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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Book section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR,

editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a single author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or technical report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study KidneyInt: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktivitelerive Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. SağlıkBilimleriEnstitüsü, DoktoraTezi. 2007.

Manuscripts accepted for publication, not published yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub ahead of print articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. DiagnIntervRadiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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RESEARCH ARTICLES

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RESEARCH / ARAŞTIRMA
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RESULTS OF VENTRICULOPERITONEAL SHUNT SURGERY USING ELECTROMAGNETIC AND OPTICAL NAVIGATION: EXPERIENCES IN 31 PATIENTS

ELEKTROMANYETİK VE OPTİK NAVİGASYON KULLANILARAK YAPILAN VENTRİKÜLOPERİTONEAL ŞANT CERRAHİSİNİN SONUÇLARI: 31 HASTADAKİ DENEYİMLER

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ABSTRACT

Objective: The ventriculoperitoneal (VP) shunt procedure is frequently performed in the field of neurosurgery to treat pathologies such as normal-pressure hydrocephalus, infection, trauma, or VP shunt dependency after subarachnoid haemorrhage. Although the precise impact of ventricular catheter placement on shunt dysfunction remains not fully elucidated, it is well established that a shunt catheter bypassing the ventricle will lead to shunt dysfunction shortly after placement. Therefore, navigation-assisted shunt surgery gains significance in order to reduce the number of cannulation attempts and for inserting the ventricle catheter in the proper position compared to free hand catheter placement.

Material and Method: This retrospective study enrolled 31 patients who underwent VP shunt placement in two different clinics by two different surgeons using electromagnetic and optical navigation between 2016 and 2023. The study population was grouped into two. In the first group, 16 patients underwent VP shunt surgery using stereotactic optical navigation and also Strata (Medtronic, Minneapolis, USA) programmable valve in Liv Hospital, İstanbul. In the second group, 15 patients were operated using EM navigation and Codman Certas (Integra Lifesciences, New Jersey, USA) programmable valve in Florence Nightingale Hospital, İstanbul.

Result: The age range of patients was 36 to 87 years, with a mean age of (73.74±9.06). Twelve of the patients participating in the study were male, and 19 were female. All patients were operated because of normal-pressure hydrocephalus. In the EM navigation group, there

ÖZET

Amaç: Ventriküloperitoneal (VP) şant prosedürü, nöroşirürji alanında sıkça yapılan bir işlemdir ve normal basınçlı hidrosefali, enfeksiyon, travma veya subaraknoid kanama sonrası VP şant bağımlılığı gibi patolojileri tedavi etmek için kullanılır. Ventrikülü bypass eden bir şant kateterinin yerleştirilmesi kısa bir süre sonra şant disfonksiyonuna yol açacağı kesin olarak bilinmektedir. Bu nedenle, navigasyon destekli şant cerrahisi, serbest el kateter yerleştirmeye kıyasla kanülasyon denemelerinin sayısını azaltmada ve ventrikül kateterini uygun konumda yerleştirmede önem kazanır.

Gereç ve Yöntem: Bu retrospektif çalışmaya, 2016 ile 2023 yılları arasında elektromanyetik (EM) navigasyon ve optik navigasyon kullanılarak iki farklı cerrah tarafından iki farklı klinikte VP şant yerleştirilen 31 hasta dahil edildi.Çalışma toplumu iki gruba ayrınları iki gruba ayırıldı. İlk grupta, 16 hastada Liv Hastanesi, İstanbul merkezinde stereotaktik optik navigasyon ve ayrıca Strata (Medtronic, Minneapolis, ABD) programlanabilir valv kullanımı yapıldı. İkinci grupta, 15 hasta Florence Nightingale Hastanesi, Istanbul merkezinde EM ve Codman Certas (Integra Lifesciences, New Jersey, ABD) programlanabilir valv kullanılarak ameliyat edildi.

Bulgular: Hastaların yaş aralığı 36 ila 87 yıl arasındaydı, ortalama yaşları (73.74±9.06) idi. Çalışmaya katılan hastaların 12'si erkek, 19'u kadındı. Tüm hastalar normal basınçlı hidrosefali nedeniyle ameliyat edildi. EM grubunda, ameliyat sonrası bilgisayarlı tomografi (BT) bulgularına ve Hayhurst tarafından tanımlanan radyolojik ölçeğe göre 7 grad I ve 8 grad II hasta bulunmaktaydı; optik navigasyon grubunda ise, 13 grad I ve 3 grad II hasta

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were 7 grade I and 8 grade II patients, and in the optical navigation group, there were 13 grade I and 3 grade II patients according to the postoperative CT findings and the radiological scale defined by Hayhurst. In three patients from the EM Navigation group, subdural effusion developed due to overdrainage in different shunt settings. In none of these three patients an additional surgical intervention was needed. There were no intraparenchymal haemorrhage or shunt dysfunction complications in our study.

Conclusion: The use of navigation in shunt surgery prevents proximal failure and reduces the complications of intraparenchymal haemorrhage and shunt dysfunction. Although the use of optical navigation requires the use of a pinned headrest and an extended preoperative preparation time, the accuracy of ventricular catheter placement is similar when using optical navigation and EM navigation, despite these disadvantages. Due to its cost-effectiveness and high accuracy, the optically guided navigation system can be used in shunt surgery, especially considering the extra cost of the electromagnetic neuronavigation system.

Keywords: Electromagnetic navigation, optical navigation, shunt dysfunction

bulunmaktaydı. EM navigasyon grubundaki üç hastada, farklı şant ayarlarında aşırı drene olmaya bağlı subdural effüzyon gelişti. Bu üç hastanın hiçbirinde ek cerrahiye gerek duyulmadı. Çalışmamızda intraparankimal kanama ya da şant disfonksiyonu komplikasyonları izlenmedi.

Sonuç: Şant cerrahisinde navigasyonun kullanımı, proksimal başarısızlığı önler ve intraparenkimal kanama ve şant disfonksiyonu komplikasyonlarını azaltır. Optik navigasyonun kullanımı, çivili başlık ve uzatılmış preoperatif hazırlık süresi gerektirse de, ventriküler kateter yerleştirme doğruluğu, bu dezavantajlara rağmen optik navigasyon ve EM navigasyon kullanıldığında benzerdir. Maliyet etkinliği ve yüksek doğruluk açısından, özellikle EM sisteminin ek maliyeti düşünüldüğünde, şant cerrahisinde optik navigasyon sistemi kullanılabilir.

Anahtar Kelimeler: Elektromanyetik navigasyon, optik navigasyon, şant disfonksiyonu

INTRODUCTION

Ventriculopertioneal (VP) shunt procedure is frequently being performed in the field of neurosurgery to treat pathologies such as normal-pressure hydrocephalus, infection, trauma or VP shunt dependency after subarachnoid haemorrhage (1). Idiopathic normal pressure hydrocephalus (iNPH) is a condition in which patients present with symptoms of gait disturbance, urinary incontinence and dementia, and the ventricles enlarge without an increase in CSF (cerebrospinal fluid) pressure and also without any secondary diseases. Every year, 6 of 100,000 people are diagnosed with iNPH (2). In VP shunt surgeries, the importance of maintaining a brief operative duration and meticulous attention to sterility cannot be overstated, as they are critical factors in preventing postoperative infections. The literature reports a positive correlation between prolonged surgical duration and the incidence of infection (3, 4).

In the United Kingdom, over 3000 VP shunt surgeries are performed annually, whereas in the United States, the number exceeds 18,000 (5). Shunt dysfunction occurs in approximately 40% of these patients within the first year, predominantly due to proximal obstruction (6-8). Although the precise impact of ventricular catheter placement on shunt dysfunction remains not fully elucidated, it is well-established that a shunt catheter bypassing the ventricle will lead to shunt dysfunction shortly after placement (9). Therefore, navigation-assisted shunt surgery is significant in order to prevent infection, reducing the number of cannulation attempts compared to free-hand catheter placement, and also surgical duration and ensuring proper catheter placement (10-12). VP shunt placement may be performed utilising either the free-hand technique or with the assistance of navigation technology. The use of stereotactic optical navigation in the placement of VP shunts is a precise technique for catheter positioning. However, it comes with disadvantages such as the requirement for the patient to be positioned in a pinned headframe and an extended preoperative preparation time (13). In the past few years, frameless navigation systems have been frequently used in neurosurgery for biopsy and catheter placement procedures due to their low complication rates and minimally invasive approaches.

This study aimed to evaluate and compare the safety and efficacy of VP shunt insertion using EM (electromagnetic) navigation and stereotactic optical navigation.

MATERIAL AND METHODS

We retrospectively examined 31 patients who underwent VP shunt placement in two different clinics by two different surgeons using electromagnetic navigation and optical navigation between 2016 and 2023. The study population was grouped into two. In the first group, 16 patients underwent VP shunt surgery using stereotactic optical navigation and also Strata (Medtronic, Minneapolis, USA) programmable valve in Liv Hospital, İstanbul. In the second group, 15 patients were operated using EM navigation and Codman Certas (Integra Lifesciences, New Jersey, USA) programmable valve in Florence Nightingale Hospital, İstanbul. Informed consent forms were obtained from all patients. This retrospective study was conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study received ethical approval from the Ethics Committee of

Gaziosmanpasa Education and Research Hospital (Date: 03.07.2024, No: 16). The age range of patients was 36 to 87 years, with a mean age of (73.74±9.06). There were 12 male patients and 19 female patients. All patients underwent surgery because of normal-pressure hydrocephalus (Table 1, 2). Postoperative control Computerised tomog-

Table 1: Characteristics of patients in the electromagnetic navigation group.

Patient	Age/sex	Indication	Proximal catheter position (Grade)	Shunt valve type	Complication
1	83/F	NPH	I	Codman	None
2	83/F	NPH	I	Codman	None
3	68/M	NPH	II	Codman	None
4	79/M	NPH	I	Codman	Subdural effusion
5	71/F	NPH	II	Codman	Subdural effusion
6	75/M	NPH	II	Codman	None
7	65/M	NPH	II	Codman	None
8	68/M	NPH	II	Codman	None
9	66/F	NPH	I	Codman	Subdural effusion
10	79/M	NPH	I	Codman	None
11	79/F	NPH	II	Codman	None
12	71/F	NPH	II	Codman	None
13	79/F	NPH	II	Codman	None
14	74/M	NPH	I	Codman	None
15	71/M	NPH	I	Codman	None

NPH: Normal Pressure Hydrocephalus, F: Female, M: Male

Table 2: Characteristics of patients in the optical navigation group.

Patient	Age/sex	Indication	Proximal catheter position (Grade)	Shunt valve type	Complication
1	74/F	NPH + Parkinson	I	Strata	None
2	87/F	NPH	I	Strata	None
3	85/M	NPH	I	Strata	None
4	74/F	NPH	II	Strata	None
5	36/F	NPH	I	Strata	None
6	82/M	NPH	I	Strata	None
7	73/F	NPH	II	Strata	None
8	70/F	Parkinson + NPH	I	Strata	None
9	67/M	NPH	I	Strata	None
10	85/F	Operated NPH	I	Strata	None
11	68/F	NPH	I	Strata	None
12	78/F	NPH	I	Strata	None
13	76/M	NPH	I	Strata	None
14	74/F	Operated NPH	I	Strata	None
15	72/F	NPH	II	Strata	None
16	74/F	NPH	I	Strata	None

NPH: Normal Pressure Hydrocephalus, F: Female, M: Male. Grade I is defined as the placement of the ventricular catheter tip within the ventricle without touching the ventricle wall, grade II as the catheter tip touching the ventricular wall or choroid plexus, and grade III as the catheter tip being located outside the ventricle and within the parenchyma.

raphy (CT) images were obtained to assess postoperative bleeding and to verify the proper positioning of the ventricular catheter. The ventricular catheter position was confirmed with postoperative CT scan, and its accuracy was evaluated using the scale mentioned by Hayhurst (9). Grade I is defined as the placement of the ventricular catheter tip within the ventricle without touching the ventricle wall, grade II as the catheter tip touching the ventricular wall or choroid plexus, and grade III as the catheter tip being located outside the ventricle and within the parenchyma. There was no intraparenchymal haemorrhage or shunt dysfunction in the study.

Surgical technique

Electromagnetic navigation

After routine preoperative anaesthesia assessment and intubation, the patient was positioned supine and the Axiem mobile emitter of StealthStation S7 (Medtronic Surgical Technologies, Louisville, Colorado, USA) was placed on the operating room table (Figure 1A). The axiem tracker device was placed on the patient's head and stabilised with sterile drape. The anatomical landmarks on the patient's face were registered into the navigation software using a navigation probe, and the reliability of the system was verified using the navigation software. Routine preoperative sterilisation procedures were performed (Figure 1B). The scalp was incised in the frontal area consistent with Kocher's point. The subcutaneous tissue was dissected and the burr hole was created using an automatic perforator. Dura was coagulated and incised. A retroauricular curvilinear incision was made and continued with subgaleal dissection to create a pocket for the shunt valve. The shunt passer was passed from the abdominal incision to the retroauricular incision using the subcutaneous route. The peritoneal shunt catheter was moved inside the passer from the abdominal incision to the retroauricular incision. The ventricular catheter was guided to the preoperatively targeted point within the ventricle using Axiem navigation (Figure 1C). Peroperatively, the ventricular catheter was monitored in real-time with the assistance of the navigation software (Figure 1D). The target point for the ventricular catheter was the ipsilateral foramen of Monro. After puncture of the lateral ventricle, the catheter was passed from the frontal incision to the retroauricular incision using the subcutaneous route. Ventricular and peritoneal catheters were connected to the valve. Incisions were closed in an appropriate manner.

Optical navigation

Rigid cranial fixation was used with a 3-pin head clamp in all cases (Figure 2A). Preoperative data were trans-

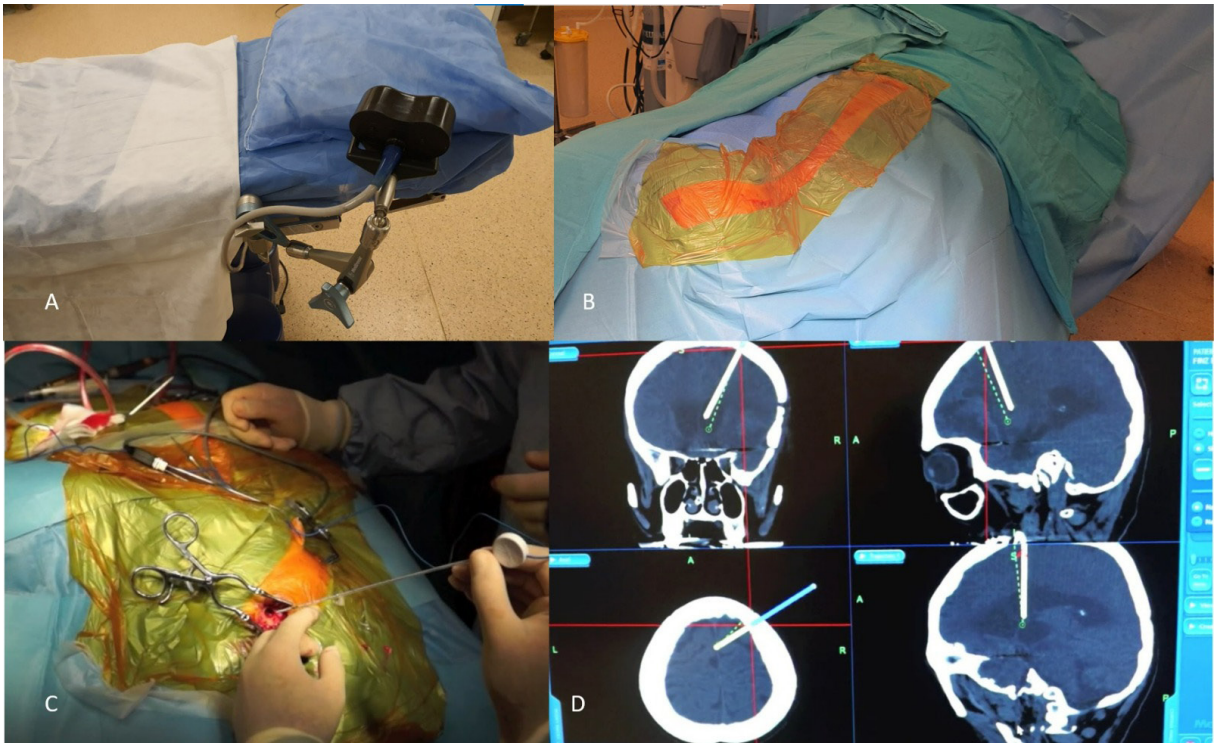

Figure 1: VP shunt insertion using EM navigation. A-B: Axiem mobile emitter of StealthStation S7 (Medtronic Surgical Technologies, Louisville, Colorado, USA) was placed on the operating room table, and the patient was positioned supine and routine sterilisation procedures were applied. C: View of the ventricular catheter with a sterile navigation stylet inserted and advanced towards the predetermined target within the ventricle. D: Peroperatively, the ventricular catheter was monitored in real-time with the assistance of the navigation software.

ferred to the navigation software, and the anatomical landmarks on the patient's face were registered into the navigation software using a navigation probe, and the reliability of the system was verified using the navigation software. After the routine sterile draping procedures, the patients were positioned supine (Figure 2C). Using the navigation software, the entry point was determined to be Kocher's point.

Instead of using a regular VP shunt insertion programme (Medtronic StealthStation S7 software), a neuronavigation-aided biopsy programme was used to increase target precision. The appropriate target (the right foramen of Monro) was marked as if it was a biopsy target. The burr hole was performed using automatic perforator, and the dura was coagulated and incised appropriately. The ipsilateral foramen of Monro was determined as the target point for the ventricular catheter. The navigation probe was inserted into the ventricle catheter and moved to the preoperatively selected target. Peroperatively, the ventricular catheter was monitored in real-time with the assistance of the navigation software (Figure 2C-2D). After the puncture of the lateral ventricle, the catheter was passed from the frontal area to the retroauricular area using the subcutaneous route. Ventricular and peritoneal catheters were connected to the shunt valve. Incisions were closed in an appropriate manner.

RESULTS

The age range of patients was 36 to 87 years, with a mean age of 73.74±9.06 years. There were 12 male and 19 female patients. All patients were operated on because of normal-pressure hydrocephalus (Table 1,2). Postoperative control CT images were obtained to assess postoperative bleeding and to verify the proper positioning of the ventricular catheter. The position of the ventricular catheter was identified by CT using the grading system mentioned by Hayhurst (9). In the EM navigation group, there were seven grad I and eight grad II patients, and in the optical navigation group, there were 13 grad I and three grad II patients according to the postoperative CT findings and the radiological scale defined by Hayhurst (9). In three patients from the EM Navigation group, subdural effusion developed due to overdrainage in different shunt settings. The preferred shunt valve Codman Certas (Integra Lifesciences, New

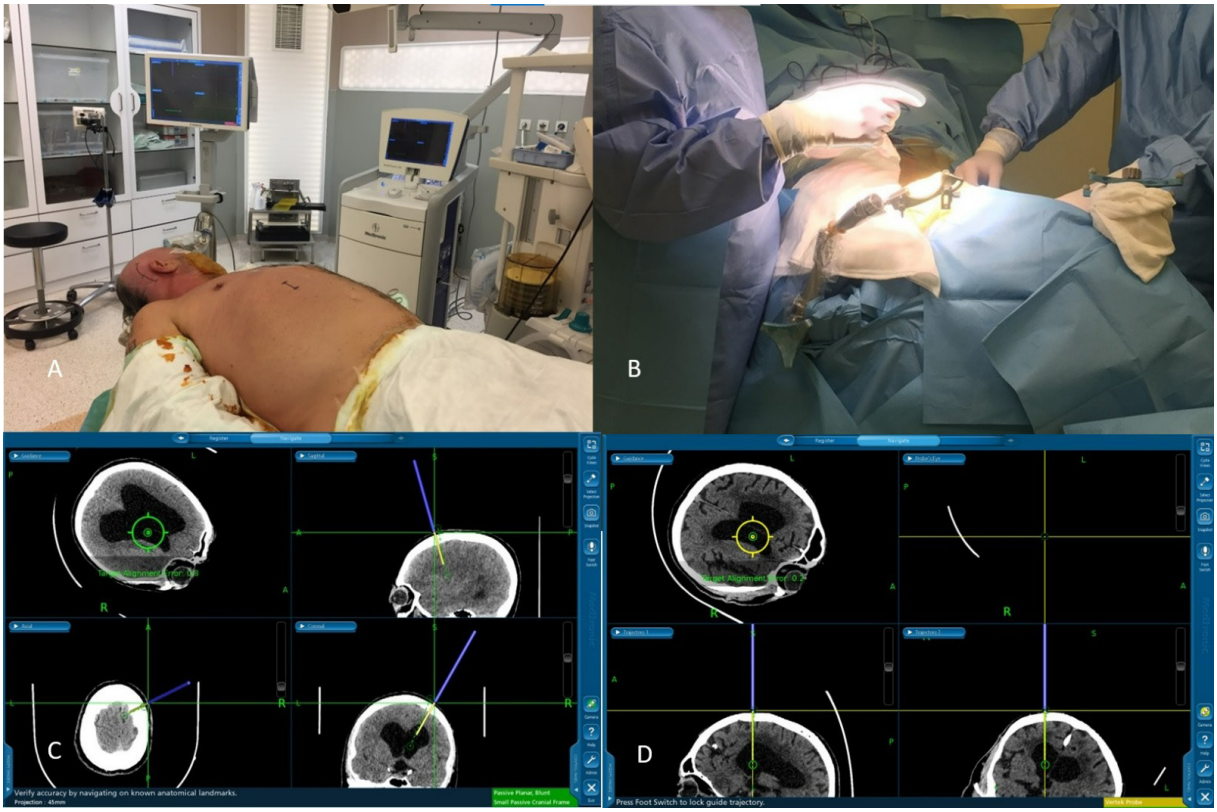

Figure 2: VP shunt insertion using optical navigation. A: Rigid cranial fixation was used with a 3-pin head clamp in all cases. B: The optical navigation system was placed on the operating room table and the patient was draped in a sterile fashion. C-D: The navigation probe was inserted into the ventricular catheter and moved to the preoperatively selected target, which was identified using navigation software. Peroperatively, the ventricular catheter was monitored in real time with the assistance of the navigation system software.

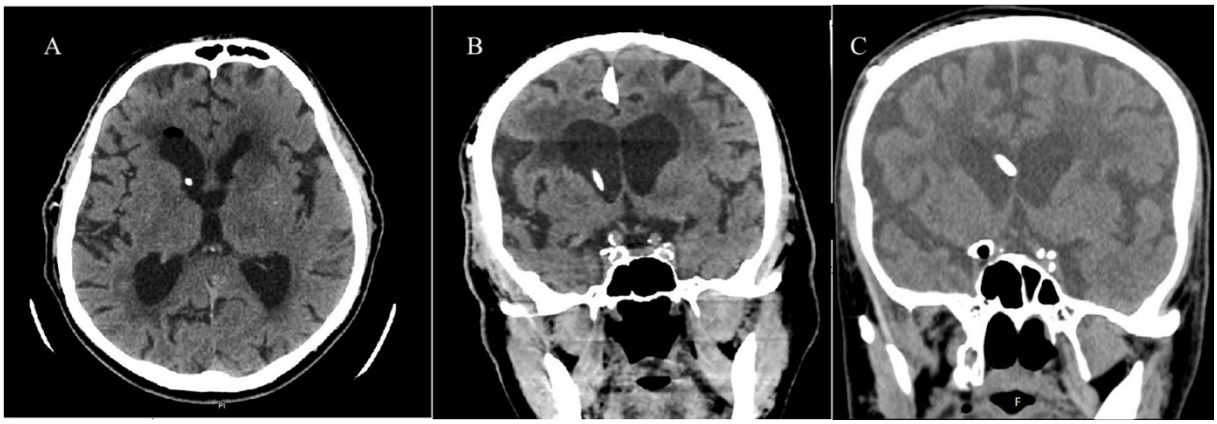

Figure 3: A: Grade I axial view non-contrast CT according to Hayhurst radiological grading, B: Grade I coronal CT scan without contrast, C: Grade II coronal CT scan without contrast

Jersey, USA) can be completely closed in this group. After the subdural effusion regressed in the control tomographies of the patients, the shunt valve was readjusted and gradually opened, and the patients' clinical symptoms improved significantly. In none of these three patients an additional surgical intervention was needed.

DISCUSSION

Due to the failures in ventricular catheter placement using the free-hand technique, the use of frameless navigation and optical navigation for ventricular catheter placement has been a subject of interest in neurosurgery, with studies aiming to assess their efficacy and safety compared to traditional techniques. The success rate of ventricular catheters placed using the free-hand technique in the literature varies between 8% and 65% (14). The failure in placing the ventricular catheter using the freehand technique is mostly referred to as proximal failure, which is the inability to place the proximal part of the catheter at the targeted point, resulting in its positioning in the parenchyma or on the ventricular wall (8, 15).

Azeem and Origitano shared their experiences with frameless navigation and the free-hand technique for ventricular catheter placement in the literature. They placed ventricular catheters using frameless navigation in 34 patients and compared the results with those of 38 patients in whom ventricular catheters were placed using the free-hand technique (16). They found that frameless navigation resulted in more accurate catheter placement, with a lower rate of proximal failures. In the study published by Hayhurst and colleagues, the free hand technique and EM navigation for ventricular catheter placement were compared, and it was found that the employment of neuronavigation significantly reduced the rates of revisions and complications (9). In our study, only 2 (6.25%) out of 32 patients were reoperated because of shunt dysfunction due to the placement of the shunt valve in the frontal area. These two patients were first operated at a different centre, so the shunt valve was placed in the frontal region instead of the retroauricular area. Shunt dysfunction may develop due to the inability to benefit from the effects of gravity because the shunt valve is placed in the frontal region instead of the retroauricular area.

VP shunts placed using EM navigation may not require fixation of the head, but their accuracy may be lower than that of shunts placed using stereotactic navigation (17-19). Although optical navigation systems have higher accuracy in comparison to EM navigation, they come with disadvantages such as the requirement for the patient to be positioned in a pinned headframe and an extended preoperative preparation time leading to infections (13). Furthermore, a visible line of sight is required between the tip of the navigation probe and the navigation camera system; otherwise, the tracking cannot be performed. Although optical stereotactic navigation has more accuracy than EM navigation and has extended preoperation time, there were no surgical infections and also no cases with malposition of the ventricular catheter in the EM and optical navigation groups in our study. In our study, we did not encounter any complications related to the use of the pinned headframe.

The correct placement of the ventricular catheter is important to minimise the risks of catheter dysfunction, intraparenchymal bleeding, and the need for repeated revision surgery (20). Navigation-assisted shunt surgery reduces the risk of catheter misplacement and risk of post operative parenchymal hemorrahage. In our study, postoperative CT scans showed that the position of all ventricle catheters were defined as Grade I or Grade II according to the CT scale mentioned by Hayhurst and there were no Grade III patients (9).

This proper positioning of ventricular catheters lead to favourable postoperative results, and there were no intraparenchymal haemorrhage or catheter dysfunction due

to proximal failure in our study. In our study, all catheters were appropriately placed on the first attempt. This result contributed to the absence of complications such as intraparenchymal haemorrhage or infection. One of the most important complications in shunt surgery is the development of a subdural effusion or slit ventricle due to overdrainage or underdrainage of the shunt valve. Therefore, the use of adjustable shunts has become important to overcome these complications (2, 21, 22). In our study, we used a Codman adjustable valve in the group where electromagnetic navigation was used, and a Strata adjustable valve in the group where optical navigation was used due to the surgeon's preferences. Shunt valves were adjusted to appropriate pressure levels to prevent the development of subdural effusion or slit ventricle due to overdrainage, considering the ventricular sizes in the control CT imaging of the patients.

The EM module (Medtronic Surgical Technologies, Louisville, Colorado, USA) has an extra cost and is not always included to the standard neuronavigation system in every institution. This extra cost may be a significant issue, despite its advantage of requiring no frame and thus resulting in a shorter preoperative preparation time. With sufficient experience, the preoperative preparation time can also be reduced for optical navigation. Thus, our navigation-assisted shunt insertion may be applied without the need for an electromagnetic neuronavigation module. In our study, in three patients from the EM Navigation group, subdural effusion developed due to overdrainage in different shunt settings. The preferred shunt valve Codman Certas (Integra Lifesciences, New Jersey, USA) can be completely closed in this group. After the subdural effusion regressed in the control tomographies of the patients, the shunt valve was readjusted and gradually opened, and the patients' clinical symptoms improved significantly. In none of these 3 patients an additional surgical intervention was needed. Although the shunt adjustment in the optical navigation group (Medtronic, Minneapolis, USA) can be adjusted to provide the minimum drainage, it cannot be adjusted to completely stop the drainage. Therefore, using a shunt valve that can be adjusted to completely stop the drainage can be advantageous in this regard. In the stereotactic frame-based biopsy technique, although the preoperative preparation period may be longer compared with the frameless biopsy techniques, similar results have been obtained in terms of permanent morbidity, mortality, and permanent neurological deficit compared with the frameless biopsy methods (23, 24). In our study, when the control tomographies were evaluated according to the positions of the ventricular catheters, Grade I and Grade II results were found according to the Hayhurst radiological staging. Although we did not have Grade III patients according to the radiological classification, Grade II patients can be prevented with the addition of ultrasound support to navigation (25).

Some limitations of our study include a relatively small number of patients, the absence of a control group operated with the freehand technique, being a retrospective study, and a lack of statistical analysis. In the future, better results can be achieved with larger studies comparing the freehand technique, navigation technique, and ultrasound-guided ventriculoperitoneal shunt placement techniques. Combining navigation-guided shunt placement with ultrasound could indeed lead to even better results.

In VP shunt surgery, incorrect placement of the ventricular catheter can lead to intraparenchymal haemorrhage and shunt dysfunction due to proximal failure. The use of navigation in shunt surgery prevents proximal failure and reduces the complications of intraparenchymal haemorrhage and shunt dysfunction. Although the use of optical navigation requires the use of a pinned headrest and an extended preoperative preparation time, the accuracy of ventricular catheter placement is similar when using optical navigation and EM navigation, despite these disadvantages. Due to its cost-effectiveness and high accuracy, the optically guided navigation system can be used in shunt surgery, especially considering the extra cost of the electromagnetic neuronavigation system.

CONCLUSION

In VP shunt surgery, incorrect placement of the ventricular catheter can lead to intraparenchymal haemorrhage and shunt dysfunction due to proximal failure. The use of navigation in shunt surgery prevents proximal failure and reduces the complications of intraparenchymal haemorrhage and shunt dysfunction. Although the use of optical navigation requires the use of a pinned headrest and an extended preoperative preparation time, the accuracy of ventricular catheter placement is similar when using optical navigation and EM navigation, despite these disadvantages. Due to its cost-effectiveness and high accuracy, the optically guided navigation system can be used in shunt surgery, especially considering the extra cost of the electromagnetic neuronavigation system.

Ethics Committee Approval: The study has ethical approval from the Gaziosmanpasa Education and Research Hospital Ethics Committee (Date: 03.07.2024, No: 16).

Informed Consent: Informed consent forms were obtained from all patients.

Peer Review: Externally peer-reviewed

Author Contributions: Conception/Design of Study- U.Ö., M.O.A., T.K.; Data Acquisition – U.Ö., M.O.A., T.K.; Data Analysis/Interpretation- U.Ö., M.O.A., T.K.; Drafting Manuscript- U.Ö., M.O.A., T.K.; Critical Revision of Manuscript- U.Ö., M.O.A., T.K.; Final Approval and Accountability- U.Ö., M.O.A., T.K.; Supervision- U.Ö., M.O.A., T.K.

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

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RESEARCH / ARAŞTIRMA
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RECONSTRUCTION OPTIONS FOR CHALLENGING PERINEAL DEFECTS

ZORLAYICI PERİNE DEFEKTLERİNDE ONARIM SEÇENEKLERİ

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ABSTRACT

Objective: Abdominoperineal resection and pelvic exenteration for the surgical treatment of advanced colorectal or gynaecological cancers can result in large perineal defects and severe surgical site morbidity. Several regional flaps can be used to treat radiation- and extirpative surgery-related wound breakdowns. This study aims to retrospectively evaluate the efficiency of different flaps used in the reconstruction of perineal defects.

Material and Method: A retrospective review of pelvic reconstructions performed between May 2021 and August 2023 was conducted, with a 6-month follow-up. Ten patients who underwent abdominoperineal resection with immediate abdominal-based flap (n=4) or thigh-based flap (n=6) reconstruction of the perineal/pelvic defect were evaluated. The two groups were compared in terms of patient characteristics, aetiology, preferred treatment, and postoperative complications.

Result: Five women and five men underwent comprehensive pelvic reconstruction. The mean age was 49.6 years (range 26–76) and mean BMI of 28.6 kg/m^2 (range 21.3–50). Five patients had previously undergone radiotherapy. In total, 11 flaps were created based on the type of perineal defect. One patient experienced a minor dehiscence (<5 cm). Two patients experienced major dehiscence (>5 cm), and required reoperation. A patient with Crohn's disease developed one intra-abdominal abscess

ÖZET

Amaç: İleri evre kolorektal veya jinekolojik kanserlerin cerrahi tedavisinde abdominoperineal rezeksiyon ve pelvik ekzenterasyon ameliyatları büyük perineal defektlere ve ciddi cerrahi alan morbiditesine neden olabilir. Radyasyon ve onkolojik cerrahiye bağlı yara iyileşme problemlerini engellemek için çeşitli bölgesel flepler yara kapatılmasında kullanılabilir. Bu çalışmanın amacı perine defektlerinin onarımında kullanılan fleplerin etkinliğinin retrospektif olarak incelenmesidir.

Gereç ve Yöntem: Mayıs 2021 ile Ağustos 2023 arasında gerçekleştirilen pelvik rekonstrüksiyon vakaları geriye dönük olarak altı aylık takip süresi ile incelendi. Toplamda 10 hasta çalışmaya dahil edildi. Bu hastalara karın temelli flepler (n=4) ve uyluk temelli flepler (n=6) kullanılarak perineal defekt rekonstrüksiyonu gerçekleştirildi. Bu iki hasta grubu hastaların demografik özellikleri, etiyoloji, tercih edilen tedavi yöntemi ve ameliyat sonrası gelişen komplikasyonlar açısından karşılaştırıldı.

Bulgular: Beş kadın ve beş erkeğe kapsamlı pelvik rekonstrüksiyon uygulandı; yaş ortalaması 49,6 (26-76 aralığı) ve ortalama vücut kitle indeksi (VKI) 28,6 kg/m^2 (21,3-50 aralığı) idi. Beş hastaya daha önce radyoterapi uygulanmıştı. Perine defektinin tipine göre toplam 11 flep uygulandı. Bir hastada yara yerinde küçük bir ayrılma (<5 cm) oldu. İki hastada yara yerinde büyük açılma oldu (>5 cm) ve yeniden ameliyat edilmeleri gerekti. Crohn has-

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because of spontaneous fistula formation. There was no vascular compromise in the flaps.

Conclusion: Repair options vary depending on the nature of the defect and extent of resection. The primary goals of reconstruction are to eliminate pelvic dead space and separate the intra-abdominal content from the perineum to prevent herniation and strangulation of the small intestines and to ensure that the perineal wound heals without complications.

Keywords: Abdominoperineal resection, flap, pelvic exenteration, perineal reconstruction, radiotherapy

talığı olan hastada spontan fistül oluşumu sonucu karın içi abse gelişti. Fleplerde kısmi veya tam kayıp izlenmedi.

Sonuç: Onarım seçenekleri defektin boyutuna ve rezeksiyonun derecesine bağlı olarak değişmektedir. Rekonstrüksiyonda öncelikli amaç pelvik ölü boşluğu ortadan kaldırmak ile karın içi içeriğin perineden ayrılmasını sağlamak, ince bağırsakların fıtıklaşmasını önlemek ve komplikasyonsuz yara iyileşmesini sağlamaktır.

Anahtar Kelimeler: Abdominoperineal rezeksiyon, flep, pelvik ekzenterasyon, perineal rekonstrüksiyon, radyoterapi

INTRODUCTION

Abdominoperineal resection or pelvic exenteration is a surgical procedure used to treat advanced colorectal cancer, locally advanced genital cancer, or perineal skin cancer. Major functional and anatomical deficits can be observed in the perineal region according to the defect (1). Furthermore, the perineal area is at higher risk of infection than other wound sites. Additional undesired patient factors such as preoperative nutritional status (albumin <2 g/dL), previous radiotherapy, accompanying diabetes mellitus, smoking, and perineal bacterial counts also affect outcomes after resection (2).

Although simple abdominoperineal defects can be treated with primary closure, local flaps, or an omental flap to obliterate the dead pelvic space, wound breakdowns continue to occur due to the aforementioned factors. In addition to wound healing, quality of life, sexual function, bladder function, and fertility are important factors to consider in pelvic reconstruction (1).

A multidisciplinary approach is required, with the participation of a reconstructive surgeon, and a wide range of local, regional, or less frequently free fasciocutaneous, musculocutaneous, and muscle flaps should be used for reconstruction, depending on the need for volume and skin replacement based on the size and location of the defect (2-4).

In this study, we would like to share our brief experience with each type of defect, including the type of flap to consider, its potential benefits and drawbacks, and the precautions to take to avoid potential complications.

MATERIAL AND METHODS

This study was approved by İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 09.02.2024, No: 3).

We conducted a retrospective analysis of medical records of pelvic reconstructions performed at the Plastic Reconstructive and Aesthetic Surgery Clinic between May 2021 and August 2023, with at least a 6-month follow-up.

Charts were reviewed for preoperative and postoperative information, and complications were noted. Minor wound dehiscence was accepted as less than 5 cm, and major wound dehiscence was defined as more than 5 cm.

Pelvic defects and preferred flaps were identified by examining anatomical subunits and associated flap characteristics. Kosutic's classification for perineal defects was used (Table 1) (5).

Complications were compared between flap types and patients who did and did not receive radiotherapy.

RESULTS

The results are summarised in Table 2. Five women and five men underwent comprehensive pelvic reconstruction, with a mean age of 49.6 years and mean BMI of 28.6 kg/m^2.

Five patients (50%) had previously received radiation therapy. One patient experienced minor dehiscence (<5 cm), which resolved with local dressing. Two patients had major dehiscence, one of whom required reoperation for dehiscence at the flap donor site, which was treated with split-thickness skin grafting (STSG); the other required an additional glutaeal rotation flap for posterior raphe closure. There was one intra-abdominal abscess in a patient with Crohn's disease due to spontaneous fistula formation. There was no vascular compromise on the vertical rectus abdominis flap (VRAM). The patient underwent serial irrigation and negative pressure wound therapy (NPWT), and an artificial mesh was extracted at the donor site. There were no hernia cases in the late period.

The abdominal-based flaps (VRAM) had a 25% complication rate, whereas the thigh-based flaps (profunda artery perforator flap [PAP flap], transverse upper gracilis flap [TUG flap], anterolateral thigh flap [ALT], and gracilis muscle flap) had a 50% complication rate.

Patients who had previously received radiation therapy had a complication rate of 60%, whereas those who had never received radiation therapy had a complication rate of 20%. No complication rate comparisons were significant.

Three female patients required partial vaginal resection with reconstruction. Three patients reported sexual activity before surgery and two reported sexual activity six months after reconstruction. One patient died after a tumour recurrence and lung metastasis within six months.

Table 1: Definition of Kosutic classification, number of patients with each type of defect, and preferred reconstruction methods

Types of defects	Definition	Number of patients	Preferred reconstruction method
Type 1a	Defects affect areas/organs anterior to the anus and up to the pubis, with no effect on the anus. The urethra is preserved.	4	ALT-VL, ALT, PAP-TUG, VRAM
Type 1b	Defects affect areas/organs anterior to the anus and up to the pubis, with no effect on the anus. The urethra is resected.	1	Gracilis flap
Type 2a	The defect affects the anus and areas posterior to it, towards the natal cleft, whereas the perineum anterior to the anal defect is not affected. Pelvic clearance is not performed.	0	-
Type 2b	The defect affects the anus and areas posterior to it, towards the natal cleft, whereas the perineum anterior to the anal defect is not affected. Pelvic clearance is not performed. Pelvic clearance is performed.	3	VRAM, PAP-TUG
Type 3	Defect includes the majority of or the entire perineum, with total pelvic exenteration performed.	2	PAP-TUG, Glutaeal rotation, VRAM

ALT: anterolateral thigh, VL: Vastus lateralis, PAP: profunda artery perforator, TUG: transverse upper gracilis, VRAM: vertical rectus abdominis

Table 2: Summary of demographic characteristics, medical history, defect type, preferred reconstruction options, and postoperative complications

Patient	Age at surgery	Diagnosis	BMI (kg/m²)	Prior RT	Defect location and type	Extirpative surgery	Reconstruction	Complications
1	39	Dermatofibrosarcoma	50	No	Mons pubis Type 1a	Mons pubis, right labia, vulva	Left-pedicled ALT-VL	Dehissence at the donor side of the STSG for defect closure
2	52	Rectum adenocarcinoma	25.9	Yes	Pelvis Type 3	APR with TPE	Left pedicled PAP-TUG flap Glutaeal rotation flap	8 cm wound separation at the anal region, right side glutaeal rotation flap
3	63	Uterine leiomyosarcoma	28.76	Yes	Pelvis Type 3	TPE	Fascia-sparing VRAM	None
4	44	Vulvar malignant mesenchymal tumour	31.18	No	Mons pubis, left vulva, left lower abdomen Type 1a	Mons pubis, left labia, vulva	Left-pedicled ALT	Tumour recurrency in 3 months- lung metastasis, ex due to tumour metastasis
5	57	Necrotising fasciitis (Fournier gangrene)	23.1	No	Scrotum posterior perineal raphe Type 1b	Penectomy, several debridement procedures	Right-pedicled gracilis flap	None
6	45	Rectal adenocancer	26.53	Yes	Pelvis and posterior raphe Type 2b	APR with TPE	Left-pedicled VRAM	Spontaneous fistula formation in the abdomen, intraabdominal NPWT, and donor site prosthetic mesh removal

Table 2: Continued

Patient	Age at surgery	Diagnosis	BMI (kg/m²)	Prior RT	Defect location and type	Extirpative surgery	Reconstruction	Complications
7	26	Rectum solider malignant fibrous sarcoma	21.3	No	Pelvis and posterior raphe Type 2b	APR with TPE	Right-pedicled fascia-sparing VRAM	None
8	76	Vulvar SCC	30.80	Yes	Pelvis and posterior raphe Type 2b	APR with TPE	Right side PAP-TUG flap	Minor wound separation and dressing application
9	48	Vulvar adenocancer	21.70	No	Mons pubis, left vulva Type 1a	Radical vulvectomy, mons pubis, labia	Right side PAP-TUG flap	None
10	56	Right groyne SCC	27.2	Yes	Right groin, fistulized lymph node Type 1a	Right ilioinguinal LND, orchiectomy, partial femoral artery excision, and reconstruction using artificial vascular graft	Right-side VRAM flap	None

BMI: Body Mass Index, RT: Radiotherapy, SCC: Squamous cell carcinoma, APR: Abdominoperineal resection, TPE: Total pelvic exenteration, ALT: Anterolateral thigh, VL: Vastus lateralis, STSG: Split Thickness Skin Graft, PAP: Profunda artery perforator, TUG: Transverse Upper Gracilis, LND: Lymphnode dissection, VRAM: Vertical Rectus Abdominis Muscle, NPWT: Negative pressure wound therapy

DISCUSSION

Radical surgical resection is the primary treatment for locally advanced rectal, gynaecologic, and urological malignancies (6-8). The most important aspect of tumour excision is to ensure microscopically complete resection (R0) with clear margins of at least 1 mm and no microscopic residual disease (4, 9). Studies have shown that the rate of local recurrence increases with resection margins. The 5-year survival rate in patients with excision of 10 mm was 80%, whereas the 5-year survival rate in patients with excision of 1 mm was 34% (10, 11). Therefore, if R0 margin resection cannot be executed by surgical excision, then neoadjuvant radiotherapy and/or chemotherapy must be considered to make the tumour resectable with R0 margins (4, 9). However, perineal wound complications were reported in 66% of patients who underwent abdominoperineal resection and primary wound closure in addition to external beam radiation therapy (12). The use of locoregional flaps decreases wound complication rates to 20%–30% (1, 13).

For Type 1 defects, several reconstruction options are available. Small or superficial defects can be closed using local cutaneous flaps or STSG. However, larger defects or previous radiation exposure necessitate the use of regional fasciocutaneous or musculocutaneous flaps (1, 14). The ALT flap has been delineated for mons and vulvar reconstruction and provides sufficient surface area and soft-tissue thickness with its long pedicle length (Figure 1) (15). The pedicle length varies between 16 and 19 cm, and the flap quickly moves under the rectus femoris and sartorius muscles (1). If necessary, a stair-step incision can be made in the rectus muscle to move the flap forward. The volume requirement determines whether a thinner fasciocutaneous flap or a bulkier musculocutaneous flap is harvested. If the defect extends to the inferior abdomen, an ALT-Tensor Fascia Lata, ALT-VL, or hemi-thigh flaps can be used as part of abdominal reconstruction (1, 16). In our opinion, the lateral thigh is a reliable source of mons pubis, inferior abdomen, and penoscrotal soft tissue reconstruction.

To achieve satisfactory surgical outcomes with vaginal reconstruction, several obstacles must be overcome. The creation of a cylindrical structure of adequate size to allow sexual intercourse and a flap of sufficient volume must obliterate the pelvic dead space to prevent perineal small-bowel herniation (1). Patient expectations and age are also important considerations.

The pedicled VRAM is typically the first choice for creating a thin cylindrical neovagina to reconstruct posterior or circumferential vaginal defects in sexually active women (1).

If VRAM is not applicable owing to previous abdominal surgery or the need for multiple ostomy placement, bilateral gracilis musculocutaneous flaps with or without omentum are a second option (13). Because gracilis flaps have limited access to reach deep into the pelvis and provide little volume to close the pelvic outlet, bilateral harvesting is frequently required. In myocutaneous gracilis flap reconstruction (TUG flap), the distal part of the skin is the least perfused and extends deep into the defect because of its unreliable vascularity. Thus, the PAP flap or combined TUG-PAP flap provides a longer distal skin island to fill the inner dead space with its dual robust blood supply (17, 18).

In nonsexually active women with combined vaginal and perineal defects, simple flap closure of the perineum without creating the neovagina may be associated with the quickest recovery and lowest incidence of complications (13). This recommendation also applies to men with perineal raphe defects, including pelvic dead space or not.

The inferior glutaeal artery perforator flap, PAP flap, and combined TUG-PAP flap are viable options for Type 2a defects. The pedicled VRAM flap is typically the first option for Type 2b defects (1).

The pedicled VRAM flap has become the workhorse flap for reconstructing extended abdominoperineal defects, such as total pelvic exenteration or Type 3 defects (19). Previously, prior abdominal surgery was considered a contraindication for harvesting VRAM flaps for abdominoperineal reconstruction (20). However, a recent series showed that previous abdominal surgery does not increase the risk of flap failure (19). Thus, our primary choice was a VRAM flap, if applicable (Figure 2). To avoid the use of artificial mesh at the donor site, the anterior rectus fascia was preserved below the rectus sheath's arcuate line, and the component separation technique was used to repair the abdominal wall fascia above the arcuate line. The inferior muscle insertion at the pubic ramus was dissected to free the pelvic outlet from tension (6).

Thigh-based flap reconstruction should be considered in patients with a violated rectus abdominis flap either from previous operations or previous ostomy placement, extensive abdominal scarring, risk of abdominal hernia, or already having an abdominal hernia in Type 3 perineal defects (Figure 3) (21). Wide abdominoperineal defects can be reconstructed using the flaps discussed in this article, either separately or combined based on their different and distinct pedicles in a single patient, depending on the defect's width, depth, and related violated anatomical subunits (1).

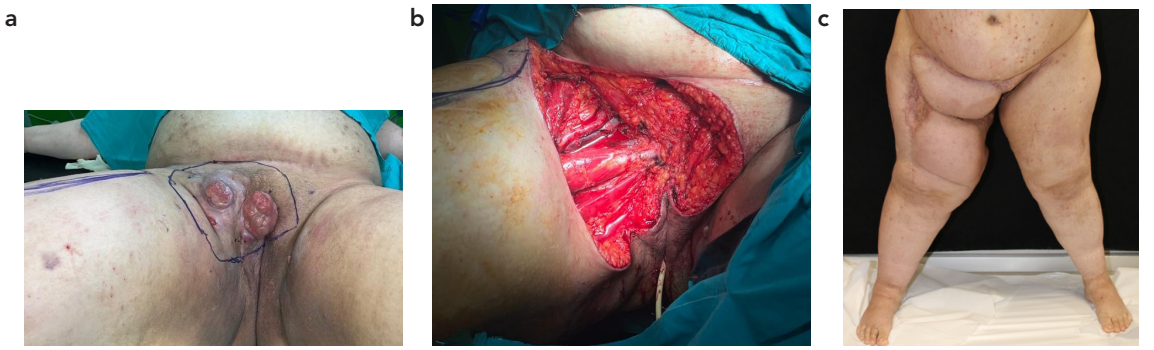

Figure 1: Patient 1: (a) 22×12 cm dermatofibrosarcoma on the mons pubis and right vulva. (b) The right femoral bundle was exposed after resection. (c) Final closure of the defect, result of the postoperative 8th month.

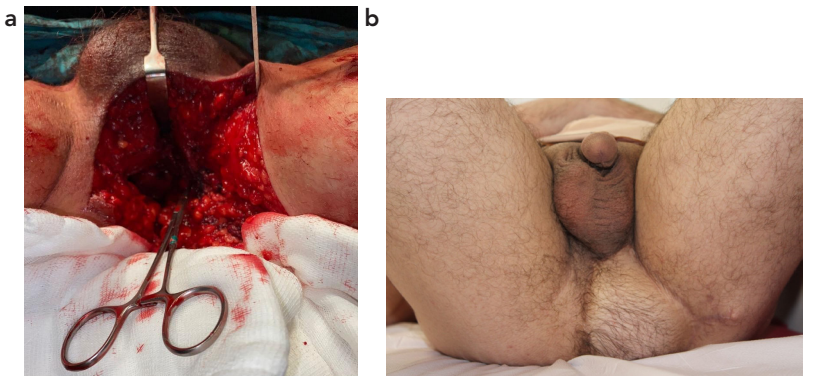

Figure 2: Patient 7: (a) Type 2b defect after the tumour excision. (b) Repair of the defect using a VRAM flap, the result of the postoperative 10th month.

Thigh flaps are associated with a higher incidence of donor-site cellulitis, recipient-site complications, pelvic abscess, and major wound dehiscence complications than VRAM flaps (42% versus 15%) (22). Most complications are caused by the relatively short arch of the thigh-based flaps. Several changes can be made to reduce the incidence of complications. First, a vertically designed PAP flap can be elevated on the first or second proximal perforator to include the distal thigh skin. The perforator can be dissected to the profunda femoris artery to increase the pedicle length (23). As a result, a longer flap can reach the pelvic outlet owing to its longer pedicle. Second, performing additional omentoplasty can eliminate pelvic dead space, thereby preventing perineal hernia in patients undergoing thigh-based flap reconstruction.

The main factor separating intra-abdominal contents from the perineal wound is the preferred flaps inset. During inset 2.0, a polydioxanone absorbable suture (Ethicon, Somerville, New Jersey) was tightly knotted from the edge of the flap to the remnant pelvic muscles or ligaments. Thus, undesirable perineal hernias and small intestinal obstructions were avoided (Figure 4).

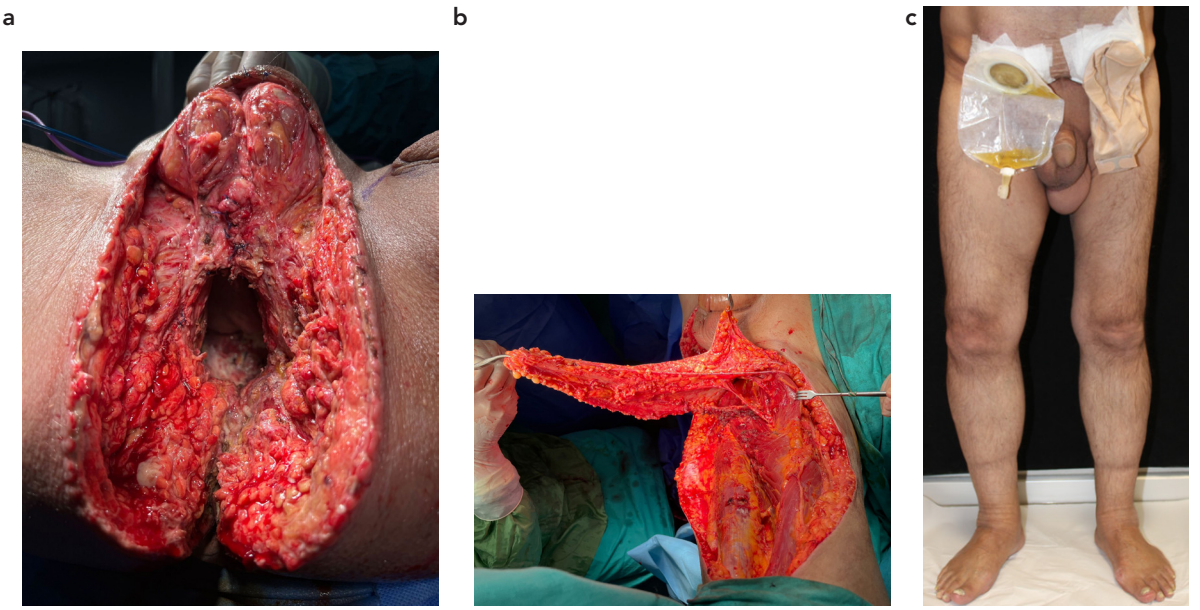

Figure 3: Patient 2: (a) Type 3 defect. (b) A combined PAP-TUG flap based on the pedicles and saphenous vein was also included in the flap to prevent venous insufficiency. (c) Final closure of the defect, result of the postoperative 14th month.

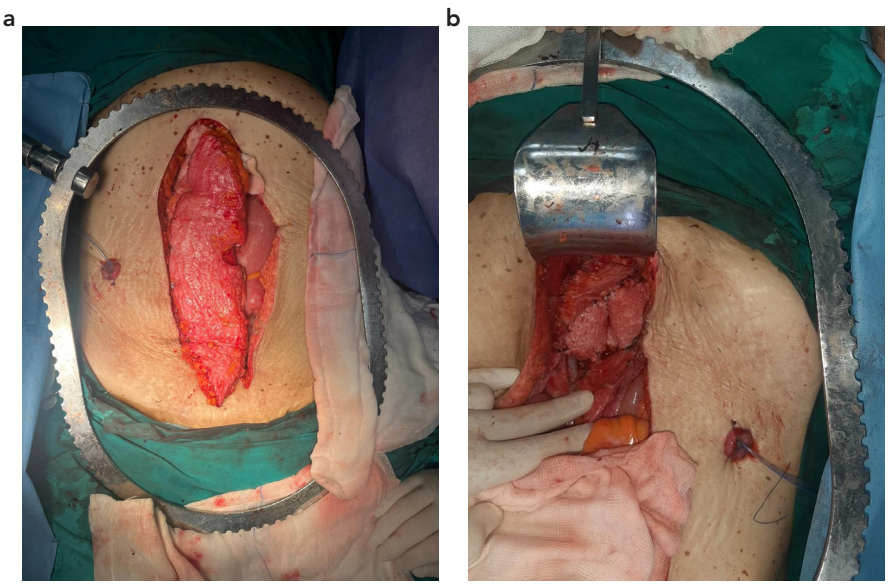

Figure 4: Patient 3: (a) De-epithelized VRAM flap. (b) The flap was sutured over the pelvic outlet.

CONCLUSION

Flap selection is based on previous surgeries, stoma requirements and placement, and the need for radiation therapy following resection. Major pelvic complications, such as abscesses, urinoma, perineal herniation, and fistulas, can be prevented by well-vascularised flap tissue, whether abdominal or thigh-based, or even omentum flap.

Therefore, an adjacent association between the oncologist and reconstructive surgeon is beneficial when planning for vaginal and perineal defect reconstruction.

Ethics Committee Approval: Ethics committee approval was received for this study from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 09.02.2024, No: 3).

Informed Consent: Informed consent was obtained from every subject.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- D.A., M.C.N., Y.Y., B.E.A., L.D.E., R.A.A.; Data Acquisition- D.A., M.C.N., M.B.D., Y.M.; Data Analysis/Interpretation- D.A., E.K., C.B.K., Ö.P., C.E.; Drafting Manuscript- D.A., Y.Y., B.E.A., L.D.E., C.B.K., Ö.P., M.B.D., Y.M.; Critical Revision of Manuscript – D.A., M.C.N., E.K., C.E., R.A.A.; Final Approval and Accountability- D.A., M.C.N., Y.Y., E.K., B.E.A., L.D.E., C.B.K., Ö.P., M.B.D., Y.M., C.E., R.A.A.; Technical or Material Support- D.A., M.C.N., Y.Y.; Supervision- D.A., E.K., C.E., R.A.A.

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RESEARCH / ARAŞTIRMA
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EFFECTS OF COLLAGEN TYPE 1 AND 3 ON CELL PROLIFERATION AND ALPHA FETO PROTEIN EXPRESSION IN A HUMAN CELL LINE MODEL OF LIVER CANCER

EKSTRASELLÜLER MATRİKS BİLEŞENLERİNDEN TİP 1 VE TİP 3 KOLLAJENİN KARACİĞER KANSERİ İNSAN HÜCRE HATTI MODELİNDE HÜCRE PROLİFERASYONUNA VE ALFA FETO PROTEİN EKSPRESYONU ÜZERİNE ETKİLERİ

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ABSTRACT

Objective: In cancer research, studies showing the behaviour of cells in different environments is important for developing new treatment methods. The microenvironment is essential for both regular and cancerous tissues. The main component of the extracellular matrix (ECM) is collagen. In HepG2 cells, a hepatocellular carcinoma cell line, the effects of Type 1 and Type 3 collagen on cell proliferation and the expression of alpha-feto protein (AFP), which is an indicator of carcinogenicity, was examined.

Material and Method: HepG2 cells were grown in Type 1 or Type 3 covered surfaces, whereas no treatment was applied to the control group. Proliferation analysis was performed via microscopic examination and cell viability assessmet kit (Cell Counting Kit-8, CCK-8). AFP was measured using a confocal microscope using immunological staining.

Result: The results showed that the viability rate of HepG2 cells growing in Type 3 collagen medium was statistically higher than in the control group ($p<0.0001$). AFP expression increased significantly in the presence of Type 3 collagen compared with the control group at the 24th hour ($p<0.05$), and at other culture times, AFP expression was seen more in Type 1 collagen culture medium.

ÖZET

Amaç: Kanser araştırmalarında, hücrelerin farklı ortamlardaki davranışlarını gösteren çalışmalar yeni tedavi yöntemlerinin geliştirilmesi için büyük önem taşımaktadır. Mikroçevre hem normal doku hem de kanserli doku için çok önemlidir. Ekstraselüler matriks (ECM) ana bileşenleri kollajenlerdir. Hepatosellüler karsinom hücre hattı olan HepG2 hücrelerinde Tip 1 ve Tip 3 kollajenin hücre proliferasyonu ve karsinojenite göstergesi olan alfa-feto protein (AFP) ekspresyonu üzerine etkisi incelenmiştir.

Gereç ve Yöntem: Proliferasyon analizi mikroskobik inceleme ve hücre canlılığı değerlendirme kiti (Cell Counting Kit-8, CCK-8) ile yapıldı. AFP ölçümleri immünolojik boyama yöntemleri kullanılarak konfokal mikroskop ile yapıldı.

Bulgular: Sonuçlar, Tip 3 kollajen ortamında büyüyen HepG2 hücrelerinin canlılık oranının kontrol grubuna göre istatistiksel olarak daha yüksek olduğunu gösterdi ($p<0,0001$). AFP ekspresyonu 24. saatte kontrol grubuna kıyasla Tip 3 kollajen varlığında anlamlı olarak artmış ($p<0,05$), diğer kültür zamanlarında ise AFP ekspresyonu Tip 1 kollajen kültür ortamında daha fazla görülmüştür.

Sonuç: Her iki tip kollajende kültüre edilen HepG2 hücrelerinin morfolojik olarak benzer bir yapıya sahip olduğu, Tip I kolla-

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Conclusion: HepG2 cells cultured in both types of collagen have a morphologically similar structure, and Type 1 and Type 3 collagen have a proliferation-enhancing effect on cancer cells. AFP, an indicator of liver cancer, is high in culture media containing Type 1 and Type 3 collagen and this finding is considered as the tendency of bad prognosis of collagen types on AFP expression.

Keywords: Alpha-fetoprotein, extracellular matrix, hepatocellular carcinoma, type 1 collagen, type 3 collagen

jen ve Tip 3 kollajenin kanser hücreleri üzerinde proliferasyonu artırıcı etkiye sahip olduğu belirlenmiştir. Karaciğer kanserinin göstergelerinden biri olan AFP, Tip 1 kollajen ve Tip 3 kollajen içeren kültür ortamlarında yüksek bulunmuş, bunun da kollajen tiplerinin AFP ekspresyonu üzerinde kötü prognoza doğru bir eğilim gösterdiğini düşündürmektedir.

Anahtar Kelimeler: Alfa fetoprotein, ekstrasellüler matriks, hepatosellüler karsinoma, tip 1 kollajen, tip 3 kollajen

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common solid tumour in incidence and third in mortality. Approximately 750,000 new cases are encountered each year. HCC causes 250,000 to 1 million deaths per year worldwide (1-4). Despite therapeutic advances, there has not been a significant improvement in the survival of patients with HCC in the last two decades. Systemic chemotherapy has not been shown to prolong survival in patients with HCC, and new approaches are critically needed to achieve a significant reduction in HCC mortality due to the failure of conventional chemotherapy. Therefore, the study of the molecular mechanism of HCC pathogenesis and the identification of new targets for molecular HCC therapy remain important (4, 5).

HepG2 cells were isolated from a 15-year-old patient diagnosed with HCC. They are frequently used to understand the mechanism of liver cancer and in therapeutic studies. Hepatoma cells are used to understand liver cancer phenotypes and the phenotypes of hepatocyte cells in healthy and diseased states (5-7).

In cancer research, studies showing the behaviour of cells in different environments is of great importance for developing new treatment methods. For cells to function normally, microenvironmental components must be in the required amount and order. The microenvironment is extremely important for both normal and cancerous tissues. One of the most important components of the microenvironment is extracellular matrix (ECM) components. The main component of the ECM is collagen (8).

When hepatocytes are cultured in vitro on flexible ECM-derived gels containing Type 1 collagen or basement membrane proteins, the culture substrate allows cells to assume different shapes and high expression of liver-specific genes. On the contrary, when hepatocytes are cultured on plastic surfaces, due to the in elasticity of the substrate, they show an undifferentiated, flattened shape, and there is a severe reduction the expression of liver-specific genes. Hepatocytes cultured in plastic or in a monolayer attached to ECM proteins such as collagen and laminin exhibit distorted morphology and liver-specific functions (9, 10). Molecular methods have shown that the level of Type 1 collagen is much higher in HCC samples than in normal liver samples (10). Type 3 collagen is the second most abundant collagen in the ECM (11).

In light of studies showing the in vitro and in vivo effects of different types of collagen in different cancer types, we aimed to examine how Type 1 and Type 3 collagen affect cell proliferation in HepG2 cells, a hepatocellular carcinoma cell line, and how collagen types affect the expression of alpha-feto protein, an indicator of carcinogenicity.

In short, we comparatively investigated the changes in the morphological and functional properties of HepG2 cells in the presence of Type 1 and Type 3 collagen compared with conventional cultured cells. The effect of collagen type on cell proliferation will be examined using a proliferation assay, and prognosis-related alpha feto protein (AFP) expression will be examined under a confocal microscope using immunohistochemical analysis.

MATERIAL AND METHODS

Cell culture

In the project; human hepatocellular cells obtained from ATCC (AmericanType Culture Collection, USA) carcinoma cell line Hep2cell. Cells were initially cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, UK) supplemented with 10% fetal bovine serum (FBS, Gibco, UK) and 1% antibiotic (penicillin-streptomycin, Pan Bitech, Germany). Cells reaching 60-70% confluent were treated with trypsin (Gibco, UK), removed from the culture dish, counted, and divided into three main groups.

The cells were seeded into uncoated, Type 1 collagen-coated, or Type 3 collagen-coated in vitro culture plates. In the classical culture medium group, HepG2 cells were seeded in 24-well culture dishes with round coverslips at a density of 70,000 cells per well and 96-well culture dishes with 10,000 cells per well. These groups of cells were fed with routine cell culture medium, and proliferation and immunohistochemical analyses were performed at the indicated experimental times. Twenty-four and 96-well culture dishes coated with Type 1 collagen (Corning-354249, USA) were kept in the incubator for at least 4 h and washed with sterile phosphate buffered saline (PBS) before seeding. Culture dishes coated

with Type 3 collagen (Genlantis-Q3HCO100,Germany) were kept in an incubator for at least 4 h as in the control group. Proliferation rates and immunohistochemical analyses of these cells were performed at the same time points as in the conventional culture control group.

Monitoring of morphological changes

A- Microscopic analysis: To evaluate changes in the morphology of HepG2 cells, live images of the cells were recorded under an inverted phase microscope (Zeiss, Primovert, Germany) during the three periods mentioned above.

B- Haematoxylin-Eosin (H&E) Staining: Cells in two separate culture media seeded on sterile round coverslips in 24-well culture dishes were fixed with 4% formaldehyde (room temperature, 20 min.) at three different analysis periods. During fixation, PBS was applied thrice for 2 min. After fixation, the cells were stained with H&E. The nucleus structure and acidic and basic changes in the cytoplasm of the cells were evaluated after staining. The stained samples were examined and photographed under a light microscope at magnifications of 200 and 400X.

Proliferation assay

In our project, the proliferation of HepG2 cells was measured in three different groups and at three different times using a commercially available cell viability assesment kit (WST-8/CCK8, Ab228554, AbCam, UK). According to the kit procedure; 10 µL of CCK-8 solution was pipetted into 100 µL volume of cell suspension seeded in 96 wells on the days to be analysed, incubated in the incubator for 1-4 hours, and then spectrophotometrically read at 460 nm wavelength in a microplate reader (BioTek Synergy H1, US) and the colour change was evaluated with the optical densities obtained.

Immunohistochemical analysis

AFP immunofluorescence (IF) staining method:

Fixed HepG2 cells for AFP staining were maintained at 4°C until immunohistochemical staining. The coverslips in the fixative were first washed with Cello-IF solution and heated to 37°C for 3x5 min. The primary AFP antibody (Thermo, US) was prepared by diluting 1/100 with Cello-IF solution, treating cells, and incubating at 37°C. Cells were then washed with Cello-IF solution and heated to 37°C for 3x5 minutes. The secondary goat anti-rabbit IgG (H+L) Dylight 488, ThermoScientific) (diluted 1/200 with Cello-IF) specific for the primary antibody was incubated at 37°C. After incubation, cells were washed with warm PBS for 3x5 minutes and covered with Hoescht core stain (33258, Sigma, Germany) + Glycerol. The preparations were kept in the dark and at 4°C until examination using a confocal microscope (Zeiss, LSM700, Germany).

Statistical analysis

The data obtained from the study were evaluated using GraphPad Prism 9 analysis programme (GraphPad Prism V. 9.01). Two-way analysis of variance (ANOVA) was used to compare the results of proliferation measurements and AFP expression in three different culture media. Statistical significance was set as $p<0.05$.

RESULTS

Morphological analysis results

HepG2 cells were examined under an inverted microscope. In the culture medium supplemented with Type 1 collagen, it was observed that the cells had a spindle structure that differed from the classical polygonal cell form, and communication between cells increased through spindle extensions. It was observed that the cells in the culture medium containing Type 3 collagen exhibited similar morphological features and the cells had spindle-like extensions. Figure 1 shows live microscopy images of the cells.

Our experimental groups were analysed morphologically using H&E staining. Accordingly, the stained preparations were examined under a light microscope, and it was shown that the control group of HepG2 cells showed the expected morphology after staining the nucleus and cytoplasm separately. The spindle-like structure of HepG2 cells in Type 1 collagen medium was clearly observed by H&E staining, and a similar morphologic structure was observed in HepG2 cells in culture medium supplemented with Type 3 collagen. It was noticed that the cytoplasm of the cells had become larger and granularized. Figure 2 shows microscope images of the cells after H&E staining.

Proliferation outcome

In order to investigate the proliferation rate of HepG2 cells in different microenvironments, spectrophotometric readings of the cells were statistically analysed in proportion to viable cells using WST-8 as described in the methods section. Accordingly, at 24 h, the rate of viable cells in media containing Type 1 and Type 3 collagen showed a statistically significant increase compared with HepG2 cells in conventional culture media ($p<0.0001$). At the 48th hour, there was an increase in the number of viable cells in all cell groups compared with the first day, and the proliferation rates of the cells in the presence of collagen were higher than those of the control group cells, whereas the increase in HepG2 cells growing in medium with Type 3 collagen was statistically significant ($p<0.0001$). At 72 hours of the culture period, it was observed that the proliferation rate in all cell groups generally decreased compared with the previous day, the proliferation rate of the cells in the collagenous medium was higher than that in the control group, and statistically, the cells in the Type 3 collagenous medium showed a significant increase compared with the control group ($p<0.01$). Figure 3 shows the results of the CCK-8 test.

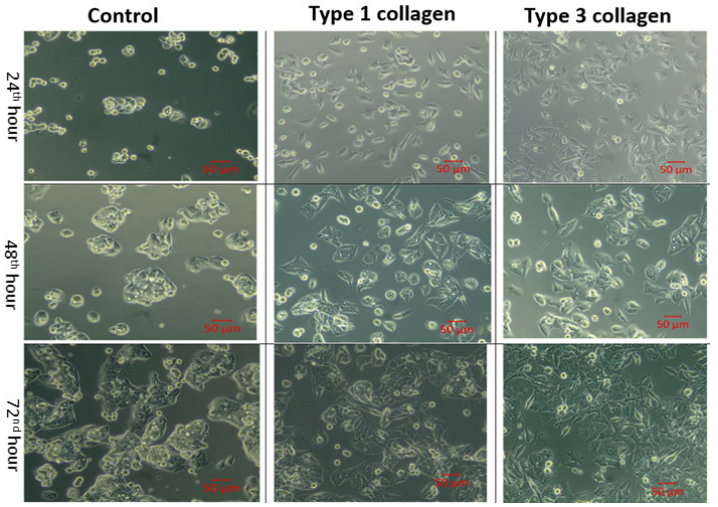

Figure 1: Live images of HepG2 cells under an inverted microscope in three different culture media and during three different culture periods. 20X magnification

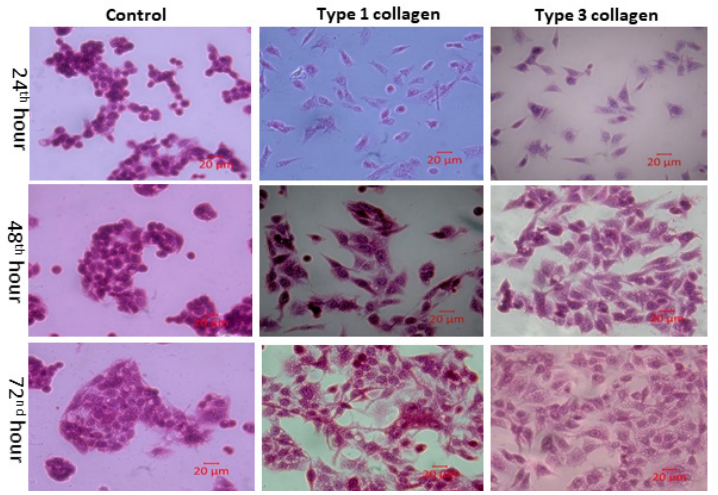

Figure 2: Cell morphological analysis using H&E staining. H&E: Haematoxylin-Eosin

Alpha fetoprotein (AFP)

It has been emphasised in various studies that AFP levels are important markers in liver cancer cells. In our project, we investigated the changes in the amount and localisation of this marker in HepG2 cells in the presence of Type 1 and Type 3 collagen under a confocal microscope (Ziess, LSM700, Germany) and performed quantitative data and statistical analysis. According to the results obtained; AFP expressed in HepG2 cells increased in media containing collagen during three different culture periods. At 24 h, the increase in AFP expression in HepG2 cells treated with Type 3 collagen medium was statistically significant compared with the control HepG2 cells ($p<0.05$). After 24 h of culture, there was an increase in AFP expression in cells treated with Type 1 and Type 3 collagen media compared with the control group, but no

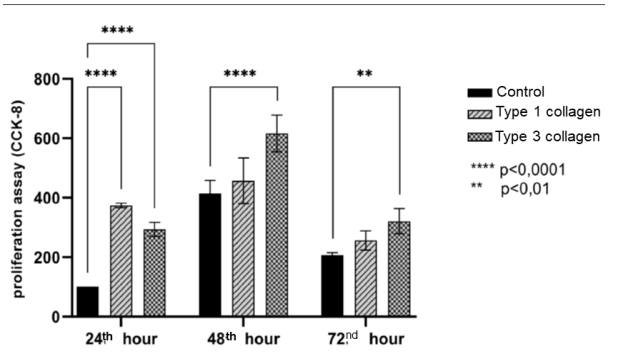

Figure 3: Proliferation analysis of HepG2 at different culture times

statistically significant difference was observed. At 72 h, there was an increase in AFP expression in HepG2 cells in culture medium supplemented with Type 1 collagen and a decrease in culture medium supplemented with Type 3 collagen, but no statistically significant difference was observed (Figure 4).

When we evaluated the confocal microscope images; specific binding with anti-AFP antibody was observed in HepG2 cells in all three culture media under a three-dimensional microscope. The AFP antibody was located in the cytoplasm close to the cell nucleus, indicating that collagen had no effect on the localisation of this antibody. Figures 5, 6, and 7 show confocal microscope images of HepG2 cells at three different culture stages in classical, Type 1 collagen, and Type 3 collagen culture media, respectively.

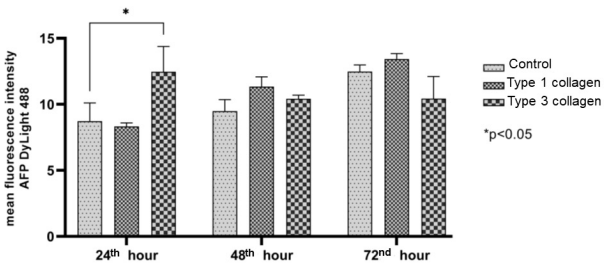

Figure 4: Bar graph showing the mean fluorescence intensity of AFP as mean ± SEM in HepG2 cells. AFP: Alpha fetoprotein

DISCUSSION

Hepatocellular carsinoma is most prominent risk factor is cirrhosis but in our country, the most common causes are viral hepatitis (Hep B and Hep C) and alcoholism (1-4). Nonalcoholic fatty liver disease, tobacco use, hemochromatosis, and diabetes are other risk factors. The incidence of COVID-19 increases with age and varies significantly depending on the geographic region. Early diagnosis of hepatocellular carcinoma significantly affects treatment and prognosis. Surgical resection and liver transplantation are the primary treatment approaches for early-stage hepatocellular carcinoma. If these methods are not available, chemotherapy is extremely useful in the advanced stage.

The microenvironment is extremely important for both normal and cancerous tissues. One of the most important components of the microenvironment is ECM components (8). The ECM consists of a non-cellular protein, glycoprotein, proteoglycan, and polysaccharide meshwork. The main component of the ECM is collagen. Collagens are often cross-linked and dispersed in such a way as to harden tissues. This elicits behavioural effects on

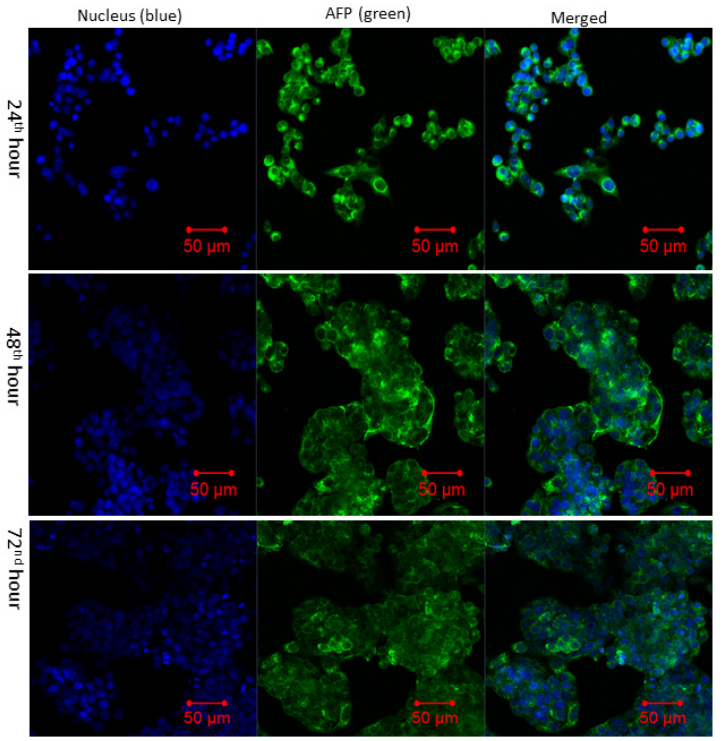

Figure 5: Confocal microscope images showing three levels of AFP expression in HepG2 cells cultured in conventional medium.

AFP: Alpha fetoprotein

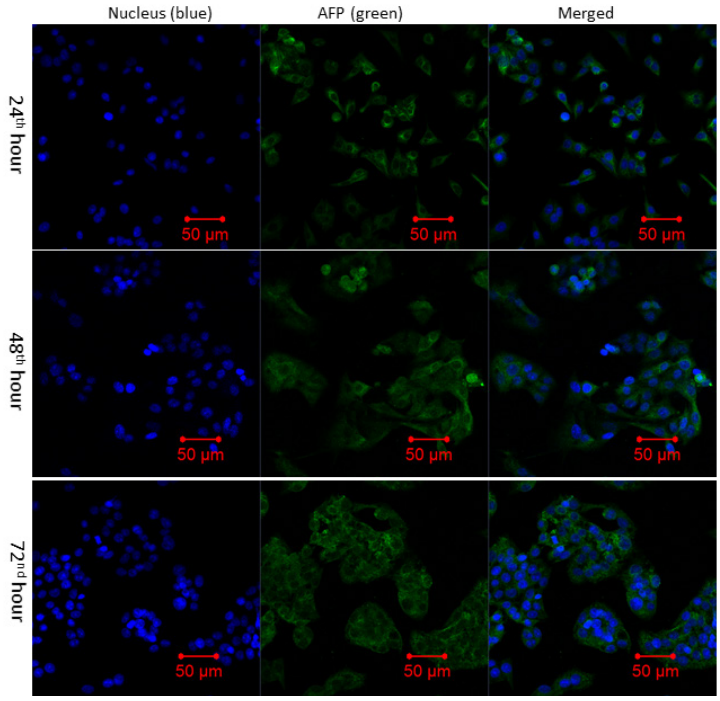

Figure 6: Confocal microscopy images of AFP expression in HepG2 cells in Type I collagen culture medium at three time points.

AFP: Alpha fetoprotein

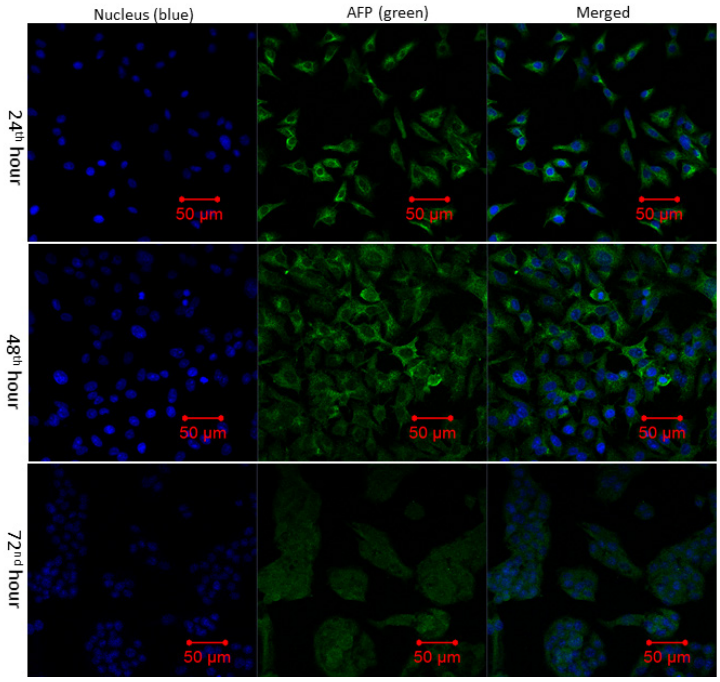

Figure 7: Confocal microscope images of AFP expression in HepG2 cells cultured in Type 3 collagen medium at three time points.

AFP: Alpha fetoprotein

surrounding tumour cells and regulates cell proliferation, differentiation, gene expression, migration, metastasis, and survival; thus, collagens directly influence the hallmarks of cancer (8, 9).

Molecular methods have shown that the level of Type 1 collagen is much higher in HCC samples than in normal liver samples (10). Type 3 collagen is the second most abundant collagen in the extracellular matrix. It is found primarily in the vascular systems, intestine, liver, skin, and lungs. Similar to Type 1 collagen, the distribution of Type 3 collagen is increased in many types of cancer (head and neck cancers, breast cancer, pancreatic cancer, colorectal cancer) (11-15).

Previous studies have shown that pancreatic cancer cells growing on Type 3 collagen exhibit increased proliferation, migration, and decreased expression of E-cadherin (16). Furthermore, Type 3 collagen plays a role in the invasion and metastasis of glioblastoma cells. These cells exhibit a high invasion and migration response when exposed to Type 3 collagen, and antibodies against Type 3 collagen inhibit these processes (17). Another study reported that Type 3 collagen altered some genes when invasive prostate cancer cells interacted with bone marrow stromal cells within the bone microenvironment. This interaction is important in demonstrating the involvement of Type 3 collagen in invasion and metastasis (18).

In a study comparing the cell spreading ability of human lung cancer cells on collagen Types 1 and 3 substrates with normal human tracheal epithelial cells; they showed that three different adenocarcinoma cell lines gradually started to contract after the initial spreading on Type 3 collagen and became round in 24 h. They suggested that their results showed that there may be a correlation between the degree of malignancy of human lung cancer cells and their ability to spread on collagen substrate and that cell spreading ability may be regulated by Type 3 collagen in some lung cancer cells (18). Wang et al. investigated Type 3 collagen expression and its roles in modulating lung carcinoma growth, viability, and apoptosis. They found that COL3A1 overexpression was associated with increased cell growth and clone formation but decreased cell apoptosis, whereas reduced COL3A1 expression led to decreased cell growth and clone formation and increased cell apoptosis (19).

In a study investigating how Type 1 collagen could restrict tumour expansion as a mechanical barrier, they found that Type 1 collagen expressed by cancer-associated fibroblasts (CAF) suppressed tumour growth by mechanically restraining tumour spread (20). In another study, they demonstrated that human breast cancer (BC) cells growing in culture media completely devoid of serum and seeded on Type 1 collagen coating exhibited a lower apoptotic rate and a decrease in Bax expression than those grown on plastic, indicating that Type 1 collagen promoted BC cell survival (21).

Collagen in the tumour microenvironment plays an important role in the regulation of tumour progression. Another study showed that Type 3 collagen, a component of tumour stroma, regulates myofibroblast differentiation and scar formation after cutaneous injury. In mouse and human breast cancer cell lines cultured at low concentrations of Type 3 and high concentrations of Type 3, it was shown that high concentrations were more effective in suppressing processes that are important in metastasis, such as surface adhesion and invasion. And it was stated that proliferation increased and cell death (apoptosis) decreased in mouse breast carcinoma cell lines in culture medium with low concentrations of Type 3 collagen. This mechanism has been mechanistically attributed to Type 3 collagen, which suppresses the procarcinogenic microenvironment by regulating stromal organisation, including the density and alignment of fibrillar collagen and myofibroblast (22).

CONCLUSION

It was determined that HepG2 cells cultured in both types of collagen have a morphologically similar structure, and Type 1 and Type 3 collagen have a proliferation-enhancing effect on cancer cells. AFP, an indicator of liver cancer, is found to be high in culture media containing Type 1 and Type 3 collagen, suggesting that there is a tendency towards poor prognosis for AFP expression of collagen types.

Ethics Committee Approval: Since the study is a cell culture study, ethics committee approval is not required.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- R.G.A., Z.A., H.M.; Data Acquisition- Z.A., B.E., G.D.; Data Analysis/Interpretation- Z.A., B.E., G.D.; Drafting Manuscript- B.E., Z.A.; Critical Revision of Manuscript – Z.A., R.G.A.; Final Approval and Accountability- Z.A., R.G.A.; Technical or Material Support- R.G.A.; Supervision- R.G.A.

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

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RESEARCH / ARAŞTIRMA
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HETEROZYGOUS PATHOGENIC *MASP2* VARIANT ASSOCIATED WITH INFANTILE GIANT CELL HEPATITIS WITH AUTOIMMUNE HAEMOLYTIC ANAEMIA IN A CHILD

OTOİMMÜN HEMOLİTİK ANEMİLİ İNFANTİL DEV HÜCRELİ HEPATİTLİ BİR ÇOCUKTA HASTALIKLA İLİŞKİLİ HETEROZİGOT PATOJENİK *MASP2* VARYANTI

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ABSTRACT

Objective: Infantile giant cell hepatitis with autoimmune haemolytic anaemia (GCH-AHA) is a rare disease characterised by giant cell and autoimmune haemolysis. The pathogenic mechanisms involve several factors, including genetic and immunological components, particularly those related to the lectin pathway of the complement system. In this study, we aimed to identify possible germline variations in patients with GCH-AHA.

Material and Method: Whole-exome sequencing (WES) was performed on a 6-month-old boy who was diagnosed with GCH-AHA. An in-house data analysis pipeline was applied to determine familial segregation using Sanger sequencing. ELISA was used for MASP2 protein detection.

Result: WES revealed a likely pathogenic heterozygous missense variant (p.(Cys618Tyr)) in the mannose-binding lectin (MBL)-associated serine protease-2 (*MASP-2*) gene. The *MASP2* variant identified in the serine protease domain was predicted to disrupt disulphide bonds. *In vitro* assays showed decreased MASP2 levels in the patient and mother compared with controls, supporting the potential pathogenicity of the variant.

ÖZET

Amaç: Otoimmün hemolitik anemili infantil dev hücreli hepatit (GCH-AHA), dev hücre ve otoimmün hemoliz ile karakterize nadir bir hastalıktır. Patojenik mekanizmalar, genetik ve immünolojik bileşenler, özellikle de kompleman sisteminin lektin yolağı ile ilgili olanlar dahil olmak üzere çeşitli faktörleri içerir. Bu çalışmada GCH-AHA'daki olası germ hattı varyasyonlarını analiz etmeyi amaçladık.

Gereç ve Yöntem: GCH-AHA tanısı konan 6 aylık bir çocukta tüm ekzom dizileme (TED) yapıldı. In house veri analizi algoritması uygulandı ve Sanger sekanslama ile ailesel segregasyon belirlendi. MASP2 protein tespiti için ELISA kullanıldı.

Bulgular: TED, mannoz bağlayıcı lektin (MBL) ile ilişkili serin proteaz-2 (*MASP-2*) geninde muhtemel bir patojenik heterozigot yanlış anlamlı varyantı (p.(Cys618Tyr)) ortaya çıkardı. Tahmin araçları bulgularına göre, serin proteaz domainde bulunan *MASP2* varyantının disülfit bağlarını bozduğu tahmin edilmiştir. In vitro testler, hastada ve etkilenen annede MASP2 seviyelerinin kontrollere kıyasla azaldığını göstererek varyantın potansiyel patojenitesini desteklemiştir.

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Conclusion: This study highlighted the association between a novel *MASP2* variant and GCH-AHA, emphasising the role of the lectin pathway in the pathogenesis of this rare disorder. The variable expressivity and incomplete penetrance observed in MASP2 deficiency underscore the complexity of genotype-phenotype correlations. Further investigations into the lectin pathway's detailed activation and its impact on GCH-AHA pathogenesis are warranted for a comprehensive understanding of the disease mechanisms.

Keywords: Infantile giant cell hepatitis, autoimmune haemolytic anaemia, complement system, MASP2, whole-exome sequencing

Sonuç: Bu çalışma, yeni bir *MASP2* varyantı ile GCH-AHA arasındaki ilişkiyi vurgulayarak, bu nadir bozukluğun patogenezinde lektin yolunun rolünü vurgulamaktadır. MASP2 eksikliğinde gözlemlenen değişken ifade ve eksik penetrasyon, genotip-fenotip korelasyonlarının karmaşıklığının altını çizmektedir. Lektin yolunun ayrıntılı aktivasyonu ve bunun GCH-AHA patogenezi üzerindeki etkisine ilişkin daha fazla araştırma, hastalık mekanizmalarının kapsamlı bir şekilde anlaşılması için önem arz etmektedir.

Anahtar Kelimeler: İnfantil dev hücreli hepatit, otoimmün hemolitik anemi, kompleman sistemi, MASP2, tüm ekzom dizilimi

INTRODUCTION

Infantile giant cell hepatitis with autoimmune haemolytic anaemia (GCH-AHA) is a progressive disorder characterised by diffuse giant cell hepatocyte transformation and autoimmune haemolysis (1, 2). Patients diagnosed within the first two years of life with elevated aminotransferase levels, acute liver injury, and haemolytic anaemia (3). Infection susceptibility is common, with 25% of patients harbouring infections such as mycoplasma, Epstein–Barr Virus (EBV), and acute otitis media.

The aetiology of GCH-AHA is poorly understood; approximately 49% of patients have idiopathic disease. The clinical presentation of GCH-AHA varies. Biliary atresia, immune dysregulation, neonatal hemochromatosis, and hypopituitarism have been seen in the patients (4). Although the genetic background is unknown, there is strong evidence of the role of humoral immunity and the complement system. Patients are Coombs-positive for immunoglobulin G positive (IgG+), implying involvement of complement protein 3 (C3) and immunoglobulins. In addition, complement-mediated damage and C5-9-mediated hepatocyte injury have been observed in children with GCH-AHA (5, 6).

The complement system is a system within the natural immune system that is activated in three ways: classical, alternative, and lectin pathways (7). When the lectin pathway (LP) is activated, the pathogen is recognised by its mannose moieties via mannose-binding lectin (MBL) and/or ficolin, and this engagement activates the MBL-associated serine proteases: MASP1, MASP2, and MASP3. MASPs cleave the complement system; autoactivation of MASP2 triggers cleavage of complement factors C4 and C2 and generates C3 convertase (8). MASP2 is the key mediator of LP because MASP2 is the only serine protease capable of cleaving C4 (9). MASP2 can initiate activation of the lectin pathway without the contribution of other proteases.

The Inborn Errors of Immunity Committee (International Union of Immunological Societies (IUIS) Primary Immune Deficiency Expert Committee) considered autosomal recessive MASP2 deficiency as an inherited complement deficiency (10). MASP2 deficiency (MIM:605102) is a rare disorder with a prevalence of <1:1,000,000. This disorder was first described in 2003 in an adult patient with severe recurrent pneumococcal infections in addition to autoinflammatory and autoimmune diseases. This patient carried the biallelic *MASP2*:p.(D120G) variant, which significantly reduced serum MASP2 levels (11). Current studies show that MASP2 deficiency can be inherited as an autosomal dominant disease. Hejazi et al. reported a heterozygous *MASP2* variant in a paediatric patient with Crohn's disease and severe tuberculosis infection (12). It has been observed that some heterozygous or homozygous variants cause significant decreases in protein levels in studies that examined the relationship between *MASP2* variants and serum or plasma enzyme levels. In addition, low enzyme levels were not observed in all individuals who carried variants of MASP2, which cause decreased enzyme levels. For example, according to the GnomAD database, the first identified variant, p.(D120G), was found frequently in the population (2.21%), indicating incomplete penetrance of this disease (11).

MASP2 protein levels are diverse among ethnic groups and populations, and variations are associated with enzyme levels (13). Low serum MASP2 levels are related to increased susceptibility to infections in several settings (14, 15). Mistegaard *et al.* identified low MASP2 levels in patients with common variable immune deficiency (CVID) and suggested that dysregulated enzyme levels may have a master or slave effect in some patients with CVID (16). In addition, *MASP2* gene variations that result in lower serum levels are associated with systemic lupus erythematosus susceptibility (17).

Here, we report a likely pathogenic heterozygous missense *MASP2* gene variant in a 6-month-old patient diagnosed with giant cell hepatitis with autoimmune haemolytic anaemia.

MATERIAL AND METHODS

Patient

A 6-month-old boy was admitted with pallor and a rapid drop in haemoglobin. He had mild jaundice and hepatosplenomegaly on admission. He was born to unrelated parents. Laboratory examination revealed direct antibody test (DAT)-positive haemolytic anaemia with mildly elevated transaminase levels (Table 1). Infectious and metabolic workup, serum immunoglobulin levels, and lymphocyte immunophenotyping were normal. Methylprednisolone 2 mg/kg/day was started. Because of early-onset autoimmune haemolytic anaemia, genetic testing was also planned to rule out underlying primary immunodeficiency. This study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 24.01.2020, No: 02). Written and oral informed consent was obtained from family members or legal representatives.

Whole-exome sequencing and data analysis

Peripheral blood samples were collected from the index case (II-1) and the parents (mother; I-2 and father I-1). DNA isolation was performed from peripheral blood using a QIAamp DNA Blood Mini kit (QIAgen Valencia, CA, USA). The amount and quality of the isolated DNA samples were evaluated using a spectrophotometry device (ND-2000, Thermo Fisher Scientific, MA, United States). Whole-exome sequencing (WES) was performed in the index case. An Agilent SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) kit was applied based on the manufacturer's protocol for a pre-captured library, and sequencing was performed in paired-end mode using the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA, US). The sequence reads were mapped to the hg19 reference genome using the Burrows-Wheeler Aligner (BWA) programme (18). Aligned data were converted to VCF format for variant calling and base quality calibrations using the Genome Analysis Toolkit (GATK) (https://gatk.broadinstitute.org/hc/en-us). Variants with a Phred score of 30 (≥Q30) confidence were called. Variants were annotated using the ANNOVAR (https://annovar.openbioinformatics.org/en/latest/) and FRANKLIN (https://franklin.genoox.com/clinical-db/home) tools. Variants were categorised based on the American College of Medical Genetics and Genomics (ACMG) classification, and pathogenic (P), likely pathogenic (LP), or variant of unknown significance (VUS) variants were included. Two different analysis pipelines were applied for variant prioritisation (Figure 1). Global filtering was applied for coding and splice region variants with minor allele frequency (MAF)<0.005, P, LP, and VUS variants according to ACMG and Clinvar records were filtered. The primary immunodeficiency (PID)-related genes with a minimum MAF<0.05 and ACMG classification were filtered using a second filtering approach. The PID-specific gene list (including 451 genes) was generated according to the International Union for Immunological Societies (IUIS) guidelines and the literature knowledge (Supplemental Table 1). *In silico* prediction tools; Combined Annotation-Dependent Depletion (CADD), Sorting Intolerant From

Table 1: Clinical characteristics of the patient and the mother.

Clinical findings	Index case	Mother	Reference values
Peripheral blood tests			
WBC (10^9xL)	28.8	9.3	5.2-12.4
Hgb (g/dl)	5.8	13.5	12-18
Plt (10^3x µL)	67.3	349	130-400
HCT (%)	16	41	37-52
MCV (fl)	88.8	85.4	80-99
AST (IU/L)	4841	NA	0-40
ALT (IU/L)	3988	NA	0-40
GGT (IU/L)	106	NA	3-25
ALP (IU/L)	442	NA	20-155
Total biluribin (mg/dL)	20.18	NA	0.3-1.2
Direct biluribin (mg/dL)	17	NA	0-0.2
Direct Coombs	AHG+3, IgG+3, C3d+3	NA	Negative
PT (sec)	12.6	NA	10.4-14
APTT (sec)	12.6	NA	21-32
INR	1.07	NA	0.85-1.15
AFP (U/L)	78.63	NA	<13
CRP (mg/L)	29.39	NA	<5
Cold agglutinin (mg/L)	Positive	NA	Negative
Complement values			
C3 (mg/L)	1.54	1.04	09.-1.8
C4 (mg/dL)	0.21	0.22	Negative
Immunoglobulin levels			
IgA (mg/dL)	31.2	127	(Ref. 19)
IgG (mg/dL)	528	1255	(Ref. 19)
IgM (mg/dL)	98.3	149.8	(Ref. 19)
IgE (mg/dL)	33.95	NA	(Ref. 19)
Autoimmune antibodies			
ANCA	Negative	NA	Negative
ANA	Negative	NA	Negative

WBC: White blood cell, Hgb: hemoglobin, Plt: platelet, HCT: hematocrit, MCV: mean corpuscular volume, AST: aspartate transferase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, PT: prothrombin, APTT: activated partial thromboplastin time, INR: international normalized ratio, AFP: alpha-fetoprotein, CRP: c-reactive protein, C3: complement component 3, C4: complement component 4, IgA: immunoglobulin A, IgG: immunoglobulin G, IgM: immunoglobulin M, IgE: immunoglobulin E, ANCA: antineutrophil cytoplasmic antibodies, ANA: antinuclear antibody, Ref: reference

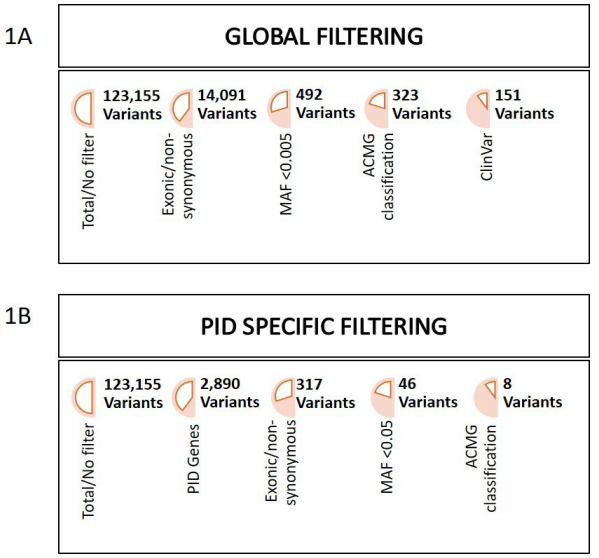

Figure 1: Variant filtering strategy for whole-exome sequencing data. **A)** Overview of global and **B)** Primary Immune Deficiency (PID)-specific variant filtering strategies with the total number of variants for each filtering step.

Tolerant (SIFT), Polymorphism Phenotyping ver.2 (PolyPhen-2), MutationTaster, and Functional Analysis through Hidden Markov Models (FATHMM), were used to predict the impact of the variants. Sanger sequencing detected the familial segregation and CLC genomics workbench 3.6.5 (QIAGEN, Aarhus, Denmark) used for analysis. Primers are available upon request.

MASP2 protein expression

Plasma samples were collected from the index case (III-5), the affected mother (II-6), and five healthy individuals with a mean age of 26 years (range: 25-34 years). MASP2 levels were determined by Enzyme-Linked Immuno-Sorbent Assay (ELISA) using a human MASP2 ELISA kit (Catalogue No: MBS2506522, MyBioSource, San Diego, USA) according to the kit's protocol. Immobilised mannan was used as a ligand to investigate the functional activity of the LP pathway. Mannan was purchased from Sigma (from Saccharomyces cerevisiae; M7504), phosphate-buffered saline (PBS) was used as solvent (10 mg/ml), and stored at 20°C. ELISA plates (microtiter plates) were coated with mannan (100 g/ml; 100 L per well) in 100 mM Na2CO3/NaHCO3 buffer (coating buffer, pH 9.6) overnight at +4°C. After each processing step, 100 µl of PBS containing 0.05% Tween-20 was used to wash the plates three times. Residual binding areas were covered by 100 µL PBS including 1% bovine serum albumin (BSA) for 1 h at 37°C. Human plasma samples diluted 1:4 using dilution buffer (kit carrier) were pre-incubated on ice for 15 min. After that, the plates were incubated at 37°C for 1 h. The minimum measurable MASP-2 concentration using the ELISA kit was 3.13 ng/mL. An enzyme-linked immunosorbent assay reader was used to measure colour intensity, and reading was performed at 450 nm. Concentration calculations were drawn as a "four-parameter logistic curve in log-log graph" paper, with standard concentration values written on the X-axis and OD values on the Y-axis. The concentration was calculated from the curve by multiplying by the dilution ratio. Comparison of MASP2 levels between the groups was performed using the Mann-Whitney U test, a non-parametric test, with GraphPad Prism version 5.01. (GraphPad Software, San Diego, California, USA, www.graphpad.com).

RESULTS

Clinical history of the index case

The patient received intravenous immunoglobulin (IVIG) at a total dose of 2 g/kg because the haemolytic anaemia did not improve. After haemoglobin levels stabilised without active haemolysis during the second month of steroid treatment, cyclosporine was added for maintenance and methylprednisolone was tapered. However, at follow-up, transaminase and bilirubin levels progressively increased (Table 1) and remained high despite discontinuation of cyclosporine and steroids. Liver biopsy was performed with a presumptive diagnosis of autoimmune or toxic hepatitis. Pathological examination revealed giant cell hepatitis. The final diagnosis was giant cell hepatitis and autoimmune haemolytic anaemia. Because the patient experienced seizures with encephalopathy and nephrotoxicity after the resumption of cyclosporine therapy, methylprednisolone was restarted. As recommended in the literature, an anti-CD20 monoclonal antibody (rituximab) at the standard dose (375 mg/m^2) was administered once weekly for six consecutive weeks in addition to steroid therapy. Eight weeks after the start of rituximab treatment, alanin aminotransferase (ALT) and aspartate aminotransferase (AST) levels returned to normal, and bilirubin levels decreased significantly to 1.3 g/dL, with normal haemoglobin levels on complete blood count (CBC) and persistence of DAT positivity (IgG 2+/C3 2+). Maintenance therapy with azathioprine was initiated, and steroid therapy was tapered and eventually discontinued. DAT became negative after two years of immunosuppressive therapy. He remained on azathioprine therapy and had no active signs of haemolysis or hepatic impairment at regular follow-up of four years after diagnosis. The father and mother had no medical history and were found to be healthy on clinical examination (Table 1) (19).

Whole-exome data analysis and *in silico* prediction

Whole-exome sequencing was performed in the index case. Global filtering was applied to the coding regions (Figure 1), MAF<0.005, nonsynonymous, and ACMG scores for the P, LP, and VUS variants (Figure 1). Filtered genes/variants (n=151) are listed in Supplemental Table 2 that were found to be irrelevant to the phenotype.

Based on the diagnosis of early-onset autoimmune haemolytic anaemia, no known pathogenic/likely pathogenic variants were found, except for the heterozygous *ABCA4*:p.(Gly172Ser) variant. Monoallelic *ABCA4* variants are associated with age-related macular degeneration (MIM:153800). The patient had no clinical symptoms at the current age, so follow-up was planned. In further analysis, the patient was screened for variants in a 451 PID-related genes list (see Supplemental Table 1), and eight candidate variants were detected (Figure 1 and Table 2). Among these, the heterozygous missense variant NM_006610.4:c.1853G>A (rs764932450) in the serine protease domain of *MASP2* was found to be relevant to clinical findings. The GnomAD frequency of the MAF was G=0.000008, ACMG class was VUS (evidence PP3 and PM2), and the aggregated prediction score was harmful. Sanger sequencing confirmed heterozygosity in the index case (III-5) and the mother (II-6) (Figure 2).

MASP2 consists of 12 exons: the serine protease (SP) domain, which is responsible for protease activity, two C1r/C1s/Uegf/bone morphogenetic protein (CUB) domains (CUB1 and CUB2), located in the heavy chain, the epidermal growth factor-like (EGF) domain, which separates the two CUB domains, and two complement control protein (CCP) domains (CCP1 and CCP2). The p.(Cys618Tyr) missense variant was found in exon 12, which encodes the serine protease domain (Figure 3A). On the 3D structure of the MASP2 protein, p.(Cys618Tyr) variant was predicted to disrupt disulphide bonds within CYS598 and CYS618 (Figure 3A). To determine the pathogenicity of the p.(Cys618Tyr) variant, SIFT, PolyPhen2, and Mutation Taster predictions were used and shown to be disease-causing, deleterious, and probably damaging, respectively. The CADD score was 24, and the variant position was also evolutionarily conserved among the species (Table 2 and Figure 3B).

Impact of the *MASP2* variant on plasma levels

To investigate the effect of the NM_006610.4:c.1853G>A;p.(Cys618Tyr) variant on active MASP2 levels, ELISA was performed on plasma samples from the index, mutated mother, and healthy controls (n=5). The MASP2 levels were 153 ng/ml in the patient and 152.3 ng/ml in the mother. The median MASP2 level in healthy control samples was 185 ng/ml (min 159-max 603 ng/ml). A decrease in MASP2 levels was found in index cases and mothers with heterozygous variation compared with healthy controls, but there was no statistical significance (p=0.12).

Table 2: The list of candidate variants after filtering based on the Primary Immune Deficiency specific filtering.

Gene	Transcript	dbSNP	AA change	Effect	Zygosity	Frequency	SIFT	PolyPhen-2	Mutation taster	CADD
ERCC4	NM_005236.3:c.2624A>G	rs1800124	p.Glu875Gly	Missense	het	G=0.013304/3345	Deleterious	Possibly damaging	D	25.08
FANCA	NM_000135.4:c.2941T>C	rs191943709	p.Cys981Arg	Missense	het	G=0.000036/9	Deleterious	Probably damaging	D	22.6
FERMT1	NM_017671.5:c.1831C>G	N/A	p.Gln611Glu	Missense	het	N/A	Tolerated	Possibly damaging	D	23.05
FERMT3	NM_031471.6:c.772G>T	rs139416960	p.Asp258Tyr	Missense	het	A=0.000012/3	Deleterious	Probably damaging	D	32
MASP2	**NM_006610.4:c.1853G>A**	**rs764932450**	**p.Cys618Tyr**	**Missense**	**het**	**G=0.000008/2**	**Deleterious**	**Probably damaging**	**D**	**24**
POLE	NM_006231.4:c.6610G>A	rs1060500871	p.Val2204Met	Missense	het	T=0.000014/2	Tolerated	Possibly damaging	D	22.8
POLE	NM_006231.4:c.6494G>A	rs5745068	p.Arg2165His	Missense	het	T=0.005893/1432	Tolerated	Possibly damaging	D	25.9
TLR3	NM_003265.3:c.1660C>T	rs121434431	p.Pro554Ser	Missense	het	T=0.000528/74	Deleterious	Probably damaging	D	24.1

Het: Heterozygous, AA: amino acid, SIFT: The Sorting Intolerant from Tolerant, PolyPhen-2: Polymorphism Phenotyping ver.2, CADD: Combined Annotation-Dependent Depletion

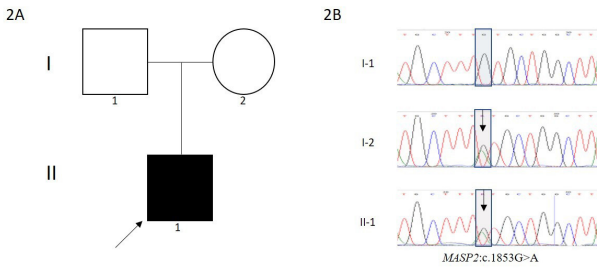

Figure 2: Family tree and segregation analysis. A) Pedigree of the *MASP2* family. B) Segregation analysis of the *MASP2* variant. The open shapes in the pedigree represent phenotypically unaffected family members, and the black square represents the affected proband. The arrows indicate the heterozgous alles of the parents.

often contributes to the pathogenesis of GCH-AHA (20, 21). Clinical findings such as hepatitis and Coombs-positive anaemia may indicate the involvement of B-cell related autoimmune dysregulation or complement deficiency. Studies have shown a high degree of complement-mediated hepatocyte injury in patients with GCH-AHA (5, 22). In this study, WES identified the heterozygous missense variant NM_006610.4:c.1853G>A in *MASP2* in a 6-month-old patient with GCH-AHA. The patient presented with hepatosplenomegaly, mildly elevated transaminase, and DAT-positive haemolytic anaemia. The pathogenic p.(Cys618Tyr) variant is located in the SP domain of the protein and is responsible for the catalytic activity of the entire molecule and may prevent the formation of the disulphide bond that binds the two polypeptide chains together in

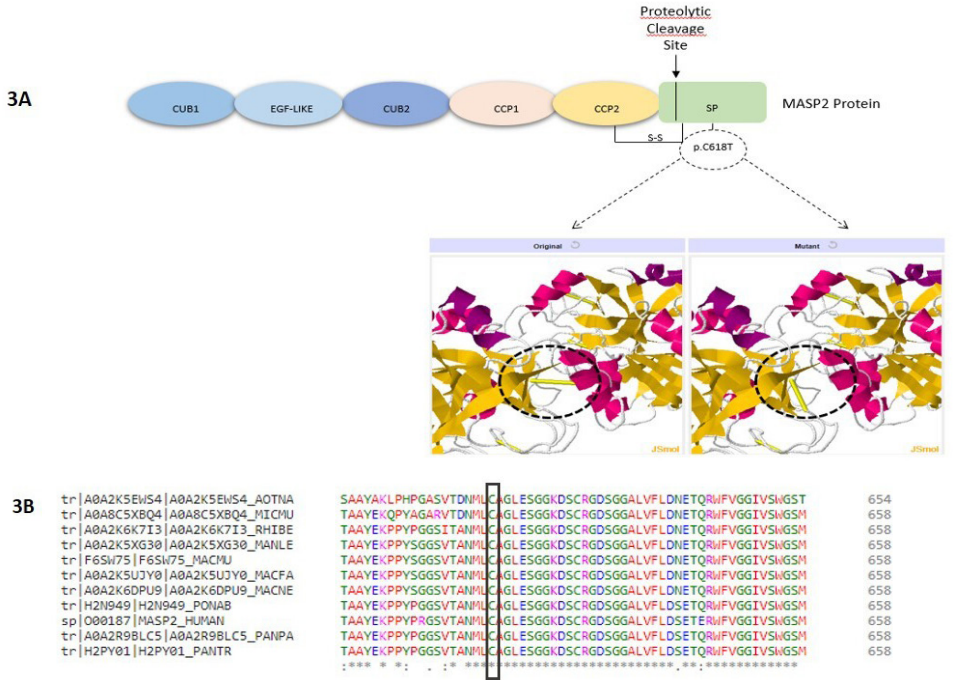

Figure 3: Localisation of p. Cys618Thr variant on the serine protease (SP) domain of MASP2 and a graphical demonstration of the wild-type and mutant forms of the MASP2 protein. 3A**)** The wild-type residue is shown to form a disulphide bond with residues CYS 598 and CYS 618 (Distance: 1.912 Å) on chain B of the wild-type structure 1q3x and the substitution disrupts this bond. 3B**)** Conservation of 618. amino acids of the MASP2 protein in primates. Conservation analysis was performed using the CLUSTAL Omega (1.2.4) multiple sequence alignment tool. The C1r/C1s/Uegf/bone morphogenetic protein 1 (CUB1) domain is an epidermal growth factor-like (EGF) domain, and the CUB2 domain is followed by two complement control protein (CCP1 and CCP2) domains and the C-terminal SP domain. The SP domain is linked to the CCP2 domain by a small linker region with S-S disulphide bonds.

DISCUSSION

Giant cell hepatitis associated with autoimmune haemolytic anaemia is associated with infection, cholestasis, and hepatic inflammation. The aetiology of this disease has not been fully elucidated, it is a factor that plays a role in immune system dysregulation. The involvement of immune-mediated findings, such as autoimmunity, most

the functionally active form of the MASP-2 protein (23). Although the mode of inheritance was autosomal dominant and segregation analysis confirmed that the mother also carried the same variation without obvious clinical symptoms, MASP2 deficiency is difficult to diagnose due to unclear genotype-phenotype correlation, low penetrance, and significant clinical heterogeneity (24).

Previous studies have shown that different mono- or bi-allelic variants of *MASP2* result in changes in serum/plasma levels and functions of MASP2 (11). Homozygous *MASP2* variants, which are believed to affect the protein, do not always cause low enzyme levels. On the contrary, some heterozygous variants can cause significant enzyme deficiency (13). The relatively low levels of MASP2 in mothers compared with healthy controls support the variable expressivity reported previously, but there is wide variability in healthy controls. Additionally, MASP2 enzyme levels were not related to age, gender, or physical activity, and there were no statistically significant differences between serum and plasma enzyme levels. Ytting et al. showed that the mean serum enzyme level in healthy controls was 416 ng/mL (ranging from 125 to 1152) and that MASP2 levels below 100 ng/mL were indicative of MASP2 deficiency in Caucasians (13, 25). To have a better understanding of the exact effects of these *MASP2* variants, the activation of the lectin pathway should be investigated in detail. GCH-AHA is a rare disorder and the genetic aetiology is unclear. Possible mechanisms include autoimmunity or dysregulation of the complement system affecting hepatocytes and erythrocytes. *MASP2* variants may contribute to the pathogenesis of GCH-AHA as a primary or secondary disease. Evaluation of enzyme levels and MBL pathway activity is the most accurate approach to assessing the clinical impact of monoallelic or biallelic *MASP2* variants.

CONCLUSION

This study presented the clinical findings and genetic analysis of an index case diagnosed with giant cell hepatitis and autoimmune haemolytic anaemia. Despite initial treatment challenges and complications, including progressive elevation of transaminase and bilirubin levels following cyclosporine therapy and subsequent seizures with encephalopathy and nephrotoxicity, our patient experienced significant improvement following a comprehensive therapeutic approach. Whole-exome sequencing revealed a heterozygous missense variant NM_006610.4:c.1853G>A (rs764932450) in the serine protease domain of MASP2, which was deemed relevant to the clinical phenotype. Through *in silico* predictions and functional assays, we elucidated the potential pathogenicity of the identified variant and its contribution to the observed phenotype.

Ethics Committee Approval: The study has ethical approval from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 24.01.2020, No: 02).

Informed Consent: Written and oral informed consent was obtained from family members or legal representatives.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- M.S., S.F., M.S.; Data Acquisition – S.F., M.S., A.K., B.I.; Data Analysis/Interpretation- M.S., S.F., M.S., A.K., Z.O., S.O., B.I.; Drafting Manuscript- M.S., S.F., M.S., A.K.; Critical Revision of Manuscript- M.S., S.F., M.S., A.K., Z.O., S.O., B.I.; Final Approval and Accountability- M.S., S.F., M.S.; Supervision- M.S.

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

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INDUCED PLURIPOTENT STEM CELL-DERIVED MICROGLIA TO STUDY NLRP3 INFLAMMASOME ACTIVATION IN ALZHEIMER'S DISEASE

İNDÜKLENMİŞ PLURİPOTENT KÖK HÜCRE KAYNAKLI MİKROGLİA HÜCRELERİNİN ALZHEİMER HASTALIĞINDA NLRP3 İNFLAMAZOM AKTİVASYONU ARAŞTIRMALARINDA KULLANIMI

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ABSTRACT

Objective: Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disease. Besides amyloid beta (Aβ) and tau accumulations, inflammation also contributes to AD pathogenesis. NLR family pyrin domain containing 3 (NLRP3) inflammasome activation in microglia is thought to be associated with AD. Since animal models of AD do not accurately reflect human pathology, in our study, human induced pluripotent stem cells (iPSCs) were differentiated into microglia, and their potential to be used in NLRP3 pathway-related mechanisms in AD was investigated.

Material and Method: iPSC cell lines of AD, isogenic, or control genotypes were differentiated into microglia and cells from different stages of the differentiation were characterized by flow cytometry, real-time quantitative polymerase chain reaction (RT-qPCR), immunocytochemistry, and Western blot. The expression of proteins associated with the NLRP3 pathway was investigated by Western blot. For functional analysis, cytokine release was assessed by Enzyme-linked immunosorbent assay (ELISA) upon NLRP3 inflammasome activators (lipopolysaccharide (LPS),

ÖZET

Amaç: Alzheimer hastalığı (AH) geri dönüşümsüz ve ilerleyici bir nörodejeneratif hastalıktır. Amiloid beta (Aβ) ve tau birikimlerinin yanı sıra, inflamasyon da AH patogenezinde rol oynamaktadır. Mikroglia hücrelerinde NLR ailesi pirin domain içeren 3 (NLRP3) inflamazomunun aktivasyonunun AH ile ilişkili olduğu düşünülmektedir. Hastalığın hayvan modelleri insandaki patolojiyi tam olarak yansıtamadığından, çalışmamızda insan kaynaklı indüklenmiş pluripotent kök hücreler (İPKH) mikrogliaya farklılaştırılarak, AH'de NLRP3 yolağı ilişkili mekanizmaların araştırılmasındaki kullanım potansiyeli incelenmiştir.

Gereç ve Yöntem: AH, izogenik ve kontrol genotipteki iPKH hücre hatları mikrogliaya farklılaştırılmış ve farklılaşmanın çeşitli aşamalarındaki hücreler akış sitometrisi, gerçek zamanlı kantitatif polimeraz zincir reaksiyonu (RT-qPCR), immünositokimya ve Western blot yöntemleriyle karakterize edilmiştir. NLRP3 yolağı ile ilişkili proteinlerin ekspresyonu Western blot ile incelenmiştir. Fonksiyonel analizler için, NLRP3 inflamazom aktivatörleri (lipopolisakkarid (LPS), Aβ) ve inhibitörü (sitokin salgılanmasını inhibe edici ilaç 3, CRID3) varlığında sitokin sa-

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Aβ) and inhibitor (cytokine release inhibitory drug 3, CRID3) treatments. The phagocytosis of pHrodo particles and Aβ were evaluated by flow cytometry, fluorescence microscopy, and live cell imaging system.

Result: In our study, we could differentiate microglia from iPSCs derived from different genotypes. These microglia cells expressed various microglia and NLRP3 inflammasome-related markers and were able to phagocytose pHrodo particles and Aβ. The stimulation of the microglia cells with LPS and Aβ caused IL-1beta and IL-18 release, while CRID3 reversed this effect.

Conclusion: Our results show that iPSC-derived microglia generated in this study recapitulate microglia functional characteristics and can therefore be used to study NLRP3 pathway-associated disease mechanisms and treatment options.

Keywords: Microglia, induced pluripotent stem cells, neuroinflammation, NLRP3 inflammasome, amyloid beta

lımı ELISA ile ölçülmüştür. pHrodo partiküllerinin ve amiloid betanın fagositozu ise akış sitometrisi, floresan mikroskobu ve canlı hücre görüntüleme sistemi ile değerlendirilmiştir.

Bulgular: Çalışmamızda farklı genotiplerdeki İPKH'ler başarılı biçimde mikrogliaya farklılaştırılmıştır. Elde edilen mikroglia hücrelerinin çeşitli mikroglia ve NLRP3 inflamazomu ilişkili belirteçleri eksprese ettiği ve pHrodo ile amiloid betayı fagosite edebildiği gösterilmiştir. Mikroglia hücrelerinin LPS ve amiloid beta ile uyarılması IL-1beta ve IL-18 salımına neden olurken, CRID3 uygulanması bu etkiyi tersine çevirmiştir.

Sonuç: Çalışmamız İPKH'den farklılaştırılan mikroglia hücrelerinin, mikroglianın işlevsel özelliklerini taşıması nedeniyle NLRP3 yolağı ile ilişkili hastalık mekanizmalarının ve tedavi seçeneklerinin araştırılmasında kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Mikroglia, indüklenmiş pluripotent kök hücreler, nöroinflamasyon, NLRP3 inflamazomu, amiloid beta

INTRODUCTION

Alzheimer's Disease (AD) is the leading cause of age-related dementia. It affects more than 50 million people worldwide (1). AD patients show progressive alterations of cognitive functions and have problems with communication, judgement, and orientation skills (2). Except for the limited number of drugs providing symptomatic treatment, there is no cure for AD (3).

The most prominent hallmarks of AD are the extracellular deposition of amyloid beta (Aβ) plaques and neurofibrillary tangles caused by the intracellular accumulation of hyperphosphorylated tau protein (4). Neuroinflammation, mediated by microglia and astrocytes surrounding Aβ plaques and neurofibrillary tangles, also contributes to the disease (5). Microglia, the innate immune cells of the central nervous system (CNS), plays a central role in brain homeostasis (6). They monitor the environment searching for pathogens and respond to inflammatory stimuli to maintain homeostasis (7). However, exposure to chronic stimuli causes microglial activation (8). Activated microglia switch to amoeboid morphology and have a number of changes such as surface markers and molecules that they release or recognize (7). They become more motile and migrate to the relevant area using chemotactic gradient clues (9).

Current studies have shown that the NLR family pyrin domain containing 3 (NLRP3) inflammasome pathway is activated in AD patients and mouse models (10, 11). NLRP3 is the best-characterized inflammasome consisting of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and caspase-1 proteins (12). The NLRP3 inflammasome pathway is activated in microglia in two steps. The first step is priming (e.g., triggered by lipopolysaccharide (LPS)), which promotes NF-kB gene transcription and pro-interleukin-1β (pro-IL-1β) and pro-IL-18 protein production, and the second step is activation (e.g., triggered by adenosine triphosphate (ATP), Aβ) (13). The activation signal leads to the assembly of NLRP3 inflammasome components, promoting the cleavage of pro-caspase-1 to active caspase-1, which further induces the production of IL-1β and IL-18 in their mature form (14). Activation of this pathway in AD mouse model leads to increased Aβ deposition, reduced Aβ phagocytosis and results in cognitive impairment (11). Several studies have shown that NLRP3 inflammasome inhibition ameliorates Aβ pathology and protects against AD in rodent models (15, 16). Therefore, NLRP3 inflammasome inhibitors have been evaluated as therapeutic targets for AD. The cytokine release inhibitory drug 3 (CRID3) is one of the most potent and selective inhibitors of the NLRP3 inflammasome (17, 18). Inhibition of this pathway with CRID3 has been considered a promising anti-inflammatory therapy in AD (19).

Animal models have helped to investigate the key mechanisms involved in the physiopathology of AD. However, these models do not fully recapitulate the characteristics of AD and therefore need to be completed (20). The use of human-derived microglia is not always possible due to the inability to obtain samples from the CNS. The discovery of induced pluripotent stem cells (iPSCs) overcame the limited possibilities of animal models and offered a unique opportunity to investigate disease mechanisms using human-derived brain cells (21). Recently, several groups have proposed different methods to generate microglia from human iPSCs (22). As iPSCs represent the pathology better, they can be eligible to investigate new treatment opportunities in AD.

The aim of this study was to characterize human iPSC-derived microglia and investigate their potential to be used in NLRP3 pathway-related neuroinflammatory mechanisms involved in AD.

MATERIAL AND METHODS

Human iPSC cell lines

All three iPSC lines were reprogrammed from skin fibroblasts derived either from a control subject (CTL) or an AD

patient (AD) carrying the ΔE9 mutation in the presenilin-1 (*PSEN1*) gene. In addition, an isogenic line (ISO) was used where the mutation ΔE9 has been corrected in the AD line.

All the iPSC lines were generated with the approval of the committee on Research Ethics of Northern Savo Hospital district (Date: 29.04.2016, No: 123) after written consent from the subjects. iPSC lines were provided by the University of Eastern Finland (UEF) based on a material transfer agreement with the German Center for Neurodegenerative Diseases (DZNE).

iPSC-derived microglia cell culture

Microglial differentiation from iPSCs was performed according to a previously published protocol (23). iPSCs were expanded in mTeSR™ Plus medium (Stem Cell Technologies, Canada) on matrigel (Corning, USA). Firstly, hematopoietic progenitor cells (HPCs) were generated from iPSCs, and then they were differentiated into microglia (Figure 1A). All the cells were incubated under 37°C and 5% CO_2 conditions in an incubator (ICO 240, Memmert, Germany).

Flow cytometry analysis

Phenotypical characterization of the cells was performed using flow cytometry. For each sample $2x10^5$ cells were used. Fluorochrome-conjugated antibodies CD11b-BV605 (1:33 (v:v), 562721, Horizon, BD, USA), CD45-APC-Cy7 (1:33 (v:v), 557833, (Pharmingen, BD), CD200R-APC (1:33 (v:v), 329308, BioLegend, USA), P2RY12-PE (1:33 (v:v), 392104, BioLegend), CD14-PE-Cy7 (1:33 (v:v), 557742, Pharmingen, BD), CD16-BV421 (1:33 (v:v), 562874, Horizon, BD), CX3CR1-FITC (1:33 (v:v), 341606, BioLegend), CD235a-PE-Cy7 (1:33 (v:v), 349112, BioLegend), CD43-APC (1:33 (v:v), 343206, BioLegend), CD34-PE (1:33 (v:v), 130-120-515, Miltenyi Biotec, Germany) or primary antibodies anti-TMEM119 (1:50 (v:v), ab185333, Abcam, UK), anti-TREM2 (1:50 (v:v), MABN755, Millipore, French) followed by labelling with secondary antibodies goat anti-rabbit Alexa Fluor 647 (1:10000 (v:v), A-21244, Invitrogen, USA) or goat anti-rat Alexa Fluor 488 (1:10000 (v:v), A-11006, Invitrogen) were used. To assess cell viability, the cells were stained with 7-AAD (Pharmingen, BD). Samples were analyzed using a flow cytometer (FACS Canto II, BD), data were analyzed using FlowJo software (BD).

Real-Time quantitative PCR (RT-qPCR)

RNA purification was performed using the RNeasy® Mini Kit (74104, Qiagen, Germany). RNA concentration and purity were determined using a spectrophotometer (NanoDropTM 2000c, Thermo Scientific, USA). cDNAs were synthesized from RNA samples using the RT2 First Strand Kit (330404, Qiagen). PCR reaction was set using cDNAs, primers of related genes and RT2 SYBR® Green Master Mix (330502, Qiagen). Table 1 indicates information about the gene-specific primer assays (human) purchased (330001, RT2 qPCR Primer Assay, Qiagen). PCR conditions were determined as 1 cycle at 95°C for 10 min, 40 cycles at 95°C for 15 s and at 60°C for 1 min using a thermal cycler (StepOnePlus, Applied Biosystems, USA). Comparative Ct quantification (ΔΔCt method) was performed using glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the endogenous control gene, and relative fold changes were calculated comparing to control samples.

Table 1: Information about the primers used in the RT-qPCR assays (330001, RT2 qPCR Primer Assay, Qiagen)

Gene symbol	Gene name	RefSeq accession number	Reference position
NANOG	Nanog homeobox	NM_024865	1774
SOX2	SRY (sex determining region Y)-box 2	NM_003106	1091
CD34	CD34 molecule	NM_001773	474
IBA1(AIF1)	Allograft inflammatory factor 1	NM_001623	349
CD14	CD14 molecule	NM_000591	463
CSF1	Colony-stimulating factor 1	NM_000757	738
CX3CR1	Chemokine (C-X3-C motif) receptor 1	NM_001337	558
TREM2	Triggering receptor expressed on myeloid cells 2	NM_018965	608
TLR2	Toll-like receptor 2	NM_003264	415
TLR4	Toll-like receptor 4	NM_138554	2695
P2RY12	Purinergic receptor P2Y, G-protein coupled, 12	NM_022788	1115
TUJ1 (TUBB3)	Tubulin, beta 3 class III	NM_006086	436
GFAP	Glial fibrillary acidic protein	NM_002055	1078
OLIG2	Oligodendrocyte lineage transcription factor 2	NM_005806	401
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	822

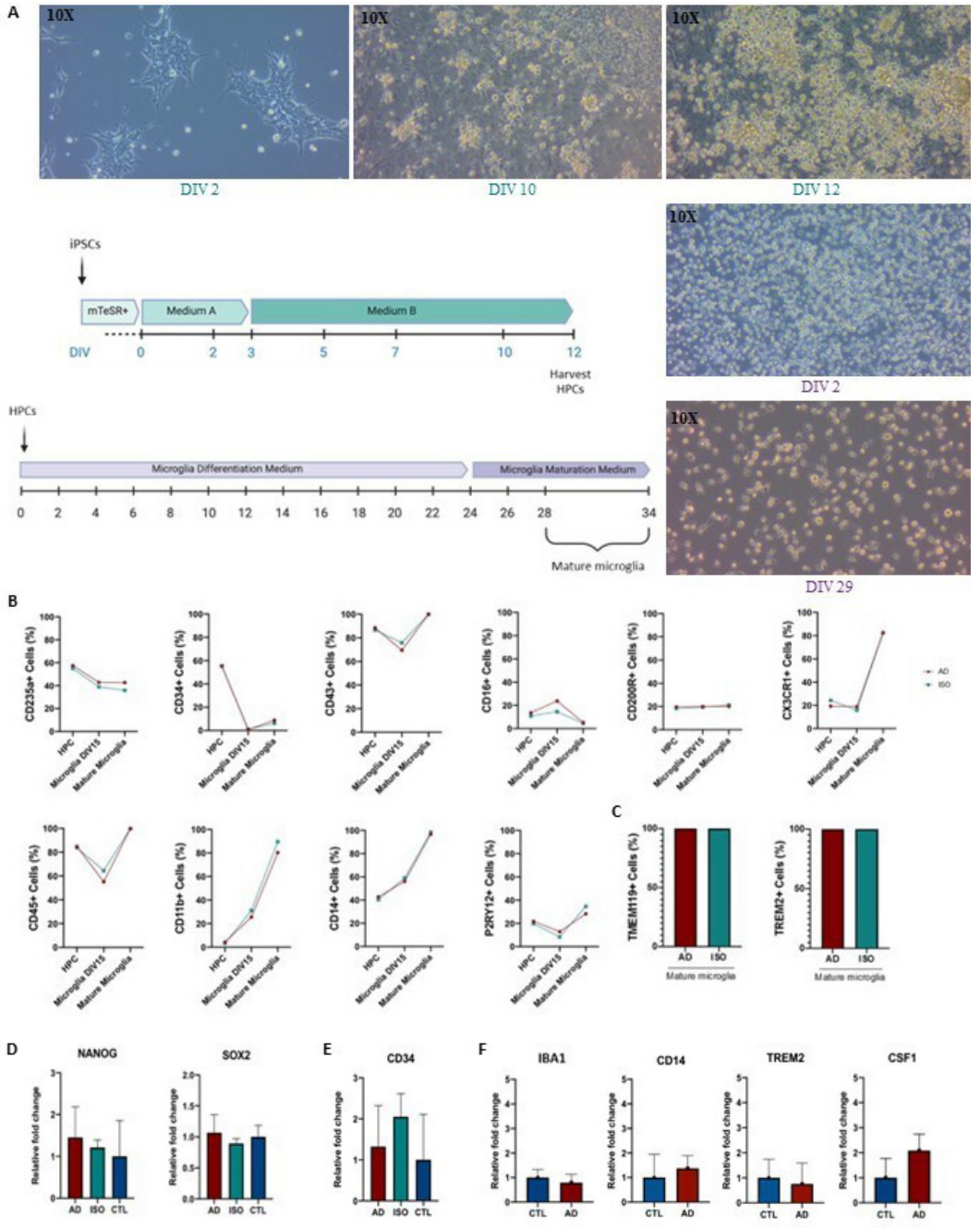

Figure 1:

Immunocytochemistry

Mature microglia were plated as $15x10^4$ cells/well on PDL-coated 8-well chamber slide. Cells were fixed with 4% paraformaldehyde, permeabilized with 3% Triton X-100 in PBS, and blocked with 3% normal goat serum (Vector Laboratories, USA). Anti-TREM2 (1:500 (v:v), MABN755, Millipore), anti-IBA1 (1:250 (v:v), 019-19741, Wako, USA), anti-TMEM119 (1:500 (v:v), ab185333, Abcam) and anti-PU.1 (1:40 (v:v), PA5-35158, Thermo Fisher Scientific) primary antibodies, and goat anti-rabbit Alexa-Fluor 488 (1:10000 (v:v), A-11008, Invitrogen) or goat anti-rat Alexa-Fluor 488 (1:10000 (v:v), A-11006, Invitrogen) secondary antibodies were used. Cells were imaged using an Laser Confocal Scanning Microscope (LSM800, Zeiss, Germany) and images were processed using Image-J software (USA).

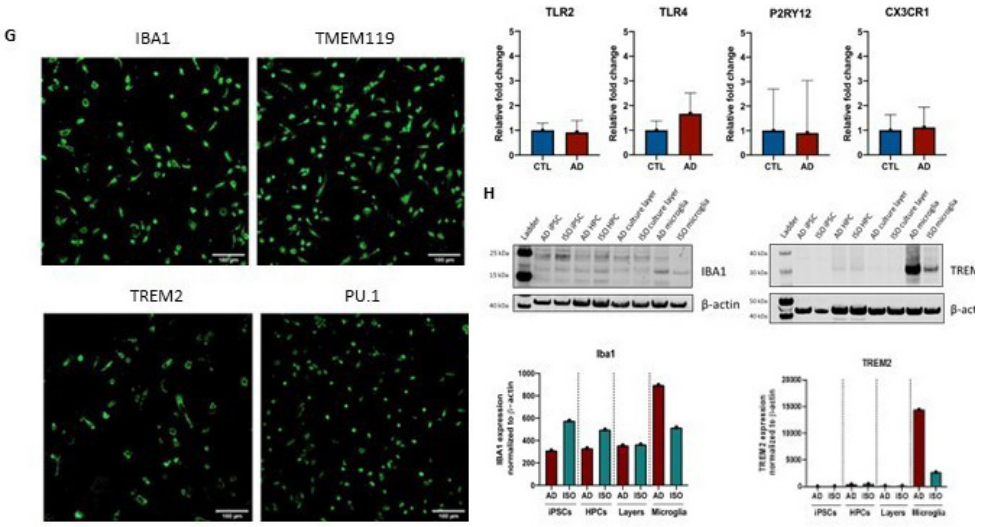

Figure 1: Continue. (A) A two-step protocol was used to obtain iPSC-derived microglia. In the first step of the protocol, HPCs were differentiated from iPSCs after 12 days. In the second step of the protocol, HPCs were driven into microglial differentiation for 24 days and after 4 days of further maturation, mature iPSC-derived microglia were obtained. (B, C) Percentages of positive cells analyzed by flow cytometry in HPCs, iPSC-derived microglia on day 15 (Microglia DIV15), and mature iPSC-derived microglia for CD235a, CD34, CD43, CD16, CD200R, CX3CR1, CD45, CD11b, CD14, P2RY12, TMEM119, and TREM2 markers (HPCs (n=3), Microglia DIV15 (n=3) and Mature Microglia (n=1)). Relative quantification of mRNA expressions of (D) pluripotency markers *NANOG* and *SOX2* in iPSCs, (E) hematopoietic marker *CD34* in HPCs, and (F) microglia-associated markers *IBA1, CD14, TREM2, CSF1, TLR2, TLR4, P2RY12,* and *CX3CR1* in iPSC-derived microglia by RT-qPCR. *GAPDH* was used as an endogenous control gene, and data are shown as fold increase relative to control samples. Experiments were carried out in triplicates. One-Way ANOVA, followed by Tukey's multiple-comparison post-hoc test (D, E) or unpaired Student's t-test (F). (G) Immunocytochemical staining of iPSC-derived microglia with IBA1, TMEM119, TREM2, and PU.1. Scale bar: 100 µm. (H) Western blots and quantification of the proteins IBA1 and TREM2 at different differentiation stages (iPSCs, HPCs, culture layers, and iPSC-derived microglia).

Western blot

Cells were lysed in RIPA lysis buffer, protein concentrations were determined using BCA Protein Assay Kit (Thermo Scientific). 20 µg proteins were separated on 4-12% Bis-Tris gels (Invitrogen) and blotted onto nitrocellulose membranes (Bio-Rad, USA) which were stained with anti-GSK3β (1:1000 (v:v), 9315, Cell Signaling Technology), anti-TUJ1 (1:1000 (v:v), MAB1195, R&D Systems, USA), anti-Nestin (1:2000 (v:v), PA5-11887, Thermo Fisher Scientific), anti-Olig-2 (1:500 (v:v), AV31464, Sigma-Aldrich, Germany), anti-ASC (1:1000 (v:v), AG-25B-0006-C100, Adipogen, USA), anti-NLRP3 (1:500 (v:v), AG-20B-0014-C100, Adipogen), anti-MyD88 (1:500 (v:v), ab2064, Abcam), anti-IBA1 (1:1000 (v:v), 17198, Cell Signaling Technology, USA), anti-TREM2 (1:1000 (v:v), ab209814, Abcam), anti-GFAP (1:1000 (v:v), MAB360, Millipore), anti-GAPDH (1:1000 (v:v), G9545, Sigma-Aldrich), anti-β-actin (1:1000 (v:v), MA5-15739, Thermo Fisher Scientific) antibodies and labelled with fluorescent near-infrared secondary antibodies IRDye 800CW Goat anti-rabbit IgG and IRDye 680LT Goat anti-mouse IgG (1:20000 (v:v), 926-32211 and 926-68020, LI-COR, USA). Proteins were visualized with an imaging system (Odyssey CLx Imaging System, LI-COR), images were analyzed using Image Studio (LI-COR). Protein levels were normalized to GAPDH or β-actin protein expressions.

Aβ preparation

Fibrillary Aβ Preparation: 1 mg HFIP (hexafluoro-2-propanol) treated $A\beta_{1-42}$ (Bachem, Switzerland) was dissolved in Dulbecco's Phosphate-Buffered Saline (DPBS) with 250 µM final concentration. Fibril formation was induced by incubating on a shaker at 1000 rpm and 37°C for 80 h, and the solution was kept at -80°C until use.

FAM-labelled Aβ Preparation: 0.5 mg fluorescently labelled $A\beta_{1-42}$ (AnaSpec, Eurogentec, USA) was dissolved in 40 mM NaOH and diluted to 221 µM with Tris HCl. After incubation at 37°C for 24 h, the solution was kept at -80°C until use.

Stimulation assays

Mature microglia were plated onto matrigel-coated plates and primed for 3 h with 100 ng/ml LPS (tlrl-eklps, InvivoGen, USA) and stimulated with 2,5 μM fibrillary Aβ for 24h. Cells were incubated in the presence or absence of 1 μM CRID3 (MCC950, Invivogen).

Measurement of cytokine secretion

Cytokines release after stimulation were measured by ELISA. Cell culture supernatants were assayed according to the manufacturer's protocol (human IL-1β, IL-18, IL-6,

CXCL12 DuoSet ELISA kit (R&D Systems)). Optical density was determined at 450 nm with a microplate reader (Infinite M200, Tecan, Switzerland), data were analyzed using GraphPad® Prism 8 software.

Phagocytosis analyses

Mature microglia were plated in Microglia Basal Medium on Matrigel-coated 24-well plates as $18x10^4$ cells/well. After incubation with 10 µg/ml pHrodo bioparticles (Invitrogen) for 30 min, 1 h, and 2 h or 0.5 µM FAM-labelled Aβ (AnaSpec, Eurogentec) for 1 h, 3 h, and 5 h, the cells were collected from the plates and added in FACS tubes. The cells were stained with 7-AAD dye (Pharmingen, BD) for 10 min. Samples were analyzed using a BD FACSCanto II flow cytometer, data were analyzed using FlowJo software.

Live cell imaging

iPSC-derived microglia were plated in Microglia Differentiation Medium on PDL-coated 8-well chambers as 10^5 cells/well and incubated overnight. The medium was changed to Microglia Basal Medium and 40 µM TAMRA-labelled Aβ (AnaSpec, Eurogentec) was added. Time-lapse images were taken during 1 h or 30 min, and videos were created from these images using Live Cell Imaging Microscopy (Eclipse Ti, Nikon, Japan) and NIS-Elements software (Nikon).

Statistical analyses

Graph Pad Prism software Version 8.0 was used. Statistical analysis was performed using one-way ANOVA or two-way ANOVA, followed by Tukey's multiple-comparison post hoc test or unpaired Student's t-test. Levels of significance were indicated as ****p<0.0001 ***p<0.001 **p<0.01 *p<0.1.

RESULTS

Characterization of iPSC-derived HPCs and microglia

iPSC-derived microglia were obtained at the end of 40 days of differentiation. In the first step, iPSCs were driven into mesodermal differentiation and then hematopoiesis was promoted. Round-shaped hematopoietic progenitor cells (HPCs) were obtained on day 12 (Figure 1A). In the second step, the collected HPCs were cultured in Microglia Differentiation Medium for 24 days. After four days of maturation in the Microglia Maturation Medium, iPSC-derived microglia were obtained (Figure 1A).

Flow cytometry showed that HPCs expressed hematopoietic markers CD43, CD235a, CD34 and also myeloid marker CD45 (Figure 1B). iPSC-derived microglia on day 15 started expressing CD11b and CD14 while hematopoietic marker expressions were decreasing. The expression of the myeloid markers CD45, CD11b, and CD14 was highly increased in mature microglia. Also, CX3CR1 and P2RY12 expressions increased when compared to HPCs. Almost all the cells of the mature microglia expressed the important microglia-associated markers TREM2 and TMEM119 (Figure 1C).

Various gene expressions of iPSC, HPC, and iPSC-derived microglia were determined by RT-qPCR. iPSCs expressed the pluripotency markers *NANOG* and *SOX2* (Figure 1D). HPCs expressed hematopoietic marker CD34 (Figure 1E), and iPSC-derived microglia expressed all the microglia-associated markers that were tested (Figure 1F).

Immunocytochemistry for IBA1, TMEM119, TREM2, and PU.1 was performed. iPSC-derived microglia were positively stained with all four markers (Figure 1G).

IBA1 and TREM2 expressions during differentiation were also analyzed by Western blot. IBA1 was slightly expressed in all stages of differentiation, but its expression was increased in iPSC-derived microglia, especially in the AD genotype (Figure 1H). TREM2 was only expressed in iPSC-derived microglia (Figure 1H). IBA1 and TREM2 proteins were expressed higher in the AD genotype compared to ISO.

Characterization of culture layers

During differentiation, iPSCs formed a layer of attached cells, which we named culture layer, and on top of which HPCs pop off (Figure 2A). We aimed to characterize culture layers after collecting the HPCs. Western blot results revealed that TUJ-1 was highly expressed in the culture layers of all genotypes (Figure 2B). However, a very low level to no expression of GFAP was detected (Figure 2B). In addition, neural progenitor cell (NESTIN) and oligodendrocyte (OLIG-2) markers were not detected in the culture layers by Western blot (not shown).

TUJ1, GFAP, and OLIG-2 expressions of the culture layers were also assessed by RT-qPCR, and similar results were obtained. TUJ1 was highly expressed in the culture layers (Figure 2C). However, GFAP and OLIG-2 expressions were not detected (not shown).

Aβ caused NLRP3-related cytokines release in iPSC-derived microglia

iPSC-derived microglia expressed ASC and NLRP3 proteins, but very low/no expression was detected in iPSC, HPC, and culture layers (Figure 3A). MyD88 protein was expressed in iPSC, culture layer, and iPSC-derived microglia at low levels, but no expression was detected in HPC samples (Figure 3A). GSK3β protein was prominently expressed in iPSC, the culture layer, and iPSC-derived microglia, but no expression was detected in the HPC samples (Figure 3A).

To characterize NLRP3 inflammasome pathway activation, ELISA analyses were performed after the stimulation assays (Figure 3B). Aβ stimulation after LPS priming caused a significant increase in IL-1β and IL-18 release (Figure 3C). In the presence of CRID3, this effect was counteract-

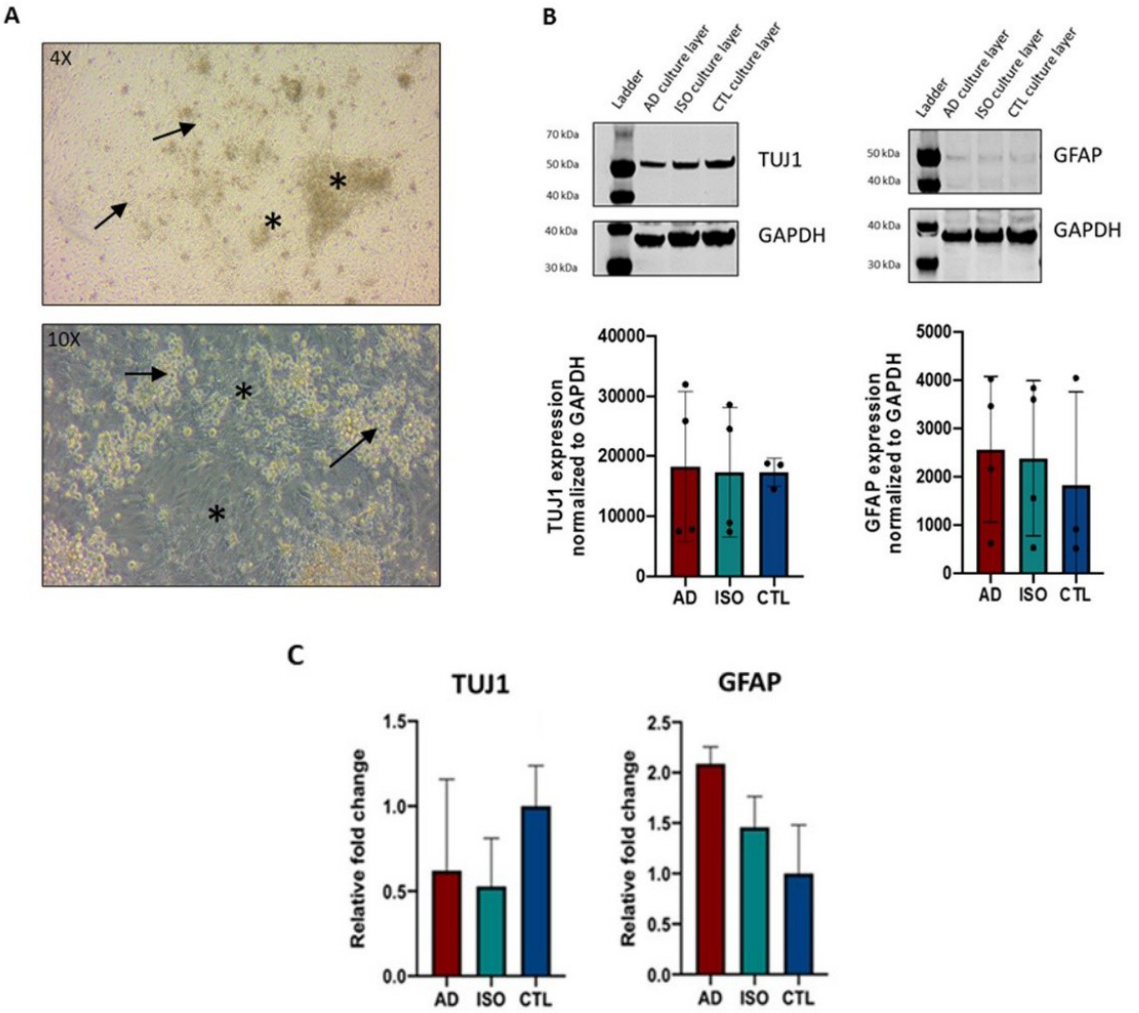

Figure 2: (A) During differentiation, iPSCs form a layer of attached cells called the culture layer (asterisks), on top of which HPCs (arrows) pop off. (B) Western blots and quantification for the proteins TUJ1 and GFAP of the culture layers (n=4). (C) Relative quantification of mRNA expressions of *TUJ1* and *GFAP* in the culture layers by RT-qPCR. *GAPDH* was used as an endogenous control gene, and data are shown as fold increase relative to the control samples. Experiments were carried out in triplicates. One-Way ANOVA, followed by Tukey's multiple comparison post hoc test.

ed. The secretions of other pro-inflammatory cytokines were also measured. LPS priming and Aβ stimulation after LPS priming increased IL-6 release, but this increase was not statistically significant (Figure 3C). Aβ stimulation after LPS priming caused an increase in CXCL12 release, and CRID3 treatment decreased this release (Figure 3C).

iPSC-derived microglia phagocytosed pHrodo bioparticles and Aβ

The phagocytosis of pHrodo bioparticles by iPSC-derived microglia was increased in a time-dependent manner (Figure 4A). Phagocytosis of pHrodo bioparticles were visualized using fluorescence microscopy (Figure 4B).

The percentages of Aβ phagocytosis by iPSC-derived microglia were high for both genotypes. (Figure 4C). iPSC-derived microglia of the AD genotype were observed to be slightly more phagocytic than those of the ISO genotype, but this difference was not statistically significant.

Aβ phagocytosis of the cells was also observed with live-cell imaging. iPSC-derived microglia of the AD genotype were observed to be more phagocytic than those of the ISO genotype (Figure 4D).

DISCUSSION

Recent studies have revealed the importance of neuroinflammation in AD and highlighted the role of microglia as one of the main regulators of neuroinflammation (24). Until recently, microglia studies were limited to animal models, biopsy of patients, or post-mortem brain samples (25). Although animal models have provided us with

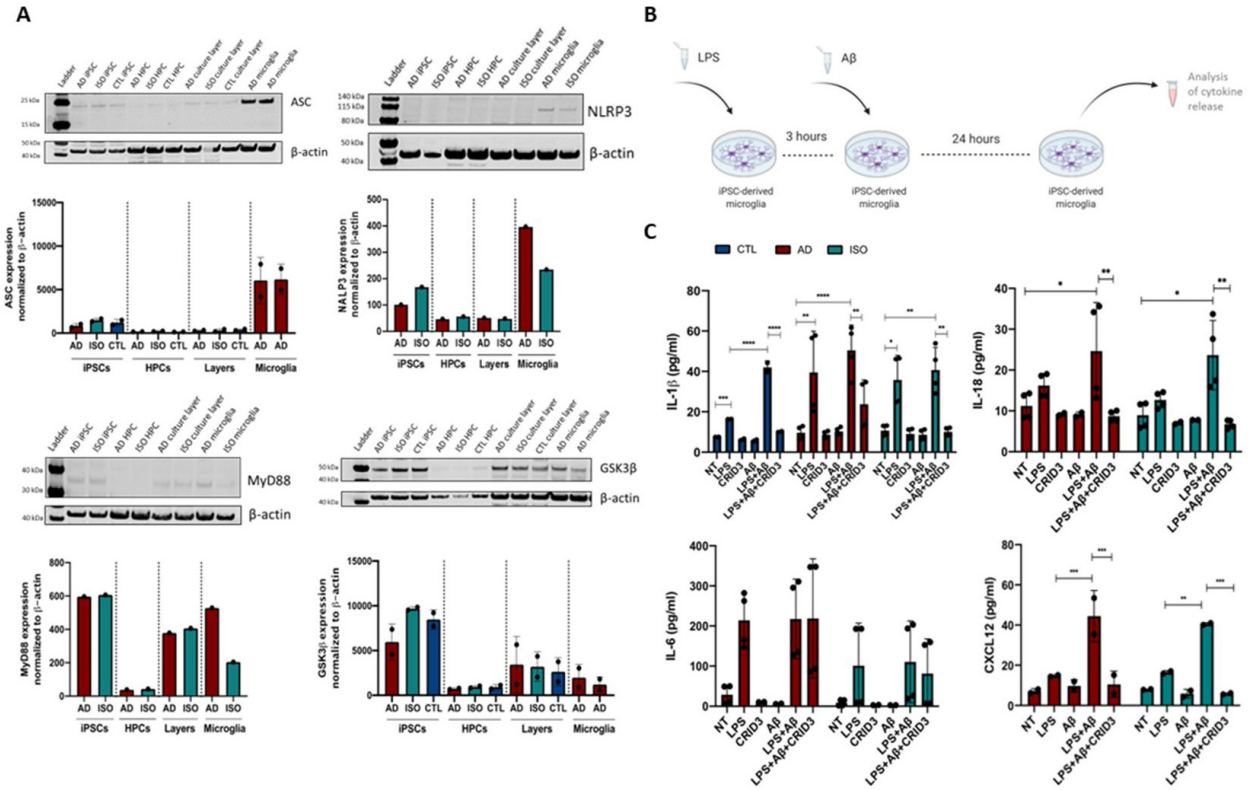

Figure 3: (A) Western blots and quantification for the proteins ASC, NLRP3, MYD88, and GSK3β of different differentiation stages (iPSCs, HPCs, culture layers, and iPSC-derived microglia); ASC and GSK3β (n=2), NLRP3 and MYD88 (n=1). (B) Scheme of stimulation experiments showing 3 h LPS priming and 24 hours Aβ stimulation of iPSC-derived microglia. (C) IL-1β, IL-18, IL-6, and CXCL12 release of iPSC-derived microglia upon LPS priming and Aβ stimulation in the presence or absence of CRID3. Experiments were carried out in duplicates (except CXCL12; n=1). Two-Way ANOVA, followed by Tukey's multiple-comparison post hoc test, *:p<0.1, **:p<0.01, ***:p<0.001, ****:p<0.0001.

very useful information regarding the pathogenesis of AD, failures in drug development studies have led to the questioning of these models. Since the etiology of AD is still not fully known, the ability of many proposed AD animal models to represent AD pathology is limited. For example, although animal models produced by the overexpression of AD-related genes recapitulate Aβ plaques, neuroinflammation, and memory loss, they fail to display neuronal death and degeneration, which are important hallmarks of AD in humans. Additionally, transcriptomic studies demonstrated substantial differences between murine and human microglia, including different aging. Most animal models fail to demonstrate the molecular signatures implicated in human AD cases, such as neuroinflammatory responses, antigen presentation, and adaptive immune responses (20, 26). Since the accessibility of human brain tissue is very limited, the number of studies on primary human microglia is few and restricted to patient samples or post-mortem tissue harvesting. Working with these samples brings problems, such as limited number of cells, post-mortem artefacts, and lack of healthy controls. Since iPSCs can be obtained from both patients and healthy individuals, provide a physiologically relevant background, can be proliferated indefinitely, and can be differentiated into desired brain cells, they present a valuable human cell source for investigating AD mechanisms by overcoming the limitations of the previous models (22).

Microglia originate from mesodermal primitive yolk sac progenitors, which give rise to erythromyeloid progenitors (EMPs) (27, 28). EMPs form yolk sac macrophages at E17 and migrate to the brain to form microglia from E31 until the blood-brain barrier is closed. Microglia that interact with other brain cells become mature and functional (22, 29). By mimicking the developmental stages, research groups have developed protocols to differentiate iPSCs into microglia. Although the basic approach is the same, each protocol has differences. As the first step of differentiation, iPSCs were directed to mesodermal differentiation using growth factors, and myeloid progenitors/erythro-myeloid progenitors were obtained. Afterwards, the cells were supported with essential growth factors and signaling molecules to promote microglial differentiation. For maturation, microglia co-cultured with neurons/astrocytes or maturation factors (CD200 and CX3CL1) were added to

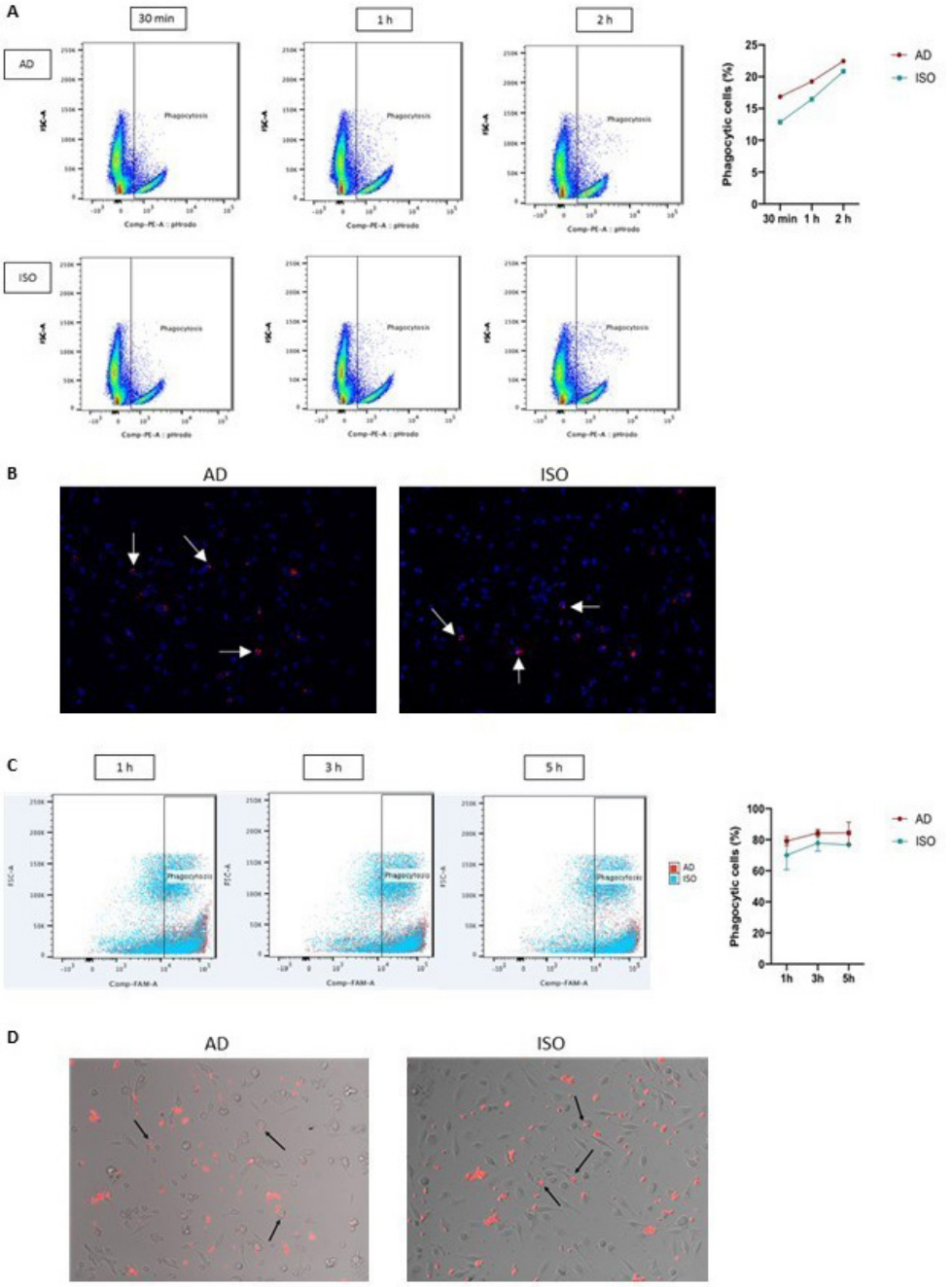

Figure 4: (A) Flow cytometry graphs showing pHrodo phagocytosis of iPSC-derived microglia after 30 min, 1 h, and 2 h incubation with pHrodo bioparticles (n=1). (B) Fluorescence microscopy images of iPSC-derived microglia showing nuclei of the cells (blue) and phagocytosed pHrodo bioparticles (red, arrows), 20X. (C) Flow cytometry graphs showing Aβ phagocytosis of iPSC-derived microglia after 1 h, 3 h, and 5 h incubation with FAM-labelled Aβ, (n=2). Two-Way ANOVA, followed by Tukey's multiple-comparison post-hoc test. (D) Live cell imaging microscopy photographs showing TAMRA-labelled Aβ phagocytosis (arrows) of iPSC-derived microglia, 20X.

the media (23, 29-31). Some methods required low oxygen conditions or the separation of cells by FACS using specific markers (30-34). As McQuade et al. suggested a simplified method that does not need hypoxic incubation, FACS sorting, or co-culture, we followed their protocol to obtain microglia from iPSCs (23).

Following the protocol, during mesodermal differentiation, iPSCs formed an attached layer of an unknown cell population, which we call culture layer, on top of which HPCs pop off. After collecting HPCs as a suspension on day 12, analysis of the culture layers revealed the expression of the neuronal marker TUJ-1 both at the mRNA and protein levels. Foudah et al. reported the spontaneous expression of neural markers, including TUJ-1, in undifferentiated mesenchymal stem cells from different sources (35). However, Bianchi et al. showed that undifferentiated iPSC colonies did not express TUJ-1 (36). TUJ-1 is known as an early neuronal marker widely used in stem cell studies to verify neuronal differentiation. In this context, TUJ-1 expression in the culture layers indicates that, although not targeted, neuronal differentiation of iPSCs may have been induced in the culture layers, and neurons may be the main supportive cells of microglial differentiation in this protocol.

One of the challenging problems of microglia research is that there is no specific marker for microglia. Some highly used common microglia markers (CD45, CD11b, IBA1) are known to be shared with other cell types like macrophages. Therefore, several studies have proposed potential markers specific to microglia. To the current knowledge, TREM2, TMEM119, CX3CR1, and P2RY12 are considered as the most specific microglia markers in human tissues (37, 38). TREM2 is involved in processes such as phagocytosis, proliferation, and chemotaxis, is a marker of tissue-specific macrophages, and is known to be expressed only by microglia in the brain. Garcia-Reitboeck et al. showed TREM2 expression in microglia differentiated from human iPSCs (39). TMEM119 is a transmembrane protein originally described as a regulator of osteoblast differentiation. Transcriptomic studies have shown that TMEM119 is expressed specifically by microglia in the CNS (40). Subsequent studies suggested TMEM119 to distinguish microglia from blood-derived macrophages infiltrating the CNS (41). CX3CR1, a chemokine receptor expressed in microglia, is involved in the recruitment of mononuclear phagocytes to the inflammation site. Since neurons express CX3CL1, the ligand of the receptor, CX3CR1-CX3CL1 signaling pathway plays an important role in microglia and neuron communication (42). P2RY12, a purinergic receptor located on the surface of microglia, detects molecules such as ATP released from damaged cells in case of danger and is involved in processes related to inflammation, cell movement and cell migration (43). In this study, we have validated that iPSC-derived microglia express the microglia-specific markers TREM2, TMEM119, CX3CR1, and P2RY12 in addition to the common microglia markers.

Microglia are responsive to inflammatory stimuli, and an exaggerated inflammatory response is involved in AD (38). Several studies have shown that inhibition of the NLRP3 inflammasome pathway improves amyloid pathology in rodent disease models and may be protective against AD (15, 16). Therefore, NLRP3 inflammasome inhibitors are considered among the therapeutic targets. CRID3 is one of the most effective and selective inhibitors of the NLRP3 inflammasome. It binds to the Walker B motif in the NACHT domain of the NLRP3 protein and puts it in a more closed and inactive conformation. This prevents ATP hydrolysis of NLRP3, thus preventing the activation of the NLRP3 inflammasome (18). Lučiūnaitė et al. showed that increased IL-1β levels upon stimulation with Aβ after LPS priming decreased after CRID3 administration in primary mouse microglia (44). Dempsey et al. stated that Aβ pathology was reduced and IL-1β level was decreased in brain homogenates upon CRID3 treatment in APP/PS1 mice (45). Mouton-Liger et al. showed that increased levels of IL-1β and IL-18 with NLRP3 activators were decreased with CRID3 treatment in primary mouse microglia (46). Clénet et al. differentiated microglia from iPSCs of Amyotrophic Lateral Sclerosis patients and healthy subjects (47). LPS treatment caused increase of IL-1β level, while CRID3 decreased this effect. In our study, we showed that iPSC-derived microglia expressed NLRP3 pathway-related proteins, and stimulation with Aβ after LPS priming resulted in the release of IL-1β and IL-18 cytokines. Moreover, in the presence of CRID3, cytokine releases were significantly reduced. Our results indicate that iPSC-derived microglia do not only express microglial markers but also show critical functional microglial features like being responsive to important stimulators in AD. Also, together with previous research, our results support that inhibition of the NLRP3 inflammasome pathway using inhibitors such as CRID3 can be evaluated as a new anti-inflammatory treatment method for AD.

Microglia recognize and phagocytose foreign particles and protein aggregates via various receptors on their surface. Our study showed that iPSC-derived microglia can successfully phagocyte pHrodo and Aβ. These data indicate that iPSC-derived microglia do not only express microglia-associated markers but also carry the phagocytic function of microglia. One of the proposed mechanisms of AD is the inability of microglia to clear Aβ deposits due to decreased phagocytic activity. However, there are controversial results regarding microglial phagocytosis in AD. In 5XFAD mice, microglia around Aβ plaques took up Aβ which is followed by microglial cell death, and dying microglia released Aβ deposits in the extracellular space, contributing to Aβ plaque growth (48). In APP/PS1 mice, deficiency of TAM receptors that function in Aβ phagocytosis of microglia resulted in fewer dense core plaques, showing that phagocytosis does not prevent the development of Aβ plaques, but rather promotes it (49). Xu et al. stated that human iPSC-derived microglia-like cells (iMGL) originating from sporadic AD lines showed higher phagocytic ability than control lines (50). However, Konttinen et al. reported dampening of phagocytosis in APOE4 iMGLs, but no change in APPswe or PSEN1ΔE9 iMGLs (33). In our study, we evaluated pHrodo and Aβ phagocytosis ability

of iPSC-derived microglia and the AD genotype seemed more phagocytic than that of the ISO genotype. Further research is needed to elucidate whether microglial phagocytosis contributes to the disease pathology in AD.

CONCLUSION

In summary, our study shows that iPSC-derived microglia provide key phenotypical and functional characteristics of microglia, such as expressing various distinctive microglial markers, being responsive to stimulant molecules, and being able to phagocytose Aβ. As iPSC-derived microglia originate from patients' own cells, this helps to overcome the limitations of AD studies related to animal models. Our results support that human iPSC-derived microglia can be a critical tool for understanding human AD pathogenesis, identifying therapeutic targets, and allowing large-scale drug screening of novel therapeutic candidates related to the NLRP3 inflammasome pathway.

Ethics Committee Approval: The study was approved by the Northern Savo Hospital (Date: 29.04.2016, No: 123).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- C.D., M.T.H., A.E.Ö.; Data Acquisition – A.E.Ö., C.D., J.K., S.L.; Data Analysis/Interpretation- A.E.Ö., C.D.; Drafting Manuscript- A.E.Ö.; Critical Revision of Manuscript- C.D., J.K., S.L., S.S., M.T.H.; Final Approval and Accountability- A.E.Ö., C.D., J.K., S.L., S.S., M.T.H.; Supervision- S.S., M.T.H., C.D.

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RESEARCH / ARAŞTIRMA
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ANTI-C1q IN SYSTEMIC LUPUS ERYTHEMATOSUS: RELATIONSHIP WITH CLINICAL MANIFESTATIONS AND DISEASE ACTIVITY

SİSTEMİK LUPUS ERİTEMATOZUSTA ANTİ-C1q: KLİNİK BULGULAR VE HASTALIK AKTİVİTESİ İLE İLİŞKİSİ

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ABSTRACT

Objective: Lupus nephritis (LN) is a detrimental consequence of systemic lupus erythematosus (SLE). The anti-C1q antibody was shown to be related to LN, or global disease activity, in various studies. Our purpose was to determine its prevalence and association with LN or disease activity in Turkish patients with SLE.

Material and Method: We conducted a cross-sectional single-centre study to investigate the clinical and laboratory findings, disease activity, and anti-C1q levels in 150 patients with SLE. The anti-C1q antibody was analyzed using an enzyme-linked immunosorbent assay and compared with 150 healthy-control patients.

Result: Lupus nephritis was present in 72 patients. The frequency of anti-C1q positivity was 17% (26/150) in patients with SLE and 3% (5/150) in control group (p<0.001). Patients with anti-C1q also had anti-Sm, direct Coombs' test, and thrombocytopenia more commonly (p=0.001, p=0.007, p=0.009 respectively). Anti-C1q was positively correlated with proteinuria, haematuria, systemic lupus erythematosus disease activity index (SLEDAI) (p<0.001), anti-dsDNA (p=0.03), and negatively correlated with C3 (p<0.001) and C4 (p=0.015). Patients with active LN had higher anti-C1q (p=0.01) and anti-dsDNA (p<0.001) titres than inactive LN patients, although in multivariate logistic regression analysis, anti-C1q was not significant for LN history. It was significant for SLEDAI severity (p=0.036).

ÖZET

Amaç: Lupus nefriti (LN), sistemik lupus eritematozus'un (SLE) tehlikeli bir sonucudur. Çeşitli çalışmalarda anti-C1q antikorunun LN veya global hastalık aktivitesi ile ilişkili olduğu gösterilmiştir. Bu çalışmada amacımız, Türk SLE'li hastalarda anti-C1q prevalansını, LN veya hastalık aktivitesi ile ilişkisini belirlemekti.

Gereç ve Yöntem: Kesitsel tek merkezli bir çalışma ile 150 SLE'li hastada klinik ve laboratuvar bulguları, hastalık aktivitesi ve anti-C1q düzeyleri değerlendirildi. Anti-C1q antikoru, ELISA (enzyme-linked immunosorbent assay) ile analiz edildi, toplam 150 kişiden oluşan hasta ve sağlıklı kontrol grubu ile karşılaştırıldı.

Bulgular: Yetmiş iki hastada LN'i saptandı. Anti-C1q pozitiflik oranı SLE hastalarında %17 (26/150), kontrol grubunda ise %3 (5/150) idi (p<0,001). Anti-C1q antikoru pozitif olan hastalarda aynı zamanda pozitif anti-Sm antikoru, direkt Coombs testi ve trombositopeni de daha sık görüldü (sırasıyla p=0,001, p=0,007, p=0,009). Anti-C1q antikoru proteinüri, hematüri, sistemik lupus eritematoz hastalık aktivite indeksi (SLEDAI) (p<0,001), anti-dsDNA (p=0,03) ile pozitif, C3 (p<0,001) ve C4 (p=0,015) ile negatif korelasyon gösterdi. Aktif LN'li hastalarda anti-C1q (p=0,01) ve anti-dsDNA (p<0,001) titreleri inaktif LN hastalarına göre daha yüksekti, ancak çok değişkenli lojistik regresyon analizinde anti-C1q LN öyküsü için anlamlı değildi. SLEDAI şiddeti açısından anlamlılık saptandı (p=0,036).

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Conclusion: Our study demonstrated a significant association of anti-C1q with SLE, proteinuria, haematuria, thrombocytopenia, general disease activity, and active LN, but not with inactive renal disease. This is the first study investigating the clinical significance of this antibody in Turkish patients. Further studies are needed to clarify the pathogenesis of lupus nephritis.

Keywords: Anti-C1q, systemic lupus erythematosus, lupus nephritis, systemic lupus erythematosus disease activity index

Sonuç: Çalışmamız anti-C1q antikorunun SLE, proteinüri, hematüri, trombositopeni, genel hastalık aktivitesi ve aktif LN ile anlamlı bir ilişkisi olduğunu, ancak inaktif böbrek hastalığı ile ilişkili olmadığını kanıtladı. Çalışmamız, bu antikorun Türk SLE'li hastalarda klinik önemini araştıran ilk araştırmadır. Lupus nefritinin patogenezini açıklığa kavuşturmak için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Anti-C1q, lupus nefriti, sistemik lupus eritematozus, sistemik lupus eritematozus hastalık aktivite indeksi

INTRODUCTION

Systemic lupus erythematosus (SLE) is characterized by various autoantibody production processes that contribute to inflammatory damage across various organ systems. Lupus nephritis (LN) is a frequent and severe condition, often indicating a worse prognosis (1). Prompt diagnosis and treatment are essential for improving LN outcomes and survival in patients with SLE (2). However, the gradual onset and unpredictable course of LN present challenges in diagnosis and monitoring. A biomarker capable of predicting LN flares before noticeable changes in proteinuria, urine sediment, or decline in kidney function that can be routinely monitored during patient visits would be invaluable for initiating treatment early and preventing significant renal damage (3).

Increased levels of anti-double-stranded DNA (anti-dsDNA) antibodies and reduced complement levels were linked to active SLE. However, their lack of specificity for renal flares has led to a search for other antibodies (4, 5). Complement activation is crucial in the development of both SLE and LN. C1q, the initial component of the classical complement pathway, participates in the removal of immune complexes formed during apoptosis from tissues (6). Although genetic C1q deficiency is linked to SLE, in most SLE patients, C1q deficiency is a secondary event associated with anti-C1q antibodies. These antibodies can impede the neutralisation of immune complexes with C1q, leading to their deposition, complement activation, and subsequent inflammation (7).

Various studies have investigated the link between anti-C1q antibodies and LN or global activity in SLE. Some of them suggested that anti-C1q antibodies are superior markers for identifying renal flares (8, 9). In contrast, others have argued that combining anti-C1q antibodies with other antibodies provides better predictive value than using anti-C1q antibodies alone (10-12). Most of these studies have stated that the absence of this antibody is related to a lower possibility of LN flares (10). A multinational study stated that anti-C1q levels were parallel to activity levels measured by the modified Safety of Estrogen in Lupus: National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) and the Systemic Lupus International Collaborating Clinics (SLICC) Renal Activity Score (RAS) (7). However, researchers have argued that anti-C1q antibodies are related to overall disease activity, not necessarily nephritis (13). Two meta-analyses aimed to resolve the conflicting findings regarding anti-C1q antibodies. Yin et al. suggested that anti-C1q antibodies could be an informative tool for forecasting LN and measuring active nephritis, whereas Eggleton et al. did not find sufficient evidence to support this association (14, 15).

In the current study, our primary goal was to assess the importance of the anti-C1q antibody in Turkish SLE patients and to analyse its connexion with LN and disease activity.

MATERIAL AND METHODS

Patients

We conducted a controlled cross-sectional study at our university hospital rheumatology outpatient clinic from January 2016 to January 2017. Our clinic serves as a tertiary referral centre for rheumatology.

One hundred and fifty consecutive SLE patients were enrolled. All patients were diagnosed with SLE, either by fulfilling at least four of the American College of Rheumatology (ACR) revised diagnostic criteria for SLE or 4 of the SLICC 2012 diagnostic criteria (16, 17).

During routine outpatient follow-up visits, we recorded demographics including age and gender. In addition, we measured levels of anti-C1q, anti-dsDNA, anti-Smith (anti-Sm), complement components C3 and C4, performed a Coomb's test, obtained a complete blood count (CBC), and analysed urine for red blood cell (RBC) casts and 24-h urine protein levels. Clinical manifestations and treatments received by the patients up to the study period were also evaluated to define their general characteristics (Table 1).

Disease activity was defined according to SLEDAI 2000 (SLEDAI-2K) criteria (18). A SLEDAI score of 0-3 was classified as inactive disease, 4-8 as mild, 9-12 as moderate,

Table 1: Clinical, laboratory findings, and treatments of SLE patients until the time of study n (%)

Photosensitivity	94 (62.7)	Anti-dsDNA (+)	36 (24)
Malar rash	71 (47.3)	Anti-Histone (+)	33 (22)
Discoid rash	13 (8.7)	Anti-Sm (+)	14 (9.3)
Oral ulcer	28 (18.7)	Anti-Sm/RNP	44 (29.3)
Alopecia	41 (27.3)	Anti-SSA (+)	57 (38)
Arthritis	101 (67.3)	Anti-SSB (+)	21 (14)
Pleuritis	25 (16.7)	Anti-Nucleosome (+)	57 (38)
Pericarditis	18 (12)	Anti-Rib P Protein (+)	21 (14)
Seizure	5 (3.3)	LAC[b]	17 (11.3)
Lupus nephritis	72 (48)	ACLA[c] IgG (+)	5 (3.3)
Proteinuria	16 (10.7)	ACLA IgM (+)	2 (1.3)
Haematuria	14 (9.3)	Steroid	148 (98.7)
Leukopoenia	24 (16)	Hydroxychloroquine	150 (100)
Lymphopenia	35 (23.3)	Azathioprine	108 (72)
Haemolytic anaemia	12 (8)	Mycophenolate mofetil	54 (36)
Thrombocytopenia	6 (4)	Cyclophosphamide	44 (29.3)
APS[a] history	24 (16)	Rituximab	15 (10)
Coomb's test (+)	25 (16.7)	Intravenous immune globulin	3 (2)
Low C3/C4	46 (30.7)	Plasmapheresis	1 (0.7)

[a]: Antiphospholipid syndrome, [b]: Lupus anticoagulant, [c]: Anti-cardiolipin antibody

and ≥12 as severe activity. Patients with moderate to severe disease activity (SLEDAI score ≥9) were considered to have active disease. Urine protein excretion ≥500 mg/day or the presence of ≥5 RBC casts per high-power field (HPF) was interpreted as active nephritis. Renal biopsies were assessed based on the revised International Society of Nephrology and Renal Pathology Society (ISN/RPS) classification (19).

The disease control (DC) group comprised 101 consecutive patients followed at the same clinic. The healthy control (HC) group included 49 individuals with no history of chronic diseases.

For anti-C1q antibody measurement, sera were collected and frozen as 100 µL samples at minus 80°C until analysis.

The Kocaeli University Ethics Committee approved the research protocol, and all participants provided written consent (Date: 11.12.2015, No: KAEK/2015/133-16/19).

Anti-C1q IgG antibodies

Enzyme-linked immunosorbent assay (ELISA) kits (ORG 549, Orgentec Diagnostika GmbH, Mainz, Germany) were utilised for anti-C1q antibody detection. Initially, sera were diluted by 1/100, introduced into the wells, and incubated for 30 min at room temperature (RT). After three washes with the wash solution, 100 µL of enzyme conjugate was introduced into the wells and incubated again at RT.

Following a 15-min incubation, each well was washed three times, and 100 µL of trimethyl benzene solution was introduced, followed by further incubation for 15 min at RT. Finally, 100 µL of stop solution was applied to the wells, and optical density was assessed at 450 nm. The results were used to determine the concentrations based on a predefined conversion method. Ten U/mL was set as the cut-off value for anti-C1q, with values ≥10 U/mL interpreted as positive per manufacturer.

Other tests

The anti-nuclear antibody (ANA) test was conducted with an indirect immunofluorescence assay (Euroimmun, Luebeck, Germany), with titres of 1:160 considered as the cut-off value. Anti-extractable nuclear antigen (ENA) antibodies were detected with an immunoblotting assay (Euroimmun, Luebeck, Germany). Anti-dsDNA antibodies were measured using ELISA (Euroimmun, Luebeck, Germany). Serum C3 and C4 levels were measured using Beckman Coulter reagents on the AU5800 analyser (Brea, California, USA). CBC measurements were performed using a Beckman Coulter DxH800 Hematology Analyzer (Brea, California, USA). The Coombs test was conducted using the Beckman Coulter Across Auto System Octom (Brea, California, USA). RBC casts were detected using a Beckman Coulter iQ200 Sprint urine microscopy system (Brea, California, USA). Twenty-four-hour urine protein levels were measured spectrophotometrically using Beckman Coulter reagents on the AU5800 Analyser (Brea, California, USA).

Statistical analysis

SPSS© 25.0 (IBM Statistical Package for Social Sciences, Corp., Armonk, NY, USA) and R© programmes were utilised for analysis. Gender, clinical and laboratory findings, and treatments patients received until the time of study were expressed as frequencies (the number of cases) and relative frequencies (percentages). Age, anti-C1q and an-

ti-dsDNA titres, proteinuria, haematuria, white blood cell (WBC), RBC, platelet (PLT) counts, and C3 and C4 levels were reported as mean values ± standard deviation (SD) in cases of normal distribution. Kolmogorov-Smirnov test was utilised to cheque distribution.

The chi-square test or Fisher exact test was used to evaluate clinical and laboratory findings based on anti-C1q antibody status. The difference in anti-C1q titres between patients and controls was analysed using the Mann–Whitney U test. Likewise, the differences in anti-dsDNA titres, proteinuria, haematuria, WBC, RBC, PLT counts, and C3 and C4 levels between patients based on anti-C1q antibody status were analysed with t-test or Mann-Whitney U test.

To evaluate the difference in anti-C1q and anti-dsDNA titres, as well as C3 and C4 levels between patients with and without LN based on disease activity, t-tests or Mann-Whitney U tests were used, based on the normality of the data.

For comparison of all four of these groups separately, ANOVA or Kruskal-Wallis test with Bonferroni analysis was utilised. Correlations between anti-C1q, anti-dsDNA, C3, and C4 were analysed using the Spearman correlation test. Furthermore, logistic regression analysis was performed to estimate the impact of anti-C1q, anti-dsDNA, C3, and C4 on LN and disease activity. A p-value less than 0.05 was defined as statistically significant.

RESULTS

SLE and control group characteristics

One hundred and fifty consecutive patients with SLE (138 female, 12 male) were enrolled. Patients' ages ranged between 19 and 82 years with a mean of 46±12.8. The average duration of disease was 74.3±51.4 months. Arthritis was the most common symptom (67.3%), followed by photosensitivity (62.7%) and malar rash (47.3%). Twenty-four patients had anti-phospholipid syndrome.

Of the 72 patients with a history of LN, only three patients did not undergo a renal biopsy. Among those who underwent biopsies, one patient's result was non-diagnostic. The pathological diagnoses were as follows: class II LN was present in 24 patients, class III in eight, class IV in 26, and class V in 10. The glomerular filtration rate (GFR) was below 60 ml/min in 12 patients.

In patients with LN, 26 had mild, four had moderate, and four had severe active disease. Patients without LN had less active disease; 13 had mild, two had moderate, and two had severe active disease. Table 1 presents the clinical and serological information, and the treatments received by SLE patients up to the time of the study.

There were 101 patients in the DC group, as follows: rheumatoid arthritis (n=85), Sjögren syndrome (n=8), adult-onset Still's disease (n=3), systemic sclerosis (n=2), and psoriatic arthritis (n=3), all meeting their respective diagnostic criteria. The HC group comprised 49 individuals without any chronic diseases. Age and gender were similar between the SLE and control groups, as detailed in Table 2.

The prevalence and titres of anti-C1q antibodies

The prevalence of anti-C1q antibodies in SLE patients was 17% (26/150) and was significantly higher than that in DC (3/101) and HC (2/49) subjects (p<0.001 calculated both combined and separately for each control group).

Additionally, compared with both control groups, the titres of anti-C1q antibodies were considerably greater in SLE patients (p<0.001) (Table 2).

Anti-C1q antibodies and clinical, laboratory findings

Patients who had anti-C1q antibodies had a greater amount of proteinuria (p=0.047) than those without this antibody, despite a similar prevalence of LN between the two groups. Additionally, these patients had more anti-dsDNA (p=0.016 for prevalence and p=0.014 for titre) and lower C3 levels (p=0.009), despite similar prevalence of low C3 across groups (p=0.131). Conversely, both the prevalence of low C4 (p=0.017) and lower C4 levels (p=0.001) were significantly higher in patients who had anti-C1q antibodies. Furthermore, these patients also had more anti-Sm antibodies (p=0.001) and Coombs' tests (p=0.007). Although thrombocytopenia was prevalent among these patients (p=0.009), platelet numbers were similar (p=0.779). Only a few patients with extremely low platelet counts could be the reason for this. Table 3 summarises clinical and laboratory features of SLE patients based on anti-C1q antibody status.

Correlations with other parameters

We observed a positive correlation between anti-C1q and SLEDAI (r=0.378, p<0.001), anti-dsDNA (r=0.178, p=0.03), proteinuria (r=0.286, p<0.001), and RBC casts (r=0.438, p<0.001). Conversely, we also found a negative correlation between this antibody and C3 (r=-0.322, p<0.001) and C4 (r=-0.198, p=0.015) (Figure 1). We did not find any correlation with WBC, lymphocyte, or PLT counts.

Table 2: Demographics and anti-C1q antibody levels in each group

	SLE group (N=150)	Controls (N=150)	AntiC1q
Female/Male	138/12	132/18	p=0.248
Age (years)	46±12.8 (19-82)	45.5±14.5 (17-76)	p=0.943
Anti-C1q (+)[b]	26 (17)	5 (3)	p<0.001*
Anti-C1q titres[c]	8.42±16.02	3.47±3.97	p<0.001*

[a]: Mean±SD (Range), [b]: n (%), [c]: Mean±SD, *:p<0.05

Table 3: Clinical and laboratory findings associated with anti-C1q antibody at the time of the study

	n (%)	n (%)	p	Odds ratio (CI)
LN history[a]	14 (53.8)	58 (46.7)	0.512	1.328 (0.5-3.1)
Proteinuria[b]	4 (15.3)	12 (9.6)	0.482	1.697 (0.5-5.7)
Haematuria[b]	5 (19.2)	9 (7.2)	0.069	3.042 (0.9-9.9)
Leukopoenia[b]	6 (23)	18 (14.5)	0.375	1.767 (0.6-4.9)
Lymphocytopenia[a]	8 (30.7)	27 (21.7)	0.324	1.597 (0.6-4.1)
Thrombocytopenia[b]	4 (153)	2 (1.6)	0.009*	11.09 (1.9-64.2)
Anti-Sm (+)[b]	7 (26.9)	7 (5.6)	0.003*	6.158 (1.9-19.5)
Coombs' test (+)[a]	9 (34.6)	16 (12.9)	0.007*	3.574 (1.4-9.4)
Anti-dsDNA (+)[a]	11 (42.3)	25 (20.1)	0.016*	2.904 (1.2-7.1)
Low C3[a]	8 (30.7)	22 (17.7)	0.131	2.061 (0.8-5.3)
Low C4[a]	13 (50)	30 (24.1)	0.017*	0.176 (0.0-1.4)
	Mean±SD	**Mean±SD**		
Proteinuria (mg/day)[d]	603.15±1014.83	2.94.77±774.46	0.047*	
Haematuria (RBC/HPF)[d]	4.31±8.21	2.23±4.11	0.484	
WBC (1000/µL)[d]	6081.54±3203.58	6666.08±2939.90	0.210	
Lymphocytes (1000/µL)[d]	1659.38±1126.85	1702.67±881.41	0.384	
Platelets (1000/µL)[c]	235984.6±107637.15	241118.9±79325.19	0.779	
Anti-dsDNA (IU/ml)[c]	230.08±322.79	77.32±216.33	0.014*	
C3 (mg/dl)[c]	87.95±36.26	108.86±27.60	0.009*	
C4 (mg/dl)[d]	26±13.57	22.09±16.06	0.001*	

[a]: Chi-square test, [b]: Fischer exact test, [c]: t-test, [d]: Mann-Whitney U test is used for analysis, *:p<0.05, CI: Confidence interval

Anti-C1q antibodies and the general disease activity

We examined the effect of anti-C1q antibodies on the disease activity. Patients who had anti-C1q antibodies exhibited higher SLEDAI scores (p=0.009). Subsequently, we stratified SLEDAI scores based on mild or moderate-severe disease activity (SLEDAI≥ 9). Patients who had anti-C1q antibodies demonstrated more active disease (p=0.004), and those with active disease displayed higher anti-C1q antibody titres (p=0.001) (Figure 2).

Moreover, multivariate logistic regression analysis highlighted the statistical significance of anti-C1q (p=0.036) and anti-dsDNA antibody (p=0.002) effects on SLEDAI severity scores. Patients who had anti-C1q antibodies had 4.5 times and those who had anti-dsDNA antibodies had 13 times active disease.

Anti-C1q antibodies and renal disease activity

Patients with LN had more disease activity (SLEDAI≥ 9) than patients without LN (p=0.003 for prevalence and p=0.002 for SLEDAI scores) (Figure 3).

Initially, we conducted separate analyses for patients with and without LN to identify any differences in characteristics or antibody profiles between the two groups. LN patients with active disease had greater anti-C1q and anti-dsDNA antibody titres than patients with inactive LN (p=0.010, p<0.001, respectively).

Among patients without LN, anti-C1q antibody titres were similar between patients regardless of activity status. However, active patients had greater anti-dsDNA antibody titres (p=0.001) and significant hypocomplementemia (p=0.019 for C3, p=0.044 for C4) (Table 4).

Subsequently, we categorised patients based on LN history and activity into four groups and analysed them using Bonferroni analysis. Patients who had active LN exhibited higher anti-C1q and anti-dsDNA antibody titres compared to patients with inactive LN and those with no LN history (p=0.013, p<0.001, respectively). In addition, they had lower C3 levels compared with both inactive patients regardless of LN status (p=0.002), and their C4 levels were lower than those of inactive LN patients (p=0.004) (Table 5).

In multivariate logistic regression analysis, we were not able to prove any effect of anti-C1q, anti-dsDNA, C3, and C4 on LN history.

DISCUSSION

LN still significantly impacts morbidity and survival in patients with SLE (20). It is imperative to identify markers for LN to forecast renal involvement, reflect clinical and pathological disease activity, monitor relapse, and guide therapeutic options (21). In SLE, organ damage results from the interplay of autoantibody production, immune complex deposition, and immune tolerance dysfunction affecting multiple organs (22). The complement system is crucial for clearing immune complexes and autoantigens produced during cell apoptosis, thereby protecting against autoimmune-mediated tissue and organ damage (23). Antibodies to C1q are among the extensively stud-

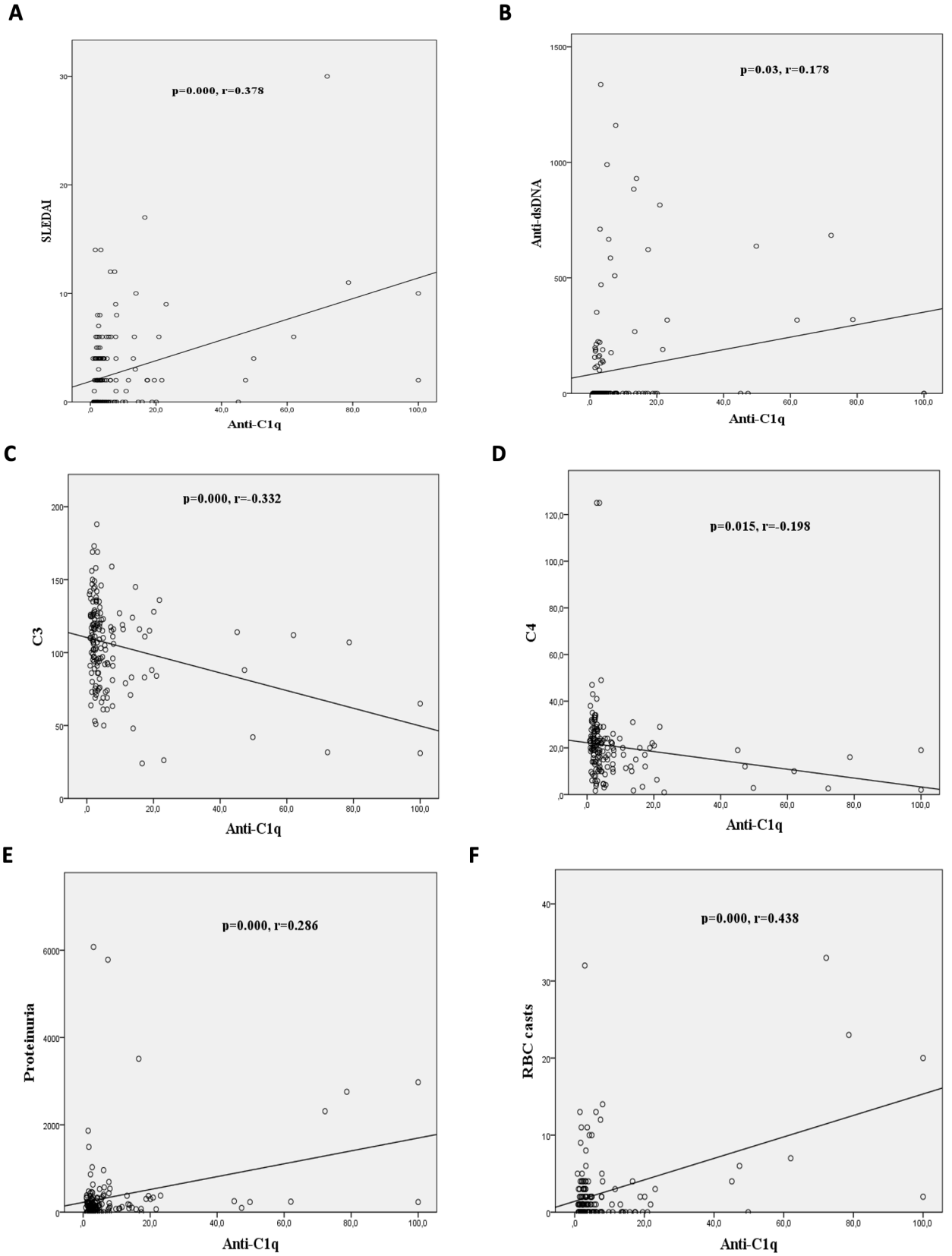

Figure 1: Correlations between anti-C1q antibody titres and A. systemic lupus erythematosus disease activity index (SLEDAI); B. anti-dsDNA; C C3; D C4; E proteinuria (mg/dl); and F Red blood cell casts/High power field (RBC casts/

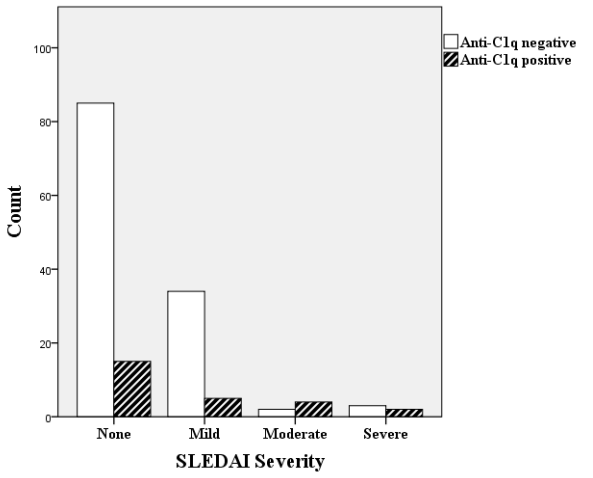

Figure 2: Anti-C1q prevalence in relation to systemic lupus erythematosus disease activity index (SLEDAI) severity. Bars indicate numbers of patients with and without anti-C1q antibody. SLEDAI severity scores; none: 0-3 points, mild: 4-8 points, moderate: 9-12 points, severe ≥12 points. Patients with anti-C1q antibody had more active disease (p=0.004).

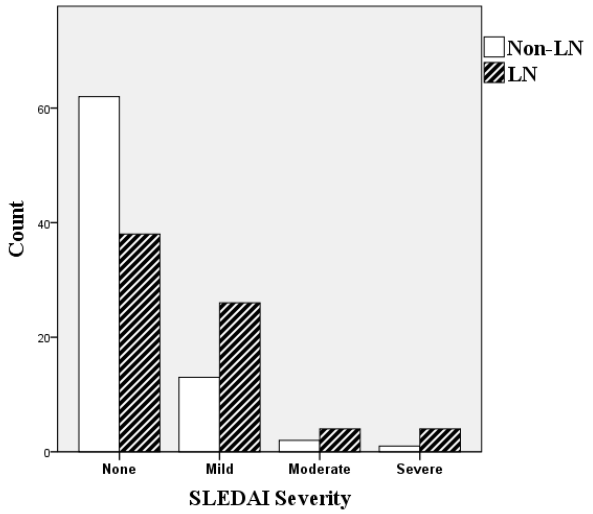

Figure 3: Lupus history in relation to systemic lupus erythematosus disease activity index (SLEDAI) severity. Bars indicate the number of patients with lupus nephritis (LN), and those without LN history (Non-LN). SLEDAI severity scores; none: 0-3 points, mild: 4-8 points, moderate: 9-12 points, severe ≥12 points. Patients with renal disease history had more active disease (p=0.003).

ied serological markers in SLE, representing a key focus in understanding these mechanisms (14, 15).

Ethnicity plays a significant role in the clinical manifestations, disease activity, organ damage, and treatment response observed in LN (24). To address these complexities, we conducted a single-centre, cross-sectional study investigating the presence of anti-C1q antibodies in Turkish patients with LN. To our knowledge, this research represents the first examination of anti-C1q antibodies in this population. Examining 150 SLE patients and 150 DC and HC subjects in total, we proved a link between anti-C1q antibodies and both the diagnosis and global disease activity of SLE. The single-centre nature of our study enabled a uniform analysis of patient data and laboratory findings. However, the prevalence of this antibody in our study (17%) was less than that reported previously, in the range of 29-60% (25).

Studies have shown that anti-C1q antibodies vanish following immunosuppressive treatment, becoming undetectable by the third month and remaining undetectable during the first year of follow-up (26). Given that most of our patients had been diagnosed and treated earlier and were in remission (average disease duration of 74.3±51.4 months, with only 7% of patients having active disease), this could explain the lower prevalence in our study.

Comparing results from different studies is challenging because of the lack of a uniform assay for anti-C1q antibody ELISA. Assays vary in terms of assay conditions and the antigen used. In our study, we used native human C1q as the antigen. A study by Jaekel et al. reported that the specificity of this assay for SLE was 89% and that for LN was 84%, but the sensitivity for SLE was 34% and that for LN was 64% (27). The high specificity but low sensitivity of our assay could also contribute to the lower prevalence of this antibody observed in our study.

In this study, patients who had anti-C1q antibodies more commonly had thrombocytopenia. Various studies have investigated the link between anti-C1q antibodies and haematologic findings. For example, a previous study reported an association between leukopoenia and this antibody (13). Armstrong et al. also emphasised the importance of anti-C1q antibodies in LN and the haematological findings of SLE (28). However, another study found no difference in findings (29). The clinical importance of thrombocytopenia in our study requires further verification.

Anti-C1q antibodies were correlated with anti-dsDNA, C3 and C4 levels, and SLEDAI, confirming its function as a valuable indicator of disease activity, as previously mentioned (11, 25). Patients with anti-C1q antibodies exhibited more active disease. In multivariate logistic regression analysis, these patients also had 4.5 times higher odds of having active disease, whereas those with anti-dsDNA antibodies had 13 times higher odds.

In our study, we observed a relationship between anti-C1q antibodies and proteinuria, haematuria, and active LN, but not with inactive LN. Although the prevalence of anti-C1q antibodies was not influenced by the history of LN, there

Table 4: Comparison of anti-C1q, anti-dsDNA antibody, C3, C4 titres according to disease activity in patients with and without lupus nephritis (LN) separately

	Active LN (n=8)	Inactive LN (n=64)	p	Active non-LN (n=3)	Inactive non-LN (n=75)	p
Anti-C1q	36.27±40.19	7.37±14.45	0.010*	13.40±9.96	6.14±9.61	0.096
Anti-dsDNA	421.13±398.43	43.42±134.71	<0.001*	861.33±513.46	91.17±211.44	0.001*
C3[a]	74.61±47.48	107.13±25.81	0.096	67.07±53.04	108.42±28.42	0.019*
C4	15.11±1482	22.40±16.27	0.065	7.30±10.40	20.21±14.53	0.044*

[a]: Only C3 is analysed with t-test, all other variables were analysed with Mann-Whitney U test, *:p<0.05

Table 5: Comparison of anti-C1q, anti-dsDNA antibody, C3, C4 titres according to disease activity and lupus nephritis (LN) history in four different groups separately

	Active LN (n= 8) (1)	Inactive LN (n=64) (2)	Active non-LN (n=3) (3)	Inactive non-LN (n=75) (4)	p	Bonferroni
Anti-C1q (U/ml)	36.27±40.19	7.37±14.45	13.40±9.96	6.14±9.61	0.013*	2<1, 4<1
Anti-dsDNA (IU/ml)	421.13±398.43	43.42±134.71	861.33±513.46	91.17±211.44	<0.001*	2<1, 2<3, 4<1, 4<3
C3 (mg/dl)[a]	74.61±47.48	107.13±25.81	67.07±53.04	108.42±28.42	0.002*	1<2, 1<4
C4 (mg/dl)	15.11±1482	22.40±16.27	7.30±10.40	20.21±14.53	0.004*	1<2, 3<2

[a]: Only C3 is analysed with ANOVA, all the other variables were analysed with Kruskal-Wallis test. Bonferroni analysis was performed, *:p<0.05.

was an association with the level of proteinuria, in line with the findings of a previous investigation by Petri et al (7). We also observed positive correlations between anti-C1q antibodies and proteinuria and haematuria.

When compared separately, patients who had active LN had higher titres of anti-C1q in comparison to patients with inactive LN and inactive non-LN. Marto et al. reported similar findings in their study: the frequency of anti-C1q antibodies was not different in patients with LN based on disease activity, but active patients had higher antibody titres (30). We were not able to show any effect of anti-C1q, anti-dsDNA, C3, and C4 in multivariate logistic regression for LN history. Our patients were more likely to represent rheumatology outpatient clinics. Most of the studies showing a relationship between anti-C1q antibodies and LN were from nephrology departments with more patients with active renal disease. As in previous studies, our results suggest that anti-C1q antibodies are related to general disease activity but not specifically to LN (29, 30).

In the meta-analysis by Eggleton et al., 31 studies were analysed to detect the accuracy of the anti-C1q antibody among patients with SLE. The authors concluded that, for distinguishing between those with and without a history of LN and the activity of patients with LN, the choice of anti-C1q antibodies as a singular diagnostic marker was not found to be useful. Post-test probabilities after a positive test were generally too low, and after a negative test, they were generally too high to be certain about the condition (15). Our findings are similar to these results.

In a recent systematic review on prognostic factors in LN and an overview of systematic reviews on the diagnostic accuracy of LN biomarkers, both sets of authors concluded that definitive biomarkers for these purposes were still lacking and further studies were needed (31, 32).

The limitations of our study include the limited number of patients with LN and the lack of repeated measures or information on disease flares because of its cross-sectional nature. In addition, being a single-centre study, the findings may not be representative of the general population of Turkish SLE patients, which could limit the generalizability of our results. Future studies could benefit from enrolling more patients with active disease, measuring anti-C1q titres before and after treatment, and obtaining repeated measures over time to better understand anti-C1q's role in diagnosing and monitoring patients with SLE.

CONCLUSION

In summary, anti-C1q antibodies are linked to SLE and overall disease activity, including active LN. Although we did not find an association with renal disease history, proteinuria was significant, and patients with active disease exhibited higher antibody titres.

Ethics Committee Approval: The study was approved by the Kocaeli University ethical committee (Date: 11.12.2015, No: 19).

Informed Consent: Consent was obtained from all participants.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- S.T., A.Y., A.Ç.; Data Acquisition- S.T., D.T.K., Ö.Ö.I.; Data Analysis/Interpretation- S.T., A.G., F.C.E.; Drafting Manuscript- S.T., A.G.; Critical Revision of Manuscript- D.T.K., Ö.Ö.I., F.C.E., A.Y., A.Ç.; Final Approval and Accountability- S.T., D.T.K., Ö.Ö.I., A.G., F.C.E., A.Y., A.Ç.

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A COMPARATIVE STUDY: PERFORMANCE OF LARGE LANGUAGE MODELS IN SIMPLIFYING TURKISH COMPUTED TOMOGRAPHY REPORTS

KARŞILAŞTIRMALI BİR ÇALIŞMA: TÜRKÇE BİLGİSAYARLI TOMOGRAFİ RAPORLARININ SADELEŞTİRİLMESİNDE BÜYÜK DİL MODELLERİNİN PERFORMANSI

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ABSTRACT

Objective: This study evaluated the effectiveness of various large language models (LLMs) in simplifying Turkish Computed Tomography (CT) reports, a common imaging modality.

Material and Method: Using fictional CT findings, we followed the Standards for Reporting of Diagnostic Accuracy Studies (STARD) and the Declaration of Helsinki. Fifty fictional Turkish CT findings were generated. Four LLMs (ChatGPT 4, ChatGPT-3.5, Gemini 1.5 Pro, and Claude 3 Opus) simplified reports using the prompt: "Please explain them in a way that someone without a medical background can understand in Turkish." Evaluations were based on the Ateşman's Readability Index and Likert scale for accuracy and readability.

Results: Claude 3 Opus scored the highest in readability (58.9), followed by ChatGPT-3.5 (54.5), Gemini 1.5 Pro (53.7), and ChatGPT 4 (45.1). Likert scores for Claude 3 Opus (mean: 4.7) and ChatGPT 4 (mean: 4.5) showed no significant difference ($p>0.05$). ChatGPT 4 had the highest word count (96.98) compared to Claude 3 Opus (90.6), Gemini 1.5 Pro (74.4), and ChatGPT-3.5 (38.7) ($p<0.001$).

Conclusion: This study shows that LLMs can simplify Turkish CT reports at a level that individuals without medical knowledge can understand and with high readability and accuracy. ChatGPT 4 and Claude 3 Opus produced the most comprehensible simplifications. Claude 3 Opus' simpler sentences may make it the optimal choice for simplifying Turkish CT reports.

Keywords: Large language model, radiology reports, readability, computed tomography, Turkish, simplifying

ÖZET

Amaç: Bu çalışmada, yaygın bir görüntüleme yöntemi olan Türkçe bilgisayarlı tomografi (BT) raporlarının sadeleştirilmesinde çeşitli büyük dil modellerinin (BDM) etkinliği değerlendirilmiştir.

Gereç ve Yöntem: Kurgusal BT bulguları kullanılarak, Tanısal Doğruluk Çalışmaları Raporlama Standartları (STARD) ve Helsinki Bildirgesi'ne uyulmuştur. Elli kurgusal Türkçe BT bulgusu oluşturuldu. Dört LLM (ChatGPT 4, ChatGPT-3.5, Gemini 1.5 Pro ve Claude 3 Opus) istemini kullanarak raporları sadeleştirdi: "Please explain them in a way that someone without a medical background can understand in Turkish". Okunabilirlik değerlendirmesi Ateşman Okunabilirlik Endeksi, doğruluk derecesi Likert ölçeğine göre yapılmıştır.

Bulgular: Claude 3 Opus okunabilirlik açısından en yüksek puanı alırken (58,9), onu ChatGPT-3.5 (54,5), Gemini 1.5 Pro (53,7) ve ChatGPT 4 (45,1) izledi. Claude 3 Opus (ortalama: 4,7) ve ChatGPT 4 (ortalama: 4,5) için Likert skorları anlamlı bir farklılık yoktu ($p>0,05$). ChatGPT 4, Claude 3 Opus (90,6), Gemini 1.5 Pro (74,4) ve ChatGPT-3.5 (38,7) ile karşılaştırıldığında en yüksek kelime sayısına (96,98) sahipti ($p<0,001$).

Sonuç: Bu çalışma, BDM'lerin Türkçe BT raporlarını tıp bilgisi olmayan bireylerin anlayabileceği düzeyde ve yüksek okunabilirlik ve doğrulukla sadeleştirebildiğini göstermektedir. ChatGPT 4 ve Claude 3 Opus en doğru sadeleştirmeleri yapmaktadır. ChatGPT 4'ün daha basit cümleleri, onu Türkçe BT raporları için tercih edilen seçenek haline getirebilir.

Anahtar Kelimeler: Büyük dil modelleri, radyoloji raporları, okunabilirlik, bilgisayarlı tomografi, Türkçe, sadeleştirme

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INTRODUCTION

Large language models (LLMs) have received considerable global attention, with numerous studies conducted worldwide. This is due to the sophisticated human-like communication and reasoning capabilities of these models (1, 2). As in many other matters, the performance of LLMs in radiological assessments, their familiarity with radiological guidelines, and their role in aiding differential diagnosis and occasionally making final decisions have recently attracted significant attention within the radiology community (3, 4).

Radiology reports, which provide a summary of radiologists' considerations and insights derived from imaging studies, are important in guiding diagnosis and treatment. They play a pivotal role in clinical practise, facilitating communication between healthcare providers and between patients and physicians. The capacity of LLMs to summarise, identify the principal ideas in texts, and interpret them has prompted an increasing interest in their potential to facilitate the simplification of radiology reports (5-8). This application would enable LLMs to enhance patients' understanding of radiology reports, alleviate their anxiety, and improve communication among healthcare providers and between patients and physicians.

This study compared how effectively different LLMs simplify Turkish CT reports, an imaging modality frequently obtained in clinical practise.

MATERIAL AND METHODS

The study included only fictional CT findings, excluding actual radiology reports, thereby negating the need for ethical board approval. The study design adhered to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) and the principles outlined in the Declaration of Helsinki (9).

The authors collaboratively generated 50 fictional CT findings in Turkish used in radiology reports. Efforts were made to ensure that these findings were representative of common scenarios in daily practise and depicted realistically (Supplementary material 1) (Table 1).

The study employed various LLMs, including ChatGPT 4, ChatGPT-3.5, Gemini 1.5 Pro, and Claude 3 Opus. The fictional findings were input into each LLM via their respective websites using the prompt, "I will write the findings from the CT report below. Please explain them in a way that someone without a medical background can understand" in Turkish (Figure 1a, 1b). Each finding was processed in a new window, as shown in Figure 1, with the default settings applied for each model. The study was conducted between April 15 and April 19, 2024.

The responses from the LLMs were evaluated using the Ateşman's Readability Index [198,825-(40,175xnumber of syllables/number of words)-(2,610xnumber of words/number of sentences)] to determine the readability levels (Table 2) (10). This analysis was performed using the publicly accessible and free website "www.readabilityindex.com." The three authors collectively evaluated the responses on a five-point Likert scale, with one representing the least favourable and five representing the most favourable, in terms of medical accuracy, consistency of recommendations, and comprehensibility. Additionally, the word count for each response was documented. The study's workflow is illustrated in Figure 2.

For the statistical analyses, we used SPSS ver.26 (IBM Corp, Armonk, NY, USA). Data distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests, while the Levene test was used to evaluate data variance. Descriptive statistics included the minimum, maximum, average, median, standard deviation, interquartile range, and percentages. To identify significant relationships between the quantitative data independent groups, we employed the Friedman and Wilcoxon tests. Spearman correlation analysis was used to examine the linearity of the correlations between the quantitative data.

RESULTS

There was no statistically significant difference between the Likert scores of Claude 3 Opus (mean: 4.7; median: 5.0) and ChatGPT 4 (mean: 4.5; median: 5.0) ($p>0.05$). However, Claude 3 Opus's Likert scores differed significantly from those of Gemini 1.5 Pro (mean: 4.3; median: 4.0) and ChatGPT-3.5 (mean: 2.8; median: 3.0) ($p<0.001$). While there was no statistically significant difference between the average Likert scores of ChatGPT 4 and Gemini 1.5 Pro ($p=0.025$; Bonferroni-adjusted p-value=0.0125), a significant difference was observed between the scores of ChatGPT 4 and ChatGPT-3.5 (mean: 2.8; median: 3.0) ($p<0.001$). The average Likert score for ChatGPT-3.5 was significantly lower than that of all other large language models ($p<0.001$).

According to Ateşman's readability index and readability levels, Claude 3 Opus had the highest average value at 58.9, followed by ChatGPT-3.5 (54.5), Gemini 1.5 Pro (53.7), and ChatGPT 4 (45.1). Although there was a significant difference in readability between Claude 3 Opus and ChatGPT 4 ($p<0.05$), there wasn't significant difference observed with other LLMs ($p>0.05$). The descriptive findings of the study are shown in Table 3.

A statistically significant difference was found in the number of words used by ChatGPT 4 (mean: 96.98) compared to Claude 3 Opus (mean: 90.6), Gemini 1.5 Pro (mean: 74.4), and ChatGPT-3.5 (mean: 38.7) ($p<0.001$). Although there was no significant difference in the word count between ChatGPT 4 and Claude 3 Opus, the average word

Table 1: A portion of the findings used as fictional Turkish and English CT findings are shown*.

1.	Sol frontalde en kalın yerinde 2 mm ölçülen subaraknoid kanama izlendi (A subarachnoid haemorrhage, measuring 2 mm at its thickest point, was observed in the left frontal region)
2.	Sol frontal lob komşuluğunda en kalın yerinde 20 mm ölçülen epidural kanama izlendi (An epidural haemorrhage, measuring 20 mm at its thickest point, was observed adjacent to the left frontal lobe)
3.	Sol frontal lob komşuluğunda en kalın yerinde 30 mm ölçülen subdural kanama izlendi (A subdural haemorrhage, measuring 30 mm at its thickest point, was observed adjacent to the left frontal lobe)
4.	Her iki frontal lobda atrofiye ikincil hemisferik kortikal sulkuslarda belirginleşme derinleşme izlendi (Enlargement and deepening of the hemispheric cortical sulci, secondary to atrophy, were observed in both frontal lobes)
5.	Sağ temporal kemikte transvers fraktür izlendi (Transverse fracture was observed at the right temporal bone)
6.	Sol plevral aralıkta en kalın yerinde 20 mm ölçülen plevral effüzyon izlendi (Pleural effusion, measuring 20 mm at its thickest point, was observed in the left pleural space)
7.	Sağ akciğer alt lobda konsolidasyon tarzında infiltrasyon izlendi (Consolidation-infiltration was observed in the lower lobe of the right lung)
8.	Her iki akciğer apekste sekel fibrotik değişiklikler izlendi (Sequelae fibrotic changes were observed at the apices of both lungs)
9.	Sol akciğer lingüler segmentte atelektatik değişiklikler izlendi (Atelectatic changes were observed in the lingular segment of the left lung)
10.	Perikardial aralıkta en kalın yerinde 11 mm ölçülen perikardial effüzyon izlendi (Pericardial effusion, measuring 11 mm at its thickest point, was observed in the pericardial space)
11.	Kardiyotorasik oran kalp lehine artmıştır (The cardiothoracic ratio is increased in favour of the heart)
12.	Pulmoner trunk 37 mm ölçülmüş olup ektatiktir (The pulmonary trunk was measured at 37 mm, indicating ectasia)
13.	Sol akciğer alt lobda 7 mm çapında solid nodül izlendi (A solid nodule, measuring 7 mm in diameter, was observed in the lower lobe of the left lung)
14.	Sol akciğer alt lobda 7 mm çapında semi-solid nodül izlendi (A semi-solid nodule, measuring 7 mm in diameter, was observed in the lower lobe of the left lung)
15.	Göğüs on-arka çapı belirgin artmıştır (The anteroposterior diameter of the chest is markedly increased)
16.	Tiroid gland boyutları belirgin artmış olup trakea sola itilmiştir (The dimensions of the thyroid gland are significantly increased, with the trachea displaced to the left)
17.	Karaciğer parankiminde steatoza ikincil diffüz dansite azalması izlendi (Diffuse decrease in parenchymal density, secondary to steatosis, was observed in the liver)
18.	Karaciğerde 10mm çapında hemanjiyom ile uyumlu periferik nodüler kontrastlanana hipodens lezyon izlendi (A hypodense lesion, measuring 10 mm in diameter and consistent with a hemangioma, exhibiting peripheral nodular enhancement, was observed in the liver)
19.	Karaciğerde 15mm çapında kontrastlanmayan öncelikle basit kist lehine düşünülen hipodens lezyon izlendi (A hypodense lesion, measuring 15 mm in diameter and not enhancing with contrast, was observed in the liver, favouring a diagnosis of a simple cyst)
20.	Safra kesesi fundus düzeyinde fokal duvar kalınlık artışı izlendi (Focal wall thickening was observed at the fundus of the gallbladder)

*:Since the findings in the study are given to LLMs in Turkish, the findings are presented both in Turkish and in brackets in English.

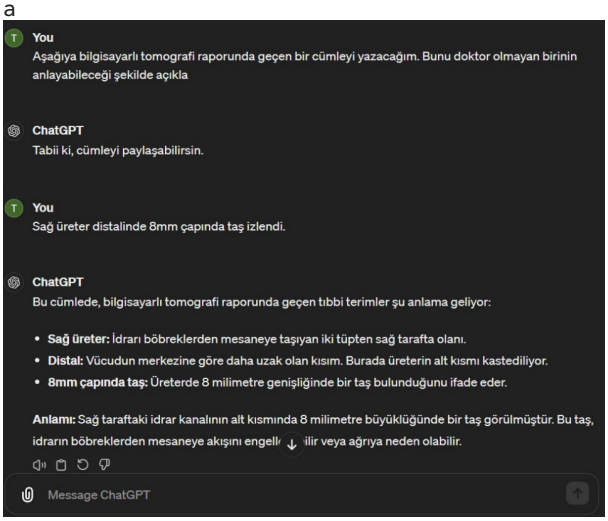
count for ChatGPT 4 and Claude 3 Opus was statistically higher than that of all other language models ($p<0.001$). Additionally, the word count for Gemini 1.5 Pro was statistically higher than that for ChatGPT-3.5 ($p<0.001$).

A linear correlation was observed between the number of words in the fictional CT findings and those generated by Gemini 1.5 Pro (correlation coefficient=0.756, $p<0.000$) and ChatGPT 4 (correlation coefficient=0.523, $p<0.000$). In contrast, no linear correlation was detected for Claude 3 Opus ($p=0.367$) and Perplexity ($p=0.552$). Additionally, a linear correlation was identified between the readability index of the fictional CT findings and the readability indices of the responses from Claude 3 Opus (correlation coefficient=0.508, $p<0.000$), ChatGPT 3,5 (correlation coefficient=0.387, $p=0.005$), and Gemini 1.5 Pro (correlation coefficient=0.331, $p=0.042$). However, no correlation was found between the readability index of the fictional CT findings and that of ChatGPT 4 ($p=0.387$).

DISCUSSION

The main result of our study is that LLMs can simplify CT reports with high readability and accuracy. The Likert scale evaluations by all three radiologists indicated a consensus that LLMs can accurately simplify CT reports in a medically sound manner. Similarly, previous studies have noted that LLMs are highly effective in simplifying and explaining radiology reports (5, 6, 10, 11). Jeblick et al. evaluated the quality of ChatGPT's simplified English radiology reports, including fictional reports, and found them to be accurate and complete, based on medical

a



b

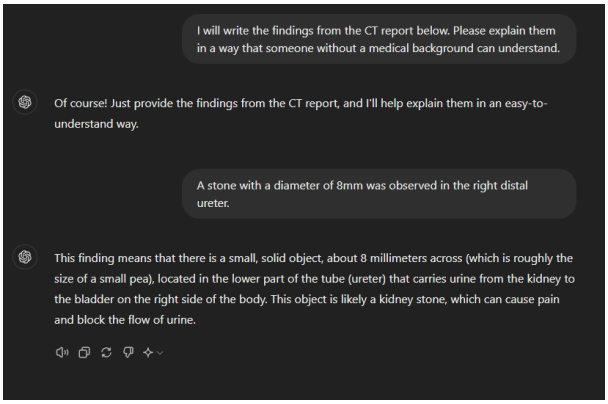


Figure 1: a) Describes the Turkish input and output process in the study through ChatGPT 4. The figure shows the Turkish version of the prompt mentioned in the methodology and the fictional finding as input. **b)** Describes the English input and output process in the study through ChatGPT 4. The figure shows the English version of the prompt mentioned in the methodology and the fictional finding as input. Explanation: Since the findings of the study are given to LLMs in Turkish, the findings are presented in Turkish in Figure 1a and in English in Figure 1b.

facts, suggesting that ChatGPT can achieve this simplification without causing any harm to patients (10).

We used Ateşman's readability index to assess how easily the simplified CT reports, produced by LLMs in Turkish, could be read (12). It measures Turkish text readability based on average syllables per word and words per sentence, with scores ranging from 1 to 100; higher scores indicate easier reading. Ateşman stressed that a text's effectiveness relies on both its readability and comprehensibility. While readability is quantitatively evaluated, comprehensibility is qualitatively assessed

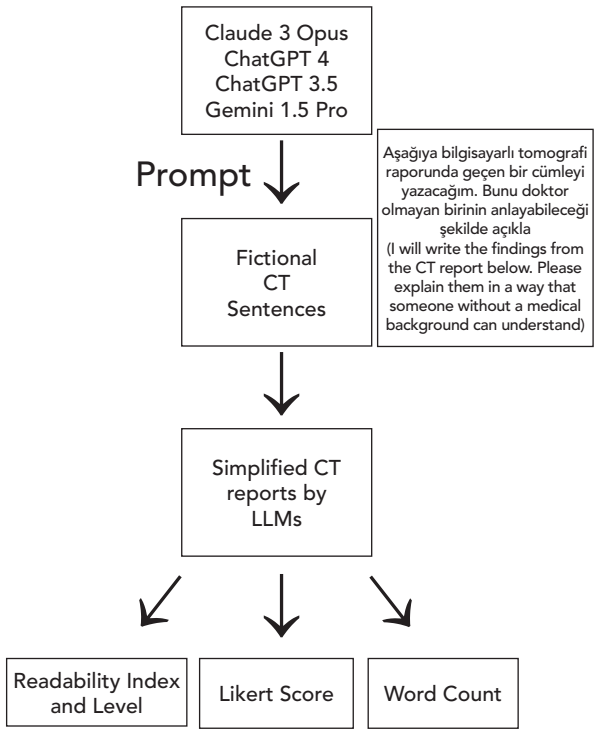


Figure 2: The study's workflow.
The prompt in the study was given to LLMs in Turkish. Thus, the prompt is presented both in Turkish and in brackets in English.

Table 2: Ateşman's Readability Index and its corresponding readability level

Index	Readability level
90-100	Easily understood by 4thgrade and below students
80-89	Easily understood by 5th or 6th graders
70-79	Easily understood by 7th or 8th graders
60-69	Easily understood by 9th or 10th graders
50-59	Easily understood by 11th or 12th graders
40-49	Easily understood by 13th or 15th-year (associate degree) students
30-39	Easily understood by bachelor's degree
<30	Easily understood by postgraduates

Table 3: Descriptive findings of the study are shown.

	Claude 3 Opus	Gemini 1.5pro	ChatGPT 4	ChatGPT 3.5
Likert Scores*				
Minimum-Maximum	4.0-5.0	3.0-5.0	3.0-5.0	1.0-4.0
Mean±SD	4.7±0.4	4.32±0.6	4.52±0.5	2.78±0.6
Median (IQR)	5.0 (0)	4.0 (0)	5.0 (0)	3.0 (1.0)
Ateşman's Readability Index				
Minimum-Maximum	33.1-79.0	23.3-68.9	34.9-72.3	21.8–79.1
Mean±SD	58.9±5.23	53.7±5.67	45.1±11.72	54.5±9.99
Median (IQR)	53.4 (13.2)	48.2 (10.9)	38.1 (8.2)	38.3 (7.1)
Readability Level				
Minimum	7-8th class	9-10th class	7-8th class	7-8th class
Maximum	Bachelor's degree	Postgraduate	Bachelor's degree	Postgraduate
Median	11-12th class	11-12th class	13-14th class	11-12th class
Word Count				
Minimum-Maximum	71-136	34-143	47-149	8-71
Mean±SD	90.66±16.77	74.42±27.26	96.98±28.39	38.74±15.9
Median (IQR)	85.0 (24.5)	69.0 (43.7)	97.5 (34.5)	40.0 (30.0)

*Likert Scores: In our study, the accuracy of the explanations, consistency, and comprehensibility of the suggestions made by the big language models were rated on a scale of 1 to 5. SD: Standard Deviation, IQR: Interquartile range.

based on the text's content. We evaluated the readability of the responses using the Ateşman's index and their comprehensibility using a Likert scale. We acknowledge that assessments by individuals without medical backgrounds would offer more valuable insights into comprehensibility. There was no significant difference between ChatGPT 4 and Claude 3 Opus in terms of Likert score, but the readability index of ChatGPT 4 was lower than all other LLMs. Claude 3 Opus had both the highest Likert score and the highest Ateşman's index among all LLMs. This shows that Claude 3 Opus uses more simple and understandable sentences to simplify CT reports by providing sufficient and accurate information. Claude 3 Opus adeptly simplifies Turkish CT reports while employing straightforward sentence structures. Hence, Claude 3 Opus may represent the optimal choice among LLMs for streamlining Turkish CT reports.

Johnson et al. simplified 750 randomly selected anonymized radiology reports with three different prompts (x-ray, ultrasound, magnetic resonance, and computed tomography reports) using ChatGPT 3.5, ChatGPT 4, Microsoft Bing, and Google Bard (now known as Gemini). The researchers reported that all LLMs produced more readable reports than the original reports (13). They also reported that the performance of each LLM was affected differently at different prompts. Although there is no generally accepted prompt for report simplification, the prompt given significantly influences LLM responses. Lyu et al. examined 62 thorax CT and 76 brain MRI reports (14). Each report had three simplified versions based on different prompts: making the report easier to understand, providing patient advice, and offering healthcare professional recommendations. They also explored how different prompts could create varied reports for patients with different education levels. Similarly, Schmidt et al. used ChatGPT 3.5 to simplify knee MRI findings of varying complexity (simple, moderate, and complex) with five different prompts (11). They showed the effect of prompts on simplifying radiology reports. In addition, their findings revealed that simplified reports were more comprehensible for patients, leading to improved patient understanding and overall satisfaction. Li et al. simplified 100 radiology reports, including different imaging modalities, using the prompt "Explain this radiology report to a patient in layman's terms: <Report Text>" and showed that simplified reports were significantly more readable and shorter (6). In order not to affect the Likert scores and readability levels of each LLM, we were careful not to include specific words that might affect the word limit and readability level of our prompt. Further studies will be instructive to show how the specific prompts given affect the readability level. In this way, the information content and readability level of the simplified reports can be adjusted by giving specific prompts according to the socio-economic level of the patients.

Our study is the first to assess how LLMs can simplify Turkish CT reports to understand people without a medical background. However, it has some limitations. The main limitation is that only radiologists scored simplified reports. Practitioners from other departments and real patients did not participate in this study, so we lacked their feedback on the simplified CT reports. Future research should include patient feedback and compare standard CT reports with those simplified by LLMs. This would

provide important insights into how understandable and useful the simplified reports are to patients. In addition, we only used fictitious findings for a single condition, not real CT reports. More complex reports that included all relevant findings might produce different results. Finally, we used only one prompt. Different prompts could produce better or worse results depending on the capabilities of the model.

CONCLUSSION

In conclusion, our study shows that LLMs can effectively simplify Turkish CT reports. Enabling patients to read and understand CT reports may help them better grasp their diagnosis and treatment, leading to improved compliance. Simplified CT reports may also enhance communication between physicians.

Ethics Committee Approval: The study does not require ethics committee approval because it only includes fictional computed tomography findings and does not use actual radiology reports.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.Ç., T.C.; Data Acquisition – E.Ç., T.C., Y.C.G.; Data Analysis/Interpretation- E.Ç.; Drafting Manuscript- E.Ç.; Critical Revision of Manuscript- T.C., Y.C.G.; Final Approval and Accountability- E.Ç.; Supervision- T.C., Y.C.G.

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RESEARCH / ARAŞTIRMA
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EFFECT OF HYPERBARIC OXYGEN THERAPY ON FASTING BLOOD GLUCOSE AND INSULIN RESISTANCE

HİPERBARİK OKSİJEN TEDAVİSİNİN AÇLIK KAN ŞEKERİ VE İNSÜLİN DİRENCİ ÜZERİNDEKİ ETKİSİ

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ABSTRACT

Objective: The subject of this study was to investigate the effect of hyperbaric oxygen (HBO2) therapy on fasting blood glucose levels and insulin resistance. The study investigated whether HBO2 therapy is effective in reducing peripheral insulin resistance in patients with and without Diabetes Mellitus (DM).

Material and Methods: The study included 27 patients with and without DM who received hyperbaric oxygen therapy at a pressure of 2.4 ATA for 120 min per session with 5 min breaks in between three 25-minute O2 periods, one session per day, five days a week, for 20 sessions. Patients were divided into two groups: those without DM and those with DM. Fifteen patients did not have DM while 12 patients had DM Type 2. Glucose, C-Reactive protein (CRP), HbA1c, C-peptide, and Homeostasis Model Assessment Insulin Resistance (HOMA-IR) values were compared at the beginning and end of treatment.

Results: In repeated measurements, there was a statistically significant decrease in the mean fasting blood glucose levels in patients with DM. Patients without DM also showed a reduction in the mean fasting blood glucose levels in repeated measurements, but this decrease was not statistically significant. There was a statistically significant decrease in the mean C-pep level in repeated measurements in patients with DM and the mean insulin level in repeated measurements in patients without DM. There was a statistically significant decrease in the HOMA-IR values calculated in repeated measurements in patients with and without DM.

ÖZET

Amaç: Bu çalışmanın konusu hiperbarik oksijen (HBO2) tedavisinin açlık kan şekeri düzeyleri ve insülin direnci üzerindeki etkisidir. Çalışmanın amacı, HBO2 tedavisinin hem Diabetes Mellitus-lu (DM) hem de DM olmayan hastalarda periferik insülin direncini azaltmada etkili olup olmadığını araştırmaktır.

Gereç ve Yöntem: Diyabeti olan ve olmayan 27 hastaya haftada beş gün, günde bir seans, 2,4 ATA basınçta, her biri aralarda 5 dakikalık hava molası verilen üç 25 dakikalık oksijen periyodu içeren 120 dakikalık 20 seans hiperbarik oksijen tedavisi uygulanmıştır. Hastalar DM olmayanlar ve DM olanlar olmak üzere iki gruba ayrılmıştır. Hastaların 15'i diyabeti olmayan, 12'si ise tip 2 DM'liydi. Tedavinin başında ve sonunda glukoz, C-Reaktif protein (CRP), HbA1c, C-peptid (C-pep) ve Model Assessment Insulin Resistance (HOMA-IR) değerleri karşılaştırıldı.

Bulgular: Tekrarlanan ölçümlerde, diyabetli hastalarda ortalama açlık kan şekeri seviyelerinde istatistiksel olarak anlamlı bir düşüş görülmüştür. DM olmayan hastalarda da tekrarlanan ölçümlerde ortalama açlık kan şekeri seviyelerinde azalma görüldü, ancak bu azalma istatistiksel olarak anlamlı değildi. DM'li hastalarda tekrarlanan ölçümlerde ortalama C-pep seviyesinde ve DM olmayan hastalarda tekrarlanan ölçümlerde ortalama insülin seviyesinde istatistiksel olarak anlamlı bir düşüş olmuştur. DM olan ve olmayan hastalarda tekrarlanan ölçümlerde hesaplanan HOMA-IR değerlerinde istatistiksel olarak anlamlı bir düşüş vardı.

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Conclusion: In our study, insulin resistance decreased in patients receiving HBO2 therapy. The fact that this decrease was also shown in patients without DM strengthens the idea that this decrease is related to HBO2 therapy. Further research is needed to gain a more comprehensive understanding of the therapeutic potential of HBO2 therapy in managing insulin resistance.

Keywords: Blood sugar level, diabetes mellitus, hyperbaric oxygen therapy, insulin resistance

Sonuç: Çalışmamızda HBO2 tedavisi alan hastalarda insülin direnci azalmıştır. Bu azalmanın DM olmayan hastalarda da gösterilmiş olması, bu azalmanın HBO2 tedavisi ile ilişkili olduğu fikrini güçlendirmektedir. HBO2 tedavisinin insülin direncini yönetmedeki terapötik potansiyelinin daha kapsamlı bir şekilde anlaşılması için daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Kan şekeri, diyabet, hiperbarik oksijen tedavisi, insülin direnci

INTRODUCTION

Hyperbaric Oxygen (HBO2) therapy is a medical treatment that involves the intermittent inhalation of 100% O2 at a pressure higher than that of the atmosphere in a closed system (1). HBO2 therapy has been a preferred treatment for decompression sickness and management of air embolism. In addition, studies have shown the benefit of HBO2 therapy in several medical conditions, including non-healing wounds, radiotherapy-induced tissue damage, and necrotising tissue infections (2-4).

Many studies have shown that HBO2 therapy causes hypoglycaemia in patients with Diabetes Mellitus (DM), and a systematic review supported this view (5). Therefore, all HBO2 therapy facilities have protocols to monitor the plasma glucose levels of patients with DM and to provide an oral replacement for those with low levels. HBO2 therapy may increase insulin sensitivity and lead to hypoglycaemia in patients with DM. Although research in this area is ongoing, there is insufficient evidence to suggest that HBO2 therapy may affect insulin sensitivity in individuals without DM.

Hypoxia has been associated with insulin resistance in white adipose tissue and skeletal muscle (6, 7). In two other studies of individuals with obesity, insulin resistance seems to be associated with hypoxia, inflammation, and oxidative stress in white adipose tissue, which are also observed in the pathogenesis of type 2 DM (8 ,9). Considering HBO2 therapy's potential to reduce inflammation and enhance antioxidant defence systems, it may be beneficial in treating insulin resistance through these mechanisms (10).

This study investigated whether peripheral insulin resistance, proposed as a mechanism explaining the hypoglycaemic effect of HBO2 treatment, is reduced in patients with and without DM.

MATERIAL AND METHODS

This study was approved by the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 24.08.2012, No: 346). Patients with and without DM who received 20 sessions of HBO2 therapy at a pressure of 2.4 ATA, each session lasting 120 minutes with 5-minute air breaks in between three 25-minute O2 periods, one session per day, and five days a week were included in the study. Patients under 18 years of age, over 80 years of age, receiving systemic steroid therapy, having a known diagnosis of hyperthyroidism, acromegaly, Cushing's syndrome, pheochromocytoma, or having undergone HBO2 therapy in the six months prior were excluded from the study. HBO2 therapy was administered in a multiplace chamber (Hypertech® Zyron12, Turkey). This chamber can accommodate up to 12 patients and one inside attendant in the main chamber. The study was conducted on patients undergoing HBO2 therapy for various conditions. The patients were divided into two groups, with and without DM, to evaluate the metabolic effects of HBO2 therapy separately within each group rather than to compare the two groups. All patients with DM had type 2 diabetes and were on insulin treatment. During the study, the insulin dose was adjusted for patients when necessary. Patients who interrupted the treatment for two days or more were excluded from the study. Informed consent was obtained from all patients and their relatives before participation in the study.

A standardised data recording form was created before the study. In this form, demographic information about the age, gender, height, and body weight of all patients was recorded, and height and weight indices were calculated. The results obtained from the laboratory measurements of the study were also recorded on this form. In our study, the C-Reactive protein (CRP) level was set as an indicator of infection stress since it's an acute phase reactant and was analysed (11).

Blood was collected from the antecubital vein of all patients who participated in the study after fasting for at least 8 h before starting HBO2 therapy (e.g., Fasting blood glucose (FBG) 1, Insulin 1, etc.), after the tenth session (e.g., FBG 2, Insulin 2, etc.) and after the 20th session (e.g., FBG 3, Insulin 3, etc.). The Biochemistry Laboratory analysed the following blood values within 1 h of collection;

- Fasting blood glucose, insulin, HbA1c, and CRP levels in patients without DM,
- Plasma glucose, C-peptide (C-pep), HbA1c, and CRP levels in patients with DM.

The Homeostatic Model assessment (HOMA) has become a widely used and reliable clinical and epidemiological tool (12, 13). Although the hyperinsulinaemic-euglycaemic glucose clamp test is considered the gold standard for measuring insulin resistance (IR), its clinical applicability is limited because it is labour-intensive and costly (14, 15). HOMA-IR is calculated using plasma glucose and insulin concentrations and is easy to perform, safe, less invasive, and less expensive (16). Its results correlate well with the euglycemic clamp test (17, 18). Because of these strengths, we calculated and applied HOMA-IR to estimate IR in our participants. HOMA version 2 software developed by the Diabetes Research Unit of Oxford University was used to calculate HOMA (19).

Statistical analysis

The measurements taken at baseline and after sessions 10 and 20, were analysed by comparing the repeated measurements and their differences. The data obtained from the study were transferred to the SPSS 22.0 (IBM SPSS Corp., Armonk, NY, USA) statistical programme. Values were obtained as mean and standard deviation. In comparing the groups within each other, Friedman's test, which allows non-parametric examination in matched groups in repeated measurements, was used because of the limited number of patients, regardless of the parametric values. "Wilcoxon Test," a non-parametric test method in paired groups, was used to determine the differences between the groups. The difference was considered statistically significant if the p-value was less than 0.05.

RESULTS

The study initially involved 37 patients. However, five patients had to be excluded from the study because they could not continue HBO2 therapy. In addition, two patients could not comply with the treatment, one patient developed claustrophobia, one patient passed away during the study, and one patient expressed a desire to leave the study. Eventually, the study included 27 patients, out of which 15 were without DM and 12 were with DM. The diagnoses for 10 patients with DM were diabetic foot, while one patient had chronic osteomyelitis and another had peripheral vascular disease. Among the patients without DM, 5 had avascular necrosis, 5 had chronic osteomyelitis, 3 had venous ulcers, 1 had Buerger's disease, and 1 had radiation cystitis. Of the patients without DM, 8 were inpatients and 7 were outpatients; of the patients with DM, 10 were inpatients and 2 were outpatients. None of the patients participating in the study had severe renal failure or cirrhosis.

Among the patients with DM, three were female and nine were male. The mean age of patients without DM was 38.67±18.3 years, and the mean BMI was 26.8±4.84 kg/m^2; the mean age of people with DM was 61.42±13 years, and the mean BMI was 25.87±3.95 kg/m^2. The demographic data of the patients included in the study are shown in Table 1.

Table 1: Demographic data of the participants

	n	%	Mean	SD	Min-max
Patients with DM	12				
Age (year)			61.42	13.1	44-79
Gender					
Male	9	75.0			
Female	3	25.0			
Height (cm)			166	8.17	157-177
Weight (kg)			71.2	10.4	53-88
BMI (kg/m^2)			25.87	3.95	22.0-33.7
Normal	7	58			
Overweight-obese	5	42			
Patients without DM	15				
Age (year)			38.67	18.3	
Gender					
Male	10	66.6			
Female	5	33.3			
Height (cm)			174	8.7	155-187
Weight (kg)			80.3	11.9	64-100
BMI (kg/m^2)			26.8	4.8	20.7-36.9
Normal	5	33.3			
Overweight-obese	10	66.6			

DM: Diabetes Mellitus, BMI: Body mass index, Normal: BMI=18.5-24.9, Overweight-obese: BMI: ≥25

The data obtained from multiple measurements are presented in Table 2. The table shows the results measured before the HBO2 therapy session, after the 10th HBO2 therapy session, and after the 20th HBO2 therapy session. The statistical significance levels of the data obtained from the study groups' repeated measurements are presented in Table 3. The statistical significance levels of the data obtained in each measurement step for the study groups are presented in Table 4.

In the patients without DM, a decrease was found in mean fasting blood glucose levels in repeated measurements. However, this decrease was not statistically significant (p=0.458). A statistically significant reduction was found in mean fasting blood glucose repeated measurements in patients with DM (p=0.002) (Table 3).

In patients without DM, a statistically significant decrease was found in HOMA-IR values calculated in repeated measurements (p=0.015) (Table 3). When the measure-

Table 2: Means and standard deviations of patients' measurements

	Patients without DM (n=15)		Patients with DM (n=12)	
Parameters	**Mean**	**SD**	**Mean**	**SD**
FBG 1 (mg/dL)	89.87	6.85	167.5	50.69
FBG 2 (mg/dL)	89.27	8.67	110.25	25.61
FBG 3 (mg/dL)	87.47	8.35	109.91	40.67
Insulin 1 (mIU/L)	11.65	7.17		
Insulin 2 (mIU/L)	10.18	4.39		
Insulin 3 (mIU/L)	8.94	3.91		
C-pep 1 (ng/ml)			2.32	1.63
C-pep 2 (ng/ml)			1.71	1.34
C-pep 3 (ng/ml)			1.57	0.88
HOMA 1	1.5	0.88	2.02	1.37
HOMA 2	1.3	0.54	1.38	1.12
HOMA 3	1.14	0.53	1.21	0.61
CRP 1 (mg/dL)	19.46	26.94	64.44	77.39
CRP 2 (mg/dL)	15.04	25.98	8.59	6.59
CRP 3 (mg/dL)	20.43	39.07	10.04	11.46
HbA1c 1 (%)	5.44	0.31	8.67	1.52
HbA1c 3 (%)	5.3	0.32	7.31	1.03

FBG: Fasting blood glucose, (FBG1: first measurement, FBG2: second measurement, etc.) C-pep: C-peptide, HOMA: The Homeostasis Model assessment, CRP: C-reactive protein, DM: Diabetes Mellitus. Note: Because the insulin level was measured in only one patient in the diabetic group and the C-pep was not measured in the non-diabetic group, neither data was compared. 1. 2 and 3 refers to pre-treatment, 10th session and 20th session measurements respectively

ments were evaluated among themselves, no statistically significant difference was found between the first and second measurement (p=0.145) and between the second and third measurement (p=0.080), while the difference between the first and third measurements was statistically significant (p=0.047) (Table 4).

A statistically significant decrease was found in HOMA-IR values calculated in repeated measurements in patients with DM (p=0.034) (Table 3). When the measurements were evaluated among themselves, the decrease between the first and second measurement (p=0.041) and between the first and third measurement (p=0.041) was statistically significant, while the decrease between the second and third measurement was not statistically sig-

Table 3: Statistical significance levels of comparision of mean levels in repeated measurement

	Patients without DM (p)	Patients with DM (p)
FBG	0.458	**0.002**
Insulin	**0.007**	
C-pep		**0.045**
HOMA	**0.015**	**0.034**
CRP	0.282	**0.002**

FBG: Fasting blood glucose, C-pep: C-peptide, HOMA: The Homeostasis Model assessment, CRP: C-reactive protein, DM: Diabetes Mellitus.

Table 4: Statistical significance levels of comparision of mean levels obtained in each measurement step

Measurement	Patients without DM (p)	Patients with DM (p)
FBG 1-2	0.700	**0.004**
FBG 2-3	0.216	0.722
FBG 1-3	0.248	**0.003**
INS 1-2	0.280	-
INS 2-3	**0.047**	-
INS 1-3	**0.041**	-
C-pep 1-2	-	**0.033**
C-pep 2-3	-	0.878
C-pep 1-3	-	0.063
HOMA 1-2	0.145	**0.041**
HOMA 2-3	0.080	0.593
HOMA 1-3	**0.041**	**0.041**
CRP 1-2	0.334	0.004
CRP 2-3	0.865	0.929
CRP 1-3	0.057	**0.004**
HbA1c 1-3	0.160	**0.002**

FBG: Fasting blood glucose, FBG1: first measurement, FBG2: second measurement, etc. INS: insulin, C-pep: c-peptide, HOMA: The Homeostasis Model assessment, CRP: C-reactive protein, DM: diabetes mellitus. Note: Repeated measurements for each data were compared in pairs: 1. 2 and 3 refers to pre-treatment, 10th session and 20th session measurements respectively

nificant (p=0.593) (Table 4).

When HOMA-IR changes were evaluated according to the BMI of the participants, no significant difference was found in the patients with DM (p=0.432) and those without DM (p=0.254).

In patients without DM, a very slight decrease was found in the mean HbA1c values before and after treatment. However, this decrease was not statistically significant (p=0.160). On contrary, a statistically significant decrease was observed in the mean HbA1c values before and after treatment in patients with DM (p=0.002) (Table 4).

During the study, one patient experienced sinus barotrauma, and two patients experienced middle ear barotrauma as a side effect of HBO2 therapy, which did not suspended treatment.

DISCUSSION

In this study, we determined the effects of HBO2 therapy on insulin resistance and plasma glucose levels in patients with and without DM. Our findings revealed that HBO2 therapy did not significantly affect the plasma glucose levels of patients without DM. Patients without DM have better glucose homeostasis to prevent drops in blood glucose, so it is unclear whether HBO2 therapy has a blood glucose-lowering effect. Further studies are needed to understand this. However, in patients with DM, we observed a rapid decline in plasma glucose levels within the first 10 sessions of HBO2 therapy, which remained low until the final session. Several other studies have also demonstrated a similar effect of HBO2 therapy in lowering plasma glucose levels (20-23).

Wilkinson et al. showed that insulin resistance decreased significantly with HBO2 therapy, but this decrease was insignificant in people without DM (24). In studies conducted on subjects with obesity, both with and without DM, it was shown that HBO2 therapy resulted in decreased insulin resistance (25, 26). In two studies comparing HBO2 therapy with normobaric O2 and hyperbaric air (27, 28), the HBO2 therapy groups showed a significant decrease in insulin resistance, whereas no decrease was found in the control groups. In our study, insulin levels measured in patients without DM and C-pep levels measured in patients with DM decreased significantly during the study period. HOMA-IR values calculated using these results showed a significant decrease in both groups. In addition to the decrease in patients without DM, no correlation was found between being overweight and the decrease in insulin resistance in both groups. This result shows that HBO2 therapy may decrease insulin resistance in non-overweight individuals. In patients without DM, HOMA-IR did not significantly decrease until after the 20th HBO2 therapy session, indicating that longer sessions may be necessary to observe the effect.

Our study found that patients with DM experienced a more remarkable improvement in their metabolic parameters during the early phase of treatment. This is because both metabolic disorders and infections were treated in addition to the HBO2 therapy at this stage. Although these patients had already recovered from their metabolic disorders and infections, their insulin resistance tended to decrease during the last 10 sessions. This continuous decline indicates that HBO2 therapy may also contribute to improving the condition in patients with DM.

Considering that insulin resistance is associated with hypoxia, oxidative stress, and inflammation, it can be hypothesised that HBO2 therapy decreases insulin resistance by increasing the anti-hypoxic, anti-inflammatory, and antioxidant defence systems (29-31). Research has revealed that in patients with Polycystic Ovary Syndrome, the activation of NF-κB and increased transcription of the TNF-α gene is due to higher production of reactive oxygen species. This, in turn, may lead to insulin resistance (32). On the other hand, HBO2 therapy has been found to inhibit the inflammatory response initiated by TNF-α and NF-κB signalling in patients suffering from hearing loss (33). Many similar connections can be found in the existing literature. In addition, in a recently published study, it was emphasised that the decrease in insulin resistance with HBO2 therapy is probably due to decreased endoplasmic reticulum stress and increased mitochondrial capacity and that HBO2 therapy probably leads to low-dose reactive oxygen radical-mediated mitohormesis in humans with type 2 DM (34).

During the first 10 sessions, it was observed that there was a significant decrease in CRP levels in patients with DM. This could be attributed to the fact that patients with DM were diagnosed with an infection-related disease, such as diabetic foot, and subsequently received antibiotic treatment. It is possible that controlling the infection made it easier to regulate blood levels in patients with DM. At this point, a decrease in CRP levels is expected to affect glucose insulin resistance. In contrast, the patients without DM did not always suffer from an underlying disease that would increase CRP. Although we cannot definitively conclude that HBO2 therapy directly caused the decrease in insulin resistance in patients with DM, this study's strength lies in demonstrating the reduction in patients without DM.

In a study in which HbA1c levels decreased significantly in patients without DM with HBO2 therapy, no significant decrease was found in both groups' fasting blood glucose and insulin levels (24). Our study found no significant reduction in HbA1c levels in patients without DM with HBO2 therapy. This may be because our study had fewer HBO2 sessions compared with the study above.

Our analysis showed that patients with and without DM experienced a metabolic response after 10 sessions, which was more pronounced in people with DM.

Our study included women, unlike other studies on this subject (25, 26, 28, 32). The study suggests further research on female-only populations and individuals without DM with high insulin resistance to understand the gender-specific effects of HBO2 therapy on insulin resistance. Future research could focus on evaluating the long-term effects of HBO2 therapy and comparing its benefits with other treatment modalities, such as exercise or pharmacological approaches. Investigating the impact of HBO2 therapy on metabolic pathways related to insulin sensitivity could provide valuable information on its underlying mechanisms. Additionally, exploring the clinical applicability of HBO2 therapy in managing insulin resistance, including its potential as an adjunctive therapy, could be beneficial.

However, our study had limitations, such as a shorter follow-up period and fewer patients than desired; the study was conducted within budget constraints, limiting our ability to perform extensive laboratory analysis. This limitation may have affected the scope and depth of the data collected. The initial study design considered only the minimum required laboratory tests and prioritised the collection of these types of data. As a result, valuable insights that could have been provided by additional laboratory tests such as haemograms, serum creatinine/eGFR, and liver function tests might have been missed. Although our study had a very short follow-up period and a very small sample size to observe significant changes in BMI, the reduction in BMI may have improved insulin resistance in both groups. The limitations of our study include the lack of follow-up on BMI. In the patients with DM, both HOMA-IR and HbA1c levels improved after HBO2. Besides the reduced inflammation/infection during HBO2, it could be related to intensive blood glucose monitoring, proper diabetic diet, additional oral antidiabetic or insulin treatments, antibiotics, or other additional treatments (e.g., quinolones and beta-blockers could cause hypoglycaemia) and reduced appetite related to infective state during the hospitalisation/HBO2 therapy. In addition, decreased haemoglobin levels during hospitalisation/HBO2 therapy due to repetitive blood samplings or surgical interventions could cause false low levels of HbA1c. In patients without DM, improved HOMA-IR may be linked to the treatment of pre-existing conditions, hospitalisation, reduced appetite, and weight loss due to critical conditions.

CONCLUSION

In conclusion, our study found that insulin resistance decreased significantly in patients with and without DM during HBO2 therapy. This suggests that HBO2 therapy may have an effect on managing insulin resistance in various patient groups, but further research is needed to gain a comprehensive understanding of its potential.

Ethics Committee Approval: The study has ethical approval from the İstanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 24.08.2012, No: 346).

Informed Consent: Informed consent was obtained from all patients and their relatives before participation in the study.

Peer Review: Externally peer-reviewed.

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FACTORS INFLUENCING INTRAUTERINE INSEMINATION OUTCOMES IN BRUNEI FEMALES

BRUNEİ KADINLARINDA İNTRAUTERİN İNSEMİNASYON SONUÇLARINI ETKİLEYEN FAKTÖRLER

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ABSTRACT

Objective: To determine the effects of female age, body mass index (BMI), hormonal profile, infertility duration and causes on pregnancy outcomes in females undergoing intrauterine insemination (IUI).

Material and Method: This retrospective study included 199 infertile females who underwent IUI from 2015 to 2019 at Raja-Isteri-Pengiran-Anak-Saleha Hospital, Brunei Darussalam. Age, BMI, hormonal profile, duration of infertility, and causes of infertility associated with pregnancy outcome during treatment were studied. Urine pregnancy test confirmed the pregnancy post-IUI. The statistical analysis tests for categorical variables were the Chi-Square test and Fisher's exact test. One-way ANOVA was used to assess numerical variables. P-values <0.05 were deemed statistically significant.

Result: The mean age of the pregnant females was 32.8±4.9 years, the mean BMI was 24.7 kg/m^2 and 12.5% of the participants had normal weight, 5.5% were overweight, and 9% were obese. Overall, 18 females (9%) achieved successful outcomes. Despite the lack of a significant relationship between BMI and pregnancy outcome, 83% of females with positive outcomes were obese. Furthermore, 72.2% of the females who were infertile for <6 years had conceived. A decreasing trend of hormones was noticed with an increasing BMI (p<0.05). Overall hormonal values did not significantly determine IUI outcome (p>0.05).

Conclusion: Successful outcomes after IUI were observed in females with unexplained or female-caused infertility. Male infertility did not influence the positive pregnancy outcome. Females with an age range of 30-39 years, duration of infertility less than six years, and BMI index of >25 kg/m^2 were able to conceive.

Keywords: Female infertility, clinical characteristics, intrauterine insemination, pregnancy outcome

ÖZET

Amaç: Bu çalışmanın amacı, intrauterin inseminasyon (IUI) uygulanan kadınlarda yaş, vücut kitle indeksi (BMI), hormonal profil, kısırlık süresi ve nedenlerinin gebelik sonuçları üzerine etkisinin araştırılmasıdır.

Gereç ve Yöntem: Bu retrospektif çalışmaya, 2015-2019 yılları arasında Brunei Sultanlığı Raja-Isteri-Pengiran-Anak-Saleha Hastanesi'nde IUI uygulanan 199 infertil kadın dahil edildi. Yaş, BMI, hormonal profil, kısırlık süresi ve nedenleri ile tedavinin hamilelik sonucu ile ilişkisi incelendi. IUI sonrası gebelik, idrar gebelik testi ile doğrulandı. Kategorik değişkenler için istatistiksel analiz testleri Ki-Kare testi ve Fisher testi kullanıldı. Sayısal değişkenler için tek yönlü ANOVA testi uygulandı. P-değerleri <0,05 ise istatistiksel olarak anlamlı kabul edildi.

Bulgular: Yaş ortalaması 32,8±4,9 ve ortalama BMI 24,7 kg/m^2 olan çalışma grubunda gebe kalanlar arasında %12,5'i normal, %5,5'i fazla kilolu ve %9'u obez idi. Tüm grupta 18 kadında (%9) başarılı sonuçlar elde edildi. BMI ile gebelik sonucu arasında anlamlı bir ilişki olmamasına rağmen, pozitif sonucu olan kadınların %83'ü obezdi. Ayrıca, altı yıldan düşük infertilite süresi olan kadınların %72,2'sinin gebe kaldığı görüldü. BMI arttıkça hormonal değerlerde azalma eğiliminin olduğu gözlendi (p<0,05). Hormonal değerlerin tümünün IUI sonucunun belirlenmesinde anlamlı olmadığı sonucuna varıldı (p>0,05).

Sonuç: İnfertilite nedeni açıklanamayan veya kadın kaynaklı olgularda IUI sonrası başarılı sonuçların alındığı gözlendi. Erkek infertilite faktörünün gebelik sonucunu etkilemediği gösterildi. Yaşları 30-39 aralığında, kısırlık süresi altı yıldan az ve BMI indeksi >25 kg/m^2 olan kadınlarda gebelik elde edilebilmiştir.

Anahtar Kelimeler: Kadın infertilitesi, klinik özellikler, intrauterin inseminasyon, gebelik sonucu

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INTRODUCTION

Infertility is the inability of couples of childbearing age to conceive after 12 months of regular, unguarded sexual interaction (1). This condition affects between 8% and 12% of couples within that age (2). The risk has been further increased by the addition of external factors, such as lifestyle and the environment. Male factors of infertility alone account for 20%–30% of cases; however, it can rise to 40% in cases of mixed infertility (3). For this reason, assisted reproductive techniques (ART) are available for treating infertility when appropriate.

Intrauterine insemination (IUI) is a relatively low-cost, effective, and least invasive assisted reproductive procedure that is often offered as a first-line treatment (4). However, it is only recommended for unexplained infertility, male sex, unilateral tubal obstruction, cervical or ovulatory dysfunction, and mild or minimal endometriosis. Several IUI studies have reported a high global pregnancy rate of a 30% chance of successful pregnancy achieved via IUI, but this rate may vary depending on the population studied (5).

Numerous previous studies have shown that maternal age, body mass index (BMI), serum hormonal levels, and the causes of infertility are factors that influence the outcome of IUI treatment (4). The duration of infertility is another factor that affects the success rate, although there is no set limit. Additional treatments, such as controlled ovarian hyperstimulation (COH), can substantially improve the success rate (6). The only noted risk factor for ovulation induction medication is ovulation hyperstimulation syndrome (OHSS), in addition to multiple pregnancies (7). Because fertility treatment involves the emotions and time of patients considering the therapy, it is crucial to weigh the benefits of IUI above every potential drawback, including the likelihood of success and its impact on the patient's mental health (8).

We hypothesised that increased female age and body mass index, prolonged duration of infertility, and a deranged hormonal profile negatively impact the success rate of IUI. Therefore, the objective of our study was to examine the effects of female age, body mass index, hormonal profile, infertility duration, and causes on pregnancy outcomes of females undergoing IUI.

MATERIAL AND METHODS

Study design and study population

This cohort retrospective study was conducted in the Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, Brunei Darussalam; after the approval obtained from Joint Research Ethics committee of the PAPRSB Institute of Health Science (IHSREC), Universiti Brunei Darussalam, and the Ministry of Health (MHREC) (Date: 09.12.2021, No:UBD/PAPRSBIHSREC/2021/83). Data on women undergoing IUI treatment from 2015 to 2019 were retrieved from the clinical database of the Brunei Health Information Management System (BruHIMS). Forty patients per year were randomly selected and de-identified into individual codes, resulting in 199 female patients.

Infertile female patients who were diagnosed with unexplained infertility, endometriosis (stage I – II), mild male factor infertility, or semen allergy and underwent IUI were included in the study.

Patients with (1) missing data, (2) increased female age (over 50 years), (3) diagnosed with bilateral tubal obstruction, (4) severe endometriosis, (5) chronic infections, or (6) receiving anti-tuberculous treatment for the last three months were excluded from the study. Additionally, severe male factor infertility (with a post-wash sperm count of <1 million/mL) was excluded from the study.

Patients were grouped into three groups according to age: 20-29 years old, 30-39 years old, and 40-49 years old. BMI data were categorized into three groups with different BMI ranges according to the South Asian Criteria for BMI: normal (18.5-22.9 kg/m^2), overweight (23.0-24.9 kg/m^2), and obese (≥25.0 kg/m^2) (9).

Based on available records and with details of the duration of infertility, females were categorised into two groups; with a cut-off of six years and also into four groups based on the infertility causes namely: unknown causes, male factor, female factor, and both. Furthermore, hormonal levels were also sorted into low, normal, or high. The reference normal serum hormone ranges were: follicular stimulating hormone (FSH): 3.5-12.5 IU/L, luteinizing hormone (LH): 2.4-12.6 IU/L, and progesterone (p): 5.02-75.9 nmol/L. Oestradiol (E2) was not included because it was not routinely measured, and sufficient data were not available for analysis.

Intrauterine insemination protocol

Ovulation induction:: All females were prepared for IUI by administering ovulation induction medications; either clomifene citrate (CC) (Y.S.P Industries (M) Sdn Bhd, Malaysia) 50–150 mg or Gonal F (GF) (Merck Serono S.p. A, Italy) 75–150 mg, or a combination of both. This regimen was started on the second day of the menstrual cycle for five days for CC and 10 days for GF. Ultrasound evaluation and relevancy blood tests were performed to monitor follicular maturation as recommended by the physician. Human chorionic gonadotrophin (HCG) (N.V. Organon, The Netherlands; Jubilant HolisterStier LLC, USA) (5000 or 10000 IU) was administered 36 hours before IUI to aid the release of mature eggs during ovulation.

Sperm sample processing: To increase the total motile sperm yield, abtinance period of 3 days (72 hours) were

applied to all male partners. The semen sample was then subjected to several processes such as sperm separation, washing, and centrifugation.

Insemination: The insemination procedure was generally performed on the day of ovulation, either naturally or by HCG induction (10).

Confirmation of pregnancy: A urinary pregnancy test was performed by the females to assess the success of the IUI procedure.

Statistical analysis

The data were organised in Microsoft Excel, and Rstudio version 1.3.1093 was used for statistical analysis. Fisher's exact test was used when >20% of the expected cell counts were <5 and categorical variables were analysed using the Chi-Square test. Quantitative data were compared by One-way ANOVA, where $p<0.05$ reflected significance.

RESULTS

The infertile females had a mean age of 32.8±4.9 years, with 62% between the ages of 30 and 39 years. Of the 199 female patients, 68.3% (n=136) had infertility for six years or less. Unexplained infertility was the leading cause of infertility (49.8%), followed by female causes (41.2%) and male factors (8%) (Table 1).

Female age and BMI were not significantly different between IUI-failed and successful patients (Table 2). Nine percent (n=18) of females had successful outcomes after the insemination. The results showed a non-significant relationship between the success rate of IUI and age and BMI ($p=0.93$ and $p=0.78$, respectively). However, out of the 18 successful outcomes, 83% were obese women.

Stratification based on BMI showed that 17 patients who had a successful IUI outcome were below the age of 40 years and only one patient above that age conceived successfully, although both differences were not statistically significant.

Out of the total females, 8% (n=16) were normal, 9% (n=18) were overweight, and 82.5% were obese (n=165), where 12.5% of the normal (2 out of 16), 5.5% of the overweight (1 out of 18), and 9% of the obese (15 out of 165) had successful IUI outcomes.

From 18 successful conceptions after IUI, 72.2% had a duration of infertility of six years, whereas the remaining 27.7% were infertile for more than six years.

A trend of decrease in progesterone, FSH, and LH levels and an increase in BMI ($p<0.05$) was observed (Table 3). The overall hormonal levels did not significantly determine the outcome of IUI ($p>0.05$); hormone levels were low in patients who experienced IUI failure.

Table 1: Demographics of infertile female patients undergoing intrauterine insemination

Variables	n	(%)	Mean (SD)
Age (years)			32.8 (4.9)
20-29	54	27.1	27.0 (1.8)
30-39	124	62.3	33.8 (2.7)
40-49	21	10.6	41.8 (2.2)
Ethnicity			
Malay	186	93.5	
Chinese	6	3.0	
Philippine	2	1.0	
Indian	5	2.5	
BMI (kg/m²)			24.7 (1.9)
Normal (18.5-22.9)	16	8.0	20.5 (1.4)
Overweight (23.0-24.9)	18	9.0	24.2 (0.5)
Obese (≥25.0)	165	83.0	29.2 (3.8)
Duration of infertility (years)			
≤6	136	68.3	3.4 (1.7)
>6	63	31.7	9.5 (2.8)
Causes of infertility			
Unknown	99	49.8	
Male factor	16	8.0	
Female factor	82	41.2	
Both	2	1.0	

SD: standard deviation, n: sample size, BMI: body mass index

DISCUSSION

The current study showed that females who had the maximum conception after IUI were between the ages of 30 and 39 years. Increasing age is a negative predictor of successful IUI (11, 12). Deterioration in ovarian reserve with increased risk of aneuploidy in oocytes is evident with advanced age. Studies have shown that women aged 40 years have a higher IUI success rate (3, 13, 14). Likewise, Ashrafi et al. did not observe any relationship between age 40 years and IUI outcomes (15). Nevertheless, our study exhibits a higher success rate of IUI in patients aged 40 years, supporting the trend in prior studies (3).

Obesity is a crucial factor, particularly with more than 50% Bruneian population being over the normal BMI range and females making up 29.5% (9). High BMI is known to be a potential risk factor for infertility because it induces anovulation, increases endometrial thickness, causes menstrual dysfunction, reduces the quality of oocytes, impairs the fertilisation capacity and alters endometrial receptivity. A common complication of obesity is anovu-

Table 2: Distribution of variables stratified according to IUI outcome

Variables	n	IUI failure		IUI success		P value
		n	Mean (SD)	n	Mean (SD)	
Age (years)						
20-29	54	49	27.0 (1.7)	5	27.2 (2.5)	0.93[a]
30-39	124	112	33.8 (2.7)	12	33.0 (2.9)	
40-49	21	20	41.9 (2.2)	1	40.0 (-)	
BMI (kg/m²)						
Normal (18.5–22.9)	16	14	20.6 (1.5)	2	20.0 (0.8)	0.78 [a]
Overweight (23.0–24.9)	18	17	24.3 (0.5)	1	23.4 (-)	
Obese (≥25.0)	165	150	29.2 (4.7)	15	29.4 (5.8)	
Duration of infertility (years)						
≤ 6	136	123	3.7 (1.5)	13	3.6 (1.9)	0.73 [b]
> 6	63	58	9.3 (2.7)	5	7.3 (0.6)	
Causes of infertility						
Unknown	99		90		9	0.57 [a]
Male factor	16		16		0	
Female factor	82		73		9	
Both	2		2		0	

[a]: Fisher's exact test, [b]: One-way ANOVA test, BMI: body mass index, IUI: intrauterine insemination

lation, which can be overcomed by administering ovulation-induction drugs to females receiving treatment (3, 16). On the other hand, increased endometrial lining observed in high-BMI patients is thought to improve the pregnancy success rate, cancelling the negative impact of obesity on uterine receptivity (10). Our study results revealed a higher proportion of obese (15 patients out of 18) females with a positive pregnancy outcome.

In our study, females with greater BMI had a higher success rate following IUI, which although negates our hypothesis, the improved success can be explained by the correction of ovulation and improvement in the hormonal profile in obese females by medicines used for ovulation induction. Researchers have documented positive IUI outcomes in obese females treated with gonadotropins, human menopausal gonadotropin and treatment with letrozole (17-20).

As reported by Wang et al., BMI is an essential factor influencing the regulation of the hypothalamic-pituitary-ovarian (HPO) axis (20). Increased free fatty acids in the serum inhibit the synthesis of gonadotrophin (Gn), resulting in the fall in serum levels of reproductive hormones like E2, LH, and P, disrupting the menstrual cycle regulation and thereby leading to anovulation. Several studies reported different results regarding BMI, and the findings were inconsistent (21). In theory, BMI may be a strong predictor of IUI outcome, as weight control is an important factor before the start of the procedure. Yavuz et al. also reported the negative influence of obesity on IUI outcomes, explaining the reduced oocyte quality, poor uterine environment, and impaired insulin sensitivity secondary to increased BMI (22). High BMI alters hormonal levels, which in turn affect the quality of the uterine endometrium (3). However, BMI was reported to be a less reliable predictor of pregnancy outcomes in obese patients. Results from our study showed that BMI influenced the outcome of the IUI procedure because 83% of the successful outcomes were females with obesity. This finding is inconsistent with the results of Whynott et al., who reported that the IUI success rate was the same compared with patients with high BMI (22).

Other factors to be discussed in this study, such as basal hormone levels, duration and infertility diagnosis, follicular count, and endometrial thickness, showed inconclusive results. Most of the females who approached the infertility clinic had a duration of infertility of 6 years. Noujua-Huttunen et al. proved in their study that the chances of successful IUI outcomes diminish with long-standing infertility (13). Tomlinson et al. observed that the success rate of IUI was higher in couples with infertility of less than four years (23). Yavuz et al. recently reported that the success rate is higher with shorter subfertility periods, indicating that the duration of infertility becomes essential when selecting IUI as a suitable treatment mode (21).

Table 3: Relationship between BMI and hormonal values in female patients with the outcome of IUI (n=199)

Hormone	BMI (kg/m²)						P-value[a]	Outcome				P-value[a]
	Normal (18.5-22.9)		Overweight (23.0-24.9)		Obese (≥25.0)			IUI failure		IUI success		
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)		n	%	n	%	
Progesterone												
Low	2	21.7 (1.4)	9	24.1 (0.6)	96	29.3 (5.2)		97	90.7	10	9.3	
Normal	14	20.3 (1.4)	7	24.6 (0.2)	62	29.1 (4.1)	<0.05	76	8	7	8.4	0.92
High	0		2	23.6 (0.1)	7	29.2 (5.6)		8	88.9	1	11.1	
Follicle stimulating hormone												
Low	0		8	24.2 (0.6)	96	29.0 (4.9)		97	93.3	7	6.7	
Normal	16	20.5 (1.4)	10	24.3 (0.5)	64	29.4 (4.7)	<0.05	79	87.8	11	12.2	0.34
High	0		0		5	30.6 (4.3)		5	100.0	0	0.0	
Luteinizing hormone												
Low	0		7	24.1 (0.6)	85	28.4 (4.6)		83	90.2	9	9.8	
Normal	15	20.5 (1.5)	9	24.3 (0.5)	61	29.7 (5.0)	<0.05	78	91.8	7	8.2	0.94
High	1	20.7	2	24.7 (0.3)	19	31.2 (4.5)		20	90.9	2	9.1	

[a]: Fisher's exact test, IUI: intrauterine insemination. The mean values are for hormone levels.

Regarding the cause of infertility, almost 50% of females with unexplained infertility underwent IUI and accounted for half of the females (n=9) who achieved successful pregnancy. Successful conception can be attributed to stimulation of the ovaries by medicines and correction of some other factors like inadequate coital frequency (24). Similar results were proposed by Noujua-Huttunen et al. (13). This contrasts with our findings, which showed no significant difference in the percentage of success rate between unexplained infertile females and female factor infertile females. The results prompt that IUI should be deliberated as the first line of approach before opting for IVF in women with unexplained infertility.

The study was limited due to the inaccessibility of data before 2012, retrospective nature, and small sample. The data do not represent all females undergoing IUI in Brunei Darussalam, as IUI treatment is also performed in other hospitals in Brunei. Therefore, the generalizability of the results was not possible. The patient records did not mention the endometrial thickness, which is another limitation of our study. Furthermore, we analysed the results based on age and BMI independently; the data was not stratifed and analyzed according to age.

CONCLUSION

IUI treatment played a significant role in treating infertile couples with ovulatory disorders or unexplained infertility. The success of IUI was largely affected by the female age (30–39 years), and the duration of infertility six years. Although conception occurred mostly in women with BMI > 25 kg/m², weight management strategies, effective counselling and personalised treatment may be advised to optimise fertility health in all infertile couples. Females with an age range of duration of infertility and a BMI index of >25 kg/m² were able to conceive.

Ethics Committee Approval: Approval was obtained from Joint Research Ethics committee of PAPRSB Institute of Health Science (IHSREC), Universiti Brunei Darussalam and Ministry of Health (MHREC) (Date: 09.12.2021, No:UBD/PAPRSBIHSREC/2021/83).

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REVIEW / DERLEME
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A BRIEF HISTORY OF THE İSTANBUL UNIVERSITY İSTANBUL FACULTY OF MEDICINE

İSTANBUL ÜNİVERSİTESİ İSTANBUL TIP FAKÜLTESİ'NİN KISA TARİHÇESİ

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ABSTRACT

İstanbul University İstanbul Faculty of Medicine, was the only medical faculty in Türkiye until the Ankara University, Faculty of Medicine was established in 1945 under Law No. 4761. Therefore, until the establishment of Ankara University Faculty of Medicine, the history of medical education in our country is a history belonging to İstanbul Faculty of Medicine. After the conquest of İstanbul, medical education was established at the Fatih Darüşşifa in İstanbul and continued to develop, being provided in institutions that opened and closed over time. This institution has undergone reforms since the establishment of Tıphane-i Âmire in 1827, which finally led to the institutionalization of the İstanbul Faculty of Medicine. Physicians from Austria and Germany made significant contributions to the modernisation of education at this medical school during the Ottoman and Republican periods. The İstanbul University İstanbul Faculty of Medicine, has supported the development of new medical faculties established in Türkiye by providing the new institutions with faculty members. Based on 2023 data, among the 128 medical faculties established in Türkiye, either by the state or private enterprise, the İstanbul University İstanbul Faculty of Medicine, has a prestigious place in Türkiye, as it was the first medical school and has always been an innovative and guiding medical institution. The school has graduates who have made significant contributions to medicine. Examples include Hulusi Behçet (1889-1948), the discoverer of Behçet's Disease, who graduated from the İstanbul School of Medicine in 1910, and Aziz Sancar (1946-), who graduated from the Istanbul School of Medicine in 1969 and shared the Nobel Prize in chemistry in 2015.

Keywords: History of İstanbul University Istanbul Faculty of Medicine, history of medical education, history

ÖZET

İstanbul Üniversitesi İstanbul Tıp Fakültesi, 1945 yılında Ankara Tıp Fakültesi kurulana dek ülkemizin tek tıp fakültesiydi. Bu nedenle Ankara Üniversitesi, Tıp Fakültesi kuruluncaya kadar ülkemizdeki tıp eğitiminin tarihi, İstanbul Tıp Fakültesi'ne ait bir tarihçedir. İstanbul'un fethi ardından İstanbul'da Fatih Darüşşifası'nda başlatılan tıp eğitimi, birbiri ardına açılıp kapanan kurumlarda verilmiş olsa da sürekli bir gelişim çizgisi izlemiş, 1827'de kurulan Tıphane-i Amire'den itibaren ise aynı kurumun zaman zaman reformlar geçirmesi ile İstanbul Tıp Fakültesinin kurulmasına yön vermiştir. Osmanlı ve Cumhuriyet dönemlerinde Avusturyalı ve Alman hekimlerin bu tıp okulundaki eğitimin modernleşmesine önemli katkıları olmuştur. İstanbul Üniversitesi Tıp Fakültesi, ülkede kurulan yeni tıp fakültelerine öğretim üyesi vererek, onların gelişimlerini de desteklemiştir. 2023 itibariyle ülkemizde devlet bünyesinde ya da özel teşebbüsle kurulmuş 128 tıp fakültesi içinde İstanbul Üniversitesi İstanbul Tıp Fakültesi, ülkenin ilk olmakla en eski geçmişe sahip, fakat daima yenilikçi ve rehber tıp kurumu olma özelliğiyle, çok prestijli bir yere sahiptir. İstanbul Üniversitesi Tıp Fakültesi'nin tıbba önemli katkılar yapan mezunları vardır. Bunlara, İstanbul Tıp Fakültesi'nin 1910 yılı mezunu Behçet Hastalığını tanımlayan Hulusi Behçet (1889-1948) ile 1969 yılı mezunu ve kimya dalında 2015 Nobel ödülünü bir meslektaşıyla paylaşan Aziz Sancar (1946-) örnek verilebilir.

Anahtar Kelimeler: İstanbul Üniversitesi İstanbul Tıp Fakültesi tarihi, tıp eğitimi tarihi, tarih

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INTRODUCTION

The *madrasas*, established following İstanbul's conquest on May 29, 1453, are considered the foundation of İstanbul University (1). The roots of the İstanbul Faculty of Medicine can be traced back to the *darüşşifa* (house of healing, the first hospital model in Turkish-Islamic culture), built by Sultan Mehmed the Conqueror and opened in 1470 within the complex bearing his name (2). In a meeting held on December 30, 1970, the Board of Professors of the İstanbul Faculty of Medicine decided to celebrate 1970 as the 500th anniversary of the establishment of the faculty, taking the date of the establishment of the *Fatih Darüşşifa* in 1470 as its basis (3). This decision expresses ownership of medical education initiated by Türkiye's ancestors after İstanbul's conquest. This study summarizes the history of the Medical Faculty of İstanbul University, the first medical school in Türkiye, and shows that teaching medicine in Türkiye, which began in madrasa and *darüşşifa* institutions, has made a continuous effort to progress by establishing successive institutions and reforming existing institutions.

Fatih Darüşşifa: Seed of the İstanbul University Faculty of Medicine

The foundations of madrasas, one of Türkiye's only institutions of higher education until the late 18th century, date back to the 9th century. Mehmed the Conqueror (1432–1481), who conquered Constantinople in 1453, had the largest madrasa in the period, called *Sekizli Medreseler* (*Sahn-ı Semân*). It consisted of eight madrasas built on both sides of the Fatih Mosque and became operational in 1470, 17 years after İstanbul's conquest. The *Darüşşifa* was built next to the four madrasas to the south (4). The most substantial evidence indicating that medical education was provided at the *Fatih Darüşşifa* was revealed by Altıntaş's research through nine archival documents, which proved the appointment of medical students between 1723 and 1783 (5).

Süleymaniye Medical Madrasa (established in 1557)

The *Fatih Darüşşifa* was destroyed by earthquakes in 1509, 1747, and 1766 (4). Therefore, Suleiman the Magnificent (1494–1566), who led the Ottoman Empire into a great era, had Mimar Sinan (Architect Sinan, 1490-1588) build the Süleymaniye Complex, which became one of the most beautiful examples of Ottoman architecture (6). The doors of the hospital were opened early in the morning, and the staff took care of in-patients and those who came for examination (7). A list of 66 books used for education at the *Süleymaniye Medical Madrasa* can be found in the Topkapı Palace Archive. Students would read books written by famous scholars such as Ibnü'l Baytâr (1197-1248), Ibn Sînâ (980-1037), Zehrâvî (936-1013), and Hacı Pasha (1339-1413) in accordance with the schedules and programmes of their teachers, take classes, and, in the afternoon, go to the sections where patients were hospitalised for practise (7). In addition to studying skeletons and bones, students were given anatomy lessons using pictures (8). There was a separate section for patients with mental disorders in the *Süleymaniye Darüşşifa*, and over time, the number of these patients cared for in the institution increased, and the hospital gradually became a *bimarhane* (psychiatric hospital). In 1873, when mentally ill patients were moved to the *Toptaşı Bimarhane*, activities at the *Süleymaniye Darüşşifa* ended (9).

An important step towards the modernisation of medical education: The Shipyard Medical School (established in 1805)

In the reign of Selim III (1789-1807), a hospital was built in Kasımpaşa Shipyard to meet the navy's need for physicians and provide patient care. In 1806, the hospital began training physicians and surgeons. This school, which brought medical instruments and books from Europe, ceased operations after political turmoil, and its building was destroyed in the Kasımpaşa Fire of 1822 (10). It is accepted that this institution, which operated for a short period, was a turning point in the Westernisation of Turkish medicine and formed the basis of the *Tıphane-i Âmire*, the first modern medical school.

The first modern medical school - *Tıphane-i Âmire and Cerrahhane-i Mâmure*

Sultan Mahmud II (1785–1839), who made great efforts to achieve progress in the Ottoman Empire in parallel with developments in the West, ordered the establishment of a military school, *Tıphane-i Âmire*, at the Tulumbacıbaşı Mansion in Vezneciler to train qualified physicians and surgeons for his newly established army (*Asâkir-i Mansûre-i Muhammediyye*) (11). This was the first medical school opened in Türkiye in the modern sense (12). The school became operational on March 14, 1827, so this date is considered the beginning of modern medical education in Türkiye and is celebrated as Medical Day (13).

Only Muslim students were accepted to this school, and medical education was scheduled for four years. Classes were taught in Turkish, Arabic, Italian, and French, and textbooks were brought from Paris. The school was mostly taught by non-Muslim teachers who had studied medicine in France and Italy (14). The dissection of cadavers was considered a sin; therefore, anatomy lessons were given using the models. A class was opened within the school to provide one year of education to students who wanted to become surgeons. On January 9, 1832, when the school *Cerrahhâne-i Mâmure* was opened in the Patients' Room of Topkapı Palace (15), the surgery class in the *Tıphane-i Âmire* was transferred to this school, and the French surgeon A.H. Sat-Deygallière (1799-?) was appointed as its head (16). In 1838, the *Cerrahhâne-i Mâmure* was moved located *Tıphane-i Âmire* on the site of today's Galatasaray High School (17).

The first modern medical school underwent reform: *Mekteb-i Tıbbiye-i Adliye-i Şâhâne* (1839)

1839 was a turning point in the history of medical education in Türkiye. As a result of Sultan Mahmud II's (1785-1839) initiative, two young military physicians, Dr. Jakob Neuner (1891-1949) and Dr. Karl Ambroise Bernard (1808-1844), graduates of the Josephinum, Vienna's Academy of Military Medicine and Surgery, and pharmacist Antoine Hoffmann were invited to Türkiye in 1839. After Bernard was appointed as the director of clinics and professor, the school was opened with a ceremony on February 17, 1839, under the name *Mekteb-i Tıbbiye-i Adliye-i Şâhâne der Âsitane-i Aliyye* (Figure 1), and it began to teach with 290 students (18). The word '*Adliye*' in the name of the school refers to the pseudonym '*Adli*' used by Sultan Mahmud II in his poems. The word '*Asitane*' was a name used for İstanbul during the Ottoman period. The name of the school was mentioned as *Mekteb-i Tıbbiye-i Adliye-i Şâhâne/Ecole Imperiale de Médecine* in the diplomas awarded to graduates.

Figure 1: Chief physician and teachers at Mekteb-i Tıbbiye-i Şâhâne. Galatasaray, 1839.
(Archives of the Department of History of Medicine and Ethics, Istanbul Faculty of Medicine)

Dr. Bernard prepared the school's curriculum based on that of the Vienna Josephinum. The French were taught intensively in the beginner class because of the increased influence of the French in Europe after the French Revolution (19). Clinical courses were taught in the hospital's patient wards. The school also had a pharmacy class with a three-year training programme and a surgery class with a three-year training programme. In 1843, a midwifery class was introduced, and in 1846, another class was opened to train caregivers. When Dr. Bernard was chief, the school had a botanical garden, a library of 1,300 books in French, and a mineral collection. *Tıphane-i Âmire* was established to train Muslim physicians for the army; however, minorities were also admitted to this school as of 1841 (19). Bernard was awarded the "Order of Honour" by Sultan Abdülmecid (1823-1861) and wrote the books of the first Ottoman Pharmacopoea, *Pharmacopoea Castrensis Ottomana*, *Botany*, *Auscultation and Percussion*, and *Bursa Thermal Springs*. His book on the hot springs in Bursa is considered the first work on Balneology in Türkiye. Bernard also worked as a physician at the Austrian Hospital in İstanbul and used the corpses there for normal and pathological anatomy lessons. Thus, dissection and autopsy were started for the first time in Türkiye. Bernard died in İstanbul in 1844 at the age of 36 due to pneumonia caused by a phlegmon in his neck. He was buried in the Italian Catholic Church in İstanbul (20).

Dr. Sigmund Spitzer (1813–1895), a physician and human anatomist from Austria, undertook the school's education management. Spitzer remained in this position until 1850. The School of Medicine has dynamically followed scientific developments emerging in the West. For example, chloroform, the effect of which was discovered in 1847 for general anaesthesia, was successfully tested on a student at *Mekteb-i Tıbbiye-i Adliye-i Şâhâne* in the presence of Sultan Abdülmecid 1 year later (21). Sultan Abdülmecid was pleased with the school's follow-up of scientific developments. He requested that final-year students take an examination at a medical school in Europe, and accordingly, four students were sent to Vienna. On January 1, 1848, these students took an exam that was conducted in front of the audience at the Vienna School of Medicine, and their superior performance in the exam was reported in the European press, increasing the reputation of the school in Europe (22). On October 11, 1848, the school was moved to the Humbarahâne Barracks in Halıcıoğlu after a fire broke out in the school's neighbourhood. On March 9, 1849, Türkiye's first medical journal, *Vekâyi-i Tıbbiye* (medical cases), and its French version, *Gazette Médicale de Constantinople* (23), were published in the school's printing house with the contributions of the students.

In 1865, during the Cholera epidemic, the school building was repurposed as a hospital, and in 1866, the school moved to Taşkışla in Demirkapı, where it would function for 27 years. During this period, the number of books written increased, and high achievers among graduates were sent to Europe for specialisation, creating future teachers.

In the 19th century, while the Ottoman Empire was fighting one war after another, developments in the West were also notable. Therefore, when Louis Pasteur discovered the rabies vaccine, Sultan Abdülhamid (1842–1918) donated 1,000 gold coins to the Pasteur Institute and sent Pasteur a medal. Zoeros Pasha (1844–1917), a professor of infectious diseases at the medical school, was in the delegation that brought these gifts. When the physicians in the delegation returned to the country after learning about the production and application of the rabies vaccine, the Rabies Vaccine Institution (*Daûlkelp Tedavihanesi*) was

opened in 1887 within the Medical School, which was operating in Demirkapı (24). In 1889, the Vaccine Institution (*Telkihhane-i Şâhâne*) produced a smallpox vaccine and distributed it throughout the country (25). A few years later, the Royal Bacteriology Laboratory (*Bakteriyolojihane-i Şâhâne*) in 1893 and the first Gynaecology Clinic (*Viladethane*) in 1894 were also opened within the school.

Struggle to switch to education in Turkish

Since education at the *Mekteb-i Tıbbiye-i Şâhâne* was provided in French, Turkish students were often unsuccessful and had to transfer to the surgery and pharmacy departments where classes were somewhat easier. In 1856, the students launched a protest, supported by some of the professors, with the aim of teaching education in Turkish, and a class called *Mümtaz Sınıf* (Estimable Class) was opened in 1856 (26). The class was closed down in 1859; however, its students did not give up their aim. Under the leadership of Kırımlı (Crimean) Aziz Bey (1840–1878), one of the graduates of the *Mümtaz Sınıf*, they worked on developing the Turkish language for medical education and founded the *Cemiyet-i Tıbbiye-i Osmaniye* society in 1866 (27). However, most professors at the *Mekteb-i Tıbbiye-i Şâhâne* were non-Muslims who had a good command of foreign languages and were adamantly opposed to Turkish education.

On January 2, 1867, the *Mekteb-i Tıbbiye-i Mülkiye* (Civil School of Medicine) was opened in one of the rooms of the military school (28). Kırımlı Aziz Bey went on to become the Dean of *Mekteb-i Tıbbiye-i Mülkiye*. With the introduction of medical education in Turkish, professors were rapidly trained, and the number of Turkish medical textbooks quickly increased (29). In 1869, graduates of the *Tıbbiye* (Medical School) were selected by examination and sent to Europe for specialisation. Those who returned after completing their specialisation were appointed professors. At the beginning of the 20th century, these physicians became the figures that shaped the health policies of the Republic of Türkiye, founded in 1923. A few notable names are Şakir Pasha (1855–1914), who studied physiology under Claude Bernard (1813–1878) in Paris and introduced the principles of experimental physiology in Türkiye. Besim Ömer Pasha (Akalın) (1862–1940), who specialised in obstetrics and gynaecology in Paris, pioneered the establishment of the Obstetrics Clinic within the school, which had not previously been allowed, and transferred modern knowledge on obstetrics. Esad Pasha (Işık) (1865–1936), who specialised in ophthalmology at the Faculty of Medicine in Paris, established a modern eye clinic with the instruments he brought from Paris, and the ophthalmoscope he developed was named after him (30). Celal Muhtar (Özden) (1865–1947), after completing his dermatology specialisation at St. Louis Hospital in Paris taught dermatology at the school, diagnosed a new skin disease, and developed a treatment method (31). Cemil (Topuzlu) Pasha (1866-1958), a highly skilled surgeon and talented physician, returned to Türkiye after his specialisation at the Paris School of Medicine and meticulously implemented asepsis and antisepsis methods at the Medical School (32).

***Gülhane Seririyat School* opened for clinical internships and construction of a new medical school building**

As the military medical school's building was outdated and inadequate, Sultan Abdülhamid II (1842–1918) ordered the construction of a new medical school building in the Haydarpaşa neighbourhood on the Asian side of İstanbul in 1893 (33). Prof. Dr. Robert Rieder (1861-1913) (Figure 3), who had been invited from Germany to make suggestions about the construction at Haydarpaşa, arrived in Türkiye on February 14, 1898, and presented a report to the Sultan on September 5, 1898. He suggested some reforms in education and suggested that German should be taught alongside French. Rieder and Dr. Georg Deycke (1865-1935), who had been invited with him, opened Gülhane Seririyat Hospital (Figure 2) with 150 patient beds on December 30, 1898, in the building where the preparatory class of the Medical School was located. This was done to ensure that physicians graduated with better clinical experience (34). Rieder was chief of the surgical clinic at Gülhane (Figure 2) and was appointed as the director of the school (35).

Figure 2: A postcard showing *Gülhane Seririyat Mektebi* (İstanbul Faculty of Medicine, Department of History of Medicine and Ethics Archive).

Figure 3: Professors and German nurses of Gülhane. Prof. Georg Deycke Pasha in the centre, Prof. Robert Rieder Pasha, on his right (İstanbul Faculty of Medicine, Department of History of Medicine and Ethics Archive).

The newly constructed, magnificent Medical School building in the Haydarpaşa district on the Asian side of İstanbul (Figure 4) was inaugurated on November 6, 1903, the birthday of Abdülhamid II (Figure 5) (33).

Figure 4: The new building of the School of Medicine in Haydarpaşa. Album of the Humble Memory of the Faculty of Medicine to the Honourable Kazım Pasha, President of the Grand National Assembly, University of Health Sciences Publication. Istanbul 2019.

Rieder Pasha injured his spine in a fall in 1902 while supervising the construction of the school in Haydarpaşa, and he returned to Germany in 1904. Deycke Pasha continued to direct Gülhane from 1904 to 1907. He was particularly interested in leprosy, tuberculosis, and dysentery and published important studies on these topics. When Deycke Pasha returned to Germany in 1907, he was replaced by Julius Wieting (1868-1922), a surgeon who took charge of the hospital until 1914. Wieting's emphasis on training military and civilian nursing staff, encouraging the production of medicines, vaccines, and serum, and organising the first scientific meeting at Gülhane were among his most important contributions (36).

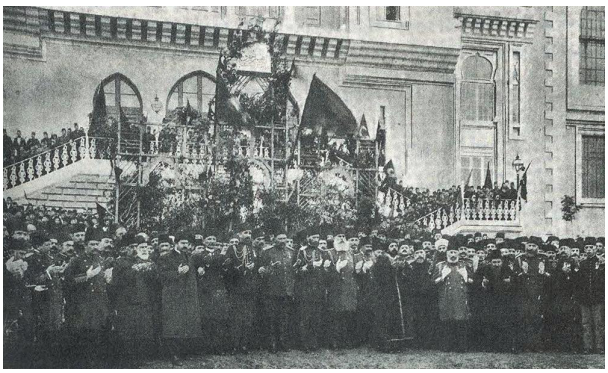

Figure 5: Inauguration ceremony for the new Haydarpaşa medical building. Besim Ömer Pasha: Nevsal-i Afiyet. Vol. 3. (Translated by İzgöer AZ) Istanbul 1904, p. 1063.

Gülhane continued to operate as a military hospital following Wieting Pasha's return to Germany. Since the Ottoman Empire was a defeated power during World War I, this building was occupied in 1918 along with the country's occupation and was turned into a military hospital for the occupiers. The Gülhane Military Hospital was later moved to Gümüşsuyu Military Hospital and later moved back to Sarayburnu following the proclamation of the Republic. After the outbreak of World War II, Gülhane moved to Ankara on July 21, 1941, and began to continue activities as the Gülhane Military Medical Practise School and would soon experience rapid development (35).

Incorporation into Darülfunun

In the second half of the 19th century, efforts were made by the Ottoman Empire to establish a European understanding of science and to create an institution of higher education that was not affiliated with religious or military institutions. The first *Darülfünun* (house of sciences), opened in 1863 with this intention, was closed after three years. The institution, which was reopened in 1870 under the name *Darülfünun-ı Osmani* was only operational until 1873. The third attempt, *Darülfünun-ı Sultani*, opened in 1874; however, it did not continue after 1881. The *Darülfünun* that would continue its activities without closing down was the *Darülfünun-ı Şâhâne*, which opened on September 1, 1900. After the declaration of Constitutional Monarchy II (1908), the title '*Şâhâne*' was removed from all institutions. Accordingly, this institution was renamed *İstanbul Darülfünun* by a regulation dated April 20, 1912. In 1900, when the *Darülfünun-u Şâhâne* was established, the Military and Civilian Medical School did not join the *Darülfünun*. On 22 May 1909, after the *Mekteb-i Tıbbiye-i Mülkiye* joined Darülfünun, the Military Medical School was also included (37).

On September 14, 1909, the Civil and Military Medical Schools merged and became the *Darülfünun-i Osmanî Faculty of Medicine* (37). In 1908, the School of Dentistry, Pharmacy, *Kabile* (Midwife), and Nursing School was established in the vacant building of the Civil Medical School in Kadırga. According to the Faculty of Medicine's Statute, this school is affiliated with the Faculty of Medicine (38).

The Medical School declares March 14 as Medical Day

The years of the First World War inevitably affected education at the Faculty of Medicine. At the end of 1914, the faculty building was converted into a reserve military hospital. Following the occupation of İstanbul by the Allied fleet on November 13, 1919, the *Darülfünun* Faculty of Medicine building was occupied in December, and the five-year British hegemony at the faculty began. The pressure on the faculty was lifted only through the victory of August 30, 1922 (39).

Darülfünun students watched with interest as Mustafa Kemal (Atatürk), the founder of the Republic of Türkiye, landed in Samsun and started a national movement throughout Anatolia. Hikmet Bey (Boran) (1901–1945), a medical faculty student, attended the Sivas Congress with the money collected by his friends and expressed his support for Mustafa Kemal (40). The students wanted to celebrate March 14, the founding day of *Tıphane-i Âmire*, the first modern medical school in our country, in 1919, as Medical Day to demonstrate their commitment to the history of their nation. For this purpose, medical students, professors, and physicians in İstanbul gathered in a movie theatre in Kadıköy on March 14, 1921, to deliver messages of independence (41). March's celebration 14 as Medical Day dates back to this event.

Publication of first issue of Journal of İstanbul Faculty of Medicine Journal

During the war years, *Darülfünun Medical Faculty Journal* was first published in March 1916 and was published in the Arabic letter until 1932 (42). During the 1933 university reforms, the journal stopped publishing for a time. In February 1937, publication began again as Year 1, Issue 1 (Figure 6), and in the Latin alphabet, which had been adopted during the alphabet revolution. The journal continues to be published, bearing the name *İstanbul Tıp Fakültesi Dergisi* (Journal of İstanbul Faculty of Medicine).


سنه : ۱ مارت ۱۳۳۲ صایی : ۱

دار الفنون

طب فاکولته سی مجموعه سیدر

طبابت ، اجزاجیلق ، دیشجیلک ، ومتعلقاتی

ایکی آیده بر نشر اولنور

10246

مندرجات :

دیزنتالین نصل تأثیر ایدر ..... [عاقل مختار]
لابورا توار تحریاتندن ..... [کمال جناب]
رفیع التکرر جریانلر ..... [شوقی]
دوائی اطراف سفلیه‌نك زیناستیقی ایله تداویسی ..... [جلال اسماعیل]
اعصاب محیطیه جروح حربیه‌سی ..... [کریم ثابت]
خیاطة عجان ومعه‌لرك التهابی ..... [آصف درویش]
هزال اعصاب ..... [راشد تحسین]
جروح ثاریه ..... [سعدالدین وداد]
مالك حاره احراضی حقنده بر مطالعه ..... [اسماعیل حقی]
شؤن ..... [تحریر هیئت اداره‌سی]

استانبول — مطبعهٔ عامره
۱۳۳۲

Figure 6: Cover of the 1st issue of the *Darülfünun Faculty of Medicine Journal*, 1916.

Admission of female students to medicine

On September 12, 1914, the *Inas* (girls) *Darülfünun* opened, and its first class graduated in 1917. In 1921, with the admission of female students to the *Darülfünun*, the *Inas Darülfünun* was closed. In 1922, as a critical move for the rights of women, female students were given the right to enrol in the Faculty of Medicine. In 1928, six of the 10 female students enrolled at the Faculty of Medicine graduated. Three students did not continue medical education, and one female student died of tuberculosis. The first female graduates became specialists in various fields. Müfide Kâzım Küley (1904–1995) studied her specialty at the Internal Medicine Clinic II at İstanbul University Faculty of Medicine between 1929 and 1933 and served as a professor in the Department of Internal Medicine at the university until 1973 when she retired. İstanbul University awarded her Doctor Honoris Causa in 1993, two years before her death (43).

Figure 7: Professors and students of *Darülfünun Faculty of Medicine* on May 20, 1924 (İstanbul Faculty of Medicine, Department of History of Medicine and Ethics Archive).

İstanbul Faculty of Medicine during the Turkish University Reform of 1933

The Medical School remained in this building on the Asian side of İstanbul for 30 years (Figure 7, 8). Some clinical professors were displeased with the distance of this magnificent new building from the centre of the city on the European side. On May 31, 1933, under Law No. 2252 on the Closure of the *İstanbul Darülfünun* and the Establishment of a New University by the Ministry of Education, the university reform process began; on July 31 of the same year, the *İstanbul Darülfünun* was closed and re-established as İstanbul University (Figure 9). The first Rector of the University was Neşet Ömer İrdelp (1881-1948), a professor-in-ordinary of internal medicine at the Faculty of Medicine. The first Dean of the İstanbul University Faculty of Medicine was Ord. Prof. Dr. Tevfik Salim Sağlam (1882-1963) (44).

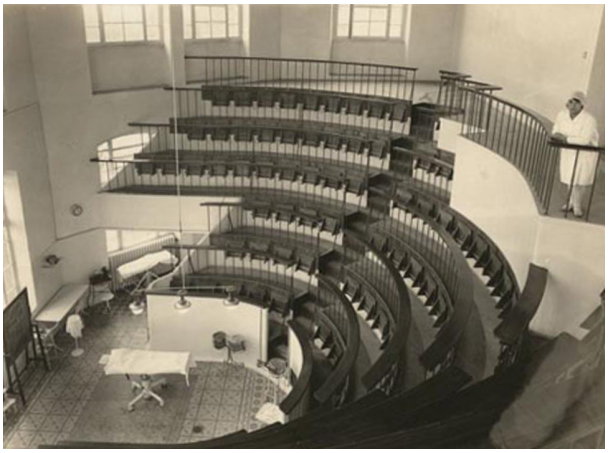

Figure 8: An example of the new medical building lecture halls in Haydarpasha. The Humble Memory of the Faculty of Medicine to His Excellency the Honourable Kazım Pasha, President of the Grand National Assembly Album Publication of the University of Health Sciences, İstanbul, 2019.

Atatürk attached great importance to İstanbul University's goal of achieving modernisation in science. As the university was reorganised, the Faculty of Medicine was moved from its building in Haydarpaşa to the European side of İstanbul. The administrative centre of the faculty (Dean's Office) and the basic science institutes were moved to the current Rectorate building in Beyazıt. According to the law establishing İstanbul University, the Faculty of Medicine was granted the right to use city hospitals for educational purposes. Clinics were distributed to hospitals in various districts of İstanbul, such as Haseki, Cerrahpaşa, Gureba, Bakırköy, and Şişli. The main clinic workload was divided among Haseki, Cerrahpaşa, and Aşağı Guraba Hospitals. During the Second World War, the warehouse buildings in the Çapa neighbourhood were repurposed as hospital clinics, and the Surgery Clinic II, which had been located in Haseki Hospital until then, was moved to these buildings, the Gynaecology Clinic II and later Internal Medicine Clinic III soon followed (45).

With the 1933 University Reform, a new teaching staff was established at İstanbul University, and some old faculty members were dismissed. Young scientists educated in Europe and world-renowned professors who had to flee Europe when Hitler came to power were assigned to vacant positions. These professors, who fled Germany, Austria, Czechoslovakia, and Hungary and took refuge in Türkiye, served as directors of institutes and clinics at the İstanbul Faculty of Medicine for many years and were part of the teaching staff (46, 47). Among the foreign teaching staff were professors such as Philipp Schwartz (Pathological Anatomy), Siegfried Oberndorfer (General and Experimental Pathology), Rudolf Nissen (Surgery), Wilhelm Liepmann (Obstetrics and Gynaecology), Erich

Figure 9: The view of İstanbul University Rectorate Building in the 1930s (İstanbul Faculty of Medicine, Department of History of Medicine and Ethics Archive).

Ruttin (ENT), Karl Hellmann (Otolaryngology, Joseph Igersheimer (Ophthalmology), Friedrich Dessauer (Biophysics, Radiology, and Radiotherapy), Max Sgalitzer (Radiology and Radiotherapy), Wilhelm Liepschitz (Biochemistry), Felix Haurowitz (Biochemistry), Zdenko Stary (Biochemistry), Tibor Peterfi (Histology and Embryology), Erich Frank (Internal Medicine), Hans Winterstein (Physiology), Julius Hirsch (Sanitation), Hugo Braun (Microbiology), and Berta Ottenstein (Dermatology). In addition, physicians, nurses, engineers, and other technical staff who had taken refuge in Türkiye were also included in the service of the İstanbul Medical Faculty. In a short time, these famous scientists from the West published textbooks in Turkish filled with modern knowledge. These professors established the German scientific tradition based on the principle that education and research are inseparable in universities and that those who cannot conduct research should not teach. They sincerely served in raising a successful generation of Turkish scientists and helped Türkiye gain well educated Turkish physicians.

The School of Pharmacy and the School of Dentistry, which had been operating as colleges within the İstanbul University Faculty of Medicine, were separated in 1962 and 1964, respectively, and became separate faculties. Over time, the number of students and faculty members at the Faculty of Medicine has increased considerably. Accordingly, with the proposal of the İstanbul University Faculty of Medicine dated January 7, 1967, the University Senate decided to establish a new faculty at a meeting on July 27, 1967. There are now two faculties of medicine at İstanbul University. The name of the faculty became "İstanbul University İstanbul Faculty of Medicine". The Faculty that would operate within the Cerrahpaşa Campus was named "Cerrahpaşa Faculty of Medicine". When the Cerrahpaşa Faculty of Medicine was established in 1967, most of the academic staff were from the İstanbul Faculty of Medicine.

Figure 10: New campus of İstanbul's Faculty of Medicine (still in construction process).
Available from: URL: https://www.hemsireaktuel.com/istanbul-tip-fakultesi-yeni-kampusu/

İstanbul University İstanbul Faculty of Medicine provided the Aegean Faculty of Medicine, founded in 1954, to Uludağ University in Bursa, founded in 1970, and Trakya University Faculty of Medicine in Edirne, founded in 1974, with important support.

CONCLUSION

When we look at the history of the İstanbul University İstanbul Faculty of Medicine, from its most distant past to the present, we see that it has always turned its face towards the West and has always followed it with dynamic interest. Efforts to modernise medical education in Türkiye were first initiated in a military medical school in 1827, and after only 40 years, a parallel civilian school was established in 1867, expanding the medical education opportunities available. The fact that physicians were invited from Austria in 1839 and Germany between 1898 and 1918, students were sent to Europe after 1869, and many European scientists forced to leave Germany and other countries after 1933 under thread took office in the İstanbul University İstanbul Faculty of Medicine are concrete examples of the fact that we have always been in close contact with Western scientists. This pioneering institution, open to innovation and committed to progress, has taught graduates who have made significant contributions to medicine. Examples include Hulusi Behçet, the discoverer of Behçet Syndrome, who graduated from İstanbul Faculty of Medicine in 1910, and Aziz Sancar, who graduated from İstanbul University İstanbul Faculty of Medicine in 1969 and shared the Nobel Prize in chemistry in 2015.

A brief history of the İstanbul University İstanbul Faculty of Medicine shows that the institution has experienced many relocation and related difficulties. The last campus was established in 1933, in the Çapa district of İstanbul, and the faculty has been there for 90 years. Today, there are 440 faculty members on the ranks of professor, associate professor, and assistant professor. In the last five years, within Dean Prof. Dr. Tufan Tükek's project framework, which aims to improve the locations of activities, new buildings have been rapidly constructed on the campus. In addition, construction of a vast medical complex in the Hasdal district of İstanbul (Figure 10) is also rapidly progressing.

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CASE REPORT / OLGU SUNUMU
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FATAL AMPHETAMINE AND METHAMPHETAMINE POISONING DUE TO BODY PACKER SYNDROME: AUTOPSY CASE

PAKET VÜCUT SENDROMUNA BAĞLI ÖLÜMCÜL AMFETAMİN VE METAMFETAMİN ZEHİRLENMESİ: OTOPSİ OLGUSU

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ABSTRACT

People who hide illegal substances in body cavities and pass them through checkpoints are called body packers. With this method, many illegal substances such as cocaine, heroin, hashish, amphetamines, methamphetamine, and ecstasy are transported, often hidden in small packages. In our study, a 27-year-old male who was found dead in his hotel room and who was determined to be a body packer after autopsy is presented. During the autopsy, a foreign substance weighing 465 grams in total, packaged in different coloured packaging materials, was detected in the patient's stomach and intestines. Some packages were found to have been opened. Because of opening of the packages, illegal substances can be absorbed and cause fatal poisoning. Therefore, relevant law enforcement officers, medical personnel, and especially forensic medicine professionals must have sufficient knowledge in the antemortem or postmortem approach to package body syndrome cases. In our study, we aimed to contribute to the literature by sharing, discussing and evaluating autopsy findings with forensic and medical documents.

Keywords: Body packer, forensic autopsy, methamphetamine and amphetamine poisoning

ÖZET

Yasadışı maddelerin paket içerisinde vücut boşluklarına saklanarak kontrol noktalarından geçiren kişiler vücut paketçisi olarak da adlandırılmaktadır. Bu yöntemle sıklıkla küçük paketler içine saklanan kokain, eroin, haşhaş, amfetaminler, metamfetamin ve ekstazi gibi çok sayıda yasadışı maddeleri taşınmaktadır. Çalışamamızda otel odasında ölü olarak bulunan ve otopsi sonrasında vücut paketçisi olduğu tespit edilen 27 yaşındaki erkek olgu sunulmuştur. Otopsi sırasında olgunun midesinde ve bağırsaklarında çok sayıda farklı renkte ambalaj malzemeleri ile paketlenmiş toplamda 465 gram ağırlığında yabancı madde tespit edilmiştir. Bazı paketlerin açılmış olduğu görülmüştür. Paketlerin açılması sonucu yasadışı maddeler emilerek ölümcül zehirlenmelere neden olabilmektedir. Dolayisıyla paket vücut sendromunu olgularına antmortem veya postmortem yaklaşım süreçlerinde ilgili kolluk görevlilerinin, sağlık personellerinin ve özellikle adli tıp profesyonellerinin yeterli düzeyde bilgi sahibi olması gerekmektedir. Çalışmamızda, otopsi bulgularını adli ve tıbbi belgelerle birlikte paylaşarak, tartışarak ve değerlendirerek literatüre katkıda bulunmayı amaçladık.

Anahtar Kelimeler: Vücut paketçisi, adli otopsi, metamfetamin ve amfetamin zehirlenmesi

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INTRODUCTION

People who carry illegal substances through checkpoints by hiding them in their body cavities are called body packers (1). To prevent leaks, these substances are placed in small packages and generally swallowed and stored in the gastrointestinal tract cavities such as the mouth, stomach, and intestines. In some cases, these packages are inserted into the rectum, vaginal canal, ear or under the skin (2, 3). With this method, many illegal substances such as cocaine, heroin, hashish, amphetamines, and ecstasy are transported, often hidden in small packages (1, 4). In this study; a 27-year-old male who was randomly detected to be a body packer during the forensic autopsy with an allegation of suspicious death was evaluated and presented in terms of forensic medicine practise. By discussing the case within the scope of the literature, we aimed to raise awareness that toxic-related deaths may occur as a result of the tearing of the packages on the bodies of body packers and the absorption of illegal substances, and to contribute to the literature.

CASE PRESENTATION

Case history and crime scene investigation

The body of a 27-year-old man was found naked, lying face down on the floor of the hotel room, and was sent to the Autopsy Centre for forensic autopsy. According to the information obtained from the research conducted before starting the autopsy; it was learned that a legal action was taken against him in another city three months ago for drug trafficking and that he did not have any history of illness in his past.

Radiological examination and autopsy findings

In the scopy taken before the autopsy, many radiopaque areas with round shapes and regular boundaries were observed in the abdomen (Figure 1). In the external examination; it was determined that there were abrasions and ecchymotic areas on the forehead, nose and lips, and blood smears inside the mouth and nostrils. In the internal examination; it was observed that the stomach was completely filled with foreign matter (possible drug substance) packed in different coloured packaging bags, the largest being 1.5 cm in diameter and the smallest being 0.8 cm in diameter. In examining the small and large intestines; foreign substances detected in the stomach were also detected in different segments of the small and large intestines, and some packages were found to be torn (Figure 2). All foreign substances detected in the digestive system were weighed as 465 g in their current packaged form. Cardiac conduction system examination was performed together with routine histopathological examinations and prominent multifocal infarct areas were observed in myocardial tissues in the left ventricle.

Toxicological examination

In the toxicological examination using the AB SCIEX 5500 QTRAP LC/MS/MS system; 776,0 ng/ml Methamphetamine, 18,4 ng/ml Amphetamine were found in blood, Methamphetamine, Amphetamine were found in urine, Methamphetamine was found in bile, Methamphetamine was found in internal organs (stomach, liver and kidney), Methamphetamine was found in stomach fragment.

Since this was an autopsy case, it was impossible to obtain consent from the case itself. Additionally, our case did not reside in our city and died while staying at a hotel

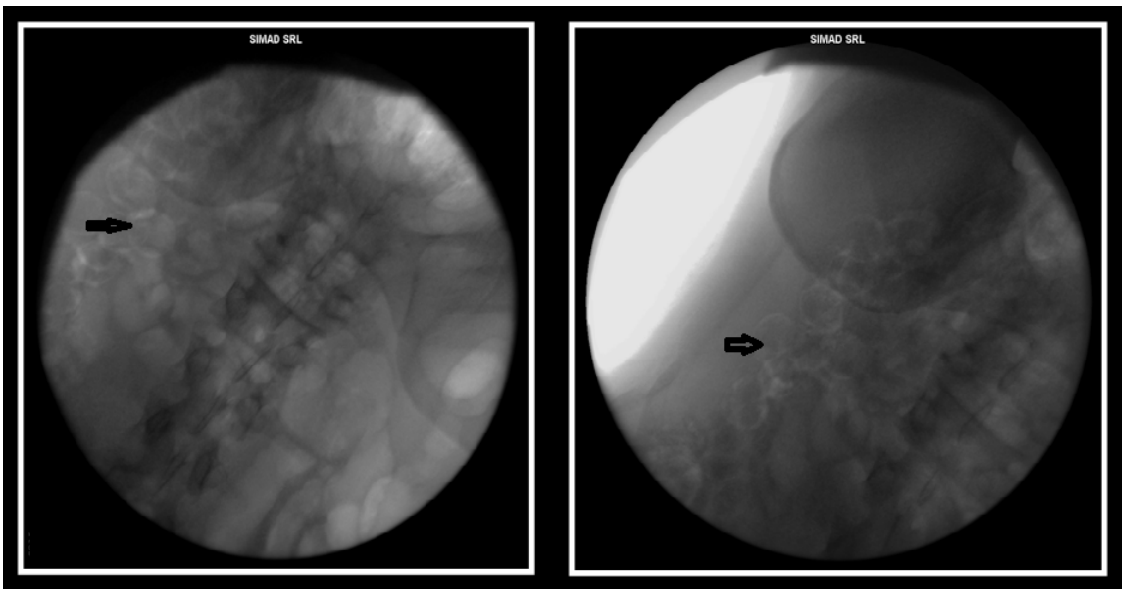

Figure 1: Numerous radiopaque areas with round shapes and regular boundaries in the scopy taken before the autopsy.

Figure 2: Colourful plastic-wrapped packages inside the stomach at autopsy.

for the day. Therefore, consent could not be obtained during the autopsy stage because the patient did not have any close relatives. The study was carried out with the approval of the Education and Scientific Research Commission of the Council of Forensic Medicine (Date: 22.02.2023, No: 21589509/2023/168).

DISCUSSION

Drug abuse is increasing day by day throughout the world and is more common in developed countries than in developing countries. Drugs maintain their regional and global importance as a major security and health problem all over the world (5). One of the complex drug trafficking methods that have been frequently preferred in recent years and that we encounter from time to time is body packers (5, 6). The probability of encountering Body Packer Syndromes in forensic practice is relatively high. For this reason, it is an issue that should be considered by forensic medicine professionals in cases of deaths that often occur without witnesses. Especially in cases where no evidence of trauma is detected, when the cause of death is determined by external examination findings without an autopsy, it is clear that the cases will be easily overlooked and the investigation will progress with difficulty. Therefore, in such cases, forensic medicine professionals should consider that the classical autopsy process should be supported by postmortem radiological examinations.

People who hide illegal substances in body cavities and pass them through checkpoints are called body packers. Illicit drug transport in body cavities was first described in 1973 and since then there has been a worldwide increase in packaged body cases (1, 7). Although the number of body packers is increasing day by day due to the constant need for quick financial gain, it is quite difficult to detect (4, 8). In our case presentation, the case of a packaged body was incidentally detected during the autopsy we performed on the allegation of suspicious death. This detection also draws attention to the importance of standardising and implementing the autopsy process with a systematic procedure.

In cases of suspicious death, a detailed crime scene investigation and a proper autopsy must shed light on the incident (9). In our study, a detailed crime scene investigation was conducted, and all evidence was collected, photographed, and recorded. No illegal substances were found in the scene. Until the radiological imaging and autopsy, no conclusion could be made that the forensic case was a body packer. This reveals that it is quite difficult to detect body packers and that radiological imaging examinations and systematic autopsy are necessary in suspicious cases (4, 8).

Radiology is one of the most important branches of modern medicine. Post-mortem imaging has led to the establishment of a bridge between radiology and forensic medicine. Forensic radiology is a new field within the forensic sciences. Postmortem imaging methods are increasingly used in conjunction with traditional autopsy in a process called "virtual autopsy" and are viewed by researchers as a complement to autopsy. Radiological methods allow easy examination of areas that are difficult to reach and incision during autopsy (10, 11). Findings obtained through these methods can be visualised and recorded and presented to judicial bodies as evidence, becoming increasingly important. The post-mortem images obtained can be recorded and the identity of the body or the cause of death can be reached by comparing with the ante-mortem images (10-12). Body packers are very difficult to detect because they do not provide accurate anamnesis during routine law enforcement searches or health examinations and are usually clinically asymp-

tomatic. Therefore, it is stated that direct abdominal radiography is a good screening tool for the evaluation of suspected body packers due to its low cost, high usability, and sensitivity between 74% and 100%. In addition, this radiological examination has a very important place in terms of evidence. Plain abdominal radiographs show, after the exclusion of nutritional contents in the stomach, small intestine, or large intestine, one or more well-defined radio-opacities (double condom sign), clear air surrounding an oval opacity, a smooth and well-shaped rectangular structure (tic-tac sign). Also, body packing should be considered when hard packages arranged parallel to each other (parallelism sign) are detected in the intestinal lumen. When direct abdominal radiographs give negative results, a low-dose abdominal CT scan is recommended if there is strong suspicion (1, 4). Before starting the autopsy for our case, radiological imaging (scopy) was performed for screening, as stated in the literature. Many radiopaque substances with round shapes and regular borders were detected in the abdomen. After this stage, an autopsy was planned with the preliminary diagnosis that the corpse was the body package carrier. Therefore, we think that it would be beneficial to routinely perform radiological imaging before forensic autopsy procedures to reach a certain preliminary diagnosis and have a more planned and systematic autopsy.

Illegal substances are often transported through the gastrointestinal tract by oral ingestion in small packages to prevent leakage. These packages are intended to be eliminated from the body through defaecation. Latex gloves, nylon bags, condoms, aluminium foil, the finger parts of surgical gloves, and balloon-like materials are often used for packaging (2, 3). With this method, approximately 1 kg of material can be transported in packages between 40 and 100 on average (8, 13). In our study, during the autopsy, a total of 465 grams of foreign matter was detected in the stomach, small and large intestines, packaged in different coloured packaging bags with a diameter of 0.8-1.5 cm. Some packages were torn. In body packers, serious poisoning and death sometimes occur because of small and large intestine obstructions, gastrointestinal perforations, rupture of the package and absorption of the illegal substance. Conservative follow-up and treatment with or without laxatives is recommended in intensive care clinics for asymptomatic patients who are found to be body packers. Endoscopic procedures are not recommended because of the possibility of perforation of the packages. Surgical removal is recommended in case of signs of toxicity, mechanical gastrointestinal obstruction, or the presence of packets in the body during long-term follow-up. Specific antidote treatment should also be applied when possible (7, 8, 13). In our case, no signs of obstruction or perforation were detected in the GIS during the autopsy, and it was determined that some packages were opened. We believe that toxic substances absorbed into the systemic circulation from packages opened during the autopsy may cause death.

Methamphetamine, an amphetamine-type stimulant of the central nervous system, is a synthetic substance produced in 1919 and can be transported by body packers. Although it is used for treating many diseases such as attention deficit hyperactivity disorder, narcolepsy, and severe obesity, the abuse of this substance has limited its clinical use (14, 15). Since methamphetamine is cheaper and easier to obtain than other illegal substances, its use worldwide is increasing day by day. Studies and United Nations reports indicate that methamphetamine use ranks second after marijuana among recreational drugs and poses a significant global health problem (15, 16). In methamphetamine overdose, tachycardia, hypertension, hyperthermia, seizures, psychosis, memory loss, hallucinations, coma, cardiopulmonary arrest, and death may occur (15-17). This case shows us, in its most concrete form, that the fatal cardiac effects of amphetamine and methamphetamine poisoning occur in line with the literature.

CONCLUSION

Drug and illegal drug trafficking is increasing day by day worldwide and has emerged as a global problem. Packages can cause blockages, perforations, especially in the gastrointestinal tract, and lethal poisoning by the absorption of their illegal content because of opening of the packages. It is very difficult to detect body packers both during routine searches by judicial law enforcement and during routine health checks. Therefore, antemortem or postmortem screening and plain abdominal radiography should be performed in all suspected cases for evidence. Whether symptomatic or asymptomatic, as soon as body packers are detected, they should be immediately admitted to health institutions with intensive care conditions and their follow-up and treatment should begin. We believe that it would be beneficial for law enforcement officers, healthcare personnel, and especially forensic medicine professionals to be trained to have detailed information about package body syndrome, which is a method of illegal substance trade.

Informed Consent: Since this study is about an autopsy case, it is impossible to obtain consent from the case itself. Additionally, the our case did not reside in our city and died while staying at a hotel for the day. Therefore, consent could not be obtained during the autopsy stage because the patient did not have any close relatives.

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Revision of Manuscript- T.V., H.Ç.K.; Final Approval and Accountability- H.Ç.K., T.V.; Technical or Material Support – T.V., M.E.; Supervision- M.E., H.Ç.K.

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LETTER TO THE EDITOR / EDİTÖRE MEKTUP
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REPLY TO LETTER TO THE EDITOR BY MEZA-ESPINOZA AND COLLEAGUES

MEZA-ESPINOZA VE ARKADAŞLARININ EDİTÖRE MEKTUBUNA CEVAP

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Dear Editor,

In this journal, we reported a rare live birth case of partial trisomy 9 resulting from a maternal translocation between chromosomes (Chr) 9 and 22 (1). The affected infant had two normal Chr9 plus a derived/dicentric Chr9 containing a complete copy of 9pter to 9q22.31 (carrying the centromere regions of both Chr9 and Chr22). Meza-Espinoza and colleagues wrote a letter to the editor about this case and the related translocation-derived gametes (2). We appreciate the contributions of the authors.

All possible gametes are shown in Table 1, and there are two options for disomy of 22 products derived from tertiary 3:1 segregation. However, these gametes are also trisomic or monosomic on the long arm of Chr9 (q22.31 to 9qter). In the case of tertiary trisomy 3:1 segregation, when normal Chr9, normal Chr22 and der(22) are transmitted to the next generation, the gamete will be disomic for Chr22 and partially trisomic for long arm of Chr9 (9q22.31 to 9qter). Trisomy of the short arm of Chr9 is a rare condition but is compatible with life, as in this case. In contrast, the trisomy of the long arm of Chr9 (the product of a tertiary 3:1 segregation) is almost always incompatible with life. In tertiary monosomy 3:1 segregation, when only der(9) is inherited, the offspring will be disomic for Chr22 but will also have partial monosomy from 9q22.31 to 9qter and will have no chance of a live birth. Two disomy 22 products derived from the segregation of interchange trisomy and interchange monosomy also exist, but the gametes will also be trisomy and monosomy for Chr9. Additionally, the second option for the adjacent 2 segregation pattern leads to partial monosomy of chromosome 9 (9pter to 9q22.31, our case region) and disomy 22.

In summary, regardless of alternate segregation, 6 disomy 22 products (seen in Table 1) could arise due to the segregation pattern of maternal translocation. However, our case is probably the only one that survived because trisomy on the short arm of Chr9 can be compatible with life.

Finally, as we mentioned in our article, the family was informed about all reproductive options, including alternate segregation (with normal and carrier fetuses) through an appropriate genetic counselling process, and preimplantation and prenatal diagnosis were recommended in subsequent pregnancies (1).

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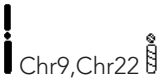
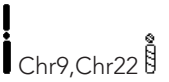
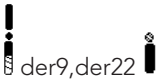
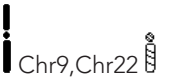
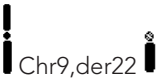
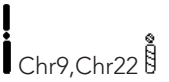
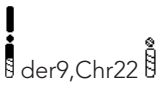
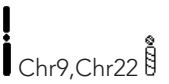
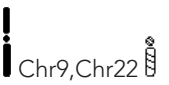
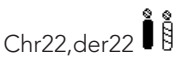
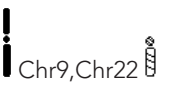
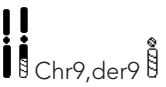
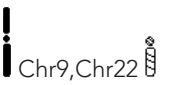
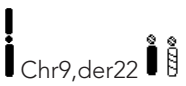
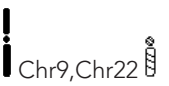
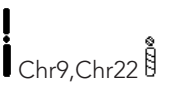
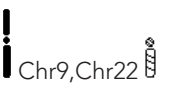
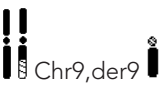
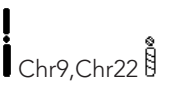
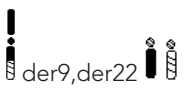
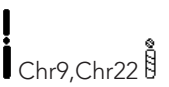
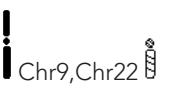
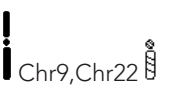
Table 1: The segregation patterns of translocation between chromosomes 9 (q22.31) and 22 (q11.1)

	MOTHER	FATHER	GAMETES
Alternate			Disomy 9–22
			Disomy 9–22 Balanced translocation carrier
Adjacent 1			Partial trisomy 9(q22.31 -> qter) and monosomy 22
			Partial monosomy 9(q22.31 -> qter) and trisomy 22
Adjacent 2			***Partial trisomy 9(pter->q22.31) and disomy 22****
			Partial monosomy 9(pter->q22.31) and disomy 22
Tertiary trisomy			Partial trisomy 9(pter->q22.31) and trisomy 22
			Partial trisomy 9(q22.31 -> qter) and **disomy 22****
Tertiary monosomy			Partial monosomy 9(pter->q22.31) and monosomy 22
			Partial monosomy 9(q22.31 -> qter) and **disomy 22****
Interchange Trisomy			Trisomy 9 and disomy 22
			Disomy 9 and trisomy 22
Interchange Monosomy			Monosomy 9 and disomy 22
			Disomy 9 and monosomy 22

*our case , **two cases of disomy 22 with partial trisomy/monosomy 9q

LETTER TO THE EDITOR / EDİTÖRE MEKTUP
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PSEUDOMYXOMA PERITONEI: A RARE ENTITY

PSÖDOMİKSOMA PERİTONEİ: NADİR BİR ANTİTE

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Dear Editor,

I write to bring an attention to a rare disease, pseudomyxoma peritonei (PMP), which was first described by Werth in 1884, yet it remains an orphan disease with limited awareness (1). The incidence of PMP is one to two patients/per million in a year that might be due to misdiagnosis (2).

PMP typically arises from a ruptured mucinous tumour, usually originating from the appendix, stomach, gall bladder, small and large intestine, fallopian tubes, ovaries, pancreas and lung, and mucin accumulates inside the peritoneal cavity and is called 'jelly belly' (1). PMP is histopathologically classified into four subtypes, based on present of tumour cells, the amount of mucin and the aggressiveness level, which are low grade, high grade, high grade with signet ring cells and acellular mucin (2).

Despite being recognised for more than a century, PMP's management is still challenging due to misdiagnosis or delayed diagnosis. This delay usually causes significant progression of the disease and adversely affects patients' outcomes. Diagnosing PMP is still challenging due to it's indolent nature with nonspesific symptoms such as abdominal discomfort and distension at the time of the disease initiation. Imaging techniques may not distinguish mucinous material from other fluid collection and may cause difficulty in obtaining a definitive histopathological diagnosis (3).

The current standard treatment of PMP is cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC), which improves patient outcomes and should be performed in specialised centres (1, 4). PMP is frequently diagnosed as an unexpected finding during emergency surgeries such as acute appendicitis or gynaecologic surgeries or imaging for other conditions. As a result, general surgeons and gynaecologists will occasionally come across such cases and they will face challenges in both diagnosis and treatment due to its rarity, lack of awareness and limited guidance on management (1). When PMP is encountered unexpectedly during surgery, surgeons should focus on taking sufficient biopsies and removing the appendix, avoiding extensive resections. After recovery and diagnostic confirmation, specialised care should be sought for further management (1).

In a multicenter study, the outcome data of 2298 patients with PMP of appendiceal origin were analyzed, and the best results were obtained with proper cytoreduction. (5). Chua et al. showed that the five-year survival rate for patients who undergo incomplete (completeness of cytoreduction) (CCR2 or CCR3), where visible residual disease remains, is 24%; while it is 85% for those who achieve CCR0 and 80% for patients with a CCR1 resection (5). Delays in diagnosis or misdiagnosis and improper treatment management after diagnosis determine the completeness of cytoreduction and therefore the prognosis in PMP patients.

In conclusion, physicians', especially general surgeons and gynaecologists who perform intra-abdominal surger-

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ies, awareness about PMP should be increased so that timely and appropriate treatment can be performed without missing diagnosis and referral to specialised centres will increase the survival of these patients.

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ERRATUM / DÜZELTME
DOI: 10.26650/IUITFD.2024.02E
İst Tıp Fak Derg 2024 / J Ist Faculty Med 2024

ERRATUM TO: THE DETERMINANTS IN THE MANAGEMENT OF PREGNANCIES COMPLICATED WITH IMMUNE THROMBOCYTOPENIA

Çiğdem KUNT İŞGÜDER, Tuğba SARAÇ SİVRİKOZ, Mehtap AKIN, Törehan ASLAN, Lütfiye SELÇUK UYGUR, Şule BİROL İNCE, İbrahim KALELİOGLU, Sevgi BEŞIŞIK, Recep HAS, Alkan YILDIRIM

In the article by Kunt İşgüder et al., titled 'The Determinants in the Management of Pregnancies Complicated with Immune Thrombocytopenia' published in the October 2023 issue of the Journal of İstanbul Faculty of Medicine, an error was inadvertently made in the authors' institutional information. After evaluating the situation with the editor and technical office, the following information has been added to the PDF file.

Çiğdem KUNT İŞGÜDER[1,2], Tuğba SARAÇ SİVRİKOZ[1], Mehtap AKIN[1], Mustafa Törehan ASLAN[3,4], Lütfiye SELÇUK UYGUR[1,5], Şule BİROL İNCE[1], İbrahim KALELİOĞLU[1], Sevgi BEŞIŞIK[6], Recep HAS[1], Alkan YILDIRIM[1]

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