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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Evaluation of the Opinions and Anxiety Levels of Female Breast Cancer Patients Receiving Outpatient Chemotherapy Regarding Fertility

Ayaktan Kemoterapi Alan Meme Kanserli Kadın Hastaların Doğurganlığa İlişkin Görüş ve Kaygı/Depresyon Düzeylerinin Değerlendirilmesi

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Abstract

Objectives: As screening and treatment for breast cancer advance, an increasing number of young patients are confronting treatment-related infertility. The aim of this study was to analyze patients' opinions on fertility, and to assess the impact of these opinions on their depression and anxiety levels.

Materials and Methods: The study was designed as a prospective, single-center cohort survey. A total of 63 female patients with breast cancer who were receiving treatment at the Outpatient Units of Medical Oncology at Ankara University were enrolled. The questionnaire included components such as patients' perspectives on fertility, demographic data, and the Hospital Anxiety and Depression Scale (HADS).

Results: The median age was 39 (minimum-maximum: 28-45). Among the patients with metastatic and early-stage breast cancer, anxiety and depression scores were similar (p=0.09). Individuals who believed that having a child posed a risk to their disease had higher HADS scores (p=0.009). Factors identified as increasing the risk of anxiety and depression included being young (<40 years old), being married, unemployment, having a child, and the fear of disease recurrence. After diagnosis, the desire to have children diminished due to concerns about disease recurrence or progression. It was found that 51% of the patients considered the information provided on fertility to be sufficient.

Conclusion: It was observed that providing information on fertility during the treatment process was sufficient for half of the patients. A significant proportion of these patients had children prior to their diagnosis. The majority of patients did not have plans for childbirth, and did not express notable concerns regarding fertility.

Keywords: Breast cancer, fertility, HADS, anxiety, depression

Öz

Amaç: Meme kanseri tarama ve tedavisi ilerledikçe artan sayıda genç hasta, tedaviye bağlı fertilite sorunlarıyla karşı karşıya kalıyor. Bu çalışmanın amacı, hastaların doğurganlık konusundaki görüşlerini analiz etmek ve bu görüşlerin depresyon ve anksiyete düzeylerine etkisini değerlendirmektir.

Gereç ve Yöntem: Çalışma prospektif, tek merkezli bir kohort araştırması olarak tasarlandı. Anket, Ankara Üniversitesi Tıbbi Onkoloji Polikliniği'nde tedavi gören 63 meme kanseri kadın hastaya uygulandı. Ankette, hastaların demografik verileri ve doğurganlık hakkında görüşlerinin yanı sıra Hastane Anksiyete ve Depresyon Ölçeği (HADS) de yer aldı.

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Bulgular: Metastatik ve erken evre meme kanserli hastalar arasında anksiyete ve depresyon skorları arasında anlamlı fark gözlenmedi (p=0,09). Çocuk sahibi olmanın hastalıkları açısından risk oluşturduğuna inanan bireylerin ortalama HADS puanı daha yüksekti (p=0,009). Anksiyete ve depresyon riskini artıran faktörler arasında genç olmak (<40 yaşında), evli olmak, işsizlik, çocuk sahibi olmak ve hastalığın tekrarlama korkusu yer aldı. Teşhis konulduktan sonra hastalığın tekrarlaması veya ilerlemesi endişesi nedeniyle çocuk sahibi olma isteğinin azaldığı izlendi. Hastaların %51'i, doğurganlık konusunda tedavi sürecinde verilen bilgilerin yeterli bulduğu belirtti.

Sonuç: Anksiyete ve depresyon riskini artırdığı belirlenen faktörler arasında genç olmak (<40 yaş), evli olmak, işsizlik, çocuk sahibi olmak ve hastalığın tekrarlama korkusu yer alıyor. Tedavi sürecinde doğurganlık konusunda bilgilendirmenin hastaların yarısı için yeterli olduğu görüldü. Aktif kemoterapi alan ve çok genç yaşta olmayan bu hastaların önemli bir kısmının tanı öncesinde çocuk sahibi olduğu, doğum planı olmadığı ve doğurganlığa ilişkin belirgin bir endişe dile getirmedikleri görüldü.

Anahtar Kelimeler: Meme kanseri, fertilite, HADS, anksiyete, depresyon

Introduction

Breast cancer is the most prevalent malignancy observed in women in Türkiye (1). Through breast cancer screening programs, early-stage detection of patients is achievable. Chemotherapy is commonly administered in operable patients diagnosed at early-stage, as well as in those with locally advanced or metastatic disease, and in cases of recurrence (2,3). Notably, 4-6% of these women are diagnosed during their reproductive years (4). Consequently, fertility-preserving strategies are implemented prior to chemotherapy in adolescent and young adult women diagnosed with cancer, particularly breast and gynecological cancers (5). Fertility-preserving approaches vary based on the social and cultural characteristics of patients, the type of cancer, and the specific treatment and chemotherapy regimens (5). The urgency to initiate cancer treatment promptly, in particular, introduces psychosocial challenges that often accompany the diagnosis (6).

Chemotherapeutic agents, a common treatment option for breast cancer, can also have detrimental effects on fertility (7). There is currently no clear data regarding how patients' and their partners' perspectives on fertility evolve during and after chemotherapy, nor on the factors influencing these views. Furthermore, the factors that determine patients' choice of individuals with whom they feel comfortable discussing and sharing fertility-related concerns following a breast cancer diagnosis remain unknown. Breast loss during the treatment process can significantly impact patients' perception of their female identity (8). When all contributing factors are considered, challenges related to sexual life in women with breast cancer may lead to marital discord and, in some cases, even divorce.

In recent years, fertility has increasingly been emphasized and, at times, used as a means of societal pressure on women (9). Such emphasis can exacerbate psychosocial challenges for women with breast cancer during their reproductive years, potentially intensifying the emotional burden of their diagnosis and treatment (10).

In our country, no research has been conducted that explores the views of women with breast cancer regarding fertility. Furthermore, only two studies in the existing literature has examined patients' perspectives on fertility and anxiety (11,12). The aim of this study was to assess the opinions and knowledge related to childbearing, as well as the depression and anxiety levels, of young female breast cancer patients who had undergone chemotherapy.

Materials and Methods

Study Design and Participants

The study was designed as a prospective, single-center cohort survey. This observational real-life study included a total of 63 breast cancer patients treated between March 1, 2017, and March 30, 2018, at the outpatient unit of Medical Oncology at Ankara University. The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki, revised in 2013, and was approved by the Ankara University Clinical Research Ethics Committee (decision no.: 12-742-17, date: 24.07.2017). Inclusion criteria were: being a woman aged 18-45, diagnosed with breast cancer, and undergoing chemotherapy and/or hormone therapy. The exclusion criterion was the presence of a neuropsychiatric condition that precluded the completion of the survey form.

Data Collection and Laboratory Measurements

The demographic characteristics of the patients, who provided written informed consent in the consultation room with a clinical psychologist at the medical oncology clinic, were assessed along with factors influencing their fertility and anxiety before, during, and after cancer treatment. These factors were evaluated through survey questions conducted via face-to-face interviews. Data related to the diagnosis, date of diagnosis, treatment modalities applied (surgery, chemotherapy, radiotherapy, hormonal therapy), active disease status, and remission status were collected from the patient database. Anxiety and depression levels were measured using the Hospital Anxiety and Depression Scale (HADS).

Assessments of Anxiety and Depression

HADS is an assessment tool developed by Zigmond and Snaith (13) to identify the risk of anxiety and depression, as well as to measure changes in their severity. The scale was specifically designed to screen for mood disorders in populations with medical conditions. It is widely used in both community and hospital settings due to its simplicity. HADS is a Likert-type scale consisting of 14 questions, with odd-numbered items assessing anxiety and even-numbered items assessing depression.

The validity and reliability of the Turkish version of the HADS were established by Aydemir et al. (14), demonstrating that the scale is effective for screening depression and anxiety symptoms in patients with physical illnesses. The scale includes two subscales: anxiety (HAD-A) and depression (HAD-D). Based on the study conducted in Türkiye, the cut-off score for the anxiety subscale was determined to be 10/11, and for the depression subscale, 7/8. Scores above these thresholds indicate individuals at risk. The scoring of each item on the scale varies accordingly. Items 1, 3, 5, 6, 8, 10, 11, and 13 on the scale indicate progressively decreasing severity, with scores assigned as 3, 2, 1, and 0. Conversely, items 2, 4, 6, 8, 10, 12, and 14 are scored in ascending order as 0, 1, 2, and 3. The total scores for each subscale are calculated by summing the scores of the relevant items. For the anxiety subscale, scores from items 1, 3, 5, 7, 9, 11, and 13 are aggregated, whereas for the depression subscale, scores from items 2, 4, 6, 8, 10, 12, and 14 are combined. The depression subscale consists of seven items, with scores ranging from 0 to 21. The possible scores on both subscales range from 0 to 21, with cut-off points for mood disorder severity classified as follows: 0-7 indicates normal, 8-10 indicates mild, 11-14 indicates moderate, and 15-21 indicates severe mood disorder (3-5).

Statistical Analysis

The appropriate sample size for the study was calculated to be 74, with 80% power and a 5% type-1 error rate. Data analysis was performed using SPSS version 17.0 software. Descriptive statistics were presented as frequencies (n) and percentages (%). Numerical variables that exhibited a normal distribution were reported as mean ± standard deviation, while those that did not follow a normal distribution were reported as median (minimum-maximum) values. The chi-square or Fisher's exact test was used for categorical variables, and the Mann-Whitney U or Kruskal-Wallis test was employed for continuous variables. The independent samples t-test was used to compare parametric variables between two groups. For comparisons involving more than two groups, the significance of differences in mean values was assessed using the One-Way ANOVA test. A 5% type I error level was adopted to determine statistical significance.

Results

Demographic Characteristics of the Patients

A total of 63 female patients participated in the study. All participants completed the study questionnaire and HADS. Among the patients, 47 (75%) were diagnosed with early-stage breast cancer, while 16 (25%) had advanced-stage breast cancer. Sixteen patients (25%) were single or divorced, and 47 patients (75%) were married. Of the patients, 31 (49%) were university graduates, whereas 32 (51%) did not hold a university degree. Additionally, 25 patients (40%) were employed, and 38 patients (60%) were not working. Forty-five patients (71%) had children, while 18 patients (29%) were childless. The characteristics of the patients are summarized in Table 1.

Patients' Opinions About Fertility

Among the survey questions posed to the patients, 7 (11%) answered "yes" and 56 (89%) answered "no" or were undecided regarding the question, "Do you plan to have children after the diagnosis?" Of the 56 patients, 43 (77%) had undergone surgery, while 13 (23%) had metastatic disease. When asked whether they were concerned that having children might pose a risk, 34 patients (22 operated, 12 metastatic) responded "yes", while 29 patients (46%) responded "no". Additionally, 48 patients (76%) indicated that they were not influenced by external opinions regarding having a sick child, whereas 15 patients reported being influenced by their family members. Among the patients who reported being influenced by their relatives or friends, 49 (78%) stated that their desires regarding having children remained unchanged, while 14 (22%) indicated that their desires had either increased or decreased. There was no statistically significant difference between early-stage and advanced-stage patients regarding their responses to questions about fertility. The patients' responses to questions concerning fertility are summarized in Table 2.

Table 1: Demographics and disease characteristics of the patients (n=63)

Characteristics	**n (%)**
Age, median (minimum-maximum), years	39 (28-45)
Female, n (%)	63 (100)
Married, n (%)	47 (75)
Education status, n (%)	
Graduated from a university	31 (49)
Working status, n (%)	
Active worker	25 (40)
The stage of breast cancer, n (%)	
Early stage cancer	47 (75)
Advanced stage cancer	16 (25)
Patients with children, n (%)	45 (71)

Table 2: The percentage of patients' who answered the following questions on fertility positively			
The patients' views	**Metastatic, (n=16) (25%)**	**Operated, (n=47) (75%)**	**p-value**
Are you worried about being in danger if you planned to have a child after your chemotherapy was completed.	12 (75)	22 (47)	0.81
Have your ideas about having a child changed?	3 (19)	11 (23)	1
Have your idea of having a child in the future changed after being diagnosed with breast cancer?	9 (56)	13 (28)	0.66
Would you like to have a child after breast cancer treatment?	3 (19)	4 (8)	0.35
Are there any influences from people around you about having a child?	10 (63)	38 (81)	0.18

Patients' Opinions About Fertility

The patients were asked whether they had been informed by the treatment team about the recommendation to avoid pregnancy during treatment. Twenty-two patients (35%) answered "no", while forty-one patients (65%) answered "yes". Of those who received information, 20 patients (31%) reported that they were informed solely by a medical oncologist, whereas 43 patients (69%) indicated that they received information from a combination of a surgeon, psychologist, and nurse. When asked about the adequacy of the information provided, 32 patients (51%) responded "yes", while 31 patients (49%) answered "no" or were undecided. Regarding whether they received information from additional sources about avoiding pregnancy and having children, 30 patients (48%) reported that they did not receive any additional information, whereas 33 patients (52%) stated that they obtained information from other sources.

No significant difference was observed between early-stage and advanced-stage patients regarding their responses to questions evaluating their opinions on the information provided about fertility. The patients' answers to these questions are summarized in Table 3.

Patients were asked whether they used any contraceptive methods before and after their diagnosis of breast cancer. Twenty-six patients (41%) reported using contraceptives, while 37 patients (59%) stated that they did not use any contraceptive methods. When inquired about the importance of having children, 25 patients (40%) indicated that it was unimportant, 10 patients (16%) were undecided, 6 patients (9%) considered it slightly important, 9 patients (14%) deemed it important, and 13 patients (21%) regarded it as very important. Additionally, among the metastatic patients, 5 (31%) considered having children unimportant, 3 (19%) were undecided, 2 (12%) considered it slightly important, 3 (19%) deemed it important, and 3 (19%) regarded it as very important. In contrast, among the operated patients, 20 (43%) viewed having children as unimportant, 7 (15%) were undecided, 4 (8%) considered it slightly important, 6 (13%) deemed it important, and 10 (21%) regarded it as very important. When asked about the importance of having children for their spouses, 13 patients (19%) considered it unimportant, 26 patients (41%) were undecided, 3 patients (5%) considered it slightly important, 10 patients (16%) deemed it important, and 12 patients (19%) regarded it as very important. Among those who considered having children very important, 33% were from the metastatic group and 67% were from the operated group.

Table 3: Patients' views on fertility information taken from the hospital	
I believe I am well informed about risks that may occur in a possible pregnancy, n (%)	32 (51)
I gathered additional information on becoming preganant from sources other than the hospital, n (%)	33 (52)

Hospital Anxiety and Depression Scale Results

The average HADS score for the metastatic group was 12 (range: 5-24), while the average HADS score for early-stage breast cancer patients was 15 (range: 1-36). No significant difference was observed between the HADS scores of the two groups (p=0.09). Twenty-six percent of the patients were identified as being at risk for anxiety, and 34% were at risk for depression. Married individuals were found to be at a higher risk for both anxiety and depression compared to single or divorced individuals (p=0.006). No significant difference in anxiety and depression was observed between patients with a university degree and those without (p=0.054). Working patients had a lower risk of anxiety and depression compared to unemployed patients (p=0.02). Although patients who wanted to have children after their diagnosis had higher HADS scores, this difference was not statistically significant compared to those who did not wish to have children or were undecided (p=0.63). There was no significant difference in HADS scores between patients who were influenced by their relatives regarding having children and those who were not (p=0.98). Among patients influenced by their relatives, no difference in HADS scores was found between those whose desires changed and those whose desires remained unchanged (p=0.63).

There was no significant difference in HADS scores between patients who were informed about pregnancy and those who were not during their treatment (p=0.76). Patients who believed

that having children would pose a risk to their disease had higher HADS scores (p=0.01). No significant difference was observed between the HADS scores of patients who used contraceptive methods before their breast cancer diagnosis and those who did not (p=0.19). Similarly, HADS scores were comparable between those who used contraceptive methods after their breast cancer diagnosis and those who did not (p=0.63). The HADS scores of patients who changed their views about having children after diagnosis were similar to those who did not change their views (p=0.95). The relationship between certain clinical features of the patients and HADS scores is illustrated in Figure 1.

Discussion

The risk of anxiety and depression was found to be comparable between patients diagnosed with early-stage and advanced-stage breast cancer. Among these patients, 26% were identified as being at risk for anxiety, and 34% were at risk for depression. Notably, patients under the age of 40 and those who were married exhibited higher levels of anxiety compared to their single or divorced counterparts. Additionally, individuals who perceived childbearing as a potential risk to their health showed higher HADS scores. Married patients with advanced-stage breast cancer experienced higher levels of anxiety compared to their unmarried counterparts. Similarly, patients with children experienced higher levels of anxiety compared to those without children. Factors associated with an increased risk of anxiety and depression include being under 40 years of age, being married, unemployment, having children, and fearing disease recurrence. A total of 51% of patients reported that they found the information provided about fertility to be sufficient, while 48% indicated that they sought additional resources for information on having children. The risk of anxiety and depression was lower in patients who were employed compared to those who were unemployed.

Studies conducted by Delgado-Guay et al. (15), Saboonchi et al. (16), and Spencer et al. (17) have identified that patients with high anxiety and depression scores are often those with chronic illnesses, particularly individuals with advanced-stage breast cancer (15-17). In the present study, no comparison was made between patients with other chronic illnesses and those diagnosed with cancer, and the sample size in the cited studies exceeded 200 patients. Additionally, Linden et al. (18), in their study involving 2,250 breast cancer patients, demonstrated that anxiety levels were three times higher compared to those in the general population. However, it is important to note that this study did not include a direct comparison with the general population.

Park et al. (19) administered the HADS to 54 women with metastatic breast cancer and found that 28% of the patients were at risk for anxiety, while 20% were at risk for depression.

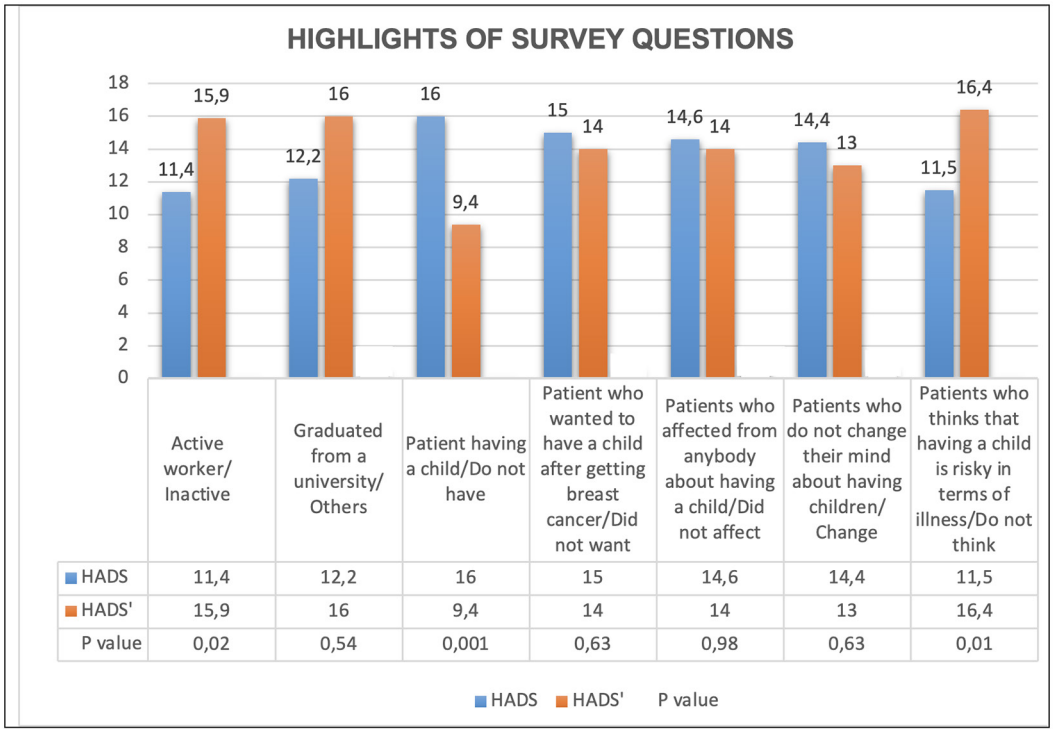

Figure 1: Some important HADS scores of the participants
*HADS: Hospital Anxiety and Depression Scale
**HADS': Hospital Anxiety and Depression Scale after informed about pregnancy
HADS: Hospital Anxiety and Depression Scale

Compared to our study, anxiety levels were higher and depression levels were lower in their cohort. Similarly, Akel et al. (20) observed that anxiety was more prevalent than depression among breast cancer patients. Both studies highlighted that factors contributing to higher HADS scores included being of White ethnicity and having a higher level of education (20).

Consistent with the findings of Linden et al. (18), our study also demonstrated that younger patients (under 40 years of age) exhibited higher levels of anxiety and depression. In a single-center study conducted by Kostev et al. (21) in Germany, the anxiety and depression levels of patients diagnosed with breast or genital organ cancer were assessed. The study found that patients, with an average age of 49.3 years, were 1.32 times more likely to experience anxiety and depression compared to individuals without cancer.

Nilsson et al. (22) demonstrated that patients with children had higher levels of anxiety and depression compared to those without children, a finding that is consistent with the results of our study. In our study, patients who perceived having children as a potential risk for disease recurrence had higher HADS scores. Similarly, in a study by Starreveld et al. (23), 267 patients who underwent breast surgery were followed for 18 months to investigate factors influencing fear of recurrence. The study found that educated, married, and optimistic women experienced less fear of recurrence. While individuals with a university education tended to have higher HADS scores compared to those without a university degree, employed patients exhibited significantly lower HADS scores compared to their unemployed counterparts. The reasons for unemployment among patients with high HADS scores were not investigated, as this was not within the scope of our study.

In the study conducted by Peate et al. (24), 120 newly diagnosed breast cancer patients were assessed regarding their future fertility perspectives, decision-making conflicts, decision regret, and treatment satisfaction. Interviews were conducted at 1 month and at 12 months. It was found that those who received assistance during their decision-making process experienced a reduction in regret over the course of the year. Patients expressed satisfaction with receiving information about effective treatments for fertility preservation. In our study, patients were surveyed shortly after diagnosis while undergoing active treatment, and their opinions were collected; however, no follow-up survey was conducted. Nevertheless, 51% of the patients indicated that the information provided regarding fertility was sufficient (as shown in Table 3).

Abe et al. (25) investigated the fertility-related perspectives of 112 breast cancer patients aged 15-40, in collaboration with gynecologic oncologists and oncologists. The study revealed that patients were inadequately informed about fertility due to concerns that fertility preservation might delay cancer treatment. This lack of information was attributed to poor communication between patients and doctors. The study emphasized the importance of oncofertility counseling. In our study, 49% of patients reported not seeking additional information about fertility, while 43% indicated that they had received information from at least one medical oncologist.

Conclusion

As a conclusion, it has been determined that the information on fertility is sufficient. Factors that increase the risk of anxiety and depression in breast cancer patients of reproductive age include being younger (under 40 years old), being married, not being employed, having children, and fear of recurrence. A significant proportion of these patients, who were undergoing active chemotherapy and were not of a very young age, had children prior to their diagnosis. The majority of patients did not have plans for childbirth, and it was observed that they did not express notable concerns regarding fertility.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki, revised in 2013, and was approved by the Ankara University Clinical Research Ethics Committee (decision no.: 12-742-17, date: 24.07.2017).

Informed Consent: Informed consent was obtained from all the participants in the consultation room with a clinical psychologist at the medical oncology clinic.

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Footnotes

Authorship Contributions

Concept: G.T., A.A., F.Ç.Ş., Design: G.T., A.A., F.Ç.Ş., Data Collection and/or Processing: G.T., Analysis and/or Interpretation: G.T., A.A., F.Ç.Ş., Literature Search: G.T., Writing: G.T., A.A., F.Ç.Ş.

Conflict of Interest: According to the authors, there are no conflicts of interest related to this study.

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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Evaluation of Retinal Layers by Optical Coherence Tomography in Patients with Acromegaly

Akromegali Hastalarında Optik Koherens Tomografi ile Retinal Tabakaların Değerlendirilmesi

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Abstract

Objectives: Acromegaly, a rare endocrine disorder caused primarily by growth hormone (GH) secreting pituitary adenomas, leads to elevated levels of growth GH and insulin-like growth factor 1 (IGF-1), resulting in systemic complications and ocular manifestations. These include increased corneal thickness, elevated intraocular pressure, retinal changes, and visual field defects. Spectral-domain optical coherence tomography (SD-OCT) enables high-resolution, non-invasive assessment of individual retinal layers. This technique provides a thickness map of the following seven different retinal layers via automatic segmentation: retinal nerve fibre layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), retinal pigment epithelium (RPE). This study employed SD-OCT to evaluate the thickness of seven distinct retinal layers in patients with acromegaly, addressing a gap in the current literature.

Materials and Methods: This cross-sectional comparative study included patients with acromegaly and age- and gender-matched healthy controls. Data collected included demographics, treatment modalities, disease activity status (remission vs. non-remission), and IGF-1 levels. A single-blinded ophthalmologist performed OCT. The thickness of seven distinct retinal layers was compared between acromegaly patients and controls.

Results: The study included 23 acromegaly patients and 18 healthy controls, with 46 and 36 eyes evaluated, respectively. The acromegaly group exhibited significantly reduced median thicknesses in the RNFL, GCL, IPL, and RPE layers compared to controls ($p<0.05$), while INL, OPL, and ONL showed no differences. Subgroup analysis revealed that INL thickness was significantly greater in non-remission patients than in those in remission ($p=0.022$).

Conclusion: Retinal layer thinning, particularly in the RNFL, GCL, IPL, and RPE, was observed in acromegaly patients compared to healthy controls. Interestingly, patients with active disease had thicker retinal layers than those in remission, suggesting a role for IGF-1 in these changes. As the first comprehensive evaluation of all seven retinal layers in acromegaly, this study highlights the need for further research to clarify these findings and the impact of IGF-1 on retinal structure.

Keywords: Acromegaly, optical coherence tomography, retinal layers, retinal nerve fiber layer, ganglion cell layer

Öz

Amaç: Akromegali, öncelikli olarak büyüme hormonu (BH) salgılayan hipofiz adenomu kaynaklı nadir bir endokrin hastalıktır ve BH ile insülin benzeri büyüme faktörü 1 (IGF-1) seviyelerinin yükselmesine bağlı olarak sistemik komplikasyonlara ve oküler bulgulara yol açar. Bu bulgular arasında kornea kalınlığında artış, intraoküler basınç artışı, retinal değişiklikler ve görme alanı defektleri yer alır. Spektral-domain optik koherens tomografi (SD-OCT), retina tabakalarının yüksek çözünürlüklü, non-invaziv değerlendirmesine olanak tanır. Bu teknikle, otomatik segmentasyon aracılığıyla yedi farklı retina tabakasının kalınlık ölçümü yapılabilir: retinal sinir lifi tabakası (RNFL), ganglion hücre tabakası (GCL), iç pleksiform

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tabaka (IPL), iç nükleer tabaka (INL), dış pleksiform tabaka (OPL), dış nükleer tabaka (ONL) ve retinal pigment epiteli (RPE). Bu çalışmada, akromegali hastalarında literatürdeki bir boşluğu doldurmak amacıyla, SD-OCT kullanarak, yedi farklı retinal tabakanın kalınlığını değerlendirmek amaçlandı.

Gereç ve Yöntem: Bu kesitsel karşılaştırmalı çalışmaya, akromegali hastaları ile yaş ve cinsiyet açısından eşleştirilmiş sağlıklı kontrol bireyler dahil edilmiştir. Demografik özellikler, tedavi yöntemleri, hastalık aktivite durumu (remisyonda veya değil) ve IGF-1 seviyeleri kaydedildi. Tüm OCT ölçümleri, tek bir oftalmolog tarafından yapılmıştır. Akromegali hastaları ile kontrol grubu arasında yedi farklı retinal katmanın kalınlıkları karşılaştırılmıştır.

Bulgular: Çalışmaya 23 akromegali hastası ve 18 sağlıklı kontrol dahil edilmiş; sırasıyla 46 ve 36 göz değerlendirilmiştir. Akromegali grubunda RNFL, GCL, IPL ve RPE katmanlarının medyan kalınlıkları kontrol grubuna kıyasla anlamlı olarak düşük bulunmuştur ($p<0,05$). INL, OPL ve ONL arasında fark gözlenmemiştir. Akromegali hastaları, remisyonda veya remisyonsuz olarak 2 gruba ayrıldığında, INL kalınlığı, remisyonsuz hastalarda anlamlı derecede daha yüksek bulunmuştur ($p=0,022$).

Sonuç: Akromegali hastalarında RNFL, GCL, IPL ve RPE katmanlarının kontrol grubuna göre inceldiği, ancak aktif hastalık grubunda bu katmanların remisyondaki hastalara kıyasla daha kalın olduğu gözlenmiştir. Bu durum, IGF-1'in retinal değişikliklerdeki rolüne işaret etmektedir. Akromegali hastalarında retinal tabakaların ayrıntılı olarak değerlendirildiği ilk çalışma olan bu araştırma ile IGF-1'in retinal yapı üzerindeki etkisini açıklığa kavuşturmak için ek geniş kapsamlı araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Akromegali, optik koherens tomografi, retinal tabakalar, retinal sinir lif tabakası, gangliyon hücre tabakası

Introduction

Acromegaly is a rare, long-term endocrine condition characterised by excessive secretion of growth hormone (GH) and insulin-like growth factor 1 (IGF-1), resulting in considerable health complications and increased risk of mortality (1). Acromegaly is nearly always caused by pituitary adenoma, most of which are macroadenomas. The disease's clinical features result from excessively increased GH and IGF-1 secretion. Chronic exposure to excessive GH and IGF-1 leads to increased cellular proliferation, organomegaly, and disturbances in metabolism. Excessive production of GH and IGF-1 in acromegaly also negatively affects the eye and ocular system. The ocular manifestations of acromegaly encompass increased corneal thickness, elevated intraocular pressure, retinal pigmentary degeneration, and visual field (VF) defects resulting from chiasmal compression by a pituitary adenoma (2-5).

Spectral-domain optical coherence tomography (SD-OCT) is a non-invasive imaging technique that captures multiple scans across the retina, producing high-resolution quantitative maps of retinal thickness. Ultrahight axial image resolution of SD-OCT provides quantitative measurement of retinal layers. In this way, instead of measuring the entire retinal thickness, individual measurements of retinal layers can be evaluated in different clinical situations.

Limited research has focused on assessing retinal layer thickness in individuals with acromegaly. This current study utilised SD-OCT to examine the thickness of seven distinct retinal layers among patients diagnosed with acromegaly.

Materials and Methods

This cross-sectional comparative study included 23 patients diagnosed with acromegaly and 18 age- and gender-matched healthy control subjects. The collected data encompassed patients' demographic details, treatment modalities received (surgical and medical therapy including somatostatin agonists and radiotherapy), disease activity status (remission versus non-remission), findings from the most recent pituitary magnetic resonance imaging (MRI), and IGF-1 levels measured during the ophthalmological evaluation. Disease remission was defined as age-adjusted normalisation of IGF-1 levels based on the most recent consensus criteria (6). Exclusion criteria included individuals with chronic ocular conditions (e.g., glaucoma, uveitis, conjunctivitis), significant lens opacities, optic disc anomalies, a history of ocular surgery or laser treatment, and systemic diseases such as diabetes mellitus, hypertension, hypothyroidism, hyperthyroidism, chronic renal failure, hepatic failure, or pregnancy.

Informed written consent was obtained from all participants before the study. The research complied with the principles of the Declaration of Helsinki and was approved by the Ankara Yıldırım Beyazıt University Clinical Research Ethics Committee (date: 28.01.2015, decision no.: 2015-30).

SD-OCT is a non-invasive imaging modality that leverages the principles of low-coherence interferometry to generate high-resolution, cross-sectional visualisations of tissue microstructures. By employing near-infrared light, the technology measures the echo time delay and intensity of backscattered light to construct images with an axial resolution in micrometres. This approach enables the acquisition of volumetric data through sequential cross-sectional imaging, which is subsequently processed to create two-dimensional and three-dimensional reconstructions. In the present study, SD-OCT was utilised to capture detailed anatomical images of the retina. This technique provides a thickness map of the following seven different retinal layers via automatic segmentation: retinal nerve fibre layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer

(INL), outer plexiform layer (OPL), outer nuclear layer (ONL), retinal pigment epithelium (RPE).

The SD-OCT scans were performed with the Heidelberg Eye Explorer (version 6) with spectral mapping software. The retinal layers from each OCT scan were measured (Figure 1). The measurements are derived from the central, inner, and outer ring subfields as outlined by the early treatment diabetic retinopathy study report (7). In this study, we focused specifically on the central subfield measurements (Figure 1).

Statistical Analysis

All participants underwent a comprehensive ophthalmological evaluation conducted by a single ophthalmologist blinded to the case status. The assessment included detailed examinations such as VF testing, auto-refraction, and OCT. Data from both eyes of patients and controls were included in the analysis.

The analysis of data was conducted using SPSS software, version 25.0. Descriptive statistics were expressed as mean ± standard deviation for variables following a normal distribution, while for non-normally distributed variables, median values were reported. Categorical variables were examined using chi-square and Fisher's exact tests, whereas continuous variables were evaluated with Student's t-test and the Mann-Whitney U test. A p-value below 0.05 was regarded as statistically significant.

Results

The study comprised 23 individuals diagnosed with acromegaly (16 women and 7 men) and 18 healthy control participants (11 women and 7 men). A total of 46 eyes from the acromegaly group and 36 eyes from the control group were evaluated. The median age of the acromegaly group was 42 years (range: 27-60), similar to the control group (p=0.072). Comprehensive patient data were documented during the ophthalmological evaluation. None of the patients exhibited VF defects. Among the acromegaly patients, 16 had undergone transsphenoidal adenomectomy, while seven had not. Eleven had received medical therapy, and five had undergone CyberKnife radiotherapy in addition to surgery. The median IGF-1 level in the acromegaly group was 353.5 (99-1454) ng/mL. Disease activity assessments revealed that 17 patients had active disease, while six were in remission. MRI findings showed that four patients had microadenomas, 11 had macroadenomas, and 8 had no residual adenomas.

The acromegaly group exhibited lower median RNFL values compared to the control group [12 µm (7-25) vs. 13 µm (9-17); p=0.028]. In terms of median GCL thickness, the patient group had a measurement of 13 µm (8-55), while the control group measured 17.5 µm (11-34) (p=0.031). The IPL measurement demonstrated a reduction in the patient group compared to the control group [19 µm (14-45) vs. 22 µm (15-34); p=0.026]. Likewise, the RPE measurements were decreased in the patient group compared to the control group [17 µm (12-21) vs. 18 µm (15-23), respectively; p=0.005]. There were no significant differences in the values of INL, OPL, and ONL between the two groups (p>0.05) (Table 1).

We assessed whether retinal thickness varied based on disease activity within the patient group. Subgroup analysis revealed that INL thickness was significantly greater in patients with non-remission, with a median value of 19 µm (range: 9-50), compared to 15 µm (range: 12-29) in the remission group (p=0.022). No significant differences were observed for the RNFL, GCL, IPL, OPL, ONL, or RPE in this subgroup analysis (p>0.05) (Table 2).

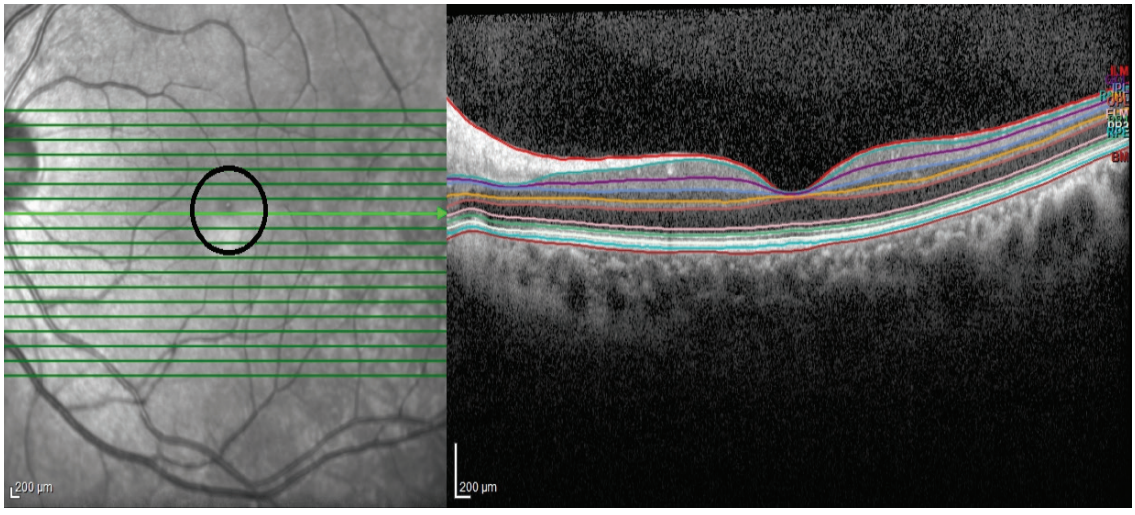

Figure 1: A segmented view of the retinal layers created using the Heidelberg Spectralis automatic segmentation analysis programme. The area within the circle is the central area

Table 1: Comparison of retinal layers in patients with acromegaly and control group

Layers (µm)	Acromegaly patients (n=46 eyes) median (min-max)	Controls (n=36 eyes) median (min-max)	p-value
RNFL	12 (7-25)	13 (9-17)	**0.028**
GCL	13 (8-55)	17.5 (11-34)	**0.031**
IPL	19 (14-45)	22 (15-34)	**0.026**
INL	18 (9-50)	19 (10-29)	0.322
OPL	23 (15-43)	23 (15-35)	0.552
ONL	90 (45-107)	87.5 (59-100)	0.974
RPE	17 (12-21)	18 (15-23)	**0.005**

Bold value: p<0.05
RNFL: Retinal nerve fibre layer, GCL: Ganglion cell layer, IPL: Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, RPE: Retinal pigment epithelium, min-max: Minimum-maximum

Table 2: Retinal layer comparison based on disease remission status in acromegaly patients

Layers (µm)	Non-remission patients (n=34 eyes) median (min-max)	Remission patients (n=12 eyes) median (min-max)	p-value
RNFL	12 (7-25)	11 (7-15)	0.093
GCL	14 (8-55)	11.5 (9-21)	0.112
IPL	20 (14-45)	16.5 (15-24)	0.063
INL	19 (9-50)	15 (12-29)	**0.022**
OPL	25 (17-43)	22.5 (15-26)	0.093
ONL	89 (45-105)	95.5 (72-107)	0.063
RPE	17 (12-21)	16.5 (14-21)	0.745

Bold value: p<0.05
RNFL: Retinal nerve fibre layer, GCL: Ganglion cell layer, IPL: Inner plexiform layer, INL: Inner nuclear layer, OPL: Outer plexiform layer, ONL: Outer nuclear layer, RPE: Retinal pigment epithelium, min-max: Minimum-maximum

When patients were stratified into three groups based on adenoma size (macroadenoma, microadenoma, and no residual adenoma) as determined by MRI during the ophthalmologic assessment, no statistically significant differences were observed across the retinal layers, including the RNFL, GCL, IPL, INL, OPL, ONL, and RPE, with corresponding p-values of 0.28, 0.35, 0.35, 0.55, 0.70, 0.20, and 0.07.

Discussion

Our findings demonstrated that RNFL, GCL, IPL, and RPE thickness were reduced in the acromegaly group compared to the healthy control group. Furthermore, retinal layers were generally thicker in the non-remission group compared to the remission group, with INL being the only layer showing a statistically significant increase in thickness.

GH-secreting pituitary adenomas are very rare and usually present with macroadenoma due to their insidious and slow course. Therefore, chiasma compressions might be seen frequently in acromegaly. Current data on ocular disease and acromegaly are controversial. A few previous studies evaluating retinal thickness in patients with pituitary adenomas have shown that the RNLF was thinner, especially in patients with chiasma compression (8-10). In a recent extensive study, Akay et al. (11) observed that the average RNFL measurements were lower in individuals with acromegaly than healthy controls. Similarly, Sahin et al. (12) reported a reduction in RNFL and IPL thickness in nearly all quadrants in acromegaly patients. These findings were attributed to a greater prevalence of macroadenomas, which are known to compress the optic tracts and optic nerve. In contrast to this attribution, Cennamo et al. (3) reported that RNFL and the ganglion cell complex, comprising the RNFL, GCL, and IPL, were thinner in patients with macroadenoma, even in the absence of optic nerve compression. In the study above, seven patients with macroadenoma had GH-secreting adenomas, six had prolactin-secreting adenomas, six presented with adrenocorticotropic hormone-secreting adenomas, and three had nonfunctional adenomas. In another study, Yazgan et al. (13) reported that RNFL thicknesses were higher in patients with acromegaly than in healthy controls. The authors attributed this surprise result to the fact that most patients were without VF defects. The authors also consider that the direct neuroprotective effects of GH on neurosensory retinal layers might increase retinal volume. In addition, many previous studies have shown no difference between acromegaly and control groups regarding RNFL value (14,15).

Our patients' absence of VF defects suggests that factors other than compression may play a more prominent role in influencing retinal layer thickness in growth hormone-secreting adenomas. Akay et al. (11) reported reduced capillary networks and microvascular atrophy in patients with acromegaly, accompanied by thinner retinal RNFL values compared to controls. This suggests that decreased retinal vascularity may contribute to thinner retinal layers. In the literature, researchers generally focused on the effects of acromegaly on the cornea. In many studies, central corneal thickness (CCT) values were higher in patients with acromegaly than in healthy controls (16,17). Bramsen et al. (17) reported that CCT and intraocular pressure were higher in patients with acromegaly than those with pituitary adenomas, which did not produce GH. These studies indirectly support the IGF-1 effect, which may clarify our study's thicker layers of non-remission patients.

Study Limitations

This study has several limitations. Firstly, the sample size was small, reflecting the acromegaly's rarity, which limits our findings' generalisability. Secondly, the variability in treatment modalities among patients may have influenced retinal layer measurements, introducing potential confounding effects.

Conclusion

In conclusion, our findings suggest that retinal layers were affected in patients with acromegaly, although the precise underlying mechanisms remain unclear. Compared to healthy controls, we observed a reduction in the thickness of retinal layers such as RNFL, GCL, IPL, and RPE in patients with acromegaly. Despite the absence of optic nerve compression in our patients, the thinning of retinal layers implies that factors such as vascular impairment may contribute to these changes. Notably, these layers were thicker in patients with active disease than those in remission, supporting the IGF-1 hormone effect on layers. This study is particularly noteworthy as it is the first to comprehensively evaluate all seven retinal layers in patients with acromegaly. However, given the limited data available in the literature, further extensive studies are warranted to explore these retinal changes and the potential impact of IGF-1 on retinal layers.

Ethics

Ethics Committee Approval: The research complied with the principles of the Declaration of Helsinki and was approved by the Ankara Yıldırım Beyazıt University Clinical Research Ethics Committee (date: 28.01.2015, decision no.: 2015-30).

Informed Consent: Informed written consent was obtained from all participants before the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.E.E.T., N.U., Concept: N.U., Design: B.E.Ö., Ç.K., Data Collection and/or Processing: B.E.Ö., İ.E.E.T., N.U., Analysis and/or Interpretation: B.E.Ö., Ç.K., R.E., B.Ç., Literature Search: B.E.Ö., Ç.K., R.E., B.Ç., Writing: B.E.Ö.

Conflict of Interest: There is no potential conflict of interest to declare.

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Blood Leukocyte Ratios: Equivocal Parameters for Allergic Diseases

Kan Lökosit Oranları: Alerjik Hastalıklar için Şüpheli Parametreler

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Abstract

Objectives: Leukocytes play crucial roles in allergic and immunologic diseases. We compared neutrophil-lymphocyte ratio (NLR), basophil-lymphocyte ratio (BLR), eosinophil-neutrophil ratio (ENR), and eosinophil-lymphocyte ratio (ELR) in 100 drug-induced urticaria-angioedema, 100 drug/venom-triggered anaphylaxis cases, and 100 healthy controls to assess their association with the severity of the allergic reaction.

Materials and Methods: Retrospective analysis of blood leukocyte counts and ratios was performed.

Results: Median NLR was 1.975 in patients (2.09 in urticaria-angioedema, 1.905 in anaphylaxis) and 1.815 in controls. NLR was significantly higher in patients (p=0.038) and notably elevated in urticaria-angioedema (p=0.008). No significant differences in NLR were found between the urticaria-angioedema and anaphylaxis groups (p=0.09) or between the anaphylaxis and control groups (p=0.342). Other leukocyte ratios showed no significant changes between patient and control groups (p>0.05).

Conclusion: NLR may indicate allergic responses but doesn't have predictive value for reaction type or severity.

Keywords: Anaphylaxis, angioedema, inflammation, leukocyte, urticaria

Öz

Amaç: Lökositler, alerjik ve immünolojik hastalıklarda önemli roller oynar. Nötrofil-lenfosit oranı (NLR), bazofil-lenfosit oranı (BLR), eozinofil-nötrofil oranı (ENR) ve eozinofil-lenfosit oranı (ELR) ölçümlerini, 100 ilaçla tetiklenen ürtiker-anjioödem, 100 ilaç/venom ile tetiklenen anafilaksi olgusu ve 100 sağlıklı kontrol olgusunda karşılaştırarak bu oranların alerjik reaksiyonun şiddeti ile ilişkilerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Kan lökosit sayıları ve oranlarının retrospektif analizi gerçekleştirildi.

Bulgular: Hastalarda median NLR değeri 1,975 idi (ürtiker-anjioödem grubunda 2,09, anafilaksi grubunda 1,905), kontrol grubunda ise 1,815 olarak belirlendi. NLR, hasta grubunda anlamlı derecede yüksekti (p=0,038) ve özellikle ürtiker-anjioödem grubunda belirgin şekilde yükselmişti (p=0,008). Ürtiker-anjioödem ve anafilaksi grupları arasında (p=0,09) ve anafilaksi ile kontrol grupları arasında (p=0.342) NLR değerlerinde anlamlı fark bulunmadı. Diğer lökosit oranları, hasta ve kontrol grupları arasında anlamlı değişiklikler göstermedi (p>0,05).

Sonuç: NLR, alerjik reaksiyonlar için bir belirteç olabilir, ancak reaksiyon türü veya şiddeti için öngörü değeri bulunmamaktadır.

Anahtar Kelimeler: Anafilaksi, anjioödem, enflamasyon, lökosit, ürtiker

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Introduction

Urticaria, angioedema, and anaphylaxis share a common pathogenesis but exhibit varying levels of severity within the spectrum of immunoglobulin E (IgE)-mediated type I hypersensitivity (1,2). Anaphylaxis often presents with skin manifestations, including flushing, urticarial plaques, and angioedema affecting the tongue, lips, eyelids, and uvula, progressing rapidly to systemic involvement in cardiovascular, respiratory, gastrointestinal, and neural systems (3).

In individuals sensitized to a specific trigger, agent-specific IgE binds to its receptor on mast cell surfaces (4). Upon re-exposure to the triggering factor, mast cells release a diverse array of mediators (4). Furthermore, these mediators from mast cells contribute to the activation of other leukocytes, such as neutrophils, eosinophils, basophils, and lymphocytes (5).

Several studies have demonstrated variations in leukocyte ratios across various conditions, including inflammatory, allergic, infectious, malignant, and cardiovascular diseases, compared to healthy individuals. It has been emphasized that these ratios serve as useful parameters for predicting disease presence, severity, or prognosis (6-8).

In this study, our aim was to evaluate the neutrophil-lymphocyte ratio (NLR), basophil-lymphocyte ratio (BLR), eosinophil-neutrophil ratio (ENR), and eosinophil-lymphocyte ratio (ELR) in cases of drug-induced urticaria-angioedema and drug- or venom-triggered anaphylaxis admitted to our clinic. We sought to elucidate the potential of these markers in identifying individuals who may exhibit mild reactions to specific triggers via type I hypersensitivity or progress to a more severe form of trigger-induced allergic reaction, namely anaphylaxis.

Materials and Methods

Patient Selection and Ethical Issues

In our retrospective study, we analyzed data from 13,753 patients aged 18 and above who visited our clinic between January 2020 and October 2023. Of these, 1,873 had urticaria and/or angioedema, and 176 had anaphylaxis based on International Statistical Classification of Diseases and Related Health Problems (ICD) codes (ICD-10) during their first visit, confirmed by World Allergy Organization criteria (9). Among those with urticaria and/or angioedema, we identified 482 cases with coinciding drug reactions, encoded with the ICD code of Y57. Excluded were 96 with chronic urticaria-angioedema and 1,295 with urticaria lacking the ICD code of Y57. From cases with possible drug reactions and urticaria and/or angioedema, 382 were excluded due to missing data or exclusion criteria.

Exclusion criteria included additional allergic diseases, immune deficiency, chronic inflammatory diseases, infectious diseases, use of anti-inflammatory or immunomodulator drugs, liver or renal diseases, malignancy, hematological disease, and pregnancy. Cases with documented urticaria and/or angioedema at admission were also excluded.

Seventy-six cases diagnosed with anaphylaxis were excluded due to undetermined triggers or meeting exclusion criteria. In total, 200 patients, comprising 100 drug-related urticaria-angioedema cases and 100 anaphylaxis cases with known triggers, were included in the study.

A study, investigating the predictive effects of BLR and ELR parameters on anaphylaxis risk, was used as a reference for the power analysis of this research (10). The study comparing anaphylaxis, urticaria/angioedema, and a healthy control group by One-Way analysis of variance (ANOVA) estimated an effect size of 0.21, a type 1 error rate of 5%, and a power of 90%. The power analysis determined a total estimated sample size of 291 for the study, with 100 patients allocated to each group to ensure equal distribution.

As healthy controls, 100 age- and sex-matched participants were selected from those admitted to our institution's general internal medicine outpatient clinic between January 2020 and October 2023 for routine health check-ups or regular occupational evaluation, encoded with the ICD code of Z00 for an encounter for a general examination. The flowchart for creating patient groups is presented in Figure 1.

Prior to commencing the research, necessary approval was obtained from the Human Research Ethics Committee of Ankara University (approval number: i09-647-23, date: 02.11.2023). Informed consent has been obtained from the participants. The investigation adhered to the Helsinki Declaration in its conduct (11).

Evaluation of Data

Age, gender, comorbidities, and a detailed history regarding the drug associated with urticaria and/or angioedema or triggering factor associated with the hypersensitivity reaction diagnosed as anaphylaxis were reviewed. The leukocyte counts and leukocyte ratios were obtained from the complete blood count test performed during the routine evaluation of urticaria-angioedema, anaphylaxis, and healthy control participants. The complete blood count test was conducted during outpatient follow-ups of patients in inactive periods where disease symptoms were not present; the aim was to evaluate whether leukocyte ratios can be used to predict the risk and severity of allergic reactions in individuals without symptoms and signs of allergy.

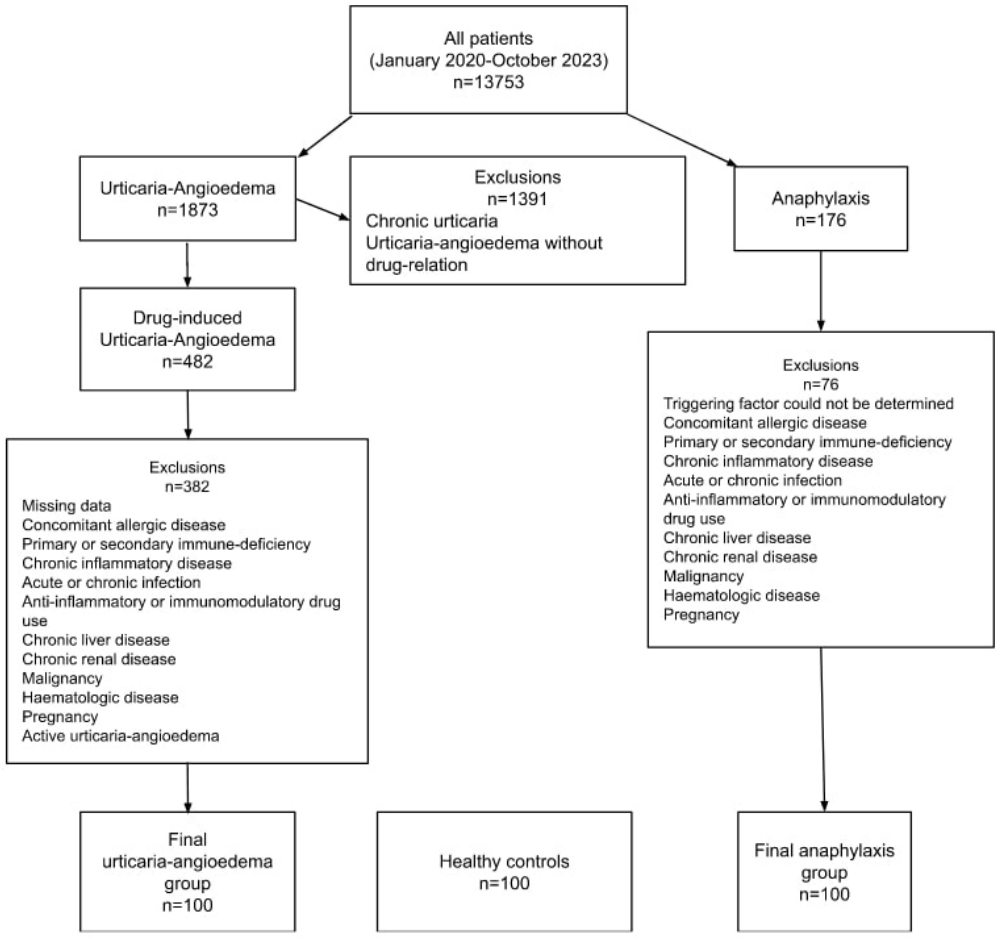

Figure 1: Flowchart for designation of patient and control groups

Statistical Analysis

Statistical analyses were performed using IBM SPSS version 27 software. Descriptive analyses were presented as frequency and percentage for categorical variables and as median (minimum-maximum) or mean ± standard deviation for continuous variables. Independent group comparisons in categorical variables were made using chi-square tests. The suitability of continuous variables to a normal distribution was examined visually (histograms and probability graphics) and using analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Two independent groups were analyzed with the Student's t-test or Mann-Whitney U test, as appropriate. For comparisons of more than two independent groups, Kruskal-Wallis test or One-Way ANOVA was used. The prognostic properties of leukocyte parameters in predicting allergic reactions were examined by receiver operating characteristics (ROC) analysis. In the presence of a significant cut-off value, the sensitivity and specificity of the test were calculated. For statistical significance, the type-1 error level was set at 5%.

Results

The percentages of female and male cases were provided, with 66% females and 34% males for 100 urticaria-angioedema subjects, 61% females and 39% males for 100 anaphylaxis subjects, and 66% females and 34% males for 100 healthy control subjects. Additionally, the mean age and age range for each group were specified, with the mean age of 40.19±13.35 years for urticaria-angioedema, 40.06±12.02 years for anaphylaxis, and 38±11.58 years for healthy control subjects. There was no significant difference between the patient and control groups in terms of gender and age (p=0.738 and p=0.445, respectively).

In the urticaria-angioedema group, the prevalence of comorbidities was as follows: hypertension in 13 cases (13%), diabetes in 12 cases (12%), hypothyroidism in 6 cases (6%), and multiple comorbidities in 12 cases (12%). Similarly, in the anaphylaxis group, the respective numbers were 10 cases (10%) for hypertension, 9 cases (9%) for diabetes, 6 cases (6%) for hypothyroidism, and 11 cases (11%) for multiple comorbidities. Statistical analysis indicated no significant difference in

comorbidity distribution between the urticaria-angioedema and anaphylaxis groups (p=0.506, p=0.489, p=1.00, p=0.825, respectively).

In the anaphylaxis group, the reaction-triggering factors were venom exposure in 54 cases (54%) and medication in 46 cases (46%). Demographic data for the patient and control groups are presented in Table 1.

The absolute neutrophil counts in the patient group were significantly higher than in the control group (p=0.006). Within the patient group, urticaria-angioedema cases exhibited significantly higher absolute neutrophil counts compared to the control group (p=0.004). No significant difference was found in absolute neutrophil counts between the anaphylaxis group and the control group or between the urticaria-angioedema group and the anaphylaxis group (p=0.055 and p=0.338, respectively).

There was a significant difference in the absolute eosinophil counts between the patient group and the control group (p=0.041). Within the patient group, absolute eosinophil counts were significantly higher in anaphylaxis cases compared to the control group (p=0.011). No significant differences were detected in absolute eosinophil counts between the urticaria-angioedema group and the control group or between the urticaria-angioedema group and the anaphylaxis group (p=0.326 and p=0.118, respectively).

On the other hand, there were no significant differences in absolute basophil and lymphocyte counts between the patient and control groups (p=0.138 and p=0.093, respectively). A comparison of the absolute leukocyte counts for the patient and control groups is presented in Table 2.

Table 1: Demographic and clinical parameters of the patient and control groups

Demographic and clinical parameters	Urticaria-angioedema (n=100)	Anaphylaxis (n=100)	Healthy controls (n=100)	p^1	p^2	p^3
Age (years) mean ± SD (range)	40.19±13.35 (18-72)	40.6±12.02 (18-67)	38±11.58 (19-59)	0.380	0.491	0.388
Gender (F/M) (n)	66/34	61/39	66/34	1.000	0.463	0.463
Comorbidities (n)	22	22	-	-	-	1.000
Hypertension (n)	13	10	-	-	-	0.506
Diabetes (n)	12	9	-	-	-	0.489
Hypothyroidism (n)	6	6	-	-	-	1.000
Multiple comorbidities (n)	12	11	-	-	-	0.825
Reaction triggers						
Venom (n)	-	54	-	-	-	-
Penicillin group antibiotic (n)	31	24	-	-	-	0.268
Non-penicillin antibiotic (n)	16	11	-	-	-	0.301
Analgesic (n)	47	36	-	-	-	0.114
Antibiotic and analgesic (n)	11	9	-	-	-	0.637
Other drugs (n)	21	11	-	-	-	0.053

p^1: Comparison between urticaria-angioedema and healthy control groups, p^2: Comparison between anaphylaxis and healthy control groups, p^3: Comparison between urticaria-angioedema and anaphylaxis groups
F: Female, M: Male, SD: Standard deviation

Table 2: Absolute leukocyte counts in patient and control groups

Leukocyte counts (median)	Urticaria-angioedema (n=100)	Anaphylaxis (n=100)	Healthy controls (n=100)	p^1	p^2	p^3	p^4
Neutrophil ($x10^9$/L) (range)	4.445 (2.1-9.37)	4.2 (2.08-8.15)	3.955 (1.89-10.8)	**0.006**	**0.004**	0.055	0.338
Basophil ($x10^9$/L) (range)	0.04 (0-0.11)	0.04 (0-0.1)	0.05 (0.01-0.16)	0.138	0.136	0.296	0.848
Eosinophil ($x10^9$/L) (range)	0.12 (0-0.8)	0.145 (0.01-1.18)	0.12 (0.01-0.58)	**0.041**	0.326	**0.011**	0.118
Lymphocyte ($x10^9$/L) (range)	2.095 (1.06-4.55)	2.22 (1.15-4.46)	2.13 (1.29-4.87)	0.093	0.935	0.213	0.205

Bold value: The absolute neutrophil counts in the patient group were significantly higher than in the control group (p=0.006). Within the patient group, urticaria-angioedema cases exhibited significantly higher absolute neutrophil counts compared to the control group (p=0.004). There was a significant difference in the absolute eosinophil counts between the patient group and the control group (p=0.041). Within the patient group, absolute eosinophil counts were significantly higher in anaphylaxis cases compared to the control group (p=0.011). The bold values represent statistical significance. p^1: comparison between patient and healthy control groups, p^2: comparison between urticaria-angioedema and healthy control groups, p^3: comparison between anaphylaxis and healthy control groups, p^4: comparison between urticaria-angioedema and anaphylaxis groups

Table 3: Leukocyte ratios in patient and control groups

Leukocyte ratios (median)	Urticaria-angioedema (n=100)	Anaphylaxis (n=100)	Healthy controls (n=100)	p^1	p^2	p^3	p^4
NLR (range)	2.09 (0.64-5.03)	1.905 (0.87-4.3)	1.815 (0.5-6.47)	**0.038**	**0.008**	0.342	0.090
BLR (range)	0.018 (0-0.05)	0.017 (0-0.064)	0.021 (0.002-0.096)	0.092	0.676	0.484	0.800
ENR (range)	0.0305 (0-0.143)	0.037 (0.003-0,27)	0.031 (0.001-0.259)	0.511	0.890	0.619	0.524
ELR (range)	0.058 (0-0.519)	0.062 (0.005-0.421)	0.056 (0.005-0.212)	0.093	0.782	0.480	0.656

Bold value: In the patient group, NLR values were significantly higher compared to the control group (p=0.038). Within the patient group, NLR values were significantly higher in urticaria-angioedema cases compared to the control group (p=0.008). The bold values represent statistical significance. p^1: Comparison between patient and healthy control groups, p^2: Comparison between urticaria-angioedema and healthy control groups, p^3: Comparison between anaphylaxis and healthy control groups, p^4: Comparison between urticaria-angioedema and anaphylaxis groups
NLR: Neutrophil-lymphocyte ratio, BLR: Basophil-lymphocyte ratio, ENR: Eosinophil-neutrophil ratio, ELR: Eosinophil-lymphocyte ratio

The NLR median values were 2.09 (0.64-5.03), 1.905 (0.87-4.3), and 1.815 (0.5-6.47) in the urticaria-angioedema, anaphylaxis, and control groups, respectively. In the patient group, NLR values were significantly higher compared to the control group (p=0.038). Specifically, within the patient group, NLR values were significantly higher in urticaria-angioedema cases compared to the control group (p=0.008). No significant difference was found in NLR values between urticaria-angioedema cases and anaphylaxis cases (p=0.09), and no significant difference was detected in NLR values between anaphylaxis cases and the control group (p=0.342).

However, there was no significant difference between the patient and control groups in terms of BLR, ENR, and ELR values (p=0.092, p=0.511, and p=0.093, respectively). A comparison of the leukocyte ratios of patients and control groups is presented in Table 3.

According to ROC analysis, NLR was identified as having a statistically significant but limited predictive feature for the development of allergic reactions, with an area under the curve of 0.573 (95% confidence interval: 0.506-0.641, p=0.038). When the threshold value for NLR was set at 1,885, the sensitivity of the test was 55.5%, and the specificity was 55%. The ROC analysis regarding NLR is visually presented in Figure 2.

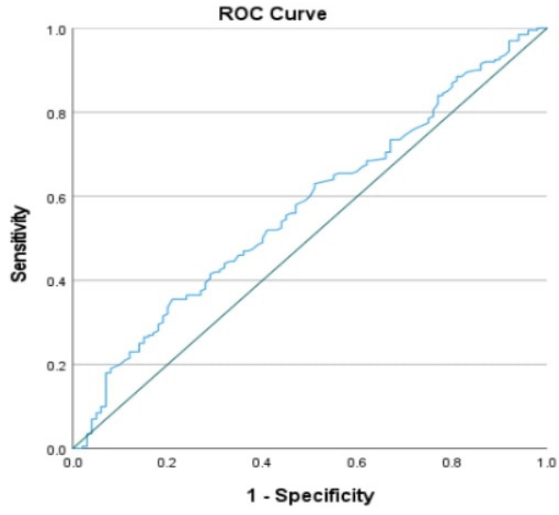

Figure 2: ROC curve of NLR
ROC: Receiver operating characteristics, NLR: Neutrophil-lymphocyte ratio

Discussion

Although urticaria and angioedema are relatively common diseases in the population, the pathogenesis of these diseases has not yet been clearly elucidated (2). Dermal mast cells secrete a wide variety of cytokines, activating various inflammatory cells such as eosinophils, neutrophils, and T lymphocytes, which contribute to disease pathogenesis (2).

In our study, we observed higher NLR values in the patient group compared to the control group; however, subgroup analyses revealed that the difference in NLR values was associated with the urticaria-angioedema group rather than the anaphylaxis group.

Several studies present inconclusive results regarding the association between NLR and various type I hypersensitivity disorders.

In a meta-analysis conducted on the relationship between NLR and asthma, NLR was found to be significantly higher in asthma cases compared to healthy controls, and it was also found to be higher in cases with asthma exacerbations compared to stable asthma cases (12). However, in a different study, no significant difference was found in NLR between asthma cases and healthy controls. Researchers attributed this discrepancy to the fact that the asthma cases included in the study had not experienced a recent attack (13).

It has been stated that in cases of allergic rhinitis, NLR was determined to be higher compared to the healthy control group, and it was correlated with the severity of the disease (14,15). However, in a different study conducted on allergic rhinitis, NLR was found to be lower in allergic rhinitis cases compared to healthy controls. Additionally, it was found to

be lower in persistent allergic rhinitis cases compared with intermittent allergic rhinitis cases (16). This difference was interpreted as indicating that the NLR could be considered as a fragile parameter since the NLR value may vary depending on additional clinical conditions, including infectious diseases that might be present in the examined patient groups that may coexist with allergic rhinitis (16).

To the best of our knowledge, our study is the first to evaluate blood leukocyte parameters in drug-related acute urticaria-angioedema cases and anaphylaxis cases with known trigger factors by comparing them with the healthy control group. However, there is also a study in which blood leukocyte rates were evaluated in anaphylaxis cases and chronic spontaneous urticaria cases by comparing them with the healthy control group, and another study evaluated NLR in anaphylaxis cases that were and were not resistant to epinephrine treatment (10,17). In the first of these studies, no significant difference was found in NLR values between anaphylaxis, chronic spontaneous urticaria, and healthy control groups. However, BLR and ELR values were found to be significantly higher in anaphylaxis cases compared with chronic spontaneous urticaria and healthy control groups (10). In the second study, NLR was found to be significantly lower in anaphylaxis cases that were resistant to epinephrine treatment compared to non-resistant anaphylaxis cases (17). However, we detected no significant relationships regarding BLR, ENR, and ELR parameters in our study.

There are several possible reasons for the differences between the data we obtained from our study and the data obtained in other studies in the literature. The presence of confounding factors that may cause subclinical inflammation, and that could not be evaluated in the participants included in our retrospectively designed study may have affected our results. Another possibility is that, while the urticaria-angioedema group in our study consisted only of drug-triggered patients, the anaphylaxis group included cases triggered by venom as well. Different triggering factors may have affected the absolute leukocyte counts and leukocyte ratios to a different extent. Last but not least, although the normal value ranges for BLR, ENR, and ELR are not defined in the literature, the normal NLR value range in healthy adult individuals has been reported as 0.78-3.53 (18). In our study, the NLR median values and the median values of absolute leukocyte counts in the patient and control groups were within the normal range defined in the literature. Thus, despite the findings obtained in our study, NLR values may not actually hold prognostic significance.

In our study, ROC analysis suggests that NLR has a statistically significant but relatively modest predictive capability for identifying individuals with allergic reactions .

Study Limitations

The main limitations of our study include being a retrospective study conducted in a single center and the relatively small number of cases included. Additionally, the lack of classification regarding the severity of the clinical picture, other than the diagnosis of urticaria-angioedema and anaphylaxis cases, is one of the limiting factors in our study. Another limitation of the present study is the lack of data regarding urticaria activity score at the time of blood sampling. It is possible to obtain more enlightening results with the help of further prospective and multicenter prospective studies.

Conclusion

There is contradictory data regarding blood leukocyte parameters in various allergic diseases in the literature. In our study, although the NLR value was higher in the patient group with a history of allergic reactions compared to the healthy control group, it was determined that this difference was not predictive of the severity of the reaction, or progression to anaphylaxis. Identifying individuals at high risk of developing type-I hypersensitivity against culprit agents is a complex task, often requiring a multi-faceted approach involving various biomarkers and clinical parameters.

Ethics

Ethics Committee Approval: Prior to commencing the research, necessary approval was obtained from the Human Research Ethics Committee of Ankara University (approval number: i09-647-23, date: 02.11.2023).

Informed Consent: Informed consent has been obtained from the participants. The investigation adhered to the Helsinki Declaration in its conduct.

Acknowledgments

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Footnotes

Authorship Contributions

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

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

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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

The Effect of Adjuvant Chemoradiotherapy Initiation Time on Prognosis in Glioblastoma Multiforme

Glioblastoma Multiformede Adjuvan Kemoradyoterapi Başlama Zamanının Prognoz Üzerine Etkisi

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Abstract

Objectives: To determine survival and investigate associated prognostic factors in glioblastoma multiforme (GBM) patients receiving adjuvant therapy.

Materials and Methods: The study population comprised patients with isocitrate dehydrogenase wild-type GBM who were enrolled between 1 September 2022 and 1 March 2024. The primary endpoint was overall survival (OS), while the secondary endpoint was progression-free survival. Comparisons between groups were conducted using the log-rank test, and multivariate analyses were performed using Cox regression.

Results: A total of 67 patients were evaluated. The median OS of patients was 19.3 months [95% confidence interval (CI) 15.1 to not reached (NR)]. A total of 60 patients (89.1%) underwent adjuvant treatment. The median OS was 31.1 months (95% CI 19.3 to NR) for patients treated within the first four weeks, 15.7 months (95% CI 15.1 to NR) for those treated within four to six weeks, and 11.3 months (95% CI 9.8 to NR) for those treated after six weeks.

Conclusion: The observed survival rate in our study was comparable to that reported in clinical trials. However, the survival rate was significantly lower in patients who received treatment at a later stage. Therefore, further studies with larger patient populations are recommended to ensure that the guidelines more accurately reflect the timing of adjuvant therapy in GBM patients.

Keywords: Glioblastoma multiforme, stupp protocol, overall survival, time to treatment

Öz

Amaç: Bu çalışmanın amacı adjuvan tedavi alan glioblastoma multiforme (GBM) hastalarında sağkalımı belirlemek ve ilişkili prognostik faktörleri araştırmaktır.

Gereç ve Yöntem: Çalışma popülasyonu, 1 Eylül 2022 ve 1 Mart 2024 tarihleri arasında kaydedilen izositrat dehidrogenaz vahşi tip GBM hastalarından oluşmaktadır. Birincil sonlanım noktası genel sağkalım, ikincil sonlanım noktası ise progresyonsuz sağkalımdır. Gruplar arasındaki karşılaştırmalar log-rank testi kullanılarak yapılmış ve çok değişkenli analizler Cox regresyonu kullanılarak gerçekleştirilmiştir.

Bulgular: Toplam 67 hasta değerlendirildi. Hastaların ortanca genel sağkalımı 19,3 aydı [%95 güven aralığı (GA) 15,1 ila ulaşılamadı (NR)]. Toplam 60 hastaya (%89,1) adjuvan tedavi uygulandı. Ortanca genel sağkalım ilk dört hafta içinde tedavi edilen hastalar için 31,1 ay (%95 GA ila 19,3-NR), dört ila altı hafta içinde tedavi edilenler için 15,7 ay (%95 GA ila 15,1-NR) ve altı haftadan sonra tedavi edilenler için 11,3 ay (%95 GA ila 9,8-NR) idi.

Sonuç: Çalışmamızda gözlenen sağkalım oranı klinik çalışmalarda bildirilenlerle karşılaştırılabilir düzeydedir. Ancak, daha geç dönemde tedavi alan hastalarda sağkalım oranı anlamlı derecede düşüktü. Bu nedenle, kılavuzların GBM hastalarında adjuvan tedavinin zamanlamasını daha doğru bir şekilde yansıtmasını sağlamak için daha geniş hasta popülasyonlarıyla daha fazla çalışma yapılması önerilmektedir.

Anahtar Kelimeler: Glioblastoma multiforme, stupp protokolü, genel sağkalım, tedaviye kadar geçen süre

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Introduction

Glioblastoma multiforme (GBM) represents the most prevalent primary central nervous system (CNS) tumor (1). In accordance with the 2021 World Health Organization (WHO) Classification of CNS tumors, GBM is characterised as isocitrate dehydrogenase (IDH)-wild-type (2,3). The median survival time for this aggressive tumor, with a median age of onset of approximately 60 years, is currently 12 to 15 months, and the 5-year survival rate is 6% (4).

The current gold standard treatment for GBM, a tumor with an adverse prognosis, comprises maximal safe resection and subsequent adjuvant therapy. This consists of temozolomide (TMZ), concurrent chemoradiotherapy (CRT), and six months of maintenance TMZ (5). The Stupp protocol has been demonstrated to enhance overall survival (OS) in patients under 70 with favorable performance status. The treatment regimen entails the concurrent administration of 75 mg/m²/day TMZ in 2 Gy fractions, with a total dose of 60 Gy, followed by six cycles of TMZ monotherapy (6). The long-term results of the trial demonstrated that survival in the TMZ arm remained superior to radiotherapy (RT) alone at both two-year (27% vs. 11%) and five-year (10% vs. 2%) follow-up points (7). In patients of an advanced age and/or with a markedly poor performance status, a supportive care approach may be considered the most appropriate course of action (8).

A number of studies have been conducted in order to ascertain the optimal timing for the commencement of adjuvant treatment in patients with various types of cancer. In patients with non-small cell lung cancer who are to receive adjuvant treatment following a curative resection, commencing treatment after a period of six weeks has been demonstrated to result in a reduction in disease-free survival (9). A study of 24,843 patients who had undergone surgery for breast cancer revealed that those who received adjuvant therapy 91 days or more after surgery exhibited a diminished OS rate [hazard ratio (HR): 1.34, 95% confidence interval (CI): 1.15-1.57] (10).

Despite adjuvant treatment currently being the standard of care, there is no consensus regarding the optimal timing for initiating treatment. The objective of this study was to ascertain the OS of patients diagnosed with GBM who received adjuvant treatment at our center and to evaluate the relationship between treatment approach, clinical characteristics, and time of treatment initiation and survival.

Materials and Methods

The present study is a retrospective cohort study that encompasses patients diagnosed with GBM at our medical center between 1 September 2022 and 1 March 2024. Patients diagnosed with an International Classification of Diseases 10th Revision code C71 (malignant neoplasm of the brain), histologically confirmed (biopsy or resection) GBM IDH-wild-type, CNS WHO grade 4, aged 18 years or older, with no previous chemotherapy or cranial RT, and no active infection, were identified through medical records as meeting the requisite criteria. Patients who did not fulfill the requisite criteria were excluded from the study.

The demographic information of the patients, including diagnosis dates and treatment initiation dates, as well as details of the treatment methods employed (surgery, chemotherapy, RT), the time elapsed between diagnosis and treatment initiation, and survival data (survival time and date of death), were defined. Additionally, data on recurrence and other treatment processes were also determined. The time to diagnosis was defined as the initial pathology date at which the primary brain tumor was identified. The early treatment group was defined as comprising those patients who commenced treatment within the first four weeks of diagnosis, while the delayed treatment group was defined as comprising those patients who commenced treatment six weeks or more after diagnosis (Figure 1).

OS defined as the time elapsed between diagnosis and death or the date of the last visit-served as the primary endpoint in this analysis. The secondary endpoint, progression-free survival (PFS), was operationalized as the time elapsed between the commencement of treatment and the date of the initial observational assessment of relapse, or death/last visit, whichever occurred first.

The time elapsed between surgical intervention and the commencement of CRT was examined as both a continuous variable and a categorical one, based on three defined time

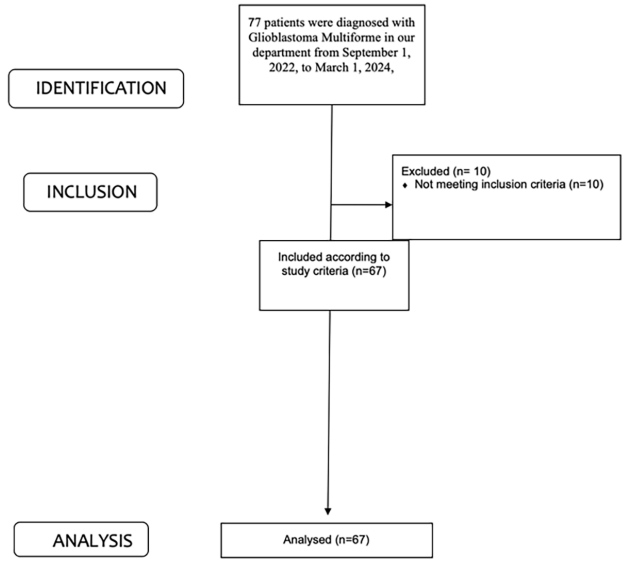

Figure 1: Patient flow diagram

intervals. The intervals were defined as follows: less than four weeks, four to six weeks, and greater than six weeks. This study was approved by the Scientific Research Evaluation and Ethics Committee of Etlik City Hospital (decision no.: AEŞH-BADEK-2024-758, date: 02.10.2024).

Statistical Analysis

In order to facilitate the analysis and presentation of the data, quantitative variables were expressed as means, with accompanying ranges. Similarly, categorical variables were described in terms of percentage frequency distributions. Kaplan-Meier survival curves were employed to estimate survival outcomes. Subsequently, intergroup comparisons were conducted utilising log-rank tests. A Cox regression analysis was employed to conduct multiple analyses. A two-tailed p-value of less than 0.05 was considered statistically significant. The statistical analyses were conducted using the BlueSky Statistics version 10.3.2 software.

Results

The mean age of the 67 patients included in the study was 60 years (range: 42-86 years). Upon analysis of the age distribution, it was observed that approximately half of the patients were under the age of 60. Of the total number of patients, 31 (46.2%) were male. Upon diagnosis, 61.2% of patients exhibited an Eastern Cooperative Oncology Group score of 0 to 1. Among the patients included in the study, 38 (56%) had pre-existing comorbidities. The most prevalent comorbidity was hypertension. A summary of the patient and tumor characteristics is presented in Table 1.

All patients included in the study underwent surgical intervention. A total resection was performed in 41 patients, representing 61.1% of the total number of patients included in the study. Over 90% of the patients were eligible for adjuvant treatment. Of the seven patients who were not eligible for adjuvant treatment, three were excluded due to age and performance status, two patients succumbed to postoperative complications, and two patients refused treatment. Of the total number of patients, 58 (86.5%) received and completed adjuvant. Two patients received only RT due to thrombocytopenia, which precluded the use of chemotherapy. The mean time to commencement of adjuvant treatment was 30 days (range 15-119 days). All patients received the standard doses of RT. During the follow-up period, 22 patients (32.8%) experienced recurrence. The estimated median PFS was 5.3 months (95% CI 3.6 to 12.6) (Figure 2a). During the follow-up period of 9.1 months, 21 patients succumbed to their disease. The estimated OS was 19.3 months (95% CI 15.1 to NR) (Figure 2b) (Table 2).

The Kaplan-Meier curves for PFS and OS were analysed according to the timing of adjuvant treatment initiation among patients. The median PFS was 7.0 months (95% CI 4.4 to NR) for 24 patients (35.8%) who commenced treatment within four weeks, 5.3 months (95% CI 3.5 to NR) for 21 patients (31.3%) who initiated treatment between four and six weeks, and 3.6 months (95% CI 2.8 to NR) for 13 patients (19.4%) who started treatment after six weeks. No statistically significant difference in PFS was observed between treatment groups (p=0.18) (Figure 3a).

Table 1: Patient, and tumor characteristics	
Age	
Median, (year)	60 (42-86)
Distibution no, (%)	
<60 ≥60	32 (47.7) 35 (52.2)
Sex no, (%)	
Male Female	31 (46.2) 36 (53.7)
ECOG PS no, (%)	
0-1 2 3 4	41 (61.2) 18 (26.8) 7 (10.4) 1 (1.4)
Comorbidty no, (%)	
Yes No	38 (56.7) 29 (43.3)
Comorbidity no, (%)	
HT DM HT + DM	20 (29.8) 8 (11.9) 10 (14.9)
Multifocal no, (%)	
Yes No	8 (11.9) 59 (88.1)
Tumorvolum (cm^3) no, (%)	
<200 200-400 >400	2 (2.9) 34 (50.7) 31 (46.2)

ECOG PS: Eastern Cooperative Oncology Group Performance Status, HT: Hypertension, DM: Diabetes mellitus

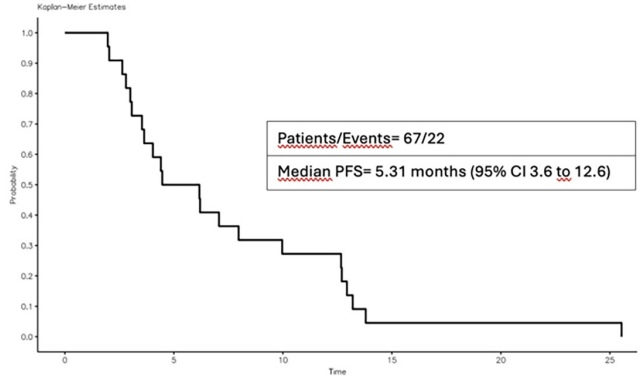

Figure 2a: Kaplan-Meier curve for PFS of the whole population
PFS: Progression-free survival, CI: Confidence interval

The median OS was 31.1 months (95% CI 19.3 to NR) for patients whose treatment was initiated within the first four weeks, 15.7 months (95% CI 15.1 to NR) for patients whose treatment commenced between four and six weeks, and 11.3 months (95% CI 9.8 to NR) for patients whose treatment began after six weeks. The OS time between the groups was statistically significant (p=0.04) (Figure 3b).

The effects of demographic and therapeutic characteristics on PFS were analysed and performance status at diagnosis (p=0.04) and intraoperative resection (total/subtotal) status (p=0.01) were statistically significant in univariate analyses. The only significant effect in multivariate analyses was intraoperative resection (p=0.03) (Table 3).

Univariate and multivariate analyses of OS revealed that performance status (p=0.001), gender (p=0.03) and time of chemotherapy initiation (p=0.03) had a statistically significant effect in univariate analysis. In multivariate analysis, the only significant effect was chemotherapy initiation time (p=0.01) (Table 4). When evaluated as post hoc analysis, a statistically significant difference was seen in all 3 groups 4 weeks/4-6 weeks, 4 weeks/6 weeks later, 4-6 weeks/6 weeks later (p<0.05).

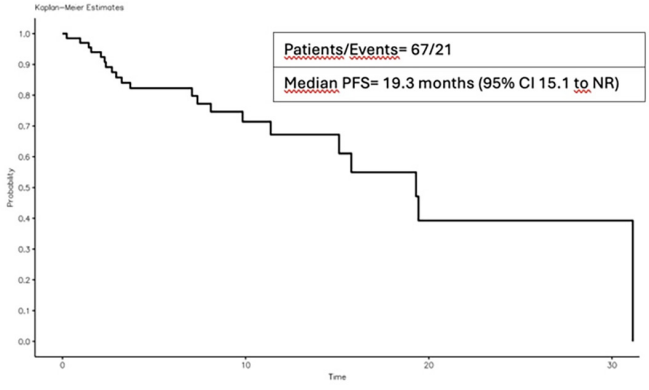

Figure 2b: Kaplan-Meier curve for OS of the whole population
OS: Overall survival, PFS: Progression-free survival, CI: Confidence interval

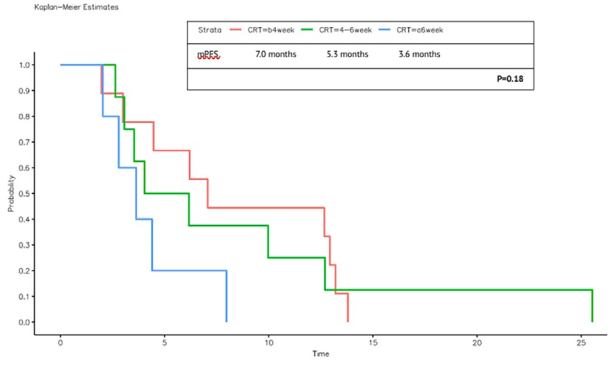

Figure 3a: Kaplan-Meier curves of PFS according to the time of initiation of adjuvant treatment
PFS: Progression-free survival, CRT: Chemoradiotherapy, mPES: Mean pulmonary end systolic

Discussion

Several factors contribute to the poor prognosis of GBM and its resistance to current therapies. The heterogeneity of GBM, the pro-tumorigenic role of the tumor microenvironment, the blood-brain barrier as a barrier to systemic treatment, and the low immunogenicity of GBM, which prevents a strong immunological response, are all factors that contribute to the poor prognosis of GBM and its resistance to current therapies

Table 2: Treatment and survival characteristics

Operation no, (%)	
Total resection Subtotal resection Biopsy	41 (61.1) 21 (31.3) 5 (7.46)
Adjuvant theraphy no, (%)	
Yes No	60 (89.5) 7 (10.4)
CRT no, (%)	
Yes No Only TMZ	58 (86.5) 7 (10.4) 2 (2.9)
RT waiting time, median (days)	30 (15-119)
RT waiting time no, (%)	
<4 weeks 4-6 week ≥6 weeks	24 (35.8) 21 (31.3) 13 (19.4)
RT dose no, (%)	
60 Gy	58 (89.5)
TMZ maintenance no, (%)	
Yes No	54 (80.5) 13 (19.4)
TMZ maintenance no, (%)	
<6 months ≥6 months	22 (40.7) 32 (59.2)
Recurrence no, (%)	
Yes No	22 (32.8) 45 (67.1)
Recurrence no, (%)	
Lokal Mutifocal	20 (90.9) 2 (9.9)
Median PFS (months)	5.3 (95% CI 3.6 to 12.6)
Exitus no, (%)	
Yes No	21 (31.3) 46 (68.6)
Median OS (months)	19.3 (95% CI 15.1 to NR)
Median follow-up (months)	9.1

CRT: Chemoradiotherapy, RT: Radiotherapy, TMZ: Temozolamide PFS: Progression-free survival, OS: Overall survival, NR: Not reached, CI: Confidence interval

(11). Furthermore, studies have demonstrated that patient characteristics (age, comorbidity, performance status), as well as surgical and adjuvant treatment-related factors (total resection, subtotal resection/biopsy), can influence OS (12-14). The tumor- and patient-related analyses, as well as the survival analyses, of our study, are in accordance with the findings of the existing literature on the subject.

The hypothesis that the growth rate of a tumor slows down with increasing tumor size may prove useful as a general rule for all tumors and may also assist in determining the optimal time to commence treatment for GBM (15). Given that radiosensitivity is inversely proportional to tumor growth rate, the commencement of RT at a later stage may result in a reduction in its efficacy (16). Nevertheless, an alternative perspective posits that hypoxia and edema in the vicinity of the surgical site in the immediate postoperative period may result in a reduction in radiosensitivity (17). In fact, the studies conducted support both of these perspectives.

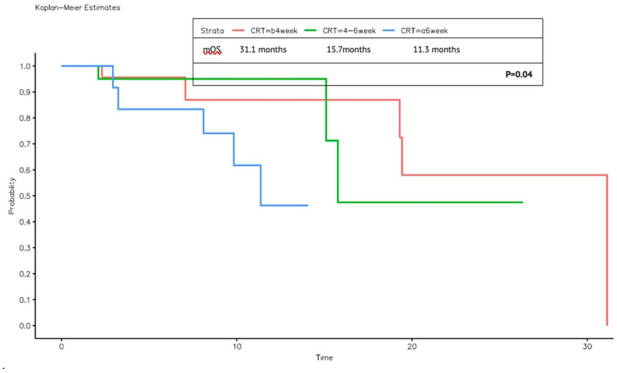

Figure 3b: Kaplan-Meier curves of OS according to the time of initiation of adjuvant treatment
mOS: Mean overall survival, CRT: Chemoradiotherapy

A study conducted in 2007 investigated the efficacy of RT in the early postoperative period. The findings indicated that, contrary to the delay observed at the time of presentation to the RT department, the delay at the time of surgery was associated with a reduction in survival (17). It is important to acknowledge that this study was conducted prior to the establishment of TMZ concurrent RT as the standard treatment. However, a recent retrospective study involving a substantial patient cohort demonstrated that adjuvant treatment initiated within the initial 35 days following total resection was associated with enhanced survival outcomes (18). However, the same improvement was not observed in residual tumors in this study. In a further large patient cohort, the early implementation of the Stupp protocol in patients with high-grade glioma was associated with a notable reduction in survival rates (19). In a separate study, a minimum interval of six weeks between surgery and CRT was associated with superior OS and PFS in patients with GBM (20). Some studies have reached the conclusion that the timing of the initiation of adjuvant treatment has no prognostic significance

Table 3: Univariate and multivariate analysis of prognostic factors for progression free survival

Prognostic factor	Patients /recurrence	Median PFS (months)	Univariate analysis p	HR (95% CI)	Multivariate analysis p	HR (95% CI)
ECOG PS						
0-1 ≥2	40/15 27/7	4.0 12.7	0.04*	0.33 (0.11-0.97)	0.07	0.31 (0.08-1.09)
Age						
<60 ≥60	32/12 35/10	3.8 6.6	0.78	0.88 (0.37-2.09)	0.99	0.99 (0.22-4.31)
Sex						
Male Female	31/11 36/11	4.4 7.0	0.15	1.97 (0.78-4.98)	0.74	1.20 (0.38-3.78)
Comorbidty						
Yes No	38/12 29/10	6.6 3.8	0.28	1.6 (0.66-3.91)	0.86	0.90 (0.30-2.73)
Operation						
Total resection Subtotal resection	41/16 21/5	4.2 13.2	0.01*	0.14 (0.03-0.64)	0.03*	0.14 (0.02-0.84)
RT waiting time						
<4 weeks 4-6 week ≥6 weeks	24/9 21/8 13/5	7.0 5.1 3.6	0.14	1.58 (0.85-2.92)	0.78	1.12 (0.46-2.72)

*Statistically significant
PFS: Progression-free survival, OS: Overall survival, CI: Confidence interval, HR: Hazard ratio, ECOG PS: Eastern Cooperative Oncology Group Performance Status, RT: Radiotherapy

Table 4: Univariate and multivariate analysis of prognostic factors for overall survival						
Prognostic factor	**Patients /exitus**	**Median OS (months)**	**Univariate analysis p**	**HR (95%CI)**	**Multivariate analysis p**	**HR (95%CI)**
ECOG PS						
0-1 ≥2	40/15 27/7	15.0 15.7	0.001*	5.05 (1.83-13.94)	0.17	2.41 (0.67-8.56)
Age						
<60 ≥60	32/12 35/10	15.0 19.3	0.27	1.65 (0.67-4.06)	0.51	1.56 (0.40-6.14)
Sex						
Male Female	31/11 36/11	15.1 15.0	0.03*	3.09 (0.11-8.58)	0.15	3.07 (0.65-14.50)
Comorbidty						
Yes No	38/12 29/10	17.7 15.0	0.53	0.75 (0.30-1.84)	0.17	2.63 (0.64-10.76)
Operation						
Total resection Subtotal resection	41/11 21/9	19.3 9.8	0.08	2.25 (0.90-5.62)	0.26	2.46 (0.50-12.09)
RT waiting time						
<4 weeks 4-6 week ≥6 weeks	24/5 21/3 13/5	31.1 15.7 11.3	0.03*	2.5 (1.04-5.99)	0.01*	3.18 (1.23-8.18)

*Statistically significant
OS: Overall survival, HR: Hazard ratio, CI: Confidence interval, ECOG PS: Eastern Cooperative Oncology Group Performance Status, RT: Radiotherapy

(21,22). The findings of our study indicate that the initiation of treatment within the first four weeks had a significant positive effect on OS.

While there is a paucity of evidence regarding the optimal timing of treatment initiation, existing studies have yielded conflicting results. Notably, only a handful of investigations have focused on O^6-methylguanine (O^6-MeG)-DNA methyltransferase (MGMT) promoter methylation, which is regarded as a potential predictor of TMZ efficacy. In a study comprising a limited number of patients, univariate regression analysis demonstrated that although MGMT methylation exhibited a borderline significant correlation with OS across the entire population (p=0.048), the initiation of RT within 24 days had a detrimental impact (23). In a separate investigation, MGMT was accessible in approximately half of the patients, and the period of adjuvant therapy exceeding six weeks was linked to diminished survival (24). It was not possible to analyse MGMT in the context of this study. Nevertheless, in the entire cohort of patients, the commencement of treatment after six weeks, irrespective of MGMT methylation status, was associated with a poorer prognosis.

In a 2016 systematic review and meta-analysis of 19 retrospective studies examining the relationship between RT treatment delay and OS in GBM patients, no statistically significant association was identified (HR: 0.98; 95% CI: 0.90-1.08; p=0.70) (25). It is important to note that the study also examined the current standard pretreatment time. The findings of our study indicate that early treatment initiation is a statistically significant predictor of OS, as demonstrated by both univariate and multivariate analyses.

Study Limitations

The study is limited by the absence of investigation into the role of promoter methylation in the pathology slides, the relatively brief follow-up period, and the lack of detail regarding the treatment options employed in the event of recurrence. Furthermore, the lack of information regarding the rationale for the prolonged adjuvant treatment process (e.g., infection or post-operative complications) represents a significant limitation of the study. Nevertheless, it is evident that this study, conducted in a recently established center and clinic, is of significant value and will inform future prospective studies.

Conclusion

A review of the literature and existing guidelines reveals a lack of consensus regarding the optimal timing for initiating adjuvant treatment in patients with GBM. The present study offers significant insights into the subject matter, given the characteristics of the patient population and the results obtained. Further prospective, multicenter studies with larger patient populations are required.

Ethics

Ethics Committee Approval: This study was approved by the Scientific Research Evaluation and Ethics Committee of Etlik City Hospital (decision no.: AEŞH-BADEK-2024-758, date: 02.10.2024).

Informed Consent: Consent was not obtained since it was a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.E.K., İ.D., G.Y., Concept: E.E.K., G.Y., Design: E.E.K., Data Collection and/or Processing: E.E.K., İ.D., G.Y., Analysis and/or Interpretation: E.E.K., İ.D., Literature Search: E.E.K., Writing: E.E.K., İ.D.

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

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

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BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

The Anti-Proliferative Effect of Genistein on Antioxidant System, Cell Proliferation, and Apoptosis: Implications for Hormone-Refractory Prostate Cancer Therapy

Genisteinin Antioksidan Sistem, Hücre Çoğalması ve Apoptoz Üzerindeki Anti-Proliferatif Etkisi: Hormon Refrakter Prostat Kanser Tedavisinde Uygulamalar

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Abstract

Objectives: This study aims to examine the effect of different genistein concentrations on apoptosis, estrogen receptor beta, and manganese superoxide dismutase (MnSOD) protein expressions in PC3 hormone-resistant metastatic prostate cancer cell lines.

Materials and Methods: Genistein concentrations of 0, 0.01, 0.1, 0.5, 1, 5, 10, and and 50 µM were applied to PC3 hormone-resistant metastatic prostate cancer cell lines for 48 hours and water soluble tetrazolium salt-1 cell proliferation assay was performed. Protein was extracted from the cells and cleaved poly (ADP-ribose) polymerase, estrogen receptor beta (ERβ), and MnSOD protein expression levels were examined using the Western blot protocol. One-way analysis of variance (ANOVA) and Student's t-test statistical analysis methods were applied to determine whether there are statistically significant differences.

Results: Genistein was found to change ERβ expression in PC3 cells depending on concentration. While ERβ protein expression increases observed at low concentrations (0.01-0.5 µM), it sometimes decreased to baseline or increased modestly at high concentrations (1-50 µM). MnSOD protein expression showed a stimulating effect on the protein expression level at high concentrations (1-50 µM).

Conclusion: This research study investigates how different concentrations of genistein affect cell proliferation, apoptosis, estrogen receptor beta, and MnSOD protein expression levels in PC3 hormone-refractory prostate cancer cells.

Keywords: Prostate cancer, genistein, apoptosis, ERβ, MnSOD

Öz

Amaç: Bu çalışmanın amacı hormona dirençli olan PC3 metastatik prostat kanser hücre serilerinde farklı genistein konsantrasyonlarının apoptoz, östrojen reseptör beta ve manganez süperoksit dismutaz (MnSOD) protein ekspresyonlarındaki etkisini incelemektir.

Gereç ve Yöntem: PC3 hormona dirençli olan metastatik prostat kanser hücre serilerine 0, 0,01, 0,1, 0,5, 1, 5, 10, 50 µM genistein konsantrasyonları 48 saat uygulanmıştır ve suda çözünebilen tetrazolyum tuzu-1 hücre proliferasyon yöntemi uygulanmıştır. Hücrelerden protein ekstrekte edilip, Western blot yöntemi kullanılarak cleaved poli (ADP-riboz) polimeraz, östrojen reseptörü beta (ERβ) ve MnSOD protein ekspresyon seviyeleri incelenmiştir. Tek yönlü varyans analizi (ANOVA) ve Student's t-test istatistiksel analiz metodları uygulanmıştır.

Bulgular: Genistein PC3 hücrelerinde konsantrasyona bağlı olarak ERβ ekspresyonunu değiştirdiği saptanmıştır. Düşük konsantrasyonlarda (0,01-0,5 µM) ERβ protein ekspresyonu artarken, yüksek konsantrasyonlarda (1-50 µM) bazen temel seviyeye düşerek ya da ılımlı artarak farklılık göstermiştir. MnSOD protein ekspresyonu ise yüksek konsantrasyonlarda (1-50 µM) protein ekspresyon seviyesi üzerinde uyarıcı etki göstermiştir.

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Sonuç: Bu araştırma çalışmasında, PC3 hormonuna dirençli prostat kanseri hücrelerinde farklı genistein konsantrasyonlarının hücre çoğalmasını, apoptozu, östrojen beta reseptörünü ve MnSOD protein ekspresyon düzeylerini nasıl etkilediği araştırılmıştır.

Anahtar Kelimeler: Prostat kanseri, genistein, apoptoz, ERβ, MnSOD

Introduction

Prostate cancer, being one of the most prevalent cancers in men worldwide, is a significant public health challenge (1). The mainstay options in the treatment of prostate cancer include traditional methods such as surgical removal, hormone ablation therapy, and radiotherapy. However, these treatments come with limitations, especially in treating androgen-independent prostate cancers that exhibit chemoresistance and radioresistance (2,3).

Recent interest has surged around genistein, a natural compound isoflavone found in soy products, due to its potential therapeutic benefits in cancer prevention and treatment. The complex methods by which genistein inhibits cell proliferation in prostate cancer involve modifying several signaling pathways that regulate cell growth and division (4).

Specifically, genistein can alter the expression of proteins involved in cell cycle regulation, halting cancer cells' uncontrolled growth (5,6). Additionally, genistein induces apoptosis, which helps eliminate damaged or malignant cells, consequently suppressing tumor growth and progression (7).

The development of prostate cancer is significantly influenced by estrogen receptor signaling, with both estrogen receptors alpha (ERα) and beta (ERβ) being implicated in the pathogenesis of the disease. The expression of these receptors in prostate cancer tissues contributes to the activation of estrogen-signaling pathways that promote cancer cell proliferation and survival (8). Genistein interacts with ERβ, mimicking estrogen's effects, which include regulating cellular growth, differentiation, and apoptosis in prostate cancer cells (9). The abnormal activation of estrogen signaling can lead to dysregulated gene expression patterns that drive tumor progression. Hence, targeting estrogen receptor signaling pathways offers promising avenues for the development of novel therapeutic strategies to be used in the treatment of prostate cancer (4).

Manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme, regulates reactive oxygen species (ROS) levels in cells by detoxifying superoxide radicals generated during cellular metabolism (10). Dysregulation of MnSOD expression in prostate cancer can lead to an imbalance in ROS homeostasis, which in turn may cause tumorigenesis (11). Some studies suggest MnSOD is one of the mediators of the antioxidant benefits of genistein on prostate cancer (12). This research study investigates how genistein affects cell proliferation, apoptosis, estrogen receptor beta, and MnSOD protein expression levels in PC3 hormone-refractory prostate cancer cells.

Materials and Methods

The cells used in the study are commercially available, so ethics committee approval is not required. The study protocol was appropriate for the Declaration of Helsinki.

Cell Culture

RPMI 1640 (Gibco, Carlsbad, CA, US) supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, US) and 1% penicillin/streptomycin (Gibco, Carlsbad, CA, US) were utilized for growing human hormone refractory-PC3 prostate cancer cells. These cells do not rely on androgen signaling for growth and survival, making them a valuable model for studying castration-resistant prostate cancer (CRPC)-an advanced stage of prostate cancer that progresses despite androgen deprivation therapy. The cells were maintained in a humidified incubator with 5% CO_2 at 37 °C. The vehicle was used as the control and around 60-70% of confluent cells were treated with varying dosages of genistein (Sigma, St. Louis, MO, US) dissolved in dimethyl sulfoxide.

Cell Proliferation Assay

Utilizing the water soluble tetrazolium salt-1 (WST-1) calorimetric cell proliferation assay kit, the impact of genistein treatment on PC-3 cell growth was examined. Cells were seeded in a 96-well plate at 1×l04 cells/well density and overnight incubated in 100 µL culture media. Afterward, the cells were treated with different concentrations of genistein (0.01, 0.1, 0.5, 1, 5, 10, and 50 µM). These concentrations were selected based on literature, our previous trial experiments. Following 48 hour, each well was treated with 10 µl of WST-1 reagent at 37 °C for 4 h. The micro-plate reader (Thermo Scientific, Multiscan GO) was used to measure absorbances at 450 nm oxide dismutase. The experiment was conducted three times.

Western Blotting

The analysis of protein expression was evaluated by Western blotting. Seeded 1×10^6 cells were subsequently treated for 48 h with 0.01, 0.1, 0.5, 1, 5, 10 and 50 µM of genistein. Following harvesting, treated and vehicle cells were lysed in 1X cell lysis buffer (Cell Signaling Technology, USA) containing diluted 1 mM PMSF (Sigma, Germany) in distilled water.

Bicinchoninic acid assay kit (Thermo Fisher Scientific, England) was utilized to measure protein concentrations in

MultiSkan GO Microplate Spectrophotometer (Thermo Fisher Scientific, England). Proteins (20 µg) were separated by sodium dodecyl sulfate-polyacrylamide gels before being transferred to a polyvinylidene fluoride membrane. Then, the blots were incubated with the primary antibodies against; Cleaved poly ADP-ribose polymerase (PARP) (Cell Signaling Technology, USA), ERβ (Invitrogen, Paisley, UK), MnSOD (Abcam, Cambridge, US) and β-actin (Cell Signaling Technology, USA). Following secondary antibody incubations, the blots were subjected to a chemiluminescence solution to visualize the specific binding. The protein bands were densitometrically measured by ImageJ (NIH, Bethesda, USA). The data was normalized by comparing the results with the expression of β-actin in every concentration group.

Statistical Analysis

Experiment findings are displayed as a mean ± standard error of the mean. Student's t-test and one-way ANOVA were applied to evaluate the group differences.

The group differences were analyzed by using one-way ANOVA and Student's t-test. The software system GraphPad Prism 7® (La Jolla, CA, USA) was used to assess all the results. A p-value ≤0.05 or p-value ≤0.0001 was accepted as statistically significant.

Results

Effects of Genistein on Cell Proliferation

Incubation of PC3 cells with 0.01 µM and 0.1 µM genistein treatments resulted in the absorbance values slightly lower than the vehicle control. However, regarding the effect of genistein on cell proliferation, incubation with 0.1 µM genistein caused a significant increase, suggesting a stimulatory effect on cell proliferation. This might be due to a biphasic dose-response relationship, where low doses of certain compounds can stimulate cell growth while higher doses inhibit it. At 0.5 µM concentration, there is a notable increase in cell proliferation compared to vehicle control, indicating a potential proliferative effect of genistein at this concentration. At 1 µM and 5 µM concentrations, the cell proliferation levels are similar to the vehicle control (Figure 1). This suggests that these concentrations do not significantly affect cell proliferation in PC3 cells, indicating a possible threshold effect where genistein neither stimulates nor inhibits cell growth significantly within this range. At higher concentrations (10 µM and 50 µM), there is a noticeable decrease in cell proliferation compared to vehicle control. This aligns with the known anti-proliferative effects of genistein at higher concentrations, likely due to its ability to induce apoptosis and inhibit cell cycle progression in prostate cancer cells.

Cleaved PARP Protein Expression

There was a moderate cleaved PARP protein expression level at vehicle control genistein concentrations with some variability in PC3 cells. At 0.01 µM genistein concentration, there was a slight increase in cleaved PARP protein levels compared to the control, indicating some apoptotic activity. At 0.1 µM genistein concentration, the levels decreased, suggesting a reduction in apoptosis. There was a significant increase in cleaved PARP protein levels at 0.5 µM genistein concentration, indicating a strong apoptotic response. At 1 µM genistein concentration, there was a decrease in cleaved PARP, indicating reduced apoptosis. At 5 µM, levels were like the control genistein concentrations while at 10 µM level, there was an increase, and at 50 µM, the levels were back to low again (Figure 2).

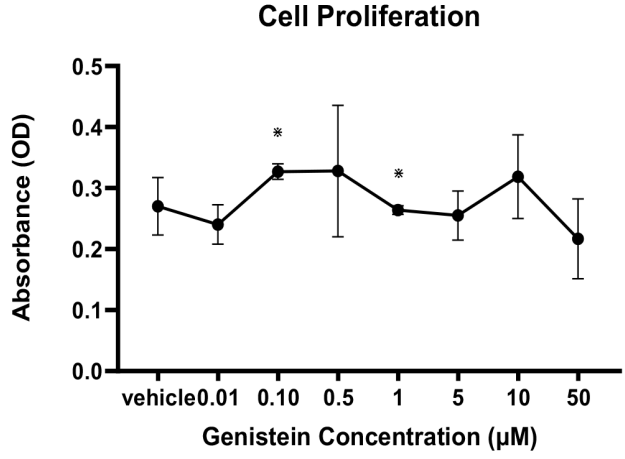

Figure 1: Cell proliferation was determined by the WST-1 assay. Triplicate wells containing 1×10^4 cells/well of PC-3 were exposed to 0.01, 0.1, 0.5,1, 5, 10 and 50 µM concentrations of genistein. Growth curves were obtained at 48 h. Error bars represent the SEM values, p<0.05

*Represents statistically significant, OD: Optical density, SEM: Standard error of the mean

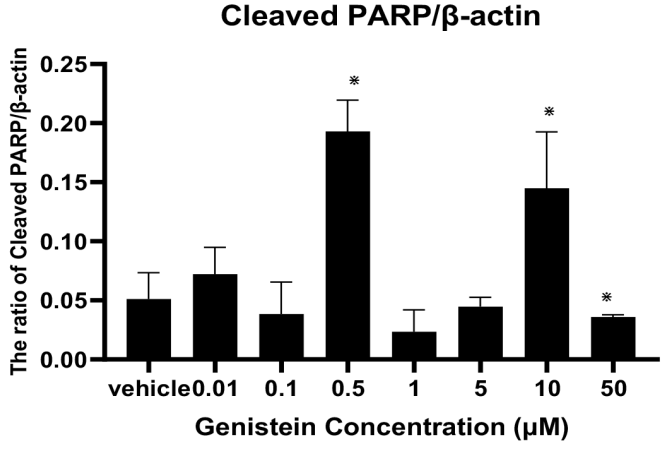

Figure 2: The cleaved PARP protein expression level was determined by Western blot. Error bars represent the SD values, p<0.0001

*Represents statistically significant, PARP: Poly ADP-ribose polymerase, SD: Standard deviation

ERβ Protein Expression

The baseline expression level of ERβ in untreated PC3 cells was approximately 0.6. At 0.01 μM genistein concentration, the expression level increased to approximately 1.2, indicating a significant upregulation of ERβ at this low concentration. At 0.1 μM genistein concentration, the expression level peaked at around 1.4, suggesting that this concentration induced the highest ERβ expression among the tested doses. At 0.5 μM genistein concentration, the expression level remained elevated at approximately 1.2, similar to the 0.01 μM treatment. At 1 μM genistein, there was a noticeable decrease in ERβ expression returning to baseline levels. At 5 μM genistein concentration, the expression level remained low at around 0.6, consistent with the vehicle control and 1 μM treatment. At 10 μM genistein concentration, the expression level increased slightly to around 0.8, indicating a modest upregulation compared to the control. At 50 μM, the highest genistein concentration, the expression level rose to approximately 1.0, showing a moderate increase in ERβ expression (Figure 3).

MnSOD Protein Expression

At 0.01 μM concentration of genistein, the MnSOD/β-actin ratio is 0.326, indicating a baseline or slightly reduced activity of MnSOD relative to β-actin protein. The MnSOD/β-actin ratio increased to 0.469 at 0.1 μM concentration, suggesting a potential stimulatory effect on MnSOD protein expression level compared to the lower concentration. At 0.5 μM concentration, the MnSOD/β-actin ratio decreased slightly to 0.424, indicating a possible reduction in protein expression of MnSOD relative to β-actin. The MnSOD/β-actin ratio increased notably to 0.519 at 1 μM concentration, suggesting a potential stimulatory effect on MnSOD protein expression level compared to lower concentrations. At 5 μM, the MnSOD/β-actin ratio continued to increase to 0.553, indicating a further potential increase. The MnSOD/β-actin ratio increased to 0.645 at 10 μM concentration, indicating a continued stimulatory effect on MnSOD protein expression level. At 50 μM concentration, the MnSOD/β-actin ratio increased further to 0.703, suggesting a potentially enhanced stimulatory effect on MnSOD activity compared to lower concentrations (Figure 4).

Discussion

There are conflicting results about the potential protective versus adverse effects of soy and genistein in breast and prostate cancer animal model studies (13-15). In our previous study, it was exhibited that genistein exerts its biphasic beneficial and harmful effects through various molecular mechanisms and pathways (16). The effects of physiological (0.01, 0.1, 0.5, 5, and 10 μM) and pharmacological (50 μM) concentrations of genistein on PC-3 cells regarding cell proliferation, apoptosis, estrogenic effects, and antioxidant defense interactions were examined in this current study. The WST-1 cell proliferation assay demonstrated a significant increase with 0.1 μM genistein treatment, indicating a stimulatory effect on cell proliferation at this low concentration. This is also demonstrated in a study by Li et al. (17) where genistein's biphasic effect on cell proliferation was exhibited, as low concentrations of the compound were shown to stimulate growth while higher concentrations inhibited it. The cell proliferation assay results showed that the cell growth levels were similar to the vehicle control at 1 μM and 5 μM genistein concentrations suggesting that these concentrations do not significantly affect cell proliferation in PC3 cells, therefore indicating a possible threshold effect where genistein neither stimulated nor inhibited cell growth significantly (18). Conversely, a noticeable decrease in cell proliferation relative

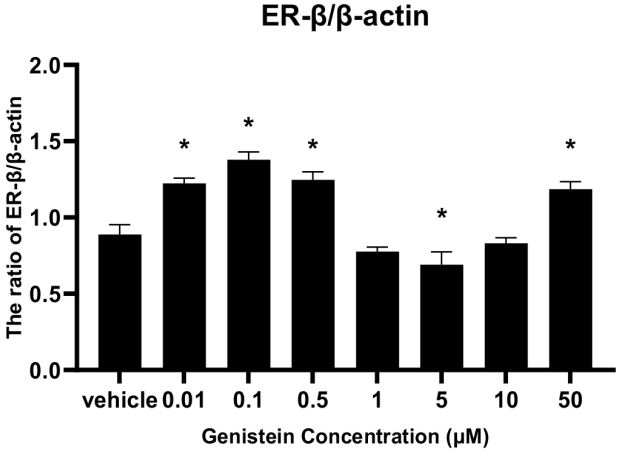

Figure 3: ERβ protein expression level was determined by Western blot. Error bars represent the SD values. p<0.0001

*: Represents statistically significant, ERβ: Estrogen receptor beta, SD: Standard deviation

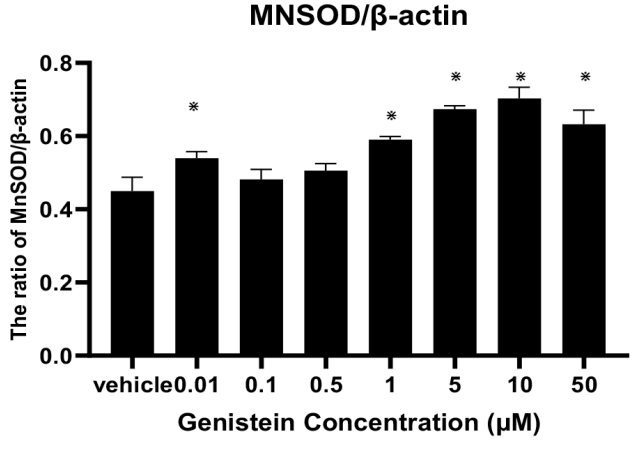

Figure 4: MnSOD protein expression level was determined by Western blot. Error bars represent the SD values. p<0.0001

*Represents statistically significant, MnSOD: Manganese superoxide dismutase, SD: Standard deviation

to the vehicle control was observed at higher concentrations (10 µM and 50 µM). This finding aligns with the known anti-proliferative effects of genistein at higher concentrations, likely owing to the compound's ability to induce apoptosis and inhibit cell cycle progression in prostate cancer cells (19). In addition to this study, the induction of apoptosis has also been shown by various other studies as one of the most reported biological activities of genistein (16,17,20). It was observed that genistein had a notable impact on apoptosis in PC3 cells through the examination of cleaved PARP protein, which is an early indicator of apoptosis. The data suggests a dose-dependent response of cleaved PARP protein levels to genistein treatments, with certain concentrations (e.g., 0.5 µM and 10 µM) showing significant increases in apoptosis markers, while others (e.g., 1 µM and 50 µM) exhibited lower levels. In terms of therapeutic potential, genistein's activation of ERβ offers a promising strategy. The ability of genistein to enhance ERβ activity suggests it could be beneficial in managing prostate cancer via the promotion of tumor suppression and reduction of metastasis. Studies have shown that loss of ERβ expression is associated with higher-grade, more aggressive prostate cancers, making the activation of this receptor a potential therapeutic target. ERβ is also known to modulate oxidative stress, a key factor affecting prostate cancer progression. Higher levels of ERβ are associated with increased expression of antioxidant enzymes, reducing oxidative stress and potentially slowing cancer progression (4,21). This study suggests that genistein modulated ERβ expression in a concentration-dependent manner in PC3 prostate cancer cells. Low concentrations (0.01-0.5 µM) significantly upregulated ERβ, while higher concentrations (1-50 µM) showed a varied response, with some concentrations returning to baseline levels or showing moderate increases indicating ERβ has anti-metastatic properties (20). By upregulating ERβ, genistein may inhibit key pathways involved in cancer cell migration and invasion, both of which are critical in the metastatic spread of prostate cancer (22). The observed upregulation of ERβ at low concentrations of genistein implies that genistein could help re-sensitize prostate cancer cells to hormonal therapies or delay the progression to hormone-refractory status by maintaining or enhancing ERβ expression and improving survival rates in return (23). A study regarding the prognostic significance of ERβ expression in prostate cancer indicated that patients with ERα-negative/ERβ-positive prostate cancer had a 5-year biochemical recurrence-free survival rate of 85.7 whose PCa tissue has ERα (-)/ERβ (+) staining results, indicating a lower risk of recurrence in this group (24). In metastatic prostate cancer, combining genistein with conventional treatments (e.g., chemotherapy, radiation) could enhance overall treatment efficiency (25). For example, the combination of genistein with AR-targeted therapies might improve outcomes in patients with CRPC by leveraging the anti-proliferative effects mediated by ERβ (4,21,23,26). Studies suggest that genistein can modulate oxidative stress pathways by improving antioxidant defenses, including upregulation of enzymes like MnSOD, to mitigate cell oxidative damage (27). Genistein's ability to enhance MnSOD activity may involve its antioxidant properties and regulation of mitochondrial function, which are crucial in maintaining cellular redox balance and protecting against oxidative stress-induced damage (28). According to the MnSOD protein expression levels, genistein concentrations have a dose-dependent effect on MnSOD/β-actin ratios in PC3 prostate cancer cell lines. Lower concentrations (0.01 to 0.5 µM) generally demonstrated variable effects on MnSOD protein expression level, with potential stimulatory or neutral effects. Higher concentrations (1 to 50 µM) consistently demonstrated an increase in MnSOD/β-actin ratio, indicating a stimulatory effect on MnSOD protein expression. Variances in measurements decreased with higher concentrations, suggesting more consistent effects at these levels. This study is in line with the results of the study conducted by Van der Eecken et al. (29) where lower concentrations of genistein had variable effects, while higher concentrations consistently increased MnSOD expression, representative of the stimulatory effect on MnSOD activity in prostate cancer cells. The modulation of MnSOD could reduce oxidative stress by enhancing cellular response, which contributes to the therapeutic potential of genistein in prostate cancer.

Studies have shown that there is a complex interaction between genistein, estrogen receptor signaling, and MnSOD in prostate cancer highlighting possibilities for the development of novel therapies.

Understanding the mechanisms through which genistein exerts its effects on prostate cancer cells could pave the way for the development of more effective treatments. Furthermore, accounting for how MnSOD and estrogen receptors contribute to the development of prostate cancer stresses the significance of targeting several pathways and variables in cancer treatments. Clarifying the interactions between genistein, MnSOD, and estrogen receptors may make it possible to create innovative treatments for prostate cancer. Furthermore, the combination of genistein with other treatments may be particularly beneficial in treating CRPC, a disease for which standard therapeutic strategies frequently prove ineffective. Moreover, genistein in combination with other therapies may be especially helpful in treating CRPC, a condition for which conventional treatment approaches are known to be ineffective.

Conclusion

In conclusion, this study highlights the biphasic effects of genistein on prostate cancer cells, demonstrating concentration-dependent modulation of proliferation, apoptosis, estrogen receptor signaling, and oxidative stress response. Findings in

this research article underscore genistein's complex molecular interactions and potential therapeutic relevance in prostate cancer, particularly in combination therapies for castration-resistant cases, warranting further investigation into its optimized clinical application.

Ethics

Ethics Committee Approval: The cells used in the study are commercially available, so ethics committee approval is not required.

Footnotes

Financial Disclosure: The author declared that this study has received no financial support.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Family-Centeredness of Special Education and Rehabilitation Services for Young Children with Special Needs in Türkiye

Türkiye'de Özel Gereksinimleri Olan Çocuklara Erken Çocukluk Döneminde Verilen Özel Eğitim ve Rehabilitasyon Hizmetlerinin Aile Merkezliliğinin Değerlendirilmesi

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Abstract

Objectives: This study aims to evaluate the family-centeredness of early childhood special education and rehabilitation services and assess families' satisfaction with these services.

Materials and Methods: This cross-sectional study included 3-36 months-old children with special needs and their families who have been followed up at Ankara University Faculty of Medicine, Department of Pediatrics, Division of Developmental Pediatrics between 2014-2017 and receiving early intervention services for at least 3 months. The "Early Childhood Development Support Services Evaluation Scale" assessed the family-centeredness of these services.

Results: A hundred and one children were included in the study. The median age of the children was 33 months, and 51.5% were male. Neurological disorders were present in 45.5%, and genetic disorders in 35.6%. Developmental evaluations showed 68.3% of children had special needs in expressive language, 64.4% in gross motor skills, and 62.4% in receptive language. At least half of the families rated the centers as "inadequate" on at least half of the scale. However, despite this, more than half of the families reported high satisfaction in terms of "completely finding what they were looking for", "choosing the institution again", and "recommending it to others.

Conclusion: This study showed that, despite finding the early intervention services for young children with special needs in Türkiye to be inadequate regarding family-centeredness, the families remain generally satisfied. This study is significant in guiding efforts to develop family-centered early intervention services and enhancing families' awareness and demands in this area, particularly in Türkiye and other similar low- and middle-income countries.

Keywords: Family-centered care, early intervention services, children, special needs, disability

Öz

Amaç: Aile merkezli yaklaşım, erken çocukluk döneminde özel gereksinimleri olan çocukların tedavisi niteliğinde olan özel eğitim ve rehabilitasyon hizmetlerinin temel taşıdır. Bu araştırmanın amacı, erken çocukluk döneminde özel gereksinimleri olan çocukların almakta olduğu özel eğitim ve rehabilitasyon hizmetlerinin aile merkezliliğinin ve ailenin kurum hakkındaki memnuniyetlerinin değerlendirilmesidir.

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Bulgular: Araştırmanın örneklemini oluşturan 101 çocuğun %51,5'i erkek olup ortanca yaşı 33 ay (25,5-43,0) saptanmıştır. Örneklemdeki çocukların %45,5'inde nörolojik, %35,6'sında genetik hastalığı mevcuttur. Ayrıntılı gelişimsel değerlendirmede çocukların %68,3'ünde anlatım dili alanında, %64,4 kaba hareket alanında, %62,4'ünde alıcı dil alanında özel gereksinimi saptanmıştır. Ailelerin en az yarısı, ölçeğin en az yarısında kurumları "yetersiz" olarak değerlendirmiştir. Ancak, buna rağmen ailelerin yarısından fazlası "aradıklarını tamamen bulma", "kurumu tekrar seçme" ve "başkalarına önerme" konularında yüksek memnuniyet bildirmiştir.

Sonuç: Bulgularımız, çocuklarının hizmet aldıkları kurumları aile merkezli hizmet açısından yetersiz olarak bildirmelerine karşılık ailelerin çoğunun kurumlarından memnun olduklarını göstermektedir. Bu araştırma, aile merkezli erken destek hizmetlerinin geliştirilmesi yanında, ailelerin bu konuda taleplerinin olgunlaştırılması konusunda Türkiye'de ve benzeri düşük-orta gelirli ülkelerde yapılacak çalışmalara ışık tutması açısından önem taşımaktadır.

Anahtar Kelimeler: Aile merkezli hizmet, erken destek hizmetleri, çocuk, özel gereksinim, engellilik

Introduction

The family-centered approach is recognized as a cornerstone in early support, rehabilitation, health, and educational services for children at high risk due to chronic physical, developmental, behavioral, or emotional conditions or those with special needs (1). This approach, widely applied in services for children with special needs over the past thirty years, has been extensively researched in high-income countries (2-4). However, it has yet to be well-known how family-centered these early support services are for young children with special needs in low- and middle-income countries.

Children with special needs are defined as those who have chronic physical illnesses, developmental, behavioral, or emotional difficulties or risks and who require more than their peers. Children with special needs include those with chronic physical illnesses, such as cerebral palsy, epilepsy, or congenital heart disease, which require ongoing medical care and rehabilitation. They may also experience developmental difficulties, including delays in speech, motor skills, or cognitive abilities. Additionally, behavioral challenges, such as difficulty following routines, regulating emotions, or interacting with peers, as well as emotional difficulties, such as trouble adapting to changes in their environment, may also be present (5). According to data from the Centers for Disease Control and Prevention (CDC), a study conducted between 2014 and 2018 found that 17% of children aged 3-17 had special needs (6). According to the 2020 Health Survey data from the Turkish Statistical Institute, the prevalence of children aged 2-14 with special needs in vision, hearing, learning, or motor skills in Türkiye was 8.2% (7). The fundamental approach for children with special needs is early intervention programs. Key components of early support programs include preventing developmental difficulties when possible, integrating prevention with early intervention, reducing risk factors, and integrating early supports with the family.

A review of 55 articles from ten high-income countries found that most families of children with special needs reported that their health and rehabilitation services were planned in collaboration with families and essentially exhibited a family-centered feature (4). Other studies conducted in high-income countries have reported that adherence to family-centered service approaches increased, improved quality of life, and positively affected parents' self-efficacy, motivation, and functionality (2,3).

Family-centered studies in low- and middle-income countries typically evaluate neonatal and pediatric intensive care units or children's hospitals. These studies have shown that health services do not adequately meet family-centered characteristics (8-11). A recent study in Türkiye assessed the family-centeredness of special education and rehabilitation services for children diagnosed with Down syndrome during early childhood. The study found high scores in "respectful and supportive care" and low scores in "provision of specific information", emphasizing the urgent need to address service deficiencies, especially among mothers with lower educational levels (12). In Türkiye, thesis research conducted in 2005 at (Ankara University Faculty of Medicine, Department of Pediatrics, Division of Developmental Pediatrics) investigated the suitability of family-centered early support services in early intervention services where children receiving special education or rehabilitation services attended. The study involved home visits with the families of 54 children aged between 10 months and ten years. According to this study, families reported inadequacies, particularly in "guidance on how to raise the child", "considering the family's opinions on what would benefit the child while preparing the educational program", and "asking the family about their feelings" (13). The extent to which the services provided to children with developmental issues are family-centered remains to be discovered, and there is no recent study to clarify whether progress has been made in this area in the last decade.

Among the early intervention programs in Türkiye, the Mother Child Education Program *[Anne Çocuk Eğitim Programı* (AÇEP)], the Portage Project, and the Small Steps Early Education Project stand out. AÇEP, although a program focused on empowering families, does not adequately cover the needs of children under the age of three or those with developmental

delays (14). The Portage Project, recognized for its applicability in home settings, has shown that families struggle to sustain the recommended practices without guidance The Small Steps Project has been evaluated as an effective program for children with developmental risks; however, it does not fully meet family-centered criteria. There is a significant need for new interventions in Türkiye to address the needs of children with developmental delays and studies to bring these issues to the forefront (15-18).

This study aims to evaluate the family-centeredness of special education and rehabilitation services received by children with special needs during early childhood and assess the family's satisfaction with the early intervention service. This study will contribute to the perspective of low- and middle-income countries, which are underrepresented in the literature, through the context of Türkiye and the population it represent.

Materials and Methods

This cross-sectional, observational study included children who admitted to Ankara University Faculty of Medicine, Department of Pediatrics, Division of Developmental Pediatrics between October 2014 and September 2017. The sample consisted of children aged 0-36 months diagnosed with special needs in at least one area and referred for special education and/or physiotherapy and rehabilitation services. The children and their families who consented to participate in the study received at least one of these services for at least three months.

Families who agreed to participate in the study underwent comprehensive, family-centered developmental assessments. This evaluation lasted approximately 1.5 hours and included a detailed history, physical examination, developmental assessment using the standard "The Expanded *Guide for Monitoring Child Development"* (19), and observational methods. The "Early Childhood Development Support Services Evaluation" Scale was used to assess the family-centeredness of the services (13). All other evaluations were conducted within routine clinical services.

Tools

The Expanded Guide for Monitoring Child Development

The Guide for Monitoring Child Development (GMCD), developed by Ertem et al. (20) and Ertem (21), addresses the lack of a standardized child development assessment tool suitable for health services (20). International validation studies, supported by the NIH, were conducted between 2010 and 2015 in Argentina, South Africa, India, and Türkiye with 12,000 children (21). The GMCD, the only internationally standardized tool of its kind, has trained experts in over 30 countries. Its extended version, the Expanded GMCD (E-GMCD), incorporates the World Health Organization (WHO), International Classification of Functioning, Disability and Health (ICF) framework and was developed in 2010 at Ankara University Faculty of Medicine, Department of Pediatrics, Division of Developmental Pediatrics. E-GMCD uses open-ended, parent-answered questions to provide detailed information on the child, family, and environment, enabling pediatricians to assess 95% of ICF domains (19). In our study, E-GMCD was used for comprehensive developmental assessment and to collect environmental data.

The ICF framework, developed by the WHO, provides a standardized approach to understanding and documenting health and disability. It emphasizes the interplay between an individual's physical and mental functions, activities, participation in daily life, and environmental and personal factors, offering a holistic perspective on health and well-being.

Early Childhood Development Support Services Evaluation Scale

In Türkiye, due to the lack of tools to evaluate the quality and quantity of services provided to children aged 0-3 with developmental issues, the "Early Childhood Development Support Services Evaluation Scale" was developed in 2005 at Ankara University Faculty of Medicine, Department of Pediatrics, Division of Developmental Pediatrics. During the development of the scale, the National Early Intervention Longitudinal Study (NEILS) (22), The Children with Special Health Care Needs (CSHCN) Screener (23), Measurement of Client Satisfaction (24), Consumer Evaluation of Child Health Services in the Non-government Sector in Hong Kong (25), and early support services evaluation scales used by Yale University (22-25) were utilized. In addition to questions derived from the global literature, open-ended questions were added by the researchers to align with our country's socio-cultural and educational system. After the draft form of the scale was created, feedback was collected from experienced experts working in the field nationally and internationally regarding how well the scale's questions and areas assessed family-centeredness. After two years of work, expert opinions, group meetings, and feedback from pilot studies led to a final version of the scale. The scale consists of 22 items under the subheadings of "family information", "sharing and supporting family feelings and thoughts", and "assessment and support of the family's socio-economic situation."

The parameters emphasized for determining satisfaction include communication and information sharing between the service provider and family, emotional support for the family, and addressing socio-economic challenges that may impact access to services.

The scales and data were completed step by step by the child's guardian under the supervision of the researchers to ensure no details were missed. All open-ended questions were

thoroughly discussed, and responses were recorded based on a mutual agreement to achieve the most accurate results. For multiple-choice questions, the most appropriate option was selected.

General Satisfaction Questionnaire

To determine the general level of satisfaction with the early intervention services, questions such as "I found what I was looking for at the early intervention services", "If I had to choose again, I would select this early intervention services", "I would sincerely recommend it to other families with similar issues", and "If I had the opportunity to go to another early intervention services, I would immediately switch" were evaluated with "yes" or "no" responses.

Statistical Analysis

Descriptive statistics were given with percentages for categorical variables and mean ± standard or median (minimum-maximum) for numerical variables. The normality of numerical data was assessed using histograms, coefficient of variation, kurtosis-skewness, Detrended plot distribution, Kolmogorov-Smirnov, or Shapiro-Wilk tests. A chi-square test was used to evaluate the associations between categorical parameters. A p-value of less than 0.05 was considered statistically significant.

Statistical analyses were performed using the SPSS 20.0 software package. The research has received approval from the Ethics Committee of Ankara University Faculty of Medicine (decision number: I2-701-17, date: 24.07.2017). All participants have signed the informed consent form and approved the study.

Results

During this study, 2,158 children applied to (Ankara University Faculty of Medicine, Department of Pediatrics, Division of Developmental Pediatrics). Of these, 122 children met the sample entry criteria, and three were excluded. Of the 119 children constituting the sample, 101 (85%) were reached and included in the study. A total of 18 children were excluded from the study: six could not be reached by phone, seven had families who did not consent to participate, and five could not attend the hospital on the appointment date.

Among the children in the sample, 51.5% were male, and the median age was found to be 33 months (25-75 percentile: 25.5-43.0). More than half of the children (58.4%) have at least one sibling. Most mothers (68.3%) and fathers (76.2%) have a high school education or higher. Consanguinity between spouses was reported in 15.8% of the families. Other sociodemographic characteristics of the sample are detailed in Table 1.

Neurological disorders were present in 45.5% of the children, and genetic disorders were present in 35.6%. Detailed developmental assessments revealed special needs in 68.3% of the children in expressive language, 64.4% in gross motor skills,

Table 1: Sociodemographic characteristics of children and their families

Sociodemographic characteristics	n (%)
Child's gender	
Male	52 (51.5)
Female	49 (48.5)
Child's age (months)	
11-24	22 (21.8)
25-36	37 (36.6)
37-48	24 (23.8)
49-60	18 (17.8)
Gestational age	
Term birth	69 (68.3)
Preterm birth	32 (31.7)
Mother's education level	
Primary school graduate	12 (11.9)
Secondary school graduate	20 (19.8)
High school graduate	42 (41.6)
College graduate and above	27 (26.7)
Father's education level	
Primary school graduate	5 (5.0)
Secondary school graduate	19 (18.8)
High school	44 (43.6)
College graduate and above	33 (32.7)
Number of siblings	
0	42 (41.6)
1	33 (32.7)
2	19 (18.8)
3 or more	7 (6.9)
Consanguineous marriage	
No	84 (83.2)
Yes	16 (15.8)
Unknown	1 (1.0)
Condition of the residence	
Apartment	89 (88.1)
Rented	49 (48.5)
Homeowner	41 (40.6)
Living in a relative's home without paying rent	11 (10.9)
Shanty house	7 (6.9)
Detached house	5 (5.0)
Presence of computer at home	
Yes	41 (40.6)
No	42 (41.6)
Presence of internet at home	
Yes	35 (34.7)
No	51 (50.5)
Presence of car	
Yes	50 (49.5)
No	51 (50.5)
Monthly income (Turkish Lira)	
Minimum wage and below	51 (50.5)
Above minimum wage	50 (49.5)

and 62.4% in receptive language. When evaluating activities and participation in life as per the ICF framework in E-GMCD, it was found that more than two-thirds of the children did not participate in family visits, and more than three-quarters did not engage in activities such as visiting parks, nature, or playing with other children. Approximately one-third of the families reported that their environment is prejudiced (Table 2).

The most frequently received services from the early intervention services were individual special education (82.2%) and physiotherapy (67.3%). 101 children attended 57 different early intervention services, evaluated with this scale. Responses coded as "never/not at all" and "rarely/seldom" were considered indicative of "insufficient" service, and the deficiencies of the early intervention services in relevant areas are presented in Table 3.

In the area of "informing families", approximately half of the families reported the early intervention services as insufficient in providing "home services when the family cannot attend due to health issues", "organizing the educational program to assist with daily activities such as eating and dressing", and "guidance on how the family should raise their child."

In the area of "sharing and supporting the family's feelings and thoughts", about half of the families found the early intervention services inadequate in providing "support for parents to share tensions or issues with their spouses", "support for parents to share new changes or difficulties affecting their lives at home", and "support for parents to share feelings of sadness, exhaustion, or helplessness."

In the area of "evaluating and supporting the family's socioeconomic status", more than half of the families considered the early intervention services inadequate in "inquiring about the family's financial difficulties", "informing about other assistance organizations the family can benefit from", "gathering information about the family's work conditions", and "creating opportunities for the family to interact with other families to share information and feelings" (Table 3).

When overall satisfaction with the early intervention service was assessed, more than half of the families (63.4%) reported that they found what they were looking for, 78.2% indicated they would choose the early intervention service again if needed, and 81.2% stated they would sincerely recommend it to other families with similar issues. However, 28.7% of the families mentioned changing early intervention services immediately if given the opportunity.

Twenty-nine families (28.7%) had previously changed early intervention services. Among those who changed early intervention services, 68.9% did so due to dissatisfaction. Reasons for changing early intervention services included address changes (10.3%), changes in specialists/educators (6.8%), the required service not being provided at the early intervention service (6.8%), and additional charges during the educational process (6.8%).

The frequency of changing early intervention services was examined based on the mother's education level (below high school vs. high school or higher; n=8, 25% vs. n=21, 30.4%, respectively), and no significant difference was observed (p=0.574). Similarly, when evaluating income groups presented in Table 1, no difference was found regarding the frequency of changing early intervention services (p=0.791). The mother's and father's education levels and the family's income levels were compared across all parameters of the Early Childhood Development Support Services Evaluation Scale, and no significant differences were found between groups (p>0.005). An interesting shared comment from two families who had changed centers emerged from the open-ended questions: "We hadn't fully evaluated how sufficient our previous center was, but we decided to change institutions, wondering if there could be a better option. If the progress or challenges in our child had been more clearly communicated to us, and if better communication had been established, we would have been able to more clearly understand how satisfied we were and how much our child benefited from that institution".

Discussion

This study has showed that at least half of the families of young children with special needs rated family centeredness of special education and rehabilitation services as inadequate in Türkiye. This study is significant in reflecting recent developments in family-centered practices in our country.

Numerous studies have been conducted in high-income countries in this area. One of the most comprehensive studies was conducted by Bailey et al. (22) in the United States in 2004, where 81% of families reported that decisions regarding the educational program were made in conjunction with them. In contrast, our study found this proportion to be 68%. The earlier initiation and progress of family-centered early intervention programs in the U.S. compared to the practices in our country may account for this difference. Research from various countries has indicated a need for more family information. For instance, in Hong Kong in 2003, Chan and Twinn (25) used a scale to assess the family-centeredness of early intervention programs for 246 children. They reported dissatisfaction with the lack of time and attention given by professionals and insufficient listening to their concerns. While only 29% of families in that study found the information provided about their child's developmental status sufficient, our study found this proportion to be approximately 50%. The discrepancy might be attributed to including younger children, better-educated mothers, and families with higher income levels in their data, which contrasts with the sociodemographic characteristics in our study.

Table 2: ICD diagnoses, areas of special needs, participation in daily life, and environmental stressors of children		
Category	**Subcategory**	**n (%)**
ICD diagnosis groups of children	Neurological disorders	46 (45.5)
	Genetic disorders	36 (35.6)
	Cardiovascular disorders	19 (18.8)
	Nephrological disorders	14 (13.9)
	Orthopedic disorders	14 (13.9)
	Respiratory system disorders	9 (8.9)
	Endocrinological disorders	6 (5.9)
	Allergic disorders	4 (4.0)
	Gastrointestinal disorders	3 (3.0)
	Immunological disorders	2 (2.0)
	Hematological disorders	2 (2.0)
	Oncological disorders	1 (1.0)
Areas of special needs	Expressive language	69 (68.3)
	Gross motor skills	65 (64.4)
	Receptive language	63 (62.4)
	Attention	53 (52.4)
	Feeding issues	44 (43.5)
	Psychological/behavioral issues affecting learning	33 (32.7)
	Sleep problems	32 (31.6)
	Fine motor skills	30 (29.7)
	Play	22 (21.8)
	Social interaction	20 (19.8)
	Self-care	19 (18.8)
Participation in daily life	Participation in visits to relatives	
	Never	68 (67.3)
	Once a week	24 (23.8)
	Twice a week	5 (5.0)
	Three times or more a week	4 (4.0)
	Participation in playgrounds and parks	
	Never	87 (86.1)
	Once a week	8 (7.9)
	Twice a week	2 (2.0)
	Three times or more a week	4 (4.0)
	Participation in nature or play with animals	
	Never	87 (86.1)
	Once a week	9 (8.9)
	Twice a week	3 (3.0)
	Three times or more a week	2 (2.0)
	Participation in play with other children	
	Never	89 (88.1)
	Once a week	7 (6.9)
	Twice a week	1 (1.0)
	Three times or more a week	4 (4.0)
Environmental and family stressors	Community prejudice	30 (29.7)
	Unemployment	17 (16.8)
	Maternal depression	17 (16.8)
	Siblings' issues	13 (12.9)
	Paternal depression	11 (10.9)
	Lack of support from relatives/friends	9 (8.9)
	Sick family member	6 (5.9)
	Family conflict	4 (4.0)
	Job/city change	4 (4.0)
	Domestic violence	1 (1.0)
ICD: International classification of diseases		

Table 3: Evaluation of family-centered approaches in early intervention services attended by children		
Category	**Subcategory**	**Inadequate**
1. Family information		
1j	Provision of home services when the early intervention services cannot be visited due to health issues	88 (87.1)
1g	Adjustment of the educational program to assist with daily activities such as eating and dressing	53 (52.5)
1e	Guidance for the family on how to raise their child	51 (50.5)
1d	Information for the family on daily care of the child	45 (44.6)
1c	Informing the family about the child's activities through an activity log	42 (41.6)
1f	Provision of useful homework to encourage family time with the child	37 (36.6)
1h	Addressing the child's health issues	35 (34.7)
1k	Consulting the family on what would benefit the child when preparing the educational program	32 (31.7)
1a	Helping the family understand the child's behavior	26 (25.7)
1l	Providing adequate answers to the family's questions at all times	23 (22.8)
1ı	Making educational sessions fun to increase child participation	17 (16.8)
1b	Informing the family about topics they need assistance with	13 (12.9)
2. Sharing and supporting family feelings and thoughts		
2f	Allowing parents to share and receive support for tensions or issues with their spouses	66 (65.3)
2e	Allowing parents to share and receive support for new changes or difficulties affecting their home life	51 (50.5)
2d	Allowing parents to share their feelings of sadness, exhaustion, or helplessness	50 (49.5)
2a	Asking parents how they feel	42 (41.6)
2b	Supporting parents' self-confidence	35 (34.7)
2c	Creating a warm environment where parents can easily share their emotions	31 (30.7)
3. Assessing and supporting family socioeconomic status		
3b	Inquiring about the family's financial difficulties	73 (72.3)
3c	Informing the family about other aid organizations they can benefit from	65 (64.4)
3a	Gathering information on the family's work conditions	61 (60.4)
3d	Creating opportunities for the family to meet with other families and share information and feelings	60 (59.4)

In a 2016 study in the United States involving 60 children with special needs, families rated early intervention services with "information about the condition" and "partnership with professionals" receiving the lowest scores (3). A 2015 study in Canada evaluated family-centeredness in early intervention services serving 143 children aged 2-18 years with special needs. Families rated the early intervention services' performance in "providing information on child-rearing" and "educating on child training" at 40% and "support in family difficulties" at 60% as inadequate (4). Similarly, our study found that at least half of the families rated these aspects as inadequate. In Italy in 2017, a study involving 382 families of children diagnosed with cerebral palsy and another study in the Netherlands in the same year involving 175 families of children with special needs highlighted "family information" as a crucial centres feature (5,6). Like many international studies, our research identified inadequacies in "information provision." A 2012 study in France with 212 children and their families identified "effective communication between professionals and families" as one of the critical components determining family satisfaction with early intervention services (7). Our study also found that the significant dissatisfaction reported in open-ended questions was due to "insufficient information and communication issues."

Comparing our results with Özdemir İncesoy and Ertem. (13) study, the first study in our country with similar objectives could provide insights into the development of family-centered services over the past 12 years. However, there are methodological differences. Özdemir İncesoy and Ertem (13) study included 54 children and 36 different early intervention services, whereas ours involved 101 children and 57 different early intervention services. The age range of the children included in Özdemir İncesoy and Ertem (13) study was ten months to 10 years and older. The information reflected by families of older children may differ from that of families with younger children. Analysis explaining the scoring differences is presented in Table 4. To compare our study's data with Özdemir İncesoy and Ertem (13), we included data where frequently applied center features were also considered inadequate. This comparison shows that both studies yielded similar results, indicating a high level of inadequacy in the family-centered features of the early intervention services where the children in the sample received services.

Table 4: Comparison of the Early Childhood Development Support Services Evaluation Scale results with the 2005 research results by Özdemir İncesoy and Ertem (13)

Category	Inadequate (%)*	Inadequate (%)	Inadequate Özdemir İncesoy and Ertem (13) (%)
1. Family information			
1j. Provision of home services when the early intervention services cannot be visited due to health issues	88	92	96
1g. Adjustment of the educational program to assist with the child's daily activities such as eating and dressing	53	75	83
1e. Guidance for the family on how to raise their child	51	83	85
1d. Information for the family about the child's daily care	47	75	73
1c. Informing the family about the child's activities through an activity log	42	64	58
1f. Providing useful assignments to help the family spend time with the child at home	37	63	65
1h. Addressing the child's health issues	35	60	67
1k. Considering the family's opinions on what would benefit the child while preparing the educational program	32	62	85
1a. Helping the family understand the child's behavior	26	67	67
1l. Always providing sufficient answers to the family's questions	23	51	29
1ı. Making educational sessions enjoyable to increase the child's participation	17	47	40
1b. Providing information on topics the family needs	13	45	38
2. Sharing and supporting family's feelings and thoughts			
2f. Allowing parents to share and receive support for tensions or issues with their partners	66	83	92
2e. Allowing parents to share and receive support for new changes or difficulties affecting their lives	51	72	85
2d. Allowing parents to share feelings of sadness, exhaustion, or helplessness with the early intervention services	50	73	77
2a. Asking parents about their feelings	42	72	81
2b. Supporting parents' self-confidence	35	61	81
2c. Creating a warm environment where parents can easily share their feelings	31	61	62
3. Assessment and support of family's socioeconomic situation			
3b. Asking about the family's financial difficulties	72	87	88
3c. Providing information about other aid organizations the family can benefit from	64	81	98
3d. Creating an environment for families to meet, share information, and emotions with others	59	79	77

In our study population, families reported high levels of dissatisfaction regarding the information provided, particularly in the area of "guidance on how to raise their child", where half of the families expressed dissatisfaction. This clearly indicates the need to improve the curricula and family consultations within early intervention programs. However, it is equally important to ensure that families are empowered to demand these services from early intervention programs and to introduce them to the scope of services they are entitled to receive. Both monitoring the quality of these programs and educating families about what constitutes quality service content are essential.

Study Limitations

The strengths of our study include its design, the requirement of a minimum of 3 months of service for inclusion in the sample, and the use of a tool specifically developed for our country. However, the study's generalizability is limited due to the families' educational backgrounds. Our study includes 101 children and their families. To address the generalizability of the findings on a national level, future studies with similar designs conducted in other regions could provide valuable contributions to the literature. In our study, there were children and families who represented the population but were excluded from the sample. Including these excluded groups in the population could have provided a more comprehensive and generalizable perspective. Therefore, the generalizability of our findings is limited by the current sample.

Conclusion

This study showed that, despite finding the early intervention services for young children with special needs in

Türkiye to be inadequate regarding family-centeredness, the families remain generally satisfied. Healthcare providers, other health professionals, advocates, and health policymakers should raise awareness among families of children with special needs about family-centered service practices and requirements. This will ensure that families' service demands are appropriately met and that satisfaction scores reflect the actual quality of services. In our country and likely in other low- and middle-income countries, it is crucial to rapidly improve the awareness of families and service providers and increase the quality of family-centered practices.

Ethics

Ethics Committee Approval: The research has received approval from the Ethics Committee of Ankara University Faculty of Medicine (decision number: I2-701-17, date: 24.07.2017).

Informed Consent: All participants have signed the informed consent form and approved the study.

Footnotes

Authorship Contributions

Concept: M.K.Y., İ.E., Design: M.K.Y., E.Ö.A., E.B.B.P., İ.E., Data Collection and/or Processing: M.K.Y., İ.E., Analysis and/or Interpretation: M.K.Y., E.Ö.A., E.B.B.P., İ.E., Literature Search: M.K.Y., E.Ö.A., E.B.B.P., İ.E., Writing: M.K.Y., E.Ö.A., E.B.B.P., İ.E.

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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Evaluation of Triglyceride-Glucose Index in Patients with Hashimoto's Thyroiditis

Hashimoto Tiroiditli Hastalarda Trigliserit-Glukoz İndeksinin Değerlendirilmesi

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Abstract

Objectives: Recently, researchers have used the triglyceride-glucose (TyG) index for the possibility of metabolic syndrome and insulin resistance. Although altered glucose and lipid metabolism have been observed in patients with Hashimoto's thyroiditis (HT), the impact of autoimmune factors on the TyG index in euthyroid HT patients has not been sufficiently studied. This research sought to assess the effects of autoimmunity on the TyG index in euthyroid HT patients.

Materials and Methods: Our research was planned as a retrospective, single-center, observational study. The study included 50 participants diagnosed with HT and 52 control groups without thyroid disease, who were followed up in the internal medicine service and outpatient clinic.

Results: The patient group had a mean age of 44.54±13.72 years, while the control group had a mean age of 33.5±13.17 years. The HT group was significantly older ($p<0.001$). No meaningful distinctions were detected regarding the gender distribution between the groups ($p=0.357$). In the HT group, the low-density lipoprotein (LDL) cholesterol level was 137.8±41.5 mg/dL, and the non-high-density lipoprotein (HDL) cholesterol level was 160.5±45.2 mg/dL, both of which were significantly higher compared to the control group (LDL: $p=0.046$, non-HDL: $p=0.029$). The TyG index showed similar results between the two groups, with measurements of 8.6±1.2 in the HT group and 8.4±1.3 in the control group ($p=0.121$).

Conclusion: In contrast to previous studies, our study focused on the impact of autoimmunity on TyG index changes in patients with euthyroid HT, taking into account the relationship with thyroid hormones. Accordingly, a similar correlation was detected between the TyG index and age in patients with and without HT. As a result, we found that the TyG index did not change due to autoimmunity in patients with euthyroid HT.

Keywords: Triglyceride/glucose index, Hashimoto's thyroiditis, autoimmunity, marker, thyroid hormone

Öz

Amaç: Son zamanlarda araştırmacılar, metabolik sendrom ve insülin direnci olasılığını tespit etmek için bir tarama yöntemi olarak trigliserit-glukoz (TyG) indeksini kullanmaya başladı. Hashimoto tiroiditi (HT) hastalarında glukoz ve lipit metabolizmasında değişiklik gözlenmesine rağmen, ötiroid HT hastalarında otoimmün faktörlerin TyG indeksi üzerindeki etkisi yeterince araştırılmamıştır. Bu araştırma, ötiroid HT hastalarında otoimmünitenin TyG indeksi üzerindeki etkilerini değerlendirmeyi amaçladı.

Gereç ve Yöntem: Araştırmamız retrospektif, tek merkezli, gözlemsel bir çalışma olarak planlandı. Çalışmaya dahiliye servisinde ve polikliniğinde takip edilen HT tanısı alan 50 katılımcı ve tiroid hastalığı olmayan 52 kontrol grubu dahil edildi.

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Bulgular: Hasta grubunun yaş ortalaması 44,54±13,72 yıl, kontrol grubunun yaş ortalaması ise 33,5±13,17 yıl idi. HT grubu anlamlı derecede daha yaşlıydı (p<0,001). Gruplar arasında cinsiyet dağılımı açısından anlamlı bir farklılık saptanmadı (p=0,357). HT grubunda düşük yoğunluklu lipoprotein (LDL) kolesterol düzeyi 137,8±41,5 mg/dL ve yüksek yoğunluklu lipoprotein (HDL) olmayan kolesterol düzeyi 160,5±45,2 mg/dL olup her ikisi de kontrol grubuna göre anlamlı derecede yüksekti (LDL: p=0,046, HDL olmayan: p=0,029). TyG indeksi, HT grubunda 8,6±1,2 ve kontrol grubunda 8,4±1,3 ölçümleriyle iki grup arasında benzer sonuçlar gösterdi (p=0,121).

Sonuç: Çalışmamızda önceki çalışmalardan farklı olarak ötiroid HT hastalarında tiroid hormonları ile ilişkisi dikkate alınarak otoimmünitenin TyG indeksi değişiklikleri üzerindeki etkisine odaklanılmıştır. Buna göre HT olan ve olmayan hastalarda TyG indeksi ile yaş arasında benzer bir korelasyon tespit edildi. Sonuç olarak ötiroid HT hastalarında TyG indeksinin otoimmüniteye bağlı olarak değişmediğini tespit ettik.

Anahtar Kelimeler: Trigliserit/glukoz indeksi, Hashimoto tiroiditi, otoimmünite, marker, tiroid hormonu

Introduction

Thyroid dysfunction may contribute to abnormal glucose homeostasis, as thyroid hormones play an important role in regulating multiple metabolic processes. Overproduction of thyroid hormones can have an impact on insulin signaling and turnover of glucose metabolism. The reduction in muscle mass among the elderly impacts peripheral glucose uptake, resulting in hyperinsulinemia and insulin resistance (1-3). Both subclinical and overt hypothyroidism have been linked to metabolic syndrome (4-11). A connection between hypothyroidism and metabolic disorders has also been revealed in the literature (12,13). Increases in thyroid stimulating hormone (TSH) levels, even at low levels, have been shown to be associated with insulin resistance (14).

Triglyceride/glucose (TyG) index determined by fasting triglyceride and glucose levels, has recently become a new indicator for metabolic syndrome (15-17). Several studies have found a correlation between elevated TyG levels and metabolic disorders (18-20).

Studies have been conducted in the literature to demonstrate the relationship between HT and dyslipidemia, and it has been found that the prevalence of dyslipidemia is increased in patients with HT (21). Moreover, in hypertension (HTN), cytokines like interleukin-1 beta (IL-1β), IL-6, and tumor necrosis factor alpha are elevated, triggering metabolic processes (22). Research has found that conditions like rheumatoid arthritis and systemic lupus erythematosus, which are autoimmune in nature, are associated with insulin resistance, largely due to inflammatory processes and an elevated TyG index (23).

The literature has not yet studied the relationship between euthyroid HT and TyG. In this study, we wanted to determine the relationship between HT and the TyG index, which is a marker of metabolic disease, in euthyroid patients. We also examined the relationship between TyG and autoimmunity in our study.

Materials and Methods

Our research was planned as a retrospective, single-center, observational study. Patients with a diagnosis of HT who were followed up in the internal medicine service and outpatient clinic with TSH (reference: 0,55-4,78 mU/L), free triiodothyronine (T3) (reference: 2,3-4,2 ng/L) and free levothyroxine (T4) (reference: 0,89-1,76 ng/dL) levels within the normal range and a control group without thyroid disease were included in the study. For the diagnosis of Hashimoto's thyroiditis (HT), antibody levels (anti-thyroid peroxidase) were checked and thyroid ultrasound was performed. The study involved patients who had high antibody levels and ultrasound findings compatible with HT. All actions in this research received approval from the Ankara Bilkent City Hospital Clinical Research Ethics Committee and were executed in line with the ethical principles established in the Declaration of Helsinki and its subsequent updates (date: 12/07/2023, approval no: E2-23-4142). The study was planned in a retrospective manner; as a result, patient consent was not collected.

The number of participants in each group was set at 45 (±10%) using the G*Power software, based on an analysis with a 95% confidence level (1-α), a 95% test power (1-β), and an effect size (d) of 0.5.

The study included 50 patients diagnosed with HT disease and 52 patients in the control group without thyroid disease, aged 18 and over, who were followed up in internal medicine services and outpatient clinics between September 2021 and March 2023. The criteria for exclusion in our study were specified as follows: age under 18, known liver disease, diabetes, HTN, chronic kidney disease, use of lipid-lowering therapy, sepsis, and abnormal thyroid function tests (TSH, T3, T4). Among the participants diagnosed with HT, there were equal numbers of those who received levothyroxine sodium treatment and those who did not. All euthyroid patients in the HT group were included in the study, regardless of whether they received treatment or not.

Various factors were assessed, including the patients' age, gender, existing comorbidities, prescribed medications, complete blood count, levels of white blood cells, neutrophils, lymphocytes, neutrophil-to-lymphocyte ratio, eosinophils, triglycerides, glucose, and lipid profile, along with TSH, T3, and T4.

Statistical Analysis

The data were analyzed utilizing SPSS software, version 25.0 (IBM Corp., 2017). The distribution of variables was checked with histogram graphs and the Kolmogorov-Smirnov test. Descriptive statistics, including mean, standard deviation, and range (minimum-maximum values), were presented. The relationship between laboratory and demographic parameters was assessed using Pearson's correlation coefficient. In patients diagnosed with HT, categorical variables such as comorbidities, demographic data, lab results, and age were compared using the chi-square test. A p-value below 0.05 was regarded as statistically meaningful.

Results

In this study, laboratory values and comorbidities were compared between patients and the control group. The results indicated that the mean age of the patient group was higher, but no considerable variations were observed in parameters such as sex, neutrophil count, and lymphocyte count. Low-density lipoprotein (LDL), non-high-density lipoprotein (HDL) cholesterol, and the TyG index was notably higher in the patient group. Significant correlations were observed between the TyG index and age, lymphocyte count, HDL, non-HDL cholesterol, and triglyceride levels. There were no cases of diabetes or coronary artery disease in either group, and the rate of HTN was alike in both groups. To conclude, while several laboratory variables were notably different across the groups, there were no substantial variations in comorbidities.

Patients with HT disease and the control group were compared according to the measured parameters in Table 1. The mean age of the patient group was higher ($p<0.001$). The proportion of females in the patient group (72%) was higher than in the control group (63.46%), but there was no statistical significance ($p=0.357$). No notable differences were observed in neutrophil and lymphocyte counts ($p=0.217$ and $p=0.164$, respectively). The neutrophil/lymphocyte ratio did not exhibit a significant difference across the groups ($p=0.079$). The hemoglobin concentrations were similar across both groups, with no considerable variation ($p=0.830$). LDL concentrations were notably elevated in the HT group ($p=0.046$). No notable difference was observed in HDL levels ($p=0.202$). The levels of non-HDL cholesterol were markedly increased in the HT group ($p=0.029$). Triglyceride and glucose levels did not show significant differences ($p=0.213$ and $p=0.543$, respectively). No meaningful variation was detected in the TyG index across the two groups ($p=0.121$).

The relationship of the TyG index with other parameters was compared between the groups in Table 2. When evaluating the relationship between the TyG index and various demographic and laboratory values in Hashimato's patients and the control group, a strong association between age and the TyG index was noted in both groups (Hashimoto: $p=0.009$, $r=0.364$; control: $p=0.003$, $r=0.407$). No notable association was detected between neutrophil count and the TyG index in either group (Hashimoto: $p=0.779$, $r=0.041$; control: $p=0.787$, $r=-0.038$). A positive correlation was found between lymphocyte count and the TyG index in the HT group ($p=0.002$, $r=0.433$), whereas no significant correlation was observed in the control group ($p=0.268$), $r=-0.156$). A negative correlation was observed between the neutrophil/lymphocyte ratio and the TyG index in the HT group ($p=0.015$, $r=-0.342$), but no meaningful correlation

Table 1: Comparison of laboratory values between patient and control groups

		Control	HT	Overall	p-value
		Mean ± SD	Mean ± SD	Mean ± SD	
Age		33.5±13.17	44.54±13.72	38.91±14.48	<0.001[1]
Sex, n (%)	Female	33 (63.46)	36 (72.00)	69 (67.65)	0.357[2]
	Male	19 (36.54)	14 (28.00)	33 (32.35)	
Neutrophil (x10^6/L)		4154.29±1516.07	3786±1474.93	3973.75±1500.08	0.217[1]
Lymphocyte (x10^6/L)		2047.5±543.88	2230.2±751.69	2137.06±657.2	0.164[1]
Neutrophil/lymphocyte ratio		2.33±1.92	1.81±0.73	2.08±1.48	0.079[1]
Hemoglobin (g/dL)		13.6±1.87	13.53±1.38	13.56±1.64	0.830[1]
Low-density lipoprotein (mg/dL)		101.35±28.73	116.16±43.24	108.61±37.14	0.046[1]
High-density lipoprotein (mg/dL)		50.77±15.9	54.52±13.46	52.61±14.8	0.202[1]
Non-high-density lipoprotein (mg/dL)		120.02±31.57	137.38±45.84	128.53±39.98	0.029[1]
Triglyceride (mg/dL)		95.98±51.2	108.36±48.33	102.05±49.96	0.213[1]
Glucose (mg/dL)		87.44±9.97	88.54±8.07	87.98±9.06	0.543[1]
Triglyceride/glucose index		8.23±0.47	8.38±0.48	8.31±0.48	0.121[1]

[1]Independent t-test [2]Chi-square test
p: variation between laboratory parameters with and without Hashimoto, HT: Hashimoto thyroiditis, SD: Standart devitation

was identified in the control group (p=0.238, r=0.167). No significant correlation was found between hemoglobin levels and the TyG index in either group (Hashimoto: p=0.235, r=0.171; control: p=0.463, r=-0.104). A borderline positive correlation was found between LDL and the TyG index in the HT group (p=0.058, r=0.270), but no significant relationship was observed in the control group (p=0.129, r=0.213). A significant negative correlation was found between HDL and the TyG index in both groups (Hashimoto: p=0.002, r=-0.433; control: p<0.001, r=-0.476). A significant positive correlation was observed between non-HDL cholesterol and the TyG index in both groups (Hashimoto: p=0.001, r=0.448; control: p<0.001 r=0.562). A strong positive correlation was found between triglycerides and TyG index in both groups (Hashimoto: p<0.001, r=0.954; control: p<0.001, r=0.916). A positive correlation was also observed between glucose levels and the TyG index in both groups (Hashimoto: p<0.001, r=0.573; control: p=0.002, r=0.417).

Comparison of comorbidities between patient and control groups was shown in Table 3. When comparing comorbid diseases between HT patients and the control group, no cases of diabetes mellitus (DM) were observed in either group. HTN was present in 3.85% of the control group and 6.00% of the HT group. However, this discrepancy was not found to be statistically significant (p=0.675), indicating that HTN prevalence was similar between the two groups. No cases of coronary artery disease were found in either group. In conclusion, there were no significant differences in the prevalence of HTN or coronary artery disease between the two groups, and no cases of DM or coronary artery disease were observed in either group.

Discussion

Even though studies in the literature suggest a link between the TyG index and thyroid function tests, there is a paucity of previous research on this relationship in patients with euthyroid HT. In our study, we aimed to investigate the potential impact of autoimmunity on the TyG in patients with euthyroid hypothyroidism, excluding thyroid function tests. In our study, we did not find a relationship between TyG index and HT. We found that there was no relationship between thyroid autoimmunity and TyG index in patients with normal thyroid function tests.

There are studies in the literature stating that the TyG index varies in DM, coronary artery disease and chronic kidney disease.

Table 2: Comparison of triglyceride/glucose index with demographic and laboratory values in patient and control groups

Triglyceride/glucose index				
	HT		Control	
	r	p-value	r	p-value
Age	0.364	0.009	0.407	0.003
Neutrophil	0.041	0.779	-0.038	0.787
Lymphocyte	0.433	0.002	-0.156	0.268
Neutrophil/lymphocyte ratio	-0.342	0.015	0.167	0.238
Hemoglobin	0.171	0.235	-0.104	0.463
Low-density lipoprotein	0.270	0.058	0.213	0.129
High-density lipoprotein	-0.433	0.002	-0.476	<0.001
Non-high-density lipoprotein	0.448	0.001	0.562	<0.001
Triglyceride	0.954	<0.001	0.916	<0.001
Glucose	0.573	<0.001	0.417	0.002

Pearson correlation test
r: Sample correlation coefficient, p: Population correlation coefficient, HT: Hashimoto thyroiditis

Table 3: Comparison of comorbid diseases between patient and control groups

		Control (n=52)	HT (n=50)	Overall (n=102)	p-value[2]
Diabetes mellitus	Absent	52 (100.00)	50 (100.00)	102 (100.00)	
Hypertension	Absent	50 (96.15)	47 (94.00)	97 (95.10)	0.675[2]
Coronary artery disease	Absent	52 (100.00)	50 (100.00)	102 (100.00)	

[2]Chi-square test
SD: Standard deviation, HT: Hashimoto thyroiditis

(8-10). Therefore, unlike other studies in the literature with such chronic diseases, we did not include these patients in our study.

Choi et al. (24) found a relationship between the TyG and TSH intervals in euthyroid patients. In our study, unlike the one conducted by Choi et al. (24), euthyroid patients were not further categorized due to the limited number of participants and the subjective determination of TSH interval values. Therefore, this relationship was not detected in our study since we did not make additional groupings. In contrast to this study, our patient population was younger, and we minimized the impact on the TyG by excluding chronic diseases.

In the literature, there is no TyG study that directly examines the relationship of euthyroid patients with autoimmune thyroiditis with the control group. Lei et al. (25) evaluated patients with autoimmune thyroid disease as predisposed to lipid metabolism disorders, comparing them with the control group. Lipid metabolism and inflammatory cytokines are thought to contribute to the pathogenesis and progression of autoimmune thyroiditis. We thought that the TyG might change by affecting lipid metabolism.

It was observed that the TyG index measured in the elderly population was closely linked to the risk of frailty (26). Loss of muscle mass in the elderly affects peripheral glucose absorption through decreased muscle mass, leading to a state of hyperinsulinemia and insulin resistance (27). In our study, we found that age and TyG index were associated independently of HT.

Our study found similar fasting blood glucose and lipid profiles in both groups. A stronger correlation between serum thyroid hormone levels and blood lipid metabolism, insulin resistance and inflammatory factors has been observed in the literature (25). In our study, unlike the literature, we examined the change in the TyG index due to autoimmunity rather than the thyroid hormone relationship by taking patients with euthyroid HT.

Similar to our study, a retrospective study by Akyüzlü and Mutlu (28) evaluated 1,280 patients and concluded that the TyG, independent of thyroid hormones, cannot serve as a marker of insulin resistance in individuals with thyroid disease. However, in this study, unlike ours, all thyroid diseases except HT constituted the patient group. We defined the normal TSH interval in our study as 0.55-4.78, while Akyüzlü and Mutlu (28) defined it as 0.5-10.

Studies examining patients with systemic autoimmune inflammatory diseases have suggested the presence of an ongoing inflammatory process (29,30). It has been demonstrated that these systemic autoimmune inflammatory diseases may contribute to the development of other conditions, including metabolic syndrome, HTN, dyslipidemia, and diabetes (31). We examined the relationship between the TyG index, which has been previously shown to be associated with metabolic syndrome, and HT, which is an autoimmune disease, and determined that this index cannot be used in HT. We thought that this result, which was different from the literature, might be related to the milder course of euthyroid HT disease compared to other systemic inflammatory diseases. The literature indicates that anti-thyroid peroxidase (TPO) antibody levels are associated with higher insulin levels in patients with a body mass index (BMI) similar to those with levels exceeding 1000 IU/mL. In the same study, consistent with our findings, no notable variation was observed among the participants with and without HT regarding glucose and lipid concentrations (32). In the research by Biyikli et al. (33), fasting glucose and triglyceride levels were notably elevated in the hypertensive group; however, the TyG index was not assessed. We believe that this discrepancy may be attributed to the exclusion criteria in our study, which accounted for additional factors that could influence fasting glucose and triglyceride levels. In addition, we evaluated that the systemic effect of HT is mostly through thyroid hormones, and in our study, this effect was minimized by including euthyroid patients. There is a need for prospective studies examining the relationship between other early diagnosed systemic autoimmune diseases and the TyG index in patients under treatment.

Study Limitations

The limitations of our study include the age variation between the groups and the inability to assess the deficiency of anti-TPO levels due to the small sample size. We recommend conducting studies examining its relationship with anti-TPO level. We suggest that patients undergo long-term follow-up, and recommend planning studies that explore the relationship between the duration of HT disease. We think that this limitation will be eliminated by prospectively recruiting a large number of patients. Another limitation of our study is the age discrepancy between the groups, as glucose levels were influenced by lipid profile changes associated with age.

We recommend conducting additional studies on autoimmune diseases with a large patient population, after excluding the parameters affecting the TyG in the literature. Because the TyG is cheap and easy, it still maintains its popularity in metabolic syndrome. The TyG index has the potential to be an important screening tool for managing these diseases. Therefore, there is a significant need for more extensive and long-term studies examining the relationship between autoimmune diseases, particularly conditions like HT, and the TyG index. Autoimmune diseases can lead to various metabolic changes due to the interaction between genetic and environmental factors, making the management of such conditions more complex. These types of studies will enhance our understanding of the TyG index's effectiveness as a marker for assessing metabolic risk factors

in autoimmune diseases and provide valuable insights for clinical practice. Moreover, the ability of the TyG index to detect metabolic syndrome and related complications early on offers an important advantage in improving patient management and preventing complications.

Conclusion

In conclusion, no changes in the TyG index were observed in euthyroid Hashimoto's patients due to autoimmunity. However, in future large-scale prospective studies, the relationship between the TyG index and thyroid autoimmune diseases may be investigated in greater detail.

Ethics

Ethics Committee Approval: All actions in this research received approval from the Ankara Bilkent City Hospital Clinical Research Ethics Committee (date: 12/07/2023, approval no: E2-23-4142) and were executed in line with the ethical principles established in the Declaration of Helsinki and its subsequent updates.

Informed Consent: Consent was not obtained since it was a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A., Concept: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A., Design: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A., Data Collection and/or Processing: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A., Analysis and/or Interpretation: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A., Literature Search: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A., Writing: O.Z., B.G., M.D., Ö.A., E.Ü., O.Ü., E.S.Ş., O.İ., İ.A.

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TEMEL TIP BİLİMLERİ / BASIC MEDICAL SCIENCES

Hypoxia Induced Downregulation of Na^+/H^+ Exchanger-1 Activity Decreases Tumor Cell Proliferation

Hipoksi ile Indüklenen Na^+/H^+ Değiş-Tokuşcusu-1 Aktivitesindeki Azalma Tümör Hücre Proliferasyonunu Yavaşlatıyor

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Abstract

Objectives: Hypoxia and acidosis are the hallmarks of proliferative tumor microenvironment which can modulate the expression and function of Na^+/H^+ exchanger-1 (NHE1) via hypoxia inducible factor 1 (Hif). Here, we investigate the severity and time dependent effects of chronic hypoxia on NHE1 activity and its correlation with cell proliferation in mouse atrium tumor derived HL-1 cells.

Materials and Methods: NHE1 activity was recorded using intracellular pH (pH_i) sensitive dye cSNARF-1 (Leica SP5). Cell proliferation was assessed by live cell movie analyzer (Nanoentek, JuLI Br®) or immunofluorescence method.

Results: According to our results, mild chronic hypoxia (2% O_2, 48 hours) or shorter duration severe chronic hypoxia (1% O_2, 24 hours) did not affect cell proliferation and NHE1 activity. In contrast, long term dimethyloxalylglycine (DMOG, Hif stabilizer) or zoniporide (NHE1 inhibitor) incubations (21% O_2, 24/48 hours) suppressed cell proliferation.

Conclusion: When our published and current results in this study interpreted together, at a critical level and duration of chronic hypoxia, Hif mediated downregulation of NHE1 activity could suppress tumor cell proliferation regardless of the well-known anti-proliferative early term direct effects of Hif. Therefore, restriction of NHE1 activity in tumor hypoxia is an important alternative target in regulating anti-proliferative action against tumor cells.

Keywords: Cell culture techniques, hypoxia, sodium-hydrogen exchangers, neoplasms

Öz

Amaç: Proliferatif tümör mikroçevresinin başlıca özelliklerinden olan hipoksi ve asidoz, Na^+/H^+ değiş-tokuşcusunun (NHE1) ekspresyon ve fonksiyonunu hipoksi ile indüklenen faktör 1 (Hif) aracılığı ile etkileyebilmektedir. Bu çalışmada, fare atrium kökenli HL-1 tümör hücrelerinde farklı süre ve şiddetlerdeki kronik hipoksinin NHE1 aktivitesi üzerindeki etkisi ve hücre proliferasyonu ile ilişkisi araştırıldı.

Gereç ve Yöntem: NHE1 aktivitesi kaydı için hücre içi pH (pH_i) duyarlı boya cSNARF-1 (Leica SP5) kullanıldı. Hücre proliferasyonu canlı hücre görüntüleme sistemi (Nanoentek, JuLI Br®) veya immünofloresan yöntemleri ile ölçüldü.

Bulgular: Bu çalışmadaki bulgularımıza göre kronik hipoksi (%2 O_2, 48 saat) veya kısa süreli şiddetli kronik hipoksi (%1 O_2, 24 saat), hücre proliferasyonu veya NHE1 aktivitesini etkilemedi. Ancak, hücrelerin dimetiloksalilglisin (DMOG, Hif birikimine yol açar) veya zoniporid (NHE1 inhibitörü) ile normokside uzun süreli inkübasyonları (%21 O_2, 24/48 saat) hücre proliferasyonunu baskıladı.

Sonuç: Daha önce elde ettiğimiz bulgular ve bu çalışmadan elde edilen sonuçlar birlikte yorumlandığında, kritik şiddet ve süredeki kronik hipokside Hif aracılı NHE1 aktivitesindeki azalma, Hif'in iyi bilinen erken dönem anti-proliferatif etkilerinden bağımsız olarak tümör hücre proliferasyonunu azaltabilir. Dolayısıyla, tümör hipoksisinde NHE1 aktivitesini sınırlandırmak tümör gelişimine karşı etki hedeflenmesinde alternatif bir yaklaşım olarak önemlidir.

Anahtar Kelimeler: Hücre kültürü teknikleri, hipoksi, sodyum-hidrojen değiş-tokuşcusu, kanser

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Introduction

Cell metabolism generates hydrogen ion (H^+) which is extruded out from the cytoplasm through intracellular pH (pH_i) regulatory proteins. Sodium hydrogen exchanger (NHE1) is a ubiquitously expressed integral membrane protein that regulates pH_i by removing a proton in exchange for an extracellular Na^+ ion (1). NHE1 is required for physiological cell proliferation as well as for tumor growth (2,3).

Hypoxic areas are frequently encountered in solid tumors due to decreased vascular growth and restricted oxygen diffusion (4,5). Oxygen deprivation stimulates hypoxia-induced factor 1 (Hif) accumulation in the cell which typically, in the presence of oxygen, is rapidly degraded by prolyl hydroxylase (PHD) enzyme. PHD enzyme is a 2-oxoglutarate-dependent dioxygenase which has been reported to be inhibited by 2-oxoglutarate analogue dimethyloxalylglycine (DMOG) leading to Hif accumulation even under normoxic conditions (6). Hif is known to affect cell proliferation at transcriptional level through regulation of cell cycle (7-10). Apart from these well-known mechanisms, Hif has also been reported to reduce DNA replication and suppress cell proliferation also through non-transcriptional pathways (11,12). Conversely, for cancer cells, Hif may contribute to increased cell growth (13).

Our aim was to investigate NHE1's role in cancer cell proliferation in chronic hypoxia. The methodology used in this study enabled us to maintain the extracellular pH constant throughout the chronic hypoxic period (acidic extracellular pH shift was prevented due to anaerobic respiration), which might be a factor itself in favoring tumor survival thereby it was eliminated (14). Moreover, we applied various hypoxia protocols that allowed us to dissect out the individual roles of Hif and its downstream target NHE1 activity in cell proliferation during chronic hypoxia. Our findings suggests that the Hif's established early term cell cycle suppressive effects might not be the major mechanism observed here. More likely, Hif suppresses cell proliferation mainly via regulating NHE1 activity at a critical hypoxic threshold. In addition, PHD and/or NHE1 inhibitors have direct effects in cardiac tumor cell proliferation. All these results point to new therapeutic targets for tumor tissue already proliferating in hypoxic environment.

Materials and Methods

Cell Culture and Induction of Hypoxia

Mouse atrial tumor-derived HL-1 cells were cultured as described previously (15). Incubation was performed under standard culture conditions (5% CO_2, 95% humidity, 37 °C) for 24-36 hours until 40-50% confluence. Grown cells were transferred to 60 mm petri dishes (two round glasses per petri dish) or to 6 wells (two fractured coverslip per well) containing fresh medium for proliferation assessment or functional experiments respectively. This maneuver increased the amount of medium per cell without changing gas diffusion parameters. Cells were then either incubated at 21% O_2 (normoxia control group) or at 1-2% (continuous hypoxia group) in an oxygen-controlled carbon dioxide incubator (Panasonic, MCO-170M-PE) for 24/48 hours. In standard culture conditions, hypoxic incubation reduces medium pH in 48 hours. By increasing buffering capacity (i.e., smaller cell density in larger volumes) medium acidification was prevented (i.e., medium pH was nearly kept constant at ~7.4) at the end of 24/48 hours of continuous normoxia/hypoxia incubation. Hypoxia mimetic agent, DMOG (1 mM) or NHE1 specific inhibitor, zoniporide (30 µM) was added directly into the medium and incubated in normoxia for 24/48 hours (16).

Superfusion of Cells and Confocal Microscopy

The immediate environment of cells was controlled by a superfusion system that provided rapid solution exchange. Solutions were delivered at 37 °C at 2 mL/min to a Perspex chamber with a coverslip bottom. Normal Tyrode solution (NT) contained 135 mM NaCl, 20 mM Hepes [4-(2-hydroxyethyl)-1 piperazine ethanesulfonic acid, pKa: 7,5], 11 mM glucose, 4.5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$. In ammonium containing solutions, NaCl was isosmotically replaced with NH_4Cl. For pH_i imaging, cells were loaded for 30 min with the pH sensitive dye SNARF (Invitrogen, 5 µM) under standard culture conditions. The dye was excited at 543 nm in a confocal microscope (TCS SP5, Leica) and the emission signal was measured at wavelengths of 580 nm and 640 nm. The ratio was converted to pH_i using a calibration curve determined by the *in situ* nigericin (K^+/H^+ ionophore, 10 µM, Sigma) technique. Cells were acid loaded by ammonium prepulse method (rapid removal of 20 mM ammonium chloride superfusion, 4-5 min). NHE1 mediated H^+-extrusion (activity) was calculated as the product of the slope of pH_i recovery (dpH_i/dt) and intrinsic buffering capacity (β_i) in NT solutions. β_i were measured separately by gradual ammonium removal and data were fitted to a previously described model (16-18).

Live or Immunofluorescence Imaging of Cell Proliferation

Live Cell Movie Analyzer and software (JuLi Br, NanoEnTek Inc.) were used for instant measurement of cell proliferation during hypoxia (19). The device was placed in the culture incubator and real-time images were collected at 10 min intervals for 48 hours. Under these conditions, the lowest O_2 level attainable was stabilized at 2%. For more severe levels of hypoxia (1% O_2, 24/48 hours), device and cable connections were removed from the incubator as to latch the inside glass door properly therefore atmospheric O_2 contamination was

minimized. In this group, cell proliferation (i.e., the total number of cells) was visualized by the nuclear stain Yo-Pro-1 using immunofluorescence method as previously described (16). Zoniporide (30 µM) and DMOG (1 mM) were added to the cell medium at normoxia to observe the effect of direct inhibition of NHE1 activity or Hif on live cell proliferation.

Statistical Analysis

The number "n" in the experiments represents NHE1 activity or proliferation measured from the cell cluster grown on round or fractured coverslips. Two-way ANOVA test (GraphPad Prism 4.0) was used for the significant effect of hypoxia, drugs and its relationship with pH_i sensitivity (i.e., a change in pH_i sensitivity) in pH_i -NHE1 activity data sets obtained from experimental groups and controls. The NHE1 activity curves were fitted to a four-parameter logistic equation curve *(fit)*. In proliferation experiments, results were presented as mean ± standard error of the mean. Two-way Student's t-test was used for the difference between groups. $p<0.05$ was considered significant.

Ethics Committee Approval

The authors declared that this research does not require ethical approval. Since the study was not conducted on humans, consent was not obtained.

Results

Hif-mediated NHE1 Inhibition Decreases Tumor Cell Proliferation at a Critical Hypoxic Threshold

According to our previous findings, incubation of HL-1 cells in severe chronic hypoxia (1% O_2, 48 hours) or in DMOG (1 mM, 21% O_2, 48 hours) markedly reduced both NHE1 activity and cell proliferation (16). While in this study, NHE1 activity and cell proliferation was not affected when cells were incubated in severe chronic hypoxic conditions for shorter duration (1% O_2, 24 hours) or kept in milder chronic hypoxia (2% O_2, 48 hours) instead (Figures 1, 2). It is well documented that either chronic hypoxia (1-2% O_2, 24/48 hours) or DMOG incubation (1 mM, 21% O_2, 24/48 hours) triggers rapid accumulation of Hif in various cancer cell lines (16,20,21). Thus, we argue that, NHE1 activity and cell proliferation may not be altered even in the presence of Hif. On the other side, inhibition of NHE1 activity using NHE1 inhibitor (zoniporide, 30 µM) had effectively inhibited cell proliferation under normoxia (21% O_2, 24/48 hours) in a Hif independent manner (Figure 2A). Hence, these findings support the idea that, at a certain level and duration of hypoxia, Hif reduces cancer cell proliferation mainly through NHE1 inhibition. Hif's eminent anti-proliferative roles acting on cell cycle proteins at transcriptional and non-transcriptional levels which are usually detected at early stages of hypoxia seem to be of minor importance under these circumstances.

Prolyl Hydroxylase Inhibition Does Not Replicate the Effects of Hypoxia on Cell Proliferation

The PHD enzyme inhibitor DMOG is widely used to mimic the effects of hypoxia under normoxic conditions (22,23). However, our findings revealed that the effects of hypoxia and DMOG incubation on cell proliferation may be dissimilar. NHE1 activity and associated cell proliferation did not change in hypoxia (1% O_2, 24 hours), whereas DMOG incubation for the same period in normoxia had profound effect on cell proliferation (Figures 1, 2).

Discussion

Hypoxia is a condition in which the oxygen concentration in organs or cells is below the normal physiological level. Hypoxia is known to modulate normal and cancer cell proliferation (24). Particularly in solid tumors, rapid proliferation leads to tumor hypoxia, increases the risk of invagination and metastasis, decreases the efficiency of treatment by suppressing anti-tumor immunity (25). Interestingly, even in the presence of elevated Hif levels, cancer cells maintain proliferation (26). Accordingly, using basic research, it is important to reveal tumor specific hypoxic response mechanisms as to develop better targeted treatment approaches against tumor growth. However, reproducibility rate of preclinical experimental data in cancer research by another's is thought to be as low as 10% (27). The inadequate quality of the preclinical data obtained has been associated with the high failure rate (~95%) of newly developed compound transition from *in vitro* validation to phase 3 testing in cancer drug research (28). To improve data reproducibility, experimental control conditions must be

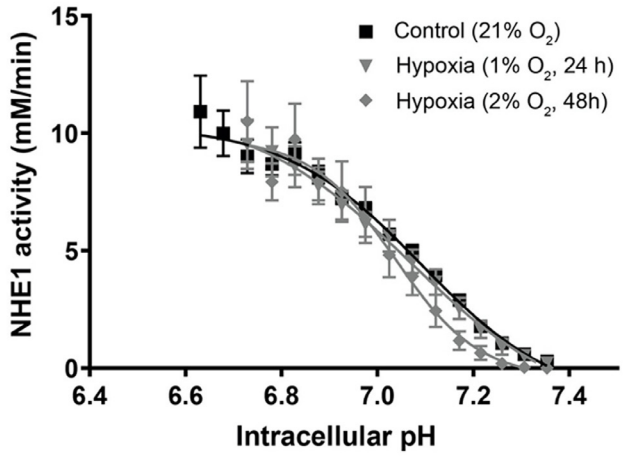

Figure 1: Intracellular pH (pH_i) sensitivity of NHE1 activity. NHE1 activity is pH_i dependent and increases at acidic pH_i. 48 h of mild hypoxia (2% O_2, ♦) or 24 h severe hypoxia (1% O_2, ▼) did not affect pHi sensitivity of NHE1 activity compared to control (■) (n: 19-24, $p>0.05$)

strictly controlled. One of the poorly controlled, fundamental variables is pH of the culture medium and biological processes are extremely sensitive to extracellular acid or base alterations. For example, extracellular pH alone is known to be effective on cell proliferation (29). Controlling medium pH in culture systems under continuous prolonged hypoxia is even more difficult because lactic acid produced as a result of increased glycolytic metabolism generally shifts medium pH to further acidic. We conducted empirical methodology in which medium buffering capacity was increased to prevent medium acidification so that single effect of hypoxia on proliferation would be observed. Continuous monitoring of the cells in hypoxia (2% O_2) for 48 hours allowed live monitoring of proliferation rate and was preferred to indirect proliferation measurement methods (e.g., ATP, MTT measurement). However, due to technical limitations (see methods section), proliferation in more severe hypoxic (1% O_2) conditions was measured using fixed preparations at the end of 48 hours hypoxic exposure (16).

Chronic hypoxia is known to affect cell proliferation through Hif dependent or independent mechanisms (30,31). In addition, Hif dependent NHE1 regulation has been shown in pulmonary artery (32), in HL-1 cells (16) and in various cancer cell lines (20). Furthermore, NHE1 protein also plays an essential role in cell proliferation through Hif independent pathway (33,34). Under these complex interactions, the aim of this study was to elucidate the unique role of NHE1 activity in cancer cell proliferation during chronic hypoxia. For this purpose, we tested the effects of duration of mild chronic hypoxia, and duration of severe chronic hypoxia on NHE1 activity and proliferation. Firstly, Hif's role in cell proliferation was dissected. Our findings and other studies (21) in HL-1 cells demonstrated that 24 hours of hypoxia (1% O_2 or 2% O_2) induces Hif accumulation where cell proliferation was not affected. These results initially indicate that, anti-proliferative effects may not be apparent in 24 hours. However, cell proliferation also did not change at prolonged exposure of mild hypoxia (2% O_2, 48 hours) (Figure 2A), possibly in the presence of the reported Hif accumulation under similar hypoxic conditions (20). Thus, in tumor derived HL-1 cells, Hif might not suppress proliferation. To mimic those effects, DMOG's effect was also tested. DMOG incubation at 24 hours was reported to induce Hif accumulation in HL-1 cells (21). Unexpectedly, DMOG incubation for 24 hours in normoxia did not mimic hypoxia results and decreased cell proliferation (Figure 2A). Accordingly, these results strengthen the idea that, DMOG does not provide an accurate replication of hypoxia and may decrease cell proliferation Hif-independently (35). DMOG is an inhibitor of 2-oxaglutarate-dependent dioxygenase enzymes which also may activate other Hif-independent signaling pathways (35,36). On the other hand, our previous findings clearly showed that proliferation decreased in a Hif dependent manner at severe chronic hypoxia (16). Therefore, we postulate that the critical hypoxia level and duration that declined cancer cell proliferation occurred only at 1% O_2 and in 48 hours. At this threshold level of hypoxia, we also previously assessed cell viability by measuring pH_i regulation as to preclude apoptotic cells under strong hypoxic stimuli (16). Nevertheless, these

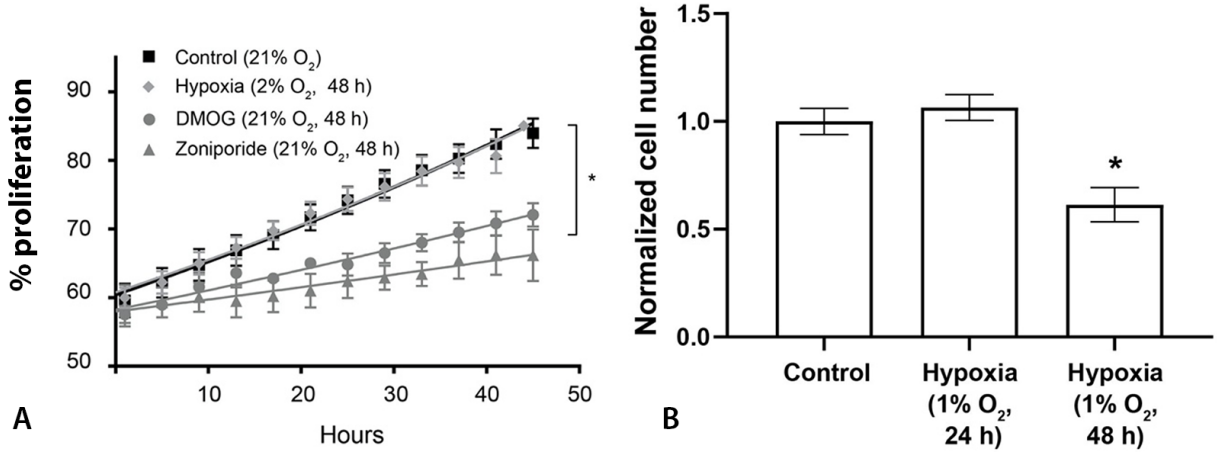

Figure 2: Image based live measurements of cell proliferation depending on confluence (area) detection or proliferation was assessed from cell counts, made from Yo-Pro-1 staining in fixed preparations. A) For live measurements, initial cell density was seeded to be ~60% confluence and then monitored in real time for 48 h in all experimental groups. At the end of the experiment, normoxic control cell confluence ■ was measured as ~80%. Incubation with DMOG (1 mM, •) or zoniporide (30 µM, ▲) for 24/48 h in normoxia decreased, whereas 2% O_2 hypoxia (♦) exposure did not alter cell proliferation. B) Due to technical limitations (see Materials and Methods), proliferation during severe hypoxia (1% O_2) were measured by immunofluorescence and cell numbers were counted from nuclei staining (2.5 µM, Yo-pro-1). Experimental groups were compared using the same confocal microscope settings (magnification) (n=3, *p<0.05, *compared to control). Note: 1% O_2 48 h data were adopted from (15) for comparison

results address at least in HL-1 cells that, Hif probably do not mainly alter cell-cycle progression directly through putative transcriptional targets where these effects were observed at the early stages (6-24 hours) of hypoxia (2% O_2) (37,38). Indeed, it appears more likely that Hif exerts influences on certain post translational pathways affecting proliferation in tumor cells. Our data emphasize the effect of hypoxia (i.e., Hif's) and NHE1 activity on cell proliferation is correlated. In other words, in the presence of Hif accumulation, 24 hours severe hypoxia doesn't alter NHE1 activity or proliferation whereas 48 hours severe hypoxia/DMOG incubation inhibits both NHE1 activity and proliferation. Thus, we propose the main effect of Hif on tumor proliferation is mediated through NHE1 activity in chronic hypoxia.

To support this, NHE1's effect on proliferation is further investigated by 48 hours zoniporide incubation in normoxia. Zoniporide incubation blocked cell proliferation at 24/48 hours. Our preliminary findings predicts that NHE1 inhibition did not necessarily disturb pH_i homeostasis in the presence of HCO_3^- ion. That is, acute zoniporide application did not alter basal pH_i showing that it's pH_i regulatory role could be compensated by HCO_3^--mediated transport (data not shown). This preliminary data makes it difficult to explain its role in cell proliferation due to alkaline shift of the steady state pH_i, induced by the blockage of NHE1 activity (2,3). Correspondingly, the pyrazinguanidine-based chemical structure of the NHE1 inhibitor zoniporide used in this study has been reported to reduce cell proliferation independently from NHE1 inhibition (39). Hence, the exact mechanism of NHE1 protein's antiproliferative action remains to be elucidated. But, NHE1 inhibitors are unlikely to inhibit PHD thereby leads to Hif accumulation in normoxia (40). As a result, we do not rule out the nonspecific actions of zoniporide or DMOG on cell proliferation, but the correlation between NHE1 regulation and cell proliferation in hypoxia still points to the importance of Hif regulated NHE1 activity. The findings of this current study and the related literature are summarized in Table 1.

Table 1: Summarized results and review of the related literature

	Hypoxia (%1 O_2)		Hypoxia (2% O_2)		DMOG (%21 O_2)		Zoniporide (%21 O_2)	
	24s	48s	24s	48s	24s	48s	24s	48s
Hif accumulation	↑*		↑#	↑**	↑#	↑*		
NHE1 activity	↔	↓*		↔		↓	↓	
Proliferation	↔	↓	↔		↓		↓	

Note: (↔) unchanged (↑) increased (↓) decreased compared to control. * The data are from (15), ** The data are from (19), # The data are from (20)

Conclusions

In summary, we propose that Hif downregulates NHE1 activity which leads to decrease in tumor cell proliferation. This relationship implies restriction of NHE1 activity as a key candidate for anti-proliferative action. Additionally, considering that NHE1 is internalized from membrane under severe chronic hypoxic conditions, NHE1 may also be an alternative target for drug delivery against tumor cells. Apart from this mechanism, inhibition of NHE1 activity with pyrazinguanidine-based compounds (such as zoniporide, amiloride) have additional anti-proliferative effects, overall suggesting them as promising compounds in cancer treatment.

Ethics

Ethics Committee Approval: The authors declared that this research does not require ethical approval.

Informed Consent: Since the study was not conducted on humans, consent was not obtained.

Footnotes

Authorship Contributions

Concept: H.B.K., Design: H.B.K., Data Collection and/or Processing: G.Ş., Analysis and/or Interpretation: G.Ş., H.B.K., Literature Search: G.Ş., H.B.K., Writing: H.B.K.

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

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SURGICAL MEDICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

Evaluation of Patients Undergoing Spinal Cord Stimulation Using the e-Health Tool

Spinal Kord Stimülasyonu Uygulanan Hastaların e-Health Tool ile Değerlendirilmesi

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Abstract

Objectives: The aim of the study is to evaluate patients undergoing spinal cord stimulation (SCS) using an online decision support system (SCS e-health tool).

Materials and Methods: In this study, data of patients who underwent SCS between 2005 and 2023 at the Department of Algology, Ankara University Faculty of Medicine, were retrospectively analyzed. The clinical characteristics of the patients were assessed using an e-health tool scoring system proposed by the panel and presented online. Furthermore, the alignment of the implanted SCS devices with the recommendations of this scoring system was evaluated.

Results: It was determined that the median SCS appropriateness score for the 117 patients who underwent SCS implantation was 7, and only 3 patients had the SCS removed during the trial period. All the patients who underwent SCS implantation in our study met the eligibility criteria for SCS according to the e-health tool algorithm.

Conclusion: We observed a much lower rate of SCS removal during the trial period compared to the literature. We believe this is attributable to the multidisciplinary evaluation conducted at our hospital by highly competent specialists from various fields who assess patients for SCS suitability.

Keywords: Spinal cord stimulation, health information systems, decision support systems, chronic pain

Öz

Amaç: Çalışmanın amacı, spinal kord stimülasyonu (SKS) için özel klinik karar destek sistemi (SCS e-health tool) ile SKS uygulanan hastaları değerlendirmektir.

Gereç ve Yöntem: Bu çalışmada, Ankara Üniversitesi Tıp Fakültesi, Algoloji Anabilim Dalı'nda 2005-2023 yılları arasında SKS uygulanan hastaların verileri retrospektif olarak analiz edildi. Hastaların klinik özellikleri, panel tarafından önerilen ve çevrimiçi olarak sunulan bir özel klinik karar destek sistemi (SCS e-health tool) puanlama sistemi kullanılarak değerlendirildi. İmplante edilen SKS cihazlarının bu puanlama sisteminin önerileriyle uyumu değerlendirildi.

Bulgular: SKS uygulanan 117 hasta için uygunluk puanının medyan değerin 7 olduğu ve deneme süresi sonunda sadece 3 hastada SKS çıkarıldığı belirlendi. Çalışmamızda SKS uygulanan tüm hastalar, çevrimiçi karar destek sistemi algoritmasına göre uygunluk kriterlerini karşıladı.

Sonuç: Deneme süresi sonunda literatüre kıyasla çok daha düşük bir SKS çıkarılma oranı gözlemledik. Bu durumun, hastanemizde SCS uygunluğunu değerlendiren, çeşitli alanlardan yetkin uzmanların yer aldığı multidisipliner konseyden kaynaklandığını düşünmekteyiz.

Anahtar Kelimeler: Spinal kord stimülasyonu, sağlık bilgi sistemleri, klinik karar destek sistemleri, kronik ağrı

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Introduction

Spinal cord stimulation (SCS) is considered a therapeutic option for managing refractory pain in conditions such as failed back surgery syndrome, postherpetic neuralgia, traumatic nerve injury, refractory angina pectoris, peripheral vascular disease, neuropathic pain syndromes (NPS), and complex regional pain syndrome (CRPS) (1,2). The efficacy of SCS is mainly based on the gate control theory. Through SCS, Aβ fibers are activated, which inhibits the excitation generated by C fibers in the same area (3). SCS is also believed to activate descending pain inhibitory pathways (2).

Appropriate patient selection and accurate identification of indications are critical for the success of SCS. Patients must be evaluated for eligibility by a multidisciplinary team of experts before the procedure. Additionally, applying an SCS requires lifelong patient cooperation and the ability to manage the device effectively (4). Eligibility criteria for SCS implantation include chronic refractory pain lasting longer than six months, the presence of objective pathology consistent with the reported pain, lack of response to conventional pain management methods, being 18 years of age or older, the ability to understand and accept the risks associated with the treatment, the capability to operate the device, not being pregnant, and the absence of emotional instability or psychiatric disorders (5).

Patients with appropriate indications for SCS are evaluated by a multidisciplinary council comprising specialists in neurosurgery, physical medicine and rehabilitation, algology, neurology, and psychiatry at our institution. Following approval by the council, a trial period of SCS is initiated for eligible patients. Patients who achieve at least a 50% reduction in pain intensity and express satisfaction with the treatment during the 7-10 day trial period are subsequently implanted with a permanent internal generator (6). In cases where patients report insufficient pain palliation during the trial period, the leads and generator are removed.

Recently, a multidisciplinary panel comprising 18 experts (including 10 anesthesiologists, 3 neurosurgeons, 3 psychologists, a specialist nurse, and a physiotherapist) from nine European countries convened to develop an e-health tool. This panel identified absolute indications and contraindications for SCS. Additionally, four main indication areas for SCS were outlined: chronic low back/leg pain (CLBP), CRPS, NPS, and ischemic pain syndromes (IPS). The panel assessed the suitability of SCS based on clinical variables associated with patients meeting these diagnostic criteria (7).

SCS has been used in our clinic for the past 15 years. After being approved by our multidisciplinary team for the trial phase, SCS leads and a temporary generator are implanted. During the trial period, if patients report inadequate pain relief or complications develop, the SCS components are removed. This study aims to evaluate the data of patients presented to the council for SCS implantation and to assess their suitability based on the e-health tool parameters.

Materials and Methods

This study was conducted at Ankara University. The study protocol was approved by the Ankara University Human Research Ethics Committee (decision no.: İ07-543-23, date: 12.09.2023) and was conducted in full compliance with the principles of the Declaration of Helsinki. Patients who were considered for SCS implantation and presented to the Ankara University Faculty of Medicine Movement Disorders Council between January 1, 2005, and August 1, 2023, were included in the study.

SCS e-health tool is accessible via www.scstool.org. A multidisciplinary panel comprising 18 experts (including 10 anesthesiologists, three neurosurgeons, three psychologists, a specialist nurse, and a physiotherapist) from nine European countries convened to develop an e-health tool. Four main indication areas for SCS were outlined: CLBP, CRPS, NPS, and IPS. The panel assessed the suitability of SCS based on clinical variables associated with patients meeting these diagnostic criteria (7). Suitability was determined using the RAND/UCLA appropriateness method (8), applied across 386 potential scenarios. Clinical variables included treatment history, the type/nature and location of pain, anatomical abnormalities, pain distribution, and response to previous procedures. For patients considered for SCS implantation, the system assigns a score on a 9-point scale based on diagnoses and clinical variables. The scoring categorizes 1-3 points as "inappropriate", 4-6 points as "uncertain", and 7-9 points as "appropriate". Additionally, the e-health tool identifies eight psychosocial factors that may influence SCS outcomes, including lack of willingness to participate, impaired coping abilities, unrealistic expectations, inappropriate levels of daily activity, social support issues, secondary gain, psychological stress/mental health concerns, and reluctance to reduce high-dose opioid use (5,7,9).

Statistical Analysis

Demographic data, including age, gender, pain etiology, pain intensity, pain characteristics, and physical examination findings, were collected from hospital records and patient files. Patients approved and not approved by the council and those who underwent trial and permanent SCS implantation were recorded. E-health tool scores for the patients were determined online via https://www.scstool.org. The demographic data and e-health tool scores of patients who underwent trial and permanent SCS were statistically compared. Additionally, any potential complications observed in these patients were documented. The data were

analyzed using IBM SPSS Statistics (Version 29.0.1 for MacOS, Armonk, NY, IBM Corp). The demographic data and e-health tool scores of patients who underwent trial and permanent SCS were analyzed by descriptives and crosstabs. Data were presented as units (n), percentage (%), mean ± standard deviation, median, minimum, and maximum.

Results

A total of 117 patients underwent SCS implantation. Our clinic's initial SCS application was identified as taking place in 2009. The mean age of the patients was 52±12.9 years (range: 18-80). The demographic and clinical characteristics of the patients are presented in Table 1. CLBP (n=95) was the most common condition among the patients, followed by NPS (n=17). The SCS appropriateness score, calculated after analyzing clinical information using the tool, had median values of 7, 7, 7.5, and 5.5 for CLBP, NPS, CRPS, and IPS, respectively. Unwillingness to reduce high-dose opioids (n=2), inadequate daily activity levels (n=1), and both (n=3) were the most common psychosocial factors associated with unfavorable SCS outcomes.

Among the 117 patients, only three patients had NPS had the device removed during the trial period. The mean age of patients with CLBP was 53.7±12 years (range: 26-80), while the mean age of patients with NPS was 45±14.7 years (range: 18-79). The demographic and clinical characteristics of patients with CLBP and those with NPS are shown in Table 2 and Table 3, respectively.

Table 1: Demographic and clinical characteristics of the patients

Variables	n=117
Gender n (%)	
Male	62 (53)
Female	55 (47)
Main indication n (%)	
Chronic low back/leg pain	95 (81.2)
NPS	17 (14.5)
IPS	3 (2.6)
CRPS	2 (1.7)
Dominant type of pain n (%)	
Neuropathic	109 (93)
Nociceptive	-
Ischemic	3 (2.6)
Mixed	5 (4.3)
Response to previous treatments n (%)	
Partial/temporary	37 (31.6)
No relief	80 (68.4)
SCS recommendation grade n (%)	
Suitable	96 (82.1)
May be suitable	21 (17.9)
SCS implantation site n (%)	
Thoracic	102 (87.2)
Cervical	15 (12.8)
Desicion after trial period n (%)	
Permanent implantation	114 (97.4)
Removal	3 (2.6)

n: Patient number, %: Percentage, CRPS: Complex regional pain syndrome, NPS: Neuropathic pain syndromes, IPS: Ischemic pain syndrome, SCS: Spinal cord stimulation

Discussion

SCS is most commonly applied to the thoracic region in our clinic for chronic low back and leg pain. According to the e-health tool, the median score was 7, and no patients were identified as unsuitable for SCS based on this tool. However, due to its insufficient effectiveness, only three patients had their devices removed during the trial period.

SCS has been a technique used in chronic pain management for over 50 years. It is applied in conditions such as NPS, CRPS, and CLBP (1,6). Consistent with the data in the literature, the most common indication for SCS in our patient population was CLBP (10).

Table 2: Demographic and clinical characteristics of patients with chronic low back/leg pain

Variables	n=95
Gender n (%)	
Male	46 (48.4)
Female	49 (51.6)
Previous spine surgery (Yes/No) n (%)	89/6 (93.7/6.3)
Main location of pain n (%)	
Leg	72 (75.8)
Back	-
Mixed (leg and back)	23 (24.2)
Dominant type of pain n (%)	
Neuropathic	92 (96.8)
Nociceptive	-
Mixed	3 (3.2)
Anatomic abnormality n (%)	
Iatrogenic nerve lesion	7 (7.4)
Spinal/foraminal stenosis	-
Recurrent disc	12 (12.6)
Scar tissue	76 (80)
Spinal insitability	-
Response to previous treatments n (%)	
Partial/temporary	28 (29.5)
No relief	67 (70.5)

n: Patient number, %: Percentage

Table 3: Demographic and clinical characteristics of patients with neuropathic pain syndromes	
Variables	**n=17**
Gender	
Male	14 (82.4)
Female	3 (17.6)
Origin of pain	
Traumatic nerve lesion	11 (64.7)
Cervical radicular pain	2 (11.8)
Phantom pain	1 (5.9)
Small fiber neuropathy	1 (5.9)
Brachial plexus injury	1 (5.9)
Arteriovenosus malformation	1 (5.9)
Dominant sypmtom	
Neuropathic	15 (88.2)
Nociceptive	-
Mixed	2 (11.8)
Spread of pain	
Leg(s)	12 (70.6)
Arm(s)	5 (29.4)
Mononeuritis	-
Response to previous treatments	
Partial/temporary	8 (47.1)
No relief	9 (52.9)
SCS implantation site	
Thoracic	13 (76.5)
Cervical	4 (23.4)
n: Patient number, %: Percentage, SCS: Spinal cord stimulation	

In the panel, patients were assessed through questions regarding their treatment history, pain type, and whether they benefited from previous interventional treatments. Based on patient-specific responses, a tool scores out of 9 was assigned. Additionally, the psychosocial status of the patients was evaluated, focusing on factors such as lack of engagement, dysfunctional coping mechanisms, unrealistic expectations, inadequate daily activity levels, problematic social support, secondary gain, psychological distress/mental health problems, and unwillingness to reduce high-dose opioid use (7). Inadequate daily activity levels and unwillingness to reduce high-dose opioids were the most observed compromising factors in our study.

SCS is a more invasive technique compared to other procedures that we perform in pain medicine and is also an expensive method in the context of our country's healthcare conditions. Since SCS is ineffective in some patients, appropriate patient selection is crucial (11). In studies, the median trial success rate has been reported to range between 72% and 82% (12). Common characteristics or factors associated with the risk of poor long-term SCS outcomes include depression, anxiety, catastrophizing, poor coping skills or self-efficacy, abnormal personality traits, inadequate pain acceptance, self-doubt, weak social support, post-traumatic stress disorder, and the presence of secondary gain. Substance use and major psychiatric disorders are also associated with poor outcomes in SCS and may even be considered contraindications for the procedure (11). Additionally, a high BMI, smoking, and high-dose opioid use at baseline are also factors that negatively impact the effectiveness of SCS (11). Typical characteristics associated with poor outcomes include substance dependence, pain catastrophizing, depression, anxiety, and several other factors (6,13). In the study, 3% of patients strongly recommended for SCS experienced failed trials, whereas 46% of patients not recommended based on the e-health tool had unsuccessful trials (9). It was reported that 308 patients (64%) had one or more psychosocial factors to consider when determining suitability for SCS. The three most commonly reported psychosocial factors were psychological distress/mental health problems (42.2%), inadequate daily activity levels (38.1%), and dysfunctional coping (27.2%) (9). In contrast, 95% of the patients who underwent SCS in our clinic had no psychosocial risk factors. The psychosocial risk factors identified in the remaining 5% were inadequate activity levels and/or unwillingness to reduce opioid use. We believe the high success rate of SCS in our clinic is due to the comprehensive psychological evaluation we perform before presenting patients to the multidisciplinary committee. Additionally, the appropriateness score of 7/9 calculated by the e-health tool for our patients underscores our focus on selecting appropriate candidates for committee evaluation.

Study Limitations

Our study has some limitations, including retrospective single-center study design and the short-term follow-up of the patients.

Conclusion

In conclusion, our clinic's workflow correlates with the SCS e-health tool. We believe that this tool can be important to reduce healthcare costs and save time.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ankara University Human Research Ethics Committee (decision no.: İ07-543-23, date: 12.09.2023) and was conducted in full compliance with the principles of the Declaration of Helsinki.

Informed Consent: Consent was not obtained since it was a retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.E.Ö., İ.A., Concept: H.A.Ü., İA., Design: H.A.Ü., İA., Data Collection and/ or Processing: H.E.A., A.B., Analysis and/or Interpretation: H.A.Ü., E.S., G.E.Ö., İ.A., Literature Search: H.A.Ü., E.S., Writing: H.A.Ü., E.S.

Conflict of Interest: There is no potential conflict of interest to declare.

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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

A Real World Study: Efficacy and Safety of Pegylated Interferon Alpha-2a in Patients with Myeloproliferative Neoplasm

Gerçek Yaşam Çalışması: Miyeloproliferatif Neoplazm Hastalarında Pegile İnterferon Alfa-2a'nın Etkinliği ve Güvenliği

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Abstract

Objectives: Several clinical studies have reported promising results in patients with essential thrombocythemia (ET) and polycythemia vera (PV) treated with pegylated interferon alpha-2a (PEG-IFN-α-2a). In this study, we evaluated the efficacy and safety of PEG-IFN-α-2a in patients with myeloproliferative neoplasms (MPNs) followed and treated in our clinic.

Materials and Methods: In this retrospective analysis, we present the outcomes of 39 patients diagnosed with MPNs (25 with ET, 13 with PV, 1 with primary myelofibrosis) who received PEG-IFN-α-2a between 2014 and 2021.

Results: The average age of the participants was 49 years, ranging from 23 to 71. Most patients (92%) had received at least one prior cytoreductive therapy. The median starting dose of PEG-IFN-α-2a was 126 mcg/week (range, 22.5-180 mcg/week), administered by self-injection. The median duration of treatment was 24 months (range, 1-77). The overall response rates in patients with PV and ET were 84.6% and 92%, respectively. The most common adverse events observed during the treatment period were fatigue (71%), myalgia (54%), and arthralgia (53%).

Conclusion: Our results suggest that PEG-IFN-α-2a remains a feasible treatment option, particularly for younger patients who wish to avoid prolonged cytotoxic therapy.

Keywords: Pegylated interferon alpha-2a, polycythemia vera, essential thrombocythemia

Öz

Amaç: Bazı klinik çalışmalarda, pegile interferon alfa-2a (PEG-IFN-α-2a) ile tedavi edilen esansiyel trombositemi (ET) ve polisitemia vera (PV) hastalarında umut verici sonuçlar bildirmiştir. Biz de bu çalışmada, kendi kliniğimizde takip ve tedavi ettiğimiz miyeloproliferatif neoplazm (MPN) tanılı hastalarımızda PEG-IFN-α-2a etkinlik ve güvenilirliğini değerlendirdik.

Gereç ve Yöntem: Bu retrospektif analizde, 2014 ve 2021 yılları arasında tanı almış ve PEG-IFN-α-2a ile tedavi edilmiş, 39 MPN tanılı hastanın (25 ET, 13 PV, 1 primer miyelofibrozis) sonuçlarını sunduk.

Bulgular: Hastaların ortanca yaşı 49 (aralık, 23-71) olup, çoğunluğu (%92) daha önce en az bir sıra farklı sitoredüktif tedaviyi almıştı. PEG-IFN-α-2a'nın ortanca başlangıç dozu 126 mcg/hafta (aralık, 22,5-180 mcg/hafta) olup, hastalar tedaviyi kendine-enjeksiyon ile uygulanmıştır. Hastalar ortanca 24 ay (aralık, 1-77) tedavi almıştır. Polisitemi vera ve ET tanılı hastalarda tüm yanıt oranları sırasıyla %84,6 ve %92 olarak bulunmuştur. Tüm hastalar arasında tedavi süresinde gözlemlenen en yaygın yan etkiler yorgunluk (%71), miyalji (%54) ve artralji (%53) olmuştur.

Sonuç: Sonuçlarımız, PEG-IFN-α-2a'nın, özellikle uzun süreli sitotoksik tedaviden kaçınmak isteyen daha genç hastalar için uygulanabilir bir tedavi seçeneği olabileceğini göstermektedir.

Anahtar Kelimeler: Pegile interferon alfa-2a, polisitemia vera, esansiyel trombositemi

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Introduction

BCR-ABL negative myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are clonal hematopoietic disorders. They are noted for thrombohemorrhagic sequelae, splenomegaly and the potential for transformation to secondary myelofibrosis, acute leukaemia or myelodysplasia (1,2). Cytoreductive therapy (typically with hydroxyurea) initiation is strongly indicated for high-risk ET and PV patients to extenuate the risk of thrombosis (1,2).

Interferon alpha (IFN-α) has been attainable over three decades and is used for treatment various cancers. Among the limited therapeutic options for MPNs, interferons continue to envolve. Nowadays, IFN has been the actual cornerstone of MPNs treatment via antiproliferative, proapoptotic, antiangiogenic and immunomodulatory effects (3). Through declared mechanisms, IFN-α depletes MPNs clones and direct apoptotic effect on hematopoietic progenitors (4). However therapeutic benefits, interferons have limited use for side effect profile and public regulatory approval difficulty.

Recently, interferons gain center of attention again with recombinant long-acting pegylated forms. Pegylated interferons (PEG-IFNs) have improved tolerability with more convenience, less-frequent injection intervals and better side effects profiles. Among all, PEG-IFN-α-2a has achieved meaningfull clinical-hematological as well as molecular and histological responses in several studies (5-8). Herein, we analyze the outcomes of PEG-IFN-α-2a theraphy in patients with MPNs at our centre.

Materials and Methods

Patients received PEG-IFN-α-2a (Pegasys, Roche) at our centre were enrolled in the study. Philadelphia chromosome-negative [Ph(-)] MPNs were identified in accordance with the World Health Organization 2008-2016 criteria. Relevant clinical data were collected from the hospital medical record system retrospectively. The participants were called-up or visited face-to-face to check data accuracy. The large majority of individuals had previously been exposed to at least one cytoreductive agent, with the exception of an off-label first-line use of PEG-IFN-α-2a.

Defination of hydroxyurea resistance/intolerance includes; insufficient depletion (platelet count >600×10^9/L; hematocrit (HCT) >45%; or continuing phlebotomy necessity; or leucocyte >10×10^9/L), progressive splenomegaly in spite of 3 months hydroxyurea ≥2 g/day or occurrence hematological or non-hematological toxicities at any dose (9).

Most patients received PEG-IFN-α-2a via subcutaneous injection once a week at a starting dose of 90 mcg. Other dosing schemes were administered to obtain appropriate response (eg, 90 µg biweekly or monthly). The dose and injection intervals were changed due to on toxicity or insufficient efficacy. The administration of treatment was continued until the patients demonstrated clinically meaningful benefits.

Regular assessments of peripheral blood tests and size of spleen through physical exams were performed at baseline and follow-up visits every 2 to 6 months. The European Leukemia Net (ELN)/International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) criteria were employed to assess the hematological responses of patients with PV and ET (10). Complete hematologic response (CHR) was characterised by normalisation of blood parameters (ET: platelets ≤400x10^9/L; PV: HCT <45% without phlebotomy, platelets ≤400x10^9/L; ET/PV leucocyte <10x10^9/L) with complete disappearance of palpable or imaging splenomegaly/symptoms without thrombotic event. A partial hematologic response (PHR) was defined as a 50% or greater reduction in the platelet count for ET or a reduction in phlebotomy by ≥ 50% or a reduction in spleen size as palpated or imaged by ≥50% in PV (10). The European Myelofibrosis Network (EUMNET)/IWG-MRT criteria were employed to determine the response in patients with MF (11,12).

The analysis of bone marrow histomorphologic data and *JAK2V617F* gene mutation was limited (10,13). Molecular complete remission (CR) required undetectable JAK2V617F levels; PR required ≥50% reduction from baseline (existing at least %20 mutant allele burden) (10,13). Only patients with a detectable JAK2V61F mutation at baseline were assessed for molecular response.

The proportion of severe adverse events was calculated. Thyroid and liver function tests were evaluated for all the participants. Data on the reduction in the need for phlebotomies, and the history of thrombotic and haemorrhagic events before and after therapy were collected. Common Terminology Criteria for Adverse Events (CTCAEs) (version 4.0) was used to classify all AEs. The study was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (date: 10.12.2021, approval no.: İ10-663-21).

Statistical Analysis

For continuous variables, the median (minimum-maximum) or interquartile range (IQR) was given. Categorical variables were presented as frequencies. Response was analysed using the intention-to-treat method. A response rate of ≥35% was considered to indicate efficacy and justify larger studies. Best responses treatment cycles were evaluated. Overall response rates (ORRs) are presented with 95% confidence intervals (CIs). A logistic regression analysis was performed to determine any relationship between CHR and patient demographic, clinical

characteristics, with adjusted odds ratios. SPSS v.20 was used for all analyses.

Results

Patients

A total of 39 patients were included in the study between 2014-2021 (PV, 13; ET, 25; MF,1). The median age of the participants was 49 years (range, 23 to 71). Eight patients (21%) were older than 60 years. The median duration from diagnosis was 52 months (range, 0-269). JAK2V617F mutation was present in 15 patients (41%). At least one previous cytoreductive treatment was given to 92% of patients. Twelve (92%) of 13 patients were resistant or intolerant to hydroxyurea (alone or with anagrelide). The vast majority of patients (87%) had a severe adverse effect of interferon. Additional demographic and clinical features are shown in Table 1.

Therapy

Patients administered PEG-IFN-α-2a for a median of 24 months (range, 1 to 77) at a median initial dose of 126 micrograms/week (mcg/wk) (range, 22.5 to 180). Of all patients studied, 7 (18%) had a dose reduction and 2 (5%) had an escalation of the dose of PEG-IFN-α-2a to achieve the optimal therapeutic benefit. After dose adjustments, the median maximum dose was 135 mcg/wk (range, 22.5-180). At last follow-up, the median dose of PEG-IFN-α-2a was 94.5 mcg/wk (range, 22.5-180).

Reasons for treatment discontinuation were treatment-emergent AEs (n=7, 39%), achieving a durable response (n=4, 22%), skip routine medical check-up due to coronavirus disease-2019 (COVID-19) pandemic (n=3, 17%), pregnancy (n=2, 11%), disease progression (n=1, 5.5 %) and death (n=1, 5.5%). The median follow-up period was 28 months (range, 1-77). At time of analysis 21 (54%) patients were still actively receiving the therapy.

Table 1: Demographic and disease characteristic of myeloproliferative neoplasm patients on PEG-IFN-α-2a

Characteristic	PV (n=13)	ET (n=25)	MF (n=1)	Total (n=39)
Median age	33 (17-57)	38 (20-61)	54	35 (17-61)
Males, n (%)	8 (62)	8 (32)	0	16 (41)
Females, n (%)	5 (38)	17 (68)	1 (100)	23 (59)
Time from diagnosis to initiation of PEG-IFN-α-2a, months	62 (21-125)	44 (0-269)	0	52 (0-269)
<3 years, n (%)	2/13 (15)	12/25 (48)	0	15/39 (38)
3-5 years, n (%)	4/13 (31)	3/25 (12)	0	7/39 (18)
≥5 years, n (%)	7/13 (54)	10/25 (40)	0	17/39 (44)
JAK2V617F-positive (%)	7/11 (64)	7/25 (28)	1/1 (100)	15/37 (41)
Median white blood cell count, $\times 10^9$ /L	7.8 (4-24)	9.5 (2.7-15.4)	8.1	8.52 (2.7-24)
White blood cell count ≥11×10^9 /L, n (%)	3 (23)	5 (20)	0	8 (21)
Median hemoglobin, g/dL	16.1 (13.5-18.5)	12.6 (9-16.6)	13.5	13.6 (9-18.5)
Median haematocrit value	48.9 (43.7-57)	41.1 (26.6-53.1)	42.6	43 (26.6-57)
Median platelet count, $X10^9$ /L	246 (174-1080)	773 (367-1582)	280	614 (174-1582)
Platelet count ≥1000×10^9, n (%)	1 (8)	5 (20)	0	6 (15)
Bone marrow results				
Fibrosis grade 0, n (%)	1/9 (11)	2/24 (8.5)	0	3/34 (9)
Fibrosis grade 1, n (%)	4/9 (45)	11/24(46)	1/1(100)	16/34 (47)
Fibrosis grade 2, n (%)	3/9 (33)	8/24 (33)	0	11/34 (32)
Fibrosis grade 3, n (%)	1/9 (11)	2/24 (8.5)	0	2/34 (6)
Fibrosis grade 4, n (%)	0	1/24 (4)	0	1/34 (3)
Suboptimal, n (%)	0	0	0	1/34 (3)
Blasts, n (%)	0	0	0	0
Significant splenomegaly, n (%)	7/13 (46.7)	8/25 (53.3)	0/1 (0)	15/39 (38.4)
Splenectomy, n (%)	0	1/25 (4)*	0	1/39 (3)
Median number of prior therapies	2 (1-3)	2 (0-3)	1	2 (0-3)

Table 1: Continued				
Characteristic	**PV (n=13)**	**ET (n=25)**	**MF (n=1)**	**Total (n=39)**
Prior therapy				
Phlebotomy, n (%)	12/13 (92.3)	3/25 (12)	0/1 (0)	15/39 (38.4)
Hydroxyurea, n (%)	11/13 (85)	21/25 (84)	1/1(100)	33/39 (85)
Anagrelide, n (%)	0	13/25 (52)	0	13/39 (33)
Hydroxyurea alone, n (%)	5/13 (38)	3/25 (12)	1/1(100)	9/39 (23)
Hydroxyurea+anagrelide, n (%)	0	4/25 (16)	0	4/39 (10)
Interferon alfa, n (%)	7/13 (54)	16/25 (64)	0	23/39 (59)
Interferon alfa alone, n (%)	1/13 (8)	0	0	1/39 (3)
Hydroxyurea+interferon alfa, n (%)	6/13 (46)	7/25 (28)	0	13/39 (33)
Anagrelide+interferon alfa, n (%)	0	2/25 (8)	0	2/39 (5)
Hydroxyurea+anagrelide+interferon alfa, n (%)	0	7/25 (28)	0	7/39 (18)
Previously untreated, n (%)	1/13 (8)	2/25 (8)	0	3/39 (8)
Causes of hydroxyurea discontinued (alone or with anagrelide)				
Resistant/ intolerant, n (%)	5/13 (38)	7/25 (28)	0	0
N/A, n (%)	-	-	1(100)	-
Causes of interferon discontinued (alone or with hydroxyurea, anagrelide or both)				
Severe AEs, n (%)	6/7 (86)	13/16 (81.25)	-	19/23 (83)
Interferon absence	1/7 (14)	2/16 (13)	-	3/23 (13)
Decrease enjection of IFN frequency	0	1/16 (6.25)	-	1/23 (4)
History of major thrombosis, n (%)	1/13 (8) Mesenteric ischemia-ACVD (1)	6/25 (24) PE (1) ACVD (1) PVT-ACVD (1) CVA (2) Venous trombosis in leg (1)	1/1 (100) PE (1)	8/39 (21)

*Before PEG-IFN 2A theraphy, PE: Pulmonary thromboembolism, ACVD: Atherosclerotic heart disease, CVA: Cerebrovascular accident, PVT: Portal vein thrombosis, N/A: Non-applicable, AEs: Advers effect, PV: Polycythemia vera, ET: Essential thrombocythemia, PEG-IFN-α-2a: Pegylated interferon alpha-2a

Toxicity

During the PEG-IFN-α-2a therapy, 92% (34/37) of patients experienced at least one AE, but most of these were grade 1 or 2. The most frequent AEs were fatigue (71%), myalgia (54%), arthralgia (53%), headache (50%), dizziness (38%) and flu-like symptoms (32%).

Nineteen percent (7) of patients experienced grade 3-4 AEs, including elevated liver function tests (n=2), hyperthyroidism (n=2), Hashimoto thyroiditis (n=1) and others (n=2) listed in Table 2. Withdrawal from treatment occurred in all of them. One patient resumed treatment after seven months with the with recovery of thyroid function. The profile of hematological AEs in all patients was grade 1-2 (Table 2). One patient discontinued treatment for one month due to grade 2 thrombocytopenia. All adverse effects were dose independent.

Response

Hematological Response

Thirty-seven patients were evaluable for response. In PV patients, 1 (8%) had a CR and 10 (77%) had a PR, resulting in an ORR of 84.6% (95% CI 82.9-86.7%). In the ET cohort, CR and PR were reported in 9 (36%) and 14 (56%) patients, respectively resulting in an ORR of 92% (95% CI 90.7-93.8%). The best ORR at any time point of theraphy was 92.3% (95% CI 91.1-94.2%) for PV patients and 96% (95% CI 95-97.4%) for ET patients. One patient with MF responded CR by EUMNET criteria and N/A by IWG-MRT criteria. Most responses were achieved within the first 3 months of treatment.

A total of 13 patients lost response (5 PV, 8 ET); nine patients due to disease progression (4 PV, 5 ET; from CR to PR); three patients after dose reduction or discontinuation due to COVID-19 pandemic or achievement of CR (1 PV, 2 ET); transformation to myelofibrosis in one patient with ET. The median time to response was 15 months (IQR, 9-24). Overall responses were still ongoing except for two patients. During the follow-up period, five patients discontinued PEG-IFN-α-2a. Four of the five patients were resumed PEG-IFN-α-2a after a median of 23 months (IQR, 12-39). Achieving a CR was not associated with age, gender, JAK2V617F allele burden, number of prior therapies, or bone marrow fibrosis grade (Table 3).

Table 2: Adverse events occurring in myeloproliferative neoplasms patients						
	PV (n=13)		ET (n=25)		MF (n=1)	
	All grades	Grade 3+	All grades	Grade 3+	All grades	Grade 3+
Hematologic						
Anemia	-	-	2 (8)	-	-	-
Lymphocytopenia	-	-	1 (4)	-	-	-
Thrombocytopenia	1(8)	-	1 (4)	-	-	-
Non-hematologic						
Abdominal pain	-	-	1 (4)	-	1 (100)	-
Alanine aminotransferase	3 (23)	2 (15)	2 (8)	-	-	-
Alopecia	3 (23)	-	6 (24)	-	1 (100)	-
Arthralgia	6 (46)	-	11 (44)	-	1 (100)	1 (100)
Bone pain	2 (15)	-	-	-	-	-
Cough	2 (15)	-	1 (4)	-	-	-
Cramp	2 (15)	-	2 (8)	-	-	-
Depression	2 (15)	-	3 (12)	-	1 (100)	-
Diarrhea	-	-	-	-	1 (100)	-
Dizziness	4 (31)	1 (8)	8 (32)	-	1 (100)	1 (100)
Edema	-	-	1 (4)	-	-	-
Epistaxis	1 (8)	-	1 (4)	-	-	-
Fatigue	10 (77)	1 (8)	13 (52)	-	1 (100)	1 (100)
Fever	3 (23)	1 (8)	3 (12)	-	1 (100)	1 (100)
Flulike symptoms	5 (38)	1 (8)	6 (24)	-	-	-
Headache	6 (46)	-	10 (40)	-	1 (100)	1 (100)
Injection site reaction	2 (15)	-	8 (32)	-	-	-
Insomnia	2 (15)	-	1 (4)	-	1 (100)	1 (100)
Irritability	1 (8)	-	6 (24)	-	1 (100)	-
Lack of appetite	2 (15)	-	1 (4)	-	1 (100)	1 (100)
Mood disorder	1 (8)	-	-	-	-	-
Myalgia	8 (62)	-	10 (40)	-	1 (100)	1 (100)
Mucositis and oral aphthae	1 (8)	-	-	-	1 (100)	-
Pruritus	1 (8)	-	4 (16)	-	1 (100)	1 (100)
Skin redness	-	-	1 (4)	-	-	-
Dry skin	-	-	1 (4)	-	-	-
Swelter	1 (8)	-	1 (4)	-	1 (100)	1 (100)
Thyrioditis	1(8)	-	2 (8)	2 (8)	-	-
Hypothyrodism	-	-	1 (4)	-	-	-
Hyperthyroidism	1 (8)	1(8)	-	-	-	-
Ecchymose	-	-	1 (4)	-	-	-
Nausea and vomiting	2 (15)	-	1 (4)	-	1 (100)	1 (100)

Data was N/A in 2 patients. PV: Polycythemia vera, ET: Essential thrombocythemia, MF: Myelofibrosis

At the time of the most recent assessment, 2 out of 13 patients (15%) had achieved a HCT level below 45% (Figure 1). In patients with PV, 12 out of 13 (92%) were receiving phlebotomy.

The median phlebotomy frequency in the year prior to therapy initiation was 4 (range, 1-48). For these patients, reduction in median number of phlebotomies by 3 (range, 1-6) per year. In addition, 7 out of 12 (58.3%) patients became phlebotomy-independent with treatment. Of the 25 patients with ET 13 (52%) had platelet normalisation (<400x10^9/L). In 2 (8%) patients with ET, the platelet counts decreased by more than 50% without normalisation (Figure 2).

Table 3: Clinical factors and association with complete hematologic response			
Risk factors	**Events/patients, n (%)**	**OR (95% CI)**	**p-value**
Age (continuous)	-	1.05 (0.97-1.1)	p=0.2
Disease type PV ET	 1/9 (11) 9/24 (38)	 3.11 (0.2-40.3) -	 p=0.38
Gender Male Female	 3/12 (25) 7/21 (33)	 1.23 (0.19-7.8) -	 p=0.82
JAK2 mutation No Yes	 8/21 (38) 2/12 (17)	 0.31 (0.34-3) -	 p=0.31
Median dose PEG received, mg/wk	90 (23-180)	1.01 (0.98-1.03)	p=0.44
Prior therapy (continuous)	-	0.85 (0.3-2.4)	p=0.77
Prior thrombosis Yes No	 1/6 (17) 9/27 (33)	 2.43 (0.19-30.5) -	 p=0.49

CHR: Complete hematologic response, OR: Overall response, CI: Confidence intervals, PV: Polycythemia vera, ET: Essential thrombocythemia, PEG: Pegylated interferon

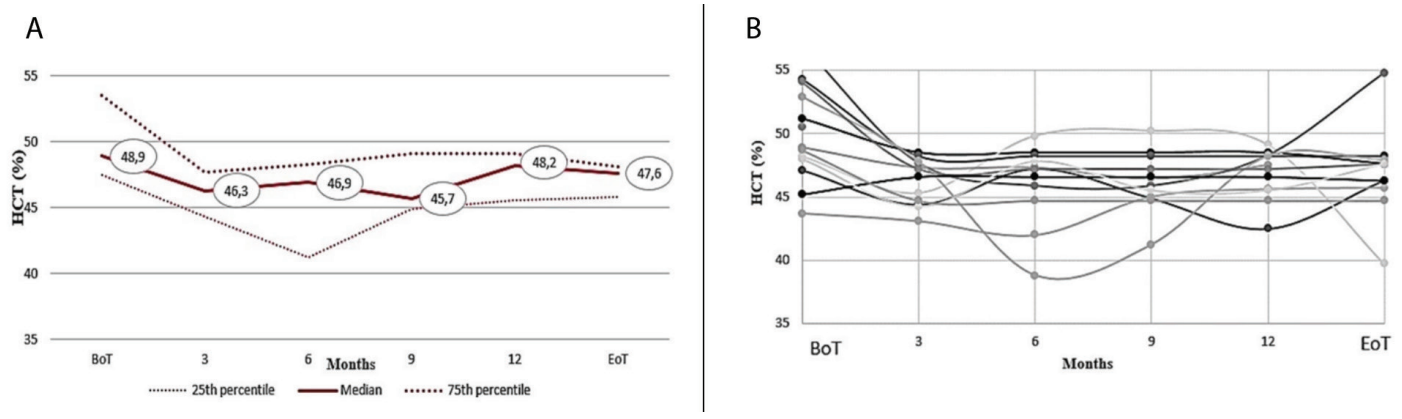

Figure 1: A) Hematocrit counts over time. Figure is shown the 25th, 50th (median) and 75th percentis for hematocrit count in patients with polycythemia vera. **B)** Hematocrit counts over time. Figure is shown the hematocrit counts in all paties with polycythemia vera

BoT: Beginning of treatment, EoT: End of treatment

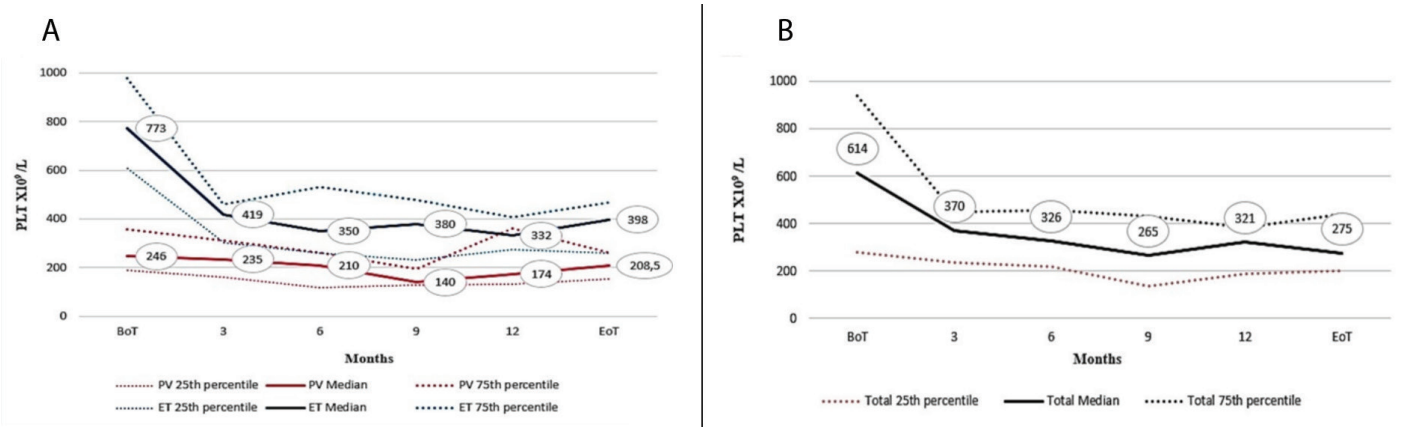

Figure 2: A) Platelet counts over time. Figure shown the 25th, 50th (median) and 75th percentis for platelet count in all patients. **B)** Platelet counts over time. Figure shown the 25th, 50th (median) and 75th percentis for platelet count in all patients with polycythemia vera and essential thrombocythemia

BoT: Beginning of treatment, EoT: End of treatment, PV: Polycythemia vera, ET: Essential thrombocythemia

Spleen Response

Of the 15 (38%) patients with palpable splenomegaly, 10 (66.7%) had a reduction of ≥5 cm. In 6 (60%) of these patients, the spleen became non-palpable.

Vascular Events

No haemorrhagic events occurred during therapy. One ET patient developed unprovoked vena femoralis thrombosis. The patient was 65 years old, had a 16-year medical history and had a PHR to 135 mcg/wk therapy within 38 months of follow-up.

Molecular and Histological Response

Molecular data was limited to JAK2V617F. Molecular response was assessable in 2 out of 7 (29%) ET patients with the JAK2V617F mutation. One achieved PR, with a JAK2V617F allele burden of 31% at baseline and 12.5-31% during follow-up. Other had a NR with stable allele burden in the range of 31-50%. A total of, 4 (10%) patients (2 PV, 2 ET) had a bone marrow response, including those with no response.

Discussion

In this study, we demonstrate that, PEG-IFN-α-2a is effective, safe and has a good tolerability in MPN patients. This analysis supports previous reports of significant clinicohematological and molecular activity also a reduction of phlebotomy, vasculer events related to PEG-IFN-α-2a. In addition, PEG-IFN-α-2a is better tolerated than IFN-α. Obviously, the benefit can be directly observed in patients discontinuing IFN-α due to severe side effects. Unlike some other studies, the majority of patients have exhausted therapeutic alternatives for the refractory nature of the disease, including an ORR of 84.6% in PV and 92% in ET patients.

Our analysis shows CHR rates of 8% and 36% in PV and ET patients, respectively, which are lower than in other studies (6,14-16). This may be due to longer disease duration. In total, 17 (44%) patients had been followed for at least 5 years before PEG-IFN-α-2a. Additionally, patients included in the study had an increased incidence of splenomegaly and vascular events. Impressively, CHR rates were observed in 38% of PV and 68% of ET patients with best response. Prolonged results from a phase 2 trial showed CHR and ORR in 75% and 80% respectively at first response, but 38% and 39% at last assessment (15). It is also important to note that the ORR in our study is higher than in this clinical trial, which may be explained by the different length of follow-up. Also, some de novo mutations, including those in e.g. DNMT3A, TET2, ASXL1,TP53, IDH1/2 have occurred during therapy, but are not targeted by PEG-IFN-α-2a (17,18). These mutant clones may contribute to the decline in response rates over time.

PEG-IFN-α-2a appeared to be well tolerated in most patients. More than two-thirds of MPN patients experienced more than one type of toxicity, even with low-doses of PEG-IFN-α-2a. The highest dose administration of 450 mcg/wk therapy was published by Quintas-Cardama et al. (6). In this study, the highest discontinuation rate was at doses above 180 mcg/wk. In our study, only 3 patients (8%) received the maximum dose of 180 mcg/wk. Therefore, our discontinuation rate was dose-independent. The overall discontinuation rate due to adverse events was 19%, which is consistent with the results of other studies with shorter follow-up (15,19,20). None of the patients required a dose reduction due to adverse events. These data suggest that the optimal dose of PEG-IFN-α-2a has not yet been established and the optimal balance between efficacy and tolerability is being investigated.

The most common grade 3-4 toxicities were liver enzyme elevations, dizziness, fatigue, fever and thyroiditis in two patients each (5%). We have reported some rare adverse events here, that may not have been reported in other studies (e.g. dry skin, ecchymosis, epistaxis, irritability, loss of appetite, mucositis and/or oral aphthae, sweating). Our study demonstrated the lowest risk of hematological toxicities associated with PEG-IFN-α-2a (15,19,20). A total of 3 patients (1 PV, 2 ET) developed autoimmune toxicities. All cases were reported as autoimmune thyroiditis. The median duration of treatment in this group of patients was 24 months. In a long-term follow-up clinical trial (almost 7 years) of PEG-IFN-α-2a, adverse effects appeared to become less frequent over time. 10-17% of patients developed new grade 3/4 toxicities following 24 months of treatment (15). For most of our patients, the incidence of adverse events decreased over time.

In the CYTO-PV study, there was a four-fold reduction in the incidence of major thrombosis in the group with a HCT of less than 45% than in the group with a target HCT of 45% to 50% (21). However, this is difficult to achieve in a short period of time. PROUD/CONTI-PV trials confirms an improved hematologic response with treatment extension in the group receiving ropeginterferon alfa-2b (22). Likewise, in a small chort study, HCT control with ropeginterferon appeared to be unsatisfactory (23). We showed here that the HCT tends to fall slowly, with 69% of patients were in the 45.5-48% HCT range. On another point, PEG-IFN-α-2a reduced the need for phlebotomies (in all but one) and >50% of PV patients became phlebotomy-free. Impressively, the number of phlebotomies required in one PV patient was reduced from 48 to 4 per year with therapy. This also contributed to the reduction in the number of imperative hospital admissions during the COVID-19 pandemic.

In recent trials, the association between leukocytosis and thrombosis has been more questionable (24). On the contrary, the median white blood cell count decreased from 12.4×10^9/L (IQR, 9-14.3) at baseline to 5.7×10^9/ L (IQR, 4.4-8.5) at the end of the study, coupled with a rapid platelet normalisation

[median value was 614×10^9/L (IQR, 280-938) at baseline and 275×10^9/L (IQR, 199-441) at endpoint]. Our experience suggests that PEG-IFN-α-2a may provide superior efficacy in ET patients. Similarly, data from the MPD-RC-111 study manifested that CHR was higher in patients with ET (43%) than in those with PV (22%) (16). This can be explained by the anti-proliferative effect of interferons and the half-life of hematopoietic stem cells (platelets<leukocytes<red blood cells).

Study Limitations

This study has limitations due to its retrospective nature. In addition, moleculer and histological remission status was not quantified in the majority of patients. In some subjects, the JAK2V617F mutation status was analysed as positive regardless of allele burden.

Recent data support that a high JAK2V617F allele burden increases the risk of venous thrombosis (25). However, we found no significant difference in the occurrence of thrombosis among patients who had or had not a JAK2 mutation status prior to PEG-IFN-α-2a treatment (1 PV, 7 ET; 4 JAK2-positive, 4 JAK2-negative). Only one venous thrombosis was recorded in a JAK2-positive case during treatment. We did not observe a link between the baseline JAK2V617F mutation status and achieving a CHR. Some reports have indicated a connection between a reduction in the frequency of the JAK2V617F variant allele and clinical response, with a particular emphasis on its significance in patients with PV (6,15,16).

Published studies have confirmed that bone marrow responses are achieved after a median treatment duration of 48 months (26). The median time to follow-up was 28 months (range, 13-47) in a limited number of patients with bone marrow non-response in our report. One of these patients also completely lost her best HR (CHR) and underwent an allogeneic stem cell transplantation for ET with myelofibrosis transformation. However, although the fibrosis grade increased by one level in a patient with PV, there is little doubt that not repeating bone marrow biopsy for confirmation, as suggested by Masarova et al. (26).

Conclusion

In conclusion, PEG-IFN-α-2a has beneficial effects in our real-world MPN patients, consistent with previous reports. Further prospective, long-term monitoring studies are required to evaluate the effects of PEG-IFN-α-2a on the dynamics and significance of clinical-hematological, molecular and morphological responses. In patients with a poor response to PEG-IFN as a single agent, PEG-IFN-based combination therapy may improve response. The acquisition of clonal genetic mutation analysis is invaluable to the efficacy of PEG-IFN and warrants investigation.

Ethics

Ethics Committee Approval: The study was approved by the Ankara University Faculty of Medicine Human Research Ethics Committee (date: 10.12.2021, approval no.: İ10-663-21).

Informed Consent: Since this was a retrospective study, patient consent was not obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practice: D.K., G.C.S., S.C.B., S.K.T., P.T., Ö.A., M.Ö., Concept: D.K., M.Ö., Design: D.K., M.Ö., Data Collection or Processing: D.K., Analysis or Interpretation: D.K., Literature Search: D.K., M.Ö., Writing: D.K.

Conflict of Interest: There is no potential conflict of interest to declare.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

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BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

Evaluation of the Diagnostic Performance of the Indirect Hemagglutination Test in the Differential Diagnosis of Cystic Echinococcosis: Single Center Experience

Kistik Ekinokokkoz Ayırıcı Tanısında İndirekt Hemaglütinasyon Testinin Tanısal Performansının Değerlendirilmesi: Tek Merkez Deneyimi

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Abstract

Objectives: The purpose of this study is to reveal the epidemiological data of cystic echinococcosis (CE) patients who applied to our hospital and to determine the diagnostic performance of the indirect hemagglutination test (IHAT).

Materials and Methods: The patients whose serum samples were routinely sent to Ankara University İbni Sina Research and Application Hospital, Central Laboratory for CE IHAT between 01.01.2023 and 01.09.2024 were evaluated. The patient's IHAT results were interpreted with clinical and laboratory findings and radiological imaging results. In the evaluation of the IHAT, the serological titer, which is 1/320 and above, is declared positive, and 1/80 and 1/160 are declared as suspicious.

Results: Serology results of 636 patients were evaluated. The positivity rate was found to be 5.97%. The age group of 0-19 had the greatest positivity percentage (19%). Age-group differences in CE seropositivity were statistically significant (p=0.011). The patient group with cystic lesions apparent in radiological imaging had the greatest seropositivity rate (11.9%). The seropositivity rate of IHAT in patients with CE-specific findings identified on radiological imaging was calculated as 39.6% when the threshold value was taken as 1/80. The seronegativity rate was found to be 95.7% in patients with no cysts detected on radiological imaging.

Conclusion: The positivity rate of IHAT was found to be low in patients with CE lesions identified on radiological imaging. Therefore, it is thought that it would be beneficial to utilize a second serological test together with IHAT to increase analytical sensitivity in routine laboratory tests.

Keywords: Cystic echinococcosis, indirect hemagglutination test, seropositivity rates, radiological imaging

Öz

Amaç: Çalışmamızın amacı hastanemize başvuran hastaların kistik ekinokokkoz (KE) epidemiyolojik verilerini ortaya koymak ve indirekt hemaglütinasyon testinin (IHAT) tanısal performansını değerlendirmektir.

Gereç ve Yöntem: Çalışma kapsamında 01.01.2023 ile 01.09.2024 tarihleri arasında KE IHAT istemi ile Ankara Üniversitesi İbni Sina Araştırma ve Uygulama Hastanesi Merkez Laboratuvarı'na rutin olarak serum örnekleri gönderilen hastalar değerlendirildi. Hastaların IHAT sonuçları, klinik ve laboratuvar bulguları ve radyolojik görüntüleme sonuçlarıyla birlikte değerlendirildi. Antikor titresi 1/320 ve üzerindeki değerler pozitif, 1/80 ve 1/160 ise şüpheli olarak belirtildi.

Bulgular: Altı yüz otuz altı hastanın seroloji sonuçları değerlendirildi. Pozitiflik oranı %5,97 olarak belirlendi. 0-19 yaş grubu en yüksek pozitiflik yüzdesine (%19) sahipti. Yaş gruplarının seropozitiflik oranları istatistiksel olarak anlamlı derecede farklı bulundu (p=0,011). Radyolojik görüntülemede kistik lezyonları görülen hasta grubu en yüksek seropozitiflik oranına (%11,9) sahipti. Radyolojik görüntülemelerde KE açısından spesifik bulguları olan

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hastalarda IHAT'ın seropozitiflik oranı eşik değer 1/80 olarak alındığında %39,6 olarak hesaplandı. Radyolojik görüntülemelerde kist saptanmayan hastalarda ise seronegatiflik oranı %95,7 olarak tespit edildi.

Sonuç: Radyolojik görüntülemelerde KE lezyonları tanımlanan hastalarda IHAT'ın pozitiflik oranı düşük bulunmuştur. Bu nedenle rutin laboratuvar testlerinde analitik duyarlılığı artırmak için IHAT ile birlikte ikinci bir serolojik testin kullanılmasının faydalı olacağı düşünülmektedir.

Anahtar Kelimeler: Kistik ekinokokkoz, indirekt hemaglütinasyon testi, seropozitiflik oranları, radyolojik görüntüleme

Introduction

Echinococcus granulosus is a zoonotic parasite that is the causative agent of cystic echinococcosis (CE). Whereas dogs are the parasite's definitive hosts, herbivorous animals, including sheep, cattle, and camels, are intermediate hosts. Humans are accidental intermediate hosts, and transmission to intermediate hosts occurs via ingestion of eggs excreted in infected dog feces through improperly washed contaminated food or hands (1). It is one of the 20 neglected tropical diseases declared by the World Health Organization (WHO). This cestode infection is widespread worldwide, but is particularly prominent in South America, Australia, China, Russia, northern and eastern Africa, and Mediterranean countries. There are more than one million people suffering from echinococcosis in the world, and 19,300 people die due to this disease every year (2,3). Cystic lesions formed during CE are the larval stages of the parasite and most commonly seen in the liver. The second most frequently involved site is the lungs, followed by the spleen, kidneys, eyes, heart, brain, and bones. Lung involvement is more prominent in children than in adults (3).

According to several reports, 3-5% of the liver cysts in the general population are detected by ultrasonography (USG) and 15-18% of them by computed tomography (CT). Liver cysts may be caused by a number of factors besides CE. It is important to carry out the appropriate diagnostic tests to make the differential diagnosis in liver cystic disease of various etiology, such as infection, inflammation, neoplasm, congenital, and trauma (4). CE is diagnosed by using a combination of serological tests and radiological imaging methods. Imaging techniques are useful in both the diagnosis and classification of the cysts. Based on imaging characteristics, the WHO informal working group on echinococcosis (WHO-IWGE) has classified cysts into five groups: active cysts are CE1 and CE2; transitional cysts are CE3 (a and b), and inactive cysts are CE4 and CE5 (5). Serological tests are required to confirm the radiological diagnosis when pathognomonic features, including membrane separation, daughter vesicle presence, and cyst wall calcification, couldn't be detected (6). The immunofluorescent antibody test (IFAT), indirect hemagglutination test (IHAT), and enzyme-linked immunosorbent assay (ELISA) can all be applied for serological diagnosis (7). In serological tests, false negativity may be observed in early-stage and late-stage cysts, cysts located outside the liver, and false positivity may occur due to cross-reactions (8-10). For this reason, it is very important to use radiology and serology in combination to support each other in the diagnosis of echinococcosis.

Our study's objectives are to put forth the epidemiological data of CE patients who applied to our hospital and to determine the diagnostic performance of the IHAT method.

Materials and Methods

In our study, patients whose serum samples were routinely sent to Ankara University İbni Sina Research and Application Hospital, Central Laboratory for CE IHAT between 01.01.2023 and 01.09.2024 were evaluated retrospectively. The patients were categorized into five groups based on age ranges, such as 0-19, 20-39, 40-59, 60-79, and 80 and above. The patient's IHAT results were interpreted together with clinical and other laboratory findings, radiological imaging results, and the reasons for the IHAT request, such as differential diagnosis of a simple cyst, confirmation of CE diagnosis, treatment evaluation, and follow-up. A single sample from each patient was evaluated, and in the case of repeated samples, the first positive sample, if any, was included in the study. Serological test results, laboratory findings, sociodemographic and clinical information, and radiological imaging results of the patients were accessed through the hospital information management system. The classification of the cysts was also documented if available in the radiological imaging report. Early cysts were defined as CE1, CE2, and Gharbi type 1 and 3 cysts; transitional cysts were defined as CE3 and Gharbi type 2 cysts; and late cysts were defined as CE4, CE5, and Gharbi type 4 cysts.

Echinococcus granulosus antibodies in serum were manually detected using a commercial kit (Hydatidose, Fumouze laboratoires, France) in order to diagnose CE. The IHA test was performed in six wells for one serum sample in our laboratory, ranging from 1/80 to 1/2500. If the sera was positive at 1/2500, it was reported as >1/1280. Values of 1/320 and higher were recorded as positive, according to the manufacturer's recommendations, and a positive serological test supported the diagnosis of CE. Titers of 1/80 and 1/160 were considered suspicious, and a test repeat is advised two to three weeks later with the same method, or better, in combination with a different serological test.

The study was approved by the Human Research Ethics Committee of Ankara University (date: 07.10.2024, desicion number: İ08-650-24).

Statistical Analysis

The IBM SPSS 24.0 package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were shown as frequency and percentage (%) for categorical variables and for quantitative variables, mean ± standard deviation if they fit a normal distribution or as median ± minimum-maximum values if they did not fit a normal distribution. The Kolmogorov-Smirnov test was used for normality distribution. The Student's t-test and Mann-Whitney U test were utilized to compare quantitative data between independent groups, and the Chi-square and Fisher's exact tests were used to compare qualitative data.

Results

Serology results of 636 patients between the specified dates were evaluated retrospectively. The average age of women was 54.7±15.3, the average age of men was 52.2±17.2, and there was no statistically significant difference between the average ages of the two groups (p=0.061). The antibody titer of 38 patients was found to be 1/320 and above, and the positivity rate was found to be 5.97%. The serology results of 17 (2.67%) patients were 1/80, and those of 11 (1.73%) patients were 1/160. The seropositivity rate among women was 5.4%, and it was 6.9% among men. The difference between the seropositivity rates according to gender was not statistically significant (p=0.417). The median age of the patients with positive serologic results for CE was 46.5 (6-85) years, whereas those with negative CE serology had a median age of 56 (1-89) years. The difference between the median ages was statistically significant (p=0.003).

Patients were categorized into five groups based on age ranges. The age group of 0-19 had the greatest positivity percentage (19%). Patients aged 80 and above (11.8%) and those aged 20-39 (8.5%) followed the 0-19 age group (Table 1). Age-group differences in CE seropositivity were statistically significant (p=0.011). The patients of whom, in the 0-19 age group, two of the patients had only lung cysts, while one patient had a liver and lung cyst and one had only a kidney cyst.

Analysis of the causes of the requests for CE IHAT revealed that cystic lesions on radiological imaging accounted for the majority of test requests (497/636, 78.1%). In 64 (10.1%) of the patients for whom serological testing was requested, radiological imaging showed nodular lesions, fatty liver, metastatic lesions, etc., but no cystic lesions. Besides, 17 patients (2.7%) were investigated for CE serology because of eosinophilia. The anamnesis of the remaining 58 individuals (9.1%) did not include any information on why the test was requested. Evaluation of IHAT results according to the reason for requesting the test was demonstrated in Table 2. In this section, where IHAT values were compared with radiological and clinical findings, those with IHAT serological test results of 1/80 and 1/160 were evaluated as seropositive unless 1/320 wasn't stated as a threshold. The patient group with cystic lesions apparent in radiological imaging had the greatest seropositivity rate (11.9%). The eosinophilia group did not demonstrate any seropositivity, and the difference among groups based on the reason for the test request was found to be statistically significant (p=0.032) (Table 2).

Moreover, the seropositivity rate was found to be 39.6% in patients with lesions specifically identified as CE in radiological imaging by observing findings such as double-wall appearance or presence of daughter vesicles. This rate was statistically significantly higher than the rate in the group of patients who had no specific findings of CE (p<0.001).

There were 61 patients whose IHAT results were negative even though CE-specific lesions were identified in their radiological imaging. Of the cysts belonging to these patients, 52 (85.3%) were found in the liver, five (9.8%) in other organs (heart, bone, muscle, brain, spleen, etc.), three (5.9%) in the liver and the kidney, and one (2%) in the liver and the lung. Of these individuals, 47 had previously received CE diagnosis, and 18 of them had undergone at least one of PAIR, surgical, and medical interventions. Furthermore, 35 of these patients had cyst staging performed using radiological imaging; nine (25.7%) had early-stage cysts, six (17.1%) had transition-stage cysts, and 20 (57.1%) had late-stage cysts.

CE radiological staging was performed in 17 of 27 patients that had CE-specific findings in the radiological reports and with CE serology test results of 1/320 and above. Of the patients,

Table 1: Evaluation of serology test results according to age groups

Age (year)		Negative	1/320- >1/1280	Total
0-19	n	17	4	21
	%	81.0	19.0	100
20-39	n	85	8	93
	%	91.4	8.6	100
40-59	n	263	18	281
	%	93.6	6.4	10
60-79	n	218	6	224
	%	97.3	2.7	100
80 and above	n	15	2	17
	%	88.2	11.8	100
Total	n	598	38	636
	%	94.0	6.0	100

five (29.4%) had early-stage cysts, six (35.3%) had transitional stage cysts, and six (35.3%) had late-stage cysts. Staging was performed in nine of 13 patients whose serological test results were found to be 1/80 and 1/160, and CE-specific findings were present in radiology. Of the patients, six (66.7%) had early-stage cysts, while three (33.3%) had late-stage cysts (Table 3).

Patients with cystic lesions in the isolated lung and the lung and bone combined had the highest CE IHAT positivity rate (100%) when the serological test findings were analyzed based on the organ involvement in radiological imaging. The involvement of the liver and lung came next, at 85.7% (Table 4). Of the 38 patients whose CE serology was found at 1/320 and

Table 2: Evaluation of serology test results according to the reason for requesting the test

The reason for test request	The antibody titer							
	Negative n (%)	1/80 n (%)	1/160 n (%)	1/320 n (%)	1/640 n (%)	1/1280 n (%)	>1/1280 n (%)	Total n (%)
No cyst in the radiological imaging	63 (98.4)	1 (1.6)	0	0	0	0	0	64 (100.0)
Presence of cyst in the radiological imaging	438 (88.1)	14 (2.8)	9 (1.8)	10 (2.0)	10 (2.0)	5 (1.0)	11 (2.2)	497 (100.0)
Spesific findings for CE	61 (60.4)	6 (5.9)	7 (6.9)	9 (8.9)	6 (5.9)	3 (3)	9 (8.9)	101 (100.0)
No specific finding for CE	377 (95.2)	8 (2)	2 (0.5)	1 (0.3)	4 (1)	2 (0.5)	2 (0.5)	396 (100.0)
Eosinophilia	17 (100.0)	0	0	0	0	0	0	17 (100.0)
Other reason	52 (89.7)	2 (3.4)	2 (3.4)	2 (3.4)	0	0	0	58 (100.0)
Total	570 (89.6)	17 (2.7)	11 (1.7)	12 (1.9)	10 (1.6)	5 (0.8)	11 (1.7)	636 (100.0)

CE: Cystic echinococcosis

Table 3: Evaluation of the stage of the cysts in comparison with the results of the serological test

		The result of IHAT		
Patients that had CE-specific findings	Stage	Negative n (%)	1/80-1/160 n (%)	1/320- >1/1280 n (%)
	Early	9 (25.7)	6 (66.7)	5 (29.4)
	Transitional	6 (17.1)	0	6 (35.3)
	Late	20 (57.1)	3 (33.3)	6 (35.3)
	Total	35 (100)	9 (100)	17 (100)

IHAT: Indirect hemagglutination test, CE: Cystic echinococcosis

Table 4: Evaluation of serology test results according to organ involvement in radiological imaging

Organ involvement	The antibody titer								
	Negative n (%)	1/80 n (%)	1/160 n (%)	1/320 n (%)	1/640 n (%)	1/1280 n (%)	>1/1280 n (%)	1/320- >1/1280 n (%)	Total n (%)
No involvement	124 (94.7)	3 (2.3)	2 (1.5)	2 (1.5)	0	0	0	2 (1.5)	131 (100)
Liver	332 (88.5)	13 (3.5)	7 (1.9)	9 (2.4)	7 (1.9)	3 (0.8)	4 (1.1)	23 (6.1)	375 (100)
Lung	0	0	0	0	0	0	2 (100)	2 (100)	2 (100)
Kidney	28 (93.3)	0	1 (3.3)	0	1 (3.3)	0	0	1 (3.3)	30 (100)
Spleen	15 (93.8)	1 (6.3)	0	0	0	0	0	0	16 (100)
Bone	3 (60)	0	0	0	2 (40)	0	0	2 (40)	5 (100)
Liver + lung	1 (14.3)	0	0	1 (14.3)	0	1 (14.3)	4 (57.1)	6 (85.7)	7 (100)
Liver + kidney	36 (97.4)	0	1 (2.6)	0	0	0	0	0	37 (100)
Liver + spleen	3 (75)	0	0	0	0	1 (25)	0	1 (25)	4 (100)
Lung + bone	0	0	0	0	0	0	1 (100)	1 (100)	1 (100)
Heart, pancreas, gall bladder, muscle, brain and other multiorgan involvement	28 (100)	0	0	0	0	0	0	0	28 (100)
Total	570 (89.6)	17 (2.7)	11 (1.7)	12 (1.9)	10 (1.6)	5 (0.8)	11 (1.7)	38 (6)	636 (100)

higher, isolated liver involvement occurred in 60.5% of cases, followed by liver plus lung involvement in 15.8% of cases and isolated lung involvement in 5.3% of cases. Table 5 shows the location of the cysts in 38 patients with positive CE serology results.

Table 5: Location of cysts detected in radiological imaging of patients with serology test results of 1/320 and above

Organ	n (%)
Liver	23 (60.5)
Liver + lung	6 (15.8)
Lung	2 (5.3)
Liver + spleen	1 (2.6)
Liver + bone	1 (2.6)
Lung + bone	1 (2.6)
Bone	1 (2.6)
Kidney	1 (2.6)
No involvement	2 (5.3)
Total	38 (100)

Discussion

Türkiye is among the endemic countries in terms of CE. Studies on echinococcosis prevalence in the population are mainly based on field studies using USG as a radiological imaging technique and/or serological tests. Besides, there are also retrospective studies that report the serological results of serum samples sent to the laboratory with the request of echinococcosis serology. The later studies are expected to report higher prevalence as the sera investigated belong to prediagnosed or suspected to have CE. In studies based on serological tests, different results were obtained depending on the geographical location of the study, the patient population selected, and the sensitivity of the method(s) used. In Türkiye, seropositivity rates in studies carried out by routine hospital laboratory findings were reported between 9.5 and 34.6% (11-18). The information about the serological studies conducted in Türkiye is given in Table 6. In our study, the CE IHAT positivity rate was found to be 5.97%, which is quite low compared to the seropositivity percentages reported in the literature. It is thought that the positivity rate may be low due to the fact that our hospital is located in Ankara, the capital of Türkiye, and patients mostly reside in urban residential areas. Diagnosis and follow-up of uncomplicated patients is possible at nearby local medical centers. Since it is a university hospital, more complicated cases that need multidisciplinary management are admitted to our hospital.

In most studies in the literature, it is stated that the percentage of CE seropositivity is higher among women (11-13,15). There are also studies, although few in number, that declare it may be more common among men (14). However, in our study, no statistically significant difference was found between the seropositivity percentages in men and women (p=0.417). A small number of studies have also been reported in the literature suggesting that the prevalence of echinococcosis serology is similar in men and women (16-18).

CE is more common in adults and is generally seen between the ages of 20-59 (11,13,14,16,19). However, it can also be detected at an early age, especially in children coming from endemic areas (20,21). Although the incubation period of the disease generally varies between 5 and 15 years, it can also be as low as one year (19,21). When the seropositivity was evaluated according to the age groups in our study, contrary to general findings in the literature, it was observed that IHAT positivity was highest in the 0-19 age group, followed by patients aged 80 and over (Table 1).

It is stated in the literature that 67% of CE is located in the lungs in children (19,22). Consistent with this, in our study, of the four patients with CE serology titer 1/320 and above in the 0-19 age group, lung was involved in two, liver and lung in one, and kidney in one. Therefore, it is important to consider the

Table 6: Studies assessing CE serology conducted in Türkiye

The number serum samples	The positivity rate (%)	The age group with the highest positivity ratio	The test method	City	Time interval	References
2009	9.5	31-45	IHAT	Erzurum	2009-2013	11
3446	32	-	ELISA	Aydın	2005-2017	12
1543	21.6	21-40	IHAT	Konya	2015-2020	13
531	14.1	20-39	IHAT	İzmir	2020-2021	14
1811	28.6	50+	IHAT	Gaziantep	2015-2022	15
454	18	31-50	ELISA, IHAT	Samsun	2005-2011	16
511	34.6	-	IFAT, IHAT	Kars	-	17
938	15.2	41-65	IHAT	Konya	2014-2018	18

IHAT: Indirect hemagglutination test, ELISA: Enzyme-linked immunosorbent assay, IFAT: Immunofluorescent antibody test

possibility of involvement in organs other than the liver when diagnosing echinococcosis, especially in young patients, and to use extensive radiological imaging to evaluate this possibility.

In adults, the liver is accounted for 60-70% of cysts, the lungs for 10-30%, and the spleen, kidney, muscle, heart, bone, brain, ovarium, and pancreas for less than 10% of the cases (3). In our study, 60.5% of patients with an IHAT antibody titer of 1/320 and higher had just liver cysts, 15.8% had liver and lung cysts, 5.3% had lung cysts, and 2.6% had kidney cysts. In 81.6% of patients, liver involvement was noted. There was multiple organ involvement in 23.7% of cases (Table 5). Compared to the studies published in the literature, our analysis revealed a greater level of extra-hepatic organ involvement (14,18).

IHAT is used as the routine serological test in the diagnosis of CE at our laboratory. Serological tests were thought to be most useful in the patient group who was reported to have cysts in the radiological imaging, but specific features for the CE disease couldn't be identified. In addition, it was determined that the patients who were reported to have cysts only in the lung and lung and bone had the highest CE IHAT positivity rate with 100%, followed by the patients with cysts in the lung and liver with 85.7% (Table 4). The common feature of these three patient groups was the lung involvement, suggesting that echinococcosis should be investigated serologically in the differential diagnosis of patients that have lung cysts detected in radiological imaging.

In our study, the serological results of the patients and, if available, USG, CT, and magnetic resonance imaging reports were evaluated together. The seropositivity rate of the patients with CE-specific lesions identified on radiological imaging was calculated as 26.7% with IHAT. Furthermore, the negativity rate of IHAT was found to be 98% in the patients having no cystic lesions in radiological imaging methods. When the threshold value of the test was taken as 1/80, the positivity rate increased to 39.6, and the negativity rate was calculated as 95.7%. The concordance between radiological imaging methods and IHAT significantly increased when the threshold value was set at 1/80 (Table 2). Six patients with antibody titers of 1/80 and seven patients with antibody titers of 1/160 had cysts with CE-specific findings in their imaging. This situation demonstrated that 1/80 and 1/60 antibody titers might likewise be significant and should be reported. The kit insert states that while values of 1/320 and higher are accepted as diagnostically significant, results of 1/80 and 1/160 should also be documented and assessed in conjunction with radiological and clinical findings (14).

The seropositivity rate of the patients with CE-specific lesions in the radiological imaging method was found to be low. To improve the analytical sensitivity of the serological method, it would be advantageous to combine IHAT with at least one of the other serological tests. The National Microbiology Standards Guide recommends using at least two of IHAT, IFAT, and ELISA tests for screening, then Western Blot for confirmation (23).

However, since performing multiple serological tests increases the cost per patient, many centers can only use a single screening test, as seen from other studies conducted in our country (11-15,18).

Of the 61 individuals with specific CE features on radiological imaging and negative serological test results, 47 had previously been diagnosed as CE. Cyst staging was performed in 35 of the patients, and 29 of them had cysts in the early or late stage (Table 3). Serological tests may give false negative results in early and late-stage cysts (7). Of the patients with CE-specific findings but negative serological test results, two patients' surgically removed cyst contents were reported to be hemangiomas, and one patients' pathology was found to be negative for echinococcosis. This demonstrates that there may also be errors in radiological imaging reports. Alveolar echinococcosis (AE) was suspected on radiological imaging of the two patients; however, no further testing had been performed to confirm. Although it is not specific for AE, the IHAT kit used for echinococcosis may produce positive results with a reduced probability. For the diagnosis of AE, specific serological tests for Em2 and Em18 should be applied (24,25). Eighteen of the patients had undergone previous surgical and/or medical treatment. All these reasons could have lowered the sensitivity of IHAT in these patients. Nevertheless, it should not be forgotten that serological tests should only be used as diagnostic aids in the diagnosis of echinococcosis.

Study Limitations

The study's limitations include retrospective design, single-center experience, and restriction of the clinical findings to the data recorded in the hospital information management system.

Conclusion

To our knowledge, this is the first study in which IHAT results were evaluated in comparison with radiological imaging findings in a center in Ankara.

The IHAT positivity rate between 01.01.2023 and 01.09.2024 in our university hospital was found to be 5.97%, which is lower compared to studies conducted in other regions. The positivity rate of IHAT was found to be low in comparison with radiological imaging methods. Reasons for a negative serological test despite having specific findings for CE included patients' having previously received surgical or medical treatment, having AE, and having early or late-stage cysts. It is thought that it would be useful to utilize a second serological test together with IHAT to aid diagnosis in patients with cysts on radiological imaging.

Ethics

Ethics Committee Approval: The study was approved by the Human Research Ethics Committee of Ankara University (date: 07.10.2024, desicion number: İ08-650-24).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Footnotes

Authorship Contributions

Concept: Ö.U.B., G.A., Design: Ö.U.B., Data Collection and/or Processing: Ö.U.B., Analysis and/or Interpretation: Ö.U.B., G.A., Literature Search: Ö.U.B., Writing: Ö.U.B., G.A.

Conflict of Interest: There is no potential conflict of interest to declare.

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SURGICAL MEDICAL SCIENCES / CERRAHİ TIP BİLİMLERİ

The Role of Hand Surgery on Quality of Life and Patient Satisfaction in Rheumatologic Patients

Romatolojik Hastalarda El Cerrahisinin Yaşam Kalitesi ve Hasta Memnuniyeti Üzerindeki Rolü

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Abstract

Objectives: Rheumatologic diseases, particularly rheumatoid arthritis (RA), often lead to hand and wrist dysfunction, significantly impairing patients' quality of life. Despite advancements in pharmacotherapy, deformities, pain, and functional losses persist, necessitating surgical interventions. This study evaluates the outcomes of hand surgeries in rheumatologic diseases, focusing on functional improvements, pain relief, and patient satisfaction.

Materials and Methods: A retrospective analysis was conducted on 17 patients with confirmed rheumatologic diagnoses, including RA, systemic lupus erythematosus, scleroderma, and ankylosing spondylitis, who underwent hand surgeries between 2021 and 2024. Functional outcomes were assessed using the sequential occupational dexterity assessment (SODA) and Duruoz Scale, along with grip strength and pain levels measured using a visual analog scale (VAS). Complications, postoperative erythrocyte sedimentation rate (ESR), and patient satisfaction were also evaluated.

Results: Soft tissue surgeries (79.2%) were more prevalent than bone surgeries (20.8%). Significant improvements were observed in functional outcomes (SODA and Duruoz Scores), grip strength, and VAS scores for general and hand-specific pain. However, ESR values remained unchanged, indicating persistent systemic inflammation. Complications occurred in 23.5% of patients, including infections, tendon adhesions, and recurrence of deformities. Patient satisfaction scores were moderate, highlighting unmet expectations.

Conclusion: Hand surgeries provide substantial functional and pain-related benefits for patients with rheumatologic diseases, but moderate satisfaction levels and high complication rates underscore the need for better preoperative counseling, implant accessibility, and multidisciplinary management. Future studies with larger cohorts are warranted to refine surgical approaches and improve outcomes.

Keywords: Rheumatologic diseases, hand surgery, rheumatoid arthritis, patient satisfaction, functional outcomes

Öz

Amaç: Romatolojik hastalıklar, özellikle romatoid artrit (RA), el ve bilek fonksiyon bozukluğuna yol açarak hastaların yaşam kalitesini olumsuz etkilemektedir. Farmakoterapideki ilerlemelere rağmen, deformiteler, ağrı ve fonksiyonel kayıplar devam etmekte olup, cerrahi müdahaleleri gerekli kılmaktadır. Bu çalışma, romatolojik hastalıklarda el cerrahisi işlemlerinin fonksiyonel iyileşme, ağrı hafifletme ve hasta memnuniyeti üzerindeki etkilerini değerlendirmektedir.

Gereç ve Yöntem: 2021-2024 yılları arasında RA, sistemik lupus eritematozus, skleroderma ve ankilozan spondilit tanılarına sahip ve el cerrahisi geçiren 17 hasta üzerinde retrospektif bir analiz yapılmıştır. Fonksiyonel sonuçlar, sıralı işlevsel el yeterliliği değerlendirmesi (SODA) ve Duruöz Ölçeği ile değerlendirilmiş; kavrama gücü ve ağrı seviyeleri görsel analog skala (VAS) ile ölçülmüştür. Komplikasyonlar, ameliyat sonrası eritrosit sedimentasyon hızı (ESR) ve hasta memnuniyeti de değerlendirilmiştir.

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Bulgular: Yumuşak doku cerrahisi işlemleri (%79,2), kemik cerrahisi işlemlerinden (%20,8) daha yaygındı. Fonksiyonel sonuçlar (SODA ve Duruöz skorları), kavrama gücü ve genel ile el bölgesine özgü ağrı VAS skorlarında anlamlı iyileşmeler gözlenmiştir. Ancak, ESR değerlerinde değişiklik olmamış ve bu durum sistemik enflamasyonun devam ettiğini göstermiştir. Hastaların %23,5'inde enfeksiyon, tendon yapışmaları ve deformite nüksü gibi komplikasyonlar görülmüştür. Hasta memnuniyeti puanları ise orta düzeyde olup, karşılanmamış beklentilere işaret etmektedir.

Sonuç: El cerrahisi işlemleri, romatolojik hastalarda fonksiyonel ve ağrıya bağlı önemli faydalar sağlamaktadır; ancak orta düzeyde memnuniyet oranları ve yüksek komplikasyon oranları, daha iyi ameliyat öncesi danışmanlık, implant erişilebilirliği ve multidisipliner yönetim ihtiyacını vurgulamaktadır. Daha geniş hasta gruplarıyla yapılacak gelecekteki çalışmalar, cerrahi yaklaşımların iyileştirilmesine ve sonuçların geliştirilmesine katkı sağlayacaktır.

Anahtar Kelimeler: Romatolojik hastalıklar, el cerrahisi, romatoid artrit, hasta memnuniyeti, fonksiyonel sonuçlar

Introduction

Rheumatologic diseases are chronic conditions that profoundly impact patients' daily lives by causing deformities, pain, and functional loss in the affected joints. Among the most common autoimmune diseases, rheumatoid arthritis (RA) is characterized by polyarticular synovitis accompanied by bone and cartilage destruction (1).

Pain is one of the most frequently reported and primary symptoms in patients with RA (2-4). When individuals living with RA are asked to identify their most significant symptom, pain is often ranked as the highest priority (2). This underscores the importance of surgical interventions as both diagnostic and therapeutic options. However, the optimal surgical procedures for specific diagnoses, their complication risks, and their impact on patients' functional outcomes and quality of life remain unclear.

In RA patients, the metacarpophalangeal, proximal interphalangeal, and wrist joints are more frequently involved than other joints in the body (5). Up to 70% of patients experience dysfunction in the hand and wrist (6), with the hand being the most commonly affected site in RA. Over the past two decades, significant advancements have been made in the pharmacotherapy of RA. However, many patients continue to experience issues in their hands. Joint damage, tenosynovitis, and tendon ruptures are common and lead to deformities that impair grip and pinch strength. Not all these deformities are suitable for surgical intervention, as patients often adapt to the gradual loss of function despite the deformity (7).

Highly motivated patients concerned about the appearance of their hands often aim to achieve improved daily activity levels and quality of life (8). With the use of methotrexate, biologic disease-modifying antirheumatic drugs (bDMARDs), or emerging targeted synthetic DMARDs (tsDMARDs), previously uncontrolled synovitis has been alleviated, and more than 50% of patients are in remission (3). However, in recent years, "painless" overuse has increased the risk of deformity, osteoarthritis, tendon rupture, and entrapment neuropathy (9).

In contemporary rheumatologic hand surgery, while the use of DMARDs has largely prevented the progression of severe deformities, difficulties in accessing arthroplasty implants have shifted focus toward palliative surgical approaches. Moreover, poor communication between rheumatologists and surgeons has weakened multidisciplinary treatment approaches, reducing referral rates of these patients to surgical units (10,11).

This study aims to explore the fundamental dynamics of hand surgery in rheumatologic diseases, evaluating commonly performed surgical techniques along with their success rates and complications. Particular attention will be given to patient-centered outcomes, including postoperative functional levels, changes in quality of life, and satisfaction with treatment. The primary goal is to assess the benefits and risks of surgical procedures targeting affected joints. The secondary objective is to evaluate the impact of surgery on quality of life and patients' individual satisfaction levels. The findings will provide valuable insights for improving current standards of practice in rheumatologic hand surgery.

Materials and Methods

Study Design and Patient Selection

This retrospective study was conducted to evaluate hand surgery practices in patients with rheumatologic conditions. A total of 17 patients who underwent hand surgery at our clinic between 2021 and 2024 were included. Inclusion criteria were a diagnosis of RA, systemic lupus erythematosus (SLE), scleroderma, or ankylosing spondylitis (AS) confirmed by rheumatologists, receipt of surgical treatment, and a minimum of 6 months of postoperative follow-up. Only primary surgical procedures were included, and revision surgeries were excluded. Furthermore, surgeries were performed on patients where the primary goal of the intervention was to achieve functional improvement and pain relief. Demographic and clinical data such as gender, age, diagnosis, disease duration, drug use (DMARDs), and education level were recorded.

Informed consent was obtained from all patients in accordance with the 1975 Declaration of Helsinki. The research

protocol was approved by the Human Research Ethics Committee of the University of Ankara (decision number: İ11-899-24, date: 13.01.2025).

Data Collection and Evaluation Process

Patient data were retrospectively obtained from medical records, operative notes, and follow-up outpatient clinic records. The parameters reviewed included

1. Demographic and clinical data: Age, gender, diagnosis type, disease duration, DMARD usage, and education level.

2. Surgical data: Total number of surgeries, types of surgeries (classified as bone surgeries or soft tissue surgeries), and the number of affected joints.

3. Functional and Pain Assessments

Sequential Occupational Dexterity Assessment

Used to evaluate hand dexterity. This included both observed tests and patient-reported questions. Observed hand dexterity was assessed using sequential occupational dexterity assessment (SODA), which measures bimanual functional abilities during daily living activities. The test comprises 12 standardized tasks performed under controlled conditions, scored by a specialist, with higher scores indicating greater hand dexterity. The structure, validity, and content of this test have been previously described (4).

Duruoz Scale

This self-reported scale consists of 18 questions designed to evaluate hand disability. The questions are divided into five categories: kitchen tasks, dressing, personal hygiene, work, and other activities. Responses were scored as follows: no difficulty (=0), very little difficulty (=1), some difficulty (=2), much difficulty (=3), almost impossible (=4), and completely impossible (=5). The total score ranged from 0 to 90, with higher scores indicating greater impairment (12).

Grip Strength

In addition to the two hand dexterity measures, precise grip strength in kilograms was measured using a Baseline hydraulic hand dynamometer (New York, USA). Grip strength was measured three times for each hand, and the average score for each hand was used.

Visual Analog Scale

This was employed for both general pain and hand-specific pain assessment. General pain was measured using a visual analog scale (VAS), while hand pain was evaluated using a separate VAS.

1. Laboratory Data

Erythrocyte sedimentation rate (ESR) was recorded in the preoperative and postoperative periods.

2. Patient Satisfaction

Patients completed a brief questionnaire to evaluate their satisfaction with the overall effects of the surgical intervention. This questionnaire included six items rated on a 4-point scale (strongly disagree, disagree, agree, strongly agree). Items are listed in Table 1. Negatively phrased items (3 and 5) were reverse scored. A total score (range: 6-24) was calculated by summing the six items (4).

Surgical Techniques

All surgeries were performed by expert hand surgeons and orthopedic surgeons following standard sterilization and anesthesia protocols. Soft tissue surgeries included synovectomy, ligament reconstruction, tenodesis and tendon transfers, capsuloplasty/interposition, calcinosis excision, ulnar nerve decompression and anterior transposition, and flap-based skin closure. Bone surgeries included arthrodesis and bone resections.

Follow-Up Process and Complication Assessment

Patients were followed postoperatively for an average of 10.29 months. All patients were assessed at baseline and during their latest postoperative outpatient visit. Complications, including infections (superficial or deep), adhesions following tendon transfers, and recurrence of deformities, were evaluated. All complications were recorded within the first three months postoperatively, and appropriate treatment approaches were determined.

Statistical Analysis

In this study, statistical analyses were performed using Jamovi version 2.3.2. The normality of data distribution was evaluated using the Shapiro-Wilk test. Descriptive statistics for numerical data are reported as mean ± standard deviation and range (minimum-maximum values). Categorical data are presented as

Table 1: Patient satisfaction scale

	Strongly disagree	Disagree	Agree	Strongly agree
I am satisfied with the results of the surgery	1	2	3	4
My hand improved because of the surgical procedure	1	2	3	4
The results of the surgery are disappointing	4	3	2	1
The surgery was a success	1	2	3	4
The surgery has been to no avail	4	3	2	1
I would undergo the same procedure again	1	2	3	4

percentages. The Wilcoxon test and Student's t-test were used to compare repeated measures on the same subjects. Statistical significance was determined at a 95% confidence interval, with $p<0.005$ considered statistically significant.

Results

The mean age of the 17 patients included in the study was 51.47 years, and 82.3% of the patients were female. Regarding diagnoses, RA was the most common, accounting for 10 patients (58.8%). Scleroderma and SLE diagnoses were observed equally (17.6% each), while AS was less frequent (5.8%). The average disease duration of 12.17 years indicated that patients had undergone chronic and long-term treatment processes. Additionally, 76.4% of patients used DMARDs, highlighting the widespread use of pharmacological treatment in this group. The demographic and clinical data are presented in Table 2.

Soft tissue surgeries (79.2%) were more common than bone surgeries (20.8%) among the 22 joint procedures performed. The conditions, procedures performed, and number of procedures are summarized in Tables 3 and 4.

Postoperative follow-up lasted an average of 10.29 months, during which significant improvements were observed in functional and pain assessment parameters. SODA, Duruoz Scale, grip strength, and VAS scores for general and hand-specific pain showed significant postoperative improvements. However, unchanged ESR values after three months suggested that inflammatory activity was independent of surgical intervention (Table 5).

The patient satisfaction survey yielded a mean score of 14.58 out of 24, indicating a moderate level of satisfaction. This finding underscores the need for better management of patient expectations.

Postoperative complications occurred in 4 patients (23.5%), including superficial and deep infections, adhesions in tendon transfers, and recurrence of deformities. These findings highlight the need for careful management of postoperative risks in rheumatologic patients.

Surgical interventions improved functional outcomes and pain levels in patients, but factors such as complications and satisfaction levels necessitate more comprehensive evaluation and refinement of treatment approaches.

Table 2: The demographic and clinical data

Age (years)	51.47±7.88 (37-66)
Gender	
Male	3 (17.6%)
Female	14 (82.4%)
Disease	
Rheumatoid arthritis	10 (58.8%)
Scleroderma	3 (17.6%)
Systemic lupus erythematosus	3 (17.6%)
Ankylosing spondylitis	1 (5.8%)
Involved joints	
Proximal interphalangeal joint	7 (31.8%)
Metacarpophalangeal joint	7 (31.8%)
Wrist joint	6 (27.2%)
Elbow joint	2 (9.1%)
Follow-up duration (months)	10.29±5.93 (6-28)
Disease duration (years)	12.17±6.26 (2-22)
DMARD therapy	
Yes	13 (76.5)
No	4 (23.5%)
Education level	
Primary education	3 (17.6%)
High school	10 (58.8%)
University	4 (23.5%)
Satisfaction	14.58±3.16 (9-20)

DMARD: Disease-modifying antirheumatic drug

Discussion

This study aimed to evaluate the effects of hand surgery in rheumatological diseases based on patient-centered outcomes, analyzing its impact on functional results, pain levels, quality of life, and patient satisfaction. The findings highlight both the benefits of hand surgery in rheumatological conditions and the challenges encountered during the process.

Table 3: The conditions, procedures performed, and number of procedures

Condition	Procedure performed	Number of procedures
Boutonniere deformity	Arthrodesis	4
Boutonniere deformity	Thompson procedure	1
Swan neck deformity	Tenodesis	2
Cubital tunnel syndrome	Anterior transposition	2
Calcinosis	Excision	2
Tendon rupture	Tendon transfer	4
Metacarpophalangeal joint involvement	Interpositional arthroplasty	1
Metacarpophalangeal joint involvement	Ligament reconstruction	1
Metacarpophalangeal joint involvement	Crossed intrinsic transfer	4
Metacarpophalangeal joint involvement	Foucher flap	1
Distal radioulnar joint involvement	Darrach procedure	1
Wrist joint involvement	Synovectomy ± ligament reconstruction	2

In this study, soft tissue surgeries were found to be more common than bone surgeries (79.2%). This suggests that, in a patient group where deformities are largely prevented, the primary goal of surgery is usually palliative treatment. The widespread use of DMARD therapy and biological agents has controlled active inflammatory processes such as synovitis, but it has also created a new patient profile characterized by "pain-free deformity" Although adequate pain relief was achieved, inflammation seems to persist, which can lead to significant destruction. This process could be referred to as "silent destruction" (13). A study examining the outcomes of painful and pain-free synovitis found that in long-standing female RA

Table 4: The surgical procedures

Patient number	Age	Gender	Rheumatologic disease	Affected joint	Surgical procedure
1	66	M	Rheumatoid arthritis	Bilateral hip and 5th finger PIP joint (boutonniere)	5th PIP arthrodesis
2	54	W	Rheumatoid arthritis	2nd finger PIP joint boutonniere	Boutonniere deformity reconstruction using PL tendon graft
3	61	W	Rheumatoid arthritis	Wrist	SL reconstruction + synovectomy + joint debridement
4	47	M	Ankylosing spondylitis	Bilateral column, bilateral sacroiliac joint and elbow	Anterior transposition of the ulnar nerve + synovectomy
5	37	W	Systemic lupus erythematosus	2nd, 3rd, 4th, 5th Fingers PIP joint (2nd, 3rd swan-neck 4th, 5th Boutonniere)	2nd -3rd Ulnar lateral band tenodesis + 4th -5th PIP Arthrodesis + synovectomy + joint debridement
6	57	W	Systemic lupus erythematosus	Bilateral elbow	Anterior transposition of the ulnar nerve + synovectomy
7	63	W	Rheumatoid arthritis	Bilateral knee and right wrist	Tendon transfer EIP to 4th EDC + tenosynovectomy
8	56	W	Rheumatoid arthritis	5th MP Joint	5th MP joint collateral ligament reconstruction with PL tendon graft + synovectomy + joint debridement
9	55	W	Rheumatoid arthritis	Wrist	Darrach procedure + joint debridement + synovectomy + EIP transfer to 3rd-4th-5th EDC
10	53	W	Scleroderma	3rd PIP joint (skin defect)	3rd PIP arthrodesis
11	41	W	Rheumatoid arthritis	Wrist	EPL rupture-EIP transfer + synovectomy
12	49	W	Rheumatoid arthritis	2nd MP joint	2. Mp joint dorsal capsule interposition arthroplasty + joint debridement
13	51	W	Scleroderma	Bilateral knee and elbow	Elbow subcutaneous calcinosis excision
14	43	W	Rheumatoid arthritis	2nd, 3rd, 4th, 5th Mp joint (ulnar drift hand)	Crossed intrinsic transfer + synovectomy + joint debridement
15	44	M	Scleroderma	2nd MP joint (skin defect)	Foucher flap
16	50	W	Systemic lupus erythematosus	Elbow	Elbow synovectomy + calcinosis excision
17	48	W	Rheumatoid arthritis	Wrist	Arthroscopic synovectomy

PIP: Proximal interphalangeal, PL: Palmaris, SL: Scapholunate, EPL: Extensor pollicis longus, M: Men, W: Women, EDC: Extensor digitorum communis

Table 5: The preoperative and postoperative evaluation of SODA, Duruoz Scale, grip strength, and VAS scores for general and hand-specific pain

	Pre-operative	Postoperative	p-value
ESR	32.11±6.67 (22-47)	33.47±12.5 (20-63)	0.705
SODA	6.52±2.85 (2-11)	9.76±2.07 (5-12)	0.000
Duruoz Score	43.64±13.52 (23-66)	28.23±13.66 (11-50)	0.000
Grip strength (kg)	12.11±2.47 (9-17)	14.17±3.5 (8-20)	0.001
Over all VAS	6.23±1.34 (4-8)	4.35±1.32 (1-6)	0.001
Hand-specific VAS	5.52±1.54 (3-8)	3.88±1.16 (2-5)	0.003

ESR: Erythrocyte sedimentation rate, SODA: Sequential occupational dexterity assessment, VAS: Visual analog scale

patients, bone erosion, ultrasound inflammatory measurements, radiographic findings, clinical deterioration, and treatment changes progressed similarly in the pain-free group within one year (14). This situation necessitates a reevaluation of surgical indications.

Regarding functional outcomes, significant improvements in both the SODA and Duruoz Scales indicate that surgery contributes to patients' daily living activities. However, the moderate level of patient satisfaction (mean score: 14.58/24) suggests that expectations from surgery were not fully met. The literature has often reported that patient satisfaction is more closely related to cosmetic outcomes rather than functional results (15). Measuring or uncovering patient expectations is an important first step in managing outcomes. The common-sense model suggests that patients' expectations are likely to evolve over time as they acquire new information and gain experiences related to their condition (16). This may cause surgical outcomes to fall short of expectations, especially in patients with high concerns about appearance. Therefore, managing patient expectations accurately before surgery is crucial.

With 20 years of experience in hand surgery, Herren ranked three interventions as clear "winners" for RA treatment based on his scoring system: ulna head resection, dorsal ten synovectomy, and 1st metacarpophalangeal joint arthrodesis (13). In our study, highly successful surgeries included proximal interphalangeal arthrodesis for boutonniere deformity (Figure 1), tendon transfer for tendon rupture (Figure 2), and dorsal wrist synovectomies (Figure 3).

Since the introduction of new medications, the types of surgical procedures have also changed. Procedures that were once common, such as wrist arthrodesis and metacarpophalangeal arthroplasties, are now rarely performed, while wrist arthroplasties and proximal interphalangeal replacements are being done more frequently (13). Limitations in implant usage in our country, especially in bone surgeries, pose a significant obstacle. Challenges in obtaining arthroplasty implants have left patients without surgeries that could provide substantial functional recovery. The prominence of palliative surgeries, instead, may indirectly affect patient satisfaction levels. The lack of implants makes it difficult to fully correct

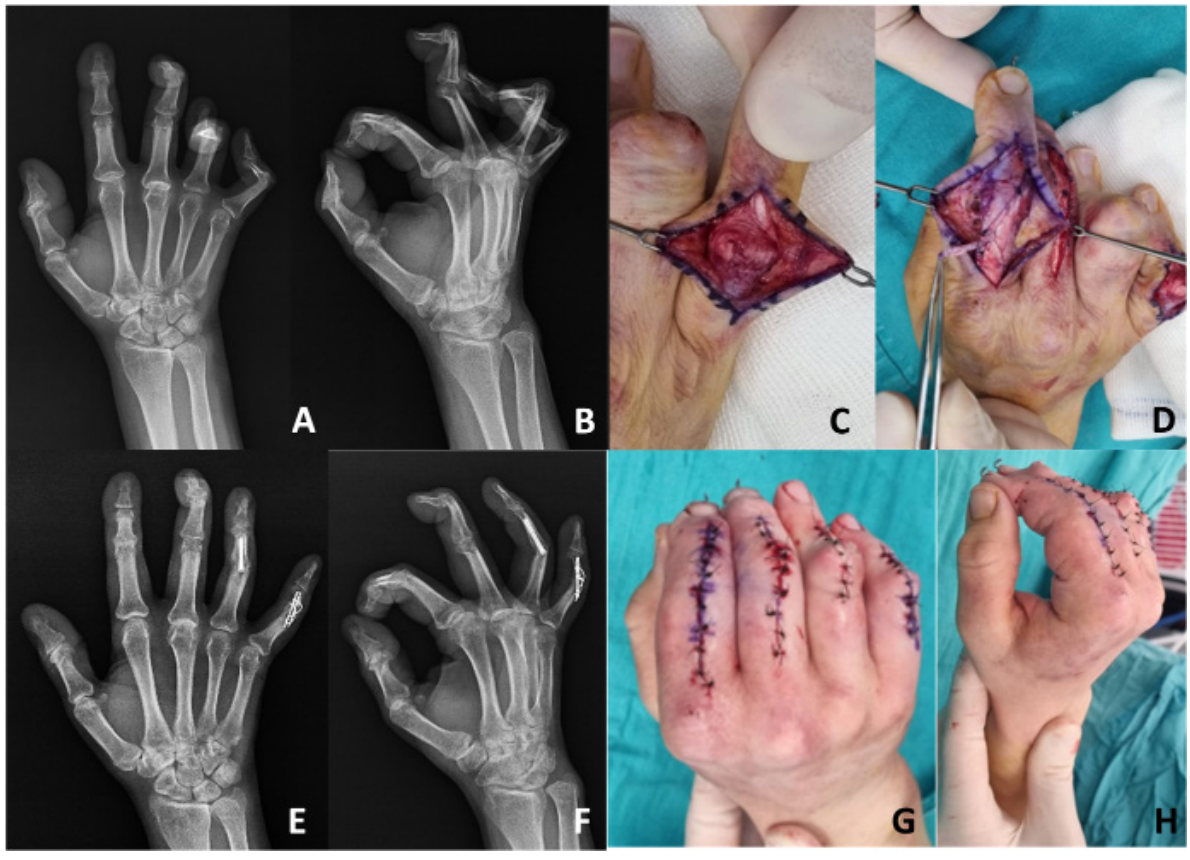

Figure 1: Preoperative findings showed swan-neck deformity in the 2nd and 3rd fingers and boutonnière deformity in the 4th and 5th fingers of the right hand. Surgical correction was achieved using lateral band tenodesis (Littler procedure) for the 2nd and 3rd fingers and PIP joint arthrodesis for the 4th and 5th fingers. Preoperative anteroposterior and oblique radiographs (A-B) illustrate the deformities, while intraoperative images (C-D) display the tenodesis technique. Postoperative radiographs (E-F) confirm the structural corrections, and clinical photographs (G-H) demonstrate the resolution of the deformities, with restored alignment and functionality

PIP: Proximal interphalangeal

deformities and limits long-term functional gains. In cases where degenerative processes, such as RA, progress rapidly, the use of appropriate implants could improve the positive effects on patients' quality of life.

The complication rate of 23.5% underscores the potential risks of surgery in rheumatological diseases. The particularly high infection rates indicate the association of rheumatological diseases with immunosuppressive treatments. In this study, patients with wound infections were receiving non-biological DMARDs, which had been discontinued one to two weeks before surgery. In this context, preoperative (such as DMARD discontinuation) and postoperative approaches for infection control should be implemented more rigorously (17). Additionally, adhesions and recurrence of deformities in tendon transfers highlight the importance of postoperative rehabilitation.

Another noteworthy finding in the study was the lack of significant changes in ESR values post-surgery. This suggests that, despite the local effects of the surgery, there is no direct impact on systemic inflammatory activity. This finding indicates that surgical treatment cannot replace drug therapies such as DMARDs, but rather plays a complementary role.

The predominance of female patients in the study group (82.3%) corresponds with the higher prevalence of rheumatological diseases in women (18). Additionally, the low

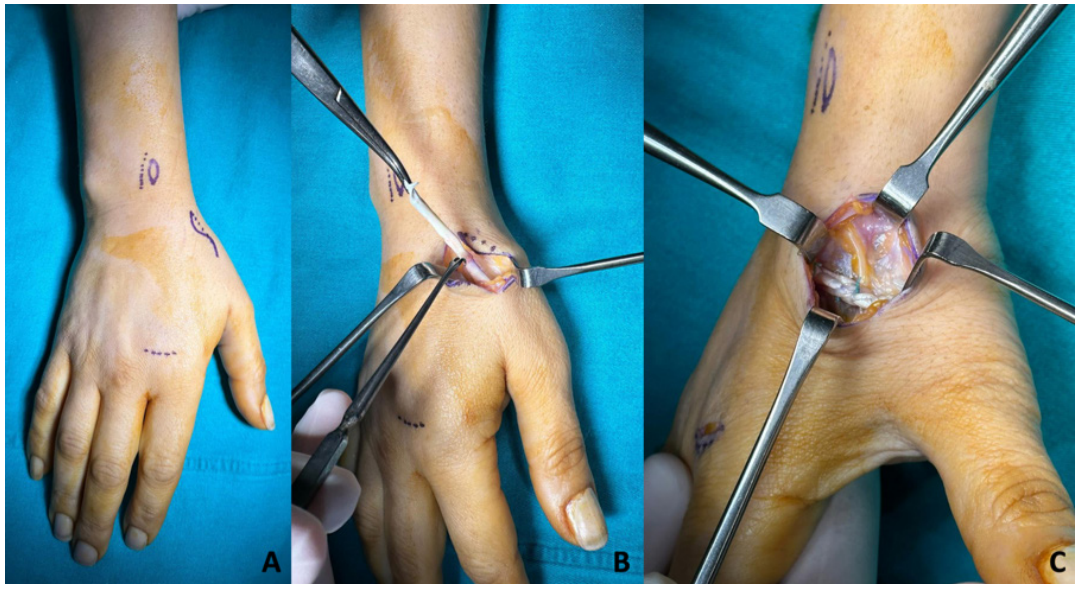

Figure 2: Tendon transfer following chronic EPL rupture. (A) Surgical incision planning. (B) Visualization of the ruptured EPL tendon. (C) Tendon reconstruction performed using the Pulvertaft weave technique
EPL: Extensor pollicis longus

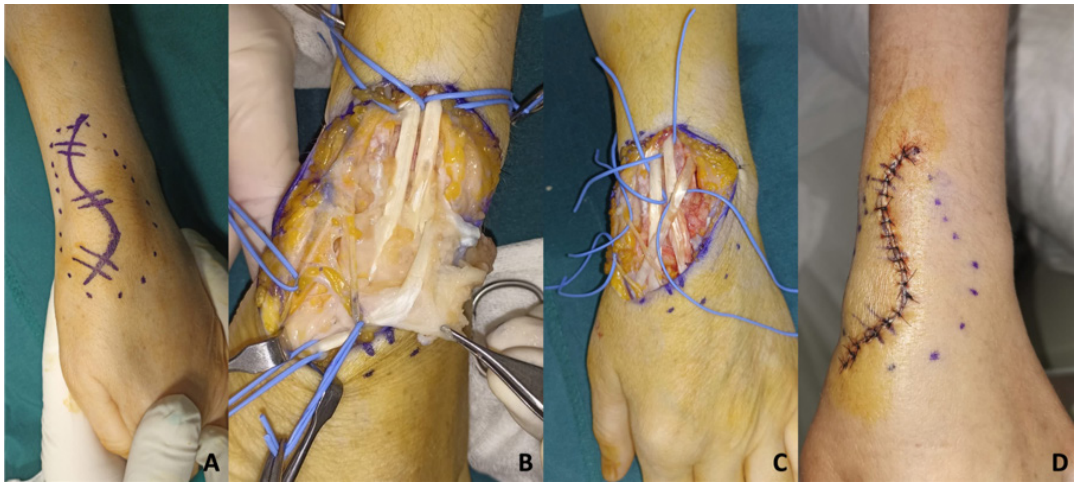

Figure 3: Dorsal wrist synovectomy. (A) Planning of the surgical incision. (B) Dense synovial tissue surrounding the extensor tendons. (C) Appearance after synovectomy. (D) Suturing of the incision line

education level (76.4% without university education) draws attention to the role of health literacy in treatment processes. A cohort study on RA in Egypt found that more highly educated patients had lower disease activity and better functional outcomes, with university education predicting lower disease activity among Egyptian RA patients (19). However, increasing patient education programs and multidisciplinary approaches could contribute to more effective evaluation of surgical outcomes.

Study Limitations

The strengths of this study include the multidisciplinary evaluation of hand surgery in rheumatological diseases and a detailed analysis of patient satisfaction and functional outcomes. However, the study has limitations, including a small sample size (n=17) and a retrospective design.

Conclusion

The findings suggest that surgery is beneficial in terms of functional and pain-related outcomes, but aspects such as patient satisfaction and complications need to be improved. Increasing access to implants, strengthening multidisciplinary approaches, and managing patient expectations are essential measures to enhance the standards of hand surgery in rheumatological diseases. Prospective studies with larger patient groups will deepen the knowledge in this field and offer more comprehensive solutions.

Ethics

Ethics Committee Approval: The research protocol was approved by the Human Research Ethics Committee of the Ankara University (decision number: İ11-899-24, date: 13.01.2025).

Informed Consent: Informed consent was obtained from all patients in accordance with the 1975 Declaration of Helsinki.

Footnotes

Authorship Contributions: Surgical and Medical Practices: U.B., Concept: U.B., Design: U.B., Data Collection and/or Processing: U.B., Analysis and/or Interpretation: M.Y., Literature Search: M.Y., Writing: M.Y.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Malignancy Associated Aortitis in a Patient with Fever of Unknown Origin

Nedeni Bilinmeyen Ateş Etiyolojisinde Malignite İlişkili Aortit Olgusu

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Abstract

Aortitis, characterized by inflammatory changes in the aortic wall, can present with various manifestations. It should be considered in the differential diagnosis of fever of unknown origin (FUO), especially in patients with aortic aneurysm. Accurate differentiation between infectious and non-infectious aortitis is essential for appropriate treatment. Clinicians must also remain vigilant regarding the potential malignancy risk in patients with vasculitis during initial evaluation and follow-up. This study presents a case of malignancy-associated large vessel vasculitis identified in a patient with FUO.

Keywords: Aortitis, large vessel vasculitis, fever of unknown origin

Öz

Aort duvarındaki enflamatuvar değişikliklerle karakterize olan aortit, kendini çeşitli bulgularla gösterebilir. Özellikle aort anevrizması olan hastalarda, nedeni bilinmeyen ateş (NBA) etiyolojisinde akılda tutulmalıdır. Enfeksiyöz ve enfeksiyöz olmayan aortitin ayırıcı tanısı, hastaların uygun tedavisi için yapılmalıdır. Ayrıca, vaskülit tanısı konmuş hastalarda malignite riski konusunda klinisyenlerin farkındalığı, başlangıç değerlendirmesi ve takip için hayati öneme sahiptir. Bu çalışmada, NBA'sı olan malignite ile ilişkili büyük damar vasküliti tanısı konmuş bir hasta sunulmuştur.

Anahtar Kelimeler: Aortit, büyük damar vasküliti, nedeni bilinmeyen ateş

Introduction

Aortitis, defined by inflammatory alterations in the aorta wall, is an important etiology of fever of unknown origin (FUO) as it can present as fever (1,2). Differential diagnosis between infectious and non-infectious aortitis must be made for appropriate treatment of patients (3). The clinical properties of vasculitis are highly variable, so the clinicians must have a strong suspicion to achieve a reliable and definite diagnosis (4). In addition to complexity of the disease, clinicians should be aware of increased risk of malignancy in these patients (5). In this study, a patient with malignancy-associated large vessel vasculitis diagnosed with FUO is presented.

Case Presentation

A 65-year-old male patient exhibited fever on the second postoperative day while hospitalized in the cardiovascular surgery ward. The patient had recently undergone thoracic endovascular aneurysm repair (TEVAR) and aortoiliac bypass surgery due to thoracic aortic aneurysm. He had a history of

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two TEVAR performed two years ago for thoracic and abdominal aortic aneurysms. Piperacillin-tazobactam and teicoplanin was empirically initiated for fever on the second postoperative day. He had no additional symptoms other than ongoing fever. Transthoracic echocardiography was normal. Antibiotherapy was discontinued at day 14, due to the absence of obvious focus of infection and culture negativity (blood and urine).

The patient was evaluated for FUO. There was no specific epidemiological risk factors for infectious diseases. Laboratory parameters indicated the presence of anemia and persistent C-reactive protein elevation not accompanied by leukocytosis and elevated procalcitonin. No pathological findings was detected in endoscopy-colonoscopy performed for the investigation of anemia etiology and malignancy screening. Positron emission tomography/computed tomography (PET/CT) which was performed to screen for malignancy and rheumatological diseases revealed increased methabolic activity in the anterior wall of the aneurysmal segment in the thoracic aorta and in the posterior wall at the level of the proximal iliac bifurcation compatible with aortitis (SUV_{max} 16.3) (Figure 1). In addition to PET/CT findings antinuclear antibody positivity was detected among rheumatological tests. The laboratory tests for brucellosis, syphilis and Q fever were negative. When all the clinical, radiological and laboratory findings were evaluated together, the patient was diagnosed with non-infectious inflammatory large vessel vasculitis/aortitis. Methylprednisolone and methotrexate was initiated by the rheumatologist. Infectious aortitis was excluded in the patient who had clinical improvement and fever response to the immunosuppressive treatment. Due to the diagnosis of vasculitis, whole body CT was performed to screen for malignancy. A nodular solid lesion with 28 mm diameter was detected in the right kidney and biopsy taken from the lesion revealed papillary renal cell carcinoma type 1.

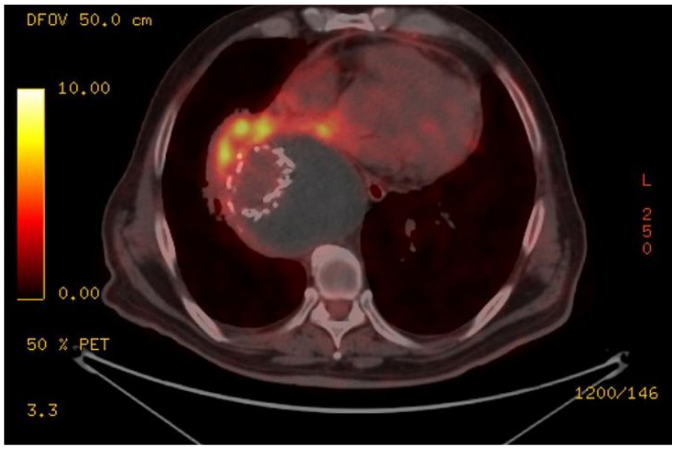

Figure 1: The abnormal methabolic activities in the anterior wall of the aort in PET/CT
PET/CT: Positron emission tomography/computed tomography imaging

The radiofrequency ablation of the lesion was performed. The patient was discharged with recovery and followed up with immunsupressive treatment for vasculitis.

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Discussion

Aortitis is often an unanticipated diagnosis in patients being investigated for an unexplained fever, chest or abdominal pain depending on the region of aortic involvement (6). As the symptoms are nonspecific a high level of clinical suspicion is necessary for an accurate diagnosis of aortitis (7). Aortitis may encompas a variety of conditions with different prognostic features (1,2). Carrer et al. (8) showed that infectious aortitis had higher mortality rates compared to non infectious aortitis. Differential diagnosis between infectious and non-infectious aortitis must be made, because there are significant differences in the treatment strategies (3). Corticosteroids and immunsuppresive agents for disease activity control is the mainstay of the treatment of non-infectious aortitis (3). Here we presented a case diagnosed with malignancy-associated non-infectious aortitis successfully treated with methylprednisolone and methotrexate.

The clinical spectrum of vasculitis can range from localized involvement to multiple organ manifestations with high morbidity and mortality. In addition to the complexity of the disease, clinicians should be aware of the increased risk of malignancy in these patients (5). Not only malignancy risk can be increased in some types of vasculitis but also vasculitis can be seen as a sign of malignancy (5). Screening for malignancy in patients with vasculitis has an important impact on patient survival as it improves the diagnosis and therapy. In this case, the patient was diagnosed with renal cell carcinoma associated vasculitis and had the opportunity to promptly obtain appropriate intervention for malignancy.

It is important for cardiovascular surgeons to be aware of the inflammatory etiology of aortic aneurysms. As surgical or percutaneous interventions including stenting and ballooning have a high risk of recurrence, vasculitic manifestations in other parts of the aorta and requirement for re-operations in vasculitis (3). Elective reconstructive surgery and endovascular interventions are most likely to succeed when performed in stable remission phases under immunosuppressive treatment (3,4). In case of urgent surgical indications like arterial vessel dissection, large aneurysm, or critical vascular ischemia, the patients should be referred to a vascular team (4). Using perioperative corticosteroids should be considered and postoperative disease activity should be controlled with close monitoring. The risk for

possible complications like anastomotic site aneurysms should be kept in mind of surgeons (3).

Conclusion

As a conclusion, the patient was diagnosed with malignancy associated large vessel vasculitis/aortitis with PET/CT as the etiology of FUO. The inflammatory changes in aortitis often manifest with fever, and the timing and outcomes of medical and surgical treatment depend on the timely and accurate diagnosis. These patients should be evaluated by a multidisciplinary team consisting of cardiovascular surgeon, infectious disease specialist and rheumatologist.

Ethics

Informed Consent: Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.M.S., G.B., E.G., G.Ç., İ.A., A.A., Concept: E.M.S., G.B., E.G., G.Ç., İ.A., M.C.S., A.G., E.Ö., A.A., Design: E.M.S., G.B., E.G., G.Ç., İ.A., A.A., Data Collection and/or Processing: E.M.S., G.B., E.G., G.Ç., İ.A., M.C.S., A.G., E.Ö., A.A., Analysis and/or Interpretation: E.M.S., G.B., E.G., G.Ç., İ.A., M.C.S., A.G., E.Ö., A.A., Literature Search: E.M.S., G.B., E.G., G.Ç., İ.A., A.A., Writing: E.M.S., G.B., E.G., G.Ç., İ.A., M.C.S., A.G., E.Ö., A.A.

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