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Case Report

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Effect of Spinal Needles used by Anesthesia Residents on Procedural Success and the Perception of Click Sensation: A Randomized Prospective Trial

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Abstract

Aim: The type and diameter of the needle used in spinal anesthesia (SA) affect the procedure's success and the sensation of clicking during a dura puncture. This study aimed to compare the effects of Quincke and pencil-point needles of the same thickness, when used by anesthesia residents new to SA application, on procedural success and the number of trials required to perceive click sensation.

Methods: This prospective randomized study included 213 adult patients undergoing elective surgery under SA, divided into six groups based on needle type and diameter: Group I: Quincke (Q)-25 Gauge (G), Group II: Q-26G, Group III: Q-27G, Group IV: Pencil-point (P)-25G, Group V: P-26G, and Group VI: P-27G. The number of interventions for SA (1-3), the attempt (1, 2, or ≥3) during which the stylet was removed and cerebrospinal fluid (CSF) flow occurred, considering that the click sensation was felt during the procedure, and the time taken for CSF appearance (<1.9 s or ≥2 seconds) were recorded.

Results: No difference was found between the groups in terms of demographic data, the American Society of Anesthesiologists risk, puncture site, number of click sensation trials, time for CSF appearance, and feasibility of the procedure ($p>0.05$). The SA success rate in the first trial ($p<0.001$) was higher when pencil-point needles were used.

Conclusion: Although the effects of spinal needles with different tip designs and diameters on the number of trials required to perceive click sensation are similar, due to the high rate of SA success in the first trial, the use of pencil-point needles is recommended for anesthesia residents new to SA application.

Keywords: Spinal needle type, spinal needle diameter, click sensation, success rate, anesthesia residents

Introduction

Spinal anesthesia (SA) is a simple, low-cost, and reliable local anesthesia method with a high success rate and is frequently used by anesthesiologists in lower abdominal and extremity surgeries (1). Based on their tip designs, we classify spinal needles as either atraumatic or conventional. Conventional needles (Quincke=Q) are most frequently used, have sharp tips, and allow injection from the tip. Atraumatic needles (pencil-point=P), on the other hand, have blunt tips and a side port that allow injection (2,3). A previous mortality study demonstrated that conventional needles cause irregular tissue resections and lead to an increase in cerebrospinal fluid (CSF) leakage, whereas atraumatic needles separate the dura tendons from each other and do not cause irregular resections (4). Therefore, atraumatic needles can reduce postdural-puncture headache (PDPH) incidence by limiting CSF leakage after lumbar puncture (5). Spinal anesthesias performed with needles having the same thickness but different tip designs have demonstrated different amounts of CSF escaping outside the dura (6,7). In general, as the diameter of the needle decreases, the SA success rate and PDPH incidence also decrease (8-10). It has been reported that the click sensation felt in dural punctures performed

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with spinal needles of different thicknesses and types may be an indicator of the success of the spinal puncture (11).

The present study aimed to compare the effects of Quincke and Pencil-Point needles of the same thickness, used by anesthesia residents new to SA application, on the success rate of the procedure and the number of trials required to perceive the click sensation.

Methods

Compliance with Ethical Standards

After obtaining permission from the local ethics committee (decision no.: 2018/20, clinicalTrials.gov identifier: NCT05704816; principal investigator: G.K., and date of registration: March 10, 2022), this prospective randomized observational study was conducted at Zonguldak Bulent Ecevit University Hospital in Turkey during November 2018-2019. Figure 1 presents the flow diagram of the study according to the Consolidated Standards of Reporting Trials 2010 (12).

Participants

The inclusion criteria of this study were as follows: patients should be between 18 and 45 years of age; they should be scheduled to undergo elective lower abdomen/extremity surgery; they should fall in the American Society of Anesthesiologists (ASA) I-II risk group; they should not have bleeding diathesis; and they should provide written consent to participate in the study. Patients for whom SA was contraindicated and those who were morbidly obese, pregnant, allergic to drugs used in this study, and had undergone previous spinal surgery and SA were excluded.

Patient Management and Data Collection

Vascular access in patients who were not premedicated was established before surgery using an 18-gauge (G) catheter, and a 10 mL/kg saline infusion was administered for 30 minutes (min). The patients were then taken to the operating room and provided with routine hemodynamic monitoring. They received 3-5 lt. min. of oxygen through a nasal cannula. The patients' demographic data (age, gender, height, and weight) and ASA I-II risk were recorded.

Spinal Anesthesia Application

The patient was asked to remain in a seated position, stretch his or her legs down the table, and keep his or her head in flexion, shoulders down, and tummy in. A straight line was drawn between the iliac crests. This line passes through the fourth lumbar vertebra's (L4) spinous process. After determining this space, the intervertebral spaces where the procedure was to be performed were located, and the physician performing the intervention evaluated the procedure's feasibility as easy, medium, or difficult. The lumbar vertebral space (L2-L3 or L3-L4) that could best be palpated was identified, and local anesthesia was subcutaneously administered by injecting 1 mL of 2% animal. Patients were randomized using a sealed envelope method into 6 groups: Group I: Q-25G, Group II: Q-26G, Group III: Q-27G, Group IV: P-25G, Group V: P-26G, and Group VI: P-27G, based on the needle type and diameter, using a sealed envelope. All spinal needles were used with a 21-G guide needle. The tip of the spinal needle was then moved parallel to the dura tendons, and the guide needle was placed along the midline of the determined intervertebral space. When the dural puncture click was perceived, the needle probe was pulled back, and free CSF flow was observed. Assuming that the click sensation due to the dural puncture was perceived, the needle probe was pulled out, and the trial number during which the CSF returned was recorded (1, 2, and ≥3). The duration from the perception of click sensation due to dural puncture and removal of the needle probe to the observation of CSF (<1.9 or ≥2 sec) was recorded using a chronometer. When the return of clear CSF was observed, the open end of the needle was turned toward the head. Spinal anesthesia was performed by injecting 2-3 mL of 0.5% hyperbaric bupivacaine into the spinal space. A successful lumbar puncture was confirmed by the observation of free CSF flow.

After the procedure, the needle was removed from the skin, and the area was closed with a sterile sponge. Following the procedure, the patient was immediately placed in the supine position. The number of interventions applied for SA (1/2/3) and the presence of paresthesia during the procedure were recorded. The "electric shock" that occurred in the leg while the spinal needle was advanced during SA was evaluated as a feeling of paresthesia. For the application, a maximum of three interventions were allowed at one location. The same cannula was used for repetitive punctures in the same patient. When the procedure was unsuccessful after three interventions, the patient was excluded from the study, and the procedure was repeated by a senior physician. If hemodynamic changes related to vagal reflexes (such as decreasing blood pressure, passing out, and having a seizure) were noted in the patient during the procedure, he/she was excluded from the study. All interventions were performed by three different anesthesia assistants who were new to SA practice but had at least 20 SA experience with spinal needles of different diameters and tip designs before the study. A physician who was experienced in SA but did not know the study asked whether the patients had headaches and backaches within the first 24 hours postoperatively and recorded them.

Approach to Postspinal Headache and Back Pain

It was ensured that the patients remained in bed for at least 4 hours (h) in the postoperative period.

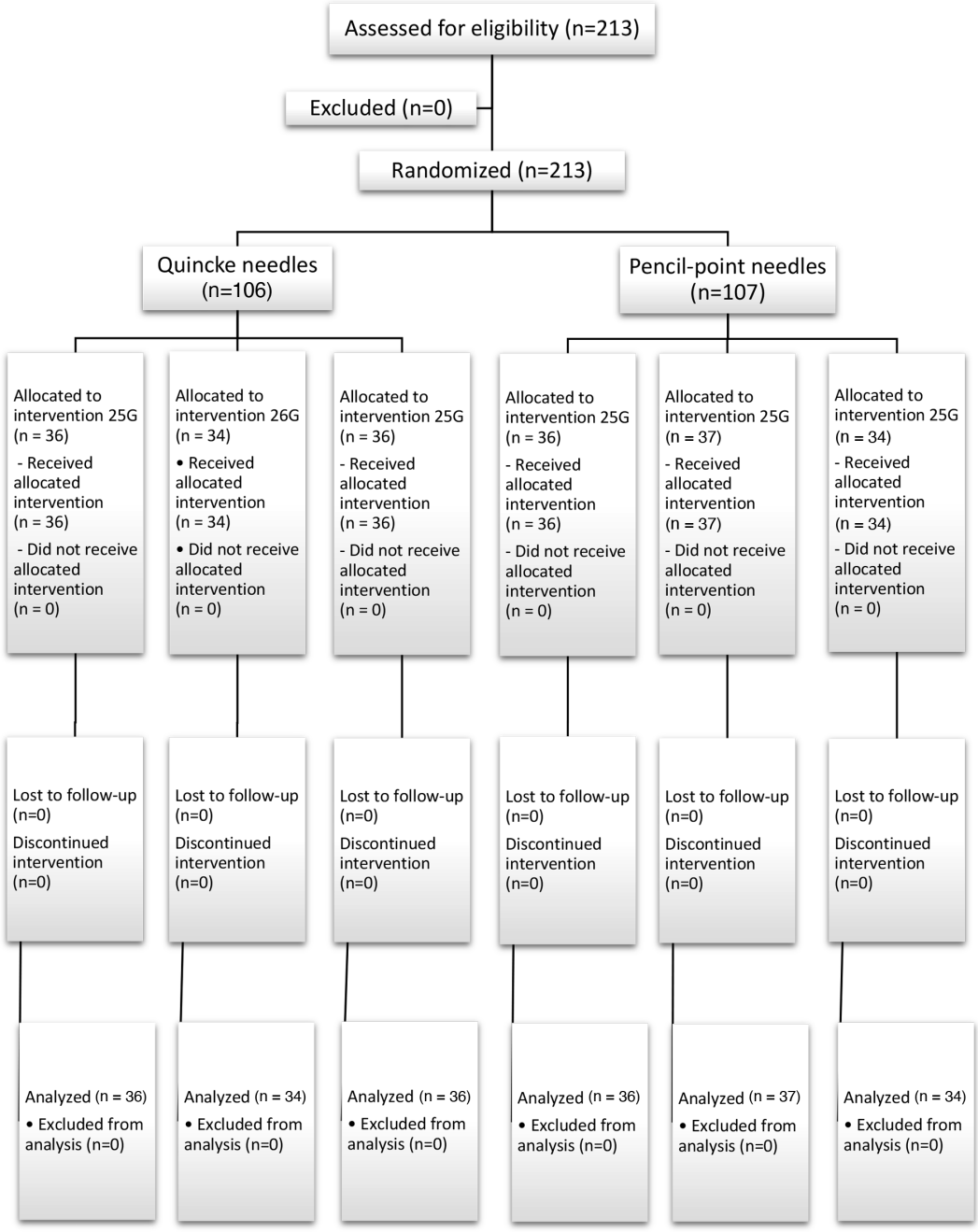

Figure 1. CONSORT flow diagram of the study

The physician investigated whether the patients had PDPH and postspinal back pain (PSBP) 24 hours after the procedure. The onset of these pains following SA and their increase with movement were accepted as sufficient. In the case of pain development, patients were recommended bed rest and an increase in fluid intake (oral and/or 2,500 mL/day IV fluid intake). Geralgine-K® tablets were administered depending on the severity of pain (light, moderate, or heavy). An epidural blood patch was administered in severe PDPH cases that did not respond to treatment.

Sample Size Calculation

In the study, in which 185 cases should be included with 95% confidence (1-α), 80% test power (1-β), and w = 0.264 impact size, 31 cases were included in each group for two different types of needles and for three different diameters (11).

Statistical Analysis

The study data were analyzed using Statistical Package for the Social Sciences Version 23.0 (IBM SPSS Inc., Chicago, IL, USA). The normal distribution of the data was checked with the Kolmogorov-Smirnov test. In the comparison of categorical variables according to needle type, the chi-square test was used. In the comparison of normally distributed data according to needle type, a one-way ANOVA was employed. For the comparison of the data without normal distribution according to needle type, the Kruskal-Wallis test was used, and multiple comparisons were analyzed with the Dunn test. The analysis results are presented as mean ± standard deviation for quantitative data. The statistical significance level was set at $p<0.05$.

Results

Demographic and Clinical Characteristics of the Participants

The study involved 213 patients. The mean age of the patients was determined to be 35.4±9.3 years. Spinal anesthesia was successfully performed on all patients, and no patients were excluded from the study. The groups were found to be similar in terms of demographic data and ASA risk (Table 1).

No difference was observed between the groups in puncture site, number of trials for click sensation, CSF observation time, or feasibility of the procedure (Table 2). It was observed that 60% of SA interventions were successful in the first trial, and the rest were successful in the second trial. Compared with Quinke needles, the first-try success rate was higher with pencil-point needles, and the highest success rate was obtained with 25G pencil-point needles ($p<0.001$). Paresthesia development was found to be more frequent with pencil-point needles during the procedure ($p=0.004$), with a rate of development of 5.6% for 27G Quincke needles and 13.5% for 26G pencil-point needles. No paresthesia was observed when using the other needle types. A total of 7 patients (n=1Q/n=6P) reported PDPH, but there was no statistically significant difference between the groups in terms of headache. PSBP developed in only 4 patients on whom a 26G pencil-point needle was used ($p=0.004$) (Table 2). Two of the four patients with moderate backaches also experienced headaches, and they were administered Geralgine-k®. Other patients

Table 1. Comparison of demographic data and ASA risk [number (n) or mean ± SD]

	Quincke			Pencil-point			
	25G (n=36)	**26G (n=34)**	**27G (n=36)**	**25G (n=36)**	**26G (n=37)**	**27G (n=34)**	**p-value**
Female/Male (n)	8/28	13/21	11/25	10/26	12/25	10/24	0.801
Age (years)	36.5±8.2	38.1±8.6	32.4±10	35.1±9.5	36.4±9.5	33.9±9.5	0.128
Height (cm)	172.2±8.5	170.4±9.7	171.9±7.5	172.1±8.8	171.3±7.4	170.4±9.6	0.910
Weight (kg)	78.4±15.8	79.1±12.2	74.6±13.2	79.1±13.8	79.1±13.5	76.9±13.4	0.678
ASA I/II (n)	19/17	12/22	18 /18	14/22	8/29	10/24	0.053

Kruskal-Wallis test statistic, Analysis of variance test statistic
SD: Standard deviation, ASA: The American Society of Anesthesiologists

Table 2. Comparison of categorical variables by needle type and diameter

	Quincke			Pencil-point			
	25G (n=36)	**26G (n=34)**	**27G (n=36)**	**25G (n=36)**	**26G (n=37)**	**27G (n=34)**	**p-value**
Procedure success 1/2	17a/19	18ab/16	15a/21	32c/4	26b/11	20ab/14	<0.001
The number of trials required to perceive click sensation 1/2/≥3	15/12/9	15/10/9	14/18/4	13/14/9	16/16/5	17/11/6	0.660
CSF flow time (sec) <1.9/≥2	14/22	11/23	8/28	17/19	8/29	8/26	0.101
Procedure's feasibility Easy/Medium/Difficult	25/7/4	22/11/1	24/12/0	24/10/2	26/10/1	22/12/0	0.401
Presence of paresthesia Yes/No	0^a/36	0^a/34	2^{ab}/34	0^a/36	5^b/32	0^a/34	**0.004**
Postspinal back pain	0	0	0	0	4a	0	**0.004**

Chi-square test statistic,
[a-c]: There is no difference between times with the same letter in a group
CSF: Cerebrospinal fluid

with headaches did not require any analgesics, and their issues were resolved on their own.

Discussion

In this study, we compared the effects of Quincke and pencil-point needles of the same thickness used by anesthesia residents new to SA application on the success of the procedure and the number of trials required to perceive click sensation. It was determined that the success rate in the first trial was higher for the 25G pencil-point needles. Patients experienced backaches more frequently when 26G pencil-point needles were used, and there was no statistically significant difference between the groups in terms of click sensation.

Opinions about the success rate in the first trial according to the type and diameter of spinal needles vary (6-10,13-16). In a meta-analysis study comparing atraumatic and conventional spinal needles, it was reported that the success rate at the first attempt was similar (13). Westbrook et al. (6) reported that puncturing the dura with pencil-point needles was more difficult and less successful than that with Quincke needles. They explained that this was because it was technically difficult to use the needle, the sensation of the needle puncturing the dura was minimal, and local anesthetic injection was challenging. Another study reported that Quincke needles can easily penetrate skin and ligaments, whereas pencil-point needles make it easier to recognize the dura mater (16). Another study demonstrated that trial number in SA depended on the experience of the practitioner (13,17,18). Krommendjik et al. (18) reported that they achieved a success rate of 81.8% in the first attempt with pencil-point needles, but the SA reported that 69% of the applications were made by assistants with different experience periods. Performance characteristics, such as failure rate, first-try success rate, and average number of attempts, have been reported to show similar efficacy when using atraumatic and conventional needles (13). In this study, the majority of SAs were completed in the first trial, and the remaining SAs were completed in the second trial. The success rate in the first trial was higher (60%) when using pencil-point needles. For 25G pencil-point needles, the success rate in the first trial was 88.9%. However, the success rate was found to be lower for 26G (70.3%) and 27G (58.8%) needles, suggesting that the SA success rate decreased with a decrease in needle diameter. We concluded that because all interventions were performed using a guide needle, it was not difficult to use pencil-point needles. Therefore, it would be appropriate for anesthesia residents new to SA applications to use pencil-point needles, as this would increase the chances of procedural success.

Opinions on perceiving click sensation based on the type and diameter of spinal needles also vary (11,18,19). Krommendijk et al. (18) reported that in SA performed with 25G Pencan needles, click sensation was perceived in 78.4% of the patients; the perception of click sensation while performing SA is an important indicator of the success of the procedure. Shutt et al. (19) reported that they perceived spinal click sensation, albeit not explicitly, in all cases performed using a 25-G pencil-point needle, and they explicitly perceived the click sensation in only a few of the cases performed using a 26-G Quincke needle. According to the results of the present study, click sensation was perceived, independent of the groups, in 42.3% (n=90) of all patients who participated in the study in the first trial, 38% (n=81) in the second trial, and 19.7% (n=42) in the third and subsequent trials. The frequent perception of click sensation in the first trial explains the success of the SA procedure. Although there was no difference between needle type and diameter in terms of perceiving click sensation, practitioners stated that they were able to perceive click sensation more clearly, smoothly, and easily when using pencil-point needles. Various studies have investigated the relationship between PSBP and needle type, diameter, and number of attempts (13,17,19,20). A meta-analysis study compared atraumatic and conventional needles and found similar backache incidences (13). Pittoni et al. (17) observed a higher rate of backache incidence among patients when using a 22G pencil-point needle (14.5%) compared with a 25G pencil-point needle (5.9%), and they stated that the reason for this was not related to the needle diameter, not the number of trials. Shutt et al. (19) could not identify a relationship between backache incidence and trial number of needles. Krommendijk et al. (18) reported a PSBP rate of 7.8%. Only 4 of the 213 patients included in this study developed backache, which shows that the rate was quite low (1.8%). We believe that the development of backache with 26G pencil-point needles is coincidental, and because of the good response of the patients to oral analgesics, they did not encounter severe backache. We believe that because the number of trials did not make a difference in backache development, the pain could be related to the use of a guide needle, and the practitioner's experience is not a factor in the development of pain. More studies that cover a larger number of patients on this issue are needed.

PDPH incidence is affected by many factors, such as needle diameter, tip design, patient position, prophylactic intravenous fluid use, bed rest, and clinician experience (3,7-10,13-25). Among the mechanisms recommended for preventing PDPH, needle tip design has been the most prominent. Studies conducted with Quincke and pencil-point needles with equal external diameters have reported less CSF loss with pencil-point needles. This was attributed to the design of the needle tip, not the needle diameter. The design of the needle has a significant

effect on PDPH (6,8,9,18,21-23). In a study comparing pencil-point needles and smaller-diameter Quincke needles, it was reported that pen-tipped needles cause headaches at a lower rate (9). Shaikh et al.'s (21) study on obstetric patients, SA performed using 25G Q, 27G Q, and 27G Whitacre point needles showed PDPH rates of 8.3%, 3.7%, and 2%, respectively. They recommended the use of a 27-G Whitacre point spinal needle during the procedure. As the diameter of the spinal needle increases, the incidence of PDPH increases; however, as the thickness of the spinal needle decreases, the difficulty of the procedure increases, which subsequently decreases the chance of success (8-10). In this study, we determined the headache incidence to be 3.2%, regardless of the age group. The higher incidence of headache development during the 24-hour postoperative period when pencil-point needles were used was surprising and contrary to what was stated in the literature. However, headaches that develop in the 24-hour postoperative period may not be evaluated as PDPH. Instead, it would be considered a headache that lasts for a short time and resolves without any intervention. From the interviews of the patients, we found that they frequently complained about headaches. In addition, due to the young age of our patients, we cannot provide clear information about whether they were mobilized in the early postoperative period and to what degree they complied with the doctor's recommendations. Because there is no difference in the number of attempts for SA, we believe that headaches are not dependent on the number of attempts or the experience of the practitioner.

Past studies have emphasized that having the same size needle is not sufficient to provide the same CSF flow, and the inner diameter is more important than the outer diameter (7,10,24,26). A recent study reported that a pencil-point needle is easy to use and does not have a low flow rate, while the flow rate of two different spinal needles with the same diameter is similar (2). It has been suggested that CSF collection takes an unreasonably long time with a needle diameter of less than 22 g (0.7 mm) (23). Krommendijk et al. (18) reported that in SA performed using Pencan needles, CSF was observed within 2 s after the procedure in 95.9% of the patients. Although the inner diameters and tip designs of the needles used in our study were different, we observed that the CSF exposure times were similar (less than 2 seconds in 30.9% of our patients).

Krommendijk et al. (18) studied the evaluations made by doctors regarding the ease of performing SA using 25G Pencan needles. They reported that 85.2% of the doctors found the procedure to be easy, 6.2% found the ease of use to be medium, 6.7% found it difficult, and 1.9% found it impossibly difficult. In this study, doctors evaluated the feasibility of the procedure. The results demonstrated that 33.3% of the doctors found the procedure easy when using pencil-point and sharp-point needles, 14% found the procedure to be feasible with the Quincke needle medium, and 2.3% found it difficult. In the case of pencil-point needles, 15% of doctors rated procedural feasibility as medium and 1.4% as difficult. We believe that needle tip design and diameter do not change the practicability of the procedure.

Paresthesia is an abnormal sensation that occurs during a spinal, epidural, or combined spinal epidural (CSE) injection or the placement of a permanent spinal needle. The reason for developing paresthesia during SA performed using pencil-point needles is the distance from the tip of the needle to the orifice. Before the orifice enters the subarachnoid space, the tip of the needle should pass through the subarachnoid space by at least 0.5 mm. It has been assumed that paresthesia caused by the needle can be produced by contact of the tip of the needle with a spinal nerve stem in the epidural space or a spinal nerve in the intervertebral foramen. The development of paresthesia is affected by various factors, such as needle tip design, use of CSE kits with long spinal needles, and the puncture technique (25-30). In theory, Sprotte needles have a higher incidence of paresthesia than Quincke needles because the interaction between the needle and tissue during lumbar puncture increases the incidence of paresthesia by causing a deviation of the needle tip. The use of guide needles has been recommended to decrease this incidence (25). In the single-shot subarachnoid technique, the removal of the probe when the needle tip is still in the interspinal ligament and its continuous forward movement until CSF is observed can decrease the incidence of paresthesia. A study that compared paresthesia development during the use of pencil-point and sharp-pointed needles reported that paresthesia was observed in 20 out of 300 (6.6%) patients, although the difference was not statistically significant (n=13 and 7, respectively) (28). In this study, paresthesia was observed when using 26G pencil-point (n=5) and 27G Quincke (n=2) needles. Paresthesia occurred in 7 of 213 (3.3%) patients; all cases of paresthesia were transient, and no neurologic complications were observed. Although all interventions were performed with accompanying guide needles, we conclude that the puncture technique may have affected paresthesia development.

Study Limitations

Our study has certain limitations. The SA procedure is standardized for at least 20 procedures, regardless of needle type and diameter, for beginners. The effect of similar or additional interventions using different diameters and types of needles on outcomes has not been evaluated.

It should also be noted that needle diameter, type, and some other factors related to the patient may also have an impact on the time it takes for CSF to appear. In addition, the results might have been different if residents of different seniority levels were involved in this study, instead of only residents who were new to SA. Another limitation is that this study was a single-center trial with a small sample size. Finally, it would be appropriate to evaluate the complications of postoperative headaches and backaches at a later time. As the strength of our study, we would like to point out that the SA procedure performed using different needle tips will be very useful for assistants who are new to SA application in terms of feeling the layers of the spinal area and gaining needle practice.

Conclusion

Although the effects of spinal needles with different tip designs and diameters on the number of trials required to perceive click sensation are similar, due to the high rate of SA success in the first trial, the use of pencil-point needles is recommended for anesthesia residents new to SA application. However, they should also be careful regarding the development of paresthesia and back pain.

Ethics

Ethics Committee Approval: The ethical approval was obtained from The Institutional Review Board of Zonguldak Bulent Ecevit University (decision no.: 2018/20, clinicalTrials.gov identifier: NCT05704816; principal investigator: G.K., and date of registration: March 10, 2022).

Informed Consent: Written informed consent was obtained from patients.

Authorship Contributions

Concept: G.K., T.O., C.B., H.A., Design: G.K., B.G.K., T.O., R.D.O., H.A., Data Collection or Processing: G.K., B.G.K., T.O., K.B., C.B., R.D.O., O.P., Analysis or Interpretation: G.K., B.G.K., C.B., O.P., H.A., Literature Search: G.K., K.B., R.D.O., O.P., Writing: G.K., B.G.K., H.A.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Factors Affecting the Publication Rate of Adult Endocrinology Theses in Turkey: A Comprehensive Bibliometric Analysis

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Abstract

Aim: Understanding the factors that influence the probability of endocrinology thesis publication can guide aspiring researchers in their academic pursuits. This study aimed to assess the publication rate of endocrinology theses and identify the factors that affect thesis publication.

Methods: Endocrinology theses between January 1980 and April 2023 were assessed. The publication rates of theses and those published in journals indexed in SCIE and Scopus were examined. The thesis topics, study design, institution, index of the journal, author's number of first-author publications, H-index, and number of publications by thesis advisors were analyzed to determine their impact on the likelihood of publication.

Results: Out of 277 theses, 142 (51.3%) of them had been published in international or national journals. One hundred seventeen (42.2%) were published in SCIE/Scopus indexed journals. A relationship was found between the thesis having a publication and that being conducted in a training and research hospital, a higher number of first-author publications, and a more recent year of the thesis. The H-index of thesis advisors for theses published in SCIE/Scopus-indexed journals was significantly higher (p=0.029).

Conclusion: The rate of publication in international peer-reviewed journals for endocrinology theses was higher than the national average. However, there are still many theses waiting to be published. Enhancing the publication rate of endocrinology theses requires a systematic approach that addresses the identified factors affecting publication probability.

Keywords: Endocrinology theses, publication rate, peer-reviewed journals, factors affecting publication, academic research

Introduction

Scientific theses are academic texts that systematically present data and analyses obtained through study. These provide experience for assistants in specialized education to assess their research skills, critical thinking abilities, analytical skills, and ability to use scientific methods (1). When examining the publication rates of specialty theses in various medical fields in Turkey, the rates range from 6.2% to 48.3% (2-6). Indeed, more than half of these are not published. Failure to publish theses in national or international peer-reviewed journals implies a waste of effort, time, resources, and intelligence, as well as a missed opportunity to disseminate knowledge. When a thesis is viewed solely as a means to complete specialized education, the quality of the study may decrease (7,8). The publication of a thesis in a peer-reviewed journal signifies that the generated knowledge is deemed acceptable within the scientific community, indicating the quality of the study (9,10).

In our country, the subspecialty program of endocrinology and metabolism requires an additional 3-year training period, which has been entered through an examination since 2007, after completing the main specialization program in internal medicine. For the specialization in internal medicine, it is mandatory to conduct a thesis; however, presenting the thesis is sufficient, and it is not mandatory to publish

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the thesis as a scientific publication. In our country, the requirement for a thesis in the subspecialty of endocrinology and metabolism was abolished in 2014. However, there are still some specialists who choose to conduct a thesis after this date. The additional training period required for subspecialization, the desire of physicians to specialize in a more in-depth manner, and their focus on a more specific patient population or medical condition may increase the academic expectations of subspecialists. There has been no study conducted in our country regarding the factors influencing the publication of endocrinology fellowship theses.

In this study, we evaluated the publication rate of endocrinology theses in international peer-reviewed journals and the factors influencing theses' likelihood of publication through a bibliometric analysis of theses published between 1980 and 2023.

Methods

Compliance with Ethical Standards

This study is a bibliometric analysis. Because the study was conducted by researching public databases on open websites and did not involve animals or humans, it does not require official ethical committee approval or informed consent. This study was conducted in accordance with the Helsinki Declaration and the registration and conditions of the National Thesis Center of the Higher Education Council.

Study Design

The Endocrinology and Metabolism subspecialty theses between January 1980 and April 2023 were scanned from the online archive of the National Thesis Center of the Higher Education Council from April 13 to April 30, 2023. The detailed search terms and fields used are shown in Figure 1. A total of 115 theses were identified after selecting "Minor Specialization in Medicine" as the search criterion. Pediatric endocrinology subspecialty theses were excluded from the study. The remaining 93 adult endocrinology subspecialty theses were included in the study. All of them were from 2000 onwards. To access theses that could not be identified through detailed searches, the membership list of the Turkish Society of Endocrinology and Metabolism on their official website was also scanned again in the National Thesis Center. An additional 184 adult endocrinology thesis data were obtained and included in the study. In total, 277 thesis data points were recorded (Figure 2).

Theses were searched on the Web of Science, Scopus, TR-Dizin, and Google Scholar. The published theses were divided into three categories: those published in international journals indexed in Science Citation Index-Expanded (SCIE) and Scopus; those published in other international journals; and those published only in national journals indexed in TR-Dizin. Publications that fit into multiple categories were hierarchically included in the upper category. The province and institution where the theses were conducted, thesis year, thesis topics, study design, publication dates of published theses, index of the journal where they were published, the author's number of first-author publications in journals indexed in SCIE and Scopus, the author's Scopus H-index, number of publications by thesis advisors in journals indexed in SCIE and Scopus, H-index of the thesis advisor, academic status of the thesis advisor, and gender of the author and thesis advisor were recorded. These parameters were compared between theses with publications in SCIE- and Scopus-indexed journals and those with publications in other indices (other international journals and national journals indexed only in TR-Dizin). The publication rates of theses and those published in journals indexed in SCIE and Scopus were examined. Since theses started to be scored for academic promotion after 2016, the publication rate of theses before and after 2016 was analyzed.

Statistical Analysis

The data analysis was performed using SPSS 25 (Statistical Package for Social Sciences). Descriptive statistics are presented as median (minimum-maximum) and mean ± standard deviation for numerical variables

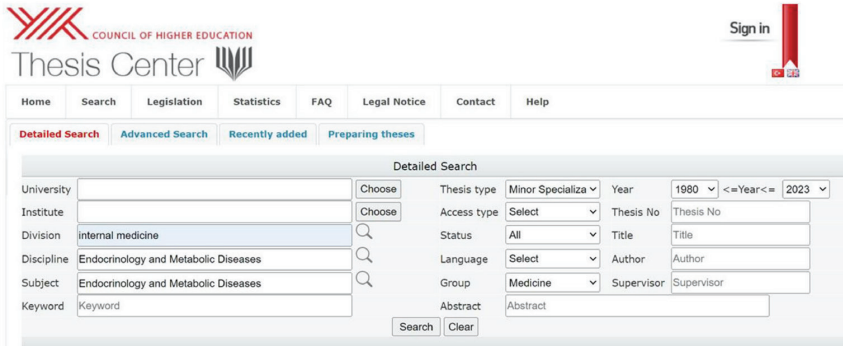

Figure 1. The search criteria used in the online archive of the Higher Education Council National Thesis Center

and as observation count and percentage (%) for nominal variables. The normality of the distribution of numerical variables was tested using the Kolmogorov-Smirnov test. The presence of a statistically significant difference in numerical variables between the two groups was evaluated using the Mann-Whitney U test. Nominal variables were assessed using the chi-square test, Fisher's exact test, and Fisher-Freeman-Halton exact test. A logistic regression analysis was conducted to determine the criteria influencing the publication of theses. The odds ratios (OR), 95% confidence intervals (95% CI), and p-values for each variable were reported. The results were considered statistically significant at $p<0.05$.

Results

A total of 277 theses were included in this study. The descriptive data are presented in Table 1. The distribution of theses by province and year is shown in Figures 3 and 4, respectively. The most common topics for theses were thyroid and diabetes (Table 1). Twenty-one (90.6%) theses were conducted at universities, while 26 (9.4%) were conducted at training and research hospitals.

Of the 277 theses, 142 (51.3%) had been published in international or national journals. Among these, 117 (42.2%) were published in SCIE/Scopus-indexed journals, 107 (38.6%) were published in SCIE-indexed journals, 8 (2.9%) were published in other international indexed journals, and 17 (6.1%) were published in national journals indexed only in TR-Dizin (Table 2, Figure 5). The time to the publication of theses was a mean of 3.8±3.3 years, with a median of 3 years (range: 0-21).

When looking at theses in 21-year intervals, between 1980 and 2000, 19 theses (33.3%) were published, while between 2001 and 2023, 123 theses (55.9%) were published. When looking at theses in 10-year intervals, the highest number of publications occurred between 2010 and 2019 (n=67, 47.2%) (Figure 6). When considering 21-year intervals, specifically between 2001 and 2023, it had the highest number of publications (n=123, 86.6%). Of the published theses (n=142), 90 (49.2%) were conducted in the three major cities (Ankara, Istanbul, and Izmir), while 52 (55.3%) were conducted in other cities.

The authors' H-index had a mean of 11.5±7.2, with a median of 11 (range: 0-51). The authors' mean number of

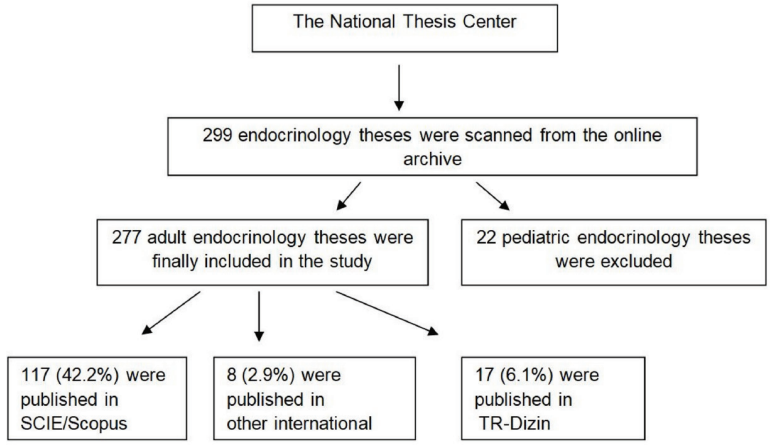

Figure 2. Flow chart of selection of endocrinolgy theses

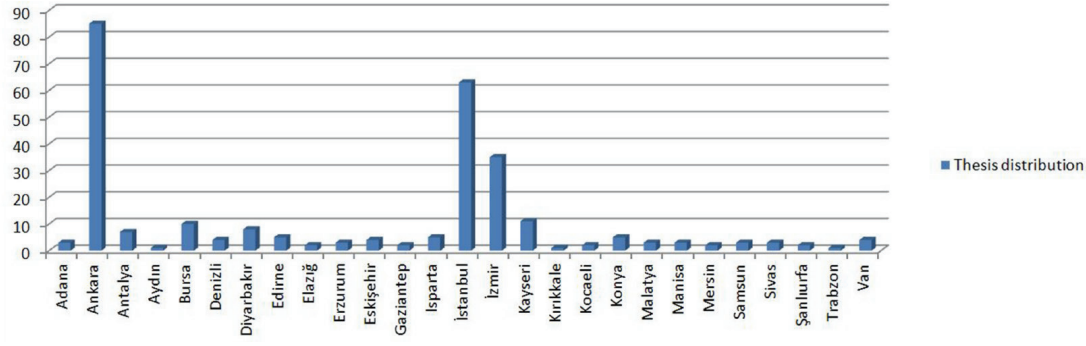

Figure 3. Distribution of thesis by provinces

Table 1. Descriptive datas of study	
Datas	
Thesis distribution by year	
1980-1989	4 (1.4%)
1990-1999	46 (16.6%)
2000-2009	110 (39.7%)
2010-2019	113 (40.8%)
2020 and beyond	4 (1.4%)
Thesis distribution by institution	
University	251 (90.6%)
Training and Research Hospital	26 (9.4%)
Thesis distribution by provinces	
Ankara	85 (30.7%)
Istanbul	63 (22.7%)
Izmir	35 (12.6%)
Kayseri	11 (4%)
Bursa	10 (3.6%)
Other provinces total	73 (26%)
Study design	
Clinical	257 (92.8%)
Experimental	20 (7.2%)
Animal	14 (5.1%)
Cell	3 (1.1%)
Sugery material	3 (1.1%)
Author gender	
Female	125 (45.1%)
Male	152 (54.9%)
Author's H-index	11 (0-51)
First-author publication	3(0-38)
Thesis advisor gender	
Female	80 (32.8%)
Male	164 (67.2%)
Thesis advisor academic title	
Professor	168 (68.9%)
Associate professor	71 (29.1%)
Assistant professor	5 (2%)
Thesis advisor's H-index	19 (1-51)
Thesis advisor publication count	67 (1-313)
Topic	
Thyroid	78 (28.2%)
Diabetes	73 (26.4%)
Hypophysis	30 (10.8%)
Obesity	20 (7.2%)
Bone metabolism	19 (6.9%)
Polycystic ovary syndrome	17 (6.1%)
Pregnancy and endocrine diseases	10 (3.6%)
Other topics total	34 (12.2%)
Data are expressed as median (interquartile range) or number (percentage)	

first-author publications was 4.6±5.1, with a median of 3 (range: 0-38).

The thesis advisors' H-index had a mean of 19.1±7.4, with a median of 19 (range: 1-51). The thesis advisors' average number of publications was 76.7±49.1, with a median of 67 (range: 1-313).

When the published and unpublished theses were compared, there were statistically significant differences in the number of first author publications, thesis year, 10-year interval, 21-year interval, and institution status ($p<0.001$, $p=0.024$, $p=0.001$, $p=0.002$, $p=0.011$, respectively) (Table 3). In the regression analysis, a relationship was found between the thesis having a publication and the thesis being conducted in a training and research hospital, a higher number of first-author publications, and a more recent year of the thesis (Table 4).

The H-index of thesis advisors for theses published in SCIE/Scopus-indexed journals was statistically significantly higher than that of thesis advisors for theses published in other international indexes or only in TR-Dizin ($p=0.029$). Additionally, the majority of these publications occurred after 2000 ($p=0.045$) (Table 5).

Discussion

In our study, the publication rate of endocrinology theses was 51.3%, with a publication rate of 42.2% in SCIE/Scopus-indexed journals. The publication of endocrinology theses was found to be associated with the thesis being conducted in a training and research hospital, a higher number of first-author publications by the author, and a more recent year of the thesis. Thesis advisors of theses published in SCIE/Scopus-indexed journals had higher H-index values, and a significant portion of these publications were from theses conducted after 2000.

In our study, we found that the overall publication rate of endocrinology theses was higher than the overall average in Turkey. de Nonneville et al. (11) demonstrated that 70% of medical oncology theses in France resulted in publications. The higher publication rate of minor

Table 2. Publication rate of endocrinology theses		
	n=277	**%**
Theses published in journals indexed in SCIE/ Scopus	117	42.2
Theses published in journals indexed in SCIE	107	38.6
Theses published in other international indexed journals	9	3.2
Theses published in national journals indexed only in the TR-Dizin	16	5.8
Number of published theses	142	51.3
Number of unpublished theses	135	48.7
Data are expressed as number (percentage)		

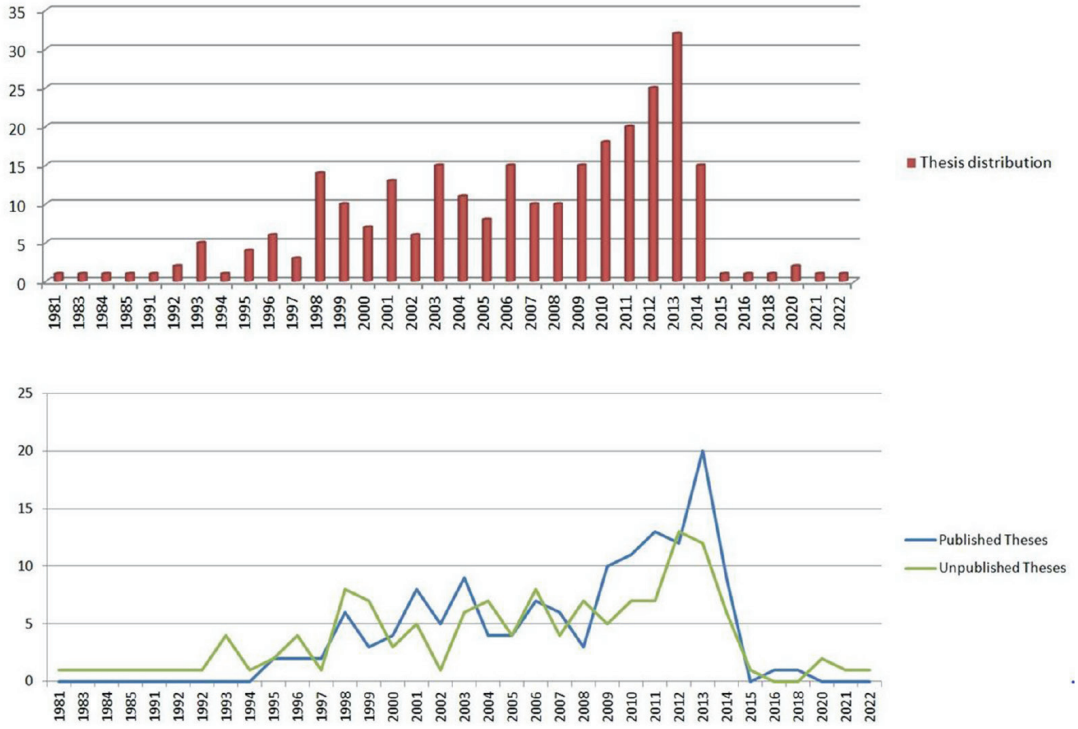

Figure 4. Distribution of theses by years and publication of theses by year

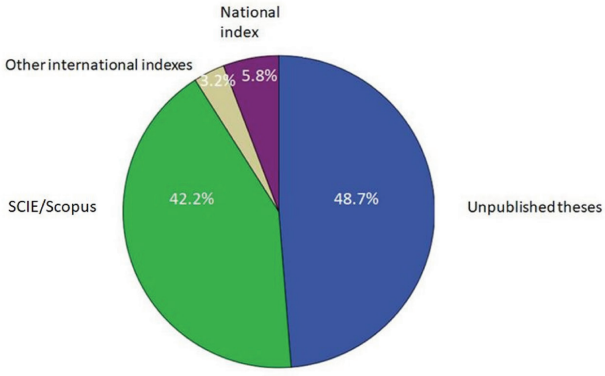

Figure 5. Publishing rates of thesis

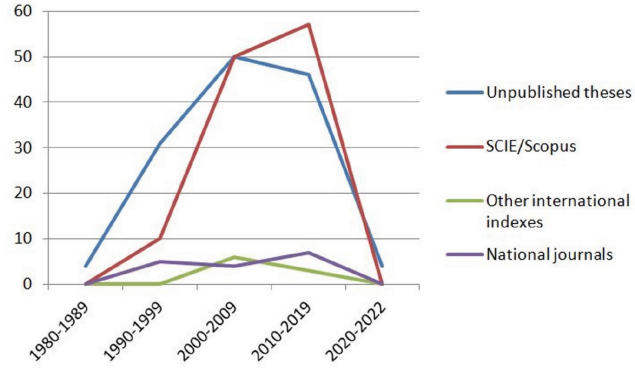

Figure 6. Theses published according to 10-year intervals

specialization theses may be due to higher academic expectations among minor specialization experts.

In 2012, Sipahi et al. (7) showed that the rate of publication of theses in national journals is higher than that in international journals. However, in our country, the publication rate of medical theses in SCIE and other international indexed journals has increased over the years. Researchers may have realized that publishing their work in international journals can enhance scientific collaboration, expand their readership, and gain greater international recognition, and they may have developed a preference for publishing in international journals as well as local journals. In addition, this can be attributed to the selection of thesis topics that contribute to the scientific literature and are internationally recognized, resulting in higher-quality publications. In addition, the influence of academic promotion criteria, which assign higher scores to studies published in international peer-reviewed journals, may have affected journal selection.

Table 3. Comparison of published and unpublished theses			
	Published theses	**Unpublished theses**	**p-value**
Study design			0.909*
Clinical trial Experimental trial	131 (92.3%) 11 (7.7%)	126 (93.3%) 9 (6.7%)	
Author gender			0.985*
Female Male	64 (45.1%) 78 (54.9%)	61 (45.2%) 74 (54.8%)	
Author's H-index	10 (1-51)	11 (0-32)	0.716$^{\delta}$
First-author publication	4 (0-38)	2 (0-26)	**<0.001$^{\delta}$**
Thesis advisor gender			0.657*
Female Male	41 (31.5%) 89 (68.5%)	39 (34.2%) 75 (65.8%)	
Thesis advisor academic title			0.731˜
Assistant professor Associate professor Professor	2 (1.5%) 40 (30.8%) 88 (67.7%)	3 (2.6%) 31 (27.2%) 80 (70.2%)	
Thesis advisor's H-index	19.5 (1-51)	19 (4-36)	0.313$^{\delta}$
Number of thesis advisor publications	67 (1-313)	64.5 (8-167)	0.256$^{\delta}$
Thesis year	2009 (1995-2018)	2006 (1981-2022)	**0.024$^{\delta}$**
10-year interval			
1980-1989 1990-1999 2000-2009 2010-2019 2020-2023	- 15 (10.6%) 60 (42.3%) 67 (47.2%) -	4 (3%) 31 (23%) 50 (37%) 46 (34.1%) 4 (3%)	**0.001˜**
21- year interval			
1980-2000 2001-2023	19 (13.4%) 123 (86.6%)	38 (28.1%) 97 (71.9%)	**0.002***
Institution province			0.333*
Three largest cities Other cities	90 (63.4%) 52 (36.6%)	93 (68.9%) 42 (31.1%)	
Institution status			0.011*
University Training and Research Hospital	122 (85.9%) 20 (14.1%)	129 (95.6%) 6 (4.4%)	

Data are expressed as median (interquartile range) or number (percentage); p<0.05
The values with p<0.05 are shown in bold.
*Chi-square
˜Fisher-Freeman-Halton Exact test
$^{\delta}$Mann-Whitney U test

Table 4. Examining the factors affecting the publication of thesis by regression analysis						
		B	**Sig. (p)**	**OR**	**95% CI for Exp. (B)**	
					Lower	**Upper**
Step 1[a]	Thesis year	0.060	0.003	1,062	1,020	1,105
	Institution status (University)	-1,206	0.015	0.299	0.113	0.794
	First-author publication	0.144	0.000	1,155	1,079	1,236
	Constant	-119,460	0.003	0.000		

OR: Odds ratio, CI: Confidence interval, Sig.: Significant

Table 5. Comparison of theses published in journals indexed in SCIE/Scopus and theses published in other international/national indexes			
	Theses published in journals indexed in SCIE/ Scopus (n=117)	**Theses Published in journals indexed other international indexes/only TR-Dizin (n=25)**	**p-value**
Study design			0.409**
Clinical trial Experimental trial	109 (93.2%) 8 (6.8%)	22 (88%) 3 (12%)	
Author sex			0.918*
Female Male	52 (44.4%) 65 (55.6%)	12 (48%) 13 (52%)	
Author's H-index	10 (1-51)	7 (1-22)	0.099$^{\delta}$
First-author publication	4 (0-38)	5 (0-12)	0.821$^{\delta}$
Thesis advisor sex			0.386*
Female Male	36 (33.6%) 71 (66.4%)	5 (21.7%) 18 (78.3%)	
Thesis advisor academic title			0.468˜
Assistant professor Associate professor Professor	1 (0.9%) 33 (30.8%) 73 (68.2%)	1 (4.3%) 7 (30.4%) 15 (65.2%)	
Thesis advisor's H-index	20 (4-51)	16 (1-36)	**0.029$^{\delta}$**
Number of thesis advisor publications	68 (5-313)	62 (1-117)	0.115$^{\delta}$
Thesis year	2009 (1995-2018)	2006 (1995-2014)	0.306$^{\delta}$
10-year interval			0.232*
1980-1989 1990-1999 2000-2009 2010-2019 2020 and beyond	- 10 (8.5%) 50 (42.7%) 57 (48.7%) -	- 5 (20%) 10 (40%) 10 (40%) -	
21-year interval			**0.045˜**
1980-2000 2001-2022	12 (10.3%) 105 (89.7%)	7 (28%) 18 (72%)	
Institution province			0.875*
Three largest cities Other cities	75 (64.1%) 42 (35.9%)	15 (60%) 10 (40%)	
Institution status			0.528˜
University Training and Research Hospital	99 (84.6%) 18 (15.4%)	23 (92%) 2 (8%)	

Data are expressed as median (interquartile range) or number (percentage); p<0.05
The values with p<0.05 are shown in bold.
*Chi-square
˜Fisher-Freeman-Halton Exact test
$^{\delta}$Mann-Whitney U test
**Fisher's exact test

Most endocrinology theses were published after 2000, indicating a relationship between thesis publication and more recent years. Several factors may have contributed to this trend, such as the recent promotion of original research in medical disciplines, the advancement of technical capabilities in training institutions for assistants, easier access to reference articles through widespread internet use, and simplified submission processes to journals, which may have encouraged authors to pursue thesis publication (12). Indeed, an increase in the usage of English and the availability of support from organizations providing foreign language assistance may have led to an increase in authors' submissions to international journals.

In different specialization fields in our country, the average time for publications derived from theses ranges from 2.8 to 3.8 years (2,6,12-15). This is a longer period of time than the two years in international settings (10). After completing specialization training in our country,

mandatory working in state hospitals with a high workload and departure from academic environments may have delayed the conversion of theses into publications.

In our study, the average publication time of endocrinology theses was found to be 3.8 years, which is longer than the average published in Turkey and the global literature. Although studies derived from endocrinology theses are published more in journals indexed in databases such as SCIE and Scopus, the longer publication time compared with the literature may be attributed to incorrect journal selection and delays in peer review processes due to the focus on journals that do not charge fees due to high application and publication fees.

In terms of publication rates and the databases in which theses are published, no significant differences were found among state universities, training and research hospitals, and private universities (14,16). In another study, it has been shown that the publication rate of theses completed in university hospitals is five times higher compared to those conducted in state hospitals (3). This difference may be due to more than a 10-year difference between the research years of the studies. In our study, the majority of theses were conducted at state universities. However, it was found that the institution associated with the publication of theses was primarily a training and research hospital. With a larger patient population and better financial resources, training and research hospitals may have an advantage in accessing the materials and technical facilities required for thesis research.

In our study, the publication rates were similar between clinical and experimental studies. In the literature, the study design has been associated with the publication status of theses (2,17). It has been shown that experimental studies are published more frequently in SCIE-indexed journals (4,13,16,18). There are also studies that found similar publication rates for clinical prospective studies and animal experiments (12,15). Both are valuable types of scientific studies. In our study, no significant difference was observed between the publication rates of clinical and experimental studies. However, it is important to note that the number of experimental studies in our study was much lower than that in clinical studies.

In our study, a decrease in the publication rate of theses after 2014 was observed (Figures 4 and 6). This decline can be attributed to the removal of the subspecialty training thesis in our country. Similar to our study, in a study where the thesis was removed from the postgraduate curriculum in India, a significant decrease in publications from those departments was observed during that period (19). When evaluating the impact of the changes in academic promotion criteria in our country in 2016 and the inclusion of publications derived from theses in the scoring system, it can be observed that most publications were from theses completed before 2016. Although it was not mandatory before 2016, the high rate of thesis publications indicates the high level of academic activity and interest among endocrinologists.

In the literature, factors such as having another article in which the author is the first author, being the first author in the thesis publication, and having a higher number of author publications have been identified as important factors in the conversion of theses into publications (2,14). The number of first-author publications, as well as metrics like the H-index, are important indicators of academic activity. In our study, the number of first-author publications was associated with thesis publication. However, the H-index of authors was similar between theses that were published and those that were not. In studies, the first authors assume significant responsibilities at various stages, from study design to publication. In multicenter or national data studies, even if the experience and knowledge of scientific publishing may not be sufficient, co-authors can have a high H-index because of the citations received. Despite the recognition of the H-index as an indicator of academic activity, authors may have few or no first-author publications. In addition, presenting the thesis as an oral presentation, which is sufficient for academic promotion, may have a negative impact on authors' motivation to publish their theses. Ultimately, in our study, the number of first-author publications, which enhances the experience of scientific publishing, was found to have a greater impact on thesis publication than the H-index of authors.

There are studies investigating the factors related to the thesis advisor in the publication of the thesis. In our study, it was observed that the thesis advisors of authors with published theses in SCIE/Scopus had higher H-index scores. This indicates the importance of the academic activity of thesis advisors in the publication of theses. However, in another study, the impact of the advisor H-index was not found to be significant (2). There are studies that show the influence of the academic title of thesis advisors (such as assistant professors) as an effective factor in the publication of theses, as well as studies that demonstrate no significant impact (2,5,14). Theses are important sources in terms of increasing the publication status of both the advisor and the author (10). Therefore, it is possible for assistant professors who serve as thesis advisors at the early stages of their academic careers to provide more support to the authors. In our study, the advisor's academic title was not found to be a significant factor influencing publication. However, most of the thesis advisors were professors, and there were very few assistant professors. Another factor, the publication count of the thesis advisor, did not have a significant effect on our study. While the H-index of thesis advisors is a significant

factor, the number of publications does not appear to have a significant effect, highlighting the importance of quality publications. However, there are studies in the literature that find the advisor's publication count to be an important factor in the publication of theses (2).

Study Limitations

The possibility of certain theses going undetected is a potential limitation of our study due to the registration of some theses uploaded to the national thesis center being recorded as "specialization in medicine" instead of "minor specialization in medicine". Despite these limitations, this is the first study that sheds light on the factors affecting the publication of adult endocrinology theses and analyzes adult endocrinology theses bibliometrically by scanning both national and international journal indexes.

Conclusion

Our study is the first to be conducted on subspecialty theses in our country. In our study, the rate of publication in SCIE/Scopus indexed journals for endocrinology theses was higher than the national average. However, there are still many theses waiting to be published. The academic expectation is higher for subspecialists who have received additional training, which is entered by an exam to specialize in more specific subjects. The publication of theses contributes to the scientific literature, disseminates knowledge and experiences, and enhances the academic recognition of healthcare professionals in our country. It is important to provide specialists with the necessary support, such as time, financial assistance, and training, to ensure the production of high-quality theses and facilitate their publication, particularly in internationally indexed journals.

Ethics

Ethics Committee Approval: This study is a bibliometric analysis. Because the study was conducted by researching public databases on open websites and did not involve animals or humans, it does not require official ethical committee approval.

Informed Consent: This study is a bibliometric analysis. Because the study was conducted by researching public databases on open websites and did not involve animals or humans, it does not require official informed consent.

Authorship Contributions

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

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Development and Validation of a Clinical Decision-Making Scale for Medical Students

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Abstract

Aim: No scale has been found in the literature that allows for determining the clinical decision-making level of medical school students in Turkey. We aimed to develop a valid and reliable scale to determine the clinical decision-making levels of medical students.

Methods: This descriptive study was conducted between October and November 2021. Interviews were conducted with 12 clinician faculty members through semi-structured interview forms created by considering expert opinions, and an item pool was created through content analysis with support from the literature. The item pool was piloted with 20 medical students, and their feedback was used to revise the scale items. The final version of the scale was administered to 332 fourth, fifth, and sixth-grade medical students undergoing clinical training. Data were analyzed using exploratory and confirmatory factor analysis, reliability analysis, and descriptive statistics.

Results: The results of the explanatory factor analysis indicated that the scale had 27 items and a three-factor structure, which explained 67,905% of the total variance. The three factors were identified as "1-defining the problem and determining its causes", "2-evaluating alternatives", and "3-individual and institutional factors". Confirmatory factor analysis results confirmed the three-factor model, and fit indices indicated a good fit between the model and data. The reliability analysis showed that the scale had high internal consistency, with a Cronbach's alpha coefficient of 0.94.

Conclusion: The study suggests that the Clinical Decision-Making Scale is a valid and reliable tool for assessing medical students' clinical decision-making competence in Turkey.

Keywords: Clinical decision-making, scale development, medical student

Introduction

Clinical decision-making, which is a type of medical decision-making, is to choose and apply the most appropriate, useful, and acceptable option among the options for solving the patient's problem (1,2). The clinical decision-making process, which is quite complex, has cognitive dimensions such as observation, interpretation, explanation, questioning, analysis, evaluation, and experience (3). Numerous factors affect the clinical decision-making process. Many factors, such as the patient's current condition, urgency, risks, existence of alternative options, chance of success, and the doctor's individual opinion, affect the clinical decision-making process. However, individual factors such as the patient's personality, values, wishes, knowledge and understanding capacity, competencies, and costs, legal and political situations, and society's perspective also affect this process (4). It is effective on decision-making personality characteristics such as the physician's self-perception, psychological state, personality structure such as emotional, shy, and excited (5), and the physician's self-confidence, autonomy, professionalism, creativity, critical and analytical thinking, problem-solving ability, and ethical values (6). Although there are many factors affecting this process, when clinical decision-making is done appropriately, the quality of patient care increases, medical errors decrease, costs decrease because resources are used effectively, and patient satisfaction increases (7).

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Undoubtedly, experience is one of the most important factors in clinical decision-making. Students' clinical decision-making skills can be acquired at every stage of their education and increase with clinical experience (8). Some studies have revealed that the perception of clinical decision-making increases with education (9,10). From this perspective, it is important to determine the clinical decision-making level of medical students and plan the necessary interventions before graduation.

No scale has been found in the literature that allows for determining the clinical decision-making level of medical school students in Turkey. Therefore, we designed this study to develop a valid and reliable scale to assess the clinical decision-making level of medical school students in Turkey.

Methods

Compliance with Ethical Standards

Ethical approval was received by Aydin Adnan Menderes University Education Research Ethics Committee decision number 2021/20-IV, and dated 06.09.2021. To collect data, written consent was obtained from the institution where the research was conducted, and verbal consent was obtained from the participants after informing them about the research. Volunteer participants were included in the study.

The Type of Research

This research is a descriptive cross-sectional study conducted between October and November 2021.

Data Collection Instrument

For the interviews to be held and the research to be applied to students, opinions were received from a measurement and evaluation specialist and a medical education specialist. After the corrections were suggested in line with expert opinion, one-on-one interviews were conducted with 12 clinician faculty members working at the Faculty of Medicine to create the item pool. Interviews were held in an appropriate time frame by making an appointment with the faculty members and lasted an average of 10 minutes. The data obtained from one-on-one interviews with faculty members was subjected to content analysis using the line-by-line reading technique, and a draft item pool of 42 items was created with the support of the literature. The draft item pool was subjected to the opinions of 10 faculty members working at the Faculty of Medicine to calculate the expert opinion score. The expert opinion method was used to determine the validity of the content. After the content validity index (CVI) and content validity ratio (CVR) coefficients were calculated after the opinions, it was determined that an additional specialist was needed, and three more medical faculty members were asked for their opinions. After the opinions, the CVR was recalculated, and six items were removed from the scale (CVR=0.778). With this 36-item version of the scale, a pilot study was conducted with 20 medical students in the fourth, fifth, and sixth grades who were not included in the study. The scale items were revised according to the feedback provided after the pilot application. Responses to the scale items (5-point Likert) were formed as "strongly disagree", "disagree", "neither agree nor disagree", "agree", "strongly agree". There is no reverse item in the scale, and a high score indicates a high level of clinical decision-making competence, whereas a low score indicates a low level of clinical decision-making competence.

Research Group

With the scale created, data were collected from 332 students studying in the 4th, 5th, and 6th grades at the same university in October-November 2021. Considering the necessity for the sample to represent the universe, Nunnually (11) stated that 300 people were sufficient, and Comrey and Lee (12) stated that the level of representation was good for 300 people. Because these classes began clinical training, we decided to select them as the research group. The minimum number of participants for factor analysis was calculated by taking at least five times the number of items on the scale (13).

Study Inclusion and Exclusion Criteria

In determining the participants in the study, attention was paid to the faculty members being clinicians and volunteering to participate in the study. In determining the students, care was taken to ensure that they were in the clinical teaching period and volunteered to participate in the study. Participants who did not volunteer and provided incomplete responses to the study data were excluded from the study.

Statistical Analysis

SPSS version 22 was used for the explanatory factor analysis (EFA) and reliability analyses of the scale, and the Lisrel 8.80 program was used to verify the factor structure. In EFA, the scale was able to separate items that do not measure the desired structure and load more than one dimension (14) and make the relevant variables some meaningful and independent factors (15). In the CFA, fit indices were examined. Factor analysis of the data was performed by the Kaiser-Meyer-Olkin (KMO) and Bartlett sphericity tests. Possible factorizations that may occur in factor analysis are determined by the Varimax rotation of the axes. The model fit was evaluated using CFA fit indices. Cronbach's alpha coefficients were calculated for reliability analysis. A Cronbach's alpha coefficient of 0.70 or higher is considered sufficient for scale and factor scores (16).

Results

Findings on Validity

Explanatory factor analysis was performed to determine how many dimensions and which items the factor structure included with the collected data. First, the KMO sampling adequacy measurement and the Bartlett sphericity test were performed to assess the support of the data for EFA. The KMO and Bartlett tests are frequently used in factor analysis of study data. The KMO measure of sampling adequacy ranges from 0 to 1, with higher values indicating better suitability for factor analysis (16). In this case, the KMO value of 0.951 suggests that the data are highly suitable for factor analysis. A significant result ($p<0.05$) suggests that the variables are correlated and suitable for factor analysis. In this case, the test statistic is very large (7872.412) with 351 degrees of freedom, and the p-value is very small (0.000), indicating that the variables are significantly correlated and suitable for factor analysis.

In Table 1, the scale items, factors, item factor loads, explained variance, and reliability coefficients are presented. In determining the number of factors, eigenvalues, scree plots, and variances explained by the factors were examined. When the slope graph in Figure 1 is examined, it is seen that the slope plot has turned horizontal starting from the third factor.

Table 1. Clinical Decision-Making Scale items, factors, item factor loadings, explained variance, and reliability coefficients

	Rotated component matrix*		
Scale items	**Factor 1 (Defining the problem and determining its causes)**	**Factor 2 (Evaluating alternatives)**	**Factor 3 (Individual and institutional factors)**
I observe to describe the patient's problems in clinical decision making	0.862		
I make inquiries describe the patient's problems in clinical decision making	0.824		
I use the patient's prior knowledge to identify the problem in clinical decision making	0.796		
I interpret the data while identifying possible causes for clinical decision making	0.756		
I consider the urgency of the patient to define the problem in clinical decision making	0.751		
I analyze all the information at my disposal when identifying possible causes for clinical decision making	0.725		
I use my knowledge gained through experience when collecting and evaluating information for clinical decision making	0.638		
I think analytically when identifying possible causes in clinical decision making	0.632		
I consider the patient's current medical condition when determining possible causes in clinical decision making	0.623		
I use my evidence-based medical knowledge to collect and evaluate information for clinical decision making	0.621		
I use my professional experience to define problems in clinical decision making	0.610		
While defining the problem in clinical decision making, the time I can spare for the patient affects my decision	0.606		
I check the compliance of my decisions with the guidelines/algorithms determined by national and international organizations		0.717	
I consider the purpose of the treatment when evaluating clinical decision making		0.715	
I consider the risk of treatment in clinical decision making		0.695	
Multidisciplinary behavior while making a decision provides more accurate clinical decisions		0.690	
My constant research/literature reading enables me to make better clinical decisions		0.677	
I can change my decisions according to the patient's economic situation		0.672	
I consider the most likely diagnoses first in clinical decision making		0.602	
I conduct research to identify possible causes of clinical decision making		0.599	
I will look to determine if there is an alternative to treatment in clinical decision making		0.543	
I consider the patient's culture when collecting information and evaluating clinical decision making			0.824
I care about the patient's values/religious beliefs when collecting information and evaluating clinical decision making			0.786

Table 1. Continued			
	Rotated component matrix*		
Scale items	Factor 1 (Defining the problem and determining its causes)	Factor 2 (Evaluating alternatives)	Factor 3 (Individual and institutional factors)
The infrastructure/opportunities of the institution for which I work affect my clinical decision			0.723
I consider institution policy when evaluating clinical decision making			0.712
I consider the legal situation (malpractice, etc.) while making an evaluation in clinical decision making			0.676
I consider the patient's place of residence when collecting and evaluating information for clinical decision making			0.597
Variance explained	55,423	8,013	4,469
Cronbach's alpha	0.956	0.934	0.875
*Extraction method: Principal component analysis. Rotation method: Varimax with Kaiser normalization. a. Rotation converged in four iterations			

In this case, the first factor explained 55.423% of the total variance, the second factor explained an additional 8.013%, and the third factor explained an additional 4.469%. Together, these three factors explain 67.905% of the total variance. The remaining PCs each explain smaller amounts of variance. It was determined that the amount of variance explained met the rule of 2/3 of the total variance proposed by Buyukozturk (16).

In factor analysis, the goal is to identify the underlying factors that explain the correlations among a set of observed variables. The matrix shows the loadings (i.e., the correlation between the variable and the factor) of each variable on each of the three identified components. The higher the loading, the stronger the association between the variable and the component. Based on this matrix, it appears that three components explain the correlations among the variables. The highest factor load of the scale, whose three-factor structure was determined, was 0.862, and the lowest factor load was 0.543. When the factors were examined, the first factor was named "defining the problem and determining its causes", the second factor was named "evaluating alternatives", and the third factor was named "individual and institutional factors". The path diagram of the Clinical Decision-Making Scale is shown in Figure 1.

Cronbach's alpha coefficients were computed for the internal consistency of the scale whose factors were settled. Because of the analysis, α=0.965 for the 27-item Clinical Decision-Making Scale, α=0,956 for the first factor, α=0.934 for the second factor, and α=0.875 for the third factor (Table 1). Item-total statistics tables were examined, and no significant increase was observed in the Cronbach's alpha coefficients of the scales when any item was removed.

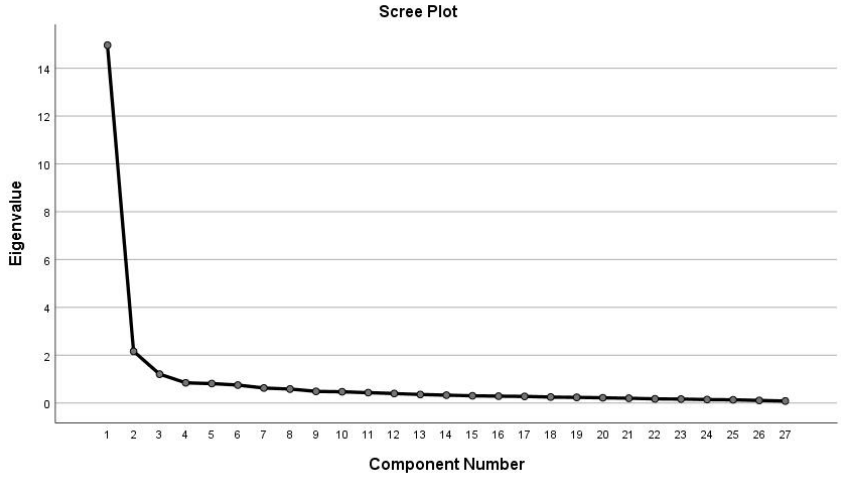

Figure 1. Scree plot of the three factors included in the Clinical Decision-Making Scale

Confirmatory Factor Analysis

Consequent to the CFA for the three-dimensional factor structure of the scale (Figure 2), the ratio of the chi-square statistic to the degrees of freedom (χ^2/df) was 3.64 (χ^2=1151.31; df=316; p=0.000); Root Mean Square Error of Approximation=0.091; Goodness of Fit Index=0.79; Adjustment Goodness of Fit Index=0.75; Comparative Fit Index=0.97; Normed Fit Index=0.96; Tucker Lewis Index=0.97; Relative Fit Index=0.96; Incremental Fit Index=0.943; Root Mean Square Residual=0.065; and Parsimony Normed Fit Index=0.75 were found. The results show that the three-dimensional factor structure of the Clinical Decision-Making Scale provides acceptable fit values (17).

Discussion

The newly developed Clinical Decision-Making Scale for Medical Students has satisfactory psychometric properties. It was determined that the scale was valid and reliable for evaluating the clinical decision-making skills of medical students. It was observed that the developed scale consisted of three factors and 27 items and explained approximately 68% of the situation to be measured. The CVI and CVR scores of the scale were found to be at an acceptable level. The internal consistency coefficient of the scale is satisfactory. The findings of the study showed that the scale is a valid tool for assessing medical students' clinical decision-making skills.

There are scales for similar purposes in the literature. The reliability of the "Clinical Decision-Making Scale in Nursing" developed by Jenkins and adapted into Turkish by Durmaz Edeer and Sarkaya (18), to determine the clinical decision-making status of nurses, was also found to be high. In addition, the four-factor structure of this scale is similar to that of the developed scale. Another scale developed with a high-level working group to determine nurses' clinical reasoning skills also has a high level of reliability, and the sub-dimensions of this scale are similar to the developed scale (19). Other studies aimed at determining the clinical decision-making status of nurses also show similar characteristics (20,21). Although the clinical decision-making situation of nurses and physicians in the field of health varies, there are common points in the decision-making process and factors affecting decision-making. There are also highly reliable studies that measure the clinical decision-making skills of physical therapy interns (22).

A highly reliable measurement tool has been developed to determine the clinical judgment competence of doctors and healthcare professionals in patients with acute asthma (23). The scale developed by urology doctors and medical students to determine differences in surgical decision-making also showed similar results to those of the research (24). Reliability analyses of the one-dimensional structure of the "Shared Decision-Making Questionnaire-Provider Version" developed by Scholl et al. (25) to determine doctors' shared decision-making behaviors also yielded similar results to the current study. Although the statistical analyses of the developed measurement tools are satisfactory, the results will be different in different study groups because of the variability of the job descriptions of medical students in the hospital. However, it is also important that the studies examined provide content aimed at understanding the clinical decision-making process of medical students. Considering the sub-dimensions of the current study, it not only determines the clinical decision-making level of medical students regarding this culture but also provides information about the clinical decision-making process.

The study showed that the scale could assess students' ability to observe, interpret, explain, inquire, analyze, evaluate, and apply clinical and biomedical knowledge. The scale also evaluated students' understanding of ethical principles and legal regulations in clinical decision-

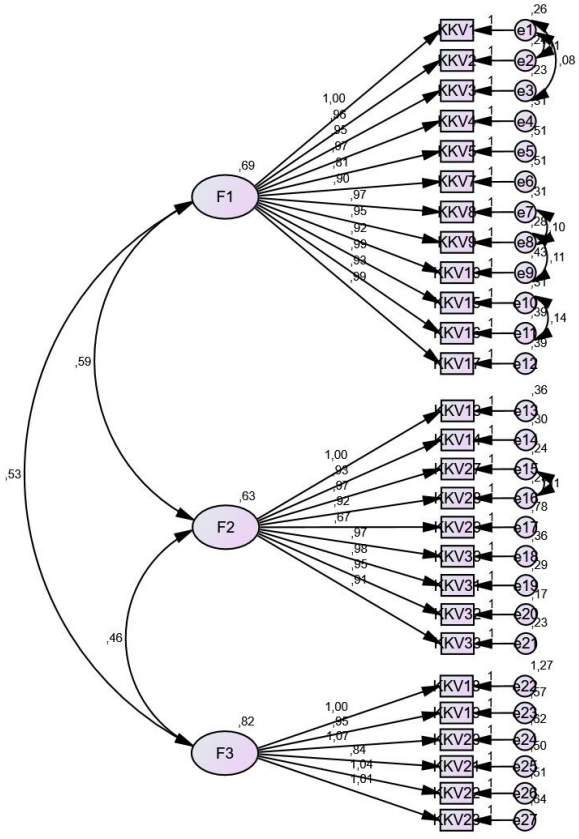

Figure 2. Clinical Decision-Making Scale path diagram

making. The results of the study indicated that the Clinical Decision-Making Scale could be used to evaluate students' competence in clinical decision-making and to identify areas for improvement in their training.

Study Limitations

This study was conducted with students from only one medical school. It can be repeated on different samples, considering regional, cultural, and educational differences, and the validity and reliability of the scale can be repeated by comparing the psychometric properties of the scale. Despite these limitations, the strengths of this study are the high number of participants, the inclusion of students at all stages of the clinical period, and the satisfactory statistical results.

Conclusion

The newly developed Clinical Decision-Making Scale for Medical Students is a valid and reliable scale that can be used to assess the clinical decision-making skills of medical students. The scale can be used to identify the strengths and weaknesses of students' clinical decision-making skills and to design educational interventions to improve their skills. These results were validated by our research group. It was thought that it would be beneficial to conduct a validity and reliability study by applying it to different cultures, societies, and research groups. It was concluded that the scale could be used in future studies.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Aydin Adnan Menderes University Educational Research Ethics Committee (approval no.: 2021/20-IV, date: 06.09.2021).

Informed Consent: Written permission was obtained from Aydin Adnan Menderes University Faculty of Medicine and verbal permission was obtained from the participants.

Authorship Contributions

Concept: H.H.U., A.S.S., Design: H.H.U., A.S.S., Data Collection or Processing: H.H.U., Analysis or Interpretation: H.H.U., A.S.S., Literature Search: H.H.U., Writing: H.H.U., A.S.S.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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MEFV Gene Mutation Analysis in Children with Immunoglobulin A Vasculitis and Its Effects on Clinical Manifestations: A Big Series from a Tertiary Center

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Abstract

Aim: Immunoglobulin A vasculitis (IgAV) is the most common vasculitis of childhood, but its pathogenesis is largely unknown, despite evidence pointing to various environmental and genetic factors. We investigated the frequency of *MEFV* gene mutations that are considered in the pathogenesis and their effect on the clinical features of patients with IgAV.

Methods: The study included 244 children diagnosed with IgAV, who underwent *MEFV* gene analyses. We recorded the demographic and clinical characteristics of the patients, along with their laboratory results. We grouped the patients based on the presence of MEFV gene mutations and MEFV variants.

Results: At least one *MEFV* mutation was detected in 89 (36.5%) patients, with E148Q being the most common (n=31, 34.8%). Age at diagnosis and the frequency of hematuria and recurrence were significantly greater among patients with *MEFV* mutations (p=0.043, p=0.008, and p=0.009, respectively). Serum IgA levels were significantly higher in patients with the M694V mutation (p=0.040).

Conclusion: The presence of *MEFV* mutations, particularly E148Q and M694V, could be associated with the development and clinical course of IgA vasculitis.

Keywords: Children, hematuria, IgA vasculitis, *MEFV* gene, recurrence

Introduction

Immunoglobulin A vasculitis (IgAV), characterized by IgA and immune complex deposition in small vessels, is the most common vasculitis worldwide (1). The primary clinical manifestations of the disease include non-thrombocytopenic palpable purpura, joint gastrointestinal (GI) tract involvement, and renal involvement (2). The etiopathogenesis of IgAV remains unclear, and genetic and environmental factors are thought to contribute to the disease (3). Nonetheless, various recent publications have focused on genetic factors, exemplified by the demonstration of the roles of polymorphisms in genes encoding cytokines and cell adhesion molecules (4-6).

Familial Mediterranean Fever (FMF) is an autoinflammatory disorder characterized by recurrent attacks of fever and serositis. It is caused by mutations in the Mediterranean Fever (*MEFV*) gene that encode pyrin, a protein involved in apoptosis, inflammation, and the secretion of cytokines (7,8). Researchers have identified over 350 mutations in the *MEFV* gene. M694V, M694I, M680I, V726A, and E148Q are the most frequently detected mutations in patients with FMF in Turkey (9,10).

Researchers mentioned that FMF may co-exist with various inflammatory diseases, including IgAV, juvenile idiopathic arthritis, Behcet's disease, inflammatory bowel disease, and polyarteritis nodosa. It has been

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established in the literature that *MEFV* gene mutations lead to an uncontrollable inflammatory reaction (11-13). Immunoglobulin A vasculitis was reported as the most common coexisting vasculitis with FMF. It has been speculated that IgAV patients with *MEFV* gene mutations have greater clinical severity and worse laboratory findings due to an exaggerated inflammatory response (11,14,15). However, the available literature on this topic is limited because of the investigation of only a few common mutations.

In the present study, we aimed to investigate the frequency of *MEFV* gene mutations in children with IgAV and to assess potential relationships between these mutations and the clinical course and laboratory findings.

Methods

Compliance with Ethical Standards

Ethical approval was obtained from the Istanbul Medeniyet University, Goztepe Training and Research Hospital Clinical Research Ethics Committee (approval no.: 2023/0059, date: 25.01.2023) before the experiment was started, and the experiment was conducted in accordance with the principles set forth in the Helsinki Declaration. Written informed consent was obtained from the legal guardians of the children.

Study Design and Population

The present study was a retrospective, cross-sectional study that was performed in a single tertiary healthcare institution from January 2005 to December 2021. Five hundred eighteen children aged 18 years who were admitted (and followed for at least 6 months) to the pediatric and pediatric rheumatology departments with the diagnosis of IgAV were assessed for inclusion in the study. Immunoglobulin A vasculitis diagnosis was made according to the consensus criteria put forth by the European League Against Rheumatism and the Pediatric Rheumatology European Society (EULAR/PRINTO/PRES 2010) (16).

Among these 518 children, we excluded subjects diagnosed with FMF before the onset of IgAV as well as those with additional chronic diseases (comorbidities), insufficient follow-up duration (<6 months), unperformed *MEFV* gene analyses, and incomplete data. A total of 244 children diagnosed with IgAV were included in the analyses. Criteria for inclusion and exclusion in the study are shown in Figure 1.

Data Collection and Disease Definitions

The patients' demographic, clinical, and laboratory data were recorded from their medical records. In all patients, symptoms, signs, and organ involvement, such as the skin, GI tract, joints, kidneys, testicles, and central nervous system, were recorded on admission and during follow-up. Cutaneous findings consisted of characteristic palpable purpura and subcutaneous edema. Gastrointestinal manifestations included vomiting, abdominal pain, invagination, and GI bleeding-defined as melena, hematochezia, or occult blood in the stool. Involvement of more than one joint but fewer than five joints was considered to demonstrate the presence of oligoarthritis. All patients were followed up for a minimum period of 6 months for renal involvement. Hematuria was defined as the presence of >5 red blood cells per high-power field in a urine examination. Mild proteinuria was defined as a spot urine protein-to-creatinine ratio of 0.2 mg/mg, whereas nephrotic proteinuria was defined in patients with a ratio of >3-3.5 mg/mg or those with absolute levels of >40 mg/m^2/h. Renal biopsy was performed in selected cases when patients presented with nephrotic-range proteinuria, persistent proteinuria of 4-40 mg/m^2/h for more than 3 months, and/or renal impairment. Skin biopsies were performed on selected IgAV patients with atypical rashes.

Patient Management

Treatment modalities, including hydration, non-steroidal anti-inflammatory drugs (NSAIDs), and the need for corticosteroid therapy and colchicine, were recorded. Treatment was planned according to existing symptoms; we routinely prescribed bed rest and NSAIDs for arthralgia and mild abdominal discomfort. Systemic steroid treatment was reserved for severe GI involvement, including severe abdominal pain, GI bleeding (GIS), progressive renal disease, and scrotal edema.

Biochemistry and Genetic Analyses

White blood cell (WBC) and platelet (PLT) count (10^9/L), hemoglobin (Hb) level (g/dL), C-reactive protein (CRP) (mg/dL), Westergren erythrocyte sedimentation rate (ESR) (mm/hr), stool blood analysis, and other laboratory parameters such as anti-streptolysin O (ASO), C3 and C4 complement levels, and serum IGA levels were determined by standard laboratory methods at the time of diagnosis. Leukocytosis was defined if the WBC count was ≥10,000/mm^3. Thrombocytopenia was accepted as a PLT level of <150,000. Elevated levels of CRP, ESR, ASO, C3, and C4 were defined as >5 mg/dL, >15 mm/h, >200 IU/mL, >170 mg/dL, and >44 mg/dL, respectively. Blood cultures, hepatitis B, hepatitis C, or human immunodeficiency virus infection serology were only performed when deemed necessary by the attending physician. Disease-related complications included the development of hypertension, invagination, and convulsions. The recurrence of disease was recorded in the medical files of the patients. Recurrence was defined as disease activation after a period of at least 3 months without signs or symptoms.

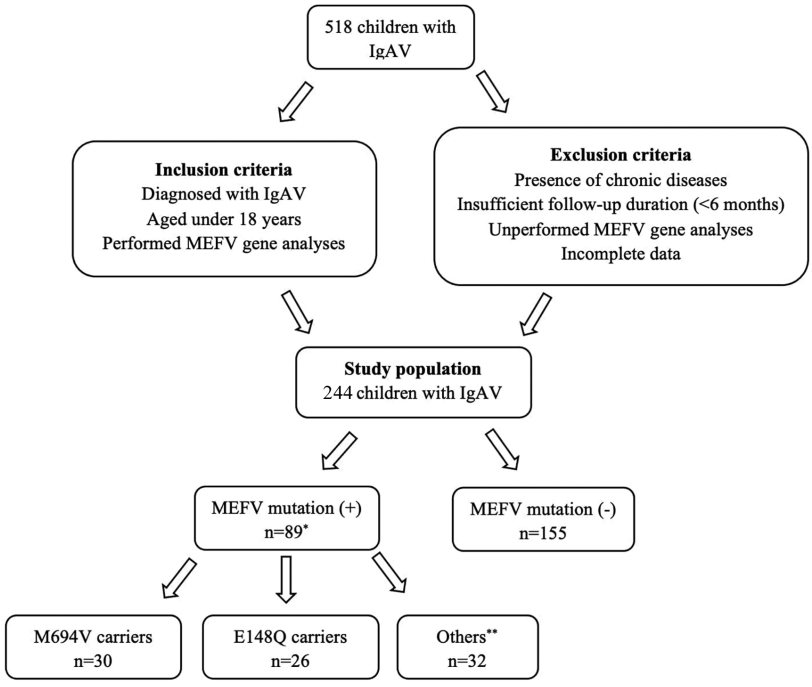

Figure 1. Criteria for inclusion and exclusion and subgroups of the study
**One patient had both E148Q and M694V mutation and was not included in the comparison analyses, **Others: R202Q, K695R, P369S, V726A, M680I, R761H*
IgAV: Immunoglobulin A vasculitis

All patients in the cohort were analyzed for sequence variants in exons 2, 3, 5, and 10 of the *MEFV* gene. For the main comparative analyses, the study population was divided into two main groups: "*MEFV* mutation carriers" (patients with mutations in at least one allele; heterozygous, homozygous, and compound heterozygous) and "non-carriers". Carrier patients were divided into three further subgroups according to their carrier status: p. M694V carriers, E148Q carriers, and "other mutation" carriers (Figure 1). We compared the demographic, clinical, and laboratory findings between the groups.

Statistical Analysis

All analyses were performed using the SPSS 23.0 statistical software package (IBM SPSS Statistics). Categorical variables were expressed as numbers (n) and percentages, whereas continuous variables were summarized as mean with standard deviation or as median with minimum and maximum where appropriate. Chi-square tests were used to compare categorical variables between groups. The normality of the distribution for continuous variables was tested using the Kolmogorov-Smirnov test (Lilliefors correction). For continuous variables that had a normal distribution, two-group comparisons were performed using the Independent Samples t-test (variances were assessed according to Levene's test, and p-values were determined according to those results). For >2-group comparisons, we used ANOVA. In the absence of a normal distribution, we used the Mann-Whitney U test for two-group comparisons and the Kruskal-Wallis test for >2-group comparisons. For the pairwise corrections of the ANOVA and Kruskal-Wallis tests, we used the Bonferroni correction, given that statistical assumptions were fulfilled. The statistical level of significance for all tests was defined as a p-value of <0.05. For power analysis, the G-Power 3.1.9.7 program was used. The power of the study was found to be 0.98.

Results

A total of 244 of the 518 children with IgAV were included in the analyses. Table 1 summarizes the demographic, clinical, and laboratory characteristics of patients with IgAV.

Distribution of *MEFV* Mutations in Patients with IgAV

At least one *MEFV* mutation was detected in 89 (36.5%) of the 244 patients with IgAV included in the study. Most were heterozygous mutations (n=71, 79.8%). E148Q was the most common mutation (n=31, 34.8%) among all *MEFV* mutations. One patient had both E148Q and M694V mutations and was not included in the comparison analyses to avoid errors in interpretations. The genotype distribution of the patients is depicted in Table 2.

Table 1. Demographic, clinical and laboratory characteristics of 244 patients with IgA vasculitis	
Parameters	**Values**
Age at diagnosis (year) median (min.-max.)	7.60 (3-18)
Time from admission to final diagnosis (days) median (min.-max.)	5.0 (1-90)
Male sex (n, %)	137 (56.1)
Distribution of clinical features during follow-up (n, %)	
Gastrointestinal system involvement	125 (51.2)
Abdominal pain/angina	94 (38.5)
Fecal occult blood	71 (30.2)
Vomiting	18 (7.4)
Macroscopic bleeding (melena/hematochezia)	7 (2.9)
Invagination	4 (1.6)
Musculoskeletal system involvement	86 (35.3)
Arthralgia	36 (14.8)
Arthritis	55 (22.5)
Oligoarthritis	31 (12.7)
Myalgia	3 (1.2)
Subcutaneous edema	72 (29.5)
Renal involvement	49 (20.1)
Hematuria	31 (12.7)
Proteinuria	36 (14.8)
Mild proteinuria	26 (10.7)
Nephrotic range proteinuria	10 (4.1)
Hypertension	5 (2)
Testis involvement (in male patients, n=137)	13 (9.5)
Fever	7 (2.9)
CNS involvement (seizure, headache)	0
Medical treatments (n, %)	92 (37.7)
Only hydration	41 (16.8)
Hydration plus NSAIDs	38 (15.6)
Hydration plus steroid	52 (21.3)
Hydration + NSAIDs + steroid	10 (4.1)
Hydration + NSAIDs + pulse steroid	2 (0.8)
Colchicine treatment	5 (2.0)
Pneumatic reduction	1 (0.4)
Cytotoxic drugs	1 (0.4)
Renal biopsy	4 (1.6)
MPGN	4 (1.6)
Laboratory parameters	
WBC (10^9/L), median (min.-max.)	10.4 (2.3-36.9)
Leukocytosis (>10.000/uL), n%	124 (52.1)
Hb level (g/dL), Mean ± Standard deviation	12.11±1.12
PLT level (10^9/L), median (min.-max.)	334.0 (128-898)
Thrombocytopenia (n%)	3 (1.3)
CRP (mg/dL), median (min.-max.)	0.91 (0.01-23.2)
Elevated CRP (n, %)	31 (13.5)
ESR (mm/hr), median (min.-max.)	32.0 (1-111)
Elevated ESR (n, %)	150 (83.3)
ASO (IU/mL), median (min.-max.)	147.0 (10-1491)
Elevated ASO (IU/mL) (n, %)	22 (46.8)
C3 level (mg/dL), Mean ± Standard deviation	145.03±31.51
Elevated C3 (n, %)	17 (17.3)
C4 level (mg/dL), Mean ± Standard deviation	27.22±10.62
Elevated C4 (n, %)	4 (4.3)
Serum IgA (mg/dL), median (min.-max.)	213.0 (46-869)
Recurrence (n, %)	27 (11.1)

Min.-Max.: Minimum-maximum, NSAIDs: Non-steroidal anti-inflammatory drugs, MPGN: Membranous proliferative glomerular nephritis, WBC: White blood cell, PLT: Platelets, Thrombocytopenia <150.000 10^9/L, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, ASO: Anti-streptolysin O, IgA: Immunoglobulin A

Comparison of Clinical Characteristics and Laboratory Findings Between *MEFV* Mutation Carriers and Non-Carrier Patients

Clinical and laboratory parameters were compared between *MEFV* mutation carriers and non-carrier patients with IgAV. Age at diagnosis, frequency of hematuria, and disease recurrence were significantly greater in *MEFV* mutation carrier patients than in non-carriers (p=0.043, p=0.008, p=0.009) respectively. Although patients with *MEFV* mutations had relatively higher WBC, CRP, and ESR levels, there was no statistically significant difference between the groups (Table 3).

Comparison of Demographic, Clinical, and Laboratory Findings of Patients with IgAV According to the Presence of *MEFV* Gene Variants

The relationship between the different *MEFV* gene variants (E148Q, M694V, and "others") and clinical characteristics is shown in Table 4. In terms of clinical characteristics, vomiting was more frequent in patients with mutations other than E148Q and M694V (p=0.026). None of the patients with E148Q and M694V mutations experienced vomiting. In terms of laboratory findings, only the serum IgA level was significantly higher in patients with the M694V mutation (p=0.040).

Discussion

Association of IgAV with *MEFV* Mutations and Their Variants

The association of vasculitis with FMF has been extensively reported in previous studies (11-13,17). The *MEFV* gene encodes the pyrine protein, which plays an essential role in inflammatory pathways by

Table 2. Distribution of *MEFV* mutations in 244 patients with IgAV	
Type of mutations	**n, %**
Non-carriers	155 (63.5)
***MEFV* mutation carriers**	89 (36.5)
E148Q	31 (34.8)
M694V	27 (30.3)
Others**	32 (36)
Homozygous mutations	**10 (11.2)**
M694V homozygote	4 (4.5)
R202Q homozygote	1 (1.1)
E148Q homozygote	5 (5.6)
Heterozygous mutations	**71 (79.8)**
E148Q heterozygote	23 (25.9)
M694V heterozygote	19 (21.4)
R202Q heterozygote	9 (10.1)
V726A heterozygote	8 (9.0)
M680I heterozygote	5 (5.6)
P369S heterozygote	3 (3.4)
K695R heterozygote	2 (2,2)
M694I heterozygote	1 (1.1)
R761h heterozygote	1 (1.1)
Compound heterozygous mutations	**8 (9.0)**
E148Q/M694V heterozygote	1 (1.1)
E148Q/P369S heterozygote	1 (1.1)
M680I/M694V heterozygote	1 (1.1)
M680I/V726A heterozygote	1 (1.1)
M694V/V726A heterozygote	1 (1.1)
P369S/R202Q heterozygote	1 (1.1)
R202Q/E148Q heterozygote	1 (1.1)
R202Q/M694V heterozygote	1 (1.1)

*One patient had both E148Q and M694V mutation and was not included in the summary to avoid errors in interpretation. **R202Q, K695R, P369S, V726A, M680I, R761H, IgAV: Immunoglobulin A vasculitis

decreasing inflammation; hence, the mutated protein causes uncontrolled inflammation and predisposes to the occurrence of vasculitis (including IgAV) (12,18). Previous research indicates that if IgAV is diagnosed in a patient belonging to an ethnic/racial group in which FMF is frequent, physicians should assess the presence of FMF symptoms (19). It has been established that the prevalence of *MEFV* gene mutations in children with IgAV is higher than that in the general population, ranging from 21% to as high as 50.7%, and it has been shown that *MEFV* mutations can affect the clinical and laboratory findings of IgAV (14,20-26). In a previous study, Ozçakar et al. (20) reported that 27 out of 80 patients (34%) with IgAV had *MEFV* gene mutations. Additionally, in a recent study from Turkey, Acarı et al. (15) reported that *MEFV* gene mutations were found in 25 out of 47 (53%) patients with IgAV, and this rate was significantly higher than that in the general Turkish population (27,28). In another study from Israel, Gershoni-Baruch et al. (21) reported that 27% of 52 patients with IgAV had at least one *MEFV* mutation. In contrast, in a recent literature review, Yokoyama et al. (29) mentioned that only five patients with IgAV-carrier *MEFV* mutations appear in Japan. In the current study, we also found that the prevalence of *MEFV* mutations in IgAV patients was significantly higher (n=89, 36.5%) than in the normal population and mostly demonstrated a heterozygous nature (n=71, 79.8%). The high prevalence of mutations in our study might be due to ethnic or racial predisposition, but it could also be explained by the likelihood of parental consanguinity. However, data concerning consanguinity were not assessed.

Research assessing the associations between *MEFV* genetic variants and IgAV has described various findings (20,21). Ozçakar et al. (20) showed that the frequencies of M694V and E148Q mutations were 20% (16 out of 80 patients) and 3.8% (3 out of 80 patients), respectively, while they were reported as 3% and 12%, respectively, in the healthy Turkish population (28). Additionally, Acarı et al. (15) reported that M694V (25%, 12 out of 47 patients), R202Q (17%, 8 out of 47 patients), and E148Q (11%, 5 out of 47 patients) were the most common detectable variants in children with IgA (15). Thus, the authors concluded that the M694V mutation was a more important predisposing factor for IgAV development, which has been supported by other studies (20,22,30). However, in another study from Turkey, it was reported that while the frequency of the M694V mutation carrier in 76 patients with IgAV was 11.7%, the frequency of the E148Q mutation carrier was 9.1% in those patients (31). Another study found that 34.8% of Turkish children with IgAV had the E148Q mutation, which is a higher frequency than that of the general Turkish population (32). In a study from China, none of the IgAV patients were found to carry the M694V mutation (33). In our study, the E148Q mutation was the most common allele; however, the frequency of the M694V mutation was also similar, indicating consistency with most of the literature.

Clinical Significance of *MEFV* Mutations and Variants in Patients with IgAV

It was reported earlier that *MEFV* mutations affect the clinical and laboratory presentation of IgAV in populations in which FMF is common (20,22,25,33,34). However, studies comparing carriers and non-carriers of *MEFV* mutations have reported conflicting results. In some of those studies, it was found that there was no relationship between the presence of *MEFV* carriers and disease characteristics in analyses including laboratory parameters,

Table 3. Comparison of demographic and clinical features and laboratory findings of patients according to *MEFV* mutation carrier state			
Parameters	***MEFV* mutation (-) (n=155)**	***MEFV* mutation (+) (n=89)**	**p-value**
Age at diagnosis (year) median (min.-max.)	7 (3-15)	8 (3-18)	**0.043**^{*,s}
Time from admission to diagnosis (days) median (min.-max.)	4 (1-45)	6 (1-90)	0.633**
Male sex (n, %)	85 (62.0)	52 (38.0)	0.587***
Clinical features during follow-up (n, %)			
Fever	3 (42.9)	4 (57.1)	0.262***
Angioedema/subcutaneous edema	47 (65.3)	25 (34.7)	0.713***
Abdominal pain/angina	63 (67.0)	31 (37.0)	0.369***
Fecal occult blood	50 (70.4)	21 (29.6)	0.166***
Vomiting	14 (77.8)	4 (22.2)	0.192***
Macroscopic bleeding (melena/hematochezia)	3 (42.9)	4 (57.1)	0.262***
Invagination	3 (75)	1 (25)	1.00***
Arthralgia	22 (61.1)	14 (38.9)	0.745***
Arthritis	31 (56.4)	24 (43.6)	0.210***
Multiple arthritis (oligoarthritis)	18 (58.1)	13 (41.9)	0.499***
Myalgia	2 (66.7)	1 (33.3)	1.000***
Hematuria	13 (41.9)	18 (58.1)	**0.008**^{***,s}
Proteinuria	18 (50)	18 (50)	0.068***
Hypertension	2 (40)	3 (60)	0.358***
Testis involvement (in male patients, n=137)	7 (53.8)	6 (46.2)	0.557***
Medical treatments (n, %)			
Only hydration	25 (61.0)	16 (39.0)	0.349***
Hydration plus NSAIDs	21 (55.3)	17 (44.7)	
Need of steroid/pulse steroids	40 (59.7)	27 (40.3)	
Colchicine treatment	1 (20.0)	4 (80.0)	
Recurrence (n, %)	11 (40.7)	16 (59.3)	**0.009**^{***,s}
WBC (10^9/L), median (min.-max.)	10.1 (2.3-30.8)	11 (5.2-36.9)	0.728****
Hb level (g/dL), median (min.-max.)	12.1 (8.6- 15.3)	11.9 (9.8-14.9)	0.208*
PLT level (10^9/L), median (min.-max.)	345.5 (136-898)	322.5 (128-674)	0.404****
CRP (mg/dL), median (min.-max.)	0.90 (0.01-15)	1 (0.01-23.2)	0.233****
ESR (mm/hr), median (min.-max.)	31.5 (1-111)	35 (2-105)	0.056*
Elevated ESR (>15), n, %	94 (62.7)	56 (37.3)	0.240****
ASO (IU/mL) level, median (min.-max.)	165 (10-866)	147 (20-1491)	0.215****
C3 level (mg/dL), median (min.-max.)	149 (33-196)	145 (10-203)	0.645****
C4 level (mg/dL), median (min.-max.)	29.2 (3-55)	25.4 (3-64)	0.496*
Serum IgA mg/dL level, median (min.-max.)	204 (46-869)	216 (84-563)	0.483****

Min.-max.: minimum-maximum, WBC: White blood cells, PLT: Platelets, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, ASO: Anti-streptolysin O, IgA: Immunoglobulin A, *Student's t-test, **One-Way ANOVA test, ***Chi-square test, ****Mann-Whitney U test, ^{s}Significant (p<0.05)

complications, outcomes, and treatment-related needs of patients (21,23,31,34,35). On the other hand, Ozçakar et al. (20) found that IgAV patients with *MEFV* carriers were younger and that *MEFV* mutations could affect clinical symptoms. Cakici et al. (14) demonstrated that arthritis, bowel angina, scrotal involvement, and recurrence were more common in patients with *MEFV* mutation positivity. In a study from Egypt, higher frequencies of arthritis, abdominal pain, GIS, hypertension, anemia, proteinuria, fecal occult blood, and recurrence were found in patients with *MEFV* mutations (36). Bayram et al. (22) reported that the frequency of scrotal involvement and WBC, ESR, CRP, and serum Ig levels were significantly higher in patients with *MEFV* mutations. Several other studies have shown significantly elevated acute phase reactants among mutation carriers (20,31,34).

Table 4. Comparison of demographic, clinical and laboratory finding of patients with IgAV according to presence of *MEFV* gene variants				
Parameters	**E148Q (n=30)**	**M694V (n=26)**	**Others* (n=32)**	**p-value**
Age at diagnosis (year), median (min.-max.)	8.75 (3-15)	8.2 (3-16)	7.7 (3-18)	0.501**
Time from admission to diagnosis (days) median (min.-max.)	6 (1-15)	5 (1-30)	5 (1-90)	0.867***
Male sex (n, %)	21 (70)	16 (61.5)	15 (46.9)	0.172****
Clinical features during follow-up (n, %)				
Fever	1 (3.3)	1 (3.8)	2 (6.3)	0.841****
Angioedema/subcutaneous edema	6 (20.0)	9 (34.6)	9 (28.1)	0.468****
Abdominal pain	7 (23.3)	12 (46.2)	12 (37.5)	0.193****
Fecal occult blood	6 (20.7)	6 (23.1)	9 (31.0)	0.637****
Vomiting	0	0	4 (12.5)	**0.026****,s
Macroscopic bleeding (melena/hematochezia)	1 (3.8)	1 (3.8)	2 (6.3)	0.841****
Invagination	1 (3.3)	0	0	0.376****
Arthralgia	4 (13.3)	5 (19.2)	5 (15.6)	0.833****
Arthritis	8 (26.7)	6 (23.1)	9 (28.1)	0.907****
Oligoarthritis	5 (16.7)	4 (15.4)	3 (9.4)	0.672****
Myalgia	1 (3.3)	0	0	0.376****
Hematuria	7 (23.3)	6 (23.1)	5 (15.6)	0.697****
Proteinuria	8 (26.7)	6 (23.1)	4 (12.5)	0.356****
Mild proteinuria	5 (62.5)	4 (66.7)	2 (50.0)	0.864****
Nephrotic range	3 (37.5)	2 (33.3)	2 (50.0)	
Hypertension	1 (3.3)	2 (7.7)	0	0.275****
Testis involvement (in male patients, n=137)	4 (19.0)	2 (12.5)	0	0.209****
Medical treatments (n, %)				
Only hydration	4 (25.0)	4 (25.0)	8 (50.0)	0.708****
Hydration plus NSAIDs	6 (35.3)	5 (29.4)	6 (35.3)	
Need of steroid/pulse steroid	9 (33.3)	9 (33.3)	9 (33.3)	
Colchicine treatment	2 (50.0)	2 (50.0)	0	
Recurrence (n, %)	4 (13.3)	5 (19.2)	7 (21.9)	0.675****
WBC (10^9/L), median (min.-max.)	10.85 (5.3-36.9)	10.1 (5.2-20.4)	11.25 (5.3-24.3)	0.760***
Hb level (g/dL), median (min.-max.)	11.6 (9.9-14.6)	11.65 (10-13.6)	12.2 (9.8-14.9)	0.276**
PLT level (10^9/L), median (min.-max.)	349.5 (223-674)	307 (128-620)	308 (215-565)	0.113**
CRP (mg/dL), median (min.-max.)	0.76 (0.1-7.99)	1.5 (0.01-23.2)	0.81 (0.01-12.8)	0.247***
ESR (mm/hr), median (min.-max.)	30 (15-91)	41 (13-105)	32 (2-103)	0.218***
Elevated ASO (IU/mL) (>200) (n, %)	1 (14.3)	1 (14.3)	5 (71.4)	0.813**
C3 level (mg/dL), median (min.-max.)	142.5 (10-193)	149.4 (69-203)	146 (98-173)	0.433***
C4 level (mg/dL), median (min.-max.)	24.5 (13-31)	23.4 (3-50)	29.8 (12-64)	0.343**
Serum IgA level (mg/dL), median (min.-max.)	413.5 (303-563)	210 (84-456)	212 (139-281)	**0.040***,s

IgAV: Immunoglobulin A vasculitis, min.-max.: minimum-maximum, *Others: R202Q, K695R, P369S, V726A, M680I, R761hNSAIDs: Non-steroidal anti-inflammatory drugs, WBC: White blood cell, PLT: Platelets, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, ASO: Anti-streptolysin O, IgA: Immunoglobulin A, **One-Way ANOVA test, ***Kruskal-Wallis test, ****Chi-square test, sSignificant (p<0.05)

Altug et al. (34) showed that ESR and CRP levels were significantly higher in patients with the IgAV-carrier *MEFV* mutation. Additionally, they noted that GI and joint involvement and subcutaneous edema were more common in IgAV patients carrying the *MEFV* mutation. Prior research has revealed that physicians should be aware of the possibility of FMF in children with intussusceptions, lower hemoglobin, higher serum IgA, and elevated PLT count; however, the study could not identify any effects of the *MEFV* mutations on recurrence rate (25). Interestingly, Gershoni-Baruch et al. (21) found that the recurrence rate was twofold higher in patients

with two mutated alleles on *MEFV* compared with those without mutations, although statistical analyses were non-significant. On the other hand, in a recent study, Acarı et al. (15) found that disease relapse was significantly higher in IgA patients who were *MEFV* carriers than non-carriers. They also mentioned that Hb levels were lower and PLT count and CRP levels were higher in IgAV patients who had *MEFV* carriers (15). In comparison to prior studies, we found that mutation carriers were significantly older than non-carriers, and our results revealed that the presence of *MEFV* mutations was significantly associated with a higher frequency of hematuria and recurrence. White blood cell, CRP, and ESR levels were similar in carriers and non-carriers, similar to a previous report by Dogan et al. (31). Further studies are needed to determine the effects of *MEFV* mutations on the laboratory characteristics of patients with IgAV.

Regarding the variants (subgroup analysis) and their clinical effects, the M694V variant was found to be associated with the clinical and laboratory findings of patients with IgAV in a previous study from Turkey (22). Ozçakar et al. (20) demonstrated that the presence of edema, arthritis, and urogenital involvement was more common in patients with M694V mutations, although E148Q mutations had no clinical significance in patients with IgAV. In later years, Bayram et al. (22) found similar results to those of Ozçakar et al. (20) in their study. A study involving Chinese patients found that the E148Q variant was associated with the severity of disease, specifically with joint abnormalities; however, the researchers did not observe any significant effects of the E148Q variant on the analyzed laboratory parameters (IgA, CRP, C3, and C4) (33). In another study from Turkey, Cakici et al. (14) reported that although *MEFV* mutations were influential on the clinical characteristics of IgAV, variants of *MEFV* were not found to have any effect on the clinical course of IgAV. Acarı et al. (15) reported that scalp edema, elevated CRP levels, and disease recurrence were more common in patients with IgAV who were carriers of the *M694V* mutation. On the other hand, they mentioned that there was no significant relationship between the long-term prognosis of the disease and renal involvement or the presence of *MEFV* mutations (15). In our study, we could not find any significant results regarding the effect of the E148Q mutation in any clinical findings, but serum IgA levels were found to be significantly higher in patients with the E148Q variation, which is in contrast to the findings of the study from China. In addition, we found that vomiting was only present among patients with "other" mutations. However, this result needs further support in larger cohorts because of the limited patient counts in this study. The E148Q variant has been considered a genetic marker in some studies (33); however, to draw conclusions regarding this matter, the functional role(s) of the E148Q variant in IgAV should be elucidated.

Study Limitations

There are some limitations to our study. First, the lack of a prospective design is a major limitation of our study. Second, we only investigated 12 well-known *MEFV* variants instead of identifying all the variants. Finally, the clinical spectrum of IgAV is similar between isolated IgAV and FMF-associated IgAV; therefore, particularly in some cases, IgAV may be an initial symptom of FMF. Despite these limitations, the strength of our study is that it includes one of the largest numbers of patients among published studies to date. The potential for such a complex relationship between these conditions demonstrates the need for prospective studies including IgAV patients (with or without *MEFV* mutations) in which longitudinal analyses are performed in larger populations.

Conclusion

Our results showed that *MEFV* mutations (especially E148Q and M694V mutations) are more frequent in IgAV patients compared with the general population, and the presence of those mutations seems to have some effects on the clinical features of IgAV patients, as demonstrated by results concerning hematuria and recurrence. Therefore, patients with IgAV, especially older children, should be followed more carefully regarding FMF development. In addition, closer follow-up for hematuria and recurrence appears to be necessary for patients with IgAV who carry those mutations. However, the different results and clinical effects observed in other studies indicate the need for further research.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Istanbul Medeniyet University, Goztepe Training and Research Hospital Clinical Research Ethics Committee (approval no.: 2023/0059, date: 25.01.2023).

Informed Consent: Written informed consent was obtained from the legal guardians of the children.

Authorship Contributions

Surgical and Medical Practices: S.Y., Z.K., M.E., Concept: S.Y., M.E., Design: S.Y., M.E., Data Collection or Processing: S.Y., Z.K., Analysis or Interpretation: O.O., Literature Search: S.Y., Z.K., Writing: S.Y., Z.K., M.E.

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Original Article

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Evaluation of Similar Genetic Pathophysiology Underlying Diabetes Mellitus and Peyronie's Disease: *WNT-2* and *TGF Beta-1* Genes

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Abstract

Aim: Some recent studies on PD have focused on the *WNT-2* and *TGF-β1* gene loci, but its genetic basis is still not clearly known. In this context, we aimed to evaluate the presence of *WNT-2* and *TGF-β1* gene expression and genetic similarity between patients with Peyronie's disease (PD) and comorbidities, especially diabetes mellitus (DM).

Methods: Between May 2020 and April 2021, 57 patients diagnosed with PD were included in this cross-sectional study. The presence of comorbidities [Dupuytren's contracture (DC), DM, hypertension (HT), dyslipidemia, and erectile dysfunction (ED)] was recorded. For genetic analysis, the *WNT-2* and *TGF-β1* genes were analyzed in the patients' serum.

Results: The mean age was found to be 50.2. 45.6% of the patients had DM, 19.1% had HT, 14% had dyslipidemia, 5.3% had DC, and 40.4% had ED. *TGF-β1* gene expression was found to be increased in all patients; *WNT-2* gene expression was found to be increased in 80.7%. When subtypes of the *TGF-β1* and *WNT-2* gene expression were analyzed, 52.6% of patients with *WNT-2* gene expression and 95.5% of patients with *TGF-β1* gene expression were found to be homozygous, and the others were found to be heterozygous. Patients with DM and PD had significantly higher homozygous *WNT-2* gene expression (p=0.03). No significant relationship was found between other comorbidities and these genes.

Conclusion: Homozygous *WNT-2* gene expression was found to be increased in PD with DM. These data could be used to explain the genetic pathophysiology of PD in diabetic patients.

Keywords: Diabetes mellitus, Peyronie's disease, *TGF-β1*, *WNT-2*

Introduction

Peyronie's disease (PD) is an acquired penile deformity characterized by hard fibrotic plaques, particularly on the dorsal surface of the penis. Its prevalence ranges from 0.3% to 13.1%. The most accepted hypothesis for the pathophysiology of PD is that recurrent trauma causes microvascular damage to the tunica albuginea. Myofibroblasts do not undergo apoptosis because of microtrauma, and collagen accumulation persists, which is the most accepted hypothesis for PD pathophysiology (1-3). Diabetes mellitus (DM), hypertension (HT), lipid metabolic disorders, ischemic cardiopathy, erectile dysfunction (ED), smoking, and excessive alcohol intake are considered to be the most prevalent risk factors for PD (4).

The genetic background of PD has been investigated using newly developed technologies to explain its etiology and pathophysiology. Patients underwent various genetic tests, including human leukocyte antigens (HLA), single nucleotide polymorphisms, karyotypic abnormalities, and gene expression variations. In particular, some recent studies on PD have focused on the

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WNT-2 and *TGF-β1* gene loci, but its genetic basis is still not clearly known (5,6).

In this study, we aimed to determine the association between the gene loci *WNT-2* and *TGF-β1*, which may be related to PD, and DM and other comorbidities associated with PD and these gene loci.

Methods

Compliance with Ethical Standards

Ethical approval was obtained from the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Istanbul Prof. Dr. Cemil Tascioglu City Hospital (approval no.: 446, and date: 29.12.2020).

Study Design

Fifty-seven informed and voluntary patients who were examined at the andrology outpatient clinic between May 2020 and April 2021 were included in this cross-sectional study. Patients with a congenital penile curvature, previous penile surgery, or penile curvature secondary to a known trauma were excluded (Figure 1). All patients in our study were evaluated for the presence of DM, Dupuytren's contracture (DC), hypertension, lipid profiles, smoking and alcohol consumption, and ED. All patients' *WNT-2* and *TGF-β1* gene expressions were examined and recorded for genetic evaluation. The international index of erectile function-5 score was used to evaluate erectile functions (7).

The expression of the *WNT-2* and *TGF-β1* genes was determined by analyzing blood samples from each patient. Gene expression analyses of *WNT-2* and *TGF-1* were performed with a real-time device (the Thermo Fisher Quants Studio 6 Pro real-time device). Individual genetic analyses were performed on each patient using both TaqMan SNP Genotyping Assays (rs1800471 and rs4730775).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) mac version 21 (SPSS Inc., Chicago, IL, USA) software was used to evaluate the research data. Descriptive statistics are presented as numbers and percentages for categorical variables and as mean, minimum, and maximum for numerical variables. Nominal variables were analyzed using the chi-square or Fisher's exact test. The statistical significance level for all parameters was 95% confidence interval, and p-value less than 0.05 was considered significant.

Results

The average age of the 57 participants was 50.23±10.17 years. 45.6% of the participants had DM (all of them had type 2 DM; no patient had type 1 DM, 19.1% had hypertension, 14% had lipid metabolism disorders, 5.3% had DC, and 40.4% had ED (Table 1).

At the genetic evaluation of the patients, it was found that the increased expression of the *TGF-β1* gene was present in all patients. An increase in *WNT-2* gene expression was found in only 46 (80.7%) patients. *WNT-2* homozygous gene expression was found to be significantly higher in patients with PD, especially those with DM (p=0.03) (Figure 2).

Contrary to *WNT-2* homozygous gene expression, no statistical significance was observed between *TGF-β1* gene expression and the presence of DM (p>0.05). There was no statistically significant difference between *WNT-2* and *TGF-1* gene expression, as well as other comorbidities associated with PD (Table 2).

Discussion

The pathophysiology of PD is still unknown, as the presence of a genetic basis has been the study's focus for many years. There are only a few studies on the relationship between PD and genetic pathologies. For many years, researchers have conducted various genetic

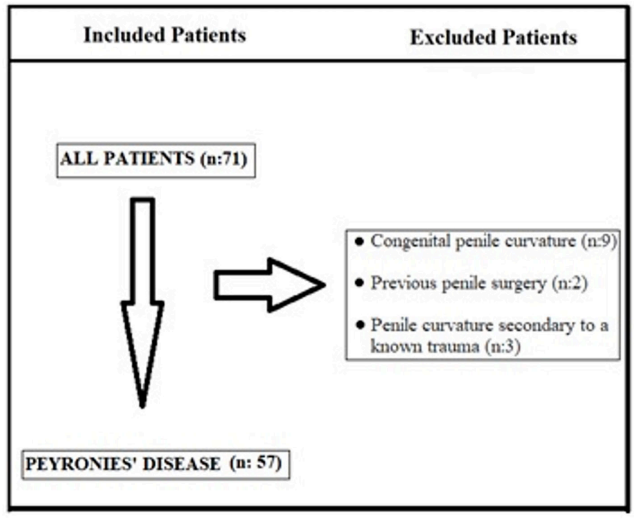

Figure 1. Flowchart of study

Table 1. Demographic data of patients	
	Mean ± SD (Min.-Max.)
Age	50.2±10.1 (30-69)
	n (%)
Diabetes mellitus	26 (45.6%)
Hypertension	11 (19.1%)
Lipid metabolism disorder	8 (14%)
Smoking	13 (22.8%)
Dupuytren's contracture	3 (5.3%)
Erectile dysfunction	23 (40.4%)
n: Number, SD: Standard deviation, Min.: Minimum, Max.: Maximum	

analyses, including autoimmunity, to explain the etiology and pathogenesis of PD.

A family history was found in 1.9% of patients with PD, and 17% of patients found that they have some genetic factors in their etiopathogenesis, according to current studies (6). A study analyzing the mutation of HLA found a genetic predisposition to PD. According to the same study, HLA was a potential predictive factor for PD. A strong association between PD and the *HLA-B27* gene was shown in autoimmunity-related studies, but no significant association was found between PD and other HLA types. In addition, other studies analyzing the relationship between idiopathic PD and HLA have shown that patients with idiopathic PD have higher HLA-B7 cross-reactions (8).

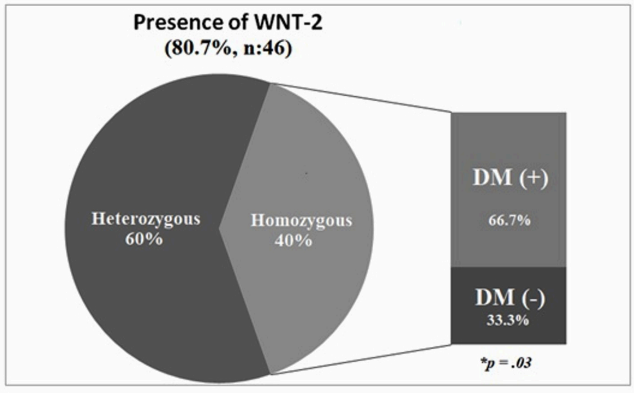

Figure 2. Relationship between *WNT-2* gene and DM
DM: Diabetes mellitus

A significant relationship was found between chromosomal abnormalities and PD in other studies. Chromosomal numerical anomalies such as Y chromosome deletion and duplication of the 7th and 8th chromosomes were found to be related to PD. Invertion [such as 46XY, inv(7)(p22q36)] and reciprocal translocation [such as 46XY, t(11;12)(q11,p11)] of chromosomes are known as other chromosomal abnormalities that were also found to be significantly associated with PD (9,10).

Several studies have focused on inherited single-nucleotide polymorphisms as the genetic cause of PD. Various hereditary single nucleotide polymorphisms such as rs1982073 (T + 29C), rs1800469 (C-509T), and rs1800471 (G915C) have been found in the *TGF-β1* gene of patients diagnosed with PD. Of all these single nucleotide polymorphisms, only the G915C polymorphism has been found to be related to PD (11).

The TGF- pathway, which is an oxidative stress and cytokine release factor, may play a role in the pathogenesis of PD. Abnormal gene expression in the TGF- pathway may lead to the formation of these abnormal fibrotic plaques (6,12,13). In another study, the expression of polymorphisms in *TGF-β1* was evaluated, and no statistically significant difference was found between *TGF-β1* and PD. According to the same study, it was found

Table 2. The presence of *TGF-β1* and *WNT-2* gene expression according to comorbidity status of patients with PD

		Presence of *WNT-2* expression (n=46, 80.7%)		Presence of *TGF-β1* expression (n=57, 100%)	
		Type of the expression			
		Homozygous	Heterozygous	Heterozygous	Homozygous
Diabetes mellitus n (%)	-	6 (33.3%)	18 (64.3%)	1 (50%)	30 (54.5%)
	+	12 (66.7%)	10 (35.7%)	1 (50%)	25 (45.5%)
	p-value	0.030*	0.086*	0.899**	0.899*
Hypertension n (%)	-	14 (87.5%)	24 (80%)	1 (50%)	45 (81.8%)
	+	2 (12.5%)	6 (20%)	1 (50%)	10 (18.2%)
	p-value	0.417*	0.887*	0.263**	0.263*
Dyslipidemia n (%)	-	13 (81.3%)	27 (90%)	1 (50%)	48 (87.3%)
	+	3 (18.8%)	3 (10%)	1 (50%)	7 (12.7%)
	p-value	0.522*	0.355*	0.136**	0.136*
Dupuytren contracture n (%)	-	14 (87.5%)	30 (100%)	1 (50%)	53 (96.4%)
	+	2 (12.5%)	0 (0%)	1 (50%)	2 (3.6%)
	p-value	0.126*	N/A	0.087**	0.087*
Erectile dysfunction n (%)	-	10 (62.5%)	18 (60%)	1 (50%)	33 (60%)
	+	6 (37.5%)	12 (40%)	1 (50%)	22 (40%)
	p-value	0.784*	0.061*	0.777**	0.777*

*Chi-square test, **Fisher's exact test
n: Number, N/A: Not applicable, PD: Peyronie's disease

that *TGF-β1* expression might help in pathogenesis, but it was not a major genetic risk factor for PD. Additionally, the expression and activity of Smad transcription factors in the *TGF-β* pathway were found to be increased in PD. Patel et al. (14) found that *TGF-β1* was more common in patients with PD (14,15). Similar to previous studies, our findings show an increase in *TGF-β1* expression in patients with PD. An increase in *TGF-β1* gene expression was observed in all patients. Upregulation of the *WNT-2* signaling pathway and high catenin levels in plaques indicate that the *WNT-2* pathway may also be related to the pathogenesis of PD (16). In immunohistochemical studies of genetic markers in Peyronie's plaques, high levels of *WNT-2* gene expression were found in the tunica albuginea of patients with PD (14,17,18). In our study, we examined the level of *WNT-2* expression in the blood samples of patients and found an increase in *WNT-2* expression of 80.7%.

Several studies on other non-genetic risk factors have analyzed the etiopathogenesis of PD. According to these studies, DM was found to be strongly associated with PD (16,19-21). In a study by Gelbard and Rosenbloom (22) evaluating the association between patients with DC and PD and the presence of DM, it was found that both DC and PD were diagnosed at a higher rate in DM patients, and that both diseases progressed more aggressively in DM patients. This relationship was found to be similar for all DM types (20). Crocetto et al. (4) showed that PD was diagnosed more frequently in patients with high insulin resistance and non-alcoholic fatty liver disease, especially in diabetic patients. It has been shown that this detected PD would progress more aggressively (4). Similarly, the higher diagnosis rate of PD in diabetic patients with a serum HbA1c level above 7 could support the hypothesis that DM and PD are associated with each other (19). None of the studies evaluating the relationship between DM and PD performed genetic analysis, and they only analyzed this relationship using retrospective data. Our study revealed a significantly higher increase in *WNT-2* homozygous gene expression in diabetic patients. Based on our findings, we hypothesize that elevated *WNT-2* homozygous gene expression may be the pathophysiological basis for the increased incidence of PD in diabetic patients. In addition, we did not find any statistically significant difference between patients diagnosed with HT, dyslipidemia, ED, and DC, which are risk factors other than DM.

Study Limitations

Our study has some limitations. First, we have a limited number of patients to analyze separately for each gene. Our findings should be supported by studies that include large patient populations. Second, in our study, the duration of comorbidities (such as DM, HT) was not analyzed. There is no published information on this subject, but there may be a correlation between comorbidity duration and gene expression. The presence of such a relationship may be investigated in the future. Third, all of the diabetes patients in our study were diagnosed with type 2 diabetes, and none of our patients had type 1 diabetes. The relationships between type 1 and type 2 DM disease subtypes and genetic expressions should be analyzed in studies with large patient populations. Another limitation is that only serum was analyzed for the presence of gene expression, and the tissue of Peyronie's plaque was not analyzed for the presence of gene expression. The results obtained from this study could be the basis for future studies that investigate the presence of gene expression at the tissue level. Despite these limitations, our study is, to the best of our knowledge, the first to evaluate the similarity of the genetic basis of PD and DM.

Conclusion

WNT-2 and *TGF-β1* gene expressions were found to be increased in PD. Although *WNT-2* heterozygous gene expression was found to be higher than that in non-diabetic patients, *WNT-2* homozygous gene expression was only found to be statistically significantly higher in patients with DM than in non-diabetic patients. According to these findings, the increase in *WNT-2* homozygous gene expression, especially in patients with DM, may play a role in the development of PD. A relationship between increased *WNT-2* gene expression and other non-diabetic risk factors (hypertension, lipid metabolic problems, and ED) could not be shown in PD. Multicenter clinical studies with a large population must support these significant relationships between increased *WNT-2* gene expression in patients with DM and PD.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Clinical Research Ethics Committee of the University of Health Sciences Turkey, Istanbul Prof. Dr. Cemil Tascioglu City Hospital (approval no.: 446, date: 29.12.2020).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: E.T., A.G., Concept: E.T., A.O., H.L.C., Design: E.T., A.O., H.L.C., Data Collection or Processing: E.T., A.G., Analysis or Interpretation: E.T., E.T.K., H.L.C., Literature Search: E.T., H.L.C., Writing: E.T., E.T.K.

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Original Article

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Diagnostic Performance of a Rapid Antigen Test for the Detection of SARS-CoV-2

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Abstract

Aim: The fight against the coronavirus disease-2019 (COVID-19) pandemic has proven crucial, necessitating the need for faster, cheaper, and more reliable detection methods. This study evaluated the performance of a rapid antigen test for the diagnosis of COVID-19 compared with reverse transcription-polymerase chain reaction (RT-PCR) results.

Methods: This prospective study included 169 participants. Two simultaneous nasopharyngeal swabs were collected from the participants. Samples were tested for severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) using the Panbio™ COVID-19 Ag rapid test (Abbott Rapid Diagnostics, Jena, Germany) and the Bio-Speedy® SARS-CoV-2 RT-PCR kit (Bioeksen, Istanbul, Turkey).

Results: Reverse transcription-polymerase chain reaction results were positive in 90 (53.2%) of 169 patients. The antigen rapid diagnostic test (Ag-RDT) was able to find 66 (73.3%) of the 90 RT-PCR positive samples as positive ($p<0.001$). In all positive samples by Ag-RDT, RT-PCR was positive. The sensitivity, specificity, negative predictive value, and positive predictive value of the Ag-RDT were 73.3%, 100%, 76.7%, and 100%, respectively. The virus detection performance of the Ag-RDT was significantly more successful in the cycle threshold ≤20 ($p<0.001$). There was no correlation between PCR positivity and the time since vaccination.

Conclusion: The Ag-RDT test can be a good option for early detection of cases and early prevention, as it is quick and easy to implement in every laboratory and even at the point of care.

Keywords: SARS-CoV-2, COVID-19, rapid diagnostic tests, reverse transcriptase polymerase chain reaction

Introduction

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), which has swept the world, is the greatest global health disaster of the century (1,2). As of January 2024, 774,469,939 cases were diagnosed worldwide (3). The clinical presentation of SARS-CoV-2 infection can be variable, ranging from asymptomatic infection to severe disease that can result in death. The most common symptoms of COVID-19 are fever and cough, fatigue, shortness of breath, and loss or change of smell and taste. Some patients also experience gastrointestinal symptoms (e.g., nausea and diarrhea), headaches, chest pain, and conjunctivitis (4,5). Coronavirus disease-2019 has symptoms similar to those of many diseases, so the need for differential diagnosis continues (6).

Diagnostic testing has played a central role in limiting the spread of infection throughout the COVID-19 pandemic (7,8). Nucleic acid amplification tests (NAAT) and antigen rapid diagnostic tests (Ag-RDT) have been commonly used to diagnose SARS-CoV-2 infection. Although reverse transcription-polymerase chain reaction (RT-PCR) tests are considered the gold standard in the diagnosis of COVID-19 in terms of sensitivity and specificity, these tests have some drawbacks, such as requiring trained personnel and specialized instruments,

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being time-consuming, and having high costs (7,9). Compared with NAAT tests, some key advantages of Ag-RDTs are simpler handling, fast turnaround time, the absence of instruments, and low cost (10). In addition, although these tests are less likely to detect the virus than PCR tests, especially in infectious cases with a high viral load, positive results are very accurate and reliable (10,11). Because these tests are portable, they can be used wherever the patient is in non-healthcare environments, such as school or home (7). Antigen rapid diagnostic tests have been widely used around the world, especially in countries where prevalence is high (12). The prevalence of disease is known to affect the positive predictive value (PPV) of tests. As the prevalence increases, the PPV also increases, but the negative predictive value (NPV) decreases (13). The hypothesis of this study, conducted at a time when SARS-CoV-2 prevalence was relatively high, was that Ag-RDT could be used instead of SARS-CoV-2 PCR.

Sensitivity and specificity are the main parameters related to the performance of diagnostic tests. However, the sensitivity of antigen tests to detect SARS-CoV-2 remains controversial. Therefore, in this study, we aimed to evaluate the performance of the Panbio™ COVID-19 Ag-RDT compared to RT-PCR, the gold standard in the diagnosis of COVID-19.

Methods

Compliance with Ethical Standards

This study was prospective, single-center cross-sectional. It was approved by University of Health Sciences Turkey, Basaksehir Cam and Sakura City Hospital, Clinical Research Ethics Committee (reference no.: 2022/370). This study was conducted in accordance with the Declaration of Helsinki. Participants were informed about the study, and written consent was obtained.

Participants and Samples

This study consists of 169 participants aged 18 years and older with a suspicion of COVID-19 who applied to the emergency service of University of Health Sciences Turkey, Basaksehir Cam and Sakura City Hospital between November 28, 2022, and December 30, 2022. All participants' contact history, symptoms, number of days since the onset of symptoms, vaccination information, and demographic data were questioned. Two simultaneous nasopharyngeal swab samples were collected from the participants for Ag-RDT and real-time RT-PCR testing.

Rapid Antigen Test

The Panbio™ COVID-19 Ag-RDT (Abbott Rapid Diagnostics, Jena, Germany) was used for the qualitative detection of specific SARS-CoV-2 antigens [viral nucleocapsid (N) protein] in nasopharyngeal samples. It contains a membrane strip pre-coated with immobilized anti-SARS-CoV-2 antibodies in the test line and a monoclonal antibody in the control line. This lateral flow test detects viral N antigens with color change as assessed by naked eye reading. The test is interpreted in 15 minutes in accordance with the manufacturer's recommendations, so that the test results of the patients are obtained in less than 30 minutes.

SARS-Cov-2 RNA Detection Using Real-Time RT-PCR

SARS-CoV-2 RNA was analyzed using a Bio-Speedy® SARS-CoV-2 double gene RT-PCR kit (Bioeksen, Istanbul, Turkey) on a CFX96 Touch System (Bio-Rad Laboratories, Inc., United States). The kit is a one-step reverse transcription and qualitative real-time RT-PCR test that provides qualitative detection of SARS-CoV-2 RNA in respiratory tract samples. The kit's limit of detection is 500 copies/mL for nasopharyngeal swab samples. It targets virus-specific open reading frame 1ab and N genes. Internal control (Human RNAseP mRNA) and negative and positive controls were used in each run. A cycle threshold (Ct) value of <36 was considered a positive result.

Statistical Analysis

Statistical analyses were performed using the SPSS Statistics v21 software (SPSS Inc., Chicago, USA). Visual (histograms) and analytical (Kolmogorov-Smirnov/Shapiro-Wilk's test) methods were used to test the normality of the distributions of continuous variables. The Mann-Whitney U test was used to compare parametric variables between groups. The chi-square test was used for categorical comparisons of nominal values between groups. The diagnostic decision-making properties of PCR Ct values in predicting Ag-RDT results were analyzed using receiver operating characteristic (ROC) curve analysis. When a significant cut-off value was observed, the sensitivity, specificity, PPV, and NPV were calculated. A p-value of <0.05 was considered significant.

Results

In this study, the median age of the 169 participants was 35 [minimum: 18 - maximum: 84; interquartile range (IQR): 26-46], and 59.2% (n=100) were male. The RT-PCR results were positive in 90 (53.2%) of 169 patients. The Ag-RDT was able to detect 66 (73.3%) as positive, while it missed 24 (26.7%) (p<0.001). In all positive samples by the Ag-RDT, RT-PCR was positive (Table 1). The sensitivity, specificity, NPV, and PPV of Ag-RDT were 73.3%, 100%, 76.7%, and 100%, respectively, was used as the gold standard.

The median Ct value of the PCR-positive samples was 19 (IQR: 16-22). A significant difference was found between the Ct values of PCR +/ Ag-RDT + samples (median Ct: 18)

and PCR +/Ag-RDT - samples (median Ct: 24) (p<0.001). Ag-RDT results according to PCR Ct values are shown in Figure 1. The Ag-RDT was positive in 85% (51/60) of those with high viral load (Ct≤20) and 50% (15/30) of those with low viral load (Ct>20). The virus detection performance of Ag-RDT was significantly more successful in the Ct≤20 (p<0.001) (Table 1). The sensitivity and specificity values of the Ag-RDT were 71% and 65%, respectively, when the ROC analysis set the cut-off value of the PCR Ct at 19.9. The area under the ROC curve was 0.76 (95% confidence interval: 0.64-0.88) and was statistically significant (p<0.001).

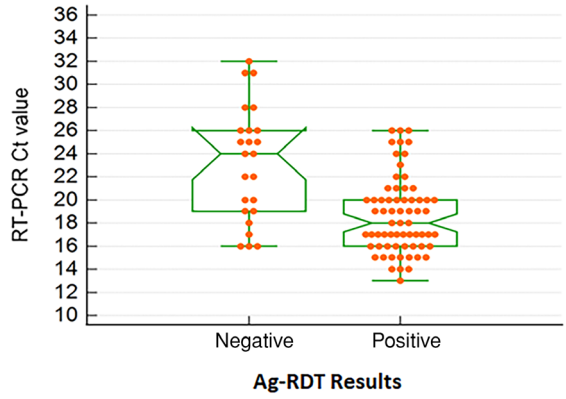

Figure 1. Comparison of SARS-CoV-2 antigen rapid diagnostic test (Ag-RDT) results with RT-PCR cycle threshold (Ct) values
SARS-CoV-2: Severe acute respiratory syndrome-coronavirus-2, RT-PCR: Reverse transcription-polymerase chain reaction

The samples were collected on the median day 3 of symptom onset. There was no significant difference in the RT-PCR and Ag-RDT test results for samples collected on days ≤4 and >4 of symptom onset (Table 2). Of the patients, 50.3% had fever, 22.5% had loss of taste or smell, and 1.8% had pneumonia (Table 3). Seven participants with a history of exposure were asymptomatic, and four of them were both the RT-PCR and Ag-RDT positive.

Out of the 153 individuals who received an average of two doses of vaccination, 112 received the Biontech vaccine, and 84 (54.9%) of these cases showed RT-PCR positive results despite the vaccination. Comorbidities were present in 24 (14.2%) study participants. The symptoms, time since vaccination, and comorbidities of the participants are detailed in Table 3.

Discussion

Given the global consequences of the COVID-19 pandemic, rapid and reliable diagnosis is crucial in identifying potentially contagious individuals, ensuring correct clinical management of patients, and taking necessary measures (14,15). The present study compared the results of a diagnostic test based on the lateral flow principle, which rapidly detects the SARS-CoV-2 N protein, with the results of RT-PCR. The RT-PCR and Ag-RDT results of 169 samples were compared, and discordant results were obtained in 24 of 90 positive samples. The Ag-RDT missed all of the discordant results, which were false negatives (Ag-RDT-/RT-PCR+). According to the statistics performed

Table 1. Evaluation of the diagnostic performance of Panbio™ COVID-19 Ag-RDT

RT-PCR	Total (n=169)	Panbio™ COVID-19 Ag-RDT		
		Antigen negative (n=103)	Antigen positive (n=66)	p-value
Negative	79	79	0	
Positive	90	24	66	
Ct, median (IQR)	90	24 (19-26)	18 (16-20)	<0.001*
Ct ≤20	60	9	51	<0.001**
Ct >20	30	15	15	

*Mann-Whitney U test, **Pearson chi-square test
COVID-19: Coronavirus disease-2019, Ag-RDT: Antigen rapid diagnostic test, RT-PCR: Reverse transcription-polymerase chain reaction, IQR: Interquartile range, Ct: Cycle threshold

Table 2. Comparison of RT-PCR and Panbio™ COVID-19 Ag-RDT results according to participants' symptom days

	Onset of symptoms the sample was collected			p-values
	≤4 days	>4 days	Total	
PCR negative	64	6	70	0.86*
PCR positive	77 (90.6%)	8 (9.4%)	85 (100%)	
Antigen negative	83	9	92	0.69*
Antigen positive	58 (92.1%)	5 (7.9%)	63 (100%)	

*Pearson chi-square test
COVID-19: Coronavirus disease-2019, Ag-RDT: Antigen rapid diagnostic test, RT-PCR: Reverse transcription-polymerase chain reaction

Table 3. Symptoms, vaccination periods and comorbidity of the participants				
	Total (n=169)	RT-PCR		
		Negative	Positive	p-value
URTI	144 (85.2%)	66 (39.1%)	78 (46.1%)	0.57*
Symptoms	158 (93.5%)	72 (42.6%)	86 (50.9%)	0.25*
Fever	85 (50.3%)	31 (18.3%)	54 (32%)	0.007*
Loss of taste/smell	38 (22.5%)	15 (8.9%)	23 (13.6%)	0.31*
Pneumonia	3 (1.8%)	1 (0.6%)	2 (1.2%)	
Vaccinated individuals	153 (90.5%)	69 (45.1%)	84 (54.9%)	0.18*
Time since vaccination				
In 1-3 months	7 (4.1%)	3	4	
In 4-6 months	46 (27.2%)	25	21	
6 months ago	79 (46.7%)	35	44	
Unvaccinated	37	16	21	
Comorbidity	24 (14.2%)	8	16 (67%)	
CLD	1	1	0	
COPD	1	1	0	
Asthma	7	2	5	
Diabetes	11	3	8	
CKF	2	1	1	
Steroid use	2	0	2	
Immunosuppression	0	0	0	

*Pearson chi-square test
URTI: Upper respiratory tract infection, CLD: Chronic lung disease, COPD: Chronic obstructive pulmonary disease, CKF: Chronic kidney failure, RT-PCR: Reverse transcription-polymerase chain reaction

by accepting RT-PCR as the gold standard, the sensitivity of Ag-RDT was 73.3%, specificity 100%, NPV 76.7%, and PPV 100%. Studies conducted in various populations and countries during the pandemic period detected sensitivity performances of Ag-RDT ranging from 24% to 93% (16). In more than 10 clinical studies involving more than 6000 subjects evaluating the performance of Panbio™ Ag-RDT, sensitivity and specificity ranges of 71.4-91.7% and 94.9-100%, respectively, were reported (17-19). Treggiari et al. (20) found the sensitivity, specificity, PPV, and NPV values of the Ag-RDT to be 66.82%, 99.89%, 97.87%, and 97.62%, respectively. In three different studies conducted in our country, the sensitivity and specificity rates were found to be 61.8% and 97.6%, 88.7 and 98.0, 70% and 100%, respectively (21-23). The sensitivity and specificity values determined in our study were consistent with those of previous studies.

Many studies have shown a clear relationship between the Ct value and the positivity rate of Ag-RDT (14,24-26). A study in China reported excellent performance of rapid tests in patients with higher viral loads, especially those with upper respiratory tract symptoms (16). In this study, Ag-RDT susceptibility was significantly higher in subjects with a high viral load (85%) than in those with a low viral load (50%). Eikelenboom-Boskamp et al. (27) conducted a study using the Panbio™ Ag-RDT and found its sensitivity to be 81%. However, given the low contagiousness of patients with a Ct value >32, they found the test's sensitivity to be 92.7% when they used 32 as the cut-off Ct value instead of 40. Indeed, it may be more advantageous to detect Ag-RDT only in the acute phase of the disease, when the viral load is high, compared with highly sensitive PCR positivity (sometimes the positivity persists for a long time). Thus, unnecessary isolation of patients who are no longer contagious, that is, with a low viral load, can be avoided (24,28). Meanwhile, there are studies showing that the sensitivity of Ag-RDTs for asymptomatic patients is significantly lower than that for symptomatic patients (4). Ag-RDT may be a good option, especially for the early diagnosis of infectious symptomatic cases and the early taking of precautions, due to its fast and easy application in every laboratory and even at the point of care, and its advantages over PCR (29).

The prevalence of the disease affects the PPV of the tests. As prevalence increases, PPV also increases, but NPV decreases. The European Commission recommends the use of Ag-RDT in a publication on COVID-19 testing

procedures because the predictive rates of Ag-RDTs are high in populations with a high prevalence of SARS-CoV-2. However, the European Centre for Disease Prevention and Control suggests using RT-PCR or a different brand of Ag-RDT to confirm positive samples in settings where the prevalence is less than 10% (29).

The Centers for Disease Control and Prevention recommends that everyone over 6 months of age, especially the elderly and immunocompromised, who are at high risk of serious illness, receive an updated COVID-19 vaccine to protect against possible serious COVID-19 disease in the fall and winter months (30). There are randomized, placebo-controlled studies showing the high efficacy of COVID-19 vaccines. However, these data may vary depending on the characteristics of the population, vaccine, and viral strain (31). In a case-control study in Germany, the two-dose vaccine efficacy was 89% overall. It was 79% in patients with more than two comorbidities and 77% in adults aged 60-75 years. The third dose increased vaccine efficacy to over 93% in all patient subgroups (32). In this study, no correlation was found between PCR positivity and the time since vaccination (vaccinated in the last 3 months, 4-6 months, and in the last 6 months). Reasons for this may include the small number of participants vaccinated in the last 3 or 6 months, insufficient vaccine doses, or the vaccines not working.

Study Limitations

The study should be interpreted with some limitations. False-negative Ag-RDT tests could not be rerun from the same samples because there were not enough tests. As different variants dominate periodically for SARS-CoV-2, this may affect the kit's performance, depending on the content of the kit. The strength of this study is that it shows that rapid diagnosis with Ag-RDT is critical, especially in cases with a high viral load (highly contagious).

Conclusion

The Panbio™ Ag-RDT kit can be good option in the rapid identification of COVID-19 patients. However, it's important to acknowledge that this qualitative test cannot completely rule out the possibility of COVID-19 infection, particularly considering the potential for false negative results. Ag-RDTs can provide significant benefits in rapid diagnosis, using the right algorithms and confirmed by RT-PCR when necessary.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Turkey, Basaksehir Cam and Sakura City Hospital, Clinical Research Ethics Committee (reference no.: 2022/370).

Informed Consent: Participants were informed about the study, and written consent was obtained.

Authorship Contributions

Surgical and Medical Practices: A.G., B.O., O.A.A., Concept: S.A., N.K., O.A.A., Design: S.A., N.K., O.A.A., Data Collection or Processing: S.A., A.G., B.O., Analysis or Interpretation: S.A., N.K., A.G., Literature Search: S.A., B.O., Writing: S.A., N.K.

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Original Article

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Effect of Blood Glucose Monitored Before Dialysis on Hypoglycemia During Dialysis in Adult Acute Hemodialysis Patients: A Multicenter Study

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Abstract

Aim: Individuals receiving hemodialysis treatment may observe glucose fluctuations due to decreased plasma glucose concentration and insulin level. The aim of this study was to evaluate pre-dialysis blood glucose measurements in acute hemodialysis patients to reduce complications that may occur during this process.

Methods: The study design was an observational-retrospective one. A total of 200 files belonging to the last 3 months in three centers were scanned between August 1, 2022, and September 30, 2022. While the files were being scanned, the 30-question "Hypoglycemia in Hemodialysis" patient form was used, and the IBM Statistical package for the social sciences 26.0 statistical program was used to evaluate the data.

Results: Hypoglycemia developed during hemodialysis in 4 of 104 patients whose blood glucose was checked before hemodialysis and in 2 of 96 patients whose blood glucose was not measured. It was determined that there was no significant difference ($p>0.05$) in terms of the rate of development of hypoglycemia between patients whose blood glucose levels were checked before hemodialysis and those who did not.

Conclusion: The study's results suggest that acute hemodialysis patients' pre-dialysis hypoglycemia does not influence the hypoglycemia that develops during the session.

Keywords: Complications, glucose, hemodialysis, hypoglycemia

Introduction

Hemodialysis is the most commonly used method for treating chronic renal failure worldwide, as well as in our country. The Turkish Society of Nephrology used epidemiologic data for the Chronic Renal Disease in Turkey Prevalence Study. According to this study, 15.7% of the Turkish population has chronic kidney disease, and 26.6% of those with this disease also have diabetes. The prevalence of chronic kidney disease in people with diabetes is 32.4%. This rate is 2.5 times higher than that of patients without diabetes (1). In diabetes, fluctuations with hyperglycemia and hypoglycemia occur (2). Hemodialysis treatment can cause glycemic fluctuations due to decreased plasma glucose concentrations and insulin levels. Therefore, especially in diabetic hemodialysis patients, control of blood glucose and adjustment of drug doses are important (3). In the study by Kang et al. (2), hypoglycemia was observed in 16.8% of diabetic hemodialysis patients and 6.9% of non-diabetic patients in the first year of dialysis. Hayashi et al. (4) reported that although dialysate containing 100-150 mg/dL glucose was used in diabetic patients receiving hemodialysis treatment, hemodialysis-induced hypoglycemia unconsciousness

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was frequently experienced, and the sensor glucose level could fall well below the dialysate glucose concentration toward the end of hemodialysis. Studies have shown that hemodialysis treatment causes changes in blood glucose levels. When the literature is reviewed, there are a limited number of studies on the control of blood glucose levels in hemodialysis patients to reduce complications (5-10).

In routine hemodialysis applications, there is no clear recommendation for routine glucose measurement before dialysis in either diabetic or non-diabetic patients. Because the response to hypoglycemia may not be sufficient in acute hemodialysis patients, identifying these patients before they develop hypoglycemia may provide early recognition of serious adverse effects and may be preventive. The aim of this study was to determine the frequency of hypoglycemia that may develop during hemodialysis in acute hemodialysis patients with and without pre-hemodialysis blood glucose monitoring, as well as to reduce the complications that may occur during this process. We anticipate that the study's results will inform the development of treatment protocols.

Methods

Compliance with Ethical Standards

Ethical approval for this study was obtained from the Publication Ethics Committee For Social Sciences and Humanities for Istanbul Beykent University (approval no.: 62746, date: 22.07.2022).The research design was an observational-retrospective study. In the study, the files of 200 acute hemodialysis patients aged 18 years and over, belonging to the last three months, in the dialysis units of a total of three centers, including a university hospital, a medical faculty, and two different training and research hospitals, were examined between August 1, 2022, and September 30, 2022. Patients were coded with the first two letters of their names, the first two letters of their surnames, and the last two digits of their date of birth (e.g., EGCE81). The 30-question "Hypoglycemia in Hemodialysis" patient form created while scanning the files was used (Figure 1).

If the blood glucose measurements of the patients were below 70 mg/dL (3.9 mmol/L) (with or without symptoms), hypoglycemia was considered. Neurogenic (tremor, palpitation, sweating, anxiety, paresthesia) and neuroglycopenic symptoms (dizziness, weakness, lethargy, delirium, confusion, seizure, coma) were considered hypoglycemia symptoms (11). Symptomatic hypoglycemia was defined as having typical hypoglycemia symptoms along with a glucose level of ≤70 mg/dL (3.9 mmol/L). Asymptomatic hypoglycemia, on the other hand, did not have typical hypoglycemia symptoms but did have a glucose level of ≤70 mg/dL (3.9 mmol/L). It was considered an event.

Statistical Analysis

The IBM Statistical package for the social sciences 22.0 statistical program was used for statistical analyses. Descriptive statistics (mean, standard deviation, median, and percentage) methods were used to evaluate the central tendency and distribution of the study variables. In the comparison of the two groups, the Student's t-test was used to compare normally distributed data for categorical variables, and the Mann-Whitney U test was used to compare non-normally distributed data. The chi-square test was used for non-categorical variables in the

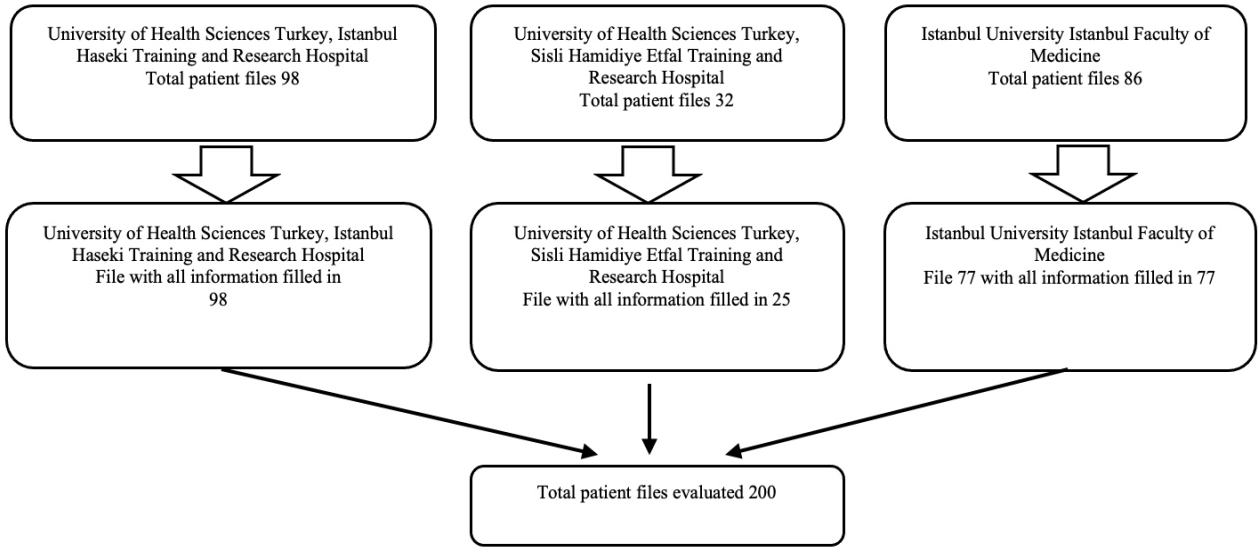


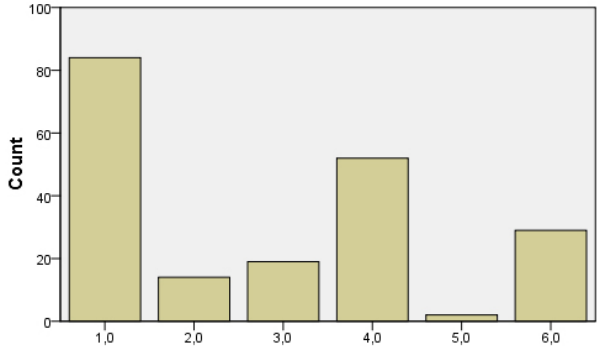
Figure 1. Flowchart of patient files included in the study

comparison of the two groups. The results were evaluated at a 95% confidence interval and a significance level of $p<0.05$.

Results

A total of 200 acute hemodialysis patient files, including 98 (49%) from University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital, 25 (12.5%) from University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital, and 77 (38.5%) from Istanbul University, Istanbul Faculty of Medicine, were reviewed, and data were collected. Table 1 displays the epidemiologic and demographic characteristics of the patients. Of the patients, 107 (53.5%) were male, and the mean age was 62±15.7 years. Seventy-seven (38.5%) patients were primary school graduates, and the most common cause of primary kidney disease was hypertensive nephrosclerosis (41%), followed by diabetic nephropathy (36.5%). When comorbidities were evaluated, 103 (51.5%) had diabetes mellitus. Thirty-seven (18.5%) had a family history of diabetes mellitus. The most common indication for acute hemodialysis was uremia (42%; Figure 2). When the antidiabetic drug and insulin use status of diabetic patients were analyzed, 42 (48%) were using oral antidiabetic drugs and 73 (70.9%) were using insulin (Table 1).

Blood glucose was checked in 104 (52%) patients before dialysis, whereas blood glucose was not checked in 96 patients before dialysis (Table 2). The mean blood glucose level of the patients whose blood glucose levels were checked was 138±54 mg/dL (minimum: 66 mg/dL, maximum: 294 mg/dL). The mean systolic and diastolic blood pressures before dialysis were 139±17 mmHg and 79±16 mmHg, respectively. During dialysis, two patients received oral nutrition, while four received oral or intravenous nutrition support. There was no significant



Uremia (1), Hyperpotassemia (2), Metabolic acidosis (3), Hypervolemia (4), Uremic encephalopathy (5), Other (6)

Figure 2. Indications for patients to undergoing acute hemodialysis

Table 1. Epidemiological and demographic characteristics of the patients (n=200)

	N (%)
Centre	
University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital	98 (49)
University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital	25 (12.5)
Istanbul University, Istanbul Faculty of Medicine	77 (38.5)
Gender	
Woman	93 (46.5)
Man	07 (53.5)
Age (average)	62±15.7
Educational status	
Illiterate	21 (10.5)
Literate	37 (18.5)
Primary school	77 (38.5)
Middle-high school	64 (32)
University	1 (0.5)
Causes of primary kidney disease	
Diabetic nephropathy	73 (36.5)
Hypertensive nephrosclerosis	82 (41)
Chronic glomerulonephritis	5 (2.5)
Autosomal dominant polycystic kidney disease	3 (1.5)
Other	22 (11)
Unknown	15 (7.5)
Diabetes	
There is	103 (51.5)
There isn't	97 (48.5)
Family history of diabetes	
There is	37 (18.5)
There isn't	39 (19.5)
Unknown	124 (62)
Indications for patients to undergoing acute hemodialysis	
Uremia	84 (42)
Hyperkalemia	14 (7)
Metabolic acidosis	19 (9.5)
Hypervolemia	52 (26)
Uremic encephalopathy	2 (1)
Other	29 (14.5)
Antidiabetic drug and insulin use status of patients with diabetes (n=103)	
Antidiabetic drug use	
There is	42 (40.8)
There isn't	61 (59.2)
Insulin use	
There is	73 (70.9)
There isn't	30 (29.1)
Descriptive statistical methods (mean, standard deviation, frequency, percentage)	

difference between the genders of the patients with and without blood glucose monitoring ($p<0.05$), whereas the patients with blood glucose monitoring were older ($p<0.05$). There was no significant difference between the urea, creatinine, albumin, and potassium values before dialysis between both groups ($p>0.05$). Diabetes was present in 58 (55.7%) of 104 patients with blood glucose monitoring, whereas 45 (46.8%) of 96 patients without blood glucose monitoring had diabetes, and the rates of diabetes were similar in both groups ($p>0.05$).

Hypoglycemia developed in 6 (3%) of 200 patients during hemodialysis. It was observed that blood glucose decreased during hemodialysis in 4 (2%) patients in the group whose blood glucose was checked before hemodialysis and in 2 (1%) patients in the group whose blood glucose was not checked. The rates of hypoglycemia during dialysis in patients with and without blood glucose monitoring were similar ($p>0.05$) (Table 3). All patients who developed hypoglycemia responded to hypoglycemia treatment.

One patient (16.7%) experienced prolonged dialysis due to hypoglycemia during hemodialysis, while six patients did not terminate their hemodialysis treatment (Table 4). Other than hypoglycemia, 38 patients developed complications during hemodialysis. The most common complication was hypotension, which occurred in 27 patients.

Table 2. Pre-dialysis blood glucose level and hypoglycemia status during hemodialysis

	N (%)
Blood glucose measurement before hemodialysis	
There is	104 (52)
There isn't	96 (48)
Development of hypoglycemia during hemodialysis	
There is	6 (3)
There isn't	197 (97)
Descriptive statistical methods (frequency, percentage)	

Table 3. Comparison of the number of patients with or without blood glucose monitoring before hemodialysis and those who developed hypoglycemia

	Developing hypoglycemia (n=6)	
	N (%)	p-value
Blood glucose level before hemodialysis (n=104)	4 (2)	0.430
Blood sugar not checked before hemodialysis (n=96)	2 (1)	
Chi-s Square test		

Discussion

This multicenter study included 200 acute hemodialysis patients, with approximately half diagnosed with diabetes, and found a 3% frequency of hypoglycemia during hemodialysis in these patients. In the study by Kang et al. (2), the rate of hypoglycemia in diabetic patients in the first 6 months of dialysis was 16.8%, whereas the rate of hypoglycemia in patients without diabetes was 6.9%. In addition, age, female gender, race, presence of a central venous catheter, lower residual renal function, and longer duration of dialysis sessions were found to affect hypoglycemia, and the risk of all-cause mortality was higher in patients with hypoglycemia. In a single-center study by Habte-Asres et al. (12) on 56 dialysis patients with diabetes, hypoglycemia was found in 23.6% of the patients. The rates of patients with hypoglycemia in this study and the literature differ. The study measured the blood glucose level during dialysis. However, the literature does not provide clear information about the timing of the glucose measurement. The discrepancy in the results could possibly be due to the timing of the blood glucose check. In addition, oral and parenteral nutrition during dialysis may have affected the results. Furthermore, in these patients, decreased caloric intake, decreased renal gluconeogenesis, and decreased insulin clearance may contribute to the increased risk of hypoglycemia. In addition, poor glycogen stores due to malnutrition, prolonged half-lives of insulin or oral antidiabetics, and the use of drugs that regulate the response to hypoglycemia (lower than normal blood glucose), such as beta blockers, may contribute to low blood glucose. In daily practice, glucose dialysate is recommended only in diabetic patients because of the risk of intradialytic hypoglycemia. Although all patients included in our study were hemodialyzed with 100 mg/dL glucose dialysate, hypoglycemia still developed in 3% of the patients. Although a rate of 3% may seem low, it may be preventive to be more careful in this

Table 4. The effect of hypoglycemia developing during hemodialysis on treatment

	N (%)
Treatment termination status of the patient who developed hypoglycemia during hemodialysis	
Yes	0 (0)
No	6 (100)
Prolongation of the treatment of the patient who developed hypoglycemia during hemodialysis	
Yes	1 (16.7)
No	5 (83.3)
Descriptive statistical methods (frequency, percentage)	

regard, considering the additional complications that may occur after hypoglycemia. In patients with hypoglycemia, intradialytic catabolism increases, and severe fatigue occurs after dialysis. When hypoglycemia is excessive, symptoms sometimes become more severe and may lead to major arrhythmias, particularly in patients with ischemic heart disease (13).

Patients who undergo hemodialysis may die due to hypoglycemia. Therefore, continuous monitoring and control of blood glucose, especially before hemodialysis, is important. Literature studies support this view (14-16).

The study's retrospective nature and exclusion of many data points from the evaluation due to their incompleteness in the patient files resulted in a smaller sample size. Studies with larger sample sizes, or those that take the number of sessions as a sample, are believed to yield more meaningful data. In addition, the percentage differences in the files selected from the centers [98 (49%) from University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital, 25 (12.5%) from University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital, and 77 (38.5%) from Istanbul University, Istanbul Faculty of Medicine] are considered the limitations of the study.

Hypotension is the most common acute complication during hemodialysis (20-30%), followed by cramps (5-20%), nausea and vomiting (5-15%), pruritus (5%), headache (5%), back and chest pain (2-5%), chills, and fever (2%). The most common complication observed in our patients included in the study was hypotension (13.5%). Hypoglycemia is not as common as these complications; however, severe hypoglycemia may prolong the duration of dialysis and cause severe neurological findings, making it a complication that requires the attention of the dialysis team regardless of its frequency.

Diabetic kidney disease is the most common cause of end-stage renal failure worldwide and in our country, and diabetes is an important risk factor for both chronic and acute kidney damage. According to the 2022 Report of the Turkish Society of Nephrology, diabetes ranks first with a rate of 36.29%, hypertension with a rate of 31.65%, and glomerulonephritis with a rate of 4.61% in the etiologies of hemodialysis patients in 2022 (17). In the present study, hypertensive nephrosclerosis was the most common cause of primary renal disease, with a rate of 82 (41%), and diabetic nephropathy was the second most common cause, with a rate of 73 (36.5%). The results of this study are different from those in the literature. This difference is believed to be due to the study's small sample size.

In our study, 84 (42%) patients were admitted to acute hemodialysis for uremia, 52 (26%) for hypervolemia, 19 (9.5%) for metabolic acidosis, 14 (7%) for hyperpotassemia (the potassium level in the blood is higher than it should be), 2 (1%) for uremic encephalopathy, and 29 (14.5%) for other indications. In the study conducted by Gülle et al. (18), the indications for emergency hemodialysis were found to be hypervolemia (31.8%), hyperpotassemia (22.7%), uremic findings (21%), and metabolic acidosis (19.2%) in order of frequency. Although the indications determined in this study and the literature are the same, the differences in the frequency of occurrence are striking. The socio-economic and cultural differences in the selected patient group, the barriers to health care access, and the status of the control group may influence the differences between the two studies.

Study Limitations

Studies with a larger sample size or a sample of more sessions are believed to yield more meaningful data. Additionally, the percentage differences in the files selected from the centers [98 (49%) from University of Health Sciences Turkey, Istanbul Haseki Training and Research Hospital, 25 (12.5%) from University of Health Sciences Turkey, Sisli Hamidiye Etfal Training and Research Hospital, and 77 (38.5%) from Istanbul University, Istanbul Faculty of Medicine] are deemed limitations of the study. Despite these limitations, the study holds value as it is the first in the literature to measure blood glucose prior to hemodialysis. This study is valuable because it raises awareness of the importance of glycemic control in hemodialysis patients.

Conclusion

Hypoglycemia developed in 6 (3%) of 200 patients during hemodialysis. Four (2%) patients who had their blood glucose checked before hemodialysis experienced a decrease in blood glucose during hemodialysis, while 2 (1%) patients did not have their blood glucose checked. There was no significant correlation between the rates of hypoglycemia during dialysis in patients with and without blood glucose monitoring. Despite the seemingly low rate of 3% and the lack of significant correlation between the two groups, this rate holds significance as hypoglycemia can potentially result in death. In routine hemodialysis practice, there is no clear recommendation for routine glucose measurement before dialysis in both diabetic and non-diabetic patients. Because the response to hypoglycemia may not be adequate in acute hemodialysis patients, identifying these patients before hypoglycemia develops may be preventive by providing early recognition of serious adverse effects. These studies should be increased, and treatment protocols and guidelines should be developed.

Ethics

Ethics Committee Approval: Approval for this study was obtained from the Publication Ethics Committee

For Social Sciences and Humanities for Istanbul Beykent University (approval no.: 62746, date: 22.07.2022).

Informed Consent: Since the data used in the study were obtained from the hospital registration system, an individual informed consent form was not required.

Authorship Contributions

Concept: I.C., E.C., V.Z.Y., Design: I.C., E.C., V.Z.Y., Data Collection or Processing: I.C., N.K., E.C., Analysis or Interpretation: N.K., E.C., Literature Search: I.C., N.K., E.C., V.Z.Y., Writing: I.C., N.K., E.C.

Conflict of Interest: No conflict of interest was declared by the authors.

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Original Article

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Evaluation of the Association Among Cerebrospinal Fluid Protein, Inflammatory Markers, and Electromyography in Pediatric Guillain-Barre Syndrome

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Abstract

Aim: Previous studies have shown that the cerebrospinal fluid (CSF) protein level correlates with the number of demyelination criteria in electromyography in adult patients with Guillain-Barré syndrome (GBS), which is a potentially life-threatening postinfectious disease. We aimed to assess the association between CSF protein level, inflammatory markers, and electrophysiological values in the diagnosis of pediatric patients to act quickly in treating GBS.

Methods: In this cross-sectional study, thirty-nine children with GBS were retrospectively analyzed from the medical records of patients who were treated as inpatients between 2013 and 2021. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, C-reactive protein, uric acid, CSF protein levels, and electrophysiological parameters of the patients on admission were recorded. Hughes disability scores (HDS) were evaluated to determine the severity of disability on admission and at the 3rd month.

Results: Cerebrospinal fluid protein was positively correlated with tibial and peroneal motor nerve distal latency (DL) and negatively correlated with tibial and peroneal sensorial nerve conduction velocities (NCV). In the acute inflammatory demyelinating polyneuropathy group, 3rd-month HDS was significantly lower than in the acute motor axonal neuropathy group. A positive correlation was found between first-admission HDS and 3rd-month HDS. There was no significant difference between the electrophysiological subgroups and inflammatory markers.

Conclusion: In pediatric GBS patients, well-standardized ranges of the tibial and peroneal motor nerves DL, as well as medial plantar and peroneal superficial NCV, may be sensitive markers. Early rehabilitation programs could prevent disability in immobile patients.

Keywords: Child, Guillain-Barre syndrome, uric acid, neural conduction, medical records

Introduction

Guillain-Barré syndrome (GBS) is a potentially life-threatening postinfectious disease characterized by rapidly evolving symptoms, which usually include ascending symmetrical weakness or paralysis, as well as hyporeflexia or areflexia. While some neurologists restrict treatment to patients with severe disease, others treat even patients with mild disease whose nerve conduction studies (NCS) are normal on electromyography (EMG) to avoid deterioration (1). Therefore, it is important to identify patients with a poor prognosis.

Because of increased blood-nerve barrier permeability, active myelin damage and increased antibody deposition result in increased cerebrospinal fluid (CSF) protein levels. Furthermore, high CSF protein levels are a marker of injury that correlates with progression and disability in adult patients with GBS (2). The literature found a correlation between the increase in CSF protein levels and the number of NCS demyelination criteria in adult GBS patients (3).

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Numerous studies have assessed the relationship between the neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), C-reactive protein (CRP), uric acid (UA), albumin, and prognosis in adult GBS patients (2-6). It has been suggested that NLR could be a prognostic factor for disability and respiratory failure in GBS patients (7). It has also been shown that high levels of UA and albumin are protective factors for patients with GBS (8). Chang et al. (6) demonstrated that UA levels in CSF were significantly increased in adult patients with acute inflammatory demyelinating polyneuropathy (AIDP), suggesting that CSF UA may be related to the pathogenesis of demyelination in patients with GBS.

The publications focused on adult patients, and we did not find any studies evaluating the effects of UA level or the correlation of specific EMG parameters and CSF protein on prognosis in pediatric GBS patients. In this study, we aimed to evaluate the association between CSF protein, inflammatory markers, and EMG in pediatric patients with GBS.

Methods

Compliance with Ethical Standards

The Clinical Research Ethics Committee of Harran University approved the study on August 16, 2021, under the number HRU/21.14.15, and it complies with the Declaration of Helsinki.

Study Design

In this cross-sectional study, electronic medical records of 39 patients with GBS who were under 18 years old and treated as inpatients at the Harran University Faculty of Medicine, Neurology, and Pediatric Neurology Departments between January 2013 and June 2021 were used. The patients diagnosed with GBS according to the Brighton criteria (9), who underwent CSF analysis and electrophysiological findings within the first 24 hours, were included in the study. The exclusion criteria were as follows: Steroid use, local or systemic infections, malignancy, and chronic diseases such as hematological, autoimmune, cardiovascular, renal, and hepatic disorders (Figure 1). Standard motor and antidromic sensory NCS were performed on at least four motor nerves (median, ulnar, tibial, and peroneal) and four sensory nerves (median, ulnar, medial plantar, and superficial peroneal). In motor nerves, distal latency (DL), amplitude, and duration of compound muscle action potential (CMAP), nerve conduction velocity (NCV), conduction block (CB), and temporal dispersion were evaluated. The F-wave minimum latency was also measured. The amplitude of the sensory nerve action potential (SNAP), peak latency, and NCV were measured in sensory nerves. The patients were electrophysiologically diagnosed with GBS according to the European Standardized Telematic Tool to Evaluate Electrodiagnostic Methods criteria (10) and distributed to the demyelinating group (AIDP), axonal group [acute motor axonal neuropathy (AMAN) and acute motor and sensorial axonal neuropathy (AMSAN)], and NCS normal group. All electrophysiological studies were conducted and evaluated by the same person.

White blood cell (WBC), neutrophil, lymphocyte, thrombocyte, CRP, UA, and albumin values, which were drawn from all patients within the first 24 hours after admission, were retrospectively recorded. Neutrophil-lymphocyte ratio was calculated as the ratio of neutrophil cell count to lymphocyte cell count, and PLR was calculated as the ratio of thrombocyte cell count to lymphocyte cell count. The disability of the patients was evaluated using the Hughes disability scale (HDS) at admission and during the control examination after 3 months. It is an accepted disability scale ranging from 0 to 6 for GBS patients and is as follows: 0/healthy, 1/minor symptoms and capable of running, 2/able to walk 5 m or more without assistance but unable to run, 3/able to walk 5 m across an open space with help, 4/bedridden or chair-bound, 5/requiring assisted mechanical ventilation for at least part of the day, and 6/death (11).

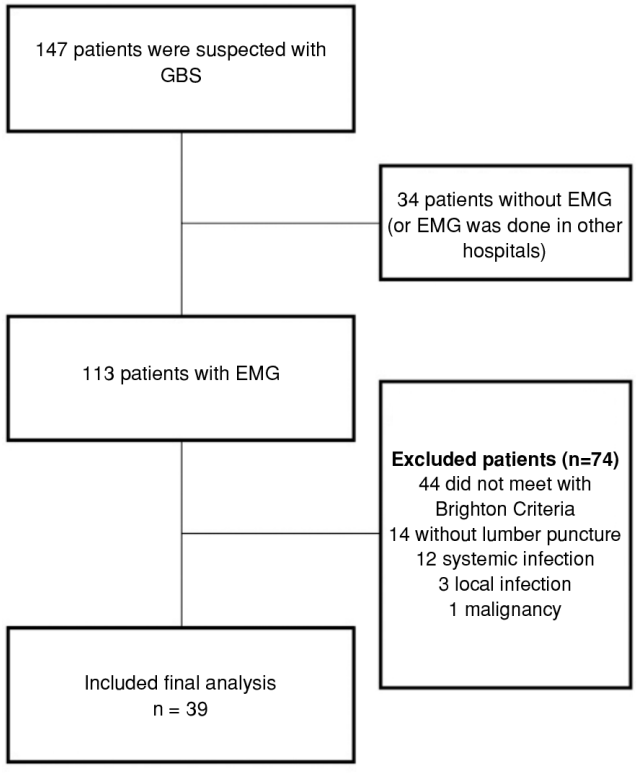


Figure 1. Flowchart of patient selection

Statistical Analysis

All statistical analyses were performed using the Statistical Package for Social Sciences for Windows version 22.0 (IBM Corp., 2013). IBM-SPSS Statistics for Windows, Version 22.0. Armonk, NY:IBM Corp.) software package. To test whether the data showed a normal distribution, the Kolmogorov-Smirnov test and the Shapiro-Wilk test were used. Student's t-test, one-way ANOVA, and Tukey's post hoc tests were used to compare normally distributed continuous variables. Categorical variables are expressed as numbers and percentages compared with the chi-square test. Differences in HDS between admission and after 3 months were examined using the Wilcoxon test. Pearson's test was used to identify correlations. A p-value of 0.05 was considered statistically significant for all comparisons and correlations.

Results

After administering the inclusion and exclusion criteria, 39 pediatric GBS patients were included in the study. Of these patients, 22 were boys (56.4%) and 17 were girls (43.6%). The mean age was 5.74±5.13 years. 13 (33.3%) patients had AMAN; 10 (25.7%) patients had AIDP; 16 (41%) had normal NCS. There were no AMSAN patients. In eight patients, we found that sensory nerves could not be obtained with decreased SNAP, prolonged DL, or reduction in NCV in the lower extremities; these patients were included in the AIDP group because they had demyelination findings in their motor nerves. EMG was performed on day 3.5 in the NCS normal group and on day 6.5 in the NCS abnormal group after complaints had started.

Fifteen patients were diagnosed with GBS with level 1 diagnostic certainty according to the Brighton Criteria. While the number of patients diagnosed with level 2 diagnostic certainty was also 15, 9 patients were diagnosed with level 3. No patients had level 4 diagnostic certainty (Table 1).

All AIDP patients had a reduction in NCV, prolonged DL, absent F-waves, or prolonged minimum F-wave latencies, 9 (90%) patients had CB. Twelve of 13 AMAN patients had absent F-waves or prolonged minimum F-wave latencies. One of the AMAN patients had a conduction block, and another had a reduction in NCV (Figure 2).

CSF protein was significantly higher in the AIDP group than in the AMAN and NCS normal groups (p<0.001). It was also higher in the AMAN group than in the NCS normal group, but this difference was not statistically significant (p=0.891).

There was no statistically significant difference between the WBC, neutrophil, leukocyte, NLR, PLR, CRP, albumin, UA, and CSF protein values and the electrophysiological subgroups of the patients. A comparison of the laboratory and clinical characteristics between the subgroups is presented in Table 2. 3rd-month HDS were significantly lower in the AIDP group compared to the AMAN group (p=0.006) (Figure 2).

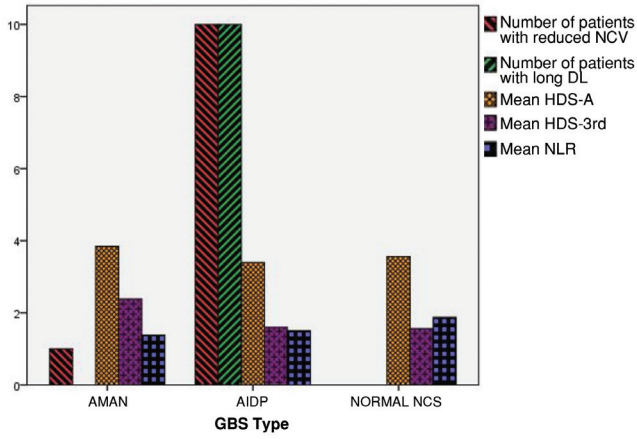
Although the 3rd-month HDS in the AMAN group was statistically higher than that in the other groups, the recovery rates at 3rd month compared with the initial HDS were significant in all three groups (p-AMAN=0.002, p-AIDP=0.007, p-NCS normal<0.001). A positive correlation was found between first-admission HDS and 3rd-month HDS (p=0.036, r=0.338). There was no correlation between the inflammatory markers and disability scores on admission or at 3rd-month (Table 3).

CSF protein was positively correlated with NCS parameters of tibial and peroneal motor nerve DL (respectively p=0.003, r=0.672 - p=0.033, r=0.564) and was also negatively correlated with NCS parameters of medial plantar and superficial peroneal sensorial NCVs (respectively p=0.041, r=0.897 - p=0.012, r=0.610).

Table 1. Diagnostic levels of patients according to the Brighton criteria between the GBS subgroups

	Brighton criteria level			
	1	2	3	4
AIDP, n (%)	9 (60)	1 (6.7)	0 (0)	0 (0)
AMAN, n (%)	6 (40)	7 (46.7)	0 (0)	0 (0)
Normal NCS n (%)	0 (0)	7 (46.7)	9 (100)	0 (0)

*Chi-square test
AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, NCS: Nerve conduction studies



GBS: Guillain Barre syndrome, AMAN: Acute Motor Axonal Neuropathy, AIDP: Acute Inflammatory Demyelinating Polyneuropathy, NCS: Nerve Conduction Study, NCV: Nerve Conduction Valocity, DL: Distal Latency, HDS-A: Hughes disability scale on admission, HDS-3rd: Hughes disability scale at 3rd-Distal Latency, HDS-A: Hughes disability scale on admission, HDS-3rd: Hughes disability scale at 3rd-month control, NLR: Neutrophil-Lymphocyte Ratio

Figure 2. Graphics of the number of the patients with reduced NCV and long DL, and mean values of HDS-A, HDS-3rd, NLR between the GBS subgroups

Table 2. Comparison of the laboratory and clinical characteristics between the GBS subgroups

	AIDP	AMAN	NCS Normal	p1	p2	p3
CSF protein	155.99±93.94	54.41±34.71	44.73±37.95	**<0.001**	**<0.001**	0.891
WBC	10.14±2.22	10.26±3.41	9.78±2.60	0.994	0.946	0.891
NEU	1.50±0.90	1.38±1.11	1.87±1.40	0.966	0.725	0.515
PLT	430.98±232.25	372.71±92.57	372.92±123.96	0.632	0.998	0.61
LYM	3.96±1.54	5.17±2.27	3.71±1.94	0.326	0.134	0.948
NLR	1.50±0.90	1.38±1.11	1.87±1.40	0.966	0.725	0.515
PLR	110.75±37.68	82.50±32.51	126.64±72.81	0.436	0.747	0.087
CRP (mg/dL)	0.07±0.06	0.21±0.33	2.69±9.77	0.998	0.564	0.55
UA (mg/dL)	3.71±0.61	3.77±0.82	3.71±1.86	0.495	0.99	0.412
Albumin (g/dL)	4.38±0.38	4.25±0.49	4.06±0.44	0.766	0.188	0.494
HDS-A	3.40±0.84	3.84±0.37	3.56±0.62	0.219	0.796	0.451
HDS- 3rd	1.60±.51	2.38±0.65	1.56±0.51	**0.006**	0.985	**0.001**

*One-Way ANOVA
Mean ± standard deviation, p1, significance between AIDP and AMAN; p2, significance between AIDP and NCS normal group; p3, significance between AMAN and NCS normal group.
GBS: Guillain Barre syndrome, AIDP: Acute inflammatory demyelinating polyneuropathy, AMAN: Acute motor axonal neuropathy, CSF: Cerebrospinal fluid, WBC: White blood cell, NEU: Neutrophil, PLT: platelet, LYM: Lymphocyte, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, CRP: C-reactive protein, UA: Uric acid, HDS-A: Hughes disability scale on admission, HDS-3rd: Hughes disability scale at 3rd-month control

Table 3. Correlation between inflammatory markers and disability scores

	HDS-A		HDS-3rd	
	r	p	r	p
NLR	-0.177	0.281	0.157	0.339
PLR	-0.042	0.8	0.37	0.822
CRP	0.117	0.477	-0.111	0.501
UA	0.028	0.867	0.23	0.158
Albumin	-0.149	0.367	-0.146	0.374

*Pearson correlation test
HDS-A: Hughes disability scale on admission, HDS-3rd: Hughes disability scale at 3rd-month control, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio; CRP: C-reactive protein, UA: Uric acid

Discussion

In the peak phase of GBS among pediatric patients, 75% are unable to walk unaided, 30% have tetraparesis, 35-50% suffer from cranial nerve involvement, and 15-20% develop respiratory and/or autonomic failure. The severity of the infiltrative period in inflammatory neuropathies determines the clinical course of GBS. Hence, many studies have investigated the underlying pathogenesis and prognostic factors (2,3,12-14). CSF total protein is highest in the demyelinating GBS subtype and correlates with disease severity (15). The CSF protein level was higher in the AIDP subtype than in the AMAN subtype in our study.

The frequency of GBS in different clinical subtypes varies according to geographical area. AIDP is the most common subtype in European countries, whereas AMAN is predominant in Asia (16). Tekgul et al. (17) demonstrated that the frequency of children with AMAN in Turkey was approximately 35%. In our study, the frequency of AMAN was found to be 33%, similar to the previous study, and it was more common than AIDP.

In addition to nine AIDP patients, one AMAN patient had a CB. Conduction block is considered an electrophysiological hallmark of AIDP; however, the low proximal amplitude seen in the acute period of AMAN might be interpreted as a pseudo-CB. Moreover, AIDP associated with severe axonal damage could be indistinguishable from the pure axonal form of GBS. Therefore, patients with prolonged distal motor latency and pseudo-CB should be followed up with EMG before GBS subtypes are determined (18).

To the best of our knowledge, CSF protein elevation has not been compared with a specific NCS in pediatric GBS patients. DiCapua et al. (3) showed that the level of CSF protein correlated with the number of electrophysiological abnormalities related to demyelination on NCS but not with any single specified criterion in adult patients with GBS. Other studies have demonstrated that CSF protein levels do not correlate with the number of electrophysiological abnormalities or any specific criterion (2,19). In our study, CSF protein levels showed a positive correlation with peroneal and tibial motor nerve DLs, but a negative correlation with medial plantar and peroneal superficial sensory NCVs in pediatric patients. According to this correlation, we suggest that the DLs of the peroneal and tibial motor nerves, as well as the medial plantar and peroneal superficial sensory NCSs in the lower extremity, may be more sensitive than other nerves to demonstrate early demyelination. However, further studies are required.

Some studies in the literature have revealed that

axonal damage is associated with a poor prognosis (20). It has been suggested that low CMAP is associated with slow recovery or poor outcomes in adults, whereas it is associated with a good prognosis in children (21). Tekgul et al. (17) demonstrated that the recovery process was slower in the AMAN group than in the AIDP group during the first 12 months, and after this duration, the recovery grades evened. In contrast, it was also shown that GBS disability scores at 6 months were higher in AIDP than in AMAN (22). Despite the contradictory data in the literature, our study revealed significantly higher HDS levels at 3 months in AMAN compared to other groups. Our patients' recovery rate was slower in the AMAN group during the first 3 months, although the rate of improvement in all groups was statistically significant.

Verma et al. (12) showed that a poor functional outcome at 6 months was associated with the axonal pattern on electrophysiological assessment and muscle weakness on admission. It has been suggested that the risk of sequelae correlates with the initial severity, especially the loss of walking ability during the acute phase of GBS (13). Barzegar et al. (23) recommended an early rehabilitation program to prevent further side effects and deconditioning secondary to immobility in pediatric patients who had some predictors such as a disability score >3, autonomic and cranial nerve involvement, and absence of CMAP. In accordance with the literature, a positive correlation was found between first-admission HDS and 3rd-month HDS in our patients. In this context, early rehabilitation programs could prevent disability in immobile pediatric patients.

The hallmarks of GBS are rapidly progressive bilateral and rather symmetric limb weakness. EMG and CSF can be used to support the diagnosis in clinically difficult cases. EMG can also be useful for differentiating the electrophysiological subtypes of GBS. However, these tests were found to be normal in the first 1-2 weeks of illness (20). The Brighton criteria from level 1 to level 4 describe the level of diagnostic certainty based on clinical presentations and additional test results. Level 1 represents the strongest level of diagnostic certainty, whereas level 4 is the weakest diagnostic level for diagnosing patients with insufficient GBS data. Level 3 represents patients who have clinical signs of GBS without CSF or EMG results in the absence of an alternative diagnosis for weakness, whereas at level 2, one of these test results supports the diagnosis (9). In our cohort, there were no patients with Level 4 diagnostic certainty because of our inclusion criteria. Among patients with normal NCS, 9 had level 3 and 7 had level 2. The European Academy of Neurology/Peripheral Nerve Society Guidelines recommend performing a second electrodiagnostic study later during the disease course, which can be helpful because abnormalities may take several weeks to develop (24). In our study, the timing of electrophysiological assessment was earlier in patients with normal NCS. The comparison of the 3rd month HDS between the NCS normal and AIDP groups revealed no significant difference, drawing attention to the importance of electrophysiological follow-up, although it is not practical.

In the literature, many studies have investigated the relationship between hematological and biochemical parameters and prognosis in adult GBS patients (2,4,5). Researchers suggested that NLR levels could be a prognostic factor in adult patients (2). Ethemoglu et al. (4) revealed that NLR was higher at the 3rd-month control in pediatric GBS patients and might be useful in estimating the course of the disease. Although decreased NLR and PLR are not associated with disease severity, they may indicate AIDP (25). It is indicated that autoimmune conditions such as GBS may stimulate the inflammatory response and thus lead to an increase in CRP production, which is significantly associated with a poor prognosis in patients with GBS. C-reactive protein can be used as a risk assessment and prognostic marker, according to Altaweel et al. (5). It has also been shown that GBS significantly reduces serum albumin levels, and this decrease is correlated with disease severity (4,8,25,26). In the current study, no correlation was found between NLR, PLR, CRP, and albumin levels in patients with electrophysiological subgroups, and there was no correlation between these parameters and prognosis.

Reduced serum UA levels have been associated with neurological diseases such as multiple sclerosis, neuromyelitis optica, Alzheimer's disease, and Parkinson's disease. Patients with GBS showed reduced serum UA levels compared with healthy controls. It has been demonstrated that CSF UA levels increased in adult patients with AIDP and suggested that it could be related to demyelination. To the best of our knowledge, although it has been shown that serum UA level is a protective factor in adult GBS patients, there are no studies evaluating serum UA in the pediatric group (6,8). Even serum UA level was not found to be associated with electrophysiological subgroups in our study, which is important because it is the first study to evaluate UA in children with GBS.

Study Limitations

Nevertheless, our study had some limitations, such as a retrospective design and a small sample. The other limitations included a short clinical follow-up and a lack of electrophysiological follow-up. Despite all these limitations, our study is valuable because it comprehensively addresses pediatric patients with GBS by considering their electrophysiological, clinical, blood, and CSF parameters. Our study contributes to the importance of EMG in the

early diagnosis of GBS in pediatric populations because it highlights well-standardized ranges of tibial and peroneal motor nerve DL, as well as medial plantar and superficial peroneal sensory NCVs.

Conclusion

Our study represents a pioneering endeavor, being the first to investigate UA levels in pediatric patients with GBS, even though our findings did not reveal a significant association between inflammatory markers such as NLR, PLR, and CRP, as well as serum UA levels and electrophysiological subgroups. In our opinion, well-standardized ranges of tibial and peroneal motor nerves DL and medial plantar and superficial peroneal sensorial NCVs, which are added to the routine study program, might be a sensitive marker in the diagnosis of pediatric GBS patients. Disability at presentation is more important than subtypes in determining prognosis, indicating the importance of early rehabilitation. Further studies with larger patient populations and serial NCSs are required.

Acknowledgements

We are grateful to our brilliant electrophysiological technician, Mehmet Fatih Ozmodanli, for his endless determination to work and support in obtaining data.

Ethics

Ethics Committee Approval: The Clinical Research Ethics Committee of Harran University approved the study on August 16, 2021, under the number HRU/21.14.15, and it complies with the Declaration of Helsinki.

Informed Consent: Retrospective study.

Authorship Contributions

Concept: D.A., O.E., T.G.D., Design: D.A., Data Collection or Processing: D.A., T.G.D., Analysis or Interpretation: D.A., O.E., M.C., Literature Search: D.A., Writing: D.A., O.E.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Effects of Pain-Related Features, Maladaptive Cognitions, Depression, and Anxiety on Pain-Related Disability: A Questionnaire-Based Cross-Sectional Study

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Abstract

Aim: The goals of this study to contribute to an evolving understanding of the interplay between cognitive and affective factors in chronic pain, providing valuable insights for advancing treatment models at the intersection of psychiatry and pain medicine. This approach may enable medical professionals to customize interventions that target the most important facets of managing chronic pain while meeting the demands of specific patients.

Methods: A questionnaire-based cross-sectional study was conducted among 440 patients with chronic pain in pain medicine clinics between February and June 2023. Demographic, health-related, and medical characteristics were comprehensively assessed alongside the Graded Chronic Pain Scale-Revised, Pain Anxiety Symptoms Scale, Pain Disability Questionnaire, Beck Anxiety Scale, and Beck Depression Scale.

Results: Beyond the range of pain severity rates, the regression model showed that elevated levels of pain overthinking, fear and avoidance beliefs, higher depression scores, catastrophizing thoughts, and nonsmoking status were significant factors impacting pain-related disability.

Conclusion: This study highlights integrative treatment modalities that address not only the physical dimensions of chronic pain but also its complex psychological aspects. A comprehensive understanding of these contributory factors provides a foundation for optimizing therapeutic approaches.

Keywords: Anxiety, chronic pain, depression, disability, maladaptive cognitions

Introduction

Chronic pain, defined by the U.S. Centers for Disease Control and Prevention as persistent or recurrent pain lasting more than 3 months, is a major public health problem affecting approximately 1 in 5 adults across Europe and the United States (1,2). Chronic pain is a complex and burdensome condition with serious consequences that can affect people's lives because of failed treatments, medication dependence, social isolation, work challenges, sleep disturbances, and emotional distress (3). Chronic painful conditions are one of the most common causes of disability (4). High-impact pain restricts professional and leisure activities in 1 in 14 adults (5). The objective of chronic pain management is multifaceted, aiming to enhance physical, emotional, and social well-being and restore optimal functionality and independence (6).

Within this intricate landscape, maladaptive cognitions emerge as influential factors, leading patients to perceive and experience more physical symptoms and behave in other ways consistent with a perception of poor health (7). Common maladaptive cognitions about chronic pain are overshining (ruminations; unable to stop thinking about pain, and difficulty focusing on other things during the pain), catastrophizing (exaggerating a negative orientation toward pain, helplessness, and magnification related to the cognitive pain experience),

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and fear/avoidance beliefs (reduced participation in physical activities, increased bedtime) (8-10). Beyond psychological implications, maladaptive cognitions can intensify physiological sensations such as tachycardia, nausea, dizziness, and shaking, amplifying the impact of pain on various aspects of patients' lives (11,12).

The goals of this study to contribute to an evolving understanding of the interplay between cognitive and affective factors in chronic pain, providing valuable insights for advancing treatment models at the intersection of psychiatry and pain medicine. In this context, the aims of the study were to reveal the relationship of pain-related features (pain localizations, pain duration, and pain severity) with pain-related disability (physical and psychosocial), maladaptive cognitions (overthinking, catastrophizing, fear/avoidance beliefs), physiological sensations, depression, and anxiety; and to develop a better understanding of which one or more of the socio-demographic, medical, cognitive, affective, and pain-related features were more predictive of pain-related disability. The need to prioritize the relative efficacy of these factors in mitigating pain-related disability drives this pursuit. This approach may enable medical professionals to customize interventions that target the most important facets of managing chronic pain while meeting the demands of specific patients.

Methods

Compliance with Ethical Standards

This study was approved by the Uskudar University Non-Invasive Clinical Research Ethics Committee (approval no.: 07 and date: 26.01.2023).

Study Design

The study adopted a cross-sectional research design in which data were collected using a questionnaire in the Pain Medicine outpatient clinic of the University of Health Sciences Turkey, Bagcilar Training and Research Hospital, from February 2023 to June 2023. Individuals with chronic pain voluntarily participated in the study. Bagcilar is a district of Istanbul, Turkey's largest province, which has received immigrants from the less developed eastern regions of Turkey with an extremely low socio-economic level and where women's participation in working life is very low. Before the research, all subjects provided written informed consent in accordance with the principles of the Helsinki Declaration. Participants were informed about the study's objectives and privacy policies. The primary inclusion criteria for the participants were: (1) patients suffering from pain in any part(s) of their body for at least three months; and (2) patients of age greater than or equal to 18 years. The following exclusion criteria were met: (1) patients who had taken psychotropics within the previous three months; (2) patients with mental retardation or severe cognitive impairment; (3) patients with new-onset (<3 months) pain symptoms; and (4) patients with cancer or severe pain requiring surgical or interventional procedures. Because of literacy problems, 11 (2%) of the participants sampled were ineligible. Of the 530 eligible participants, 66 (12.5%) refused to participate, and 24 (4.5%) failed to respond, leaving 440 participants (83% of those eligible) as study respondents (Figure 1).

Psychiatric Rating Scales

Depression

The Beck Depression Scale (13) is a self-report tool that evaluates the physical, emotional, cognitive, and motivational symptoms of depression and gauges its severity. It comprises 21 items, each of which corresponds to a depression-specific behavioral pattern. The scale's validity and reliability have been studied in Turkish (14), and the instrument has been used in several studies and in clinical practice. Cronbach's alpha coefficient for the Beck depression scale in this study was 0.71.

Anxiety

The Beck Anxiety Scale (15) is a 21-item self-report scale that assesses the general symptoms of anxiety. A validity and reliability study of the scale was conducted in Turkish (16). Cronbach's alpha coefficient for the Beck Anxiety Scale in this study was 0.81.

Pain-Related Features (Pain Duration, Pain Localizations, Pain Severity, Maladaptive Cognitions, and Pain-Related Disability)

Pain Duration

Participants were questioned if they felt "pain or discomfort all the time or on and off" and if "the current pain had persisted for more than 3 months". Participants who agreed with both of these criteria were classified as having chronic pain. This definition is congruent with that of the International Association for the study of pain (17).

Pain Localizations

A body drawing of the anterior and posterior views of the human body was used to determine the localization of pain. On the diagram, the human body was divided into 12 parts (head, face, and mouth; neck and arm; shoulder and upper arm; low back; low back and legs; only legs; dorsal region; knee; hip; sacrum; abdomen; chest).

The Graded Chronic Pain Scale-Revised

The Graded Chronic Pain Scale-Revised (GCPS-R) (18) is a seven-item questionnaire that assesses pain severity as well as interference with daily activities. The GCPS-R is organized in a hierarchical manner that allows responders to be classified into mild, moderate, or high-impact chronic pain. The scale has been translated into Turkish

(19). Cronbach's alpha coefficient for the total GCPS-R was 0.74 in this study.

The Pain Anxiety Symptoms Scale

The Pain Anxiety Symptoms Scale (PASS-20) (20) is used to assess maladaptive cognition related to pain. The PASS-20 measures overthinking, catastrophizing, fear/avoidance beliefs, and physiological sensations to identify pain-specific anxiety symptoms. Every item is scored on a frequency scale ranging from 0 (never) to 5 (always). The overall score ranged from 0 to 100, with higher scores indicating greater pain-related anxiety. We used the Turkish version of the PASS-20 (21) which has demonstrated satisfactory levels of internal reliability in the present study (α=0.88 for overthinking, α=0.75 for fear/avoidance, α=0.85 for catastrophizing, α=0.84 for physhological sensations, and α=0.91 for total score).

Pain Disability Questionnaire

The Pain Disability Questionnaire (PDQ) (22) is a brief, 15-item measure to assess perceived disability in two aspects: functional abilities (nine items) and psychosocial abilities (six items). Each item is rated on a visual analog scale, which is scored on a 10-point scale, and the total functional disability ranges between 0 and 150. In this study, the Cronbach's α values were 0.92, 0.89, 0.84 for total scores, functional abilities, and psychosocial abilities, respectively.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, Ill., USA) was used to analyze the study data. Frequency, percentage, mean, and standard deviation were used to express descriptive statistics. The skewness and kurtosis values of the numerical variables for the univariate normal distribution were evaluated by considering the ±1.5 (12). Multiple linear regression analyses used the Enter method to evaluate the PDQ's total scores as dependent variables. In multiple linear regression analyses, Durbin-Watson values were between 1.957, tolerance values were between 0.406 and 0.908, and variance inflation factor values were between 1.101 and 2.466. With these values, it was observed that there were no autocorrelation or multicollinearity problems in the regression analysis. The scale's reliability was assessed using internal reliability coefficients (Cronbach's alpha). The cut-off for significance was used as $p<0.05$.

Results

As shown in Table 1, the sample included 329 women (74.8%). The average (standard deviation) age and average body mass index were 42.95±11.18 years and 27.59±5.24 kg/m^2, respectively.

Table 2 shows the participants' pain localizations. The majority of patients reported more localized lower back and leg pain (60.2%), neck and arm pain (45.2%), and

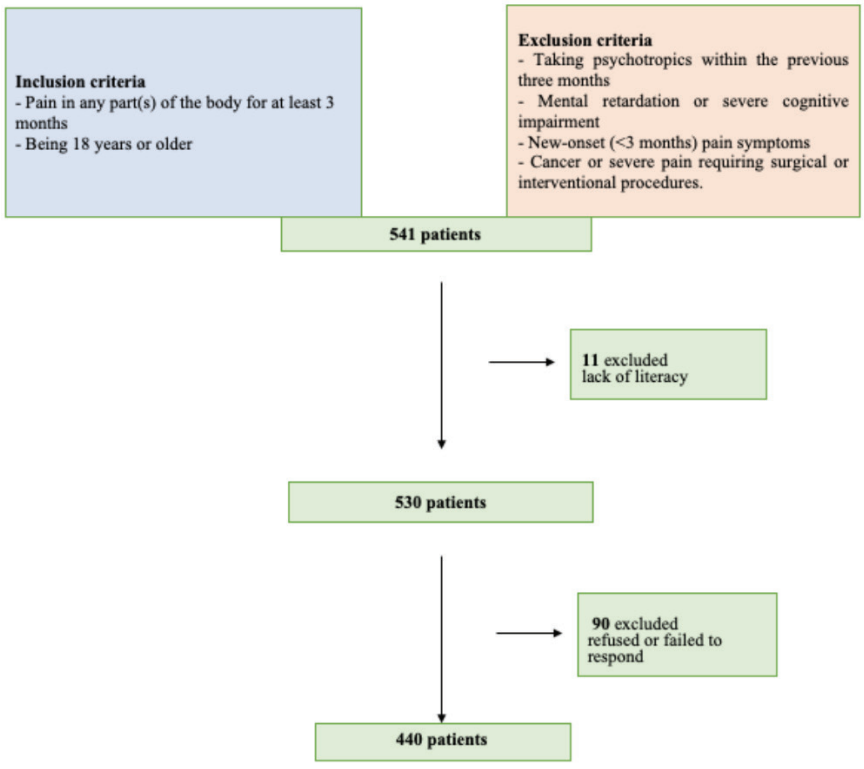

Figure 1. Study design

head, face, and mouth pain (16.8%), whereas others experienced more diffuse pain in multiple regions (13.6%).

Table 3 presents the relationship of pain-related features with PASS-20, PDQ, Beck anxiety, and Beck depression scores. There was a significant positive relationship between pain duration and the total and psychosocial sub-dimension scores of the PDQ ($p<0.05$ for each).

Table 4 shows the relationships between the measures and their subdimensions. There was a significant positive relationship between the total and sub-dimension scores of the PDQ and the total and sub-dimension scores of the PASS-20 ($p<0.01$ for each). Similarly, a coherent correlation was found between the total and sub-dimension scores of the PDQ and the scores of the Beck Anxiety and Beck Depression Scales ($p<0.01$ for each). There was also a strong correlation between the total and sub-dimension scores of the PASS-20 and the scores of the Beck Anxiety and Beck Depression Scales ($p<0.01$ for each).

Table 5 presents multiple linear regression analyses of variables predicting pain-related disability. In this regression analysis, it was found that the model was significant ($F=21.600$, $p<0.001$) and the independent variables explained 54% of the variance. In order of importance, the predictors of the pain-related disability were; PASS-Overthinking ($\beta=0.238$, $t=4.917$, $p<0.001$), higher scores of GCPS-R ($\beta=0.216$, $t=5.833$, $p<0.001$), PASS - Fear/avoidance ($\beta=0.214$, $t=4.995$, $p<0.001$), higher scores of the Beck Depression Scale ($\beta=0.130$, $t=2.684$, $p=0.008$), PASS-Catatostrophising ($\beta=0.112$, $t=2.157$, $p=0.032$), and no smoking ($\beta=-0.093$, $t=-2.460$, $p=0.014$).

Table 1. Socio-demographic characteristics and medical diseases of the participants

Gender, n (%)	
Male	111 (25.2%)
Female	329 (74.8%)
Age, Mean ± SD	42.95 (11.18%)
BMI, Mean ± SD	27.59 (5.24)
Marital status, n (%)	
Single	108 (24.5%)
Married	332 (75.5%)
Employment, n (%)	
Unemployed	277 (63%)
Employed	163 (37%)
Education status, n (%)	
Primary education	166 (37.7%)
High school	246 (55.9%)
University	28 (6.4%)
Smoking	278 (63.2%)
Alcohol consumption	372 (84.5%)
Physical diseases, n (%)	
Diabetes mellitus	53 (12%)
Hypertension	77 (17.5%)
Thyroid disorders	62 (14.1%)
Heart diseases	13 (3%)
Other physical diseases	85 (19.3%)

SD: Standard deviation, BMI: Body mass index

Table 2. Pain localizations of the partcipants

	n
Head, face, and mouth, n (%)	74 (16.8%)
Neck and arm, n (%)	199 (45.2%)
Shoulder and upper arm, n (%)	148 (33.6%)
Low back, n (%)	143 (32.5%)
Low back and leg, n (%)	265 (60.2%)
Leg, n (%)	32 (7.3%)
Knee, n (%)	102 (23.2%)
Hip, n (%)	116 (26.4%)
Dorsal region, n (%)	136 (30.9%)
Sacrum, n (%)	79 (18%)
Abdomen, n (%)	16 (3.6%)
Chest, n (%)	23 (5.2%)
Diffuse pain, n (%)	60 (13.6%)

Table 3. Relationship of pain-related features with measures

	Pain severity (GCPS-R)	Pain duration (months)	Number of pain localizations
PDQ-Total	0.445**	0.109*	0.041
PDQ-Functional	0.438**	0.087	0.063
PDQ-Psychosocial	0.390**	0.122*	0.004
PASS-Total	0.325**	0.086	0.027
PASS-Overthinking	0.330**	0.058	0.018
PASS-Fear/avoidance	0.300**	0.051	-0.034
PASS-Catastrophizing	0.257**	0.090	0.004
PASS-Physhological sensations	0.154**	0.073	0.098*
Beck Depression Scale	0.125**	0.043	0.052
Beck Anxiety Scale	0.091	0.039	0.060

Pearson correlation analysis was used to evaluate the relationships between scale scores.
PDQ: Pain Disability Questionnaire, PASS: Pain Anxiety Symptoms Scale, GCPS-R: Graded Chronic Pain Scale-Revised
*$p<0.05$, **$p<0.01$

Discussion

The present study was designed to determine the effect of pain-related features on pain-related disability, pain-related anxiety, and depression and anxiety symptom severity in patients with chronic pain and to identify the factors that predict pain-related disability.

Pain severity, which encompasses various indicators of chronic pain, was significantly correlated with functional and psychosocial pain-related disability, depression, and all maladaptive cognitions, as revealed in this study. According to a study of National Health Interview Survey results, most people with high-impact chronic pain face challenges such as being "unable to work" and experiencing limitations or restrictions in two or more essential daily activities (23). Consistent with the literature, this study found that pain severity was one of the most significant factors in pain-related disability. As pain becomes more severe, it is more likely to impact a person's ability to function and participate in various aspects of life (5). Pain severity can influence the development and maintenance of maladaptive cognition. Individuals with more severe pain are more likely to develop negative thoughts and beliefs about their pain, leading to increased distress and disability (24). Conversely, maladaptive cognitions can also influence the perception of pain severity, amplifying the subjective experience of pain (25).

Patients with chronic pain commonly experience symptoms of anxiety and depression, which are associated with the broader impact of widespread musculoskeletal pain on daily activities (26,27). A noteworthy finding of this study was that, while depression emerged as a significant predictor of pain-related disability, anxiety did not exhibit a similar effect. Consistent with our results, a previous study demonstrated that depression symptoms, rather than anxiety symptoms, mediate the relationship between pain and disability (28). While anxiety can also influence pain perception and coping strategies, its direct impact on pain-related disability may be less pronounced than that on depression. Anxiety symptoms such as worry, fear, and hypervigilance may heighten pain perception and arousal, but they may not necessarily lead to the same degree of functional impairment as the cognitive, behavioral, and neurobiological factors associated with depression. Depression often involves negative cognitive patterns that can intensify perceptions of pain and increase disability. These maladaptive thought patterns may contribute to a sense of helplessness and decreased motivation to engage in activities, leading to greater functional impairment. Moreover, individuals with depression may be more likely to withdraw from social interactions and physical activities, which can intensify pain-related disability over time.

Rumination, defined as repetitive thinking about one's feelings and concerns, contributes to distorted thoughts and unhealthy coping mechanisms that reinforce overshining patterns (29). Several prior studies have identified a link between overthinking and pain-related disability in chronic pain sufferers (30,31). In accordance with the present results, previous research has consistently demonstrated that overshining is a significant predictor of the severity of patients' disabilities and is highly associated with pain severity assessments (32). Similar to our results, a previous study (9) found that rumination was more strongly associated with disability than other cognitions, explaining 18% of the variance in disability for patients off work due to chronic pain. Rumination tends to amplify the perceived severity of pain. Individuals who engage

Table 4. Relationships between the measures and their sub-dimensions

	1	2	3	4	5	6	7	8	9	10
PDQ-Total (1)	1									
PDQ-Functional (2)	0.956**	1								
PDQ-Psychosocial (3)	0.913**	0.753**	1							
PASS-Total (4)	0.660**	0.616**	0.623**	1						
PASS-Overthinking (5)	0.592**	0.560**	0.549**	0.837**	1					
PASS: Fear/avoidance (6)	0.545**	0.517**	0.504**	0.750**	0.554**	1				
PASS: Catastrophizing (7)	0.557**	0.506**	0.547**	0.875**	0.673**	0.532**	1			
PASS: Physhological sensations (8)	0.415**	0.390**	0.388**	0.731**	0.435**	0.352**	0.554**	1		
Beck Depression Scale (9)	0.349**	0.329**	0.325**	0.388**	0.273**	0.199**	0.355**	0.406**	1	
Beck Anxiety Scale (10)	0.304**	0.285**	0.286**	0.430**	0.304**	0.195**	0.408**	0.455**	0.702**	
Mean	79.54	49.97	29.56	38.15	11.72	11.62	10.16	4.63	23.60	26.30
SD	34.69	21.54	15.43	15.58	4.94	4.45	5.30	4.74	12.52	14.06

Pearson correlation analysis was used to evaluate the relationships between the scale scores.
PDQ: Pain Disability Questionnaire, PASS: Pain Anxiety Symptoms Scale, SD: Standard deviation
*p<0.05, **p<0.01

Table 5. Multiple linear regression analysis of variables predicting pain-related disability			
Variables	**β (95% CI for B)**	**t**	**p-value**
Age	-0.003 (-0.272; 0.253)	-0.073	0.942
Gender	-0.060 (-11.419; 1.837)	-1.421	0.156
BMI	0.055 (-0.138; 0.869)	1.426	0.155
Employment	-0.034 (-8.334; 3.465)	-0.811	0.418
Occupation	-0.060 (-3.084; 0.370)	-1.545	0.123
Education status	-0.054 (-7.399; 0.977)	-1.507	0.132
Marital status	0.036 (-2.991; 8.773)	0.966	0.334
Diabetes mellitus	-0.014 (-9.262; 6.302)	-0.374	0.709
Hypertension	-0.002 (-7.332; 6.985)	-0.048	0.962
Thyroid disorders	-0.058 (-12.625; 0.977)	-1.683	0.093
Heart diseases	-0.024 (-19.112; 9.146)	-0.693	0.489
Other physical diseases	0.006 (-5.688; 6.679)	0.158	0.875
Smoking	**-0.093 (-11.979; -1.337)**	-2.460	0.014
Alcohol consumption	0.043 (-2.715; 10.938)	1.184	0.237
Pain duration	0.043 (-0.019; 0.082)	1.219	0.223
PASS: Overthinking	**0.238 (1.003; 2.338)**	4.917	<0.001
PASS: Fear/avoidance	**0.214 (1.013; 2.329)**	4.995	<0.001
PASS: Catastrophizing	**0.112 (0.065; 1.402)**	2.157	0.032
PASS: Physical sensations	**0.068 (-0.122; 1.111)**	1.575	0.116
Beck Depression Scale	0.130 (0.096; 0.623)	2.684	0.008
Beck Anxiety Scale	0.010 (-0.226; 0.273)	0.187	0.852
GCPS-R	**0.216 (9.963; 20.090)**	5.833	<0.001
Number of pain localizations	0.029 (-0.681; 1.629)	0.806	0.420

CI: Confidence interval, BMI: Body mass index, PASS: Pain Anxiety Symptoms Scale, GCPS-R Graded Chronic Pain Scale-Revised
Variables highlighted with bold predict pain-related disability.

in rumination may hyperfocus on their pain sensations and imagine the worst possible outcomes related to their pain condition, leading to an exaggerated perception of pain intensity. This heightened attention to pain can further contribute to distress, feelings of helplessness, hopelessness, and disability.

Negative pain beliefs and/or illness knowledge might lead to worst-case scenarios during actual or anticipated painful experiences (33). A recent study indicated that pain catastrophizing was the highest among people with generalized pain (34). This study confirmed that greater fear-avoidance beliefs and catastrophizing were significant predictors of pain-related disability (35,36). A similar finding was reported in a review of non-operative pain treatment among individuals with chronic pain, where higher baseline fear-avoidance beliefs correlated with greater disability and a lower likelihood of returning to work at 1-month, 6-month, and 1-year follow-ups (37). Individuals who catastrophize pain may experience prolonged periods of pain due to the amplification of pain signals and inhibition of pain modulation mechanisms, ultimately contributing to greater functional impairment over time. Catastrophizing may cause avoidance of activities or excessive reliance on passive coping mechanisms. The causal link between fear, catastrophizing, and pain-related disability appears to be a typical "chicken and egg" problem. If catastrophizing and fear drive pain-related disability, could severe disabling pain lead someone to think about catastrophizing and feel scared? It is obvious that more studies are needed to determine the details of the relationship between these variables.

Study Limitations

Several limitations of our study indicate that generalizing from these findings should be done with caution. First, the cross-sectional study design limits the establishment of causal links. For example, we cannot rule out the possibility that higher pain intensity or catastrophic thoughts about pain may increase pain-related disability levels, or vice versa. Second, self-reported measures are vulnerable to response bias and may overstate shared method variance, despite being reliable and valid. Furthermore, depression and anxiety were

assessed on the basis of symptom severity rather than particular diagnostic criteria. Third, pain characteristics were not included. Finally, the fact that we focused on patients with chronic pain who were treated at pain specialty clinics limits the generalizability of our findings to other populations. Longitudinal studies in a large primary care context are required to shed more light on how maladaptive cognition and depression affect pain-related disability independently and interactively.

Despite these limitations, this study has notable strengths. The comprehensive use of validated scales and a diverse set of measurements enhances the robustness of our findings. The large sample size and meticulous consideration of various contributing factors further support the reliability of our results. In addition, the focus on maladaptive cognitions, depression, anxiety, and their interplay in chronic pain contributes novel insights to the existing literature.

Conclusion

Understanding the cognitive, mental health, and pain-related predictors that contribute to the existence of pain-related disability is crucial for comprehensive assessment and effective management of individuals experiencing chronic pain. This study highlights the need for multidimensional interventions targeting not only medical management but also psychological well-being to alleviate the burden of chronic pain and improve the overall quality of life for those affected by this pervasive condition.

Ethics

Ethics Committee Approval: This study was approved by the Uskudar University Non-Invasive Clinical Research Ethics Committee (approval no.: 07 and date: 26.01.2023).

Informed Consent: Before the research, all subjects provided written informed consent in accordance with the principles of the Helsinki Declaration.

Authorship Contributions

Concept: E.S., E.Y.Z., A.S., Design: E.S., B.G., E.Y.Z., Data Collection or Processing: E.S., A.S., Analysis or Interpretation: B.G., Literature Search: E.S., Writing: E.S.

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Case Report

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Blastoid Variant Mantle Cell Lymphoma with Amplified *IGH/CCND1* Fusion: A Unique Case and Current Literature Review

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Abstract

Mantle cell lymphoma (MCL) is a subtype of mature B-cell non-Hodgkin lymphoma (NHL). Most cases exhibit *CCDN1/IGH* translocation; however, reports indicate that amplification of the fusion gene is extremely rare. A 72-year-old male patient diagnosed with an MCL blastoid variant was referred to us. Amplified *CCDN1*(11q13)/*IGH*(14q32.3) fusion genes were observed in 75% of the interphase nuclei of the patient. We present the first case of blastoid variant MCL with multiple *IGH/CCND1* fusion signals by interphase fluorescence in situ hybridization. Our patient exhibited an aggressive course and passed away on the second day of treatment.

Keywords: Mantle cell lymphoma, *IGH/CCND1*, fusion, amplification, fluorescence in situ hybridization

Introduction

Mantle cell lymphoma (MCL) is a subtype of mature B-cell non-Hodgkin lymphoma (NHL), accounting for approximately 7% of adult NHL. Mantle cell lymphoma, which is mostly seen in older men, exhibits clinical and genetic heterogeneity (1). Mantle cell lymphoma tends to disseminate to the bone marrow, blood, lymphoid tissues, and extranodal sites, particularly the gastrointestinal tract (2). Morphologically, there are three types of MCL: classical, pleomorphic, and blastoid variants (1). Most cases carry t(11;14)(q13;q32)/*CCND1/IGH*, which juxtaposes the *IGH* locus with the *CCND1* gene, resulting in *CCND1* overexpression. Deregulation of *CCND1* expression increases the G1–S transition in the cell cycle and induces tumor development (3).

More than half of the cases with *IGH/CCND1* translocation have additional chromosomal rearrangements, particularly: del(1p), +3, del(6q), del(7q), +7, -8, del(9p), +12, del(13q), del(17p) +18, + mar (4). Moreover, MCL cases have shown abnormalities in the *TP53* and *ATM* genes (5).

We report a case of blastoid variant mantle cell leukemia with multiple *IGH/CCND1* fusion signals detected by interphase fluorescence in situ hybridization (FISH). To our knowledge, this is the first case showing amplification of the *IGH/CCND1* gene in blastoid variant mantle cell lymphoma.

Case Presentation

A 72-year-old male patient was diagnosed with bladder papillary urothelial carcinoma. A 2×2 cm lymphadenopathy was detected in the left supraclavicular area. The complete blood count showed leukocytosis (White blood cell 21.5×10^9/L; normal range 4.5-11×10^9/L) and anemia (Hemoglobin 10.6 g/dL; normal range 12-16 g/dL). A computed tomography scan of the abdomen revealed suggestive lymphoma, thickening of the terminal ileum wall, as well as paraaortic and mesenteric

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lymphadenopathy. Only weight loss and constitutional type B symptoms were present, with no reports of fever or night sweats. The patient was referred to the hematology clinic. However, he initially refused all the recommended strategies; until a few weeks later, the patient consented to a colonoscopy and a biopsy at the suspicious tumor site. Pathology revealed a blastoid variant of the MCL in a biopsy of the right colon. Immunohistochemical evaluation showed that tumor cells were positive for CD20, CD5, BCL-2, and cyclin D1 in only 40% of cells. BCL-6 was positive and negative for SOX 11, LEF-1, CD10, CD3, and CD23 staining (Figure 1). The Ki-67 proliferation fraction was determined to be 80%, which is associated with a poor prognosis.

The patient, who was found to have bone marrow involvement on positron emission tomography and was evaluated as stage IV according to the Ann-Arbor staging system, was referred to our department of medical genetics. Fluorescence in situ hybridization analyses were performed using an *IGH/CCND1* plus translocation, dual-fusion probe (Cytocell, LPH 072) for *CCND1/IGH* rearrangements on the bone marrow aspirate sample. *CCND1*(11q13)/*IGH*(14q32.3) translocation and amplified fusion genes with three to six yellow signals were observed in 75% of the interphase nuclei (Figure 2A). Additional interphase nuclei FISH analysis was performed using an *MYC*(8q24) break-apart probe (Cytocell, LPH 010), *ATM*(11q22.3) (Cytocell, LPH 011), and TP53 (17p13.1) deletion probe (PrimeFISH, LS 17-003) (Figure 2B-D). They revealed a 23% heterozygous deletion in the *TP53* gene, a 100% homozygous deletion in the *ATM* gene, and no amplification or rearrangement in the *MYC* region. Chromosome analysis, which was performed using the G-banding technique on short-term (24 hours) cultured bone marrow cells, showed 47,XY,+mar [2]/46,XY [5]. The patient was planned to undergo R-CHOP (Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone) chemotherapy but died on the second day of rituximab treatment because of tumor lysis syndrome.

Discussion

Mantle cell lymphoma is caused by the abnormal proliferation of mature B cells. In the clinical course of the disease, the blood, bone marrow, and other systems, especially the gastrointestinal tract, might be involved (2). Both conventional cytogenetics and FISH analyses are commonly performed on patients. Our patient, who had gastrointestinal MCL involvement, underwent these analyses. *MYC* copy number changes and rearrangements are widely observed in aggressive variants of MCL and are associated with a poor prognosis. However, there was no rearrangement or copy number change in the *MYC* gene in our patient (6-9). In addition, *ATM* and/or *TP53* gene deletions are expected in these cases. *ATM* is a tumor suppressor gene, and its deletion is thought

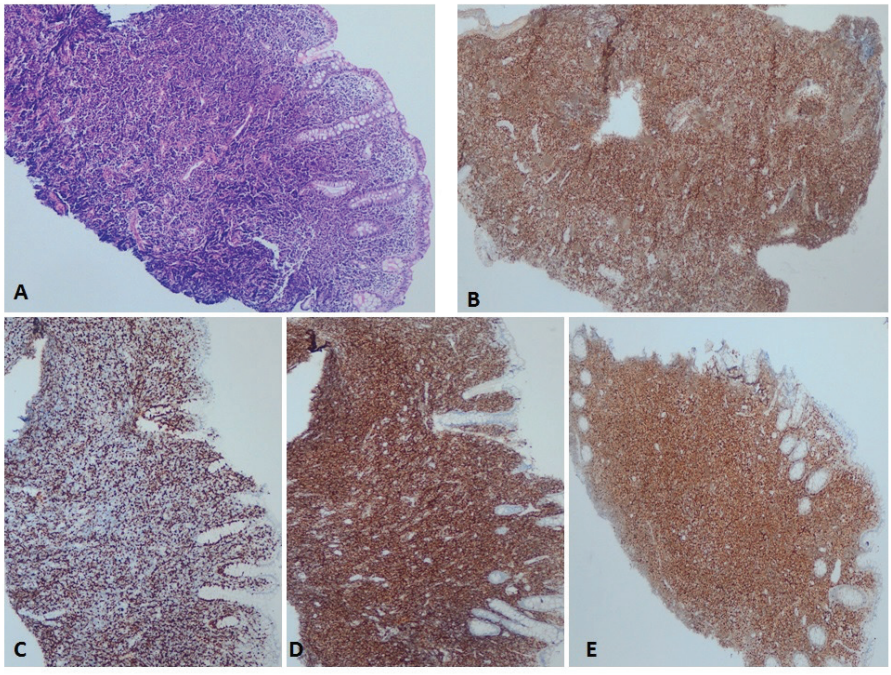

Figure 1. Morphology and immunocytochemistry of the right colon biopsy specimen: **A)** Hematoxylin&Eosin (H&E) staining and positive immunohistochemical stains of **B)** Cyclin D1, **C)** Ki-67, **D)** CD5, **E)** BCL-2, all of them are consistent with MCL (A-E, ×200 μm)
MCL: Mantle cell lymphoma

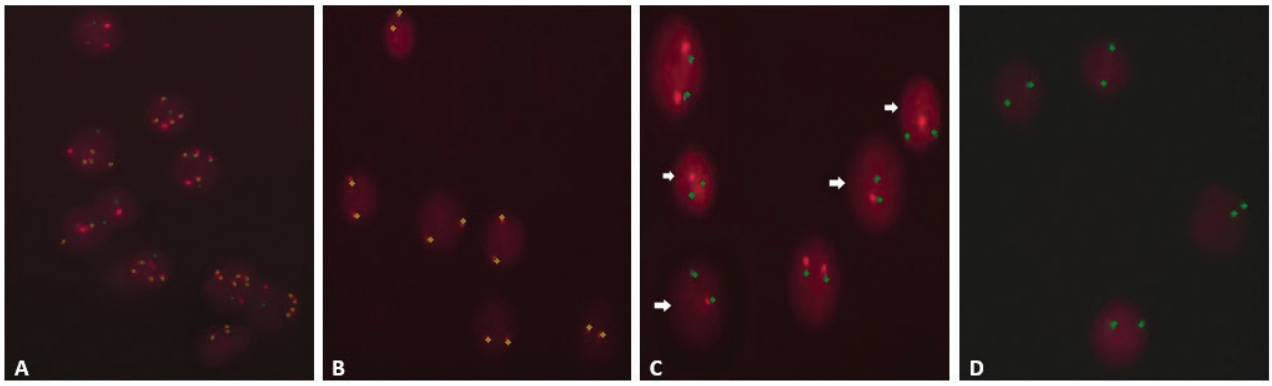

Figure 2. Fluorescence in situ hybridization (FISH) analysis. **A)** Using the *IGH/CCND1* plus translocation, dual-fusion probe in the bone marrow aspirate; the presence of the t(11;14)(q13;q32) and amplification of the *CCND1-IGH* fusion gene were observed. **B)** *MYC* FISH using the dual-color break-apart probe showed no amplification or rearrangement of the *MYC* gene. **C)** Heterozygous deletion with *TP53* probe was seen in a number of nuclei (indicated by arrows) with one red and two green signals. **D)** Homozygous deletion of the *ATM* gene was observed in all nuclei having solely green signals

to be effective in the onset and progression of the disease. Schaffner et al. (10) demonstrated that *ATM* gene deletion was found in 58% (7/12) of MCL patients. Similarly, *TP53* gene deletions are frequently observed in MCL cases and are associated with aggressive diseases. It was observed in 29 of 176 (16%) MCL patients that were unresponsive to intensive chemoimmunotherapy (9,10). We found a 23% heterozygous deletion in the *TP53* gene and a 100% homozygous deletion in the *ATM* gene in our patient. We believed that these changes in the patient could have contributed to his early death following the diagnosis.

To the best of our knowledge, this is the first report of amplification of the *IGH/CCND1* gene in the blastoid variant of mantle cell lymphoma. In the literature, five cases of *IGH/CCND1* fusion amplification have been presented. The diagnosis for them was one each of classical type MCL, pleomorphic type MCL, anaplastic multiple myeloma, and two cases of plasma cell leukemia (1,5-7,11).

The first case reported was a 58-year-old female patient with splenomegaly and lymphocytosis. She received an MCL diagnosis after splenectomy, and FISH analysis detected *IGH/CCND1* fusion amplification. The patient had a complex karyotype and a heterozygous deletion in the *TP53* gene region. The patient began treatment with chlorambucil and prednisolone, but died within 6 months, despite the average life expectancy of MCL cases being 2.5-4.5 years (5).

The second case was a 64-year-old male patient with bone pain, splenomegaly, and enlarged lymph nodes on the left side of his neck. He was diagnosed with plasma cell leukemia on bone marrow examination and had a complex karyotype and amplification of the *IGH/CCND1* fusion gene. He died very soon after the initiation of intensive chemotherapy treatment (6).

The third case was a 78-year-old male patient with night sweats, fatigue, dyspnea, and palpable masses in the groin and arm. Doctors diagnosed the patient with MCL, observing intense lymphoid cell infiltration in the bone marrow. The bone marrow cytogenetic examination detected a complex karyotype including t(11;14), and the FISH analysis detected *IGH/CCND1* fusion amplification in approximately 90% of the interphase cells. The patient also displayed monosomy 13 and *MYC* gene amplification. *TP53, ATM*, and *BCL2* gene deletion, rearrangement, and variation analyses were normal. Despite aggressive chemotherapy and radiotherapy, the patient deceased within approximately 15 months (1).

The fourth case was a middle-aged adult with dyspnea, bruising, anemia, and leukopenia. The patient's bone marrow was hypercellular, and 90% of the cells were composed of plasma cells. Her diagnosis was plasma cell leukemia. Fluorescence in situ hybridization analysis observed *CKS1B* (1q) amplification, heterozygous deletion of 13q, and *IGH/CCND1* fusion amplification, but conventional cytogenetic analysis failed. Although the patient received combination chemotherapy, she died within approximately 1 year (7).

The other case from the literature was a male patient with weight loss and low back and rib cage pain. The tomography revealed lytic lesions, and the bone marrow aspirate revealed 46.4% pleomorphic plasma cells, leading to the diagnosis of multiple myeloma. Conventional cytogenetics resulted in normal. Fluorescence in situ hybridization analysis revealed *IGH/CCND1* fusion amplification, *CKS1B* amplification, 13q deletion, and *TP53* gene deletion. This case also had a poor clinical course (11).

Here we report the sixth patient with *IGH/CCND1* fusion amplification reported in the literature and the third one

with mantle cell lymphoma. To the best of our knowledge, this is the first case of blastoid variant MCL with an amplified *IGH/CCND1* fusion gene. It is important to present cases with rare molecular cytogenetic abnormalities to enhance our understanding of their possible effects on prognosis and contribute to the literature.

Ethics

Informed Consent: Informed consent was obtained.

Authorship Contributions

Surgical and Medical Practices: E.G., H.H.E., A.T., Concept: E.G., Design: E.G., Data Collection or Processing: E.G., H.H.E., A.T., Analysis or Interpretation: E.G., S.S., Literature Search: E.G., S.S., Writing: E.G., S.S.

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

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