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## THE EFFECT OF TRADITIONAL MAT EXERCISES VERSUS REFORMER PILATES AND HAMMOCK YOGA ON PAIN, ENDURANCE, BALANCE, DISABILITY, AND QUALITY OF LIFE IN INDIVIDUALS WHO HAD CHRONIC BACK PAIN

### KRONİK BEL AĞRISI OLAN BİREYLERDE GELENEKSEL MAT EGZERSİZLERİ İLE REFORMER PİLATES VE HAMAK YOGANIN AĞRI, DAYANIKLILIK, DENGE, ENGELLİK VE YAŞAM KALİTESİ ÜZERİNDEKİ ETKİSİ

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#### ABSTRACT

**Objective:** The aim of this study to examine the effects of traditional mat exercises, Reformer Pilates (RP) and Hammock Yoga (HY) approaches on pain, endurance, balance, disability, and quality of life of individuals who had Chronic low back pain (LBP).

**Method:** 60 individuals who had Chronic LBP were participated and randomly attended into 3 groups as RP group (n=20, mean age:31.85±8.89 years), HY group (n=20, mean age:29.90±6.70 years), and mat group (n=20, mean age:30.40±8.21 years). All exercise training was applied twice a week for 4 weeks (45 minutes). The pain was evaluated with VAS and McGill, endurance was evaluated with plank tests and single leg hip bridge test, static balance was assessed with standing on single leg, and dynamic balance was assessed with the Star Excursion Balance Test. The Oswestry Disability Questionnaire and World Health Organization Quality of Life (WHOQOL-Bref) were used for disability and quality of life measurements, respectively.

**Results:** Improvements were observed on a group basis in all evaluated parameters (p<0.05). In the mat group, the McGill score improved more than the HY group, and Oswestry and WHOQOL-Bref improved more than the HY and RP groups (p<0.05). The performance of standing on single leg improved in the HY group more than the mat group (p<0.05).

**Conclusion:** Traditional mat exercises can be preferred primarily to reduce disability and improve the quality of life in individuals with Chronic LBP in a short time. The HY can be added to the content of individual exercise programs for the development of balance.

**Key Words:** Low Back Pain, Pilates Training, Yoga, Disability, Quality of Life

#### ÖZ

**Amaç:** Bu çalışmanın amacı geleneksel mat egzersizleri, Reformer Pilates (RP) ve Hamak Yoganın (HY) yaklaşımlarının kronik bel ağrılı (KBA) bireylerin ağrı, dayanıklılık, denge, engellilik ve yaşam kalitesi üzerindeki etkilerini incelemektir.

**Yöntem:** KBA'sı olan 60 birey çalışmaya katıldı ve rastgele randomizasyon yöntemi ile RP grubu (n=20, ortalama yaş:31.85±8.89 yıl), HY grubu (n=20, ortalama yaş:29.90±6.70 yıl) ve mat grubu (n=20, ortalama yaş:30.40±8.21 yıl) olmak üzere 3 gruba ayrıldı. Tüm egzersiz eğitimleri 4 hafta boyunca haftada iki kez (45 dakika) uygulandı. Ağrı VAS ve McGill ile endurans plank testi ve tek bacak kalça köprüsü testi ile statik denge tek ayak üzerinde durma testi ile dinamik denge yıldız denge testi ile değerlendirildi. Engellilik Oswestry Özürlülük Anketi ile yaşam kalitesi ise Dünya Sağlık Örgütü Yaşam Kalitesi (WHOQOL-Bref) ile değerlendirildi.

**Bulgular:** Değerlendirilen tüm parametrelerde gruplar bazında gelişme saptandı (p<0.05). Mat grubunda McGill skoru HY grubuna göre, Oswestry ve WHOQOL-Bref skorları ise HY ve RP gruplarına göre daha fazla iyileşti (p<0.05). Tek ayak üzerinde durma performansı HY grubunda mat grubuna göre daha fazla gelişti (p<0.05).

**Sonuç:** Geleneksel mat egzersizleri KBA'sı olan bireylerin engelliliğini azaltmak ve yaşam kalitesini kısa sürede artırmak amacıyla öncelikle tercih edilebilir. Denge gelişimi için bireysel egzersiz programlarının içeriğine HY eklenebilir.

**Anahtar Kelimeler:** Bel Ağrısı, Pilates Eğitimi, Yoga, Engellilik, Yaşam Kalitesi

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## INTRODUCTION

Low back pain (LBP) is one of the leading musculoskeletal problems worldwide impacting healthcare system and causing socio-economic burden [1]. It can affect people of all ages in the community, but it is common in individuals between the fourth and fifth decades of life [2,3]. Low back pain is divided into 3 subclasses according to the duration of the symptoms as Acute (lasting only a few weeks), Subacute (lasting about 6 to 12 weeks), and chronic (lasting more than 12 weeks) [4,5]. Clinicians cannot make a specific diagnosis in approximately 90% of chronic LBP cases, and for this reason, it is classified as non-specific chronic LBP [6]. Approximately 33% of individuals who have LBP say that they face permanent pain and activity restriction after one year [7]. Many people who have this disability are more limited in daily living activities such as walking, running, and bending over compared to healthy people [1]. In addition to the unpleasant feeling, chronic pain also affects the quality of life, cognitive and emotional state of the individual [8]. When the etiology of chronic LBP is examined, it is seen that it is not only a mechanical problem, but physical and psychosocial factors also play major roles [1,8,9].

Traditional mat exercises (ground-based strength and stretching exercises) were effective in the management of chronic LBP as an extremely valuable approach to preventing movement limitation, controlling existing pain, and regaining motor functions [10]. Reformer Pilates is another valuable exercise approach for the treatment of LBP. The only difference from Pilates is that it is used as an auxiliary tool. Reformer Pilates consists of a sliding platform working with the help of a pulley system specific to the use, allowing the individual to apply certain resistances, and providing the opportunity to exercise sitting, standing, or lying down. Pilates activates the lumbopelvic muscles, reduce the load on the spine and supports functional movement as an effective factor in reducing pain [11]. Yoga is a mind-body exercise discipline that includes both physical and mental aspects of pain with core strengthening, flexibility, relaxation, and breathing modalities [12,13]. The Hammock Yoga, on the other hand, is performed with a silk hammock, which is an auxiliary equipment just like Reformer Pilates. The most distinctive characteristic that makes Hammock Yoga different from other exercises is the spine traction provided by the upside-down posture, and the smooth and shiny silk hammock allows strengthening exercises [14]. The Hammock Yoga aims to reduce the load on the spine by upside-down postures that defy the laws of physics.

It was shown that Pilates exercises are more effective than other conservative modalities (e.g., resting, thermal agents such as ice-heat, analgesics, and Nonsteroidal Anti-Inflammatory Drugs) used in the treatment of chronic LBP [15]. It was found that yoga exercise is quite effective for individuals with chronic LBP who do not engage in any other exercises [16]. In another study, it was proven that yoga exercise reduces pain, the need for analgesics, and disability, and also increases the spinal mobility [17]. When the literature was reviewed, no study was detected examining the effectiveness of Hammock Yoga and Reformer Pilates in people who have chronic LBP and compares it with traditional mat exercises. The present study was conducted to examine the effects of traditional mat exercises, Reformer Pilates, and Hammock Yoga on pain, endurance, balance, disability, and quality of life in individuals with chronic LBP.

## METHOD

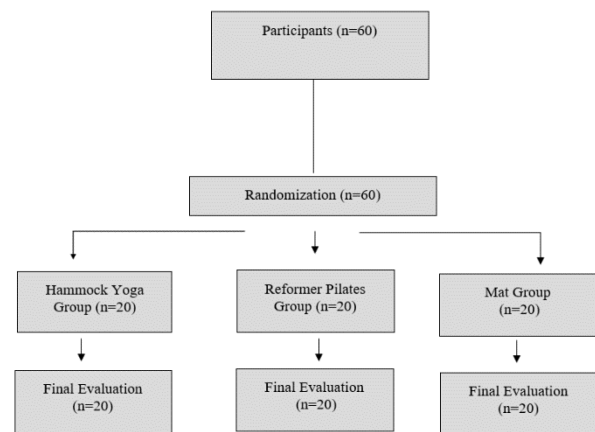
### Study Design and Participants

This parallel, 3-group, randomised control trial design was conducted in 60 patients aged 25-60 years with persistent low back pain in the previous 12 months. The study was carried out in a private health clinic in Gaziantep between April 2022 and June 2022. Participants with a low back pain intensity of 40 mm or worse in the previous 6 months as demonstrated by VAS (0-100 mm) were included in the study.

The diagnosis of chronic LBP was made by a physical medicine and rehabilitation specialist with 13 year of experience in the clinical assessments, laboratory testing and radiological screening. Patients underwent a detailed assessment that include motor and sensory functions, special diagnostic testing. A suspicion occurred base on clinical assessment and anamnesia, the blood tests and EMG were used for clarity.

Participants with a history of surgery in the lumbar region in the last 1 year, inflammatory arthritis, axial spondyloarthropathies, radiculopathy or polyneuropathy, malignancy, performed regular physical activity, systemic or physiological disorders, and those who were using steroids and anti-inflammatory drugs in the physiotherapy program were excluded from the study.

Participants were recruited in Gaziantep through direct referral from primary care clinicians, social media and advertisements. Patients meeting the inclusion criteria were divided into 3 groups (n=20 Mat, n=20 Reformer Pilates, n=20 Hammock Yoga) using a closed envelope randomisation method. The same clinician repeated the baseline assessment and the final assessment after 8 sessions (4 weeks). Only pain severity (VAS) was assessed at baseline, and in the 1st, 2nd, 3rd, and 4th weeks (total of 5-time intervals). No one dropped out of any group while studying (Figure 1).



**Figure 1.** Study flow chart

### Assessments

The sociodemographic and physical characteristics of the participants (i.e., ages, heights, body weights, pain complaints, and physical activity levels) were recorded before the study.

### Pain

McGill Short Form was used at the beginning and end of the study to evaluate the qualitative characteristics of pain. The language validity of the McGill Pain Scale was conducted by Yakut et al. [18]. The scale helps to learn about the sensory, emotional, and intensity component of pain. Fifteen items in the scale help define pain (11 sensory descriptors and 4 emotional descriptors). The participants were asked to rate their pain as 0=none, 1=less, 2=moderate, and 3=extreme, according to the intensity level. The total score was obtained by summing the scores given [18].

A Visual Analog Scale (VAS, 0-100mm) was used to evaluate pain intensity 5 times (at the beginning, weekly (in the first, second, and third weeks), and at the end of the study).

### Endurance

The endurance of the trunk muscles was evaluated with Plank Tests and Single Leg Bridge Tests. Oral information about the tests was given to the individuals and a demonstration was made by the physiotherapist before the test.

## Plank Tests

The prone plank test position was initiated on the prone bridge over the forearm and toes and the individuals were instructed to position their elbows just below their shoulders with their fingers reaching forward. Feedback was given to keep the spinal region in a neutral position so that they were in a proper alignment from head to heels. Once the correct position was taken, the physiotherapist started the stopwatch, and the duration they could stand until the position was broken was recorded [19].

The Lateral Plank Test was used bilaterally on the right and left sides. The participants were asked for a side-lying position, stand on forearms with arms perpendicular to the ground. Then, wanted elbows flexed at 90o, put the other hand on the waist, extending lower extremities with both feet on top of each other. When the individuals took this position, the timer was started and they were asked to maintain this position as much as possible, if they could not, the test was terminated and the time was recorded [19].

## Single Leg Bridge Test

The participants were asked to go to the bridge position with their supine hands-free at their sides and hips in the air, and while they were in this position, they were asked to keep the hip and knee flexion of the tested side and take the other leg into the air with the sole on the ground, and maintain the neutral position of the pelvis. When the test position was taken, the physiotherapist initiated the stopwatch and recorded the time until the participant broke the pelvis position [20].

## Balance

### Static Balance

Before starting the test, the physiotherapist knelt behind the participant and helped the participants to find the neutral pelvis position by placing their hands on the iliac crest. The participants were then asked to pull their knees towards the abdomen and maintain this position while keeping their hands on their waists without disturbing the neutral pelvis [21]. The time that passed until the position was broken was calculated and this test was repeated three times in total for both the right and left legs, and the maximum time was recorded in seconds.

### Dynamic Balance

Developed by Gray in 1995 to evaluate dynamic balance, the Star Excursion Balance Test was used in the evaluation of dynamic balance. A total of 8 lines (anterolateral, anterior, anteromedial, medial, posteromedial, posterior, posterolateral, and lateral) of 1m length were drawn on a flat surface at 45o their centers converging. The individuals were then asked to reach the farthest point possible in each line with the tip of the other foot and the foot to be tested in the middle of the star [22]. A resting period of 5 seconds was given after each stretch. If the balance was disturbed while reaching, the participants could not touch the lines, or gave full weight with their feet, the attempt was rejected and repeated. The distances that the individuals could reach and touch were recorded, and the test was repeated 3 times to record the maximum score.

## Disability

Disability (e.g., walking, sitting, standing, pain intensity, and sleeping) were evaluated with the Oswestry Disability Index, which consisted of 10 sections. A total score was obtained by scoring a Likert-type design ("0" - "5" in each section. An increase in the score indicated an increased disability [23].

## Quality of Life

The World Health Organization Quality of Life-Bref (WHOQOL-Bref) Questionnaire was used to evaluate the quality of life. The validity and reliability study of it was conducted by Eser et al. The questionnaire has 5 sections (Psychological Health, Physical Health, General Health, Social Relations, and Environmental Health). The

total score was obtained for each question with the Likert-type scoring. The higher score was implied the higher quality of life [24].

## Exercise Training

An exercise program was created for the Reformer Pilates, Hammock Yoga, and Mat groups, 2 days a week, for 4 weeks, in a total of 8 sessions. While the exercises of the Reformer Pilates and Hammock Yoga groups were given as individual sessions by the physiotherapist, the Mat group exercises were applied as a home program.

The exercise sessions include 10-minute warm-up exercises, main exercise section (30 minutes each) and 5-minute cool-down exercises, respectively. All exercises were done as a set with 10 repetitions in the first week, 12 repetitions in the second week, and 15 repetitions in the last 2 weeks. The exercise intensity (the number of repetitions and the level of movement) was increased gradually. In choosing the exercise, the purpose was to work the transversus abdominis, multifidus, and gluteus muscles both eccentrically and concentrically, and to protect the spinal stabilization and create awareness during the movement. All exercises were performed with breathing coordination. Hammock Yoga and Reformer Exercises were explained by the physiotherapist in detail to understand the movement and starting position correctly, and the participants were then asked to perform the movements. In the Mat group, the exercises were visually given with detailed explanations on a piece of paper, and they were checked by telephone every week. The participants of Reformer Pilates and Hammock Yoga groups were also asked to wear sports clothes to move freely, and attention was paid to the ventilation of the exercise room.

The exercise program was planned for each group in the following order (Figure 2).

### Warm-up

- Hip flexor stretches
- Flexing hip adductors
- Hip extensor stretches
- Footwork series

### Force Series

- Squat
- Bridge exercise
- Abdominal series
- Plank
- Back extensors strengthening series
- Shoulder posture

### Cool-down

- Gastrosoleus stretching
- Flexing the hip extensors
- Piriformis stretching
- Yawning in a mermaid

## Ethical Approval

Ethics Committee Approval was obtained on 13.04.2022 with the number 2022/037 from Hasan Kalyoncu University Faculty of Health Sciences Non-Interventional Research Ethics Committee. Informed and signed consent forms were obtained from the volunteers who met the inclusion criteria of the study.

## Statistical Analysis

The minimum total number of participants needed for the study was calculated as 53 ( $\alpha=0.05$ ) to determine the expectation that there would



be a significant difference between three different groups at the large effect level ( $f=0.75$ ) with a power of 0.95. The G-Power Program version 3.9.1.7 was used in the power analysis.

Statistical analysis of the data was made with the SPSS version 23 program. Whether the data were normally distributed or not was tested with the Kolmogorov-Smirnov Test. The Kruskal Wallis Test was used for the comparison of the non-normally distributed data between the groups, and the ANOVA Test was used for normally distributed data. The Mann-Whitney U-Test was used to determine from which group the difference stemmed. The Two-Way ANOVA was used to evaluate the effects of exercise and time. The effect size ( $\eta^2$ ) was defined as small (0.2), medium (0.5), and large (0.8). The Bonferroni Forward Statistics were used to find the sources of the differences. Continuous variables were given as Mean±Standard Deviation and categorical variables as percentages and numbers, and  $p<0.05$  was taken as the statistical significance level.



**Figure 2.** Some example for bridge exercises from the Hammock Yoga (a), Mat (b) and Reformer Pilates (c) sessions

## RESULTS

Randomisation was carried out with 60 patients who were eligible for the study and no patients dropped out of the study. The attendance of the individuals was 100%. The mean age of the individuals was  $31.85\pm 8.89$  for the Reformer Pilates group,  $29.90\pm 6.70$  for the Hammock Yoga group, and  $30.40\pm 8.21$  for the Mat group, respectively. The physical characteristics of the individuals (i.e., age, weight, height, and BMI scores) are given in Table 1. The groups were similar in terms of age, weight, height, and BMI scores ( $p>0.05$ ).

The baseline pain score of the Mat group was found to be higher than the Hammock Yoga group. Also, the Oswestry Disability Questionnaire and the WHOQOL-Bref Questionnaire scores were higher than both groups ( $p<0.05$ ). Time, group, and group x time effects were observed in McGill and Oswestry scores ( $p<0.05$ ). McGill's pain score decreased more in the Mat group when compared to the Hammock group ( $p<0.05$ ). The disability score showed more improvement in the Mat group when compared to the other groups ( $p<0.05$ ). In terms of the WHOQOL-Bref scores, only time and group effects were found and the Mat group showed more improvement than the other two groups ( $p<0.05$ ). There was a time effect (pain decreased in all groups) in the pain intensity (VAS) measurements at 5 different times during the study ( $p<0.05$ ) but there was no group and group x time ( $p>0.05$ ) (Table 2).

In the Star Excursion Dynamic Balance Evaluation on the right leg and left leg before the treatment, the posteromedial direction value of the Mat group was higher than the Reformer group ( $p<0.05$ ). The time effect was observed in all balance parameters but the group effect was only present in the left single-foot balance test ( $p<0.05$ ). The group x time effect was detected in right-left single-foot balance measurements, right star test anterior-posterior-posterolateral-lateral directions and left anterior-anteromedial-posterior-lateral directions, it was significant only in left single-foot balance value in the Bonferroni advanced statistical analysis. Balance on a single left foot improved more in the Hammock group than in the Mat group ( $p<0.05$ ) (Table 3).

Only the right single-leg hip bridge score was different between the groups before the treatment in the endurance tests ( $p<0.05$ ). The right

single-leg hip bridge score of Mat group was better than the Hammock Yoga group ( $p<0.05$ ). Time effect was detected in all endurance parameters ( $p<0.05$ ). Group effect was not detected in any parameters ( $p>0.05$ ). While the group x time effect was detected in the prone plank and right single-leg hip bridge parameters, no difference was detected between the groups in the Bonferroni advanced statistical analysis ( $p>0.05$ ) (Table 4).

## DISCUSSION

The present study investigated the effectiveness of traditional mat exercises in people with chronic LBP despite Reformer Pilates and Hammock Yoga approaches. Traditional mat exercises were found to be more effective than Hammock Yoga and Reformer Pilates in reducing pain, disability and improving quality of life in the short term. Hammock Yoga training further improved the static balance.

In the literature, it was found that many researchers stated that weakened core muscles (especially M. Transversus Abdominis and M. Multifidus) cause low back pain because they cannot provide spinal stability [1]. Lee et al. showed that the frequency of low back pain may be caused by insufficient and imbalance of core muscle strength, uncontrolled neuromuscular structure, and biomechanics of the spine [25]. Although the causes of low back pain are often weakness in the core area, there are also studies reporting that there are other factors (age, sex, smoking, obesity, depression/anxiety) involved [26,27]. In a randomized controlled study, 3 different exercises were applied to 44 people who had low back pain (Lumbar Stabilization, Dynamic Strengthening, and Pilates). The pain, core strength, and disability scores improved in the three exercise groups during 3 weeks, but the improvement in the lumbar stabilization group was greater than in the other groups. It was argued that the reason for this was that the exercises chosen in the Pilates were dynamic, and that abdominal hollowing could provide more transverse abdominis and multifidus activation in the lumbar stabilization exercises [9]. In our study, Reformer Pilates and mat exercises may have activated the core more than Hammock Yoga, which may have had a different effect on pain reduction. In hammock yoga, it can be difficult to retract and hold abdomen in during gravitational movements. Also, we think that the our individuals might have developed anxiety or fear against various gravitational movements during Hammock Yoga. It may also be possible that Pilates Exercises and mat exercises consisted of more horizontal movements and reduced the pain by affecting the compressive and separating forces of the spinal region. It is already known that 4-week Pilates Training has significant effects in reducing pain in the long term [15].

There is no consensus in the literature on the reduction of disability. Reformer Pilates has been shown to reduce disability and pain in workers with chronic back pain [11]. However, Lim et al. reported that the Pilates were more effective for reducing pain when compared to alternative treatments, but it was ineffective for reducing disability [28]. Pereira et al. concluded that the Pilates did not reduce either pain or disability [29]. However, La Touche et al. found that the Pilates reduced pain and disability in patients with low back pain. [10]. Similarly, as in the Lim et al. study, we found that Reformer Pilates reduced pain but had no effect on disability and quality of life. Some methodological differences (population, timing and exercise principle, etc.) may explain our conflicting results with other studies. On the other hand, the improvement in pain and disability in the mat exercise group may have been due to the mat exercise being more familiar, increasing attendance for patients in this group.

The primary cause of low back pain is generally seen as insufficient endurance of the lumbar extensors and the abdominal muscles are neglected. But the most important thing is to train the lumbar and abdominal muscles together because it works together synchronously for stabilization and reduces the load on the spine. Trunk endurance training, which is used for patients with chronic back pain, has positive effects on the balance, pain, and flexibility [30].

**Table 1.** Descriptive variables for groups

Variables	Hammock Yoga (n=20)		Reformer Pilates (n=20)		Mat (n=20)		f	p
	X±SD	(Min-Max)	X±SD	(Min-Max)	X±SD	(Min-Max)		
Age (year)	29.90±6.70	(21-44)	31.85±8.89	(24-55)	30.40±8.21	(21-48)	0.322	0.726
BW (kg)	60.85±12.72	(45-102)	67.75±18.75	(48-130)	67.80±14.49	(44-100)	1.326	0.274
Height (cm)	164.00±6.49	(150-176)	166.15±10.85	(148-197)	165.25±6.74	(153-176)	0.341	0.713
BMI (kg/m <sup>2</sup> )	22.58±4.14	(15.9-35.3)	24.27±4.39	(18.3-33.5)	24.76±4.66	(17.5-32.3)	1.400	0.255

kg: Kilogram; cm: Centimeter; BW: Body Weight; BMI: Body Mass Index; \*p<0.05; One-way ANOVA test

**Table 2.** Comparison of pain, disability and quality of life

Variables	Hammock Yoga (n=20)		Reformer Pilates (n=20)		Mat (n=20)		ANOVA Effect Size (η <sup>2</sup> )			Pairwise comparison (Bonferroni) Groups
	Pre-test	Post-Test	Pre-test	Post-Test	Pre-test	Post-Test	Exercise	Time	Exercise-time	
McGill (score)	13.65±11.36	8.00±6.54	17.55±9.38	7.95±4.31	25.70±14.00 <sup>b</sup>	8.50±7.61	<b>0.29*</b>	<b>0.47**</b>	<b>0.15*</b>	H<M
Oswestry (score)	19.05±5.01	15.35±4.23	24.50±9.16	14.80±7.50	32.60±11.07 <sup>b,c</sup>	19.60±7.76	<b>0.28**</b>	<b>0.46**</b>	<b>0.14*</b>	H=P<M
WHOQOL-Bref (score)	92.50±13.23	96.80±10.79	90.30±12.73	96.85±9.42	79.25±13.72 <sup>b,c</sup>	91.15±13.79	<b>0.16*</b>	<b>0.20**</b>	0.04	H=P<M
<b>VAS (score)</b>										
Vas Pre-test	5.95±1.79		6.30±1.66		6.55±2.14					
Vas 1.week	4.40±1.79		4.60±1.57		5.05±2.33					
Vas 2.week	3.30±1.49		3.60±1.57		4.10±2.13		0.04	<b>0.90**</b>	0.05	NS
Vas 3.week	1.95±1.50		2.20±1.20		2.85±1.90					
Vas Post-test	1.25±1.29		1.20±1.01		1.65±1.53					

WHOQOL-Bref: World Health Organization Quality of Life scale; VAS: Visual Analog Scale; NS: Non-significant; \*p<0.05; \*\*p <0.01; Data are presented as mean±SD; Pre-test differences between groups (Mann-Whitney U test); <sup>a</sup>p<0.05 Hammock vs. Reformer; <sup>b</sup>p<0.05 Hammock vs. Mat; <sup>c</sup>p<0.05 Reformer vs. Mat.

**Table 3.** Comparison of static and dynamic balance

Variables	Hammock Yoga (n=20)		Reformer Pilates (n=20)		Mat (n=20)		ANOVA Effect Size (η <sup>2</sup> )			Pairwise comparison (Bonferroni) Groups
	Pre-test	Post-Test	Pre-test	Post-Test	Pre-test	Post-Test	Exer.	Time	Exer. -time	
SLS-Right (sec)	67.30±51.54	92.85±70.64	70.20±40.88	113.90±45.68	105.40±49.26 <sup>c</sup>	120.60±53.70	0.07	<b>0.43**</b>	<b>0.11*</b>	NS
SLS-Left (sec)	56.70±34.99	82.40±49.24	71.00±37.23	124.70±58.65	102.20±51.06 <sup>c</sup>	122.90±55.93	<b>0.13*</b>	<b>0.55**</b>	<b>0.19*</b>	H>M
<b>SEBT Test Right (cm)</b>										
Anterior	70.60±11.71	80.90±11.83	73.40±9.26	82.40±8.37	80.10±17.16	82.40±13.55	0.04	<b>0.42**</b>	<b>0.15**</b>	NS
Anteromedial	66.10±12.20	74.85±12.72	69.50±10.22	79.00±9.37	75.15±15.37	82.05±13.91	0.08	<b>0.50**</b>	0.02	NS
Medial	60.90±14.09	71.05±12.69	63.05±11.24	73.70±10.85	69.85±17.47	77.40±13.24	0.06	<b>0.46**</b>	0.02	NS
Posteromedial	58.95±12.37	69.15±13.26	60.75±11.07	74.00±12.51	69.05±15.80 <sup>e</sup>	75.15±11.37	0.07	<b>0.54**</b>	0.09	NS
Posterior	64.30±11.34	75.60±13.75	66.70±9.61	77.15±12.68	73.15±14.95	75.15±12.75	0.02	<b>0.40**</b>	<b>0.16**</b>	NS
Posterolateral	70.25±15.09	77.15±15.42	72.50±10.92	81.75±10.88	70.15±18.18	70.95±14.68	0.04	<b>0.23**</b>	<b>0.11*</b>	NS
Lateral	69.30±16.46	77.70±14.78	75.85±13.96	83.40±12.56	71.50±19.14	70.80±15.61	0.05	<b>0.19**</b>	<b>0.13*</b>	NS
Anterolateral	72.80±14.85	83.35±14.62	78.10±15.36	89.05±12.96	75.05±18.75	80.70±15.03	0.03	<b>0.50**</b>	0.07	NS
<b>SEBT Test Left (cm)</b>										
Anterior	71.85±12.14	83.20±11.97	76.05±10.64	87.25±10.78	78.70±15.06	82.95±13.77	0.02	<b>0.59**</b>	<b>0.16**</b>	NS
Anteromedial	70.05±14.61	82.65±13.64	80.30±11.81	90.10±9.93	77.30±14.67	82.05±12.80	0.09	<b>0.51**</b>	<b>0.12*</b>	NS
Medial	72.15±12.67	79.80±11.51	78.80±11.94	89.10±9.53	76.95±16.43	81.65±12.69	0.07	<b>0.46**</b>	0.07	NS
Posteromedial	68.75±11.82	77.65±11.55	74.15±11.71	85.65±10.40	75.35±16.04	80.25±12.59	0.06	<b>0.48**</b>	0.09	NS
Posterior	68.55±11.48	77.90±9.14	70.55±11.27	82.20±9.24	76.75±13.95	81.55±10.08	0.06	<b>0.51**</b>	<b>0.10*</b>	NS
Posterolateral	63.40±13.68	73.45±10.45	68.95±11.38	80.10±9.60	70.65±17.48	76.70±13.61	0.05	<b>0.45**</b>	0.05	NS
Lateral	59.75±14.14	70.30±11.69	61.60±12.94	75.50±13.17	68.75±18.22	73.55±11.34	0.03	<b>0.56**</b>	<b>0.16**</b>	NS
Anterolateral	66.80±13.30	78.05±11.80	71.35±13.00	84.85±12.87	75.05±15.99	86.45±26.83	0.05	<b>0.42**</b>	0.01	NS

SLS: Single leg standing; SEBT: Star excursion balance test; Sec: second; cm: Centimeter; Exer: Exercise; NS: non-significant; \*p<0.05; \*\*p<0.01; Data are presented as mean±SD; Pre-test differences between groups (Mann-Whitney U test); <sup>a</sup>p<0.05 Hammock vs. Reformer; <sup>b</sup>p<0.05 Hammock vs. Mat; <sup>c</sup>p<0.05 Reformer vs. Mat.

**Table 4.** Comparison of endurance

Variables	Hammock Yoga (n=20)		Reformer Pilates (n=20)		Mat (n=20)		ANOVA Effect Size ( $\eta^2$ )			Pairwise comparison (Bonferroni)
	Pre-test	Post-Test	Pre-test	Post-Test	Pre-test	Post-Test	Exer	Time	Exercise- time	Groups
<b>Forward Plank (sec)</b>	46.80±17.35	56.20±21.56	44.40±24.37	66.35±29.90	40.25±19.00	56.55±17.68	0.02	<b>0.55**</b>	<b>0.11*</b>	NS
<b>Right Lateral Plank (sec)</b>	18.70±7.60	24.50±12.91	17.45±11.40	25.85±12.14	16.60±11.12	20.80±11.58	0.02	<b>0.44**</b>	0.06	NS
<b>Left Lateral Plank (sec)</b>	18.55±10.35	24.05±13.61	23.95±13.70	32.60±15.15	17.75±13.84	23.30±12.14	0.07	<b>0.40**</b>	0.03	NS
<b>Right SLHB (sec)</b>	35.85±15.44	56.40±18.67	43.05±20.10	65.60±29.89	57.90±25.79 <sup>b</sup>	68.60±24.99	0.09	<b>0.68**</b>	<b>0.15*</b>	NS
<b>Left SLHB (sec)</b>	39.20±21.12	58.95±21.67	46.80±21.91	70.65±29.96	55.50±20.24	69.05±20.63	0.07	<b>0.60**</b>	0.07	NS

Sec: Second; Exer: Exercise; SLHB: Single Leg Hip Bridge; NS: non-significant; \* $p < 0.05$ ; \*\* $p < 0.01$ ; Data are presented as mean±SD; Pre-test differences between groups (Mann-Whitney U test); <sup>a</sup> $p < 0.05$  Hammock vs. Reformer; <sup>b</sup> $p < 0.05$  Hammock vs. Mat; <sup>c</sup> $p < 0.05$  Reformer vs. Mat.

In a randomized study, Mat Pilates and Reformer Pilates improved abdominal endurance similarly during 8 weeks [31]. Similarly, endurance improved in all our groups and they did not have superiority over each other, which may have occurred because the exercises were targeted similar muscle groups, and the exercise duration and volumes were the same in all groups.

In the present study, Hammock Yoga improved static balance more than traditional mat exercise programs. It may be the result of the increase in postural control because of the change of the gravitational center by starting most of the exercises horizontally from the ground and continuing vertically in Hammock Yoga and Reformer Pilates groups. In previous studies that investigated the effects of Pilates Exercises on balance, dynamic and static balance results increased when compared to the control group [32,33]. In healthy elderly women, the dynamic balance improved when compared to the control group, who did nothing in Pilates Exercise, which took 60 minutes in total, 3 days a week for 3 months [34]. These results were also demonstrated by weekly training in adult healthy individuals [35]. In another study, which was designed to monitor the progress of balance ability in patients with hemiplegia, exercise training with Pilates and a suspension apparatus, similar to hammocks used here and appealing to similar purposes. These exercise protocols were applied 3 days a week for 30 minutes, and at the end of 8 weeks, exercises with suspension apparatus in hemiplegic patients improved the balance ability [36]. In another study that aimed to draw attention to the effects of exercises with suspension apparatus, elite football players participated in a total of 16 sessions, 2 days a week, and it was reported that closed kinetic chain exercises with Hanger apparatus improved balance and reduced chronic back pain [31]. We did not understand in detail why static balance, unlike dynamic balance was improved in the Hammock Yoga than mat exercises, and could not analyze it fully. It may be assumed that Hammock Yoga exercises provide more upper extremity activation and that may increased static balance.

### Limitations

If modalities (e.g., EMG, etc.) could be used to evaluate transverse abdominis muscle activation objectively or evaluate the strength of the core muscles in the present study, our findings could be analyzed better. The mat exercises were followed with a home program, this could be occurred a bias. Some of the participants might have developed anxiety or kinesiophobia because all participants in the Hammock Yoga group experienced this type of exercise for the first time. In this respect, investigating the fear in the individuals while performing some movements that could be considered acrobatic so it could enable us to understand this.

### CONCLUSION

It may be more effective to direct and prefer traditional mat exercises that are already known by patients in reducing pain and disability and improving the quality of life in people who have chronic back pain. All three exercise training modalities can be used to develop core endurance, but Hammock Yoga can be considered a priority for developing balance.

**Ethical Approval:** 2022/037 Non-Interventional Research Ethics Committee of Hasan Kalyoncu University

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## FOOD INSECURITY STATUS AND RELATED FACTORS IN MINIMUM WAGE WORKERS ASGARİ ÜCRET ÇALIŞANLARINDA GIDA GÜVENCESİZLİĞİ DURUMU VE İLİŞKİLİ FAKTÖRLER

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### ABSTRACT

**Objective:** This study was conducted to determine the food insecurity and nutrition status of individuals working for minimum wage and to identify associated factors.

**Method:** The study was carried out with 189 voluntary females and 197 males working for the minimum wage in various workplaces in the Üsküdar district of İstanbul between February 01, 2022, and March 31, 2022. Descriptive characteristics, dietary habits and food consumption frequency with quantity were inquired and recorded for all participants. In addition, the Household Food Insecurity Access Scale was administered to individuals. All data were collected through face-to-face interviews. Energy and nutrient intakes were determined utilizing the BeBIS 8.2 program. The obtained data were analyzed using the IBM SPSS 22.0 software package. The significance level was accepted as  $p<0.05$ .

**Results:** According to the results, out of the total 386 participants, 52.1% were food secure, while 47.9% were not. The majority of food insecure individuals (45.4%) were university graduates. It was observed that the participants who were food secure, consumed more dairy products ( $p=0.040$ ), fruits and vegetables ( $p<0.01$ ) compared to food insecure individuals. In contrast, animal protein sources ( $p=0.010$ ), fats ( $p=0.001$ ), and the bread and cereal group ( $p<0.001$ ) were found to be consumed more by food insecure individuals. Significant associations were identified between food insecurity and age, additional income for nutrition, and smoking status. A unit increase in age was determined to decrease food insecurity by 0.52 units ( $p=0.015$ ).

**Conclusion:** Determining the food insecurity status of individuals working for minimum wages as well as addressing diseases related to nutrition will be a crucial step in preventing inadequate and imbalanced nutrition. Making healthy foods more affordable or increasing the overall living standard of individuals is necessary for the continuation of proper nutrition and health.

**Key Words:** Minimum Wage, Nutrition, Food Insecurity

### ÖZ

**Amaç:** Bu çalışma asgari ücretle çalışan bireylerin gıda güvencesizliği ve beslenme durumunun saptanması ve gıda güvencesizliği ile ilişkili faktörlerin belirlenmesi amacıyla yapıldı.

**Yöntem:** Çalışma 01 Şubat 2022-31 Mart 2022 tarihinde, İstanbul ili Üsküdar ilçesinde çeşitli iş yerlerinde asgari ücret karşılığında çalışan, gönüllü 189 kadın ve 197 erkek ile yürütüldü. Çalışmaya katılan tüm bireylerin tanımlayıcı özellikleri, beslenme alışkanlıkları ve besin tüketim sıklığı miktarı sorgulanıp kaydedildi. Ayrıca bireylerden Hanehalkı Gıda Güvencesizliği Erişim ölçeğini doldurmaları istendi. Tüm veriler yüz yüze toplandı. Enerji ve besin ögesi alımları BeBIS 8.2 programı ile belirlendi. Elde edilen verilerin analizinde IBM SPSS 22.0 paket programı kullanılarak anlamlılık düzeyi  $p<0.05$  kabul edildi.

**Bulgular:** Çalışmaya katılan toplam 386 asgari ücret çalışanın %52.1'inin gıda güvenceli, %47.9'unun gıda güvencesiz olduğu belirlendi. Gıda güvencesiz bireylerin çoğunluğu (%45.4) üniversite mezunuydu. Gıda güvenceli asgari ücret çalışanlarının süt grubu ( $p=0.040$ ) ile sebze ve meyve grubu ( $p<0.01$ ) besinleri gıda güvencesiz bireylerden daha fazla tükettiği; hayvansal protein kaynakları ( $p=0.010$ ) ile yağ ( $p=0.001$ ), ekmek ve tahıl grubunu ( $p<0.001$ ) ise gıda güvencesiz bireylerden daha fazla tükettiği saptandı. Gıda güvencesizliği ile yaş, ek beslenme geliri ve sigara içme durumu arasında anlamlı ilişki olduğu bulundu. Bir birimlik yaş artışının gıda güvencesizliğini 0.52 birim azalttığı belirlendi ( $p=0.015$ ).

**Sonuç:** Asgari ücretle çalışan bireylerin gıda güvencesizliği durumunun belirlenmesi yetersiz ve dengersiz beslenmenin ve beslenmeye bağlı oluşabilecek hastalıkların önüne geçilmesinde önemli bir adım olacaktır. Sağlıklı besinlerin daha uygun fiyatlı hale getirilmesi veya refah düzeyinin yükselmesi doğru beslenme ve sağlığın devamı için gereklidir.

**Anahtar Kelimeler:** Asgari Ücret, Beslenme, Gıda Güvencesizliği

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## INTRODUCTION

Food security refers to the physical, social, and economic access to sufficient, safe, and nutritious food by individuals, that meets their food preferences and dietary needs, enabling them to lead an active and healthy life [1]. Conversely, the absence of this condition is defined as food insecurity [1]. The prevalence of moderate or severe food insecurity, predominantly affecting women and rural dwellers worldwide, is reported to be 29.6% (2.4 billion people) [2]. While this figure has remained stable over the past two years, it has been observed that an additional 291 million people are experiencing food insecurity compared to the period before the COVID-19 pandemic [2]. Studies on food insecurity indicate its association with adverse health outcomes and conditions such as increased prevalence of obesity and compromised dietary quality [3-6].

Food insecurity is closely linked to socioeconomic status, with individuals of lower socioeconomic status having a higher likelihood of experiencing food insecurity [7]. In a study where the majority of participants had an annual household income of less than \$20,000, it was found that approximately 75% of them experienced low or very low food insecurity [8]. Another study revealed that households with a monthly income of \$140.86 were ten times more likely to experience food insecurity compared to those with a monthly income of \$234.77 [9].

Minimum wage is defined as the wage paid for a day's work, intended to cover the essential needs of an employee such as food, housing, clothing, health, transportation, and culture, based on prevailing prices [10]. However, issues such as the minimum wage definition focusing solely on the needs of the worker rather than including the needs of dependents, regional variations in the cost of living, high taxation on minimum wage, and increases in wages lagging behind inflation, pose numerous social and economic challenges for minimum wage workers [10].

In a study conducted in Scotland by Newell et al. (2014), it was demonstrated that the minimum wage was insufficient to meet the basic needs of a four-person household or a single mother with three children, requiring these households to compromise on their diets to cover other expenses [11]. A study examining the relationship between wage-setting policies and food insecurity across 139 countries found that the likelihood of moderate or severe food insecurity was 31% in countries without or with low minimum wages, 29% in countries with moderate minimum wages, and 25% in countries with high minimum wages [12]. Furthermore, research conducted on Canadian families with children found that a one dollar increase in the minimum wage was associated with a 0.8% to 1% decrease in the risk of experiencing food insecurity [13].

In Turkey, the proportion of individuals working for minimum wage is 50% in the industrial sector, 53.9% in the construction sector, and 39.1% in the service sector [14]. It is anticipated that earning below the amount required for a four-person household's kitchen expenses (the hunger threshold) would hinder access to adequate nutrition and result in a decline in dietary quality. This study aims to determine the food insecurity status of individuals working for minimum wage and to correlate it with their nutritional status.

## METHOD

### Study Design

This study is descriptive and cross-sectional in nature.

### Population and Sample of the Study

The data for this study were collected between February 01, 2022, and March 31, 2022. The study was conducted with a total of 386 voluntary individuals, including 189 females and 197 males, employed at minimum wages, in various workplaces such as construction sites, private healthcare centers, cafes, markets, hair salons, etc., selected through random sampling method in the Üsküdar district of Istanbul

province. The sample size of the study was calculated with a 95% confidence interval, 5% margin of error, and a prevalence value of 50% in the unknown population (n=385). Participants who voluntarily declared that they were earning minimum wages were randomly selected at their workplaces and times.

### Data Collection Instruments

The study data were collected through face-to-face interviews conducted at the participants' workplaces using a survey form, a Food Frequency Questionnaire, and the Household Food Insecurity Access Scale (HFIAS).

*Survey Form:* This form queried participants' demographic information and dietary habits, as well as their self-reported height and weight.

*Food Frequency Questionnaire:* This questionnaire inquired about the frequency and quantity of food consumption over the past 30 days. It included a total of 103 food items categorized under headings such as dairy and dairy products, meat-eggs-legumes, bread and cereals, vegetables and fruits, fats-sugars-sweets, beverages, and other items. Frequencies were categorized as "every meal", "every day", "once a week", "2-3 times a week", "3-4 times a week", "5-6 times a week", "once in fifteen days", "once a month", and "never". The food consumption frequency form was entered into the Nutrition Information System (BeBIS 18.2) program. Total daily energy and nutrient intakes were obtained. Energy and nutrient intakes were determined utilizing the BeBIS 8.2 program.

*Household Food Insecurity Access Scale (HFIAS):* This is an 18-item scale designed to measure the degree of food insecurity over the past four weeks (30 days). It was developed by the Food and Nutrition Technical Assistance (FANTA) project and partners to distinguish food-secure households from food-insecure ones [15]. The Turkish validity and reliability study was conducted by Bor (2018), with a Cronbach's Alpha value of 0.876 [16], indicating good reliability of the questionnaire. The items of the questionnaire inquired about experiencing food insecurity situations by participants (no=0 points, yes=1 point) through nine questions, each of which also asked how often this situation occurred in the past four weeks (rarely=1 point, sometimes=2 points, often=3 points). Individuals were classified into four categories based on the degree of food insecurity: food secure, mildly food insecure, moderately food insecure, and severely food insecure. The total score of the scale is determined by summing the scores of the responses given to the questions, and an increase in the total score indicated an increase in the severity of food insecurity [15]. The highest score that could be obtained from the scale was 27 and the lowest score was 0.

### Ethical Approval

The ethical approval of the study was obtained by the Health Sciences University Hamidiye Scientific Research Ethics Committee with the decision number 21/761 on 31.12.2021. The study was conducted in accordance with the Declaration of Helsinki and Informed Voluntary Consent was obtained from all individuals participating in the study.

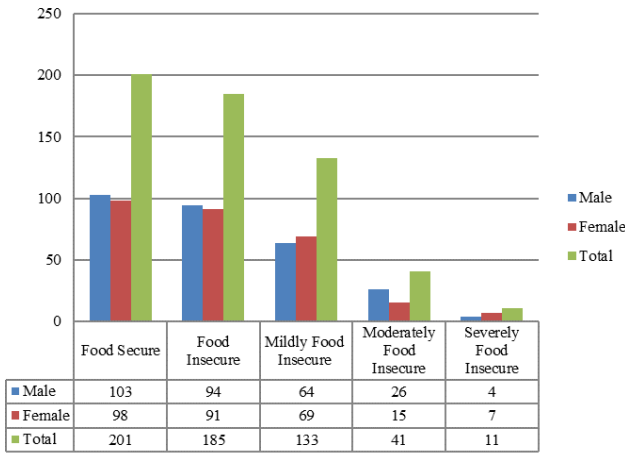
### Statistical Analysis

The statistical analyses of the data obtained from the research were conducted using SPSS 22.0 software. Descriptive statistical analyses (Mean, Standard Deviation, Median, Frequency, Ratio, Minimum, Maximum) were performed, and the distributions of the data were evaluated using the Shapiro-Wilk Test. For comparisons between two groups of quantitative data that did not follow a normal distribution, the Mann-Whitney test was utilized, while the Kruskal-Wallis test was employed for comparisons involving three or more groups. For quantitative data showing a normal distribution, the Student t-test was used for comparisons between two groups, and the Oneway ANOVA test was applied for comparisons involving three or more groups. To determine the relationship between qualitative data, the Chi-square test

was utilized, and logistic regression analysis was employed to identify independent variables affecting the dependent variable. Statistical significance was evaluated at the  $p < 0.05$  level for all analyses.

**RESULTS**

Of the individuals participating in the study, 49% (n=189) were females, 51% (n=197) males, with a mean age of  $30.45 \pm 9.09$  years. The mean Body Mass Index (BMI) was  $24.13 \pm 4.04$ . Out of the total 386 minimum wage workers in the study, 201 (52.1%) were food secure, while 185 (47.9%) were food insecure (Table 1). Among the food insecure, 71.9% were classified as mildly food insecure (Figure 1).



**Figure 1.** Food insecurity among minimum wage workers and distribution by level of food insecurity

Regarding the participants, 43.5% were university graduates, and 24.2% were married. Individual monthly expenditure on nutrition ranged mostly between 1000-1499 TL for the majority (42.2%) of individuals, and more than half (67.9%) received nutritional assistance outside their income. It was observed that 58.8% of individuals skipped at least one main meal, with 22.5% skipping meals due to economic reasons (Table 1).

**Table 1.** Descriptive characteristics of minimum wage workers

Variables	Total (n=386) n (%)	Food Secure (n=201) n (%) / $\bar{X} \pm SS$ (M')	Food Insecure (n=185) n (%) / $\bar{X} \pm SS$ (M')	P
<b>Gender</b>				
Female	189 (49.0)	98 (48.8)	91 (49.2)	*0.932
Male	197 (51.0)	103 (51.2)	94 (50.8)	
<b>Age</b>				
18-24	126 (32.6)	71 (35.3)	55 (29.7)	*0.663
25-34	161 (41.7)	82 (48.8)	79 (42.7)	
35-44	57 (14.8)	27 (13.4)	30 (16.2)	
45-60	42 (10.9)	21 (10.4)	21 (11.4)	
<b>Educational attainment</b>				
Primary school	30 (7.8)	15 (6.5)	15 (8.1)	*0.085
Primary education	31 (8.0)	23 (11.4)	8 (4.3)	
High school	157 (40.7)	79 (39.3)	78 (42.2)	
University	168 (43.5)	84 (41.8)	84 (45.4)	
<b>Marital status</b>				
Married	94 (24.2)	48 (23.9)	48 (25.9)	*0.403
Single	268 (68.9)	138 (68.7)	122 (65.9)	
Divorced	27 (6.9)	15 (7.5)	15 (8.1)	

<b>Chronic disease</b>				
Yes	59 (15.3)	23 (14.4)	30 (16.2)	*0.626
No	327 (84.7)	172 (85.6)	155 (83.8)	
<b>Smoking</b>				
Yes	144 (37.3)	86 (42.8)	58 (31.4)	*0.017*
No	191 (49.5)	96 (47.8)	95 (51.4)	
Sometimes	51 (13.2)	19 (9.5)	32 (17.3)	
<b>BMI (kg/m<sup>2</sup>)</b>				
Underweight (<18.5)	27 (7.0)	14 (7.0)	13 (7.0)	*0.997
Normal (18.5-24.9)	209 (54.1)	108 (53.7)	101 (54.6)	
Overweight (25.0-29.9)	122 (31.6)	64 (31.8)	58 (31.4)	
Obesity ( $\geq 30.0$ )	28 (7.3)	15 (7.5)	13 (7.0)	
<b>Housing status</b>				
Owned by	74 (19.2)	44 (21.9)	30 (16.2)	*0.016*
Belongs to a family member	157 (40.7)	68 (33.8)	89 (48.1)	
Tenant	155 (40.2)	89 (44.3)	66 (35.7)	
<b>Nutrition budget (Monthly wage spent on food/individual)</b>				
<500 TL	28 (7.3)	18 (9.0)	10 (5.4)	*0.251
500-999 TL	96 (24.9)	54 (26.9)	42 (22.7)	
1000-1499 TL	163 (42.2)	84 (41.8)	79 (42.7)	
$\geq 1500$ TL	99 (25.6)	45 (22.4)	54 (29.2)	
<b>Do you have any other source of free food for your diet other than your income (Aid, vineyard-garden, etc.)?</b>				
Yes	264 (67.9)	161 (80.1)	100 (54.1)	*a<0.001**
No	125 (32.1)	40 (19.9)	85 (45.6)	
<b>Skipping main meals (<math>\geq 1</math>) (n=227)</b>				
Economic reasons	51 (22.5)	19 (15.3)	32 (31.1)	*a0.005*
Not economically motivated	176 (77.5)	105 (84.7)	71 (68.9)	
<b>Is there a catering service at work?</b>				
Yes	208 (53.9)	111 (55.2)	97 (52.4)	*a0.583
No	178 (46.1)	90 (44.8)	88 (47.6)	
<b>Age</b>	30.45 $\pm$ 9.09	29.91 $\pm$ 9.38 (183.35)	31.03 $\pm$ 8.75 (204.53)	*b0.062
<b>BMI (kg/m<sup>2</sup>)</b>	24.13 $\pm$ 4.04	24.26 $\pm$ 4.05 (197.34)	23.99 $\pm$ 4.02 (189.33)	*b0.481
<b>Number of main meals (per day)</b>	2.37 $\pm$ 0.57	2.32 $\pm$ 0.59 (186.15)	2.42 $\pm$ 0.55 (201.49)	*b0.124
<b>Food insecurity score</b>	3.15 $\pm$ 4.56	0.07 $\pm$ 0.25 (101.29)	6.50 $\pm$ 4.66 (293.68)	*b<0.001**

\*Mean Rank, <sup>a</sup>:Chi-square, <sup>b</sup>:Mann-Whitney U test, \* $p < 0.05$ , \*\* $p < 0.001$ .

Factors associated with food insecurity among the participants were examined (Table 2). According to binary logistic regression analysis, food insecurity was found to be higher among minimum wage workers under the age of 25 (18-24 years).

An increase of one unit in age was associated with a 0.52 unit decrease in food insecurity ( $p=0.015$ ). Furthermore, having additional nutritional income significantly reduced the likelihood of experiencing food insecurity ( $p < 0.001$ ), with an Odds Ratio (OR) of 0.291. Another variable found to be associated with food insecurity was smoking status. Non-smokers were 1.692 times more likely to experience food insecurity compared to smokers ( $p=0.025$ ). Factors associated with food insecurity were determined to be age, smoking status, and the presence of additional nutritional income.

**Table 2.** Factors associated with food insecurity among minimum wage workers

Variables	B	Wald	OR	p	%95 CI
Gender (Male)	0.069	0.081	1.071	0.776	0.67-1.72
Age ( $\geq 25$ ) †	-0.654	5.917	0.520	0.015*	0.31-0.89
Education (University) ‡	0.396	2.863	1.486	0.091	0.94-2.35
Marital status (Married)	0.096	0.135	1.101	0.713	0.67-1.84
Disease status (None)	-0.307	1.002	0.736	0.317	0.40-1.34
Housing status (Does not pay rent)	0.275	1.509	1.316	0.219	0.85-2.04
Smoking status (No)	0.526	5.038	1.692	0.025*	1.07-2.68
Supplementary nutrition income (Available)	-1.236	26.627	0.291	<0.001**	0.18-0.47
Food at work (Available)	0.365	2.565	1.441	0.109	0.92-2.25
BMI (kg/m <sup>2</sup> )		0.045		0.997	
Poor (<18.5)	0.122	0.042	1.130	0.837	0.35-3.62
Normal (18.5-24.9)	0.043	0.010	1.044	0.921	0.44-2.46
Overweight (25.0-29.9)	0.049	0.012	1.050	0.914	0.44-2.54
Constant	0.236	0.161	1.266	0.688	

B:Beta, OR:Odds Ratio, CI:Confidence Interval, BMI:Body Mass Index, Logistic regression analysis, \* $p < 0.05$ , \*\* $p < 0.001$ , † Age group was analyzed in two groups as under 25 years and 25 years and above. ‡ Education group was analyzed in two groups as high school and below and university graduates.

The comparison of food consumption by food secure and food insecure minimum wage workers, according to food groups, is presented in Table 3. It was observed that the consumption of plant protein sources and confectioneries did not differ significantly between food secure and insecure individuals. However, dairy products ( $p=0.040$ ) and fruits and vegetables ( $p < 0.01$ ) were consumed more by food secure individuals compared to food insecure ones. Conversely, animal protein sources ( $p=0.010$ ), fats ( $p=0.001$ ), and cereals and bread ( $p < 0.001$ ) were consumed more by food insecure individuals.

The portions of food groups consumed and the percentages of meeting the recommendations of the Turkish Dietary Guidelines (TÜBER), by food secure and food insecure individuals are presented in Table 4 and Figure 2. None of the individuals in the study met the recommended intake of dairy products according to TÜBER. There was no significant difference between food secure and insecure individuals, as far as percentage of meeting the recommended intake of dairy products was concerned ( $p=0.079$ ). However, food secure individuals met 19.5% of the recommended intake of fruits and vegetables, food insecure individuals met only 15.1%, and the difference between the groups was statistically significant ( $p < 0.001$ ). The meat consumption of both groups met the recommended intakes, in fact consumption was a little bit higher in food insecure individuals. However, the difference was not statistically significant ( $p=0.075$ ). The portions of cereals and bread consumed by food secure individuals was 116.3% of the recommended intake, while food insecure individuals was 157.6%, difference between them being statistically significant ( $p < 0.001$ ).

The average daily energy and nutrient intake and the extent to which recommended dietary allowances (RDA) energy and nutrient requirements were met according to food security status of individuals were examined and presented as an additional table (Supplementary Table A-B).

## DISCUSSION

Food insecurity is a global public health issue that predominantly affects low-income families [17]. If not detected early and addressed, it can lead to various health problems in the future [3-6]. In a study conducted in Turkey, the current status of food security was examined across four dimensions (availability, accessibility, utilization, and stability), revealing that food security could not be ensured across all

dimensions [18]. The fact that the minimum wage in Turkey is below the hunger threshold suggests that food insecurity is inevitable for families of four living on minimum wage [19]. In a study aimed at determining the food insecurity status among minimum wage workers and assessing their nutritional status, it was found that 52.1% ( $n=201$ ) of minimum wage workers were food secure, while 47.9% ( $n=185$ ) were food insecure.

In a study conducted with seasonal agricultural workers, who were part of the at-risk group for food insecurity due to their low-wage jobs, family size, income, and having children were identified as factors associated with food insecurity [20]. In this study, age, smoking status, and having additional nutritional income were found to be associated with food insecurity. The risk of experiencing food insecurity was 0.5 units lower among minimum wage workers under the age of 25 (18-24 years) compared to individuals over 25 years old. Another study found that the rate of individuals experiencing mildly and severely food insecurity was higher among those under 25 (19-25) years as compared to those in the 26-30 age group [21]. In this study, the prevalence of food insecurity among non-smoking participants was 1.7 times higher compared to smoking participants. These results contradict some other studies in the literature [22,23]. It is speculated that the high taxes on tobacco in Turkey may contribute to this inconsistency.

Food insecurity affects nutritional status of individuals. A study examining the relationship between the prevalence of food insecurity and food consumption found that the rate of food insecurity was 41% in households with individuals under the age of 18, compared to 26.4% in households where all individuals were over 18 [24] years of age. In the same study, it was observed that fruit and vegetable consumption in households experiencing food insecurity was lower compared to food-secure households. Similarly, in this study individuals experiencing food insecurity consumed fewer fruits and vegetables ( $p < 0.001$ ,  $Z=-3.684$ ) compared to food secure individuals (Table 3). It is known from previous studies that individuals experiencing food insecurity consume less fruits and vegetables (0.44 fewer portions of fruit and 0.43 fewer portions of vegetables per day) [25]. In a study conducted among mothers with children under the age of 18, it was found that mothers experiencing food insecurity consumed more bread and grains, eggs, sweets, and sugary beverages compared to food secure mothers, while consuming fewer nuts, dairy products, fruits, and alcoholic beverages [26]. In this study, the consumption of bread and grains ( $p < 0.001$ ,  $Z=-4.096$ ), fats ( $p=0.001$ ,  $Z=-3.335$ ), and confectioneries ( $p=0.313$ ,  $Z=-1.009$ ) was higher among food insecure individuals compared to food secure individuals (Table 3). This may be due to the fact that the cost per calorie of fruits and vegetables is higher than the cost per calorie of cereals, oils and sweets, as shown in another study [27]. Some studies have linked this to a higher prevalence of obesity and BMI in individuals experiencing food insecurity [28-29]. However, in contrast to these studies, our study did not find a statistically significant difference in BMI between food secure and food insecure minimum wage workers. It may be considered that the fact that the anthropometric measurements of individuals were not taken by researchers were self-reported by participants may have influenced this result.

Moderately food insecurity is associated with a decrease in diet quality, diversity, or desirability, while severely food insecurity is associated with disrupted eating patterns and decreased energy and nutrient intake [30]. In this study, it is thought that the higher energy intake among food insecure individuals may be due to the majority of individuals (94%) experiencing mildly or moderately food insecurity. Low-income individuals have been reported to have stated that the type and quality of food were affected rather than the quantity or frequency of consumption [31]. Hutchinson et al. (2022) did not find a statistically significant relationship between food insecurity and the proportion of energy derived from fat, carbohydrates, sugar, and saturated fat [32]. In this study, it was observed that the percentage of energy derived from carbohydrates was higher in food insecure individuals compared to food secure individuals, with no significant



difference in the percentage of energy derived from fat Supplementary Table A-B). Additionally, contrary to the study by Hutchinson et al. (2022), this study found that food insecure individuals consumed more fiber.

This may be due to the fact that Turkey is an agricultural country, where legumes are relatively cheaper than fruits and vegetables per calorie.

**Table 3.** Comparison of food security and precarious minimum wage workers according to their consumption of food groups

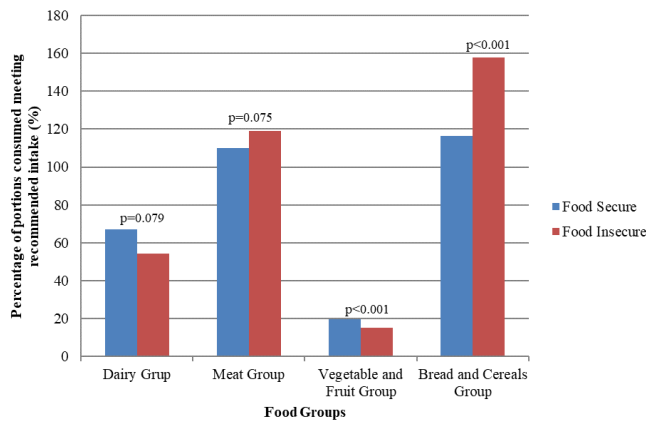
Food Group	Food Secure (n=201)			Food Insecure (n=185)			Z	p
	$\bar{X}\pm SS(M^f)$	Lower value	Upper value	$\bar{X}\pm SS(M^f)$	Lower value	Upper value		
Milk (g)	262.4±206.15 (204.68)	0.0	1618.0	213.2±145.86 (181.35)	0.0	1134.0	-2.052	0.040*
Meat (g)	216.6±126.00 (182.32)	6.0	702.0	234.4±121.12 (205.65)	28.0	733.0	-2.052	0.040*
Animal (g)	167.0±107.90 (179.55)	0.0	623.0	188.3±108.46 (208.65)	0.0	674.0	-2.560	0.010*
Vegetable (g)	49.6±44.72 (197.13)	0.0	283.0	46.1±42.61 (189.56)	0.0	234.0	-0.666	0.505
Vegetables and Fruit (g)	388.9±244.01 (213.57)	0.0	1500.0	301.5±1025.0 (171.69)	0.0	1025.0	-3.684	0.000**
Bread and Cereals (g)	374.8±186.32 (171.19)	22.0	991.0	472.7±1170.0 (217.74)	73.0	1170.0	-4.096	0.000**
Oil (g)	49.8±30.20 (175.33)	0.0	157.0	61.1±134.0 (213.24)	1.0	134.0	-3.335	0.001*
Confectionery (g)	38.3±39.70 (188.00)	0.0	308.0	39.7±376.0 (199.47)	0.0	376.0	-1.009	0.313

f: Mean Rank, Mann-Whitney U test, \*p<0.05, \*\*p<0.001.

**Table 4.** Comparison of food group intakes of food-insecure and food-insecure minimum wage workers according to the recommendations of Turkey-specific nutrition guidelines

Food Group	Food Secure (n=201)				Food Insecure (n=185)				p
	$\bar{X}\pm SS (M^f)$	Lower value	Upper value	%‡	$\bar{X}\pm SS (M^f)$	Lower value	Upper value	%‡	
Milk (servings)	2.02±1.53 (203.06)	0.00	9.42	67.2	1.63±1.02 (183.11)	0.00	9.18	54.3	0.079
Meat (servings)	3.02±1.75 (183.78)	0.20	9.93	109.9	3.28±1.77 (204.06)	0.36	9.02	119.2	0.075
Vegetable-Fruit (servings)	0.97±0.61 (213.57)	0.00	3.75	19.5	0.75±0.45 (171.69)	0.00	2.56	15.1	<0.001*
Bread and Cereals (servings)	5.82±3.13 (169.80)	0.23	14.29	116.3	7.88±4.27 (219.25)	1.00	17.93	157.6	<0.001*

f: Mean Rank, ‡: Percent Coverage, Mann-Whitney U test, \*p<0.001.



**Figure 2.** Percentage of food groups consumed by food-secure and food-insecure individuals meeting the recommendation according to the Turkey Specific Dietary Guidelines (TÜBER). Statistical analysis was performed using Mann-Whitney U test.

Food insecurity is inversely related to income status. However, not every poor individual experience food insecurity. For example, approximately 65% of households near the poverty line were reported to be food secure [33]. This situation may have been associated with the implementation of certain supplementary nutrition programs. In this study, 80% of food secure individuals received nutritional assistance, supporting this hypothesis. In the United States, the implementation of supplementary nutrition assistance programs (SNAP) for unemployed adults resulted in a 2.2% reduction in food insecurity among low-income households [34]. In another study, it was

found that individuals not benefiting from SNAP were 81% more likely to report food insecurity [35].

**Limitations**

The study data were collected from individuals working with minimum wage in Üsküdar district of Istanbul province. It cannot be generalized to all minimum wage earners. In addition, the body weight and height of the participants were not measured by the researchers but were taken on the basis of self-declaration by individuals.

**CONCLUSION**

According to the results of this study conducted with minimum wage workers, the prevalence of food insecurity was 47.9%. The risk of food insecurity was found to be associated with age, supplementary nutrition income and smoking habits. Although not statistically significant, individuals with food insecurity consumed more bread and cereals, fat and meat group foods and confectionery. In addition, it was observed that the consumption of vegetables, fruits and milk group of all minimum wage earners who participated in the study did not meet the recommendations of TÜBER. Therefore, it is necessary to evaluate minimum wage workers in terms of food insecurity and to ensure access to affordable healthy foods in order to prevent health problems that may occur due to inadequate and unbalanced nutrition.

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Supplementary Tables

**Table A.** Average daily energy and nutrient intake of minimum wage workers and comparison of averages according to food security status

Variables	Total (n=386)		Food Security (n=201)		Food Insecurity (n=185)		*p
	$\bar{X}\pm SS$	Lower-Upper Value	$\bar{X}\pm SS$	Lower-Upper Value	$\bar{X}\pm SS$	Lower-Upper Value	
Energy (kcal)	2673.1±1100.15	738.91-9085.41	2546.21-1094.54	738.91-9085.41	2810.97-1092.5	819.75-5803.28	0.011*
Carbohydrate (g)	307.6±137.83	47.00-969.25	289.24-140.27	47-969.25	327.63-132.62	78.19-762.38	0.002*
Carbohydrate (%)	46.6±7.74	23-72	45.64-8.13	23-65	47.6-7.163	27-72	0.025*
Protein (g)	93.2±40.18	23.03-305.77	90.01-40.39	28.58-305.77	96.65-39.77	23.03-257.97	0.044*
Protein (%)	14.3±2.78	8-28	14.58-3.16	8-28	14.01-2.27	8-21	0.170
Oil (g)	116.6-51.43	22.35-438.35	112.04-50.17	25.43-438.35	121.51-52.45	22.35-296.17	0.054
Oil (%)	39.0-7.76	14-65	39.63-8.19	22-65	38.27-7.21	14-59	0.168
Fiber (g)	31.1-14.90	6.12-110.30	29.41-14.51	6.12-110.3	32.82-15.13	7.99-81.39	0.014*
Alcohol (g)	0.4-1.33	0.00-11.33	0.48-1.53	0-11.33	0.37-1.05	0-8.47	0.392
PUFA	32.2-18.20	3.30-107.71	29.13-17.144	3.3-107.71	35.616-18.75	4.5-73.84	0.001*
Cholesterol (mg)	420.8-215.32	2.31-1863.16	412.95-217.42	21.52-1501.12	429.36-213.28	2.31-1863.16	0.072
Vitamin A (meg)	1911.7-2157.81	21.00-21619.25	1816.85-2309.05	21-21619.25	2014.76-1981.57	137.16-9887.66	0.240
Carotene	3.9-3.31	0.00-33.16	4.39-3.72	0-33.16	3.39-2.7	0.17-16.64	<0.001**
Vitamin E (mg)	31.3-18.18	2.88-81.42	27.3-16.01	2.88-79.43	35.68-19.39	3.59-81.42	<0.001**
Thiamine (mg)	1.3-0.57	0.38-5.99	1.29-0.62	0.43-5.99	1.31-0.51	0.38-3.56	0.253
Riboflavin (mg)	1.9-0.93	0.35-8.56	1.89-1.02	0.46-8.56	1.83-0.8	0.35-6.07	0.820
Pyridoxine (mg)	1.9-1.15	0.40-8.94	1.99-1.36	0.4-8.94	1.74-0.84	0.47-6.12	0.567
Folate (mg)	347.4-167.93	82.10-1740.01	347.33-188.18	128.84-1740.01	347.4-143.24	82.1-1151.63	0.312
Vitamin C (mg)	89.3-57.87	0.00-557.01	100.30-67.08	0-557.01	77.43-42.93	4.08-290.03	<0.001**
Sodium (mg)‡	3270.6-1752.35	491.26-11526.16	3032.02-1593.88	515.76-11526.16	3529.89-1879.89	491.26-10516.2	0.018*
Potassium (mg)	2986.2-1227.02	948.44-12615.06	3023.95-1368.11	1083.89-12615.06	2945.09-1054.75	948.44-7752.23	0.962
Calcium (mg)	1048.7-432.30	251.71-3800.74	1067.92-471.11	415.61-3800.74	1027.73-385.92	251.71-3761.41	0.996
Magnesium (mg)	409.8-161.03	116.79-1403.25	404.7-175.29	130.83-1403.25	415.41-144.2	116.79-960.52	0.116
Phosphorus (mg)	1500.5-644.54	417.41-5689.20	1491.77-699.53	421.37-5689.2	1510.02-580.65	417.41-4561.96	0.261
Iron (mg)	13.2-5.94	2.99-41.79	12.62-6.00	2.99-41.79	13.77-5.83	3.29-34.11	0.210
Zinc (mg)	13.2-5.53	3.47-42.28	13.05-5.92	4.45-42.28	13.39-5.075	3.47-37.44	0.154

PUFA: Polyunsaturated fatty acid, ‡:Dietary salt consumption is not included, a:Mann Whitney U test, \*p<0.05, \*\*p<0.001.

**Table B.** Distribution of minimum wage workers meeting recommended daily allowance (RDA) energy and nutrient requirements and relationship analysis

Variables	Total (n=386)			Food Security (n=201)			Food Insecurity (n=185)			*p
	Insufficient (<%67)	Adequate (%67-110)	Excess (>%110)	Insufficient (<%67)	Adequate (%67-110)	Excess (>%110)	Insufficient (<%67)	Adequate (%67-110)	Excess (>%110)	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Energy (kcal)	94 (24.4)	253 (65.5)	39 (10.1)	51 (25.4)	133 (66.2)	17 (8.5)	43 (23.2)	120 (64.9)	22 (11.9)	0.515
Carbohydrate (g)	170 (44)	204 (52.8)	12 (3.1)	97 (48.3)	96 (47.8)	8 (4.0)	73 (39.5)	108 (58.4)	4 (2.2)	0.920
Protein (g)	66 (17.1)	217 (56.2)	103 (26.7)	35 (17.4)	111 (55.2)	55 (27.4)	31 (16.8)	106 (57.3)	48 (25.9)	0.918
Oil (g)	50 (13)	198 (51.3)	138 (35.8)	25 (12.4)	105 (52.2)	71 (35.3)	25 (13.5)	93 (50.3)	67 (36.2)	0.914
Fiber (g)	103 (26.7)	198 (51.3)	85 (22)	65 (32.3)	98 (48.8)	38 (18.9)	38 (20.5)	100 (54.1)	47 (25.4)	0.025*
Cholesterol (mg)	51 (13.2)	128 (33.2)	207 (53.6)	27 (13.4)	77 (38.3)	97 (48.3)	24 (13.0)	51 (27.6)	110 (59.5)	0.060
Vitamin A (mg)	38 (9.8)	110 (28.5)	238 (61.7)	16 (8.0)	64 (31.8)	121 (60.2)	22 (11.9)	46 (24.9)	117 (63.2)	0.192
Vitamin E (mg)	23 (6)	106 (27.5)	257 (66.6)	16 (8.0)	58 (28.9)	127 (63.2)	7 (3.8)	48 (25.9)	130 (70.3)	0.146
Thiamine (mg)	59 (15.3)	219 (56.7)	108 (28)	34 (16.9)	113 (56.2)	54 (26.9)	25 (13.5)	106 (57.3)	54 (29.2)	0.627
Riboflavin (mg)	26 (6.7)	141 (36.5)	219 (56.7)	13 (6.5)	79 (39.3)	109 (54.2)	13 (7.0)	62 (33.5)	110 (59.5)	0.498
Pyridoxine (mg)	43 (11.1)	173 (44.8)	170 (44)	22 (10.9)	94 (46.8)	85 (42.3)	21 (11.4)	79 (42.7)	85 (45.9)	0.718

<b>Vitamin C (mg)</b>	112 (29)	178 (46.1)	96 (24.9)	50 (24.9)	84 (41.8)	97 (33.3)	62 (33.5)	94 (50.8)	29 (15.7)	<0.001**
<b>Calcium (mg)</b>	74 (19.2)	245 (63.5)	67 (17.4)	44 (21.9)	117 (58.2)	40 (19.9)	30 (16.2)	128 (69.2)	27 (14.6)	0.082
<b>Magnesium (mg)</b>	54 (14)	218 (56.5)	114 (29.5)	33 (16.4)	113 (56.2)	55 (27.4)	21 (11.4)	105 (56.8)	59 (31.9)	0.295
<b>Phosphorus (mg)</b>	2 (0.5)	73 (18.9)	311 (80.6)	1 (0.5)	42 (20.9)	158 (78.6)	1 (0.5)	31 (16.8)	153 (82.7)	0.584
<b>Iron (mg)</b>	112 (29)	139 (36)	135 (35)	63 (31.3)	78 (38.8)	60 (29.9)	49 (26.5)	61 (33.0)	75 (40.5)	0.089
<b>Zinc (mg)</b>	31 (8)	156 (40.4)	199 (51.6)	20 (10.0)	85 (42.3)	96 (47.8)	11 (85.9)	71 (38.4)	103 (55.7)	0.177

Cramer's V correlation test, \* $p < 0.05$ , \*\* $p < 0.001$ .



## THE ROLE OF ERYTHROPOIETIN IN A RAT MODEL OF RENAL ISCHEMIA/ REPERFUSION INJURY

### RENAL İSKEMİ/REPERFÜZYON HASARLI SIÇAN MODELİNDE ERİTROPOETİNİN ROLÜ

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#### ABSTRACT

**Objective:** A powerful activator of erythroid progenitor cells, erythropoietin (EPO) is markedly elevated during hypoxia. A major cause of renal cell death is renal ischemia caused by artery blockage or organ transplantation, and reperfusion exacerbates the damage. The study aimed to investigate the effect of EPO treatment on renal injury following ischemia and reperfusion (I/R).

**Method:** Thirty rats assigned to five groups of six rats each as control, EPO, ischemia, ischemia/reperfusion (I/R) and I/R+EPO. The renal tissue samples were evaluated in terms of hematoxylin-eosin (H&E) staining for histopathological changes, immunopositivity of Beclin-1 for autophagy, and the TUNEL assay for apoptosis.

**Results:** The H&E staining showed the impairment in the tubular epithelium, glomerular and peritubular hemorrhage in the renal tissues of I/R group. Less histopathological changes were observed in I/R + EPO group. Renal tissue Beclin-1 immunopositivity and TUNEL positive cells were significantly increased in the I/R group compared with the others (p<0.05). Treatment with EPO decreased the number of the TUNEL positive cells and Beclin-1 expression.

**Conclusion:** The data showed that EPO treatment could be effective in reducing renal injury following renal I/R and alleviate histomorphological damage.

**Key Words:** Apoptosis, Autophagy, Erythropoietin, Histopathology, Renal ischemia/reperfusion

#### ÖZ

**Amaç:** Eritroid progenitor hücrelerinin güçlü bir aktivatörü olan eritropoietin (EPO), hipoksi sırasında belirgin şekilde yükselmektedir. Arteriyel blokaj veya organ naklinin neden olabileceği renal iskemi böbrek dokusundaki hücre ölümünün başlıca sebebi olmakla birlikte reperfüzyon oluşan bu hasarı arttırmaktadır. Bu çalışmada eritropoietin uygulanmasının iskemi/reperfüzyon (I/R) sonrasında böbreklerde oluşan hasar üzerindeki etkisini araştırmayı amaçladık.

**Yöntem:** Otuz adet rat her grupta altı adet olacak şekilde kontrol, EPO, iskemi, iskemi/reperfüzyon (I/R) ve I/R+EPO gruplarına ayrıldı. Böbrek doku örnekleri histopatolojik incelemeler için hematoksilin-eozin (H&E) ile boyandı, otofajiyi tespit edebilmek için Beclin-1 proteininin immünoekspresyonu ve apoptoz için TUNEL yöntemi kullanılarak değerlendirildi.

**Bulgular:** H&E boyama sonucunda I/R grubuna ait böbrek dokularında böbrek tübüllerinin epitelinde bozulma, glomerüller ve peritübüler kanama olduğu tespit edildi. I/R+EPO grubunda histopatolojik değişikliklerin azaldığı gözlemlendi. Renal Beclin-1 immünoekspresyonu ve TUNEL pozitif hücrelerin I/R grubunda diğer gruplara kıyasla istatistiksel olarak anlamlı düzeyde arttığı (p<0.05) saptandı. EPO uygulamasının Beclin-1 ekspresyonunu ve TUNEL pozitif hücre sayısını azalttığı tespit edildi.

**Sonuç:** Bu çalışma ile elde ettiğimiz veriler doğrultusunda eritropoietin tedavisinin renal iskemi/reperfüzyon sonrası böbrek hasarını azaltmada ve histomorfolojik hasarı hafifletmede etkili olabileceğini düşünmekteyiz.

**Anahtar Kelimeler:** Apoptoz, Otofaji, Eritropoietin, Histopatoloji, Renal iskemi/reperfüzyon

#### INTRODUCTION

The balance of salt and water, as well as blood pressure, are crucial functions of the kidneys. Ischemia/reperfusion (I/R) injury which usually follows sepsis, infarction, or organ transplantation, is caused by limited blood flow and oxygen to tissues. It causes induction of cytokines, chemokines, and reactive oxygen species, which lead to tissue damage [1].

Acute renal failure, a prevalent clinical disease associated with high mortality and morbidity despite major advances in supportive care, is primarily caused by I/R damage [2].

Renal tubular cells' morphologic response, which includes loss of cell polarity, cell death, dedifferentiation of viable cells, proliferation, differentiation, and restoration of a normal epithelium, is dependent on the degree and intensity of ischemia [3,4].

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Numerous research have studies on different triggers to slow the progression of injury processes because acute ischemia injury causes excessive apoptotic cell death. Except for supportive therapy, there isn't an efficient therapy right now. Therefore, it is necessary to find effective therapeutic intervention options for renal I/R injury.

Erythropoietin (EPO) a key protein in the production of erythrocytes is a hypoxia-inducible hematopoietic hormone. It primarily promotes angiogenesis and neovascularization, which improve blood flow. In response to hypoxia, the kidney and the fetal liver both create EPO. Erythropoietin's biological effects are mediated via its binding to erythropoietin receptors (EPOR), and the fact that EPOR is present in renal mesangial, glomerular, and tubular epithelial cells suggests that the potential role of EPO in the kidney [5]. EPO has a variety of protective actions, including anti-inflammatory, anti-oxidant, and anti-apoptotic [6].

Erythropoietin also exhibits powerful tissue-protective effects against I/R injury in a wide range of organs including, the heart [7], liver [8], and central nervous system [9].

The cellular process of removing useless and unhealthy components is known as autophagy. Autophagy makes easier for these unnecessary cellular components to be recycled and destroyed by lysosomes [10]. Autophagy is a mechanism for preserving cellular energy and acts as an adaptive response to negative stress in certain disease processes. This process is necessary to encourage cell survival and inhibit disease progression [11].

Indeed, due to the molecular interactions between autophagy and cell death mechanisms including apoptosis and necrosis, autophagy can both prevent and aid in cell death, depending on the nature and duration of the stress [12,13]. This autophagy paradox-destroys on one hand, protects on the other-indicates that the dynamics and role of autophagy during renal IR injury are not well understood [14].

The renal I/R injury's pathophysiology is complex, multifaceted, and controlled by numerous, interrelated molecular pathways and signaling cascades. The current study's objective was to examine how EPO administration affected cell apoptosis, autophagy, and renal tissue damage in the rat I/R injury model.

## METHOD

### Experimental Design

We used 30 female Wistar albino rats (200-220g at 8 to 10 weeks old) for this study. Rats were maintained in laboratory under controlled environmental conditions, ad libitum access to water and standard pellet food, and housed according to a 12 h light–dark cycle.

The animals were divided into five groups (n=6).

*Group I (control):* The control group rats were sacrificed and their kidney tissues were removed under anesthesia without any procedure.

*Group II (EPO):* In this group, rats were intraperitoneally (i.p) injected with only of human recombinant EPO (Eprex; Janssen-Cilag AG Sihlbruggstrasse, Switzerland) (1500 IU/kg/day) [15] without occlusion.

*Group III (Ischemic group):* Right nephrectomy applied quickly under anesthesia. After that, ischemia was induced by utilizing an atraumatic microvascular clamp to occlude the left renal artery and vein for 30 minutes.

*Group IV (I/R group):* The rats were subjected to clamping-induced ischemia for 30 minutes in the I/R group, after the clamping was relaxed, it underwent reperfusion for 3 hours [16].

*Group V (I/R+ EPO group):* In the I/R+ EPO group, the rats were subjected to clamping-induced ischemic injury for 30 min; then 1500 IU/kg/day of EPO were i.p. injected to the rats prior to reperfusion.

### Surgical Procedure

Each rat was anesthetized with intramuscular ketamine hydrochloride at 50 mg/kg body weight (Keta-Control, Cat No: 210034, Doğa İlaç, Turkey) and xylazine hydrochloride at 10 mg/kg body weight (Xylazibio, Cat No. 825827A Bioveta, Türkiye).

Renal I/R damage was performed according to previous study briefly; right nephrectomy was completed quickly under anesthesia. After that, ischemia was induced by utilizing an atraumatic microvascular clamp to occlude the left renal artery and vein for 30 minutes. After the clamping was relaxed, it underwent reperfusion, and the change in kidney color was used to validate blood flow. The abdomen is closed in two layers with 2-0 sutures.

After 3-hours of reperfusion the rats were sacrificed by using servical dislocation method under anesthesia and immediately the kidney tissues were removed.

### Histological Evaluation

Renal tissues were fixed in a 10% formalin solution for 24–48 hr for histological examination. The tissues were washed under tap water for 6 hours, then pass through a gradually increased alcohol for 1 hour, then put in xylene for 15 minutes. After that, samples of renal tissue were cut into 5 µm sections using a microtome (Leica RM2245, Germany), after being embedded in paraffin blocks. Then the sections were subjected to deparaffinization and dehydration, pass through decreased alcohol series. Then the slides were stained with hematoxylin (Mayer's hematoxylin, Merck) in 30 seconds and eosin in 15 seconds. The slides were photographed under a light microscope (LeicaDM4000, Wetzlar, Germany).

Sections were examined in groups and graded according to the extent of cortical involvement on a scale of 0 to 4 ( 0=normal; 0.5=small damage within focal areas; 1=<10% damage in the cortex; 2=10 to 25% damage in the cortex; 3=25 to 75% damage in the cortical area; and 4=>75% damage in the cortical area [17]. All scores were added up and displayed on a graph as average values.

### Immunohistochemical Analysis

Slides were deparaffinized, placed in xylene, then rehydrated using ethanol in increasing concentrations. Antigen retrieval was carried out using citrate buffer in a microwave and the tissues were then blocked in blocking serum (UltraV Block, ScyTek Laboratories, Utah, USA). The slides were incubated with Beclin1 (anti-Beclin1 antibody, ab13847, Abcam, Cambridge, UK ) primary antibody, 1:50 dilution with PBS, overnight. The primary antibody was incubated on tissues for five minutes before washed twice with PBS., and then, incubated with biotinylated anti-mouse secondary antibody (BA-9200; Vector Laboratories, Burlingame, CA ) for 10 minutes at room temperature. Following the application of the secondary antibody, the tissues were rinsed three times in five minutes with PBS and then incubated for ten minutes with streptavidin peroxidase and then taken into PBS. DAB substrate kit solution was applied for reaction. The slides were counterstained with hematoxylin (Merck, ScyTek Laboratories ) and covered with entellan.

The slides were examined and photographed under the light microscope (LeicaDM4000, Wetzlar, Germany) As previously mentioned, H-SCORE analyses were utilized to evaluate the immunohistochemistry results [18].

### TUNEL assay

Apoptotic cells were stained by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique using an apoptosis detection kit (In Situ Cell Death Detection Kit, Roche, Mannheim, Germany). Deparaffinized paraffin sections were rehydrated and treated in a microwave with 10 mM citrate buffer twice for 5 min each, allowed to cool for 20 min. Endogenous peroxidase activity was suppressed with 3% hydrogen peroxide following three

PBS washings. Following equilibration buffer incubation for 10–15 s, the sections underwent TdT enzymatic labeling of nuclear DNA strand breaks in a humidified chamber at 37 °C for 60 min. The typical labels were revealed by adding an alkaline phosphatase-converter with subsequent staining with the chromogenic substrate. Counterstaining was performed with Mayer’s hematoxylin (Merck, ScyTek Laboratories). TUNEL-positive cells’ distribution was counted and reported as earlier [19].

**Ethical Approval**

The study was approved (Approval code:672) on 04.07.2023 by the animal ethics committee of Kobay Company (Türkiye).

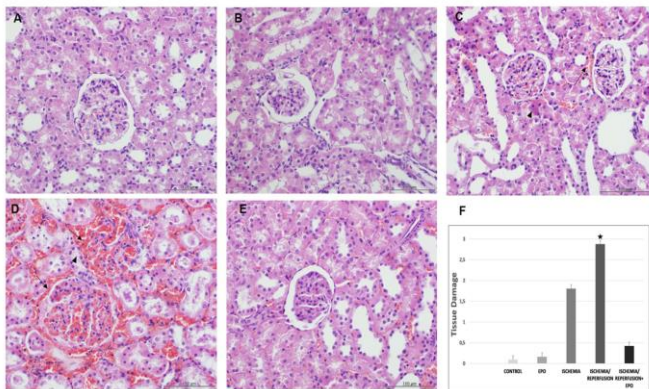
**Statistical Analysis**

Statistical analysis was performed by one-way ANOVA followed by Dunnett’s test and the Mann Whitney U test by using Sigma Plot 12 (Jandel Scientific Corp., San Rafael, CA ). P<0.05 was used to indicate that the data were statistically significant. The mean and standard error (SEM) of three independent experiments are used to represent all data.

**RESULTS**

**Histomorphological Changes**

The histological changes of renal tissue samples from each group of rats were seen (Figure 1). The renal sections in the control revealed a healthy histological structure with glomerular structure and tubular cells (Figure 1A). The EPO group exhibited no histological changes in renal morphology (Figure 1B). Peritubular and glomerular hemorrhage was observed in the ischemia and I/R groups (Figure 1C and D). Furthermore, renal sections of the I/R group showed extensive tubular degeneration which included tubular dilatation, tubular necrosis, vacuolization of tubular epithelial cells, and loss of brush borders compared to control and EPO groups. The I/R+EPO group had less histopathological alterations in renal tissue (Figure 1E) when compared with ischemia and I/R groups. Renal tissue damage was significantly increased in I/R group and decreased with the EPO treatment (Figure 1F).

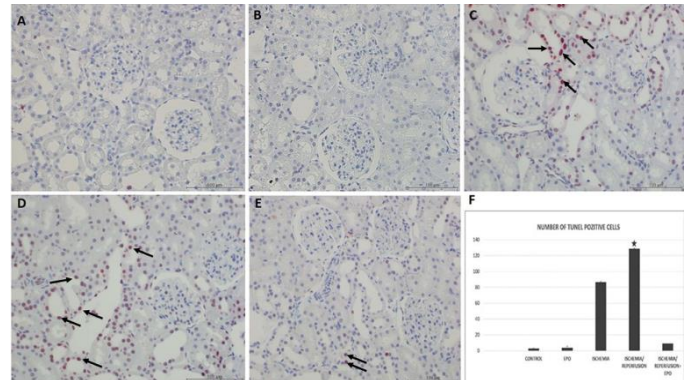


**Figure 1.** Histomorphological evaluation of renal tissues of rats induced I/R injury. A: Control group; the normal renal tissue structure. B: EPO group; exhibited no changes in renal morphology. C: Ischemic group; peritubular hemorrhage (arrow) and vacuolization of the tubular epithelium (arrowhead) was observed. D: I/R group; showed extensive peritubular and glomerular hemorrhage (arrow), tubular dilation, and loss of brush border (arrowhead). E: I/R+EPO group; histomorphological changes were significantly attenuated treated with EPO. (Stained with H&E; scale bar, 100 µm.; magnification, 400x) F: Quantitative analysis of tissue damage data are means±SEM. \*p<0.05.

**TUNEL Assay Results**

The apoptosis in renal I/R damage was evaluated using TUNEL labeling. In the renal tissues from the control and EPO groups, there were no detectable TUNEL positive cells (Figure 2A and B). On the other hand, several TUNEL labeled cells were observed in ischemia

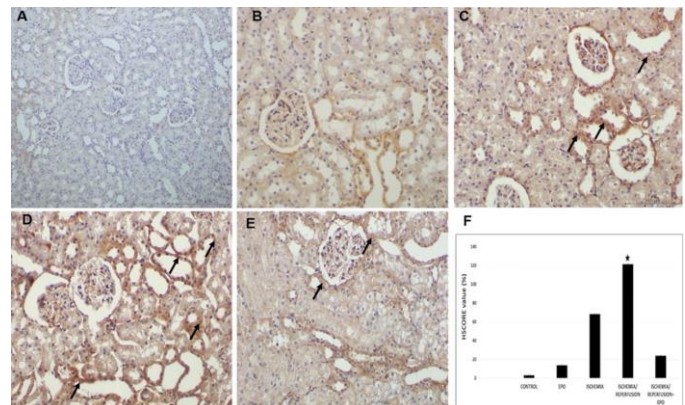
and I/R groups (Figure 2C and D) compared to control (Figure 2A) and EPO (Figure 2B) groups. The proportion of TUNEL-positive cells was much lower in the I/R+ EPO group (Figure 2E) compared to ischemia (Figure 2C) and I/R (Figure 2D) groups. In the I/R group, the number of TUNEL positive cells was significantly increased (p<0.005) compare to the other groups (Figure 2F).



**Figure 2.** TUNEL staining in renal tissue samples of rats induced I/R injury. No TUNEL positive cells in Control (A) and EPO group (B). Several TUNEL positive cells were detected in renal tissue samples of rats induced ischemia (C) and I/R injury group (D). A few TUNEL positive cells (arrowa) were detected in the renal tissue samples of rats from I/R+EPO group (E). (Scale bar, 100 µm;magnification 40X). F : Quantitative analysis of TUNEL-positive cells. The number of the TUNEL positive cells was significantly increased in the I/R group compare to other groups. Data are means±SEM. \*p<0.05.

**Immunohistochemistry Results**

To determine how autophagy influences renal I/R injury we investigated the autophagic protein Beclin 1 expression using immunohistochemistry. There was not any specific immunostaining of Beclin 1 in rat kidney tissues from control and EPO groups (Figure 3A and B).The immunoeexpression of Beclin 1 was observed in the ischemic rat kidney (Figure 3C). Additionally, the immunostaining of Beclin 1 was higher in I/R group (Figure 3D) compared to other groups. The immunohistochemical images, which revealed that pretreatment with EPO decreased the expression levels of Beclin1 in the renal tissue samples of I/R+ EPO (Figure 3E) when compared to those of ischemia (Figure 3C) and I/R groups (Figure 3D). The immunoeexpression level of Beclin 1 was significantly increased (p<0.005) in the I/R group compare to others (Figure 3F).



**Figure 3.** The immunoeexpression of Beclin 1 in renal tissue samples of rats induced I/R injury. A:Control group: here was not any staining. B:EPO group. C:Ischemia group: Modarate staining of Beclin 1was observed. D:I/R group showed strong immunostaining of Beclin 1. E:I/R+EPO group: Low Beclin 1 staining was observed. (scale bar, 100 µm, magnification, x400); F:H-SCORE values of Beclin1. The expression levels of Beclin were significantly increased in the I/R

group and decreased in the I/R+EPO group. Data are expressed as SEM. \* $p < 0.05$ .

## DISCUSSION

In the current study we investigated the effects of EPO on the I/R injury in the rat experimental model. Although there has been studies in the literature about this issue we focused on firstly to figure out the connection between apoptosis and the autophagy in the renal I/R injury. Secondly we aimed to determine the effects of EPO on this pathway. Therefore we investigated the histomorphological changes, the apoptotic cells and the autophagic pathway.

The findings of the current study revealed that renal I/R damaged affect the histologic architecture of the kidney. When comparing the I/R group to the control and ischemic groups, there was a significant increase in apoptotic TUNEL positive cells and autophagic marker Beclin-1 expression in the renal tissue. Both the quantity of TUNEL positive cells and the expression of Beclin-1 were reduced by EPO treatment. Although ischemia already causes damage, reperfusion may result in a wide range of issues. It could result in patients life-threatening situations when related to systemic issues. The oxygen reactions following reperfusion causes tissue injury and initiates a chain reaction of damaging cell results in inflammation, cell death, and finally organ insufficiency [20].

The ability to maintain intracellular calcium homeostasis and the ability to regenerate ATP, following reperfusion are the factors that ischemia reversibility [21].

Several experimental I/R models have demonstrated the protective effects of EPO, through a number of pathways, including the control of microvascular damage, the reduction of tubulointerstitial damage, anti-inflammatory and anti-apoptotic actions, and reduced fibrocyte accumulation [22].

The possible renoprotective effects of EPO on renal I/R model rats were examined in the current study. The histomorphological results revealed that I/R caused severe damage to renal tissue particularly diffuse congestion and tubular injury that was ameliorated with the EPO treatment. In the kidney with IRI, tubular necrosis and interstitial infiltration of inflammatory cells are typical clinical features. Inflammatory responses to I/R injury include neutrophil and macrophage infiltration, oxidative stress, and increased production of inflammatory cytokines [23].

According to reports, EPO protected the kidney from I/R by reducing the amount of tissue congestion and inflammatory cell infiltration [24]. The results of the current study are analogous to those of an earlier investigation into the underlying molecular mechanisms of renal I/R established in EPO-treated rat models.

The generation of red blood cells is regulated by the glycoprotein cytokine erythropoietin, which is mostly produced by the kidney. Apart from its well acknowledged impact, numerous investigations have demonstrated that EPO functions as a factor that protects tissue [25-28].

EPO has been used not only in renal I/R but also in the recovery of I/R injury in other tissues. Guneli and co-workers [29] have investigated the possible protective effects of EPO against intestinal I/R injury in rats. TUNEL labeling was used to identify apoptotic cells and the histological evaluation was performed to determine the level of the injury. Intestinal tissue was damaged by I/R compared to the sham demonstrated by an increase in TUNEL-positive cells.

One type of cell death that helps remove dying cells from groups of proliferating or developing cells is called apoptosis, sometimes referred to as programmed cell death [30]. On the other hand, in pathological conditions, apoptosis activation leads to extensive and fast cell death, which causes tissue malfunction.

Some researchers has preferred to use EPO combination with other subjects for example Banaei et al. [31] have investigated the anti-apoptotic effect of EPO and melatonin on renal I/R injury in rats. The study's findings demonstrated that I/R enhanced the number of TUNEL-positive cells. The tubular epithelium's renal impairment was corroborated by the IR group's histopathological findings. TUNEL positive cells and histological alterations were reduced by EPO and melatonin treatment.

In hospitalized patients, acute kidney injury (AKI) is the most prevalent ailment. An efficient treatment strategy for ischemia/reperfusion-induced AKI (IR-AKI) is essential because it plays a significant role in end-stage disease. A strong erythroid progenitor cell stimulant, EPO is markedly elevated in hypoxic environments. Using an IR-AKI male C57BL/6 mice model, Kwak et al. [32] have investigated the renoprotective properties of EPO. The study's findings demonstrated that EPO considerably reduced tissue damage and renal dysfunction brought on by IR-AKI. In mice given EPO, apoptotic cell death and oxidative stress were considerably decreased.

In the current study we can clearly say that the I/R injury in renal tissue results in the increasing of apoptotic cells which were confirmed by the increase number of TUNEL positive cells. This findings is consistent with other research showing that renal I/R starts a convoluted chain of events that ultimately cause damage and the necrotic and apoptotic death of renal cells [33]. Apoptosis during renal I/R markedly increased and EPO treatment before reperfusion reduces I/R-induced apoptosis in renal tubular cells. Based on TUNEL labeling, our findings showed that EPO administration protects renal tissue against I/R-induced apoptosis.

To maintain intracellular homeostasis, damaged organelles and macromolecules are degraded and recycled by the conserved multistep process known as autophagy. The autophagic pathway is comprised of four distinct sections: (i) initiation; (ii) engulfment of mature structures by a double-membrane structure, resulting in the generation of autophagosomes; (iii) autolysosome formation through the fusion of autophagosome and lysosome; and (iv) the ultimate process, which involves the degradation and recycling of the engulfed structures [34]. Beclin 1, p62, and microtubule-associated protein light chain 3B (LC 3B) have all been shown to be essential components of the autophagy process [35].

Under stressful circumstances such as hypoxia, nutrition, and growth-factor deficiency, the autophagy pathway is activated. Recent research has shown that basal autophagy in the kidney is essential for maintaining the proximal tubules' homeostasis. Renal function was impaired by the deletion of important autophagy proteins, which also raised p62 levels and oxidative stress. Autophagy deletion in the proximal tubules increased tubular damage and renal function in AKI mice, emphasizing the renoprotective effects of autophagy in AKI [10].

In both healthy and damaged tissue, autophagy is a self-degradative process that involves the turnover and recycling of cytoplasmic components. In the early phases of programmed cell death, autophagy has been demonstrated to be protective, but in some circumstances, it can potentially encourage apoptosis. Bendix and collages have examined erythropoietin's impact on autophagy signaling in a developing rat brain using an in vivo oxygen toxicity model. The study showed that in the developing brain, high oxygen levels cause up-regulation of Beclin 1 indicators for autophagic cell death and these alterations are neutralized by EPO treatment [36].

Beclin-1 starts the production of phagophores, which are the progenitors of autophagosomes, when autophagy is started [37]. Zhang et al. have focused on how autophagy affects renal I/R damage. Kidney tissue samples were graded histopathologically. Light chain 3 (LC3), Beclin-1, p62, and the quantity of autophagic vacuoles were indicators of autophagy. TUNEL was used to induce apoptosis, and caspase-3



was expression was assed by immunohistochemically. Following renal I/R damage, autophagy was triggered. Unlike our findings; the study show that autophagy may be triggered during I/R injury and may even be beneficial in cases of kidney injury [38]. Due to the close reliance of their constituent cells on the preservation of normal oxidative metabolism, neurologic, renal, and cardiovascular tissue are especially vulnerable to I/R injury [39].

In rats that have undergone cerebral I/R, Huang et al. have examined the impact of post-conditioning ischemia on the expressions of the hippocampus neuron autophagy-related proteins LC3-II and Beclin-1. The findings of the investigation showed that cerebral ischemia post-conditioning increased the expressions of Beclin-1 and LC3-II, two proteins linked to autophagy [40].

Another investigation of ischemic myocardium has been published in the literature. Autophagy seems to help with survival in this situation, but it can also cause cell death during reperfusion. The length and severity of stress, as well as the degree of autophagy in cardiac tissue, appear to have an impact on autophagy's overall function. Ventricular fibrillation (VF) is one of the main side effects of reperfusion intervention. The study have examined for any potential relationships between VF and autophagy. Researchers have demonstrated that the fibrillated myocardium had increased levels of Beclin-1 and LC3B-II/LC3B-I ratio, two autophagy indicators, compared to tissue from nonfibrillated hearts [41].

Autophagy is most frequently used by cells and is mainly regulated by Beclin-1 and ubiquitin [42,43]. Actually, a known biomarker for the presence of autophagy in biological samples is the Beclin-1 protein [44].

To learn more about function of autophagy we investigated the renal expression of Beclin-1. The immunexpression of Beclin-1 was increased remarkably in the I/R group compare to other groups. Similarly some studies in the literature we also found the increase expression of Beclin 1 in ischemia and ischemi reperfusion groups. Additionally Beclin 1 expression was decreased with the treatment of EPO compare to ischemia and I/R groups. Therefore we can cleary say that I/R injury trigger the autophagy. Although recent studies reported that autophagy has renoprotective effects, interestingly, our results showed autophagy activation following I/R was suppressed by EPO treatment ,based on the immunohistochemistry staining of decreased Beclin-1 levels.

### Limitations

This study has potential methodology limitations including sample size, surgery process.

### CONCLUSION

Histological analysis performed in our work demonstrated that I/R led to tubular epithelial cell enlargement, pyknotic nuclei, and congestion. Dilated Bowman's space and glomerular atrophy were additional effects of renal I/R. Treatment with EPO reduced the histological alterations linked to renal IR injury.

Additionally;in the current study we determined the correlation between the apoptosis and the autophagy depends on the findings of TUNEL and immunohistochemistry assay results. The proliferation of TUNEL positive cells in the ischemia and I/R groups, compared to other groups, indicates that an increase in apoptosis and EPO showed its anti-apoptotic effects depends on the decreased the TUNEL positive cells with the treatment of EPO in I/R+EPO group.

As well as an excessive elevation in autophagy due to the higher immunexpression of autophagic marker Beclin 1 following I/R injury. According to immunohistochemical labeling of reduced Beclin-1 levels, EPO therapy prevented higher autophagic activation after I/R. We thought that EPO suppressed the excessive activation of autophagy.

In conclusion, EPO treatment ameliorated the severe damage of renal tissue caused by I/R injury and also enhanced kidney histomorphology at cellular level, lowered apoptosis, and suppressed the excessive autophagic activation. Taken together the results of the current study may contribute to the literature with different aspects.

**Ethical Approval:** 2023/672 Animals Ethics Committee of Kobay Company (Türkiye)

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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**Author Contribution:** **Concept:** BK,EA; **Design:** MFB,EA,US; **Data collecting:** MFB, EA,US; **Statistical analysis:** BK; **Literature review:** BK; **Writing:** BK; **Critical review:** BK,MFB,EA,US.

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## INVESTIGATION OF THE RELATIONSHIP BETWEEN COGNITIVE LEVEL AND UPPER EXTREMITY FUNCTIONS IN PATIENTS WITH CHRONIC STROKE

### KRONİK İNME Lİ HASTALARDA KOGNİTİF DÜZEY İLE ÜST EKSTREMİTE FONKSİYONLARI ARASINDAKİ İLİŞKİNİN İNCELENMESİ

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#### ABSTRACT

**Objective:** Cognitive impairment and loss of upper limb functions are common after stroke and these two components can influence each other in anatomical and functional contexts. In this study, it was aimed to examine the relationship between cognitive level and upper extremity functions in patients with chronic stroke.

**Method:** The study included 39 individuals diagnosed with stroke, in the chronic phase and with a mean age of 61.33±12.71 years, who were evaluated at Pamukkale University Hospital. Standardized Mini Mental Test (SMMT) and Stroop Test Basic Sciences Research Group (TBAG) Form were used to evaluate the cognitive levels of individuals. Fugl Meyer Upper Extremity Rating Scale, Frenchay Arm Test and Box-Block Test were used to evaluate upper extremity functions.

**Results:** When the findings of the study were examined, statistically significant relationships were found between the Standardized Mini Mental Test and the Fugl Meyer Upper Extremity Rating Scale, Frenchay Arm Test and Box-Block Test ( $p<0.05$ ). Between SMMT and 'Flexor synergy' and 'Normal reflex activity', which are subtitles of Fugl Meyer Upper Extremity Rating Scale a statistically significant correlation was found ( $p<0.05$ ). In addition, between the subtitles of the Stroop Test TBAG form 'Chapter 3 Error' and the subtitles of the Fugl Meyer Upper Extremity Evaluation Scale 'Reflex Activity'; statistically significant relationships were also found between 'Chapter 2 Correction' and 'Reflex activity', 'Extensor synergy' and 'Non-synergy movement' ( $p<0.05$ ). No statistically significant correlation was found between the other parts of the Stroop Test TBAG Form and the scales assessing upper extremity functions ( $p>0.05$ ).

**Conclusion:** The results obtained from this study show that there is a relationship between the cognitive level and upper extremity functions in patients with chronic stroke. More efficiency can be obtained by taking ocnsider these two components as two components that may affect each other in the evaluation stages of patients and may help to create more personalized and successful programs.

**Key Words:** Chronic Stroke, Cognitive Level, Upper Extremity, Function

#### ÖZ

**Amaç:** İnme sonrası kognitif bozukluk ve üst ekstremitte fonksiyonlarında kayıplar yaygındır ve bu iki bileşen anatomik ve fonksiyonel bağlamda birbirini etkileyebilmektedir. Bu çalışmada kronik inmeli hastalarda kognitif düzey ile üst ekstremitte fonksiyonları arasındaki ilişkinin incelenmesi amaçlandı.

**Yöntem:** Çalışmaya Pamukkale Üniversitesi Hastanesi'nde değerlendirilen inme tanısı almış, kronik evrede ve yaş ortalaması 61.33±12.71 olan 39 birey dahil edildi. Bireylerin kognitif düzeylerini değerlendirmek için Standardize Mini Mental Test (SMMT) ve Stroop Testi Temel Bilimler Araştırma Grubu (TBAG) Formu kullanıldı. Üst ekstremitte fonksiyonlarını değerlendirmek için Fugl Meyer Üst Ekstremitte Değerlendirme Ölçeği, Frenchay Kol Testi ve Kutu- Blok Testi kullanıldı.

**Bulgular:** Çalışmanın bulguları incelendiğinde, Standardize Mini Mental Test ile Fugl Meyer Üst Ekstremitte Değerlendirme Ölçeği, Frenchay Kol Testi ve Kutu-Blok Testi arasında istatistiksel olarak anlamlı ilişkiler bulundu ( $p<0.05$ ). SMMT ile Fugl Meyer Üst Ekstremitte Değerlendirme Ölçeği'nin alt başlıkları olan "Fleksör Sinerji" ve "Normal Refleks Aktivite" arasında istatistiksel olarak anlamlı bir ilişki bulundu ( $p<0.05$ ). Ayrıca Stroop Testi TBAG formunun alt başlıkları olan "Bölüm 3 Hata" ile Fugl Meyer Üst Ekstremitte Değerlendirme Ölçeği'nin alt başlıkları olan "Refleks Aktivite" arasında; "Bölüm 2 Düzeltme" ile "Refleks Aktivite", "Ekstansör Sinerji" ve "Sinerji Dışı Hareket" arasında da istatistiksel olarak anlamlı ilişkiler bulundu ( $p<0.05$ ). Stroop Testi TBAG Formunun diğer bölümleri ile üst ekstremitte fonksiyonlarını değerlendiren ölçekler arasında istatistiksel olarak anlamlı bir ilişki bulunmadı ( $p>0.05$ ).

**Sonuç:** Bu çalışmadan elde edilen sonuçlar kronik inmeli hastalarda kognitif düzey ile üst ekstremitte fonksiyonları arasında ilişki olduğunu ve rehabilitasyon sürecinde kognitif terapiler ile üst ekstremitte fonksiyonlarını geliştirmeye yönelik tekniklerin birlikte uygulanarak tedaviden daha fazla verim alınabileceğini gösterdi.

**Anahtar Kelimeler:** Kronik İnme, Kognitif Düzey, Üst Ekstremitte, Fonksiyon

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## INTRODUCTION

Stroke, cerebrovascular accident (CVA); it is a serious syndrome that causes damage to a certain region of the brain due to problems in the blood circulation, and clinical findings are seen according to the affected brain region. It may develop due to stroke, ischemia or hemorrhage. Symptoms settle quickly, vary in severity, and can result in death [1,2]. The cause of stroke, the portion supplied by the affected artery in stroke, and the size of this portion affect the clinical findings accompanying the stroke [3]. Loss of balance and coordination, impaired gait, sudden headache of unknown origin are the most common acute clinical findings [4].

Cognitive impairment is common after stroke, and studies have shown that this rate is 35% in the first 3 months and 32% in the next 3 years [5,6]. Depending on the location and severity of the lesion, the severity and type of cognitive impairment may also vary in stroke patients. Loss of patients' memory, attention, concentration, problem solving, thinking and decision-making abilities are frequently encountered problems. It is accepted by some authors that visual-spatial perception and apraxia are among cognitive problems. In order to provide an effective and successful rehabilitation, patients must be able to perceive commands, give the necessary responses and have the capacity to learn. Loss of these abilities will negatively affect the rehabilitation process [6-9].

The most common neurologic involvement after stroke is in upper extremity motor functions, and studies show that upper extremity dysfunction is present in 85% of patients [10]. If stroke severity is severe, only 15% of patients may experience improvement in hand functions [11]. Studies have shown that upper extremity neurologic dysfunction after stroke showed that recovery was in the first 3 months [3].

Studies conducted in recent years show that cognitive and physical areas are closely related [12], but neurorehabilitation focuses more on the motor system and the cognitive aspect of motor movements is put into the background [13]. Attention is very important for perception – cognition ability, and attention losses in stroke patients cause decreases in motor functions. Disturbances in the motor mechanism of the pyramidal system also lead to upper extremity functional losses in stroke patients [14]. Motor dexterity of the hemiparetic upper extremity has been shown to be associated with increased activation of the prefrontal areas involved in working memory and visuospatial transformations [15]. These results suggest that cognitive aspects of motor control are associated with modulation of activity in the common motor cortical area and associated areas [16].

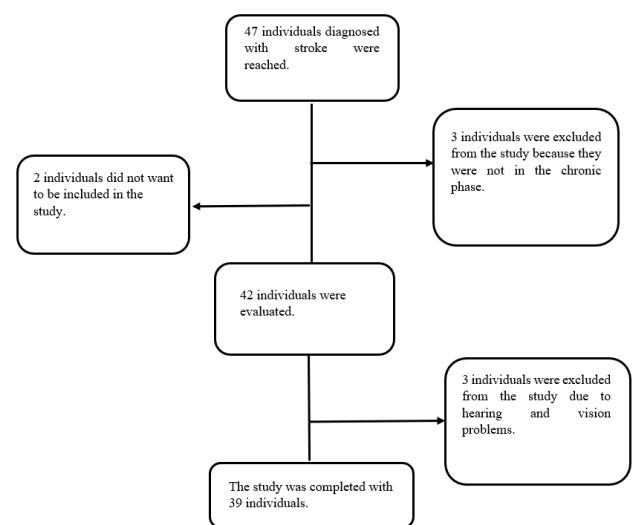
Studies on the rehabilitation process of stroke patients have shown that cognitive processes and arm motor development have a positive relationship and that deficits in memory and executive attention negatively affect upper limb motor recovery of patients [17,18]. Although research in recent years has increasingly focused on the effect of cognitive performance on motor performance [19], many studies on upper extremity rehabilitation after stroke have not considered cognitive skills. The ICF (International Classification of Function) definition, which is the basis for planning rehabilitation processes, considers body structure and functions, activity and participation parameters, and cognitive and motor processes as processes that affect each other [20]. Considering these relationships between upper extremity performance and cognitive skills, it is emphasized that these parameters should not be considered separately in rehabilitation and evaluation processes.

Studies have focused on the effects of cognitive rehabilitation approaches on motor function, and there are few studies on upper extremity functions, more specifically. Our study was planned based on the hypothesis that cognitive processes and upper extremity performance are related in chronic stroke patients. Our aim is to contribute to the literature and to give clinicians an idea about the evaluation process of patients by considering these parameters.

## METHOD

### Research Design and Population

The population of the study, which was planned as a descriptive and cross-sectional study, consisted of stroke patients evaluated at Pamukkale University Hospital between August 2020 and April 2021. 39 individuals, 12 women and 27 men with a mean age of  $61.33 \pm 12.71$  years who were diagnosed with stroke were included in the study. While the inclusion criteria of the individuals in the study were to be volunteers, to have had an ischemic or hemorrhagic stroke, to be diagnosed with CVA at least six months ago, to have no more than one stroke, to have no communication problems, and to be able to read and write, the exclusion criteria were; having hearing loss, receiving additional treatment for rehabilitation, having an orthopedic disorder, having undergone recent surgery, having additional neurological disease other than stroke, having a psychological disorder, having a previous disease that may affect his cognitive level, and not meeting the inclusion criteria. The inclusion processes of the individuals participating in the study are shown in Figure 1.



**Figure 1.** The scheme of selection of individuals to work

### Sample Size Calculation

G\*Power package software program (G\*Power, Version 3.1.9.7, Franz Faul, Universität Kiel, Germany) was used to calculate the required sample size for the study. Considering the upper extremity function parameter in a similar study [21], it was calculated that a sample of 34 people was required to obtain 90% power with an effect size of  $d=0.20$ , type I error of  $\alpha=0.05$  and type II error of  $\beta=0.10$ . The sample selection of the study was completed with 39 chronic stroke patients who met the inclusion criteria using simple random sampling method.

In addition to the demographic data of the individuals participating in the research; history of stroke, type of stroke, personal and family history, height and weight, use of assistive devices, smoking, alcohol use, dominant and affected extremities, and existing comorbidities were also recorded. All individuals were evaluated in Pamukkale University Hospital.

### Outcome Measures

#### Cognitive State

The cognitive levels of the individuals participating in the study were evaluated with the Standardized Mini Mental Test and the Stroop Test TBAG Form.

*The Standardized Mini-Mental Test (SMMT)* created by Folstein et al. in 1975, is a practical and easy-to-apply test for the assessment of cognitive and dementia disorders [22]. The reliability and validity studies of the Turkish form of SMMT were performed by Gungen et

al. in 2002. SMMT; It consists of five parts: orientation, recording, attention and calculation, recall and language tests. The total score is 30, those who score 24 points or less are at risk for cognitive impairment [23,24].

The Stroop Test was developed by Stroop in 1935 and is a neuropsychological test that measures focused attention and information processing speed. The test is based on measuring the reader's reaction time in the face of this confounding effect when the color of the written word and the color the word expresses are different [25]. In this study, the Stroop Test TBAG form, adapted to Turkish culture and proven to be valid and reliable, was used, which was developed within the scope of the Stroop Test BİLNOT Battery. Stroop Test TBAG Form is applied with four cards and consists of five parts. Color names printed in black on the 1st card, color names printed in color on the 2nd card (the color in which the word is written on this card and the color the word denotes are different), the circles printed in different colors on the 3rd card, and the neutral words printed in color but without a color name are included on the 4th card. The 2nd card with the color names printed in color is used both in the 2nd part and in the 5th part. In the evaluation, the duration of each section, the number of errors and corrections are recorded [26].

**Upper Extremity Functions**

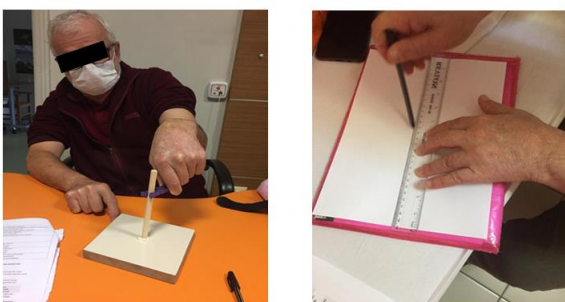
Upper extremity functions of the individuals participating in the study were evaluated with Fugl Meyer Upper Extremity Rating Scale, Frenchay Arm Test and Box Block Test (BBT).

The Fugl Meyer Upper Extremity Rating Scale (FMA-UE) is a frequently recommended scale, especially for use in stroke patients, and is based on measuring performance [27, 28]. The test consists of reflex activity, flexor and extensor synergy, combined synergistic movements, non-synergy movements, normal reflex activity, wrist and hand evaluation, coordination and speed evaluation. It consists of 33 items in total and each item is scored between 0 and 2 points (0: unable, 1: partially able, 2: fully able). The total score is 66. It is easy to apply, it does not require much equipment, the items in the house are sufficient and it takes about 30 minutes [29, 30].

The Frenchay Arm Test is based on measuring the functional abilities of the affected upper extremity. The test consists of five simple upper extremity tasks. In case of successful completion of the tasks, 1 point is awarded and the total score is 5. The patient tries to do the following five items using the affected upper extremity:

- 1- Fixing the ruler
- 2- Keeping a cylinder
- 3- Raising the glass
- 4- Attaching a latch to the bar
- 5- Combing hair [31].

The application of the Frenchay arm test to individuals is shown in Figure 2.



**Figure 2.** The application of the Frenchay arm test to individuals (Attaching a peg to a stick- Fixing the ruler)

The Box-Block Test was developed to assess rough manual dexterity and is a very simple, practical and quick test to use. For the test, a box and small wooden blocks are used, which is divided into two equal areas right in the middle. Small wooden blocks are all placed on one side of the box. For the test, the patient is told to throw the wooden blocks in one section into the other section as quickly as possible and 60 seconds are given. The test is applied to both the dominant and non-dominant hand and the number of wooden blocks thrown to the opposite side gives the total score. The test is particularly suitable for patients with limited grip and dexterity [32]. The application of the Box-Block test to the individuals is shown in Figure 3.



**Figure 3.** Application of the Box-Block test to the individuals.

**Ethical Approval**

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Pamukkale University Non-Interventional Clinical Research Ethics Committee on 29/07/2020 (No:14). All participants were informed about the study before participating in the study and the necessary written consent was obtained.

**Statistical Analysis**

Statistical analysis of the data was performed using IBM SPSS version 26. The conformity of the data to the normal distribution was examined using the Shapiro Wilk test, histograms and probability pilots. Normally distributed data were showed as mean- ± standard deviation (SD), non-normal distributions were showed as median (minimum-maximum), and ordinal variables were showed as frequency and percentage. Spearman correlation analysis was used to examine the relationship between the data. P<0.05 was accepted for statistical significance. Correlation analysis results were classified as follows: 0.00-0.20 (poor correlation), 0.21-0.40 (fair correlation), 0.41-0.60 (moderate correlation), 0.61-0.80 (good correlation), and 0.81-1.00 (very good correlation) [33].

**RESULTS**

A total of 39 individuals, 12 women (30.8%) and 27 men (69.2%), with a mean age of 61.33±12.71 years, aged between 43 and 87 who were diagnosed with stroke were included in the study. Demographic data of individuals are shown in Table 1.

**Table 1.** Demographic data of individuals

Variables	Mean±SD	Median	Minimum-Maximum
Age (years)	61.33±12.71	60	43-87
Length (cm)	166.38±6.62	167	152-180
Body weight (kg)	78.74±13.73	80	52-110
BMI(kg/m <sup>2</sup> )	28.49±5.13	28.30	18.64-41.91
Disease duration (month)	20.10±2.37	14	7-66

SD:Standart deviation, BMI:Body mass index.

Twenty of the subjects had left extremity involvement, 19 had right extremity involvement, and 32 had ischemic stroke and 7 had hemorrhagic stroke. Other descriptive characteristics of individuals are shown in Table 2.

**Table 2.** Descriptive characteristics of individuals

Variables		n	%
Gender	Female	12	30.8
	Male	27	69.2
Type of stroke	Ischemic	32	82.1
	Hemorrhagic	7	17.9
Effectuated side	Right	19	48.7
	Left	20	51.3
Smoking	Yes	18	46.2
	No	21	53.8
Alcohol use	Yes	1	2.6
	No	38	97.4
Use of assistive devices	Yes	24	61.5
	No	15	38.5
Total		39	100

n: Number, %: Percent

According to the Standardized Mini Mental Test, the cognition levels of the cases; 6 of them were severe dementia (15.4%), 9 of them were mild dementia (23.1%), and 24 of them were normal (61.5%). The findings related to the numerical data obtained from the Standardized Mini Mental Test and Stroop Test TBAG Form test results, Fugl Meyer Upper Extremity Assessment Scale total score and sub-headings, Frenchay Arm Test and Box Block tests of the individuals included in the study are shown in Table 3 and Table 4.

**Table 3.** Findings related to cognition level assessment scales of individuals

Scales	X±SD	Median	Min-Max
SMMT	23.79±5.31	26	11-29
Stroop Part 1- Time (s)	22.56±6.72	23.15	9-37.15
Stroop Part 2- Time (s)	28.27±7.33	26.20	13.10-46.11
Stroop Part 3- Time (s)	29.64±7.81	28.70	13.56-50.77
Stroop Part 4- Time (s)	37.19±7.59	37.40	21.50-52.65
Stroop Part 5- Time (s)	43.23±9.32	43.75	24.70-69.10
Stroop Part 1- Error	0.12±0.65	0	0-4
Stroop Part 2- Error	0.38±1.16	0	0-6
Stroop Part 3- Error	0.48±0.75	0	0-2
Stroop Part 4- Error	0.66±1	0	0-4
Stroop Part 5- Error	0.74±0.96	0	0-3
Stroop Part 1- Correction	0.25±0.63	0	0-3
Stroop Part 2- Correction	0.38±0.74	0	0-3
Stroop Part 3- Correction	0.41±0.67	0	0-2
Stroop Part 4- Correction	0.56±0.82	0	0-3
Stroop Part 5- Correction	1.35±1.01	1	0-4

X: Mean, SD: Standard deviation, Min: minimum; Max: maximum; S: Second

As a result of the correlation analysis, a statistically fair level of significant and positive correlation was found between the Standardized Mini Mental Test (SMMT) and the Fugl Meyer Upper Extremity Rating Scale, Frenchay Arm Test and Box-Block Test (affected extremity) (respectively; r:0.327, p:0.042; r:0.327, p:0.042; r:0.326, p:0.043). In addition, a moderately significant and positive relationship was found between SMMT and Flexor synergy and Normal reflex activity, which are sub-headings of the FMA-UE (r:0.470, p:0.003 and r:0.401, p:0.011, respectively). A statistically fair level of significant and negative correlation was found between the

**Table 4.** Findings related to the upper extremity rating scales of individuals

Scales	X±SD	Median	Min-Max
Fugl-Meyer Upper Extremity Rating Scale (Total)	40.20±16.78	43	7-66
Reflex activity (FMA-UE)	3.33±1.32	4	0-4
Flexor synergy (FMA-UE)	7.71±2.75	8	1-12
Extensor synergy (FMA-UE)	4.35±1.59	4	1-6
Combined synergistic movements (FMA-UE)	3.71±2.06	4	0-6
Non-synergy movements (FMA-UE)	3.20±1.92	3	0-6
Normal reflex activity (FMA-UE)	0.97±2.15	0	0-6
Wrist assessment (FMA-UE)	5.66±3.60	7	0-10
Hand assessment (FMA-UE)	8.30±4.05	8	0-14
Coordination and speed (FMA-UE)	3.07±1.36	3	1-5
Frenchay Arm Test	3.64±1.67	4	0-5
Box-Block Test (affected upper extremity)	24.94±14.25	26	0-55
Box-Block Test (unaffected upper extremity)	49.28±14.42	49	21-84

FMA-UE: Fugl-Meyer Upper Extremity Rating Scale

Stroop Test Chapter 3 Error section and the reflex activity, which is one of the sub-headings of FMA-UE (r:-0.391, p:0.014). On the other hand, a statistically fair significant and negative correlation was found between the Section 2 Correction section of the Stroop Test and the reflex activity and non-synergy movement sections of the FMA-UE, and a moderately significant and negative relationship between the extensor synergy section (r:-0.384, respectively). p:0.016; r:-0.389, p:0.015; r:-0.564, p<0.001; r:-0.389, p:0.015). There was no significant relationship between the other parts of the Stroop test and the upper extremity rating scales (p>0.05). The findings of the correlation analysis of the data are shown in Table 5.

**Table 5.** Correlation analysis findings of the data

Variables	Fugl Meyer UERS	Frenchay Arm Test	Box and Block Test (affected)	Box and Block Test (unaffected)
SMMT	r:0.327* p:0.042	r:0.327* p:0.042	r:0.326* p:0.043	r:0.229 p:0.161
Stroop P1-time	r:-0.220 p:0.179	r:-0.156 p:0.342	r:-0.246 p:0.131	r:-0.036 p:0.827
Stroop P2-time	r:-0.87 p:0.597	r:-0.196 p:0.232	r:-0.187 p:0.255	r:-0.052 p:0.751
Stroop P3-time	r:0.092 p:0.576	r:-0.051 p:0.760	r:-0.177 p:0.280	r:-0.002 p:0.989
Stroop P4-time	r:-0.113 p:0.495	r:-0.190 p:0.246	r:-0.199 p:0.225	r:-0.023 p:0.889
Stroop P5-time	r:-0.008 p:0.962	r:-0.074 p:0.653	r:-0.113 p:0.493	r:-0.155 p:0.346
Stroop P1-error	r:-0.189 p:0.249	r:-0.017 p:0.918	r:-0.096 p:0.560	r:0.102 p:0.536
Stroop P2-error	r:-0.250 p:0.125	r:-0.105 p:0.526	r:-0.274 p:0.091	r:0.006 p:0.973
Stroop P3-error	r:0.009 p:0.956	r:0.078 p:0.636	r:0.115 p:0.485	r:-0.153 p:0.352
Stroop P4-error	r:0.065 p:0.693	r:-0.077 p:0.640	r:0.070 p:0.673	r:0.177 p:0.282
Stroop P5-error	r:0.139 p:0.398	r:0.103 p:0.533	r:0.248 p:0.128	r:0.217 p:0.184
Stroop P1-correction	r:-0.051 p:0.757	r:-0.035 p:0.832	r:0.015 p:0.925	r:0.013 p:0.936
Stroop P2-correction	r:-0.347* p:0.031	r:-0.291 p:0.073	r:-0.278 p:0.087	r:-0.177 p:0.282
Stroop P3-correction	r:0.038 p:0.818	r:-0.180 p:0.274	r:-0.182 p:0.266	r:0.173 p:0.294
Stroop P4-correction	r:0.126 p:0.446	r:0.099 p:0.550	r:0.203 p:0.214	r:-0.028 p:0.865
Stroop P5-correction	r:0.128 p:0.436	r:-0.031 p:0.850	r:0.104 p:0.527	r:-0.123 p:0.456

SMMT: The Standardized Mini-Mental Test, Stroop P: Stroop Part, r: correlation coefficient, \*p<0.05.

## DISCUSSION

In the recent study in which we examined the relationship between cognitive level and upper extremity functions in patients with chronic stroke, we found that there was a relationship between general cognitive level and upper extremity functions, but there was a limited relationship between cognitive information processing and attention skills and upper extremity functions. These results support our study hypothesis in general.

Imaging studies examining the relationship between cognition and upper extremity function in stroke patients show that movement-related activity in the premotor and prefrontal cortex is dependent on the cognitive context [34]. Another similar physiologic basis is that the area occupied by the upper extremity in the motor and sensory homunculus is greater than the lower extremity. Especially in middle cerebral artery injuries, since this artery perfuses the areas corresponding to the upper extremity and face regions in the homunculi, the effect will be greater in these areas. Therefore, any damage to the cortex will affect upper extremity functions more [35].

In a study that evaluated upper extremity functions with the Box-Block Test in acute stroke patients, similar to our study, and examined its relationship with cognition, it was shown that cognitive performance is effective on arm motor skills [36]. Similar to our study, Roh et al. examined the relationship between cognition and upper extremity performance in chronic stroke patients and the effects of these parameters on activities of daily living. Their results showed that the two parameters were related to each other and to the performance in daily living activities [37].

The results of the recent study support our hypothesis based on this neurophysiological basis and show that there is a relationship between general cognitive level and upper extremity functions.

There are four studies examining the relationship between the affected extremity and cognitive functions in stroke patients, and these studies found correlations between both attention and visuospatial functions and extremity functions [38- 41]. The relationship between sustained attention and affected upper extremity functions has also been demonstrated in patients with right hemisphere involvement, especially two years after stroke [39]. In addition, an association was found between affected hand motor skills and basic attention and reasoning in the acute period [38]. Levin et al. showed that executive function performance has a more significant effect on upper extremity function than other cognitive abilities in stroke patients [42]. In another study, this was attributed to the fact that executive functions play a major role in the body's ability to make the necessary adjustments to changing environmental conditions as a result of functional movements [43]. In this recent study, we found associations between information processing speed and selective attention abilities and upper extremity parameters in patients with chronic stroke that are difficult to generalize. The reasons for this result can be varied. To assess executive functions and selective attention, we used the Stroop Test, a cognitive test consisting of different colors and words in which abilities are assessed in units of duration, error and correction. The test is a commonly used test to assess selective attention and executive functions in stroke patients and in upper extremity injuries in order to examine the effect of cognitive performance on motor performance [44,45]. We included stroke patients with a wide age range and varying levels of visual and reading skills, which may have affected their performance on the Stroop Test. The SMMT, another test we use to assess cognitive level, is one of the most well-known tests for the assessment of general cognitive level, memory and dementive disorders. Our findings showed that there were significant correlations between all parameters evaluating the upper extremity and SMMT. This may be attributed to both the physiological basis of our study and the way the test was adapted. The majority of the SMMT was administered in a question and answer format between the assessor and the patient, which facilitates the completion of the test for the patient. Although findings of the recent study support similar studies in the

literature, there is a need to find stronger associations using different measures specific to these abilities in order to make stronger interpretations.

Recovery in upper extremity functions after stroke is more difficult compared to lower extremity. This is due to the fact that upper extremity functions are more complex. From a functional point of view, upper extremity especially hand functions involve difficult fine motor skills such as manipulation, grasping and holding; lower extremity functions include skills such as keeping the body in balance and in an upright position and walking [11,46]. Upper extremity movements require more cognitive components as they require more attention, concentration and a more goal-oriented approach compared to lower extremity movements. Therefore, losses at the level of cognition affect upper extremity functions more. In our study, we used three different tests to evaluate upper extremity functions. There are many studies using these tests in stroke patients [47-49]. The Fugl Meyer Upper Extremity Assessment Scale is a comprehensive scale that considers upper extremity functions in the context of symptoms, similar to a neurological examination. The Frenchay Arm Test evaluates upper extremity functions based on functional skills in daily life. Box-Block Test is an easily applicable and understandable test that evaluates gross motor skills of the hand. In the design of the study, it was aimed to comprehensively evaluate upper extremity functions by using these three tests. One of the aspects that makes our study different from the relevant literature is that it provides a more holistic view of upper extremity functions by using different tests that evaluate more than one function, rather than a single test.

## Limitations

Our study has some limitations. The first of these was that the age range of the patients included in the study was wide and therefore their various physical abilities showed a wide range of variation. This caused the results of the tests to be very variable. Another limitation was that it was not taken into account that whether the patients were right or left dominant could affect their cognitive and arm skills.

The literature focuses on the contribution of using various cognitive strategies during the treatment process to the development of upper extremity function rather than the evaluation phase. We think that the results of recent study will be beneficial especially in terms of giving clinicians a preliminary idea about the patients in the evaluation process, providing a more individualized treatment program for patients, and using more accurate techniques during rehabilitation.

## CONCLUSION

The results of recent study show that there is a significant relationship between general cognitive level and upper extremity function in patients with chronic stroke.

Clinicians and researchers may consider these two components as two components that may affect each other in the evaluation stages of patients and may help to create more personalized and successful programs. Planning rehabilitation programs by taking cognition and upper extremity performance into consideration can make the process more goal oriented and effective in terms of intervention-time interaction.

**Ethical Approval:** 2020/14 Non-Interventional Clinical Research Ethics Committee of Pamukkale University

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## INVESTIGATION OF CLINICAL FACTORS AFFECTING PERCEIVED PAIN INTENSITY IN FEMALE PATIENTS WITH KNEE OSTEOARTHRITIS

## DİZ OSTEOARTRİTLİ KADIN HASTALARDA ALGILANAN AĞRI ŞİDDETİNİ ETKİLEYEN KLİNİK FAKTÖRLERİN İNCELENMESİ

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## ABSTRACT

**Objective:** The present study aimed to identify the clinical variables influencing perceived pain intensity at rest, during activity, and at night in female patients with knee osteoarthritis (OA).

**Method:** One hundred-six female patients with knee OA (mean age, 58.50±9.48 years; mean BMI, 30.73±5.53 kg/m<sup>2</sup>) were included. The Visual Analogue Scale (VAS), active range of motion (AROM), strength of the iliopsoas, gluteus medius, quadriceps femoris, and hamstring muscles, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and Short Form-12 (SF-12) were outcome measures. A multivariate or univariate regression analysis was conducted to examine the relationship between the VAS ratings and AROM, muscle strength, WOMAC, SF-12, and Kellgren-Lawrence classification.

**Results:** The mean values for VAS-rest, VAS-activity, and VAS-night were 3.02±2.32, 6.62±1.96, and 3.89±2.72, respectively. A significant correlation was found between VAS-rest and quadriceps femoris muscle strength, as well as the WOMAC score. Similarly, VAS-activity showed a significant association with hip flexion and knee extension AROM, quadriceps femoris muscle strength, Grade 3 or Grade 4 OA, physical component score of SF-12, and WOMAC score (p<0.05). There was also a significant association between VAS-activity and age ( $\beta$ : -0.194, 95%CI: -0.043 - 0.021, p=0.04).

**Conclusion:** The quadriceps femoris muscle strength and functional level of the patients with knee OA significantly predict both pain intensity at rest and during activity. In addition, pain intensity during activity was found to be associated with hip flexion and knee extension AROM, Kellgren-Lawrence grading, the physical component of quality of life, and age.

**Key Words:** Function, Knee, Osteoarthritis, Pain, Regression

## ÖZ

**Amaç:** Bu çalışmanın amacı, diz osteoartriti (OA) olan kadın hastalarda dinlenme anında, aktivite sırasında ve gece algılanan ağrı yoğunluğunu etkileyen klinik değişkenleri belirlemektir.

**Yöntem:** Diz OA'lı 106 kadın hasta (ortalama yaş, 58.50±9.48 yıl; ortalama VKİ, 30.73±5.53 kg/m<sup>2</sup>) çalışmaya dâhil edildi. Sonuç ölçümleri Vizüel Analog Skala (VAS), aktif eklem hareket açıklığı (AEHA), iliopsoas, gluteus medius, kuadriseps femoris ve hamstring kas kuvveti, Western Ontario ve McMaster Üniversiteleri Osteoartrit İndeksi (WOMAC) ve Kısa Form-12 (SF-12) idi. VAS skorları ile AEHA, kas kuvveti, WOMAC, SF-12 ve Kellgren-Lawrence sınıflandırması arasındaki ilişkiyi incelemek için çok değişkenli veya tek değişkenli regresyon analizi yapıldı.

**Bulgular:** VAS-dinlenme, VAS-aktivite ve VAS-gece için ortalama değerler sırasıyla 3.02±2.32, 6.62±1.96 ve 3.89±2.72 idi. VAS-dinlenme ile kuadriseps femoris kas kuvveti ve WOMAC skoru arasında anlamlı bir ilişki bulundu. Benzer şekilde, VAS-aktivite ile kalça fleksiyonu ve diz ekstansiyonu AEHA, kuadriseps femoris kas kuvveti, Evre 3 veya Evre 4 OA, SF-12 fiziksel komponent skoru ve WOMAC skoru arasında anlamlı bir ilişki bulundu (p<0.05). VAS-aktivite ile yaş arasında da anlamlı bir ilişki vardı ( $\beta$ : -0.194, %95CI: -0.043 -0.021, p=0.04).

**Sonuç:** Diz OA'lı hastaların kuadriseps femoris kas gücü ve fonksiyon seviyesi, hem istirahat hem de aktivite sırasındaki ağrı şiddetini anlamlı olarak öngörmektedir. Ayrıca aktivite sırasındaki ağrı yoğunluğunun kalça fleksiyonu ve diz ekstansiyonu AEHA, Kellgren-Lawrence derecelendirmesi, yaşam kalitesinin fiziksel bileşeni ve yaş ile ilişkili olduğu bulundu.

**Anahtar Kelimeler:** Fonksiyon, Diz, Osteoartrit, Ağrı, Regresyon

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## INTRODUCTION

Osteoarthritis (OA) is the most common joint disease in individuals over 60 years of age globally [1]. OA, seen in 10% of older adults, is a dynamic process affecting all joint structures, especially cartilage and bone tissue [2]. Although the cause of OA remains unclear, it most commonly affects the knee and hip joints [2-4]. The current evidence indicates that it depends on many factors that affect cartilage homeostasis [5]. The most important risk factor for OA is the female sex [4,6]. It is suggested that some features often found in females, such as the increased prevalence of obesity, reduced muscle tone, and heightened joint hypermobility, contribute to varied degrees of joint instability [3-5]. This instability may create a favorable environment for the occurrence of recurrent microtrauma, ultimately resulting in irreparable joint damage [3-5]. Other risk factors include obesity [7], genetic characteristics [8], excessive physical work, sports, or occupational work [9], previous knee joint trauma [10], vitamin D deficiency [11], and chondrocalcinosis [12].

Patients with knee OA commonly describe symptoms like joint pain, joint stiffness, and reduced joint functionality, which can ultimately lead to disability [13]. Pain and disability often occur when there are functional limitations, structural alterations in the subchondral bone, cartilage degradation, and the involvement of surrounding soft tissue [14]. Patients often experience two types of pain: occasional but typically severe or intense pain and persistent background pain characterized by aching sensations [15,16]. The progression of pain associated with osteoarthritis (OA) can be observed through several stages. In the initial stages, patients typically experience pain that is triggered by physical activity. As the condition advances, this discomfort gradually becomes more persistent and is punctuated by intermittent, intense pain [16,17]. In the more advanced stages of the disease, pain can be continuous, disrupting sleep patterns at night, and causing deterioration in the patient's psychological health. Social and recreational activities, subsequently reducing the health-related quality of life [17].

When reviewing the literature, many valuable studies focus on the impact of various treatment approaches on knee OA pain, but few studies investigate the pain intensity and influencing factors in untreated patients with knee OA. Therefore, the present study aimed to identify the clinical factors that influence the perceived pain intensity at rest, during activity, and at night in female patients with knee OA.

## METHOD

### Study Design and Setting

The present study was a cross-sectional study conducted in İstanbul, Türkiye.

### Participants

All participants with knee OA who consulted the orthopedic clinics of İstanbul University and Avrasya Center for Orthopedics between February 2022 and August 2023 were recruited to the study after undergoing an assessment that considered the predefined criteria for participation. Orthopedic surgeons performed a clinical examination and radiological imaging assessment to diagnose knee OA of grades 2 to 4 based on the Kellgren and Lawrence (1957) scale [18]. Volunteer participants who met the clinical criteria for diagnosis of knee OA, as outlined by the American College of Rheumatology standards (pain in the knee and at least three of the following: age >50 years, stiffness <30 min, crepitus, bony tenderness, bony enlargement, and no palpable warmth) were included [19]. The criteria for exclusion were as follows: (1) a confirmed diagnosis of neurologic diseases, rheumatoid arthritis, radiculopathy, peripheral neuropathy, or psychiatric diseases; (2) a documented history of knee surgery or intraarticular corticosteroid injection in the last 6 months; (3) disclosed use of oral or topical analgesics for knee pain in the last 6 months; (4) participation in a lower limb physical therapy program in the last six-months; (5)

inability to read and write; (6) inability to understand and follow simple instructions; and (7) presence of visual or hearing impairments.

### Sample Size Calculation

The sample size was calculated by using the G\*Power 3.1.9.2 sample size calculation program. The calculations were based on a moderate effect size ( $\rho^2=0.13$ ), an alpha level of 0.05, a 95% confidence interval, the desired power of 80%, and the number of predictors ranging from 1 to 4 [20]. The parameters result in a sample size of 60-103 participants. A total of 110 patients were invited to the study.

### Outcome Measures

Outcome measures included perceived pain intensity, active range of motion (AROM), muscle strength, functional status, and health-related quality of life. All eligible individuals were questioned regarding their age, body mass index (BMI), dominant extremity, affected extremity, education, and occupation. The Charlson Comorbidity Index was used as a metric for assessing the presence of comorbidities.

The *Visual Analogue Scale (VAS)* was used for the perceived pain intensity under three conditions: at rest (VAS-rest), during activity (VAS-activity), and at night (VAS-night). This assessment was made using a 10 cm line scale ranging from no pain (0) to severe pain (10). The score was determined by measuring the distance on a 10 cm line [21].

*Active Range of Motion:* The measurement of AROM for hip flexion, knee flexion, and knee extension was conducted according to the methodology outlined by Norkin and White [22], utilizing a digital goniometer (Baseline Evaluation Instrument® Fabrication Enterprises, Inc). All participants were provided with verbal instructions regarding the testing technique. The affected side was measured three times, with a 30-second interval between tests, and the average value was computed.

*Muscle Strength:* The isometric muscle strength of the iliopsoas, gluteus medius, quadriceps, and hamstring muscles was assessed using the methodology outlined by Mentiply et al. [23], employing a hand-held dynamometer (The Lafayette Instrument Company, Lafayette, Indiana, model 01160). All participants were provided with verbal instructions regarding the testing technique. Each limb was measured three times, with a 30-second interval between tests, and the average value was computed.

The *Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC)* is a reliable and valid tool for assessing outcomes in patients with knee OA. It consists of 24 items and is divided into three main categories. The items are rated on a 5-point Likert scale ranging from none to extreme. The WOMAC score ranges from 0 to 96, with higher values indicating lower function levels [24,25].

The *Short Form-12* is a reliable and valid tool for assessing subjective health-related quality of life perception. It consists of 12 items, including seven items related to physical components (PCS-12) and five items related to mental components (MCS-12). Both scores range from 0 to 100, with higher values indicating a better health-related quality of life [26,27].

### Ethical Approval

The study protocol was approved by the Non-Interventional Clinical Research Ethics Committee at Haliç University (approval number 2021-147 on June 24, 2021). The study was carried out in accordance with the Declaration of Helsinki. The trial was registered on ClinicalTrials.gov with the registration number NCT05238857. Before the data collection, all participants provided written informed consent.

### Statistical Analysis

Statistical Package for Social Science (IBM SPSS Statistics New York, USA) version 20.0 was used to perform statistical analyses. Descriptive statistics are presented as mean±standard deviation for

continuous data, whereas binary and categorical variables are shown as numbers and frequencies. Regression analysis was performed to examine the relationship between VAS-rest, VAS-activity, and VAS-night, as well as potential factors that may contribute to pain severity in patients with knee OA. A multivariate linear regression analysis was conducted to examine the relationship between AROM, muscle strength, SF-12, VAS-rest, VAS-activity, and VAS-night. The association between WOMAC, age, VAS-rest, VAS-activity, and VAS-night was analyzed with univariate regression analysis. Analysis for Kellgren-Lawrence classification was conducted with logistic regression due to being a categorical variable. The significance level was set at  $p < 0.05$ .

## RESULTS

One hundred ten participants with a clinical diagnosis of knee OA were assessed for eligibility, and a total of 106 voluntary participants were included in the study. The sociodemographic and clinical characteristics of the participants are presented in Table 1. The mean scores were  $3.02 \pm 2.32$  for VAS-rest,  $6.62 \pm 1.96$  for VAS-activity, and  $3.89 \pm 2.72$  for VAS-night. The mean WOMAC score was  $50.54 \pm 16.61$ , the mean PCS-12 score was  $36.42 \pm 8.74$ , and the mean MCS-12 score was  $44.29 \pm 8.72$ .

**Table 1.** Sociodemographic and clinical characteristics of the participants (n=106)

Characteristics		
Age (years)		58.50±9.48
BMI (kg/m <sup>2</sup> )		30.73±5.53
CCI (score)		2.83±2.24
WOMAC (score)		50.54±16.61
	<25	12 (11.3)
BMI (n,%)	25-30	37 (34.9)
	≥30	57 (53.8)
	Unilateral	52 (49.1)
Affected extremity (n,%)	Bilateral	54 (50.9)
	Illiterate	5 (4.7)
	Primary school	59 (55.7)
Education (n,%)	Secondary school	18 (17.0)
	High school	12 (11.3)
	College/University	12 (11.3)
	VAS-rest	3.02±2.32
Pain Intensity (cm)	VAS-activity	6.62±1.96
	VAS-night	3.89±2.72
	Iliopsoas	9.03±2.84
Muscle Strength (kg.N <sup>-1</sup> )	Gluteus medius	7.22±2.83
	Quadriceps femoris	8.99±2.05
	Hamstring	8.61±4.46
Kellgren-Lawrence Classification (n,%)	Grade 2	24 (22.6)
	Grade 3	56 (50.0)
	Grade 4	29 (27.4)
Dominant Side (n,%)	Right	102 (96.2)
	Left	4 (3.8)
Occupation (n,%)	Active worker	17 (16.0)
	Housewife	68 (64.2)
	Retired	21 (19.8)
Active Range of Motion (°)	Hip flexion	110.34±17.16
	Knee flexion	116.54±17.03
	Knee extension	-5.04±2.87
Short Form-12 (score)	PCS-12	36.42±8.74
	MCS-12	44.29±8.72

BMI:Body Mass Index, CCI:Charlson Comorbidity Index, MCS-12:Mental Component Summary score of SF-12, PCS-12:Physical Component Summary score of SF-12, SF-12:Short Form-12, VAS:Visual Analogue Scale, WOMAC:The Western Ontario and McMaster Universities Arthritis Index.

Linear regression analysis between the AROM, muscle strength, and VAS scores is shown in Table 2. A significant correlation was found between VAS-rest and quadriceps femoris muscle strength. VAS-activity was associated with hip flexion and knee extension AROM, and quadriceps femoris muscle strength ( $p < 0.05$ ). However, VAS-night was not found to be associated with AROM or muscle strength ( $p > 0.05$ ).

Linear regression analysis between disease-related outcome measurements, age, and VAS scores is shown in Table 3. VAS-rest was found to be associated with the WOMAC score ( $p = 0.021$ ). VAS-activity was found to be associated with PCS-12 score, WOMAC score, and age ( $p < 0.05$ ). In addition, there was a significant association between VAS-activity and having Grade 3 or Grade 4 knee OA. However, VAS-night was not found to be associated with disease-related outcome measurements or age ( $p > 0.05$ ).

## DISCUSSION

The findings of the present study revealed that perceived pain intensity during rest was correlated with quadriceps femoris muscle strength and WOMAC score, whereas perceived pain intensity during activity was correlated with age, hip flexion, and knee extension AROM, quadriceps femoris muscle strength, having a Grade 3 and Grade 4 knee OA, physical health-related quality of life, and WOMAC score. The correlation ranged from mild to strong. Current evidence indicates that sleep disturbance is associated with altered pain processing, with the severity of knee OA further exacerbating the problem [28, 29]. However, an association between the outcome measures of the current study and the perceived pain intensity at night was not found. Following a focus group study on night pain in knee OA, it was reported that simple dichotomous questions at a single time point or pain severity measures like a VAS might not accurately measure night pain in knee OA [30]. In our study, the possible relationship between night pain and clinical variables may not have been revealed due to our pain assessment method.

The predisposing factors for perceived pain intensity in patients with knee OA were always a topic of interest since the findings would allow clinicians to shape their rehabilitation programs accordingly. Literature suggests that there are many links between the pain of the patient and numerous factors. For instance, in one of the latest articles on this topic, Bjerre-Bastos et al. [31] reported that pain at rest was significantly correlated with the WOMAC score in patients with knee OA. Moreover, the association between pain at activity and WOMAC was stronger than the association between pain at rest and WOMAC. Our findings were in line with the results reported in the study by Bjerre-Bastos et al. [31]. This result is reasonable considering that activities, especially those involving weight-bearing, trigger biochemical mechanisms causing pain [31, 32]. Another rationale for this notion could be that the data from the literature indicate that patients with early OA have more pain in weight-bearing conditions than at rest, and the association between pain at rest and the WOMAC score weakens as the disease progresses [33]. These findings also support the association found in the current study between pain during activity and having Grade 3 and 4 levels of knee OA, health-related quality of life, and age.

A recent study indicated that aging reduces pain sensitivity for lower pain intensities [34]. Besides, pain during activity is significantly associated with a decline in health-related quality of life, particularly in the elderly [35]. The experience of chronic pain tends to increase with age, particularly in the context of osteoarthritis, and pain can lead to decreased quality of life, with the latter being further affected by functional limitations [36]. Therefore, reducing knee pain is crucial for improving functionality and health-related quality of life in patients with knee OA. Furthermore, literature findings collectively suggest that perceived pain intensity in patients with knee OA was correlated with weaker quadriceps femoris muscle strength, and this association was especially prominent in females [37] because of the quadriceps

**Table 2.** Linear regression analysis between the active range of motion, muscle strength, and VAS scores

	VAS-rest		VAS-activity		VAS-night	
	$\beta$ (95% CI for B)	<i>p</i>	$\beta$ (95% CI for B)	<i>p</i>	$\beta$ (95% CI for B)	<i>p</i>
<b>Model-1: ROM</b>						
Hip flexion	-0.082 (-0.309-0.136)	0.444	-0.281 (-0.065-0.001)	0.033*	-0.120 (-0.065-0.027)	0.412
Knee flexion	-0.028 (-0.042-0.035)	0.846	-0.002 (-0.032-0.031)	0.991	-0.097 (-0.060-0.029)	0.494
Knee extension	-0.047 (-0.212-0.135)	0.664	0.197 (-0.008-0.278)	0.040*	0.118 (-0.090-0.314)	0.274
<b>Model-2: Muscle Strength</b>						
Iliopsoas	-0.140 (-0.341-0.102)	0.288	-0.156 (-0.302-0.076)	0.215	-0.003 (-0.270-0.263)	0.982
Gluteus medius	0.236 (-0.122-0.518)	0.222	-0.094 (-0.339-0.206)	0.876	-0.160 (-0.540-0.227)	0.420
Quadriceps femoris	-0.095 (-0.104-0.008)	0.021*	-0.258 (-0.583-(-0.061))	0.016*	-0.077 (-0.427-0.200)	0.475
Hamstring	-0.176 (-0.107-0.028)	0.251	0.107 (-0.037-0.078)	0.488	0.147 (-0.042-0.120)	0.348

\* $p < 0.05$ , VAS-rest: Visual Analogue Scale score at rest, VAS-activity: Visual Analogue Scale score during activity, VAS-night: Visual Analogue Scale score at night.

**Table 3.** Linear regression analysis between disease-related outcome measurements, age, and VAS scores

	VAS-rest		VAS-activity		VAS-night	
	$\beta$ (95% CI for B)	<i>p</i>	$\beta$ (95% CI for B)	<i>p</i>	$\beta$ (95% CI for B)	<i>p</i>
<b>Model-3: Kellgren-Lawrence Classification</b>						
Grade 2	-	-	-	-	-	-
Grade 3**	0.966 (0.806-1.158)	0.711	1.288 (1.013-1.638)	0.004*	0.926 (0.797-1.077)	0.321
Grade 4**	1.152 (0.947-1.402)	0.157	1.874 (1.178-2.105)	0.002*	1.036 (0.873-1.230)	0.685
<b>Model-4: SF-12</b>						
PCS-12	-0.048 (-0.066-0.041)	0.636	-0.314 (-0.158-(-0.038))	0.002*	-0.144 (-0.077-0.012)	0.155
MCS-12	0.123 (-0.021-0.086)	0.227	-0.003 (-0.061-0.059)	0.103	0.165 (-0.008-0.082)	0.975
<b>Model-5: WOMAC</b>						
Score	0.224 (0.005-0.058)	0.021*	0.551 (0.046-0.084)	<0.0001*	0.062 (-0.022-0.042)	0.525
<b>Model-6: Age</b>						
	-0.152 (-0.341-0.102)	0.123	-0.194 (-0.043 - 0.021)	0.041*	-0.161 (-0.540-0.227)	0.334

\*\*Reference category is Grade 2, \* $p < 0.05$ , MCS-12: Mental Component Summary score of SF-12, PCS-12: Physical Component Summary score of SF-12, SF-12: Short Form-12, VAS-rest: Visual Analogue Scale score at rest, VAS-activity: Visual Analogue Scale score during activity, VAS-night: Visual Analogue Scale score at night, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

femoris muscle strength and this association was especially prominent in females [37] because of the quadriceps femoris muscle atrophy, neuromuscular alterations, and inflammatory processes [31]. However, it is not clear whether quadriceps femoris muscle strength is the result or the reason for pain in patients with knee OA. For instance, Bokaeian et al. [32] reported that strength training for quadriceps femoris muscle did not result in improvement in pain and functional status in patients with knee OA, suggesting that quadriceps femoris muscle strength was not associated with pain. On the other hand, DeVita et al. [33] showed that 12 weeks of strengthening program decreased the pain and increased the function in patients with knee OA, suggesting an increase in quadriceps femoris muscle strength would result in an improvement in knee pain. Our findings conclusively suggest that quadriceps femoris muscle strength was associated with both pain at rest and pain during activity. There is conflicting evidence from the literature and differences among the studies, but a recent paper may explain this reason. According to a recent article by Alshahrani et al. [31], the association between muscle strength of quadriceps femoris and pain was not a causative relationship. Rather, pain was a mediator between muscle strength and postural control, thus affecting overall functional mobility [31]. These findings underscore the importance of addressing pain and muscle strength in the management of knee OA to improve functional mobility. Another important finding of the current study was that AROM in hip flexion and knee extension was associated with pain during activity,

however, this association was weak. Although the association between OA and AROM has already been well-known for years [34], the knowledge regarding the association between knee extension ROM

and pain was just recently broadened by a paper stating that decreased knee extension ROM was observed during gait in patients with OA [35]. The potential consequences of a reduction in normal terminal knee extension can significantly impact knee mechanics during ambulation and weight-bearing activities [36]. Similarly, hip flexion ROM was also stated as a determinant of increased pain in patients with hip and/or knee OA [37]. The loss of ROM was particularly underlined since this could result in the development of secondary OA and/or increased pain, especially during daily tasks [38]. Therefore, it is important to target both strengthening and stretching training when treating patients with knee OA [39].

### Limitations

This study has some limitations that should be mentioned. Firstly, all participants were female, which impedes the generalization of the results. Secondly, pain at rest, during activity, and at night was assessed with a single-item pain-intensity measure. However, the VAS is a reliable scale, with the smallest errors in the measurement of knee OA pain [38]. Another limitation of this study was that gluteus maximus muscle strength, and hip internal and external rotation ROM were not evaluated. Restricted joint mobility in the external rotation of the hip was found to be an important determinant of impairment in patients with OA [39].

### CONCLUSION

To summarize, our findings pointed out that hip flexion and knee extension AROM, quadriceps femoris muscle strength, and age are significant predictors of pain intensity during activity. In addition, pain intensity during activity was also associated with the physical

component of quality of life and function. The present findings and the evidence suggest that enhancing the AROM in knee extension and hip flexion, along with augmenting the strength of the quadriceps femoris muscle, is important for the rehabilitation of knee OA, particularly in terms of decreasing the perceived pain intensity at rest and during activity. However, none of the factors investigated in this study were associated with the pain experienced at night. Therefore, further studies are still required to determine the possible predisposing factors for night pain in patients with knee OA.

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