerative abilities, differentiation potential, and responsiveness to tissue damage (95). This reduced function contributes to age-related tissue degeneration, impaired healing processes, and diminished capacity for tissue regeneration and repair in aging individuals (96).

Aging-related changes in MSC Function

Age-related changes in MSCs include a shift in differentiation potential, favoring adipogenesis over osteogenesis, which contributes to conditions such as osteoporosis (97). The decline in the ability to form new bone cells and cartilage impacts the integrity of the skeletal system, leading to an increased risk of fractures, osteoarthritis, and other degenerative joint disorders (98).

Molecular mechanisms underlying MSC Aging

***Mitochondrial dysfunction*:** Aging MSCs exhibit diminished mitochondrial function and heightened production of reactive oxygen species (ROS) (99). This increase in ROS leads to oxidative stress, causing cellular damage and impairing MSC functionality (100). Consequently, the reduced ability to maintain and repair tissues contributes to age-related degeneration and diminished regenerative capacity (101).

***Senescence and SASP*:** As MSCs accumulate senescence in aging tissues, they release pro-inflammatory factors through the senescence-associated secretory phenotype (SASP) (102). This release disrupts the local tissue microenvironment, promoting inflammation and impairing the regenerative capacity of tissues, thereby contributing to age-related tissue degeneration and reduced healing and repair processes (103).

***Epigenetic modifications*:** Age-related changes in DNA methylation and histone modifications alter the expression of key genes involved in MSC differentiation (104). These epigenetic changes reduce the cells' ability to undergo osteogenesis (bone formation) and chondrogenesis (cartilage formation), leading to a diminished regenerative capacity in aging tissues and impaired skeletal health (105).

***Altered niche interactions*:** As the bone marrow and other MSC niches age, they undergo inflammatory changes that disrupt the local environment (106). This inflammation impairs the ability of MSCs to effectively respond to signals for tissue repair, reducing their regenerative function and contributing to age-related tissue degeneration and delayed healing (107).

3.3. Neural stem cells (NSCs)

NSCs are specialized cells located in specific areas of the adult brain, primarily in the subventricular zone and the hippocampus (108). These regions are key to neurogenesis, the generation of new neurons and glial cells, which are essential for brain function (109). NSCs are crucial for maintaining cognitive abilities, supporting learning and memory, and aiding in the brain's recovery from neurological damage (110). Unfortunately, as individuals age, the activity of NSCs decreases significantly, leading to a decline in neurogenesis (111). This reduction is a major factor contributing to age-related cognitive decline, impairments in memory, and an increased susceptibility to neurodegenerative conditions such as Alzheimer's and Parkinson's diseases (112). Understanding NSC dynamics is therefore vital for developing therapies to combat these age-associated neurological issues (113).

Decline in neurogenesis and cognitive function

As individuals age, the rate of neurogenesis declines due to reduced NSC proliferation, increased cellular senescence, and shifts in differentiation that favor the production of glial cells over neurons (114). This decline is associated with impairments in cognitive functions such as learning and memory. Additionally, age-related neuroinflammation, marked by the activation of microglia and elevated cytokine levels, further exacerbates the reduction in NSC function and neurogenesis (115). These factors contribute to diminished brain plasticity and may increase the risk of cognitive decline and neurodegenerative diseases in older individuals (116).

Molecular mechanisms contributing to NSC aging

***Telomere Shortening*:** Like other stem cells, NSCs undergo telomere shortening as they age, which limits their ability to proliferate (117). Telomeres, protective caps at the ends of chromosomes, gradually shorten with each cell division (118). In NSCs, this telomere erosion reduces their regenerative capacity, impairing their ability to generate new neurons and glial cells (119). As a result, the brain's ability to repair and maintain itself diminishes over time, contributing to cognitive decline and an increased risk of neurodegenerative diseases (120). Telomere shortening is thus a key factor in the aging of NSCs and the overall decline in brain plasticity (121).

***DNA damage and senescence*:** The accumulation of DNA damage and the onset of cellular senescence in NSCs significantly impede their regenerative capacity, contributing to brain aging (122). Over time, NSCs experience increased DNA damage due to oxidative stress and other age-related factors (123). This damage triggers cellular senescence, a state in which cells lose their ability to divide and function properly (124). Senescent NSCs not only fail to regenerate neurons and glial cells but also release pro-inflammatory factors that promote a neurodegenerative environment (125). This shift exacerbates age-related cognitive decline and increases the risk of neurodegenerative diseases, such as Alzheimer's and Parkinson's (126).

***Epigenetic dysregulation*:** Changes in histone modifications and DNA methylation in aging NSCs) can influence the expression of key genes involved in neurogenesis and neuronal differentiation (127). These epigenetic alterations may disrupt normal gene regulation, leading to impaired brain cell development and reduced neuroplasticity, contributing to cognitive decline and age-related neurological conditions (128). Understanding these changes is crucial for developing potential interventions to support healthy brain aging (129).

***Mitochondrial dysfunction*:** Aging NSCs show impaired mitochondrial function, resulting in decreased energy production and heightened oxidative stress (130). This dysfunction negatively impacts neurogenesis, reducing the ability to generate new neurons and maintain brain health (131). Understanding these changes in mitochondrial efficiency is essential for developing strategies to mitigate age-related declines in cognitive function and support neural regeneration (132).

3.4. Satellite cells

Satellite cells are a type of adult stem cell located between the basal lamina and sarcolemma of skeletal muscle fibers (133). These cells are essential for the repair, regeneration, and maintenance of skeletal muscle, especially after injury or damage (134). When muscles are injured, satellite cells become activated, proliferate, and differentiate into myoblasts, which fuse with damaged muscle fibers to aid in repair (135). However, as satellite cells age, their regenerative potential diminishes, leading to impaired muscle repair and regeneration (136). This decline contributes significantly to sarcopenia, the age-related loss of muscle mass and strength, which is a major cause of reduced muscle function in the elderly (137). The reduced efficiency of satellite cells in aging muscles is linked to cellular senescence, alterations in the stem cell niche, and changes in signaling pathways, all of which reduce their ability to respond to muscle damage (138). Understanding these mechanisms is the key to developing interventions for age-related muscle loss (139).

Decreased in satellite cell activity

With age, the number of quiescent satellite cells decreases, and their capacity to activate and proliferate in response to muscle injury declines (140). This reduced responsiveness leads to a weakened muscle repair process. Moreover, aged satellite cells often show an impaired ability to differentiate into mature myofibers, which further hinders muscle regeneration (141). As a result, muscle healing becomes delayed and incomplete, contributing to a decline in muscle function over time (142). These age-related changes in satellite cell behavior are key factors in the development of sarcopenia, the progressive loss of muscle mass and strength in the elderly (143).

Molecular pathways involved in the decline of muscle regeneration

***Cellular Senescence*:** The increased senescence of satellite cells reduces their ability to effectively participate in muscle repair, weakening the regeneration process (144). Additionally, the senescence-associated secretory phenotype (SASP) released by these aging cells disrupts the muscle microenvironment, promoting inflammation and further impairing muscle regeneration (145). This dual impact of cellular aging contributes to delayed healing and reduced muscle function, exacerbating age-related conditions like sarcopenia (146).

***Notch and Wnt Signaling dysregulation*:** Age-related changes in signaling pathways, such as Notch and Wnt, negatively impact the activation and differentiation of satellite cells, impairing their ability to regenerate muscle tissue. The Notch pathway, essential for satellite cell activation, becomes less efficient with age, while increased Wnt signaling promotes differentiation into fibrotic tissue instead of healthy muscle. These imbalances disrupt the muscle repair process, contributing to weakened muscle regeneration and increasing the risk of sarcopenia and muscle function decline in aging individuals.

Mitochondrial dysfunction and metabolic shifts: Aged satellite cells exhibit reduced mitochondrial function and altered metabolic profiles, which significantly limit their capacity for effective muscle regeneration (78). Mitochondria, the powerhouse of the cell, play a crucial role in energy production required for cell proliferation and repair (147). With aging, mitochondrial dysfunction leads to decreased energy availability, impairing satellite cell activation and their ability to undergo proper differentiation (148). Additionally, metabolic shifts in aged satellite cells contribute to reduced efficiency in muscle regeneration, further slowing the repair process and promoting muscle degeneration, which exacerbates age-related conditions like sarcopenia (149).

***Inflammatory environment*:** Chronic low-grade inflammation, known as "Inflammaging," occurs in aged muscle tissues and has a detrimental impact on satellite cell function and muscle regeneration (150). This persistent inflammatory state is characterized by elevated levels of pro-inflammatory cytokines, which disrupt the muscle microenvironment (151). Inflammaging impairs the activation, proliferation, and differentiation of satellite cells, limiting their ability to effectively repair muscle damage (152). Over time, this inflammation-induced dysfunction accelerates muscle degeneration, contributing to age-related muscle loss (sarcopenia) and reduced muscle strength (153). Managing chronic inflammation may be key to improving muscle regeneration and mitigating the effects of aging on skeletal muscle (154).

3.5. Intestinal stem cells (ISCs)

ISCs reside at the base of the intestinal crypts and play a vital role in maintaining the integrity of the gut lining (155). They continuously replenish the epithelial cells, ensuring efficient nutrient absorption and acting as a barrier against pathogens. However, as individuals age, the regenerative capacity of ISCs declines (156). This reduced functionality contributes to a weakening of the intestinal barrier, slower cell turnover, and compromised epithelial repair (157). Consequently, the aging gut becomes more susceptible to inflammation, infections, and gastrointestinal disorders, such as inflammatory bowel disease and colorectal cancer (158). Diminished ISC activity also affects nutrient absorption efficiency, potentially leading to

nutrient deficiencies (159). Understanding the age-related changes in ISCs is crucial for developing targeted therapies to preserve gut health, enhance intestinal regeneration, and reduce the risk of age-associated gastrointestinal conditions, thereby improving overall well-being in the aging population (160).

Impact of aging on ISC turnover and gut health

As ISCs age, their function declines, leading to slower renewal of the intestinal epithelium and increased permeability of the gut barrier (161). This deterioration raises the risk of gastrointestinal disorders, including inflammatory bowel disease (IBD) and colorectal cancer (162). Additionally, aging impacts the differentiation potential of ISCs, causing a shift in cell fate decisions. There's a tendency for aged ISCs to favor the production of secretory cells—like goblet and Paneth cells—over absorptive cells, which can compromise nutrient uptake and overall gut function (163). This imbalance in cell types contributes to a less efficient and more vulnerable intestinal lining. Understanding these age-related shifts in ISC behavior is essential for developing strategies to maintain gut health, prevent gastrointestinal diseases, and optimize nutrient absorption in older adults, thereby supporting better overall health and quality of life as the population ages (164).

Molecular mechanisms of ISC Aging

***Telomere attrition and DNA damage*:** Aging ISCs undergo telomere shortening and accumulate DNA damage, reducing their ability to proliferate effectively (165). This decline in regenerative capacity impacts the maintenance of the intestinal lining, leading to compromised gut function (166). Understanding the mechanisms of telomere attrition and DNA damage accumulation in ISCs is crucial for developing interventions to support gut health and counteract age-related gastrointestinal issues (167).

***Mitochondrial dysfunction*:** In aged ISCs, the buildup of dysfunctional mitochondria hampers energy production and elevates reactive oxygen species (ROS) levels (168). This oxidative stress reduces the cells' regenerative potential, compromising the maintenance of a healthy intestinal lining (169). Addressing mitochondrial dysfunction in aging ISCs is key to enhancing their regenerative capacity and supporting overall gut health (170).

***Niche changes*:** With age, the ISC niche experiences alterations, including shifts in key signaling pathways like Wnt, Notch, and BMP (171). These changes disrupt the delicate balance of ISC self-renewal and differentiation, leading to impaired gut regeneration and altered cell fate decisions (172). Understanding how aging impacts these signaling molecules is crucial for developing strategies to maintain ISC function and promote healthy gut aging (173).

3.6. Embryonic stem cells (ESCs)

ESCs are pluripotent cells derived from the inner cell mass of blastocysts, capable of differentiating into any cell type in the body. Their remarkable self-renewal and differentiation potential make them invaluable for regenerative medicine, disease modeling, and developmental biology research (174). However, despite their inherent plasticity, ESCs are not entirely immune to aging-related changes, particularly when maintained in long-term culture. Over time, ESCs may experience a decline in functionality, including reduced proliferation capacity, altered differentiation potential, and epigenetic instability. Understanding these age-related changes is crucial for optimizing their use in clinical applications, ensuring that ESC-derived therapies remain effective and safe for regenerative treatments (175).

Impact of aging on embryonic stem cell potency and differentiation

As ESCs age, either *in vivo* (in early developmental stages) or in vitro (during prolonged culture), their ability to self-renew and differentiate efficiently can diminish. One of the key observations is that aged ESCs exhibit slower proliferation rates, possibly due to accumulated cellular stress or epigenetic modifications (176). Additionally, their differentiation potential may become skewed, leading to a preference for certain cell lineages over others. This bias could compromise their utility in generating specific tissues for regenerative therapies. Another critical factor is epigenetic drift—changes in DNA methylation and histone modifications that alter gene expression patterns, potentially leading to loss of pluripotency or abnormal differentiation. These age-related shifts highlight the need for improved culture techniques and interventions to maintain ESC quality over extended periods (177).

Molecular mechanisms underlying ESC aging

***Telomere attrition and genomic instability*:** ESCs normally maintain their telomeres through high telomerase activity, which prevents the shortening typically seen in somatic cells during replication. However, prolonged in vitro culture or exposure to cellular stress can lead to gradual telomere erosion and DNA damage accumulation (178). When telomeres become critically short, cells enter senescence or apoptosis, impairing their regenerative potential. Additionally, genomic instability from DNA damage further compromises ESC function, reducing their ability to self-renew and differentiate efficiently. Understanding and mitigating telomere attrition in ESCs is crucial for maintaining their long-term therapeutic potential (179).

***Mitochondrial dysfunction and oxidative stress*:** While ESCs primarily rely on glycolysis for energy production, mitochondria play a vital role during differentiation. As ESCs age, mitochondrial efficiency declines, leading to the accumulation of defective mitochondria (179). This dysfunction results in increased production of reactive oxygen species (ROS), causing oxidative damage to proteins, lipids, and DNA. Elevated ROS levels not only impair ESC pluripotency but also disrupt differentiation capacity. Strategies to enhance mitochondrial quality control, such as antioxidant supplementation or mitophagy activation, could

help preserve ESC functionality in regenerative applications (180).

***Epigenetic alterations*:** Aging ESCs undergo significant epigenetic changes, including DNA methylation shifts and histone modifications, which alter gene expression patterns. These changes can lead to the silencing of pluripotency genes (e.g., *Oct4*, *Nanog*) or aberrant activation of differentiation pathways (181). Epigenetic drift contributes to reduced stem cell stability and increases the risk of uncontrolled differentiation or senescence. Epigenetic reprogramming techniques, such as transient exposure to Yamanaka factors, may help reset these modifications and restore youthful gene expression profiles in aged ESCs (182).

***Niche signaling pathway disruptions*:** The stem cell niche provides critical signals that regulate ESC self-renewal and differentiation. With aging, changes in key signaling pathways—such as Wnt, BMP, and FGF—can disrupt the balance between stemness and differentiation (183). For example, altered Wnt signaling may promote spontaneous differentiation, while dysregulated BMP activity could bias lineage specification. Optimizing culture conditions to mimic a youthful niche, including the use of growth factor cocktails or 3D scaffolds, may help maintain ESC potency and function over extended periods (184).

3.7. Induced pluripotent stem cells (iPSCs)

iPSCs are reprogrammed somatic cells that regain pluripotency through the introduction of key transcription factors (Oct4, Sox2, Klf4, c-Myc). While iPSCs share many characteristics with ESCs, including self-renewal and differentiation potential, they also exhibit unique aging-related challenges (185). Over time, both the original somatic cell age and the reprogramming process can influence iPSC functionality. Understanding these age-associated changes is critical for ensuring the reliability of iPSCs in regenerative medicine, disease modeling, and drug discovery (186).

Impact of aging on iPSC reprogramming efficiency and function

Aging significantly affects iPSC generation and function. Older donor cells carry epigenetic changes and DNA damage that reduce reprogramming efficiency and often leave residual age-related markers in resulting iPSCs. These aged iPSCs show three main limitations: (1) lower reprogramming success due to epigenetic barriers and cellular senescence, (2) inconsistent differentiation potential across cell lineages, and (3) increased genomic instability raising safety concerns (187). These challenges are particularly problematic for developing therapies for age-related diseases, where patient-specific iPSCs would ideally come from elderly donors. Current research focuses on improving reprogramming techniques, enhancing epigenetic resetting, and implementing stricter quality controls to overcome these age-related limitations in iPSC technology (188).

Molecular mechanisms underlying iPSC aging

***Epigenetic memory and incomplete reprogramming*:** iPSCs often retain epigenetic marks from their original somatic cell type, a phenomenon known as "epigenetic memory." This residual memory can bias differentiation toward the donor cell's lineage, limiting their plasticity (189). Incomplete reprogramming is more common in aged cells due to accumulated DNA methylation and histone modifications. Advanced reprogramming techniques, such as prolonged factor expression or small molecule treatments, may help erase these aging signatures (186).

***Mitochondrial dysfunction and metabolic shifts*:** Reprogramming resets cellular metabolism from oxidative phosphorylation to glycolysis, but aged iPSCs may retain mitochondrial abnormalities. Dysfunctional mitochondria can increase reactive oxygen species (ROS), contributing to oxidative stress and impairing iPSC self-renewal. Strategies to enhance mitochondrial clearance (mitophagy) or provide metabolic support could improve iPSC quality (190).

***Genomic instability and DNA damage accumulation*:** Aged somatic cells often harbor pre-existing DNA damage, which can persist after reprogramming. Additionally, the reprogramming process itself can induce genomic stress, leading to mutations or chromosomal abnormalities. Monitoring and selecting high-quality iPSC clones is crucial for clinical applications, particularly in aging research (190).

***Telomere dynamics and replicative senescence*:** While reprogramming extends telomeres through telomerase reactivation, iPSCs derived from aged cells may have shorter initial telomeres, potentially affecting long-term culture stability. Ensuring proper telomere maintenance is vital for sustaining iPSC proliferation and differentiation capacity (190).

3.8. Epithelial stem cells (EpSCs)

EpSCs are tissue-specific adult stem cells responsible for the continuous renewal and repair of epithelial tissues, which form critical barriers between the body and its external environment. Found in the skin, gastrointestinal tract, respiratory system, mammary glands, and other epithelial-rich organs, these stem cells maintain tissue homeostasis by balancing self-renewal and differentiation (26). Their primary role is to replace damaged or dying epithelial cells, ensuring proper barrier function, nutrient absorption (in the gut), pathogen defense (in the skin and airways), and wound healing (191).

Impact of Aging on EpSC function

Aging profoundly disrupts the regenerative capacity of EpSCs, leading to progressive tissue dysfunction across multiple organ systems. The decline in EpSC activity manifests through both cell-intrinsic changes and alterations in the stem cell niche, ultimately compromising tissue integrity and repair mechanisms (192).

Molecular mechanisms underlying EpSC aging genomic instability

Aged EpSCs accumulate DNA damage from telomere shortening and oxidative stress. Declining repair mechanisms lead to persistent DNA damage, triggering senescence or apoptosis. This depletes functional stem cells while increasing cancer risk (193).

Epigenetic dysregulation: Aging alters DNA methylation and histone marks, silencing stemness genes (e.g., *Lgr5*, *p63*) and disrupting chromatin accessibility. Non-coding RNA imbalances further impair regeneration, locking EpSCs in dysfunctional states (194).

Mitochondrial dysfunction: Damaged mitochondria accumulate due to failed quality control, increasing ROS and reducing ATP production. NAD+ depletion worsens this metabolic crisis, impairing EpSC proliferation and tissue repair (195).

Proteostasis collapse: Aged EpSCs lose protein-folding capacity as chaperone systems decline. Impaired autophagy and proteasome function allow toxic protein aggregates to accumulate, triggering chronic ER stress and senescence (195).

Niche degradation: The stem cell microenvironment stiffens with cross-linked ECM proteins while growth factor signaling (Wnt, BMP) becomes imbalanced. Senescent niche cells secrete inflammatory factors (IL-6, TGF-β) that suppress EpSC function (196).

Table 1. Different types, source, characteristics and role in aging of stem cells

Stem Cell Type	Source	Characteristics	Role in Aging	References
Embryonic Stem Cells	Blastocyst (early-stage embryo)	Pluripotent, unlimited self-renewal, can differentiate into any cell type	Declines with age; potential for regenerative medicine to counteract aging effects	(197)
Induced Pluripotent Stem Cells	Reprogrammed somatic cells (e.g., skin cells)	Pluripotent, genetically reprogrammed, avoid ethical concerns of ESCs	Used to model aging diseases; potential for autologous cell therapy in aging	(198)
Mesenchymal Stem Cells	Bone marrow, adipose tissue, umbilical cord	Multipotent, support tissue repair, immunomodulatory properties	Reduced regenerative capacity contributes to osteoporosis, arthritis, and frailty	(199)
Hematopoietic Stem Cells	Bone marrow, umbilical cord blood	Multipotent, give rise to blood and immune cells	Decline in function leads to anemia, immune senescence, and increased cancer risk	(200)
Neural Stem Cells	Brain (subventricular zone, hippocampus)	Multipotent, generate neurons and glial cells	Decline leads to cognitive impairment, neurodegenerative diseases (e.g., Alzheimer's)	(201)
Epithelial Stem Cells	Skin, gut lining, other epithelial tissues	Maintain and repair epithelial barriers, high turnover	Dysfunction leads to thinning skin, poor wound healing, and gastrointestinal decline	(202)
Intestinal Stem Cells	Crypts of Lieberkühn (small intestine)	Rapidly dividing, maintain gut epithelium, Lgr5+ marker	Decline leads to impaired gut barrier, reduced nutrient absorption, and increased susceptibility to infections	(203)
Satellite Cells (Muscle Stem Cells)	Skeletal muscle (under basal lamina)	Unipotent, repair and regenerate muscle fibers	Reduced activity causes sarcopenia (age-related muscle loss) and impaired recovery	(204)
Cardiac Stem Cells	Heart tissue (niche-dependent)	Limited regenerative capacity, can form cardiomyocytes and vascular cells	Insufficient repair contributes to heart failure and age-related cardiac decline	(205)

4. The stem cell niche and its role in aging

The stem cell niche is a unique microenvironment that plays a critical role in regulating stem cell behavior by providing structural support, essential nutrients, and biochemical signals (206). This niche controls key processes such as self-renewal, differentiation, and overall maintenance of stem cells (207). However, as stem cells age, the niche undergoes significant changes that can adversely affect stem cell function (208). These age-related alterations in the niche, including shifts in extracellular matrix composition, changes in signaling molecules, and increased inflammation, contribute to the decline in the regenerative capacity of stem cells (209). Additionally, systemic factors such as circulating hormones, immune cell activity, and metabolic signals also influence the aging of stem cells (210). Together, these local and systemic changes impact the stem cells' ability to repair and regenerate tissues, accelerating age-related degeneration (211). Understanding these dynamics is crucial for developing targeted interventions to slow down stem cell aging and promote healthy tissue maintenance (212).

4.1. Changes in the stem cell microenvironment (Niche)

The stem cell niche consists of diverse cellular and acellular elements that work together to support stem cell function (211). These include the extracellular matrix (ECM), niche cells,

signaling molecules, and mechanical cues—all of which create a dynamic environment influencing stem cell behavior (213). As the body ages, significant alterations occur in these niche components (214). The ECM may stiffen or degrade, niche cells can change in number or function, signaling molecules may become imbalanced, and mechanical properties of the tissue can shift (215). These changes can disrupt the communication between stem cells and their microenvironment, leading to a decline in stem cell maintenance and regenerative capacity (216). The cumulative impact of these age-related modifications in the niche contributes to diminished tissue repair and increased susceptibility to age-associated diseases, highlighting the importance of preserving niche integrity to support healthy aging (211).

Extracellular matrix alterations

The extracellular matrix (ECM) plays a critical role in supporting the stem cell niche, influencing stem cell behavior through its biochemical signals and mechanical properties (217). As aging progresses, the ECM undergoes changes in composition and stiffness, often linked to increased collagen cross-linking and the buildup of advanced glycation end products (AGEs) (218). These modifications can significantly alter the mechanical environment of the niche, impacting stem cell function (219). For example, a stiffer ECM has been found to drive MSCs towards a senescent state, reducing their ability to self-renew and differentiate effectively (220). This age-related shift in ECM properties can lead to impaired tissue regeneration and increased susceptibility to age-related conditions (221). Understanding the impact of ECM alterations on stem cell health is crucial for developing strategies to counteract the negative effects of aging and maintain tissue functionality (222).

Changes in niche cell populations

The stem cell niche is composed of various supporting cells, including fibroblasts, endothelial cells, and immune cells, which release signaling molecules that regulate stem cell behavior and maintenance (210). These niche cells create a microenvironment that is crucial for the proper functioning of stem cells (223). However, with aging, significant changes occur within the niche (224). The populations of these supporting cells shift, and their secretory profiles are altered, leading to disruptions in the regulation of stem cell activity (213). For instance, in the bone marrow niche, aging affects two key cell types, osteoblasts, which form bone, and adipocytes, which store fat (225). Changes in these cells negatively impact HSCs), the stem cells responsible for generating blood and immune cells (226). As a result, aged HSCs exhibit reduced regenerative capacity and altered differentiation patterns, contributing to a decline in immune function and the body's ability to repair tissues (156). These age-related changes in the niche play a critical role in the overall decline of tissue homeostasis and regeneration observed in older individuals, linking the aging process to impaired stem cell function and increased vulnerability to diseases associated with aging (227).

4.1.3 Inflammatory signals and "Inflammaging"

Chronic low-grade inflammation, or "inflammaging," is a hallmark of aging that disrupts the stem cell niche (228). Pro-inflammatory cytokines like interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) accumulate in aged tissues, disturbing the signaling balance needed for proper stem cell function (229, 230). This pro-inflammatory environment contributes to cellular senescence, impaired tissue regeneration, and altered stem cell differentiation. Inflammaging thus creates conditions that undermine the regenerative capacity of tissues (231). For example, increased levels of TNF-α in the aging muscle niche are linked to reduced satellite cell activity, which impairs muscle repair (232). This illustrates how chronic inflammation in aged tissues negatively impacts stem cell activity, accelerating tissue decline and contributing to age-related functional deterioration (233). Inflammaging is therefore a key factor in the reduced regenerative ability of tissues and organs commonly observed with aging (234).

4.2. Systemic factors and their influence on stem cell aging

The aging process affects not only the local environment of the stem cell niche but also systemic factors that regulate stem cell function across the entire body (235). Circulating factors, such as hormones, growth factors, and cytokines, play an essential role in maintaining stem cell activity, mediating repair, and supporting tissue regeneration (236). With age, these systemic factors become imbalanced, leading to a decline in stem cell function and regenerative capacity (237). Hormones like growth hormone and insulin-like growth factor-1 (IGF-1), which support tissue repair, decrease with age, negatively influencing stem cell maintenance and differentiation (238). Similarly, pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) accumulate in the bloodstream, further disrupting stem cell function across various tissues (239). This systemic shift in circulating factors contributes to the overall decline in tissue regeneration and the increased susceptibility to age-related diseases (240). For example, the decline in muscle repair seen in aging individuals is linked not only to local changes in the muscle niche but also to reduced levels of circulating regenerative factors (241). These systemic changes, combined with local niche deterioration, create a multi-faceted challenge for stem cell function, leading to impaired tissue maintenance and repair during aging (237, 238).

The Role of circulating factors in stem cell aging

Systemic factors in the blood, through endocrine signaling, play a crucial role in regulating stem cell function, and their decline with age significantly impacts tissue regeneration (242). Hormones like insulin-like growth factor-1 (IGF-1) and growth hormone (GH) decrease as we age, impairing the regenerative potential of stem cells in various tissues, including

muscle and bone (243). These hormones are vital for maintaining stem cell activity and tissue repair, and their reduced levels directly contribute to the body's diminished ability to heal (244). Additionally, age-related declines in estrogen and testosterone negatively affect MSCs and muscle satellite cells, leading to decreased bone density and impaired muscle regeneration (245). Estrogen plays a key role in bone health, and its reduction contributes to osteoporosis, while lower testosterone levels hinder muscle repair (246). Together, the decline in these systemic factors compromises stem cell function, driving tissue deterioration and contributing to age-related health issues (247).

Parabiosis experiments and implications for systemic regulation of aging

Parabiosis, a technique where the circulatory systems of two animals are surgically joined, has been used to study the effects of systemic factors on aging and stem cell function (248). Experiments involving heterochronic parabiosis (pairing of young and old animals) have shown that exposure to a young circulatory environment can rejuvenate aged stem cells and improve tissue function (249). For example, in heterochronic parabiosis studies, aged muscle satellite cells exposed to young systemic factors exhibited increased regenerative capacity and a reversal of age-related decline (250). Conversely, young stem cells exposed to an old circulatory environment showed signs of premature aging (251). These findings suggest that systemic factors play a significant role in regulating stem cell aging and that modifying these factors could be a potential therapeutic approach to rejuvenate aged stem cells (252).

Age-related changes in the hematopoietic and NSC niches

Specific stem cell niches, such as those for hematopoietic and neural stem cells, undergo distinct age-related changes that affect stem cell function (12, 88, 253).

Hematopoietic stem cell niche: The bone marrow niche for HSCs experiences structural and functional changes with age, including increased adipogenesis and altered osteoblastic activity (254, 255). These changes contribute to a decline in HSC function, leading to myeloid skewing and immune senescence (256). The aged HSC niche also exhibits increased inflammation and oxidative stress, further impairing stem cell function (92, 254).

Neural stem cell niche: The neurogenic niches in the aged brain show decreased levels of growth factors such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), which are essential for NSC maintenance and neurogenesis (257). In addition, increased neuroinflammation and activation of microglia in the aging brain negatively impact NSC function, contributing to cognitive decline and an increased risk of neurodegenerative diseases (258).

Extracellular vesicles and their role in stem cell aging

Extracellular vesicles (EVs), including exosomes and microvesicles, are released by cells and serve as carriers of signaling molecules like proteins, lipids, and RNA, playing a vital role in intercellular communication (259). They can influence stem cell function by transferring these bioactive molecules between cells (260). However, with aging, the composition and function of EVs change, potentially contributing to stem cell dysfunction (261). For instance, aged EVs may carry pro-inflammatory signals that promote cellular senescence and impair tissue regeneration. This shift can exacerbate the decline in regenerative capacity seen in aging tissues (262). In contrast, EVs derived from younger organisms have shown promise in rejuvenating aged stem cells by delivering regenerative factors that enhance their function (263). These findings highlight the dual role of EVs in either promoting or mitigating age-related stem cell decline, depending on their source and molecular content (264).

5. Therapeutic approaches to combat stem cell aging

As research into age-related stem cell decline advances, a range of therapeutic strategies have been developed to combat aging impact on stem cells (265). These methods aim to restore the regenerative abilities of aging stem cells, rejuvenate tissues, and promote healthier, extended lifespan (266). Key approaches include dietary interventions, which leverage nutrition to enhance stem cell function; genetic and epigenetic reprogramming, which modifies DNA and gene expression to reverse aging markers; and therapies targeting cellular senescence to remove or alter dysfunctional cells (267). Other strategies involve stem cell transplantation to replace damaged cells, tissue engineering to repair or regenerate organs, and mitochondrial-targeted therapies, focusing on improving cellular energy production (268). Collectively, these innovative approaches aim to slow or reverse the detrimental effects of aging, offering potential pathways to healthier aging (269).

5.1. Caloric restriction and dietary interventions

Caloric restriction (CR) and other dietary interventions have been widely researched for their potential anti-aging effects (269-271). CR entails reducing calorie intake without causing malnutrition and has been shown to extend lifespan and delay the onset of age-related diseases in various organisms, including mammals (272). The anti-aging benefits of CR on stem cells are mediated through its influence on key cellular pathways involved in metabolism, stress response, and nutrient sensing (271, 273). These pathways help regulate how cells respond to energy levels, environmental stressors, and nutrient availability, all of which play critical roles in aging (274). By activating these mechanisms, CR enhances cellular repair processes, reduces oxidative damage, and improves the regenerative capacity of stem cells (275). This maintenance of stem cell function is crucial for tissue homeostasis and slowing the decline associated with aging (272, 276). As a result, CR offers a promising approach to promoting healthy aging, not only by extending lifespan but also by preserving the regenerative potential of stem cells, delaying the onset of age-

related dysfunction (277).

Influence of caloric restriction on stem cell aging

Caloric restriction (CR) has been shown to improve the function of several stem cell populations, including HSCs, neural stem cells (NSCs), and MSCs (271). In HSCs, CR enhances function by reducing oxidative stress and preventing the accumulation of DNA damage, both of which are key contributors to stem cell aging (270). This preservation of HSC function supports blood and immune system regeneration, which typically declines with age. For NSCs, CR has been found to increase neurogenesis—the production of new neurons—and improve cognitive functions, including learning and memory (272, 273). These effects are mediated through the modulation of key signaling pathways, such as the insulin/IGF-1 and AMP-activated protein kinase (AMPK) pathways, both of which play critical roles in cellular metabolism and stress response (274). By influencing these pathways, CR helps maintain NSC activity, which is essential for brain health and function during aging. Similarly, CR also benefits MSCs by promoting their regenerative potential, aiding in the maintenance of bone, cartilage, and muscle tissues (275, 276). Collectively, these effects of CR on stem cell populations highlight its broad potential in preserving tissue homeostasis and combating the detrimental effects of aging (272).

Role of nutrient-sensing pathways

Key nutrient-sensing pathways, such as AMP-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), and sirtuins, play crucial roles in mediating the effects of CR on stem cell aging.

AMPK: Activation of AMP-activated protein kinase (AMPK) during caloric restriction (CR) boosts mitochondrial biogenesis and reduces oxidative stress, both of which contribute to improved stem cell function (278). By promoting the formation of new mitochondria, AMPK helps enhance energy production and cellular health, while lowering oxidative stress protects cells from damage and aging. This dual effect supports the maintenance and regenerative capacity of stem cells, enabling better tissue repair and overall function, which are key factors in healthy aging (278, 279). AMPK activation through CR thus plays a crucial role in sustaining stem cell vitality (280, 281).

mTOR: Inhibition of the mTOR (mechanistic target of rapamycin) pathway has been linked to increased stem cell self-renewal and delayed stem cell aging (282). The mTOR pathway plays a key role in regulating cell growth and metabolism, and its suppression helps reduce cellular stress and aging-related damage (283). Rapamycin, a well-known mTOR inhibitor, has demonstrated potential in enhancing the regenerative capacity of aging stem cells. By inhibiting mTOR, rapamycin promotes stem cell maintenance and improves their ability to regenerate tissues, which is crucial for maintaining tissue health during aging (284). This has made mTOR inhibition an attractive therapeutic target for promoting longevity and mitigating age-related decline in stem cell function (285, 286).

Sirtuins: NAD+-dependent deacetylases, known as sirtuins, are activated by caloric restriction (CR) and play a vital role in regulating cellular metabolism, stress response, and longevity (287). Sirtuin activation enhances mitochondrial function, improving energy production and cellular health, while also reducing inflammation in aging stem cells. This dual action is crucial for preserving stem cell function and delaying age-related decline (288). By boosting mitochondrial efficiency and limiting chronic inflammation, sirtuins help maintain stem cells' regenerative potential, supporting tissue repair and overall health during aging. This makes sirtuin activation an important mechanism in CR's anti-aging effects (289).

Potential of dietary interventions

In addition to caloric restriction, other dietary approaches such as intermittent fasting, ketogenic diets, and supplementation with specific nutrients have shown potential in delaying stem cell aging and improving tissue regeneration (278, 279). Intermittent fasting and ketogenic diets influence nutrient-sensing pathways, promoting cellular repair processes and enhancing the regenerative capacity of stem cells. Supplementing with nutrients like nicotinamide riboside, which boosts NAD+ levels, further supports these effects by activating sirtuins and improving mitochondrial function. These dietary strategies help modulate key pathways involved in metabolism and stress response, offering a non-invasive method to rejuvenate aging stem cells and improve tissue repair (280). By targeting the fundamental mechanisms of cellular aging, such approaches may provide effective means to promote healthy aging and combat age-related tissue degeneration (270).

5.2. Genetic and epigenetic reprogramming

Genetic and epigenetic reprogramming strategies aim to reverse age-related changes in gene expression, restoring a more youthful state in aging stem cells. By resetting the epigenetic landscape, these approaches can rejuvenate stem cells, enhancing their ability to regenerate tissues and maintain tissue homeostasis (256). A groundbreaking development in this field is the reprogramming of somatic cells into iPSCs, which has transformed regenerative medicine. iPSCs can differentiate into various cell types, offering the potential to replace damaged or aged tissues (126). This technology has opened new possibilities for treating age-related diseases by providing patient-specific cells for repair and regeneration. By reversing cellular aging markers and restoring stem cell function, genetic and epigenetic reprogramming represents a promising approach to combat aging and improve longevity in therapeutic settings (65).

Induced pluripotent stem cells (iPSCs)

iPSCs are created by reprogramming somatic cells through the introduction of specific transcription factors, such as Oct4,

Sox2, Klf4, and c-Myc. This process resets the cells' epigenetic landscape, effectively rejuvenating them and restoring their ability to differentiate into any cell type, a property known as pluripotency (118). iPSC technology holds great promise for generating patient-specific stem cells, offering new possibilities for personalized regenerative therapies. However, several challenges must be overcome for its clinical application. Issues such as the risk of tumorigenesis, genetic instability, and immune rejection pose significant hurdles (225). Tumor formation can result from the reprogramming process, while genetic instability might affect cell safety. Additionally, immune rejection could occur despite the patient-specific nature of iPSCs. Addressing these challenges is crucial for safely harnessing the full potential of iPSCs in treating diseases and promoting tissue regeneration (118).

Partial reprogramming to rejuvenate aging cells

An emerging strategy in regenerative medicine is "partial reprogramming," which involves the transient expression of reprogramming factors to rejuvenate aging cells without reverting them to a pluripotent state (90). This approach has shown promise in reversing cellular senescence, improving mitochondrial function, and restoring the regenerative potential of various stem cell types. For instance, the application of partial reprogramming using the Yamanaka factors—Oct4, Sox2, Klf4, and c-Myc—has successfully rejuvenated aged muscle and NSCs, significantly enhancing their regenerative capabilities (244). By inducing a temporary state of reprogramming, this method can restore cellular health and function while avoiding the risks associated with full pluripotency, such as tumorigenesis (58). As research continues to unfold, partial reprogramming may emerge as a powerful tool for combating age-related decline and improving tissue regeneration, paving the way for innovative therapies in age-related diseases (237).

Epigenetic therapies

Epigenetic therapies aim to target age-associated changes in DNA methylation and histone modifications, restoring youthful gene expression patterns. These therapies involve the use of inhibitors of histone deacetylases (HDACs) and DNA methyltransferases (DNMTs), which have shown potential in reversing age-related epigenetic alterations and improving stem cell function (125). By modifying the epigenetic landscape, these inhibitors can enhance the ability of stem cells to regenerate tissues and maintain homeostasis. Additionally, compounds like resveratrol, known for their ability to activate sirtuins, also play a role in modulating the epigenetic environment. Sirtuin activation can improve mitochondrial function and reduce inflammation, further supporting stem cell maintenance (237). Together, these epigenetic therapies offer a promising avenue for addressing age-related decline in stem cell functionality and may contribute to healthier aging and enhanced tissue repair. As research progresses, these approaches could lead to innovative treatments for age-related diseases (105, 146).

5.3. Senolytics and senescence-targeting therapies

The accumulation of senescent cells in aging tissues significantly contributes to tissue dysfunction and impaired regeneration. These cells, while no longer dividing, can adversely affect their environment through the senescence-associated secretory phenotype (SASP), which releases inflammatory factors and disrupts neighboring cell function (267). Senolytics are a class of drugs designed to selectively eliminate senescent cells, effectively reducing their detrimental effects on tissue health. By removing these cells, senolytics can enhance tissue function and promote regeneration. Additionally, senescence-targeting therapies aim to modulate the SASP, mitigating its harmful impact without necessarily eliminating the senescent cells (290). By addressing the underlying mechanisms of cellular senescence, both senolytics and SASP-modulating therapies hold promise for improving tissue health, restoring regenerative capacity, and potentially extending healthy lifespan. Continued research into these strategies could lead to innovative treatments for age-related diseases and enhance the overall quality of life in aging populations (291, 292).

Development of senolytic drugs

Several senolytic agents, including dasatinib, quercetin, and fisetin, have demonstrated effectiveness in reducing the burden of senescent cells in animal models of aging. These drugs specifically target key survival pathways in senescent cells, promoting their selective elimination while sparing healthy cells (291). The removal of these dysfunctional cells is linked to significant improvements in tissue function, reduced inflammation, and enhanced stem cell activity. By clearing senescent cells, senolytics can help restore the regenerative capacity of tissues and improve overall health. In preclinical studies, treatments with these agents have shown promise in mitigating age-related decline, suggesting potential applications in clinical settings for age-related diseases (290). As research continues, the development and optimization of senolytic therapies may offer a novel approach to promote healthy aging and enhance the quality of life for aging populations. Further studies will be crucial to determine the long-term effects and safety of these therapies in humans (292).

Targeting the SASP to improve regenerative capacity

The senescence-associated secretory phenotype (SASP) is marked by the secretion of pro-inflammatory cytokines, growth factors, and proteases that disrupt the tissue microenvironment and impair stem cell function. This inflammatory milieu negatively impacts neighboring cells and can lead to further aging-related decline (290). Therapies designed to modulate the SASP, such as JAK/STAT inhibitors and NF-κB blockers, have shown promise in reducing inflammation and enhancing the regenerative potential of aging tissues. By targeting the SASP, these therapies may improve the niche environment for stem cells, facilitating better tissue repair and regeneration. Consequently, modifying the SASP could restore normal tissue function and promote

healthier aging (291). This approach highlights the importance of addressing the underlying mechanisms of cellular senescence to improve stem cell activity and overall tissue health, potentially leading to innovative strategies for treating age-related diseases and enhancing quality of life in older individuals. Continued research in this area is essential for clinical applications (292).

5.4. Stem cell transplantation and tissue engineering

Stem cell transplantation and tissue engineering present promising strategies for rejuvenating aging tissues and addressing age-related degenerative diseases. By harnessing the regenerative capabilities of stem cells, these approaches aim to restore tissue function and improve patient outcomes (293). Advances in stem cell biology have deepened our understanding of stem cell properties, enabling the development of more effective therapies. Innovations in biomaterials have significantly enhanced the delivery and integration of stem cells into damaged tissues, improving the overall efficacy of stem cell-based interventions (106). Tissue engineering combines scaffolding materials with stem cells to create artificial tissues that can replace damaged ones, supporting cell survival and providing the necessary microenvironment for stem cell differentiation and function. Additionally, 3D bioprinting technologies allow for the precise construction of complex tissue structures that mimic natural tissues (211). Together, these advancements offer exciting possibilities for developing effective therapies to combat the effects of aging, potentially making stem cell transplantation and tissue engineering integral components of regenerative medicine and improving the quality of life for older adults (100).

Autologous stem cell transplantation

Autologous stem cell transplantation involves utilizing a patient's own stem cells to regenerate damaged or aged tissues, which significantly reduces the risk of immune rejection and other complications associated with allogeneic transplants (244). This approach capitalizes on the patient's natural regenerative capacity, making it a safer and more personalized treatment option. Techniques for autologous transplantation typically include the isolation and expansion of various types of stem cells, such as MSCs, HSCs, or iPSCs derived from the patient's own tissues (237). Following the expansion process, these stem cells can be transplanted back into the patient to promote tissue repair and regeneration. This method has shown promising potential in treating a range of conditions, including osteoarthritis, cardiovascular diseases, and neurodegenerative disorders, where conventional therapies may fall short (243). By harnessing the body's own stem cells, autologous transplantation not only enhances the likelihood of successful integration and regeneration but also paves the way for personalized medicine approaches that cater to individual patient needs, ultimately improving clinical outcomes and quality of life (202).

Tissue engineering and creation of rejuvenated niches

Tissue engineering integrates stem cells, scaffolds, and growth factors to develop functional tissue constructs capable of replacing or repairing damaged tissues. This interdisciplinary approach utilizes biomaterials designed to mimic the natural extracellular matrix, which plays a crucial role in supporting cell attachment, proliferation, and differentiation (220). By closely resembling the physiological environment, these biomaterials enhance the integration and function of transplanted stem cells, thereby improving tissue regeneration outcomes. Moreover, engineering rejuvenated niches by incorporating young systemic factors or anti-inflammatory agents into the scaffolds can significantly augment the regenerative capacity of aged stem cells. These factors help create a more favorable microenvironment that mitigates age-related decline, promoting better cell survival and functionality (212). As a result, engineered tissues not only support the healing of damaged areas but also potentially restore the overall health of aging tissues. This innovative approach holds great promise for treating various degenerative diseases, enabling the development of personalized therapies that harness the body's regenerative potential and improve the quality of life for patients with age-related conditions (199). With ongoing research and technological advancements, tissue engineering is poised to revolutionize regenerative medicine and offer effective solutions for tissue repair and replacement (294).

5.5. Mitochondrial-targeted therapies

Mitochondrial dysfunction is a hallmark of aging that significantly contributes to the decline in stem cell function. As mitochondria play a crucial role in energy production and cellular metabolism, their impairment can lead to reduced ATP levels, increased oxidative stress, and compromised cellular health (71). Strategies focused on improving mitochondrial function, such as the use of antioxidants or compounds that enhance mitochondrial biogenesis, hold promise for restoring the regenerative capacity of aging stem cells. By mitigating oxidative stress and enhancing energy production, these approaches can rejuvenate stem cells, promoting their ability to repair and regenerate tissues effectively (92). Additionally, interventions that target mitochondrial health may also improve the overall function of aged tissues, contributing to healthier aging. As research advances in this area, enhancing mitochondrial function in aging stem cells could become a key therapeutic strategy for combating age-related decline and promoting tissue regeneration in various degenerative diseases (253).

Enhancing mitochondrial function

Several approaches have been explored to improve mitochondrial function in aging stem cells:

Mitochondrial biogenesis: Activating pathways that promote mitochondrial biogenesis, such as AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated

receptor gamma coactivator 1-alpha (PGC-1α), can significantly enhance mitochondrial function and energy production in aging stem cells (237). AMPK serves as an energy sensor that, when activated, initiates process to boost mitochondrial mass and efficiency. Similarly, PGC-1α is a key regulator of mitochondrial biogenesis, driving the expression of genes involved in energy metabolism (70). By stimulating these pathways, it is possible to rejuvenate aging stem cells, improve their regenerative capacity, and enhance overall cellular health, ultimately contributing to healthier aging and tissue repair (61).

Antioxidant therapies: The use of antioxidants, such as N-acetylcysteine (NAC) and mitochondrial-targeted antioxidants like MitoQ, can effectively reduce oxidative damage and enhance stem cell maintenance. NAC acts as a precursor to glutathione, a powerful antioxidant that helps neutralize reactive oxygen species (ROS) and mitigate oxidative stress within cells (295). This reduction in oxidative damage is crucial for maintaining the health and functionality of stem cells, particularly as they age. MitoQ, on the other hand, is specifically designed to target mitochondria, delivering antioxidant protection directly where it is most needed. By reducing oxidative stress in these organelles, MitoQ can help preserve mitochondrial function and support energy production in aging stem cells (296). Together, these antioxidants offer promising strategies for improving stem cell viability and regenerative capacity, ultimately contributing to better tissue repair and healthier aging. Continued research into their effects may lead to innovative therapies for age-related degenerative diseases (297).

NAD+ restoration: NAD+ levels decline with age, significantly impacting mitochondrial function and cellular metabolism. This reduction in NAD+ is associated with various age-related health issues, including diminished stem cell activity. Supplementation with NAD+ precursors, such as nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), has demonstrated the ability to restore mitochondrial function and enhance stem cell activity in animal models of aging (298). These precursors facilitate the biosynthesis of NAD+, which plays a crucial role in energy production and the regulation of cellular processes. By replenishing NAD+ levels, NR and NMN can improve mitochondrial health, boost metabolic efficiency, and enhance the overall regenerative capacity of aging stem cells (299). This approach highlights the potential of NAD+ supplementation as a therapeutic strategy to combat age-related decline, offering promising avenues for improving tissue repair and promoting healthier aging in humans. Continued research is essential to fully understand the benefits and mechanisms of these NAD+ precursors in stem cell biology and aging (288).

Mitochondrial replacement therapy: Mitochondrial replacement therapy (MRT) is an innovative approach that involves replacing dysfunctional mitochondria in aging cells with healthy mitochondria. This technique has shown promise in enhancing mitochondrial function and reversing age-related decline in stem cell activity (300). By providing healthy mitochondria, MRT can help restore energy production and reduce oxidative stress, which are crucial for maintaining cellular health and function. Although MRT is still in the experimental stages, preliminary research suggests it could serve as a potential future strategy for rejuvenating aged stem cells and improving tissue regeneration (301). This could have significant implications for treating age-related diseases and promoting healthier aging. As the understanding of mitochondrial dynamics and their role in cellular aging continues to evolve, MRT may emerge as a transformative therapeutic option. Continued research and clinical trials will be essential to evaluate the safety and efficacy of this approach, paving the way for its potential application in regenerative medicine and anti-aging therapies (302).

6. Challenges and future directions

The development of therapeutic approaches aimed at counteracting age-related declines in stem cell function holds great promise for regenerative medicine; however, several significant challenges must be addressed to translate these strategies into clinical practice (92). One major hurdle is ensuring the safety and efficacy of treatments, particularly when dealing with stem cell manipulation and transplantation, as concerns about tumorigenesis, genetic instability, and immune rejection must be thoroughly evaluated to minimize risks. Additionally, the complexities of the aging process complicate the identification of effective therapeutic targets, given that age-related changes are multifaceted and involve numerous cellular pathways (303). Another challenge lies in the scalability and reproducibility of stem cell therapies, making the development of standardized protocols for stem cell isolation, expansion, and differentiation crucial for consistent outcomes. Furthermore, effectively delivering these therapies to the appropriate tissue sites in a controlled manner remains a significant obstacle (247). Future directions to overcome these challenges include advancing technologies such as gene editing, improved biomaterials for stem cell delivery, and refined methods for assessing treatment safety (303). Collaborative research efforts among scientists, clinicians, and regulatory bodies will be essential to foster innovation and ensure the successful translation of stem cell rejuvenation therapies into clinical practice for aging populations (301).

6.1. Overcoming hurdles in stem cell rejuvenation

Despite the promising results of various interventions aimed at rejuvenating aging stem cells, several technical and biological challenges remain.

Tumorigenesis and safety concerns

The potential for tumorigenesis is a significant concern in stem cell-based therapies, especially with genetic and epigenetic reprogramming techniques. Introducing pluripotency factors to

reprogram somatic cells carries the risk of inducing uncontrolled cell proliferation and malignant transformation (155). This risk is compounded by the possibility of generating teratomas, which are tumors that can arise from pluripotent stem cells. Similarly, the use of senolytic drugs, which aim to eliminate senescent cells, may inadvertently promote the survival of pre-cancerous cells that could evade treatment (53). Such outcomes highlight the need for a careful assessment of the risks and benefits associated with these therapies. Developing safer reprogramming methods, including more targeted approaches that minimize the potential for malignancy, is essential for advancing stem cell research (119). Ongoing studies should focus on identifying biomarkers for early detection of tumorigenesis and implementing rigorous safety protocols to ensure the long-term safety and efficacy of stem cell-based therapies (28).

***Immune rejection and allogeneic* transplants**

Stem cell therapies frequently involve the transplantation of cells derived from donors, known as allogeneic transplants, which can lead to immune rejection and other immune-related complications. While autologous stem cell therapies, which utilize the patient's own cells, may reduce the risk of rejection, they are often time-consuming and costly (106). Additionally, the age-related decline in the function of autologous stem cells can limit their therapeutic potential. These challenges underscore the need for innovative solutions in stem cell therapy. Developing immunomodulatory strategies could help create a more favorable environment for transplant acceptance, minimizing the risk of rejection (304). Furthermore, employing gene-editing techniques to reduce the immunogenicity of allogeneic stem cells may enhance their compatibility and effectiveness in diverse patient populations (305). By addressing these immunological challenges, researchers can improve the feasibility and outcomes of stem cell therapies, ultimately advancing the field of regenerative medicine and expanding treatment options for various degenerative diseases (306).

Stem cell survival and integration in aged tissues

The effectiveness of stem cell-based therapies relies heavily on the ability of transplanted cells to survive, integrate, and function within the aged tissue environment. However, the aged niche is often characterized by chronic inflammation, oxidative stress, and impaired signaling, which can significantly hinder the survival and regenerative potential of transplanted stem cells. These adverse conditions create a challenging environment that diminishes the effectiveness of stem cell therapies (305). To improve therapeutic outcomes, enhancing the local microenvironment through niche engineering is crucial. This can involve creating a supportive scaffold that mimics the natural extracellular matrix or delivering factors that promote a healthier environment. Additionally, implementing anti-inflammatory treatments can help reduce chronic inflammation, allowing transplanted cells to thrive (269). Preconditioning of stem cells prior to transplantation, such as exposing them to mild stressors or specific growth factors, may also enhance their resilience and functionality once integrated into the aged tissue (307). By addressing the challenges posed by the aged niche, these strategies could significantly improve the success of stem cell therapies in regenerative medicine (308).

Long-term efficacy and sustainability

While several interventions show promise in reversing age-related declines in stem cell function, the long-term efficacy and sustainability of these approaches remain uncertain. It is crucial to determine whether these therapies can provide lasting benefits or if repeated treatments will be necessary to maintain their effects (309). Understanding the duration of the therapeutic impact is essential for evaluating the overall feasibility and practicality of these interventions in clinical settings. Furthermore, it is vital to investigate the potential side effects and unintended consequences associated with long-term use of these therapies (310). Prolonged interventions could lead to unforeseen complications, such as altered cellular behavior, immune responses, or even the promotion of tumorigenesis (311). Thorough research and long-term studies are needed to assess the safety profiles of these treatments and ensure they do not compromise patient health. By addressing these critical questions, researchers can work towards developing safe, effective, and sustainable therapies that genuinely enhance stem cell function and promote healthy aging (312).

6.2. Personalized medicine and stem cell therapies

The future of regenerative medicine lies in personalized approaches that take into account individual variations in genetics, lifestyle, and environmental factors. Tailoring stem cell therapies to the unique needs of each patient could significantly improve therapeutic outcomes and minimize adverse effects.

Patient-specific stem cells

The use of patient-specific stem cells, particularly iPSCs derived from an individual's somatic cells, presents a significant advancement in personalized regenerative therapies. This approach enables the generation of autologous transplants that are less likely to be rejected by the immune system, thereby reducing complications associated with immune rejection often seen in allogeneic transplants (313). Moreover, iPSCs hold immense potential for correcting disease-causing mutations through genetic modification, offering a potential cure for genetic disorders. By reprogramming somatic cells into a pluripotent state, iPSCs can be differentiated into various cell types needed for therapy, tailored specifically to the patient's requirements (314). This not only enhances the likelihood of successful integration and function within the recipient's tissue but also empowers patients with personalized treatment options that target the underlying causes of their conditions. As research progresses, the application of patient-specific iPSCs could revolutionize

the field of regenerative medicine, paving the way for innovative therapies that improve patient outcomes and quality of life (315).

Targeting individual aging pathways

Aging is a complex and multifactorial process, and the rate of stem cell decline can vary significantly among individuals. This variability highlights the need for personalized medicine approaches that target specific aging pathways, such as telomere maintenance, epigenetic alterations, and mitochondrial dysfunction (225). By focusing on these individual factors, tailored interventions can be developed to more effectively address age-related diseases. Furthermore, the advancement of biomarkers to assess the biological age of stem cells and predict their regenerative potential could greatly enhance the design of individualized therapies (316). These biomarkers would enable clinicians to evaluate the functional capacity of a patient's stem cells, guiding treatment decisions and optimizing therapeutic outcomes. By integrating personalized approaches with biomarker assessments, healthcare providers can create targeted strategies that enhance stem cell function, improve tissue regeneration, and ultimately promote healthier aging (317). As research continues to uncover the intricacies of aging and stem cell biology, the potential for personalized interventions in regenerative medicine will become increasingly feasible and impactful (318).

6.3. Ethical and clinical considerations

The application of stem cell-based therapies for aging raises several ethical and clinical challenges that must be carefully considered to ensure responsible and equitable use.

Ethical challenges in stem cell research

Ethical concerns surrounding the use of stem cells, particularly ESCs, have been a longstanding issue in regenerative medicine. The derivation of ESCs involves the destruction of human embryos, raising significant questions about the moral status of the embryo and the implications of such actions (319). While iPSCs and adult stem cells provide alternative sources that circumvent these ethical dilemmas, they are not without their own set of concerns. Issues regarding the long-term safety of these technologies remain a critical topic of discussion, particularly in light of potential risks associated with genetic modifications or unforeseen side effects (320). Additionally, the possibility of misuse of stem cell technologies, such as genetic enhancement or anti-aging treatments aimed at life extension, raises ethical questions about the implications for societal inequality and the definition of a "normal" human lifespan (321). As the field of stem cell research continues to advance, it is essential to engage in ongoing ethical deliberation and establish robust regulatory frameworks to ensure responsible use of these powerful technologies, balancing innovation with moral considerations (322).

Access to stem cell therapies

As stem cell therapies become more advanced, ensuring equitable access to these treatments will present a significant challenge. The high costs associated with developing and delivering personalized regenerative therapies may restrict availability to a limited subset of the population, raising concerns about equity in healthcare (301). If these therapies remain accessible only to those who can afford them, they could exacerbate existing health disparities and create a divide between socioeconomic groups. Policymakers and healthcare systems must prioritize strategies that promote inclusive access to these innovations, ensuring that advances in stem cell research benefit a broad range of patients, regardless of their financial situation (322). This may involve developing funding models, subsidizing treatment costs, and fostering partnerships between public and private sectors to support research and delivery. Additionally, efforts should be made to raise awareness and educate communities about available treatments (237). By addressing these challenges proactively, the healthcare system can strive to provide equitable access to stem cell therapies, promoting justice and fairness in the evolving landscape of regenerative medicine (322).

Regulatory and Clinical Trial Challenges

Bringing stem cell therapies to market necessitates rigorous regulatory oversight to ensure their safety and efficacy. Clinical trials for stem cell-based treatments encounter several challenges, including patient heterogeneity, the complexity of stem cell biology, and the requirement for long-term follow-up to assess outcomes and potential side effects (323). The diverse responses among individuals can complicate the evaluation of treatment effectiveness and safety. Additionally, the intricate mechanisms governing stem cell behavior and their interactions with the host environment require careful consideration in trial design. As such, regulatory agencies must adapt to the unique characteristics of stem cell therapies, moving beyond traditional frameworks to establish appropriate guidelines for their evaluation and approval (324). This may involve developing specialized protocols that account for the specific risks and benefits associated with stem cell interventions, as well as facilitating adaptive trial designs that allow for modifications based on emerging data (325). By ensuring that regulatory processes are tailored to the distinct nature of stem cell therapies, agencies can help expedite the development of safe and effective treatments while maintaining high standards of patient safety (326).

6.4. Emerging technologies in stem cell research

The field of stem cell research is rapidly advancing, with new technologies offering the potential to overcome current limitations and open new avenues for rejuvenation and regenerative medicine.

CRISPR and genome editing

CRISPR-Cas9 and other genome-editing technologies have revolutionized the field of genetic engineering by providing precise tools for editing the genome. These advanced technologies enable researchers to correct genetic mutations,

enhance stem cell function, and mitigate the risk of tumorigenesis associated with stem cell therapies (141). The ability to perform targeted genome editing in stem cells holds immense promise for personalized medicine, allowing for the customization of treatments tailored to the unique genetic profiles of individual patients. This can be particularly beneficial in treating genetic disorders, where specific mutations can be corrected at the genomic level, potentially offering a cure rather than merely managing symptoms (302). Furthermore, genome editing can enhance the safety and efficacy of stem cell therapies by ensuring that cells used in treatment are genetically optimized for better integration and function within the host environment. As research progresses, the integration of CRISPR-Cas9 and similar technologies into stem cell therapies could lead to groundbreaking advancements in regenerative medicine, transforming the landscape of treatment options available for various diseases and conditions (293).

Single-cell RNA sequencing

Single-cell RNA sequencing (scRNA-seq) has emerged as a powerful tool that allows researchers to examine gene expression at the level of individual cells. This technology provides critical insights into the heterogeneity of stem cell populations and the molecular mechanisms underlying aging (109). By analyzing the gene expression profiles of individual stem cells, scRNA-seq can identify specific subpopulations that are more susceptible to age-related decline, revealing the distinct biological pathways and stress responses that characterize these cells (90). This detailed understanding can guide the development of targeted therapies aimed at rejuvenating these vulnerable stem cell populations, potentially improving tissue regeneration and restoring functionality in aged tissues (110). Moreover, scRNA-seq can help uncover biomarkers associated with stem cell aging, facilitating the design of personalized treatment strategies tailored to the unique characteristics of an individual's stem cell landscape (262). As researchers continue to explore the implications of scRNA-seq in stem cell biology, its potential to inform therapeutic interventions for age-related diseases becomes increasingly evident, paving the way for advancements in regenerative medicine (92).

Organoids and 3D bioprinting

Organoids, which are miniature, self-organizing tissue structures derived from stem cells, have emerged as invaluable tools for studying tissue development, disease modeling, and drug testing. These "mini-organs" can effectively recapitulate the cellular architecture and function of human tissues, providing a robust platform for personalized medicine applications (208). Their ability to mimic the in vivo environment makes organoids particularly useful for understanding disease mechanisms and testing therapeutic interventions in a controlled setting. Additionally, advancements in 3D bioprinting technology enable the fabrication of complex tissue constructs, further enhancing their application in tissue engineering and regenerative therapies (327). By integrating stem cells, biomaterials, and bioengineering techniques, researchers can create functional tissues and organs that can be used for transplantation or to model human diseases more accurately. This innovative approach holds great promise for addressing the challenges of organ shortages and developing personalized therapeutic strategies, ultimately contributing to the future of regenerative medicine and improving patient outcomes (328). The combination of organoids and bioprinting is poised to revolutionize our understanding of human biology and the development of effective treatments for a range of conditions (329).

6.5. Potential breakthroughs in reversing age-related stem cell decline

Future research may lead to breakthroughs that can effectively reverse age-related declines in stem cell function, potentially extending healthy lifespan and improving regenerative outcomes.

Discovery of new molecular targets

Ongoing research is poised to uncover new molecular targets involved in stem cell aging and tissue regeneration. By identifying key regulators of stem cell function, including transcription factors, signaling pathways, and metabolic enzymes, scientists can gain deeper insights into the mechanisms driving stem cell behavior and aging (76). These discoveries may pave the way for the development of novel therapeutic agents specifically designed to target these pathways, aiming to rejuvenate aging stem cells and enhance their regenerative capacity. For instance, targeting specific signaling pathways that influence cell fate decisions could improve stem cell viability and functionality in aged tissues (66). Additionally, understanding the metabolic shifts that occur during stem cell aging could inform strategies to optimize energy production and reduce oxidative stress in these cells. As researchers continue to explore the intricate molecular landscape of stem cells, the potential for innovative therapies that harness these insights grows, offering promising avenues for improving tissue regeneration and combating age-related decline (28). Such advancements could ultimately transform the field of regenerative medicine, leading to effective interventions for a range of age-associated diseases (128).

Combining multiple therapeutic approaches

Combining different therapeutic approaches, such as caloric restriction, senolytics, mitochondrial-targeted therapies, and genetic reprogramming, may yield synergistic effects that significantly enhance stem cell rejuvenation and tissue repair. A multi-faceted strategy that simultaneously targets various aspects of aging could prove more effective than single interventions alone (53). For instance, caloric restriction can improve cellular metabolism and reduce oxidative stress, while senolytics can eliminate senescent cells that hinder tissue regeneration. Mitochondrial-targeted therapies can enhance energy production and reduce damage from reactive oxygen

species, and genetic reprogramming can restore youthful gene expression patterns in aging stem cells (330). By integrating these approaches, researchers may be able to create a comprehensive therapeutic regimen that not only rejuvenates stem cells but also creates a more favorable microenvironment for tissue repair. This holistic strategy acknowledges the complexity of aging and aims to address it from multiple angles, ultimately leading to improved outcomes in regenerative medicine and potentially extending healthy lifespan (331). As research advances, the development of combination therapies could represent a significant leap forward in our ability to combat age-related decline and enhance the body's regenerative capabilities (332).

Development of "Youth Factors" for rejuvenation

The identification of "youth factors" present in young organisms that promote tissue regeneration could pave the way for developing rejuvenating therapies. For instance, proteins or extracellular vesicles derived from young plasma may hold the potential to reverse age-related decline in stem cell function and improve tissue health (333). Parabiosis experiments, which involve connecting the circulatory systems of young and aged animals, have demonstrated that young systemic factors can rejuvenate aged tissues, highlighting the role of these factors in enhancing regenerative processes. However, while these findings are promising, the isolation and clinical application of specific youth factors require further investigation to understand their mechanisms and therapeutic potential fully (334). Researchers must work to identify the precise molecules responsible for these rejuvenating effects and explore how they can be effectively delivered in clinical settings. Additionally, addressing challenges related to safety, dosage, and long-term effects will be essential for translating these discoveries into viable therapeutic strategies (335). As research progresses, the development of therapies based on youth factors could revolutionize approaches to combatting age-related decline and promoting healthier aging (336).

7. Conclusion

The decline in stem cell function with age plays a central role in the progressive deterioration of tissue homeostasis, repair, and regeneration. Understanding the molecular mechanisms underlying age-related changes in stem cells and their niches is crucial for developing effective strategies to combat age-related diseases and promote healthy aging. This review highlights the complex interplay between intrinsic factors, such as telomere shortening, DNA damage, epigenetic changes, and mitochondrial dysfunction, and extrinsic factors, including alterations in the stem cell niche and systemic signals, that drive stem cell aging. Intrinsic factors contribute to the cellular aging process by compromising stem cell viability and functionality, while extrinsic factors can create an unfavorable microenvironment that hinders stem cell activity. By elucidating these interconnected mechanisms, researchers can identify potential therapeutic targets and devise interventions aimed at rejuvenating aging stem cells and restoring tissue regeneration. Ultimately, advancing our understanding of stem cell aging is essential for developing innovative approaches to enhance health span and mitigate the impact of aging on overall well-being. This knowledge could lead to transformative therapies that address the root causes of age-related decline and improve the quality of life for aging populations. Stem cell biology has the potential to transform the landscape of aging medicine and pave the way for a future where regenerative therapies extend not just lifespan but health span.

Conflict of interest

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Review Article

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Investigation of the effect of a cognitive rehabilitation program on neuroplasticity in stroke patients

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Abstract

This review aims to examine the effectiveness of various cognitive rehabilitation approaches used in stroke-related cognitive dysfunctions, with a particular emphasis on their impact on neuroplasticity, thereby contributing to increased awareness in this field. This study is an integrative review, referring to the retrospective systematic scanning of articles on the subject. The PICOS criteria have structured the study design to ensure methodological standards. An electronic search strategy was used to determine identify the studies targeted by the research. A search was conducted using six electronic databases: PubMed, Cochrane Library, Google Scholar, ResearchGate, Web of Science, and Scopus. All studies conducted between 2014 and 2024 were included. We only included articles with full text. Only articles with full texts available were included. Stroke is a leading cause of illness and death globally. Many survivors face cognitive issues that lower their quality of life. This review examines strategies to enhance neuroplasticity in stroke rehabilitation. Interventions such as computer-assisted cognitive training (CACT), physical exercise, virtual reality (VR), transcranial direct current stimulation (tDCS), and transcranial magnetic stimulation (TMS) have demonstrated potential in therapeutic applications. When combined with physical exercise, their efficacy is further enhanced. Non-invasive brain stimulation has been shown to facilitate improvements in executive functions and daily activities, while VR-based training contributes to rehabilitation. Overall, a multimodal approach that promotes neuroplasticity can significantly enhance cognitive functions and overall quality of life. Future research should focus on evaluating these interventions across diverse populations to optimize and refine treatment strategies.

Keywords: stroke, cognitive rehabilitation, recovery, neuroplasticity

1. Introduction

Stroke is a leading cause of death and disability, affecting 116.4 million people globally. Up to 80% of stroke survivors face physical, motor, language, sensory, behavioral, visual, and cognitive challenges (1,2). These include difficulties with memory, attention, and awareness. Delays in rehabilitation can worsen recovery. Treatments administered during periods of high neuroplasticity yield better results. A key question is whether we can extend the natural neuroplasticity boost after a stroke. Cognitive exercises may aid individuals with mild cognitive impairment and help prevent decline in healthy seniors (3). These exercises include computer training, memory techniques, puzzles, strategy games, and physical activity. Regular practice is crucial.

Previously, it was believed brain cells couldn't renew after birth. However, 20th-century studies have shown that brain cells can repair, form new connections, and even create new neurons, albeit in limited quantities (4). Neuroplasticity refers to the changes in neurons and their connections that occur due to environmental factors. It has two types: functional and structural. Functional neuroplasticity enables undamaged areas of the brain to assume the functions of damaged areas. Structural neuroplasticity involves changes in neuron networks due to learning or experience. Such changes can include alterations in dendrite structures, the formation or elimination of synapses, and even the birth or death of neurons (5).

Stroke is a common neurological disorder characterized by loss of brain function (6). Stroke is the third most common cause of death in developed countries after coronary heart disease and cancer (7,8). Its incidence is increasing worldwide, and its economic burden is also increasing (9). According to estimates, by 2030, approximately 80% of all stroke cases will occur in low- and middle-income countries (10). The prevalence of stroke in low and middle-income countries varies between 0.3% and 2.1%. In a developing middle-income country like Turkey, there is no definitive nationwide registry for stroke, and precise prevalence data are not available

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(11,12). Accurate data on stroke are limited, and there are only two published studies in Turkey, which determined that the prevalence of stroke in the ≥45 age group ranged between 0.9% and 4.1% (13,14). Although clinical symptoms are diverse (15), permanent motor deficits (including weakness, lack of coordination, and decreased mobility) and cognitive disorders (such as impairments in information processing and executive function) are typically observed (16,17). Stroke, a leading cause of disability worldwide, can impact all levels of the International Classification of Functioning; that is, it can affect body structures and functions, activity, and participation domains (18,19).

A review of the literature reveals that studies conducted with individuals who have experienced a stroke predominantly focus on the effectiveness of physical rehabilitation interventions targeting functional abilities such as gait, fine and gross motor skills, balance, and coordination. However, in addition to motor impairments, stroke survivors frequently experience deficits in cognitive functions, including executive functions, attention, and memory. In this context, this review aims to examine the effectiveness of various cognitive rehabilitation approaches used in stroke-related cognitive dysfunctions, with a particular emphasis on their impact on neuroplasticity, thereby contributing to increased awareness in this field.

1.1. Neuroplasticity

The concept of plasticity is derived from the Greek word "plastics," meaning "to shape" or "to give shape." Livingston first defined the concept of neuroplasticity in this way (24,25). In the past, it was thought that neural cells were not renewed after birth and diminished over time. However, research conducted in the 1900s revealed that brain cells can repair themselves throughout life, that new neurons can be formed, albeit to a limited extent, and that this process occurs in various brain regions. The discovery in 1998 that the adult human brain can produce new brain cells altered our understanding of the human brain. It sparked a renewed interest in the brain's plasticity throughout life (25,26). Neuroplasticity refers to the brain’s ability to alter its structure and function in response to internal or external stimuli. Through neuroplasticity, individuals can acquire new cognitive or physical skills throughout their lives and regain lost abilities. The brain's inherent capacity primarily drives the effectiveness of both physical and mental rehabilitation. While neuroplasticity is often more pronounced in younger individuals, it persists throughout life. Even in older adults, this capacity can help prevent both physical and cognitive decline (20,21).

The extent of neuroplasticity varies among individuals due to genetic differences. A key factor in neuroplasticity is the brain’s ability to produce neurotrophic factors, which support neuronal growth and function. In particular, research has shown that the production of brain-derived neurotrophic factor (BDNF) increases with aerobic exercise, thereby enhancing motor learning and memory (34, 22). Consequently, it is now well-established that physical activity enhances cognition, while cognitive activity can improve motor function. Given that stroke patients often experience both mental and physical impairments, they are ideal candidates for rehabilitation. The role of neuroplasticity in restoring lost function has been demonstrated in various studies employing different rehabilitation strategies in this patient population (35,23). However, the effective integration of cognitive rehabilitation into traditional motor rehabilitation remains unclear. This section will discuss the key mechanisms underlying neuroplasticity that contribute to functional recovery.

Rehabilitation-based Neuroplasticity Mechanisms

Neurogenesis

Neurogenesis is considered a component of structural neuroplasticity. Neurogenesis is the process by which stem cells differentiate into neurons. It primarily occurs in the hippocampus of the brain. Here, stem cells develop into new neurons and support cells, particularly with the acquisition of new experiences. The hippocampus is crucial for enabling the brain to adapt to cognitive demands. In adults, this process shows remarkable flexibility (26). Neurons are formed and selected to survive in a small brain area. Neurogenesis primarily occurs in the subventricular zone and the subgranular zone of the hippocampus. Environmental factors regulate it. Enriched environments, exercise, learning, and antidepressants boost neurogenesis. However, chronic stress and aging hinder it (27).

Changes in Axonal and Dendritic Branches

Neuronal circuits grow and shrink through changes in axonal and dendritic morphology. New dendritic branches form "trial synapses." Only the ones with proper input survive. Neuroplasticity enhances dendrites, forms new synapses, and adjusts existing ones. It can even create new neurons, aiding stress resistance. Dendrites are the most adaptable part of a neuron. Their changes indicate neuroplastic development. Synaptic connections drive these changes, which are further enhanced by environmental cues (28).

Synaptic Connections

In early development, synapse density in the human cortex increases to approximately twice that of adults. Brain growth later reduces this density. Synaptic pruning continues into the third decade, ensuring that only the most effective synapses remain. This process is vital for adapting to stimuli. Brain connections are dynamic, adjusting to needs. Activities that promote neuroplasticity enhance synaptic connections and communication pathways (30).

Re-learning after Brain Damage

Brain injury recovery methods fall into two main types:

1. Preventing further loss of function.
2. Restoring or compensating for lost function.

The first method is crucial. Early treatment might not

entirely prevent long-term issues. Thus, understanding post-injury brain changes is vital. The brain forms new connections through learning (31). It adapts by coding experiences and altering circuits. Learning requires specific changes in the nervous system. These changes, known as neuroplasticity, depend on behavior, sensory input, and thought. Healthy brains adapt by reorganizing. In damaged brains, undamaged areas take over. Learning helps adapt after injury (32). People develop new strategies to cope with lost functions.

Use and Improve

Animal studies have shown that long-term training enhances brain plasticity. For example, monkeys learned to use their fingers to obtain food, increasing the finger area of the motor cortex. Likewise, mice that reached for food showed increased activity in the distal forepaw areas of the motor cortex. Post-injury training also aids recovery. It enhances performance and brain plasticity. Research now targets intentional training (33).

Repetition of Learned Experiences

Learning a new behavior requires repetition for lasting brain changes. Firstly, you need to learn a skill and then keep practicing it. This repetition makes the behavior stick, even when not in use. It's crucial for rehab. It helps patients maintain what they've gained in therapy and continue to improve (39).

Density of Repetitions

Both the duration and intensity of training affect brain plasticity. In tests, animals doing 400 reaching tasks daily had more synapses in the motor cortex. In contrast, those doing just 60 tasks showed no such increase (40).

1.2. Cognitive Rehabilitation

Cognitive rehabilitation is a therapeutic approach aimed at restoring lost cognitive functions or slowing progressive decline in individuals with neurological conditions such as traumatic brain injury, stroke, Alzheimer's disease, Parkinson's disease, or multiple sclerosis (36). Cognitive rehabilitation can be categorized into two types: compensatory and restorative (reference). In stroke patients, deficits in attention, long-term memory, executive functions, and visuospatial abilities are commonly observed (37). Additionally, emotional lability, depression, and anxiety are significant psychosocial challenges. Cognitive rehabilitation plays a crucial role in addressing these deficits in stroke patients. Moreover, improvements in these mental functions can enhance participation in motor rehabilitation and increase motivation, ultimately contributing to overall health and the recovery of daily functional abilities (41). After a stroke, the brain can repair itself. This relies on the plasticity of the remaining nervous systems. Such adaptations aid recovery and are essential in rehabilitation. Boosting this adaptability is crucial in post-stroke rehab (42).

With these objectives in mind, we aimed to write a review by examining research articles that investigate the effectiveness and application of cognitive rehabilitation methods in stroke. Specifically, we focused on approaches considered adequate through neuroplasticity, including virtual reality (VR), transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS), computer-assisted cognitive training (CACT), and cognitive rehabilitation combined with exercise.

2. METHOD

This study is an integrative review that involves a retrospective systematic review of articles on the subject.

2.1. Study design

PICOS (Population, Intervention, Comparison, Outcomes, Study design) standards were used to determine the study design (Table 1).

Table 1. PICOS Method in Determining Study Design

P Population	Participants with stroke
I Intervention	Cognitif rehabilitation (CACT, tDCS, TMS, virtual reality, cognitive exercises)
C Comparison	of Groups Pre-test-post-test without a control group, comparative with a control group, comparative without a control group
O Outcomes	Cognitive functions
S Study design	Interventional study, single and double blind randomized controlled trials

2.2. Search strategy

An electronic search strategy was used to determine the studies targeted by the research. A search was conducted using six electronic databases: PubMed, Cochrane Library, Google Scholar, ResearchGate, Web of Science, and Scopus. All studies conducted between 2014 and 2024 were included. We only included articles with full text. The search yielded 36 studies. Seven randomized controlled trials met our criteria. The keywords "cognitive exercise," "stroke," "rehabilitation, "recovery," and "neuroplasticity" were used as search terms. The search terms were related to cognitive rehabilitation, which was the central theme of the study. The second stage consisted of associating with neuroplasticity. In these stages, the words "and," "not," and "or" were used to ensure that studies combining the topics were addressed.

2.3. Study selection and data collection process

Four researchers (BÜ, YY, GA, SGÖ) independently reviewed the titles and abstracts of the studies to determine whether the studies met the inclusion criteria. The studies that met the requirements were recorded, and the full texts were evaluated.

3. Neuroplasticity in post-stroke cognitive rehabilitation

3.1. Computer-Assisted Cognitive Training (CACT)

CACT, using computers, tablets, and phones, has surpassed traditional methods. It enhances skills like memory and problem-solving through interactive exercises. Unlike other methods, CACT is accessible, comprehensive, and personalized (43). Its game-like structure makes it engaging. It's also safe, affordable, and scalable, crucial for preserving cognitive functions in older adults (44). CACT is vital in treating post-stroke cognitive impairment. A review by Fava-Felix et al. (2022) found it boosts recovery, especially in more

educated patients. Gil-Pagés et al. (2022) reported that supervised home CACT improves recovery in patients with chronic stroke (45). Fava-Felix et al. (2022) reported that the 'Reh@Task' program was implemented three times per week for one month, whereas in another study, the 'BrainHQ' program was administered two or three times per week for a duration ranging from 12 to 18 months. These interventions demonstrated that computer-based cognitive training (CACT) particularly supports cognitive improvement in patients with higher educational levels. Similarly, Gil-Pagés et al. (2022) utilized the 'Guttmann, NeuroPersonalTrainer' application once daily over six weeks in their randomized, double-blind study. They reported that home-based, supervised CACT supports functional recovery in patients with chronic stroke. CACT focuses on enhancing cognitive skills, such as memory and problem-solving, through practice and repetition using engaging and interactive exercises. Distinguishing itself from other cognitive training approaches, CACT is notable for being accessible, comprehensive, and adaptable to individual needs. With its game-like structure, CACT provides a motivating experience for participants and is considered a safe, cost-effective, and scalable method. Therefore, it plays a significant role in preserving cognitive functions, particularly in older adults.

3.2. Combining Physical and Cognitive Training

Combining physical and cognitive training is more effective than either alone. Yeh et al. (2022) demonstrated that combining aerobic exercise with CACT significantly enhances cognitive functions. Bo et al. conducted a study with 225 stroke patients, randomly assigning participants into four groups: an exercise group, a cognitive training group, a combined exercise and cognitive training group, and a control group. The combined group demonstrated significantly better results in the mental rotation test compared to the other groups. Similarly, Bo et al. (2019) found significant cognitive improvements in stroke patients with vascular cognitive impairment when using this combination (46, 47).

3.3. Combination of Transcranial Direct Current Stimulation (tDCS) and Cognitive Training

Transcranial Direct Current Stimulation (tDCS) is a noninvasive method that adjusts brain activity. Developed in 2000 for clinical use, it shows promise in treating neurological conditions. tDCS works by altering neuronal activity. It affects sodium and calcium channels and boosts NMDA receptor activity (48). This process changes neural activity and increases cortical excitability. Liu et al. (2021) found that tDCS, combined with cognitive training, improved executive functions and daily living skills in individuals who had experienced a stroke (49). Similarly, Chen et al. (2024) reported that tDCS, along with mental training, helped stroke patients with unilateral neglect (50). When applying tDCS in patients with stroke, potential physiological side effects should be carefully considered. These side effects may include mild skin redness, itching, a burning sensation, headache, dizziness, and fatigue. Furthermore, contraindications must be considered, such as a history of epilepsy, the presence of a pacemaker or other implanted electronic devices, and active skin infections or open wounds (48).

3.4. Combination of rTMS and Cognitive Training

Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive brain treatment. It uses magnetic pulses to stimulate or inhibit specific neurons. Typically, rTMS sessions occur daily or weekly. The duration depends on the patient's condition and response. This study focused on brain areas associated with cognition, including the dorsolateral prefrontal cortex, motor cortex, and parietal cortex. Treatment varies in frequency (e.g., 1 Hz, 10 Hz), intensity, and number of sessions based on individual needs. Li et al. (2023) demonstrated that combining rTMS with cognitive training benefits individuals with post-stroke cognitive impairment. It enhances cognition, executive functions, and working memory. However, further research is needed to confirm these results and clarify the role of rTMS (51). When applying repetitive transcranial magnetic stimulation (rTMS) in patients with stroke, potential adverse effects should be carefully considered. Common physiological side effects may include headache, dizziness, and discomfort at the site of stimulation. Additionally, contraindications must be considered, such as a history of epilepsy or seizures, the presence of metal implants in the brain, pacemakers or other implanted electronic devices, and severe anxiety disorders or other significant psychiatric conditions (51).

3.5. Combination of Virtual Reality and Cognitive Training

VR, as one of the new technological alternatives to traditional rehabilitation methods, offers an interactive and experiential environment that can be utilized to practice activities of daily living. Additionally, the fully immersive or augmented reality environments provided by VR enable individuals to engage in both physical and cognitive tasks in a safe setting. For example, VR facilitates the simultaneous practice of motor skills such as walking, stepping, and grasping, along with cognitive functions like attention, memory, and executive functions, making it a preferred rehabilitation tool. VR is used in conjunction with traditional methods to treat cognitive disorders following a stroke. It creates interactive environments that help train cognitive skills. A study by Huang (2022) found that VR training improved the active range of motion and daily activities in stroke patients compared to conventional occupational therapy alone. These sessions consisted of 16 intervention sessions, each lasting 60 minutes per day, 2 to 3 days a week. In addition, they used the commercial immersive VR headset developed by HTC VIVE (HTC Corporation) in this study (52,53). VR-based rehabilitation approaches are increasingly being utilized to support motor and cognitive recovery in individuals with stroke. The cost-effectiveness of VR systems is attributed to their potential to reduce overall healthcare expenditures in long-term rehabilitation processes. Home-based VR systems,

in particular, can minimize the need for frequent hospital and

clinic visits, offering economic benefits for both healthcare systems and patients. However, the initial investment costs associated with advanced VR devices and software can be relatively high (38).

In terms of accessibility, the development of portable and user-friendly VR devices has facilitated their use among individuals from diverse socioeconomic backgrounds. Nevertheless, regional and economic disparities in access to technology persist as significant barriers to the widespread implementation of this technology (52). In conclusion, the primary advantages of using VR for cognitive and motor rehabilitation are its engaging nature, the ability to motivate patients through real-time feedback, which ensures sustained patient participation, and the provision of a safe environment.

The studies conducted on cognitive rehabilitation in individuals with stroke are summarized in Table 2.

Table 2. Summary table of studies referenced in the review about cognitive rehabilitation

Study	Participants	Intervention Group(s)	Comparison Group(s)	Outcomes	Primer Outcome
Yeh et al. (2022) Single blind randomized controlled trial	56 stroke patients	Computerized cognitive training (n = 18) 60 min/day, 3 days/week, for a total of 12 weeks	Aerobic exercise training (n = 18) Sequential combination of aerobic exercise and computerized cognitive training (n = 20) group	1. MoCA 2. Wechsler Memory 3. Scale-Third Edition 4. The Stroop color-word test 5. Timed Up and Go test 6.6-Minute Walk Test 7.Functional Independence Measure	The combined training group showed significant improvement in MoCA ($P < .05$) two sub-tests in WMS-III (both Ps < 0.05)
Gil-Pagés et al. (2022) Double-blind, randomized, crossover clinical trial	40 stroke patients	Computerized cognitive training (CCT) (n=18) A set of 1-h sessions, five sessions per week for 6 weeks. A series of cognitive exercises focusing on attention, memory, and executive functions was conducted in each session.	sham intervention (n=22)	1. Patient Competency Rating Scale (PCRS) 2. Rating Scale for Attentional Behavior (RSAB) 3. Prospective and Retrospective Memory Questionnaire (PRMQ) 4. Behavior Rating Inventory of Executive Function – Adult Version (BRIEF-A)	Significant mean differences in intervention group PCRS (p = 0.02) PRMQ (p = 0.01
Bo et al. (2019) Single-blind Randomized controlled trial	225 stroke patients	Groups (1): Physical exercise (n=56; 50-minute session) Group (2): Cognitive training (n=57; 60-minute session) Group (3): Combined intervention of physical exercise and cognitive training (n = 55; 50-minute session)	Group (4) control groups (n=57; 45-minute session) All participants received training for 36 sessions, three days per week, for 12 weeks.	1. Trail Making Part B, 2. Stroop, forward digit span 3. Mental rotation tests	The combined training group (e.g., mental rotation, 17.36% vs. 0.87%, $P = 0.002$)
Liu et al. (2021) Randomized controlled trial	50 stroke patients	Group 1: real tDCS (n=25) Left dorsolateral prefrontal cortex (DLPFC): tDCS was applied continuously at an intensity of 2.0 mA. 5 sessions per week for 4 weeks (20 min)	Group 2: sham tDCS (n=25)	1. Wisconsin Card Sorting Test (WCST) 2. Stroop Color-Word Test (SCWT) 3. Digital Symbol Test (DST) 4. Montreal Cognitive Assessment (MoCA) 5. Mini-mental State Examination (MMSE)	WCST, SCWT, DST, MMSE, and MoCA in the real-tDCS group were significantly higher than the sham-tDCS group ($p<0.05$)

Chen et al. (2024)	72 stroke patients	tDCS group (n = 18) CACT combined with tDCS group (n = 18) 20-minute treatment 15 times a week for three consecutive weeks	Conventional cognitive training (CCT) group (n = 18) CACT group (n = 18) 20-minute treatment 15 times a week for three consecutive weeks	1. MoCA 2.Instrumental Activities of Daily Living Scale (IADL) 3.Transcranial Doppler ultrasound (TCD) 4. Breath-holding index (BHI)	MoCA and IADL scores significantly increased after treatment (P< 0.01) in each group.
Huang et al. (2022) Single-blind Randomized controlled trial	30 stroke patients	Virtual reality training (VRT) (n=15) 16 sessions of intervention for 60 min/day, 3 days/week (HTC VIVE)	Conventional occupational therapy (COT) (n=15) 16 sessions of intervention for 60 min/day, 2 to 3 days/week	1.İnterleukin 6 (IL-6) 2.Brain-derived neurotrophic factor (BDNF) 3.Active range of motion of the upper limb 4.Fugl-Meyer Assessment for upper extremity (FMA-UE)	Signifcant time efects in serum IL-6 (p =0.010) Clinical assessments FMA-UE(p<0.05) in VRT group
Li W. et al. (2022) Double blind randomized controlled trial	58 stroke patients	TMS group (n=28) Left dorsolateral prefrontal cortex: 100MT TMS 50 Hz burst repeated 5 Hz (On/Off time) 5 days of the week Total 10 sessions	Sham group (n=30)	1.Mini-mental state examination (MMSE) 2.Oxford cognitive screen 3.Event-related potential P300	The TMS group exhibits more significant changes in semantic comprehension and executive function (p < .05).

4. Discussion

This review examines methods that promote brain recovery after a stroke. It was found that CACT, physical exercise, tDCS, rTMS, and VR can all be beneficial. CACT is the most popular and accessible method, especially helpful for educated patients. Combining CACT with physical exercise may enhance cognitive functions. Brain stimulation methods, such as tDCS and rTMS, can improve specific cognitive issues when combined with mental training. VR offers a more engaging environment for cognitive training post-stroke (44,52). In terms of cost, CACT is the most affordable method (44). tDCS and rTMS are more expensive and require specialized training. VR is also costly due to its equipment needs. Each method has its pros and cons. CACT is easy to access and motivating, but its effects might be limited. Physical exercise enhances health and cognitive functions, but it may not be suitable for everyone. tDCS and rTMS target specific brain areas and aid recovery but may have side effects. VR is interactive and immersive, increasing motivation, but is limited by cost and accessibility (52). Effective cognitive rehabilitation after a stroke needs teamwork. Physiotherapists are key players. They blend cognitive and physical exercises, plan programs, oversee tDCS and rTMS treatments, and design VR exercises. While neuroplasticity-based methods show promise, they can be hard to implement. Ongoing education is crucial. Therapies must be tailored using assessment techniques. Regular sessions are essential for recovery. Addressing ethical issues and maximizing therapy benefits is vital as neurorehabilitation evolves (46, 49, 50).

The lack of standardized protocols across clinical studies complicates the interpretation of treatment efficacy. To address these challenges, it is essential to provide comprehensive training for healthcare professionals, ensure individualized treatment approaches tailored to patients' needs, and develop cost-effective and user-friendly systems. Multidisciplinary collaboration and adherence to ethical standards, particularly regarding data privacy in remote applications, are also critical for the effective integration of these technologies into clinical practice.

Furthermore, existing studies have not specified which rehabilitation methods are more effective for different stroke subtypes (e.g., left vs. right hemisphere, anterior vs. posterior circulation, minor vs. significant stroke). It is well known that motor and cognitive impairments vary across different stroke groups. There are gaps in the literature regarding the optimal dosage and application of these interventions. Additionally, the long-term outcomes of cognitive rehabilitation have not been thoroughly addressed. These observed gaps in the literature could serve as a guide for future research, providing valuable insights for clinicians and academics working in this field.

Utilizing neuroplasticity-based methods in post-stroke rehabilitation can improve cognition and enhance quality of life. Techniques such as computer training, exercise, and specific brain stimulation aid recovery, either alone or in combination. Each has its pros and cons. The best choice depends on the patient's unique needs. In stroke rehabilitation, the application of advanced neurorehabilitation techniques such as VR, TMS, tDCS, and CACT presents several challenges. High initial costs, limited accessibility in low-resource settings, and the need for technical expertise can

hinder the widespread adoption of these technologies. Additionally, patient-related factors such as age, cognitive impairments, and acceptance of technology may impact usability and adherence. Potential side effects, including headache, dizziness, and skin irritation, particularly with TMS and tDCS, necessitate careful patient selection and monitoring. Most studies focus on specific cognitive skills. Future research should aim for broader programs that enhance various cognitive areas and relate to daily life. Examining diverse patients and methods will refine understanding and treatment plans.

A team approach is vital in stroke rehab. Experts from different fields should collaborate. They ensure cognitive exercises are included, keep patients motivated, and follow treatment plans. Neuroplasticity-based methods show promise in improving cognition and quality of life. Further research could lead to more effective, personalized treatments.

Conflict of interest

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Ethical statement

The study employed a review design. Ethics committee approval is not required for reviews. The studies included in the evaluation were cited in the article.

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Review Article

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Endogenous opioid system in pain management: Mechanisms, influences, and clinical implications

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Abstract

This manuscript discusses the role of the endogenous opioid system in pain management and its potential clinical applications. The endogenous opioid system is a network of naturally produced painkillers, known as opioid peptides, and the receptors to which they bind. The main components of this system are endorphins, enkephalins, and dynorphins. Opioid receptors (mu, delta, and kappa) interact with these peptides to regulate various physiological and psychological processes, such as analgesia, euphoria, and stress responses. The article provides detailed explanations of the biosynthesis, secretion, and receptor activation mechanisms of opioid peptides. Additionally, the effects of stress, exercise, and alternative treatment methods (acupuncture, meditation) on the endogenous opioid system are discussed. In clinical applications, dysfunction of the endogenous opioid system is noted to be associated with the risk of opioid misuse in chronic pain patients. The article also presents recommendations for future research to develop more effective pain management strategies.

Keywords: endogenous opioid system, pain management, opioid peptides

1. Introduction

1.1. The Importance of Pain Management

Pain is defined as a complex sensory and emotional experience that arises in response to tissue damage in the body and adversely affects an individual's quality of life (1). Its impact on public health is extensive; chronic pain can limit individuals' ability to perform daily activities, leading to a loss of workforce, social isolation, and psychological problems (2). Individuals suffering from chronic pain may require continuous medical treatment, imposing a significant economic burden on healthcare services (3). Moreover, pain, which significantly reduces the quality of life, can lead to additional health problems such as sleep disorders, depression, and anxiety (4). Therefore, developing effective pain management strategies is crucial for protecting public health and improving individuals' quality of life (5).

1.2. Definition of the Endogenous Opioid System

The endogenous opioid system is a complex network formed by opioid peptides, which are the body's naturally produced painkillers, and the receptors to which they bind. The primary components of this system include endorphins, enkephalins, and dynorphins. Endorphins are peptides that are particularly released in response to stress and pain and have effects similar to those of morphine (6). They are known to be released during intense exercise, painful stimuli, and stressful situations, creating a sense of well-being by alleviating pain. Enkephalins are short-chain peptides that play a role in pain modulation and neurotransmission, and they are particularly concentrated in the spinal cord. They are spread throughout the central nervous system and exhibit analgesic effects by inhibiting pain signals (7). Dynorphins are opioid peptides with high affinity and are particularly effective in chronic pain conditions. They play a significant role in the stress response and addiction mechanisms and can cause dysphoric effects by interacting with kappa opioid receptors (8). These components interact with opioid receptors to regulate various physiological and psychological processes such as analgesia, euphoria, and stress responses (9). Opioid receptors are distributed throughout the central and peripheral nervous systems and are crucial in modulating pain signals (10). The endogenous opioid system has been shown to play an important regulatory role in both acute and chronic pain management and in other psychological conditions such as addiction and stress (11).

1.3. Mechanisms of the Endogenous Opioid System

1.3.1. Opioid Receptors

Opioid receptors are G-protein-coupled receptors that play a critical role in pain modulation and many physiological

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functions. These receptors are classified into three main types: mu (μ), delta (δ), and kappa (κ) receptors. Mu receptors are associated with effects such as analgesia, euphoria, and respiratory depression, and they are widely distributed in the brain, spinal cord, and gastrointestinal (9). These receptors mediate the effects of morphine and similar opioid drugs(10). Delta receptors are linked to pain control and mood regulation and are typically concentrated in the brain and spinal cord (12). Activation of delta receptors is seen as a promising target in chronic pain treatment. Kappa receptors are associated with dysphoria, hallucinations, and some analgesic effects and are particularly found in the brain, spinal cord, and certain peripheral tissues. Activation of kappa receptors plays a significant role in stress and addiction mechanisms (8).

1.3.2. Synthesis and Secretion of Endogenous Opioid Peptides

The synthesis and secretion of endogenous opioid peptides are carried out through complex biochemical processes. The biosynthesis of these peptides begins with the gene expression of precursor proteins, which are then cleaved into active opioid peptides by proteolytic enzymes (13). For example, large precursor proteins such as proopiomelanocortin (POMC), proenkephalin, and prodynorphin are converted into active peptides like β-endorphin, enkephalin, and dynorphin, respectively. These peptides are stored in the vesicles of nerve cells and released into the synaptic cleft to function as neurotransmitters (14). The release process occurs via calcium-dependent exocytosis and is triggered by the electrical stimulation of the nerve cell (15). After secretion, opioid peptides bind to opioid receptors on target cells, activating intracellular signaling pathways (16). During the metabolism stage, these peptides are inactivated by enzymatic pathways and broken down into small peptides or amino acids, thus controlling the duration and intensity of the signal (17).

1.3.3. Activation of Opioid Receptors

The activation of opioid receptors triggers various physiological and pharmacological effects by activating cellular signaling pathways. Opioid receptors are G-protein-coupled receptors, and their activation causes the dissociation of G-proteins into α, β, and γ subunits (18). This dissociation leads to the inhibition of the adenylate cyclase enzyme, a decrease in cyclic AMP (cAMP) levels, and a reduction in protein kinase A (PKA) activity (19). Consequently, intracellular calcium levels decrease and potassium channels open, causing hyperpolarization of the cell membrane, reducing neuronal excitability, and inhibiting the transmission of pain signals (20). Additionally, opioid receptor activation can affect mitogen-activated protein kinase (MAPK) pathways, which regulate various cellular processes such as cell growth, differentiation, and apoptosis (21). Also, internalization and desensitization of receptors occur via beta-arrestin, playing an important role in tolerance and addiction mechanisms associated with long-term opioid use (22). These complex signaling pathways are crucial not only in pain control but also in regulating many systemic effects such as mood, respiration, and gastrointestinal motility (9).

1.4. Role of Endogenous Opioids in Pain Management

1.4.1. Effects of Endogenous Opioids on Pain Perception

Endogenous opioids are biochemical substances that modulate pain perception and provide analgesic effects. These opioids are naturally produced in the body and interact with mu, delta, and kappa receptors to alleviate pain. These interactions produce potent analgesic effects in pain management, guiding the development of more effective analgesics in clinical pain management (23). Additionally, endogenous opioids play a role in regulating cell growth and inflammation in various conditions, such as gastrointestinal and liver diseases (24). This versatile role of the endogenous opioid system allows it to have a broad range of effects in pain management.

1.4.2. Stress Response and Endogenous Opioids

Stress can trigger the activation of the endogenous opioid system, significantly impacting pain perception. For instance, during acute stress, the release of endogenous opioids like endorphins increases, helping the body cope with stress and reducing the sensation of pain. However, under chronic stress, the functioning of this system may be impaired, leading to an increased perception of pain. Furthermore, it has been found that stress can contribute to dysfunction in the opioid system, affecting emotional responses in chronic pain patients (25).

1.4.3. Exercise and the Endogenous Opioid System

Exercise is another crucial factor that increases the release of endogenous opioids, thereby producing analgesic effects. During physical activity, the release of endogenous opioids like beta-endorphin increases, reducing pain sensation post-exercise. Moreover, exercise therapy contributes to the modulation of endogenous opioids in managing post-stroke pain, supporting the analgesic effects of exercise (26).

1.5. Clinical Applications and Research

1.5.1. The Endogenous Opioid System and Pain Management

The endogenous opioid system plays a significant role in pain management. Opioids such as beta-endorphin and enkephalins bind to mu, delta, and kappa receptors, providing analgesia. These mechanisms guide research to develop more effective pain relief treatments in clinical applications (23). Clinical studies have shown that dysfunction of the endogenous opioid system is associated with the risk of opioid misuse in chronic pain patients (27). Additionally, long-term opioid treatment has been found to lead to a decrease in sex hormone levels and gonadal dysfunction in patients with cancer-related pain, correlated with opioid dosage and cortisol concentrations (28).

1.5.2. Placebo and the Endogenous Opioid System

The placebo effect is an important factor in pain management and

is largely modulated by the endogenous opioid system. When a placebo is administered, the body releases natural opioids like endorphins, reducing pain perception (29). This mechanism can enhance patients' responses to pain relief treatments and be used to increase treatment efficacy in clinical applications. The placebo effect works by triggering the release of endogenous opioids that bind to opioid receptors, explaining the strong analgesic effects of placebo.

1.5.3. Pharmacological Approaches

Opioid agonists and antagonists are pharmacological agents widely used in pain management. Opioid agonists provide pain relief by binding to endogenous opioid receptors. For example, morphine and similar drugs bind to mu-opioid receptors, providing potent analgesia (30). However, long-term use of opioid agonists can lead to tolerance, dependence, and other side effects. Therefore, opioid antagonists are used in opioid addiction and overdose treatment. These drugs bind to opioid receptors, blocking the effects of opioids, and thus can be lifesaving in treating opioid overdoses (31).

1.6. The Endogenous Opioid System and Alternative Treatment Methods

1.6.1. Acupuncture and the Endogenous Opioid System

Acupuncture is a treatment method that provides analgesic effects by activating the endogenous opioid system. This mechanism plays a significant role in explaining the effects of acupuncture on psychological conditions and behaviors (32). Acupuncture promotes the release of endogenous opioids in the brain, increasing T-lymphocyte transformation function and immune responses (33). Furthermore, the rapid effect of acupuncture in treating depression highlights the role of the endogenous opioid system in this treatment method (34). These findings support the effectiveness of acupuncture in the treatment of chronic pain.

1.6.2. Meditation and mindfulness

Meditation and mindfulness practices are effective alternative treatment methods for pain management. The analgesic effects of mindfulness meditation are provided through the endogenous opioid system. For example, the significant reduction in pain scores after mindfulness meditation indicates that this effect is related to endogenous opioid pathways (35). However, some studies have shown that the pain-relieving effects of mindfulness meditation are not dependent on the endogenous opioid system (36). These conflicting results suggest that more research is needed to better understand the complex mechanisms of meditation and mindfulness practices in pain management.

1.6.3. Nutrition and the Endogenous Opioid System

Nutrition can affect the activity of the endogenous opioid system. Endogenous opioid peptides play a crucial role in regulating feeding behavior. For example, opioid antagonists such as naloxone and naltrexone can reduce the intake of flavored water and food, while opioid agonists can increase the intake of certain foods (37). Additionally, nutrition can modulate the regulation of opioid receptors and peptides in the central and peripheral nervous systems, influencing pain relief and reward processing functions (38). These findings highlight the potential effects of nutrition on the endogenous opioid system and, consequently, its role in overall health.

1.7. Future Perspectives and Research Directions

1.7.1. New Treatment Methods

The endogenous opioid system stands out as a significant target in developing new treatment methods for pain management. The molecular and neuroanatomical features of this system form the basis for new pharmacological and non-pharmacological treatment methods. For example, pharmacological agents such as low-dose naltrexone (LDN) and opioid growth factor (OGF) hold promise in the treatment of various chronic diseases (39). Furthermore, the effects of genetic and epigenetic regulations on the opioid system contribute to the development of personalized treatment strategies (40). These new approaches have the potential to offer more effective and safer treatment methods for opioid addiction and other chronic pain conditions.

1.7.2. Combined Treatments

The integration of the endogenous opioid system with other pain management strategies can enhance the effectiveness of combined treatments. For example, the combination of transcranial direct current stimulation (tDCS) and placebo significantly activates the endogenous μ-opioid system, providing meaningful analgesic effects in pain management (41). Additionally, non-pharmacological approaches such as acupuncture can increase the release of endogenous opioid peptides, helping alleviate pain (42). These combined treatment strategies provide more effective pain management, especially in complex conditions such as chronic pain and neuropathic pain (30).

1.7.3. Genetic and Molecular Research

Research on the genetic and molecular level of the endogenous opioid system allows us to better understand its functioning and role in pain management. Genetic studies reveal the specific roles of opioid receptor genes in various physiopathological conditions. For example, genetic deletion of opioid receptors provides important insights into the role of the opioid system in chronic pain conditions (43). Additionally, modern gene editing technologies such as CRISPR/Cas9 help us understand the dynamics of endogenous μ-opioid receptors under continuous opioid stimulation, providing insights into cellular responses (44). These types of research contribute to identifying new treatment targets and developing personalized pain management strategies.

2. Conclusion

This article has examined the critical role of the endogenous opioid system in pain management and its potential clinical applications. Key findings include the biosynthesis and secretion of endorphins, enkephalins, and dynorphins, their interactions with opioid receptors, and how these processes play a role in analgesia and stress management. Additionally, the effects of stress, exercise, and alternative treatment methods on the endogenous opioid system were detailed.

Optimizing the endogenous opioid system in clinical applications for pain management is important. Specifically, targeting opioid receptors and regulating this system in chronic pain patients can reduce the risk of opioid misuse. Non-pharmacological approaches such as acupuncture and mindfulness can enhance the activation of this system, providing analgesic effects. It is recommended to update clinical protocols to include these approaches.

Future research should focus on better understanding the endogenous opioid system at the genetic and molecular levels. Using modern gene editing technologies such as CRISPR/Cas9, the dynamics of opioid receptors and their roles in chronic pain conditions should be examined. Additionally, the efficacy and safety of new pharmacological agents such as low-dose naltrexone and opioid growth factor should be investigated. These studies will contribute to developing personalized pain management strategies.

Conflict of interest

No conflict of interest to declare.

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Authors' contributions

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A comprehensive review on Chandler's syndrome: Pathophysiology, diagnosis, management, and future perspectives

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Abstract

Chandler's Syndrome, a rare subtype of the iridocorneal endothelial (ICE) syndrome spectrum, is marked by corneal endothelial abnormalities, iris atrophy, and secondary glaucoma. This narrative review synthesizes current evidence on its clinical features, pathophysiological mechanisms, diagnostic modalities, and therapeutic approaches. Predominantly affecting middle-aged Caucasian women, Chandler's Syndrome typically presents unilaterally with corneal edema, visual impairment, and subtle iris alterations. Aberrant endothelial cell proliferation and migration lead to peripheral anterior synechiae (PAS), intraocular pressure (IOP) elevation, and progressive corneal decompensation. While the etiology remains incompletely understood, herpes simplex virus (HSV) and Epstein–Barr virus (EBV) have been postulated as potential viral triggers. Morphological changes, including endothelial cell metaplasia and ectopic membrane formation, further impair aqueous humor outflow and corneal transparency. Diagnosis relies on slit-lamp biomicroscopy, specular and confocal microscopy, and ultrasound biomicroscopy (UBM). Accurate differentiation from posterior polymorphous corneal dystrophy (PPCD) and Fuchs' endothelial dystrophy is critical. Management strategies encompass pharmacological IOP control and surgical interventions such as trabeculectomy, glaucoma drainage devices, Descemet stripping endothelial keratoplasty (DSEK), or penetrating keratoplasty (PK). Literature for this review was identified through PubMed, Scopus, and Google Scholar using relevant search terms, with inclusion based on clinical applicability and peer-reviewed validity. Future studies should prioritize elucidation of viral involvement and endothelial pathobiology to guide targeted therapeutic innovations.

Keywords: Chandler syndrome, corneal diseases, Iridocorneal endothelial syndrome, ultrasound biomicroscopy, keratoplasty

1. Introduction

Chandler's Syndrome, a rare variant of iridocorneal endothelial (ICE) syndrome, is characterized by corneal endothelial abnormalities in conjunction with angle and iris changes (1). First described by Chandler in 1956, it is now classified among the three distinct clinical subtypes of ICE syndrome, alongside Essential Iris Atrophy and Cogan-Reese Syndrome (2,3). ICE syndrome constitutes a non-hereditary, proliferative endotheliopathy marked by peripheral anterior synechiae (PAS), elevated intraocular pressure (IOP), and progressive endothelial decompensation (4). Essential Iris Atrophy, in particular, manifests with significant iris alterations, including pupil distortion and stromal thinning, frequently leading to secondary glaucoma due to progressive angle closure (5,6).

ICE syndrome is typically sporadic and predominantly affects Caucasian women aged 20 to 50 years (7). Chandler's Syndrome accounts for approximately 50% of ICE cases and is often associated with prominent corneal edema and comparatively milder iris changes relative to the other subtypes (8). Although the underlying etiology remains undetermined, a viral hypothesis has gained traction, with herpes simplex virus (HSV) and Epstein–Barr virus (EBV) implicated in its pathogenesis. Elevated HSV DNA levels detected in corneal endothelial specimens from affected individuals support this association (9).

Early diagnosis of Chandler's Syndrome is challenging due to minimal iris involvement and variable IOP elevation in the initial stages (8). Differentiation from conditions such as posterior polymorphous corneal dystrophy (PPCD) is critical, given PPCD's typical bilateral, hereditary presentation versus the sporadic, unilateral nature of Chandler's Syndrome (10). Histopathological findings often reveal endothelial cells exhibiting epithelial-like morphology, consistent with aberrant differentiation potentially triggered by viral agents (11).

While ICE syndrome is predominantly unilateral, bilateral involvement has been reported in up to 10% of cases. These bilateral or atypical presentations, including rare occurrences in male patients, challenge the conventional understanding of strict unilaterality (12–20). In Asian populations, structural predispositions—such as narrower anterior chamber angles—

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heighten the risk of primary angle-closure glaucoma (PACG) in the contralateral eye, underscoring the need for vigilant bilateral assessment, particularly among older individuals (21–25).

Current management of Chandler's Syndrome emphasizes IOP regulation and corneal preservation. Surgical options, including penetrating keratoplasty, may be considered in advanced disease, though outcomes are often compromised by graft failure and ongoing endothelial dysfunction (26). Ongoing progress in surgical techniques and exploration of targeted therapies remain essential to improving long-term prognosis. This narrative review consolidates contemporary knowledge on the pathophysiology, clinical spectrum, diagnostic challenges, and therapeutic strategies associated with Chandler's Syndrome. By bridging established clinical data with emerging research, this review aims to assist clinicians in the recognition and comprehensive management of this rare ocular condition, while highlighting key priorities for future investigation.

2. Methodology

This review was conducted using a narrative approach to synthesize and interpret current literature on Chandler's Syndrome, a subtype of iridocorneal endothelial (ICE) syndrome. A comprehensive literature search was performed using electronic databases including PubMed, Scopus, and Google Scholar. The search strategy incorporated a combination of keywords such as "Chandler's Syndrome," "iridocorneal endothelial syndrome," "ICE syndrome," "corneal edema," "glaucoma," "endothelial cell transformation," and "Descemet's membrane." Boolean operators (AND/OR) were used to optimize search combinations.

Articles published in English up to April 2025 were considered for inclusion. Priority was given to peer-reviewed original research articles, clinical studies, review papers, and histopathological reports that provided insight into the epidemiology, pathophysiology, clinical manifestations, diagnostic imaging, and management of Chandler's Syndrome. Case reports, conference abstracts, editorials, and non-English language publications were excluded unless they contributed unique or illustrative information on rare features or variants. Reference lists of key articles were manually screened to identify additional relevant studies.

3. Clinical Manifestations

Chandler's Syndrome typically manifests as unilateral visual disturbances resulting from corneal endothelial dysfunction. Affected individuals often report morning visual haze, reduced visual acuity, and perception of halos, symptoms that are especially prominent upon awakening due to nocturnal corneal fluid accumulation and subsequent light scatter (14, 27–29). These visual phenomena are primarily attributable to corneal edema, with secondary glaucoma playing a lesser role in the initial symptomatic phase. Clinical evaluation reveals subtle iris alterations, which may complicate early diagnosis. Some patients present with changes in pupil morphology, including corectopia, warranting further ophthalmologic assessment (29).

Although mild iris atrophy may be observed, classic features such as full-thickness iris holes—typical of other ICE variants—are infrequently encountered in Chandler's Syndrome (2). The pupils generally retain a round or mildly oval configuration in this subtype. Considerable phenotypic variability characterizes the syndrome. Although classically unilateral, bilateral but asymmetrical involvement has been reported, with one study documenting only two bilateral cases among 21 individuals with ICE syndrome (30). Rarely, overlapping features of two ICE subtypes may coexist within the same patient, further complicating clinical classification and subtype distinction. Slit-lamp biomicroscopy typically reveals key features, including a "hammered-silver" endothelial appearance (Fig.1.) and peripheral anterior synechiae (PAS) (Fig.2.) (8,31). Additional findings may include corneal guttae and stromal iris atrophy, both of which contribute to elevated intraocular pressure over time.

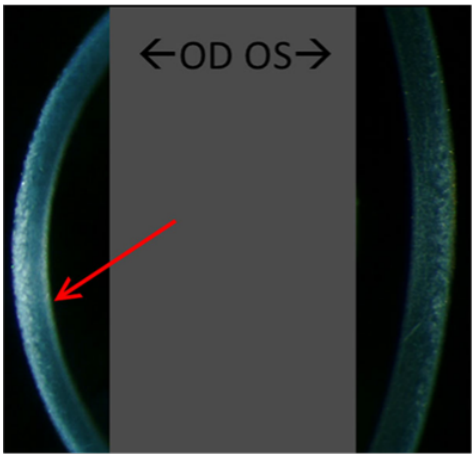

Fig. 1. Slit-lamp photograph showing the characteristic "hammered silver" appearance of the corneal endothelium in Chandler's Syndrome (33)

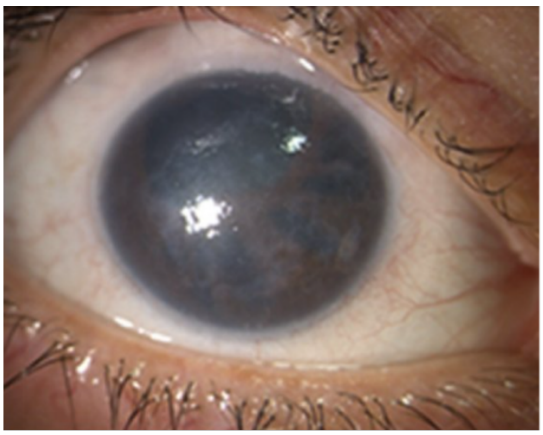

Fig. 2. Moderate corneal edema, polycoria, and peripheral anterior synechiae in a patient with Chandler's Syndrome (40)

Table 1. Comparative analysis of corneal manifestations: Chandlers syndrome vs. ICE subtypes

Chandler's Syndrome	ICE Subtypes
Primary corneal edema with "hammered silver" or "beaten bronze" endothelial appearance (32)	Less prominent corneal edema, may develop secondary to elevated IOP (33)
Bullous keratopathy and corneal endothelial decompensation as predominant features (32)	Corneal changes typically secondary to iris and angle abnormalities (34)
Microcystic edema even at normal intraocular pressures (33)	Corneal pathology usually associated with advanced glaucomatous damage (34)
Corneal thickness increases with decreased endothelial cell density and hexagonal cell ratio (32)	Corneal involvement less severe and often follows iris manifestations (34)

Table 2. Comparative analysis of iris and pupillary abnormalities: Chandlers syndrome vs. ICE subtypes

Chandler's Syndrome	ICE Subtypes
Mild to absent iris changes, minimal iris atrophy (32, 33)	Severe corectopia, iris hole formation, polycoria, ectropion uveae (33)
Minimal pupillary distortion or displacement (33)	Multiple pedunculated nodules on anterior iris surface, heterochromia (35)
Iris findings less common, majority of patients show no iris changes (33)	Robust and progressive iris findings over time (33)
Preservation of iris architecture in early stages (36)	Tan pedunculated nodules with adjacent iris atrophy, ectropion uveae (37)

Table 3. Comparative analysis of glaucomatous manifestations: Chandlers syndrome vs. ICE subtypes

Chandler's Syndrome	ICE Subtypes
Elevated intraocular pressure often presenting feature (36)	Glaucoma development through extensive synechial angle closure (34)
May present with normal pressures initially due to corneal pump dysfunction (33)	Pressure elevation typically follows iris and angle structural changes (35)
Glaucomatous damage may be masked by corneal edema (36)	Visual field defects often correlate with extent of iris atrophy (34)

The diagnosis of Chandler's Syndrome (CS) is frequently delayed due to its characteristically subtle iris changes, which lack the pronounced features observed in progressive iris atrophy or Cogan-Reese syndrome, such as iris holes or nodular formations (32, 38). These mild signs—most commonly corectopia or stromal thinning—are often overlooked during routine slit-lamp evaluations, especially in the early stages of disease (33). Diagnostic uncertainty is further compounded by the typically modest intraocular pressure (IOP) elevations observed in CS. Median IOP values are generally lower than those associated with other ICE subtypes, which may contribute to delayed recognition of glaucomatous progression (38). Moreover, corneal edema—a hallmark clinical feature of CS—does not consistently correlate with IOP levels, potentially leading to underestimation of disease severity and the risk of secondary glaucoma (33, 38). In atypical presentations, including bilateral cases, CS may be misdiagnosed as other ocular conditions such as Fuchs' endothelial dystrophy, herpetic keratouveitis, or developmental anomalies like Axenfeld-Rieger syndrome, further complicating clinical classification (32, 34). These diagnostic challenges highlight the importance of adjunctive imaging modalities. Techniques such as specular and confocal microscopy, along with ultrasound biomicroscopy, are instrumental in detecting endothelial abnormalities and angle membranes that may not be evident with standard examination methods (39).

4. Pathophysiology

The central pathogenic mechanism in Chandler's Syndrome (CS) involves aberrant behavior of corneal endothelial cells, which undergo epithelial-like transformation (33, 40). Transmission electron microscopy has revealed that these transformed endothelial cells develop desmosomes, microvilli, and intracytoplasmic filaments (40, 41). These phenotypically altered cells migrate posteriorly beyond Schwalbe's line, forming a contractile basement membrane that encroaches upon the trabecular meshwork and iris (33, 40), leading to peripheral anterior synechiae (PAS) and secondary angle-closure glaucoma. In contrast to congenital corneal dystrophies, CS is characterized by postnatally acquired endothelial changes, with prenatal Descemet's membrane appearing histologically normal (42). This observation supports a non-congenital pathogenesis that distinguishes CS from conditions such as posterior polymorphous dystrophy (44). Two prevailing hypotheses attempt to explain PAS development in CS: the "Spontaneous Hole Formation Theory," which posits that iris holes facilitate synechiae formation (5, 44), and the "PAS-First Theory," suggesting that PAS formation leads to subsequent pupil distortion (6, 45). Both models attribute anterior segment remodeling to endothelial degeneration and ectopic membrane proliferation (46).

Ultrastructural variability among CS specimens has been noted. Some studies describe attenuated endothelial cells lacking epithelial features (10), whereas others report keratin-positive, filament-rich cells with epithelial-like morphology (41). These findings blur the boundary between CS and posterior polymorphous corneal dystrophy (PPMD) (10, 47). Immunohistochemically, affected endothelial cells stain positively for cytokeratins K7 and KL1 (48), but are negative for glial, vascular, and neuroendocrine markers (49, 50). Early disease stages are characterized by enhanced barrier function and tight junction formation (51), while advanced stages show endothelial necrosis, intercellular gaps, and marked morphometric irregularity (9). A fibrous layer developing

between Descemet's membrane and the endothelium further contributes to corneal opacity and progressive vision impairment. Although CS may clinically resemble Fuchs' endothelial dystrophy, its unilateral presentation and lack of hereditary transmission aid in its distinction (52, 53). Additional ultrastructural markers identified in CS include liposomal junctions and pigment granules (54).

5. Corneal and Iris Endothelial Alterations

Endothelial cell metaplasia in CS results in abnormal collagen synthesis and the formation of a fibrotic, retro-Descemet membrane (55–57). These structural changes contribute to Descemet's membrane lamination, thickening, and progressive corneal decompensation (58). Affected endothelial cells display dynamic, motile phenotypes—characterized by microvilli, ruffled borders, and elongated filopodia—that may reflect a maladaptive reparative response (54, 56, 59). On the iris surface, these ectopic endothelial cells distort anterior segment architecture, thereby facilitating PAS formation and angle narrowing. Resultant obstruction of aqueous humor outflow and elevated intraocular pressure (IOP) contribute to glaucomatous optic neuropathy, in line with the clinical trajectory observed in other ICE syndrome variants (56).

6. Viral Etiology

Polymerase chain reaction (PCR)-based studies have identified herpes simplex virus (HSV) DNA in up to 64% of corneal endothelial specimens from ICE syndrome patients, including those with CS, when compared to control samples (40, 60). HSV DNA has also been detected in aqueous humor, providing further support for viral involvement in the pathogenesis of corneal endothelial metaplasia (60). Electron microscopy corroborates these findings, revealing epithelial-like endothelial features such as desmosomes and microvilli—hallmarks of virus-induced cellular reprogramming (40). While Epstein-Barr virus (EBV) and varicella-zoster virus (VZV) have also been sporadically linked to CS (40, 43, 61), their exact pathogenic roles remain unclear. The typically unilateral and sporadic presentation of CS aligns with a hypothesis of postnatal viral acquisition (40). Despite ongoing speculation regarding viral etiology, no randomized controlled trials have demonstrated therapeutic benefit from antiviral agents in CS. Consequently, antiviral therapy remains investigational at this stage (43).

7. Diagnostic tools and their specific detection capabilities

The diagnosis of Chandler's Syndrome relies on a combination of clinical examination and targeted diagnostic modalities. Slit-lamp biomicroscopy typically reveals diffuse or microcystic corneal edema, which may be present even in the setting of normal intraocular pressure. A hallmark feature is the characteristic "hammered silver" or "beaten bronze" appearance of the corneal endothelium, reflecting abnormal cellular morphology (33, 36). Corectopia, though often subtle, may also be noted. Gonioscopic evaluation is critical, demonstrating broad-based peripheral anterior synechiae (PAS) that extend above Schwalbe's line—considered pathognomonic for ICE syndrome—as well as membrane formation obscuring angle structures (33, 36, 39). Tonometric assessment reveals variable intraocular pressure elevation; although pressure may remain within normal limits during early disease, progressive endothelial dysfunction and angle compromise frequently result in secondary glaucoma (36, 40). These findings underscore the importance of comprehensive anterior segment evaluation in patients presenting with unexplained unilateral corneal edema and iris abnormalities.

Functional and structural assessments play a critical role in evaluating glaucomatous progression in Chandler's Syndrome. Visual field testing may reveal early functional deficits, typically presenting as mild generalized constriction or superior nasal field defects, consistent with glaucomatous optic neuropathy. These changes, though sometimes subtle, provide important evidence of optic nerve compromise (36, 62). Optical coherence tomography (OCT) complements these findings by offering high-resolution structural imaging of the optic nerve head. It enables precise evaluation of optic disc cupping and retinal nerve fiber layer thinning, both of which are indicative of glaucomatous damage (62). Together, these modalities support early detection and longitudinal monitoring of glaucoma in the context of Chandler's Syndrome.

Advancements in ocular imaging technologies have significantly enhanced the diagnostic precision and disease monitoring of Chandler's Syndrome. Techniques such as specular microscopy, confocal microscopy, and ultrasound biomicroscopy (UBM) allow detailed visualization of endothelial abnormalities and anterior segment changes not evident on routine examination (Table 4).

Table 4. Advanced imaging technologies

Diagnostic Tool	Specific Findings in Chandler's Syndrome
Specular Microscopy	Reduced corneal endothelial cell density; light-dark reversal characteristic of ICE; dysmorphic endothelium; "epithelium-like" transformation of corneal endothelium (62, 63).
Confocal Microscopy	"Epithelium-like" transformation of corneal endothelium; irregularly shaped cells with hyperreflective nuclei; ICE-cells visualization on corneal endothelium (39, 40, 64).
Ultrasound Biomicroscopy (UBM)	Membrane extending from corneal endothelium to anterior iris surface causing traction; structural changes of anterior chamber angle; bridge-shaped synechiae; membrane-like mounds in iridocorneal angle (39, 40, 65).
Anterior Segment OCT (AS-OCT)	ICE membrane visualization; differentiation between true PAS and iridocorneal touch; assessment of trabecular meshwork involvement; increased lens vault (66, 67).
Pachymetry	Corneal thickness measurements to assess edema severity (63).

8. Management and Treatment

The initial therapeutic approach in Chandler's Syndrome emphasizes intraocular pressure (IOP) reduction and symptomatic relief, particularly for morning visual haze. Topical hypertonic saline may offer temporary corneal deturgescence, thereby improving visual clarity. First-line pharmacologic agents typically include aqueous humor suppressants, such as beta-blockers (e.g., timolol) and carbonic anhydrase inhibitors (e.g., dorzolamide). While effective in lowering IOP, these agents should be prescribed cautiously, as they may exacerbate underlying corneal edema.

Prostaglandin analogs are generally avoided due to their potential to reactivate latent herpesviruses, a concern supported by virologic hypotheses in Chandler's Syndrome pathogenesis (29). The application of Minimally Invasive Glaucoma Surgery (MIGS) in Chandler's syndrome remains largely unexplored in published literature. Available evidence suggests that Schlemm's canal-based procedures, including Trabectome and iStent devices, are not feasible in most ICE syndrome patients due to continued endothelial membrane proliferation that compromises long-term efficacy (68). Future research should focus on developing novel surgical approaches specifically designed for the unique challenges presented by ICE syndrome variants, as conventional MIGS techniques remain unsuitable for this patient population.

Management of secondary angle-closure glaucoma presents particular challenges, especially in patients with high lens vault and shallow anterior chambers. In such anatomical contexts, phacoemulsification may mitigate non-pupillary block mechanisms that contribute to angle narrowing. Clinical evidence indicates that patients with greater lens vaults experience more substantial postoperative IOP reduction, thereby supporting early lens extraction as a viable strategy in select cases (70-72).

Table 5. Treatment summary

Category	Mild Disease	Moderate Disease	Severe/Refractory Disease
Corneal Edema Management	Topical hypertonic saline drops and ointments (33).	Combination therapy with aqueous suppressants (33).	Endothelial keratoplasty (DSEK/DSAEK) (69).
IOP control	Beta blockers, alpha agonists, carbonic anhydrase inhibitors (33, 40).	Combination topical therapy (40).	Trabeculectomy with antifibrotic agents (33, 68).
Surgical Intervention	Medical management (40).	Trabeculectomy with mitomycin-C (33).	Glaucoma drainage devices (tube shunts) (33, 68).

9. Conclusion

Chandler's Syndrome, a rare and under-recognized variant of the iridocorneal endothelial (ICE) syndrome spectrum, presents distinct diagnostic and management challenges due to its subtle clinical features and progressive nature. Characterized by abnormal endothelial cell behavior, anterior segment remodeling, and secondary glaucoma, its pathophysiology implicates both cellular metaplasia and possible viral triggers. Advances in imaging modalities and surgical techniques have enhanced diagnostic accuracy and therapeutic outcomes; however, delayed diagnosis and treatment failure remain common. Current management remains largely supportive, with no definitive therapies targeting the underlying endothelial dysfunction. Ongoing research into the molecular and virologic basis of the disease is essential to inform targeted interventions. Early recognition, individualized management strategies, and long-term monitoring are critical to preserving visual function and quality of life in affected patients. This review underscores the need for increased clinical awareness and interdisciplinary collaboration to optimize outcomes in this complex and visually debilitating disorder.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

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A rare cause of acute urinary retention: Meningitis-retention syndrome caused by varicella zoster virus

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Abstract

A 33-year-old male patient presented to the urology clinic with complaints of headache, fatigue, and acute urinary retention. Physical examination revealed paresthesia/hypoesthesia in the S3–S5 dermatomes and decreased anal sphincter reflex. Blood and urine studies were performed, with results within normal limits. No abnormalities were observed in the urinary system on abdominal computed tomography. Cranial magnetic resonance imaging showed meningeal thickening and contrast enhancement. Lumbar puncture results reported a positive varicella zoster virus DNA. After the diagnosis, he was given intravenous acyclovir, and the symptoms gradually improved.

Keywords: meningitis-retention syndrome, varicella zoster virus, acute urinary retention

1. Introduction

Acute urinary retention (AUR) is a urological emergency characterized by the sudden inability to void urine voluntarily. AUR occurs with a male-to-female incidence ratio of 13:1, and its primary causes include obstructive, iatrogenic, inflammatory, and neurogenic factors (1). Obstructive factors are the most prevalent causes, with benign prostatic obtruction accounting for 53% of cases (2).

Varicella zoster virus (VZV) is a DNA virus from the Herpesvirus family. Following the initial varicella (chickenpox) infection, VZV establishes latency in the nervous system, which can remain dormant for years. Reactivation of the virus can be prompted by factors such as emotional stress, immunosuppression, acute or chronic illnesses, exposure to the virus, and malignancies (3). When latent VZV reactivates, it can cause inflammatory lesions in the sensory root ganglia, the meninges, and occasionally the spinal cord (4).

In this report, we present a case of acute urinary retention in a young patient triggered by the reactivation of VZV, for which informed consent was obtained from the patient.

2. Case Presentation

A 33-year-old male patient presented to the urology clinic with complaints of headache, fatigue, and inability to urinate. The patient was found to be in urinary retention. An indwelling urinary catheter was inserted, and 1100 mL of clear urine was drained immediately. The patient was hospitalized to investigate the etiology of his AUR. The patient stated that he had no history of instrumentation for AUR and did not experience any lower urinary tract symptoms or benign prostatic obstruction. The patient had no history of chronic illnesses or previous surgeries. The physical exam was significant for paresthesia/hypoesthesia in the S3–S5 dermatomes and decreased anal sphincter reflex. There was no dermatomal rash. Urine microscopy and serum creatinine, total prostate specific antigen (TPSA), white blood cell (WBC), C-reactive protein levels were normal. Urine culture was subsequently negative for bacterial growth. No stones were detected on a non-contrast computed tomography scan performed to investigate suspected urinary tract stones.

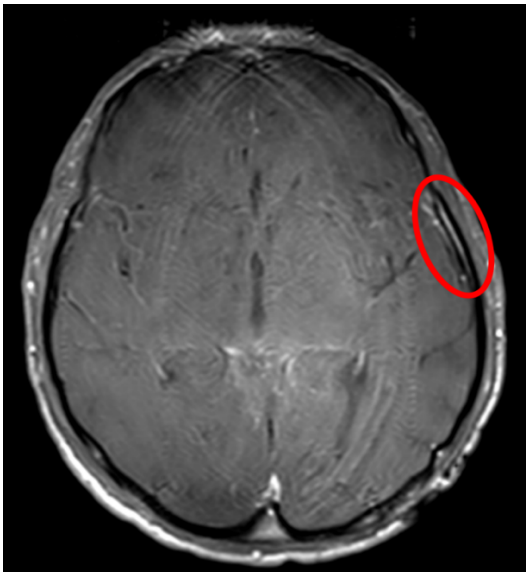

Fig. 1. Cranial MRI showing meningeal thickening and contrast enhancement

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Considering the patient's primary neurogenic etiology, a neurology consultation was requested. The electromyography was normal. Cranial contrast-enhanced magnetic resonance imaging (MRI) showed meningeal thickening and contrast enhancement (Fig.1), no abnormalities were observed in the spine on MRI. Because of the MRI findings, his cerebrospinal fluid was examined for confirmation of the diagnosis. The lumbar puncture findings were consistent with aseptic meningitis, and VZV DNA was positive by polymerase chain reaction (PCR) assay. While investigating the causes of the virus reactivation, the anti-HIV antibody test was reported positive. Subsequently, the HIV RNA test also came back positive, confirming the diagnosis of HIV.

The final diagnosis was meningitis-retention syndrome (MRS). After the diagnosis, acyclovir 750 mg x 3 / day for the VZV and a combination of nucleozide/nucleotide reverse transcriptase inhibitors and integrase strand transfer inhibitors for the HIV were promptly started. Two weeks after the treatment, the patient failed to trial of void and required clean intermittent catheterization. The urinary stream was good nine weeks after the treatment, with 20 cc post-void residual urine.

3. Discussion

We report a case of AUR caused by aseptic meningitis. Urinary retention occurring in the setting of aseptic meningitis is referred to as MRS, first described by Sakakibara in 2005 (5). MRS is a rare condition, furthermore due to VZV is rare. Neurological complications affecting the peripheral and central nervous systems arise in 0.1% to 0.75% of individuals with VZV infection (6). In the last review in March 2023, Pellegrino et al. reported 29 MRS cases (7). After reviewing the literature, the present, we found further 4 MRS cases (8-11) except our case. In most cases, the subtle nature of encephalitic symptoms and findings, along with neurophysiological studies generally being within normal limits, makes diagnosing MRS challenging. MRS is considered a self-limiting condition, with no evidence indicating that any treatment significantly alters its clinical progression (7). The underlying pathological mechanisms have yet to be fully elucidated. However, clinical observations have documented elevated basal myelin protein levels in several cases, which may indicate an inflammatory demyelinating process primarily involving the sacral spinal segments. Hypotheses suggest that mechanisms analogous to those observed in acute disseminated encephalomyelitis (ADEM), potentially initiated by viral infections, may contribute to this pathology (12).

The patient's symptoms and findings were non-specific for both VZV and encephalitis. Infection with the VZV typically presents with a skin rash. However, as in our case, the typical skin rash is not observed in approximately 40% of meningoencephalitis cases caused by VZV (13). Therefore, VZV should be included in the differential diagnosis of aseptic meningitis, even without a skin rash.

Lesions in the upper motor neurons affecting the brain or spinal cord result in an underactive detrusor, especially during the acute shock phase. An underactive detrusor is considered the primary cause of voiding dysfunction in neurological disorders (5). Since we determined that the primary cause of AUR was an underactive detrusor and that the results of a urodynamic study would not alter the treatment plan, we chose not to perform the study.

Acyclovir is administered in cases of VZV encephalitis due to its potential for enhanced efficacy. Although VZV accounts for only approximately 5% of encephalitis cases, early empirical treatment is essential, necessitating a careful balance with toxicity monitoring (14). Based on this rationale, we initiated acyclovir therapy for the patient.

This case highlights the rare but significant association between aseptic meningitis and AUR, identified as MRS. The reactivation of VZV in the absence of a typical skin rash, as well as the simultaneous diagnosis of HIV, further complicates the clinical presentation and emphasizes the need for a thorough diagnostic workup, including cerebrospinal fluid analysis and PCR testing for VZV. Although we know MRS is a self-limiting disease, we believe that etiology-specific treatment would benefit once the underlying cause is identified. Further studies are necessary to understand the pathophysiology of MRS better and to establish standardized treatment protocols.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: E.K., M.G., Design: E.K., Data Collection or Processing: O.K., Analysis or Interpretation: E.K., Literature Search: E.K., M.G., Writing: E.K., O.K.

Ethical Statement

This case report is not required ethical decision.

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Case Report

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Utilization of ultrasound to confirm lesions and perform paravertebral blocks for painful pleural metastasis treatment – A report of two cases

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Abstract

Pleural metastasis-related pain poses a significant challenge in patients with cancer, often leading to severe discomfort and impaired quality of life. Thoracic paravertebral block is an effective regional anesthesia technique for managing such pain, particularly when performed under ultrasound guidance. This report illustrates the use of ultrasound for identifying metastatic lesions and guiding thoracic paravertebral blocks in two patients with pleural metastasis. The first case involved a 76-year-old woman with severe left upper back pain secondary to lung cancer metastasis. Ultrasound confirmed the presence of a lesion at the T5 level, and ultrasound-guided thoracic paravertebral block provided immediate and substantial pain relief, thereby improving sleep quality. The second patient was a 72-year-old man with intractable left upper back pain. Ultrasonography revealed a metastatic lesion at T6, and subsequent thoracic paravertebral block led to significant pain reduction and improved pain management with reduced opioid use. Both cases highlight the effectiveness of ultrasound in accurately locating metastatic lesions and determining the appropriate spinal level for thoracic paravertebral block, resulting in significant pain relief and improved quality of life. These findings underscore the importance of precise anatomical localization and the potential of ultrasound-guided thoracic paravertebral block as a valuable tool for managing pain associated with pleural metastases.

Keywords: Ultrasonography; thoracic paravertebral block; pleural neoplasms; intractable pain

1. Introduction

Pain caused by pleural metastasis poses a significant challenge in advanced malignancies, leading to considerable morbidity and reduced quality of life. Effective pain management is crucial for alleviating suffering and improving overall functionality (1). Thoracic paravertebral block (TPVB) is a valuable regional technique for thoracic pain, reducing pain, opioid use, and systemic side effects (2).

Traditionally, TPVB is performed using anatomical landmarks and loss-of-resistance techniques, which increases the risk of inaccurate needle placement. Ultrasound guidance has revolutionized regional anesthesia via precise localization of the paravertebral space, improving needle placement accuracy and reducing complications such as pneumothorax and pleural puncture (3). Ultrasonography is crucial for identifying lesions and guiding the TPVB to the appropriate spinal level in pleural metastases with localized pain. Correctly targeting the spinal nerves ensures optimal clinical outcomes and safety. Despite these advantages, few studies have explored the role of ultrasound-guided TPVB in pleural metastasis. This report highlights two cases where ultrasound was used for metastatic lesions to determine the appropriate spinal level for TPVB in patients with painful pleural metastases.

2. Case Presentation

2.1. Case 1

A 76-year-old woman with non-small cell lung cancer presented with intractable left upper back pain for two months. The pain, described as sharp and electric shock-like, worsened in the supine position and disrupted her sleep. Her pain score fluctuated between 2 and 8 on the numeric rating scale (NRS). Physical examination revealed T5 dermatome tenderness and allodynia, suggesting intercostal nerve irritation from pleural metastasis as the primary pain source. To confirm the presence of pleural metastasis and accurately determine the spinal level for TPVB, ultrasound imaging (LOGIQTM E10, GE Healthcare, USA) was utilized. Ultrasonography confirmed a metastatic lesion adjacent to the T5 vertebra (Fig. 1), corroborating prior computed tomography (CT) findings (Fig. 2). Consequently, TPVB was performed at the T5 level corresponding to the identified lesion. Ultrasound-guided TPVB was conducted with the patient in the prone position using a transverse in-plane approach to visualize the needle as

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it entered the left-sided paravertebral space at T5. Ten milliliters of 0.2% ropivacaine combined with 5 mg dexamethasone was injected into the paravertebral space. Approximately 20 min after the procedure, the patient reported a significant reduction in pain from 5/10 to 0/10 on the NRS. Numbness was observed in the sensory distribution of the left T5 dermatome. On the day of the procedure, the patient experienced a marked reduction in pain intensity, ranging from 1 to 5 out of 10 on the NRS. The patient was able to lie in the supine position without discomfort, with significantly improved sleep quality. Additional TPVBs were performed over the following month without further pain reduction. However, the patient continued to report sustained improvements in pain levels and sleep quality following subsequent cancer treatment.

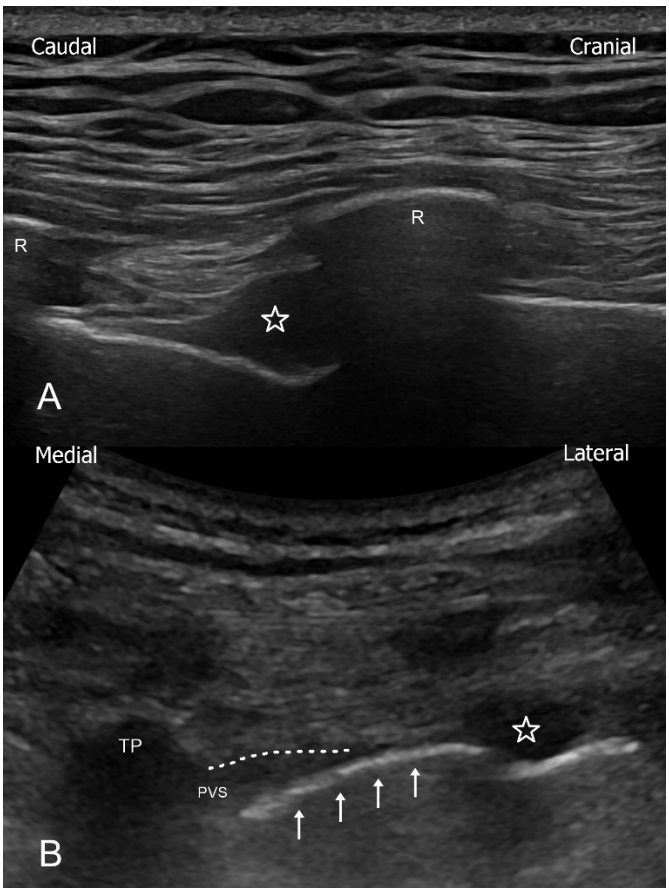

Fig. 1. Ultrasound imaging for localization of pleural metastasis adjacent to the T5 vertebra (Case 1). (A) Parasagittal ultrasound image showing rib shadows (R) and the hypoechoic metastatic lesion (☆) near the T5 vertebral level. (B) Transverse ultrasound view demonstrating the metastatic lesion (☆) in the paravertebral space (PVS), deep to the transverse process (TP). The dotted line indicates the parietal pleura, and the white arrows mark the trajectory of the intended needle path for TPVB

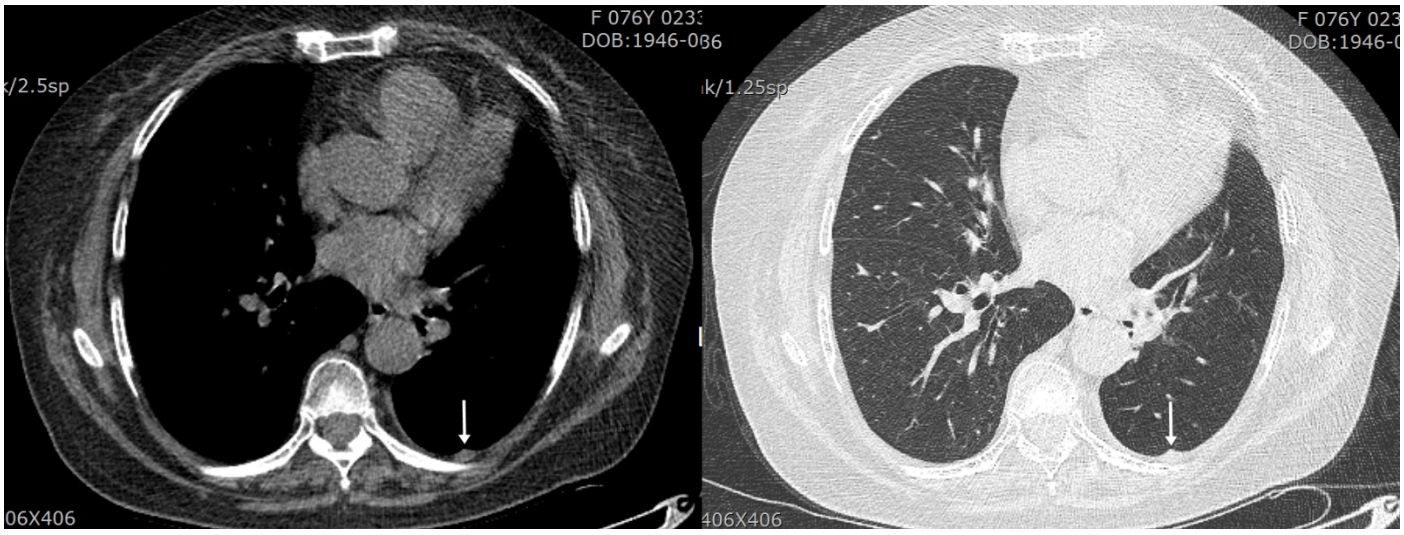

Fig. 2. Axial CT images revealing pleural metastasis (Case 1). (Left) Axial non-contrast-enhanced chest CT image showing a pleural-based soft tissue mass (white arrow) adjacent to the T5 vertebra on the left hemithorax. (Right) Lung window setting of the same axial level, highlighting the pleural lesion (white arrow) with adjacent parenchymal distortion

2.2. Case 2

A 72-year-old man with non-small cell lung cancer and pleural metastasis experienced severe left upper back pain for four months, described as burning and electric shock-like, with sleep-disrupting tingling sensations. Despite opioid use, the patient reported persistent severe pain, rated 8/10 on the NRS, with recent opioid resistance. Ultrasonography confirmed a metastatic lesion adjacent to the T6 vertebra, aligning with the prior CT results. Imaging revealed a distinct metastatic lesion in the pleura adjacent to the T6 vertebra (Fig. 3). Ultrasound-guided TPVB at T6 was performed, leading to immediate pain relief and numbness in the left T6 dermatome. Over the following months, the breakthrough pain intensity and frequency decreased by 50%, allowing effective pain management with oral analgesics and continued cancer treatment without additional blocks.

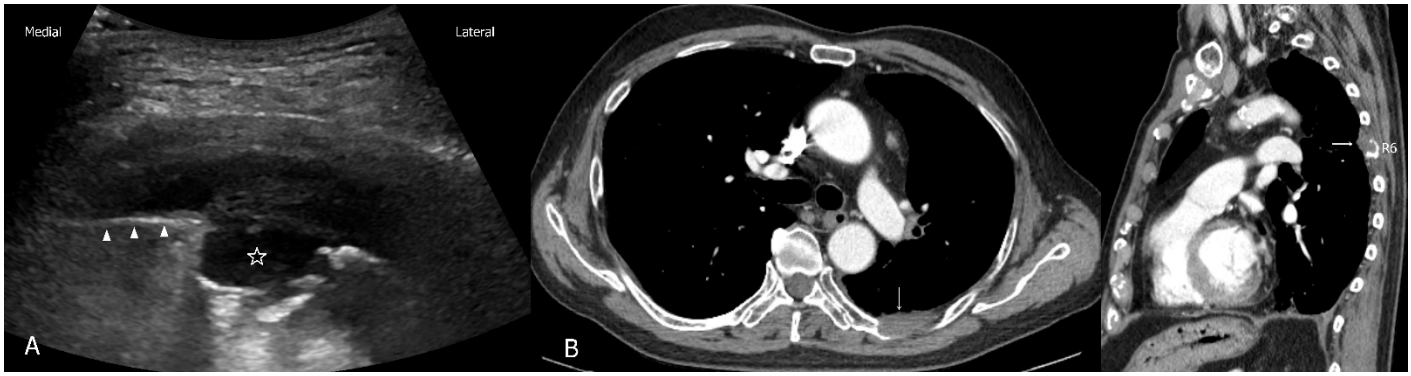

Fig. 3. Imaging findings (Case 2). (A) Ultrasound image showing a hypoechoic metastatic lesion (☆) adjacent to the transverse process at the T6 level; white arrows mark the trajectory of the intended needle path for TPVB. (B) Axial and sagittal computed tomography (CT) images show a pleural-based metastatic lesion adjacent to the left T6 vertebra (white arrows)

3. Discussion

This case report highlights the significance of ultrasound guidance in managing pain associated with pleural metastasis in patients with TPVB. Unlike traditional imaging techniques, ultrasound provides real-time visualization of the paravertebral space, enabling precise identification of metastatic lesions and accurate determination of spinal levels for needle placement. These features improve the safety profile of the procedure and enhance pain relief. Moreover, the immediate and significant pain reduction observed in both cases highlights the importance of meticulous anatomical localization and targeted intervention.

Cancer pain involves mixed mechanisms involving inflammatory, neuropathic, and ischemic processes, rendering treatment challenging (4). In pleural metastasis, localized pain on the affected side often results from direct tumor invasion of the pleural surface, causing inflammation and irritation of the parietal pleura (5). The parietal pleura is innervated by the somatic intercostal nerves. Any trauma or inflammation in this region leads to pain localized to the corresponding cutaneous nerve (6). This irritation commonly manifests as sharp, stabbing, or electric shock-like pain, often worsened by deep breathing or positional changes (5). TPVB provides ipsilateral, segmental, somatic, and sympathetic nerve blockade across contiguous thoracic dermatomes by delivering local anesthetics and anti-inflammatory agents into the paravertebral space and interrupting sensory, motor, and autonomic signal transmission, thereby inhibiting pain perception (7).

Ultrasound-guided TPVB is a well-established technique in regional anesthesia; however, it is traditionally performed based on anatomical landmarks. Reliance on these landmarks, however, does not always ensure alignment with actual pathological spinal segments (8), especially in patients with metastatic lesions. This misalignment can lead to suboptimal analgesia and an increased risk of complications. In this context, our report highlights the significant utility of high-resolution ultrasound in identifying and precisely localizing metastatic lesions in the thoracic region. Ultrasound enhances the precision of needle placement and facilitates targeted drug delivery by directly visualizing the pathological segments. Compared with existing studies, such as Malik's 2014 report (9) on ultrasound-guided paravertebral neurolytic blocks for lung cancer with pleural invasion, our case report emphasizes how ultrasound can overcome the limitations of landmark-based techniques by providing real-time imaging of both normal and pathological anatomies.

While ultrasound can determine the target level in cases of chest wall pathologies, such as rib fractures and pleurisy (10), its utility in complex anatomical scenarios may be limited. However, in the aforementioned cases, ultrasound was essential for confirming thoracic metastatic lesions and facilitating targeted drug delivery, highlighting its potential for addressing the challenges posed by conventional methods. Despite the acoustic challenges posed by skeletal structures, ultrasound has demonstrated its invaluable role in complex anatomical settings by providing precise and reliable visualization to guide interventions. This underscores the diagnostic and therapeutic value of TPVB in improving its accuracy and safety in cases of pleural metastasis.

A key limitation of this study is the absence of a long-term pain control assessment, which is needed to determine the lasting effectiveness of analgesia. While high-resolution

ultrasound proved valuable in detecting tumor invasion of the pleura and chest wall (11), reliance on ultrasound alone without corroborative imaging may affect accuracy, especially in complex cases. Further research is needed to evaluate the broader application, long-term efficacy, and standardized protocols of ultrasound-guided TPVB for pleural metastases.

In conclusion, ultrasonography is crucial for accurately identifying metastatic lesions and guiding TPVB in pleural metastases, thus enabling precise targeting for effective analgesia. Ultrasound-guided TPVB is safe and effective for managing oncological pain in patients with pleural involvement.

Ethical Statement

This study was approved by the institutional review boards of the Inje University Haeundae Paik Hospital, Republic of Korea; a waiver of consent was obtained (Number: HP IRB 2024-05-040 Date: 2024.5.31).

Conflict of interest

The authors declare no conflict of interest.

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None to declare.

Authors' contributions

Concept: J.H.K., D.O., Design: J.H.K., D.O., Data Collection or Processing: J.K., B.L., Analysis or Interpretation: S.H.M., M.J.K., Literature Search: Y.H.P., Writing: J.H.K., D.O.

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Case Report

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Posterior reversible encephalopathy syndrome in pregnancy: A case report with review literature

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Abstract

Posterior reversible encephalopathy (PRES) is an uncommon condition that causes edema in the white matter of the posterior fossa of the brain along with headache, altered consciousness, visual impairment, and occasionally seizures. The diagnosis and course of treatment for a PRES case involving a patient who was 24 weeks pregnant are discussed in this article along with relevant literature.A 21-year-old patient who was 24 weeks pregnant suddenly lost her vision, had high blood pressure, hemolysis, raised liver enzymes, low platelet count, and an emergency cesarean section was used to deliver the baby. Magnetic resonance imaging (MRI) in the early postoperative phase showed vasogenic edema in the occipito-parietal region's white matter. In addition to antihypertensive drugs and cortisol, the patient received intravenous (IV) hydration treatment. She was discharged on the 10th postoperative day with his complaints and laboratory values within normal limits. In the existence of neurological symptoms in PRES disease, strong suspicion, and brain imaging are required for early diagnosis. Treatment is available before a lasting neurological disability arises with an early diagnosis.

Keywords: posterior reversible encephalopathy, pregnancy, neurological symptom, magnetic resonance imaging

1. Introduction

Posterior reversible encephalopathy syndrome (PRES) is a rare neuroradiological clinical condition that was first described by Hinchey in 1996. This syndrome includes brain capillary leak syndrome, reversible posterior leukoencephalopathy, reversible outside syndrome, posterior cerebral edema syndrome, and hypertensive Even though it is known as encephalopathy, PRES is the term that is most frequently used. In this instance, patients who arrive complaining of headaches also have altered mental status, epileptic convulsions, altered eyesight, and radiological abnormalities that point to posterior subcortical edema. Pregnancy accounts for around 6-20% of PRES syndrome cases, however, because not all cases undergo neuroradiological imaging, the real frequency of PRES syndrome is unclear. in PRES Although its origin is unknown, thrombotic thrombocytopenic Purpura is linked to acute hypertension, encephalopathy, renal failure, sepsis, autoimmune illnesses, immunosuppressive medication, and chemotherapy, as well as HIV syndrome, blood transfusions, and electrolyte imbalances. The most prevalent definition of PRES syndrome is preeclampsia with or without eclampsia during and after pregnancy (1). Although its pathogenesis is not fully understood, endothelial dysfunction is recognized as a typical mechanism for the degradation of the blood-brain barrier (2).

PRES is reversible in individuals with isolated eclampsia and does not arise spontaneously. Delays in treatment may result in irreversible brain damage in the afflicted areas (1). Because many acute neurological disorders include PRES in their differential diagnoses, brain magnetic resonance imaging (MRI) is used to make differential diagnoses with a high level of clinical suspicion. The optimal way to make a diagnosis is to use T2/fluid-attenuated inversion recovery (FLAIR) sequences on MRI to reveal hyperintense lesions in the parieto-occipital and frontal lobes (primarily at the cortical-subcortical junction), which are typically symmetrically positioned posteriorly (3).

In this article, the diagnosis and treatment process of a 21-year-old patient with a 24-week pregnancy who was diagnosed with PRES by MRI due to acute neurological findings following preeclampsia and HELLP (hemolysis, elevated liver enzymes, low platelet) syndrome, is presented in the light of literature.

2. Case Presentation

The 21-year-old female patient, who had a child with Gravida 2, was 24 weeks pregnant based on her previous menstrual period. The patient was hospitalized at the Gynecology and Obstetrics Clinic after complaining of headache and vision

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impairment and receiving a preliminary diagnosis of HELLP syndrome.

The patient was found to have a moderate overall health status, a 9 Glasgow Coma score, and a blood pressure reading of 170/110 mmHg. The patient began receiving Nidilat (nifedipine 10 mg) and intravenous (IV) magnesium sulfate (MgSO4) loading and maintenance therapy for tuberculosis at an outside facility. In the blood tests of the patient, BUN, urea, and creatinine were normal. Other parameters were as follows: albumin: 3.3 g/dL (3.5-5.2), AST: 824 IU/L (0-32), ALT: 448 IU/L (0-33), LDH: 2312 IU/L (135-214), total bilirubin: 1.6 mg/dl (0-1.2), calcium: 6.94 mg/dl (8.6-10), sodium: 121 mmol/L (135-145), potassium: 5.42 mmol/L (3.5-5.1), magnesium: 3.47 mg/dl (1.6-2.6), fibrinogen: 148.7 mg/dl (200-400), hemoglobin 13.7 g/dL (12.5-16), hematocrit: 40.9% (37-47), platelet: 77000 (150000-400000), white blood cell (WBC) 9.5x103/uL (4-10.5), C-reactive protein (CRP): 30 mg/L (0-5), complete urinalysis (UA) 3+++ proteinuria was detected. PT, APTT, and INR were normal. The ultrasound revealed a viable fetus at 24 weeks gestation, a breech presentation, a placenta fundus location, and adequate fluid. The patient was diagnosed with HELLP syndrome, and an emergency procedure was scheduled. Under spinal anesthesia, a single live female infant, measuring 29 cm in length, 515 grams in weight, and 21 cm in circumference around the head, was born via cesarean section (C/S abdominal). For close observation, the patient was brought to the intensive care unit. Intubation of the patient was not necessary. IV fluid therapy, IV calcium, IV Dexamethasone, IV antifibrinolytic (Herajit 250 mg), IV antihypertensive furosemide amp IV, oral 12.5 mg (Carvedilol (Beta Blocker)+, cleane 0.6 1x1, MgSO4 2 grams/hour maintenance therapy, analgesic, and IV calcium was administered to the patient. Amlodipine (5 mg) was started as a treatment. The critical care unit's departments of neurology, cardiology, and infectious diseases assessed the patient. Metronidazole 500 mg three times per day IV and Cefbactam 1 g three times per infectious disease guidelines were initiated. During the neurology evaluation, speaking, comprehension, cooperation, and consciousness were normal. Words are formed with meaningful replies, despite the difficulty. The patient did not exhibit any facial asymmetry, dysarthria, lateralizing impairment, or extremity-jerking epileptic episodes. The pupils were isochoric. The patient had diffusion magnetic resonance imaging (MRI) and cranial computed tomography (CT), with a tentative diagnosis of PRES.

A cardiology consultation was also requested for the patient to control her blood pressure. BP: 135/90 mmHg; pulse rate: 125 beats per minute for the patient. The patient received Dilatrend 12.5 mg 1x1 on recommendations from the field of cardiology. On the second surgical day, the patient was transferred from the intensive care unit to the gynecology and obstetrics clinic. The patient's vital signs were clear: TA was 140/80, pulse was 95 beats per minute, and overall condition was mild. Cardiology, neurology, and infectious disease guidelines were followed for administering IV antibiotics, IV Prednol, IV hydration therapy, low-molecular-heparin, and antihypertensives (Furosemide amp; oral 12.5 mg Carvedilol (Beta Blocker) + 5 mg Amplodipine (Monovas)). The cerebral CT scan revealed no signs of bleeding (Fig. 1).

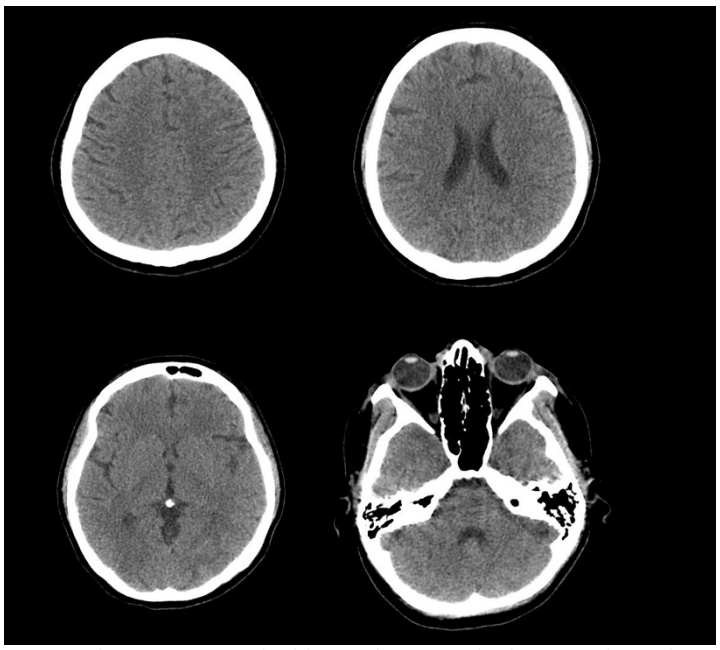

Fig. 1. There was no cerebral hemorrhage seen in the non-enhanced computed tomography scans

In cranial diffusion MRI, there were increases in the T2-WI/FLAIR signal at the parahippocampal level, more pronounced on the left, and the T2-WI/FLAIR hyperintense signal in the temporo-occipital deep white matter and the right internal capsule posterior crus (Fig. 2).

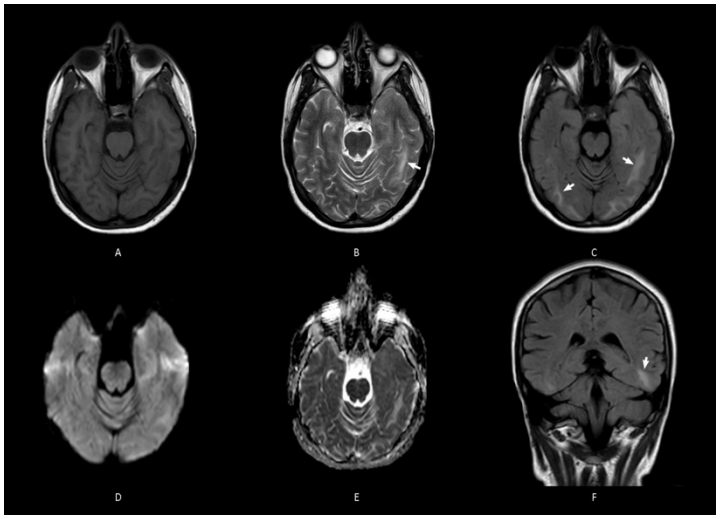

Fig. 2. Shows cranial magnetic resonance imaging demonstrating parieto-occipital region PRES findings. Axial (A), coronal (C), and axial (F) plan FLAIR sequences (arrows); (B) hyperintense signal property on T2-WI; (A) hypointense on T1-weighted imaging (WI). Diffusion weighted imaging (D) and apparent diffusion coefficient images (E) did not exhibit any diffusion restriction

Three days have passed since the surgery. Overall health was good, and consciousness was evident. It measured 135/90 mmHg for blood pressure (BP) and 95 beats per minute for pulse. Along with cranial MRI results and BP follow-ups, the patient was consulted once again with the ward's neurology and cardiology departments. It was advised to continue taking oral 12.5 mg Carvedilol, stop taking Monovas, and check BP during the cardiology visit. The neurology consultation recommended electrolyte monitoring, magnetic resonance venography (MR venography), and continuation of the existing course of treatment. Vital signs and overall health were monitored for the patient. On the fourth and fifth postoperative days, the overall condition was satisfactory. Oral intake is possible, and consciousness is evident. Following BP checks, average readings were 130/80 mmHg, the average pulse was 85 beats per minute, and room air po2 was 97. Liver enzyme levels were found to be relatively normal. As directed by neurology, the patient had an MR venography evaluation, and the results showed that the venous structures were normal. It was determined that the left transverse sinus was hypoplastic (Fig. 3).

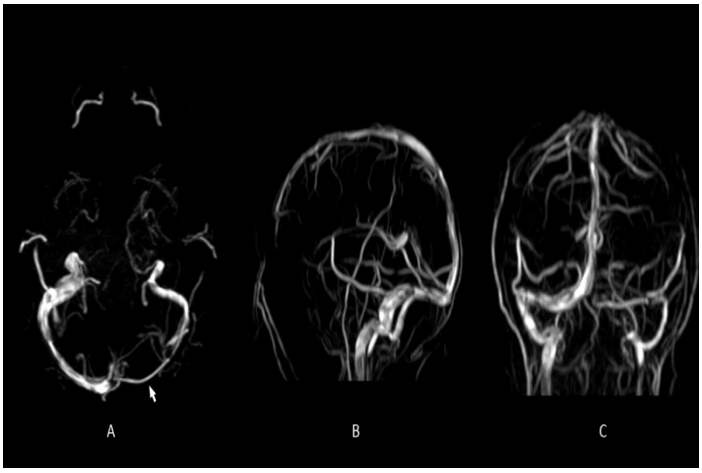

Fig. 3. (A) sagittal, (B) coronal, and (C) axial plans. The superior sagittal sinus, sinus rectus, galena vein, internal cerebral vein, right transverse sinus, bilateral sigmoid sinuses, and internal jugular veins were all shown to be patent on MR venography pictures. A hypoplastic left transverse sinus was noted (arrow)

During the follow-up with the patient, vital signs were stable and BP values were measured within normal limits. The patient's laboratory results on the 7th postoperative day were 15 IU/L for AST and 51 IU/L for ALT, respectively. The patient's laboratory results on the 7th postoperative day were 15 IU/L for AST and 51 IU/L for ALT, respectively. Fever: 34 C, BP: 140/90 mmHg, pulse: 95/min. Regarding the release, the patient had another consultation with neurology. A follow-up visit to the outpatient clinic was advised upon discharge, as neurology had no further recommendations. On the 11th postoperative day, the patient's overall condition was good; awareness was clear; BP: 130/80 mmHg; pulse: 90/min; fever: 36.9 °C; AST: 25 IU/L; ALT: 40 IU/L; and CRP: 21 mg/L were measured. Antihypertensive medication was administered to the patient. On the 11th postoperative day, the patient was discharged with her neurological problems entirely cured, and a check-up was referred to the gynecology, obstetrics, and neurology outpatient clinic.

3. Discussion

Eclampsia has been linked to several conditions, including PRES syndrome, hemolytic uremic syndrome, collagen vascular diseases, immunosuppressive medications, acute porphyria, pheochromocytoma, primary aldosteronism, thermal injury, hypercalcemia, blood transfusion, and situations involving stimulant medications like phenylpropanolamine, ephedrine, pseudoephedrine, and scorpion poisoning (4).

The neurological condition known as PRES is characterized by vasogenic edema, mainly in the parieto-occipital lobe, headache, visual abnormalities, epileptic seizures, and decreased consciousness (5). Even though pregnancy is not the main cause of PRESS, treating preeclampsia and eclampsia requires an understanding of the pathophysiology of PRESS. The pathogenesis of preeclampsia, eclampsia, and PRES syndrome share significant similarities. The pathophysiological process of PRESS has been the subject of several ideas, but it is generally associated with poor cerebrovascular autoregulation. Hyperperfusion results from rapidly increasing hypertension that surpasses the capacity of the cerebrovascular autoregulation system. Hypoperfusion is another benefit of vasospasm, which arises in hypertension to preserve brain perfusion. Endothelial damage occurs in the blood-brain barrier in both circumstances. The blood-brain barrier is disrupted by this illness, allowing plasma and macromolecules to permeate the interstitial space. Additionally, by altering the feeding arterioles' lumen diameter, certain hazardous inflammatory cytokines may indirectly compromise the blood-brain barrier. Vasogenic edema is usually the result of PRES in the parieto-occipital white matter, frontal and temporal lobes, and posterior fossa. It is unclear, therefore, why the posterior region of the brain is overly involved (1, 2). The posterior half is assumed to be more involved because of inadequate autoregulation brought on by the region's predominant vascular circulation as a result of the absence of sympathetic input. Additional theories of PRES have linked immunosuppressive therapy, particularly cyclosporine, excessive medication dosages, aluminum overload, hypomagnesemia, and hypercholesterolemia. The release of endothelin, prostacyclin, and thromboxane A2, which directly cause damage to vascular endothelial cells, are other potential pathways (4).

PRES is clinically seen with nonspecific symptoms that appear acutely within a few hours or days. Approximately 28–94% of the cases that develop encephalopathy include confusion, dizziness, and in 39%, decreased visual accuracy, diplopia, visual field disorders, cortical blindness, color

blindness, Visual symptoms such as vision abnormalities and visual hallucinations, epileptic seizures affecting 74–87% of the cases and developing within 24-48 hours, and widespread headaches are observed in approximately half of the patients. Many acute neurological conditions are included in the differential diagnosis of PRES. The key to diagnosis is high suspicion; communication between clinicians and radiologists is important (6).

Confirming the diagnosis of PRES and assessing its severity can be aided by a brain MRI scan. High-resolution CT is therefore recommended, particularly in the posterior fossa structures. However, the initial method of acute imaging is CT scanning, which is also used to diagnose PRESS. Symmetric hemisphere vasogenic edema affecting the subcortical white matter and frequently extending to the overlaying region is a characteristic seen on CT and MRI scans (6). However, unlike in the literature, it is stated that imaging methods are less sensitive than computed tomography (CT) MRI in the diagnosis of PRES, and T2/FLAIR sections are more sensitive than routine MRI sequences in detecting subcortical and cortical lesions. Similarly, Shaikh et al. stated in their study that 44% of PRES cases were diagnosed with MRI in cases with normal CT (4). In these patients, an MRI is initially useful in ruling out conditions such as cerebral hemorrhage or infarction; nevertheless, an MRI is required to confirm the diagnosis (7). The cerebral spinal fluid investigation is not required until MRI results are specific for diagnosis, and lumbar puncture should be avoided when findings indicate increased intracranial pressure (6). In the current case, the patient had simultaneous cranial CT and brain diffusion MRI scans. Although the tomography was regarded as normal, a conclusive diagnosis was confirmed using an MRI.

The treatment guidelines for PRES are primarily based on consensus, and there have been no randomized trials comparing various treatment modalities. To remove or counteract the triggering element, early identification and therapy are crucial (6). The goals of treatment for PRES syndrome are symptomatic and center around controlling or removing the underlying cause. Because plasma exchange removes systemic inflammatory mediators, it may be helpful in PRES (8). In individuals with acute hypertension, it's critical to treat the patient with IV fluids, address electrolyte imbalances, and reduce blood pressure. The ideal range for mean arterial pressure is 105–125 mmHg. First-line antihypertensive therapy medications include nimodipine, nicardipine (5–15 mg/hour), and labetalol (2–3 mg/minute). Second-line medicines include sodium nitroprusside, hydralazine, and diazoxide. Since nitroglycerin may exacerbate cerebral edema, it is not advised for PRES patients. Early delivery is crucial for people who are pregnant. Benzodiazepines, sodium valproate, levetiracetam, or phenytoin can be administered as antiepileptics in situations of status epilepsy (6, 9). In the case presented, IV fluid therapy and electrolyte therapy were started early in the patient's life.

About 75–90% of PRES cases recover completely without sequelae. Life-threatening side effects, such as hemorrhage or infarction, could, however, sporadically occur. In extreme circumstances, patients would require an intensive care unit, which could result in fatalities or long-term impairments. Three to six percent is said to be the death rate. Preventing brain damage and death by removing the etiological cause with an early diagnosis is the most crucial aspect of treating PRES. Retrospective investigations have shown that 4% of patients experience PRES recurrence, with autoimmune diseases, sickle cell crises, hypertensive episodes, and renal failure disorder being the most common causes (6,9,10).

In conclusion, PRES is a neurological disease characterized by headaches, visual disturbances, changes in consciousness, and epileptic seizures. To confirm the diagnosis in clinically suspected instances, T2-WI and FLAIR MRI sequences should be evaluated. Despite being a benign disease, there is a chance that postponing diagnosis and treatment could result in irreversible brain damage. Even though most therapies are symptomatic, it is important to begin treating the etiological cause as soon as possible

Ethical Statement

None.

Conflict of interest

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Authors' contributions

Concept: E.K.Ç, H.S., Design: E.K.Ç, H.S., Data Collection or Processing: E.K.Ç, H.S., Analysis or Interpretation: E.K.Ç, H.S., Literature Search: E.K.Ç, H.S., Writing: E.K.Ç, H.S.

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Pediatric difficult airway management: Key strategies and case insights

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Abstract

Pediatric difficult airway management poses significant challenges due to unique anatomical and physiological features, particularly in neonates and infants. We reported the case of a 3-month-old, 5200-gram male infant with dysmorphic features, including anencephaly, macrocephaly, microphthalmia, micrognathia, and nasal anomaly, who underwent ventriculoperitoneal shunt surgery. Following preoxygenation and induction with inhalation anesthesia and 4 mg/kg IV propofol, successful intubation was achieved using video laryngoscopy with a size 3.0 endotracheal tube. The surgery was completed without complications, and the patient was safely extubated in the intensive care unit. This case underscores the importance of individualized strategies and proficiency with video laryngoscopy for the safe and effective management of pediatric difficult airways.

Keywords: pediatric difficult airway, airway management, video laryngoscopy

1. Introduction

Pediatric difficult airway is a critical clinical condition with the potential for severe complications such as cardiac arrest and brain death. Although its incidence is lower in pediatric patients than adults, it is higher in neonates and children under one year. The incidence of difficult intubation is reported as 0.24–4.7% in infants and 0.07–0.7% in older children (1,2). Pediatric anatomical and physiological differences complicate airway management. Risks such as respiratory depression, hypoxia, and bradycardia are amplified due to an immature nervous system, high metabolic rate, and heightened parasympathetic activity. Anatomical factors, including a prominent head and neck, high larynx, narrow cricoid cartilage, and U-shaped epiglottis, further increase challenges (3). Syndromic or dysmorphic features, obesity, and oromaxillofacial surgeries are additional risk factors for a difficult airway (2).

2. Case Presentation

We presented the case of a 3-month-old, 5200-gram, full-term male infant with multiple dysmorphic features, including anencephaly, macrocephaly, microphthalmia, micrognathia, and nasal anomaly, scheduled for ventriculoperitoneal shunt placement. Preoperative laboratory evaluations were within normal limits; however, nasal endoscopy revealed significant nasal obstruction. Upon arrival in the operating room, standard American Society of Anesthesiologists (ASA) monitoring was applied, including electrocardiography, non-invasive blood pressure, and pulse oximetry. Preoxygenation with 100% oxygen was initiated Anesthesia induction was initiated with sevoflurane due to the unavailability of IV access. Following successful IV cannulation with a 24G scalp vein catheter, 4 mg/kg IV propofol was administered, followed by 0.6 mg/kg IV rocuronium for neuromuscular blockade. Tracheal intubation was successfully performed on the first attempt using video laryngoscopy with a 3.0-mm endotracheal tube, and correct placement was confirmed by capnography. Bilateral breath sounds were confirmed on auscultation, and the tube was secured at 9 cm at the lip. The patient was mechanically ventilated with appropriate parameters to ensure normocapnia and adequate oxygenation. The surgical procedure was uneventful. After the completion of surgery and appropriate preparation, the patient was safely extubated in the intensive care unit.

Considering the patient's primary neurogenic etiology, a neurology consultation was requested. The electromyography was normal. Cranial contrast-enhanced magnetic resonance imaging (MRI) showed meningeal thickening and contrast enhancement (Fig.1), no abnormalities were observed in the spine on MRI. Because of the MRI findings, his cerebrospinal fluid was examined for confirmation of the diagnosis. The lumbar puncture findings were consistent with aseptic meningitis, and VZV DNA was positive by polymerase chain reaction (PCR) assay. While investigating the causes of the virus reactivation, the anti-HIV antibody test was reported positive. Subsequently, the HIV RNA test also came back positive, confirming the diagnosis of HIV.

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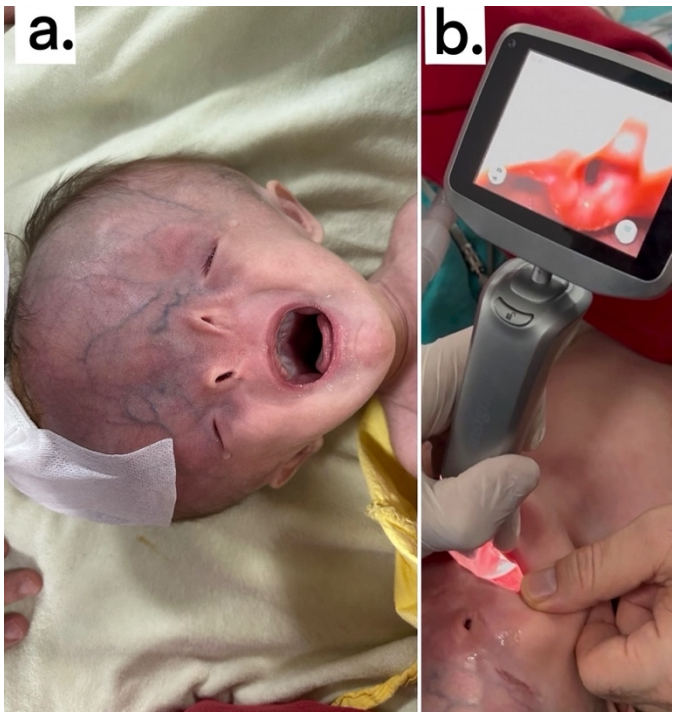

Fig. 1. (a) A 3-month-old male infant with dysmorphic features, including anencephaly, macrocephaly, microphthalmia, micrognathia, and nasal anomaly, presenting for ventriculoperitoneal shunt surgery. **(b)** Successful intubation performed using video laryngoscopy (VL) with a size 3.0 endotracheal tube while maintaining spontaneous respiration, demonstrating the view of the glottis on the VL screen

The final diagnosis was meningitis-retention syndrome (MRS). After the diagnosis, acyclovir 750 mg x 3 / day for the VZV and a combination of nucleozide/nucleotide reverse transcriptase inhibitors and integrase strand transfer inhibitors for the HIV were promptly started. Two weeks after the treatment, the patient failed to trial of void and required clean intermittent catheterization. The urinary stream was good nine weeks after the treatment, with 20 cc post-void residual urine.

3. Discussion

Key considerations for difficult airway management include preoxygenation, which extends safe apnea time. Apneic oxygenation techniques should become routine, particularly in infants, who desaturate more rapidly (4). In pediatric patients, general anesthesia is often required, with a preference for maintaining spontaneous respiration. However, laryngospasm is a notable risk during light anesthesia. Although sugammadex permits safe muscle relaxant use, its role in cannot-intubate, cannot-ventilate scenarios remains unclear (4). Difficult mask ventilation is rare in pediatric patients (2.8%-6.6%), and mask ventilation is generally effective. When this fails, supraglottic airway devices can be used (2,4,5). In anticipated difficult airways, VL is recommended as the first-choice tool. In unanticipated difficult airway scenarios, VL should be used after the failure of direct laryngoscopy (4). Fiberoptic bronchoscopy, while effective in adults, poses challenges in pediatric cases due to equipment size and sensitivity to secretions, limiting its applicability (5). The complex airway anatomy necessitated advanced planning and the use of VL, which has been consistently supported in the literature as a superior technique for pediatric difficult airway management. Lingappan et al. (6) demonstrated that VL improves intubation success rates and reduces complications compared to direct laryngoscopy in neonates with challenging airways. Similarly, Park et al. (7) highlighted the efficacy of GlideScope® VL in increasing intubation success and minimizing airway trauma in pediatric patients with difficult intubations. In this case, VL enabled clear visualization of the glottis, allowing for successful intubation without complications. For more severe airway difficulties, Burjek et al. (8) emphasized the utility of combined techniques, such as supraglottic airway with fiberoptic intubation, which provides continuous ventilation and oxygenation during airway management. While such combinations enhance safety, they require additional time and personnel, making them less feasible in emergent scenarios. This case underscores the critical role of VL in managing pediatric patients with difficult airways. The successful outcome highlights the importance of individualized preparation, appropriate technique selection, and adherence to evidence-based practices to minimize complications and optimize patient safety. In patients with craniofacial dysmorphism, such as anencephaly and micrognathia, airway visualization may be significantly impaired due to anatomical distortion, further supporting the value of VL in these populations. Extubation is a critical phase in managing difficult pediatric airways. Low-weight patients (<10 kg) face a higher risk of failed extubation, with a reported failure rate of ~5%. Extubation should be conducted with an experienced team and appropriate equipment. Deep sedation during extubation is not advised; patients should be fully awake with restored spontaneous respiration (2,9). In conclusion, effective management of pediatric difficult airways requires individualized planning and a structured approach. This case demonstrates that video laryngoscopy can enable safe, first-attempt intubation in neonates with complex craniofacial anomalies. Individualized airway planning and early use of VL are essential in similar scenarios. Limiting attempts minimizes complications, and proficiency with equipment, techniques, and algorithms is essential for optimal outcomes.

Conflict of interest

The authors declared no conflict of interest.

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None to declare.

Authors' contributions

Concept: E.S.O., C.K., B.D., Design: E.S.O., C.K., B.D., Data Collection or Processing: E.S.O., C.K., B.D., Analysis or Interpretation: E.S.O., C.K., B.D., Literature Search: E.S.O., C.K., B.D., Writing: E.S.O., C.K., B.D.

Ethical Statement

This case report is not required ethical decision.

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2-MAIN TEXT:

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b) Review: Systematic review or meta-analysis on data from the literature and critical analysis of the present state of knowledge should be represented with appropriate headings.

c) Case Report: Introduction; Case Report; Discussion; informed consent; conflict of interest; references.

The main text should contain the following items:

Abstract: This should provide a concise description of the purpose of the report or summary of the review and should not exceed 300 words. Avoid abbreviations, unless they are used universally.

Keywords: Provide at least 4-6 keywords. Only use terms that are include in the Medical Subject Headings - MeSH (http://www.ncbi.nlm.nih.gov/mesh).

Introduction: The objectives of the research should be clearly stated in this section. Relevant background information and recent published studies should be described concisely, and be cited appropriately.

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Ethical statement: Research carried out on human participants must be in compliance with the Helsinki Declaration as revised in 2013. (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The written consent of each subject should be

taken. Therefore, for any research studies or evaluations involving human participants (including students, residents, fellows and faculty members), authors need to provide information regarding ethical approval for the research presented in the manuscript. A statement to this effect must be incorporated into the ‘‘Materials and Methods’’ section and should include the name of the approving committee (e.g., Institutional Review Board, Ethics Committee on Human Research, etc.), the name of the institution at which approval was granted and a reference number where appropriate. When experimental animals are used, the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), or with the animals for experimental procedures.

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Funding:

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Acknowledgments:

Author acknowledgments should be written in the third person ("The authors wish to thank...") and written permission should be obtained from all individuals who are listed in the Acknowledgments section of the manuscript.

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