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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

PRİMER İMMÜN TROMBOSİTOPENİDE ARTMIŞ RETİKÜLER LİF DERECESİ

INCREASED RETICULAR FIBER GRADE IN PRIMARY IMMUNE THROMBOCYTOPENIA

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ÖZET

AMAÇ: Primer immün trombositopenide (İTP) kemik iliği fibrozu, trombopoeitin reseptör agonist tedavileri sırasında fibrozisli vakalar bildirildiğinden ilgi odağı haline gelmiştir, ancak hastaların tanı anında fibrozis durumunu değerlendiren az sayıda çalışma vardı. Bu çalışmanın amacı İTP'li hastalarda kemik iliği fibrozisinin özellikle tedaviye yanıt ve prognoza etkisini değerlendirmektir.

GEREÇ VE YÖNTEM: Hastaların kemik iliği retikülin lif derecesi, hemoglobin, trombosit, yaş, cinsiyet, komorbiditeleri, başvuru sırasında hepatit ve otoimmün belirteçleri, yanıt, remisyon durumu ve remisyon süresi ve tedaviler hastaların tıbbi dosyalarından kaydedildi ve her parametre, İTP'li 53 hastada retiküler lif derecesi ile bir ilişki açısından değerlendirildi.

BULGULAR: Hastaların %79,3'ünde kemik iliği retikülin içeriği derece 1 veya daha fazlaydı. Kemik iliği retiküler lif derecesi ile tanı anında toplam kan sayımı, birinci, ikinci ve üçüncü basamak tedaviye yanıt süreleri, tedavi sonrası trombosit sayıları ve iki tedavi hattı arasındaki süre, yaş, cinsiyet, komorbidite varlığı, antinükleer antikor pozitifliği ve yanıt oranı ve süresi arasında anlamlı bir ilişki bulunmadı. Tanı anındaki trombosit sayısı ile yaş arasında anlamlı ve pozitif korelasyon ($p<0,05$), tanı anındaki beyaz kan ve nötrofil sayısı ile yaş arasında istatistiksel olarak anlamlı ve negatif korelasyon vardı ($p<0,05$).

SONUÇ: İTP'li hastalarda ilk kez daha yüksek dereceli fibrozis bulundu. ITP ve otoimmün fibrozis arasındaki bağlantıyı doğrulamak için kemik iliği biyopsilerinin takip edildiği prospektif çalışmalara ihtiyaç vardır.

ANAHTAR KELİMELER: Primer immün trombositopenide (İTP), Retiküler lif, Prognoz.

ABSTRACT

OBJECTIVE: Bone marrow fibrosis in primary immune thrombocytopenia (ITP) has become a centre of attention since cases with fibrosis were reported during trombopoeitin receptor agonist therapies but, there have been few studies evaluating the fibrosis status of the patients at diagnosis. The aim of the study was to evaluate the impact of marrow fibrosis on especially response to treatment and prognosis in patients with ITP.

MATERIAL AND METHODS: Bone marrow reticulin fiber grade, haemoglobin, platelets, age, sex, co-morbidities of the patients, hepatitis and autoimmune markers on admission, response, remission status and duration of remission and treatments were recorded from medical files of the patients and each parameter was evaluated for an association with reticular fiber grade in 53 patients with ITP.

RESULTS: 79.3% of patients had marrow reticulin content grade 1 or more. No significant correlations were found between bone marrow reticular fiber grade and total blood count at diagnosis, response times to the first, second- and third-line treatment, platelet counts after treatment and time between two treatment lines, age, gender, presence of comorbidity and antinuclear antibody positivity and response rate and time. There was a significant and positive correlation between platelet count at diagnosis and age ($p<0.05$) and, there was a statistically significant and negative correlation between white blood and neutrophil count at diagnosis and age ($p<0.05$)

CONCLUSIONS: For the first time, higher grade of fibrosis was found in patients with ITP. Prospective studies with follow-up bone marrow biopsies are needed to validate the link between ITP and autoimmune fibrosis.

KEYWORDS: Primary Immune Thrombocytopenia (ITP), Reticular fiber, Prognosis.

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INTRODUCTION

Primary immune thrombocytopenia (ITP) is an autoimmune disorder that is associated with autoantibodies to platelets and in relation to that, a platelet count of < $100x10^9$ L. It has a chronic course especially in adults and its incidence is 3.3/10000 (1). The clinical presentations are highly variable; there may be a sudden onset of bleeding episodes such as petechia and non-palpable purpuras and low platelet counts (<20000 μL); however, in most of the patients, the disease tends to have an insidious onset with relatively high platelet counts. Treatment is usually indicated when bleeding occurs, or platelet counts are below $20x10^9$. Corticosteroids are the first line treatment option in ITP; despite good response rates (80%), many of the patients eventually relapse (2). Splenectomy has been a good choice of second line therapy for decades with good five-year response rate of 60-70% but, post-operative complications, risk of developing infections, thromboembolism and pulmonary hypertension has made the option fallen out of sight with fewer than 25% of patients with relapsed ITP have been receiving this treatment option (3 - 5). Rituximab and immunosuppressives are other alternatives; Rituximab especially induces good response rates at the beginning but on follow up; five-year response rate is 20% (6) ,so, alternative therapies were explored so as to achieve a durable response in ITP. Thrombopoietin receptor agonists (TRA), Eltrombopag and Romiplostim, have been shown to be active in refractory ITP. In extend study, Eltrombopag therapy induces an 85% response rate and durable platelet counts more than $50x10^9$ could be achieved with treatment (7). On the other side, 303 patients who had received Romiplostim in trials were reviewed and Romiplostim usage showed a significant increase of platelet levels in 75% of patients (8) so, both Romiplostim and Eltrombopag became useful options in the treatment of refractory ITP. Adverse effects were usually mild and transient; they did't seem to be related to significant morbidity; however, thromboembolic disease and bone marrow fibrosis were reported. Myelofibrosis wasn't found to be a common event with TRA; most studies did only find a slightly increased risk with usually complete resolution of fibrosis when stopping TRA temporarily, even in some patients, fibrosis resolved completely despite ongoing TRA therapy (3, 8 - 11). Bone marrow fibrosis is caused by increased deposition of reticulin fiber, a special form of collagen, in bone marrow. Bone marrow reticular fiber grade is evaluated by pathologists with special scoring methods, and thus, information about the fibrotic structure in the bone marrow can be obtained. The etiology of bone marrow fibrosis includes hematopoietic and non-hematopoietic malignancies and chronic myeloproliferative disorders. Bone marrow fibrosis in ITP became a centre of attention since cases with fibrosis have been reported during TRA therapies but, in ITP, there were few studies evaluating the fibrosis status of the patients at diagnosis and these studies found no correlation with disease severity (12 - 14). The diagnosis of ITP does not require bone marrow biopsy especially in younger adults; so, in many centres there is not enough data to evaluate the bone marrow findings in ITP. We aimed to study the impact of marrow fibrosis on especially response to treatment and prognosis in patients with ITP who were treated in our centre.

MATERIALS AND METHODS

Newly diagnosed two hundred sixty-four patients with ITP who were followed up in Suleyman Demirel University Medical Hospital between 2006 and 2018 were at first included and retrospectively evaluated in this study. Bone marrow reticulin fiber grade, haemoglobin, platelets, age, sex, co-morbidities of the patients, hepatitis and autoimmune markers on admission, response, remission status and duration of remission and treatments were recorded from medical files of the patients. Co-morbidities were grouped as hypertension, diabetes, atherosclerotic heart disease, chronic renal failure, congestive heart disease, cerebrovascular disease and hyperlipidemia. Formalin-fixed, paraffin-embedded bone marrow biopsies obtained at the diagnosis were available for all of the patients. Reticulin fiber grade was evaluated with the Thiele grading scale by an expert pathologist working in the field of hematopathology. According to this scale, bone marrow fibrosis was defined according to reticulin content of bone marrow as described by Thiele (Grade 0-3) (15). Response to a treatment

was defined as a platelet value over 30000x103/µL and loss of treatment response was defined as platelet levels below 30000x103/µL. Duration of remission is defined as the time between first- and second-line therapy. The duration of treatment response was recorded as days and, if the response to the previous line treatment was lost, each new treatment modality and platelet counts and how long the patient remains in remission with these treatments had also been recorded. We then evaluated whether marrow fibrosis had any relation to platelets, hepatitis markers, autoimmune markers, co-morbid diseases on admission and, the relation to response to specific treatment, remission duration and effect on multiple line therapy. Each treatment modality was evaluated separately. We excluded the patients without definitive medical recordings and after those exclusions 53 patients were included in the study.

Ethical Committee

Informed consent form was obtained from all patients. The study was approved by Ethical Committee of Suleyman Demirel University Medical Faculty on 10.06.2021 with number 222. The study was carried out according to the Declaration of Helsinki.

Statistical Analysis

Was done using SPSS 24.0 IBM SPSS Statistics for Windows (version 24.0. Armonk, NY). For descriptive statistics, mean and standard deviation were given for continuous data, and numbers and percentages were given for discrete data. In univariate analysis, $p<0.05$ were considered significant. Chi-square and Fisher exact test were used in the analysis of categorical data, mean comparison was used in the analysis of quantitative data; t test in regular distribution; Mann- Whitney U test for those with irregular distribution; Spearman correlation test was used for correlation analysis.

RESULTS

20 male and 33, female participated in our study. 30.1% of the participants were 65 years and older; 32 (60.3%) did not have comorbidities. Of the comorbidities, 30.1% of the participants had hypertension, 24.5% had diabetes mellitus, 11.3% had coronary artery disease, 3.7% had chronic kidney failure, 1.8% had a history of cerebrovascular accident and 7.5% had hyperlipidemia. For bone marrow reticulin fiber grade of the patients; 20.8% of them were grade 0, 28.3% were grade 1, 37.7% were grade 2, and 13.2% were grade 3 **(Table 1)**. 79.2 % of patients had grade 1 or more reticular fiber and 50.9 % of patients had a reticular fiber grade 2 or more.

Table 1: Characteristics of the Participants and Values at Diagnosis

		n	%
Age	<65	37	69.9
	65	16	30.1
Gender	Male	20	37.7
	Female	33	62.3
Comorbidities*	None	33	62.2
	Hypertension	16	30.1
	Diabetes Mellitus	13	24.5
	Coronary Artery Disease	6	11.3
	Chronic Renal Failure	2	3.7
	History of Cerebrovascular Disease	1	1.8
	Hyperlipidemia	4	7.5
Reticular Fiber Grade	0	11	20.8
	1	15	28.3
	2	20	37.7
	3	7	13.2
Total Blood Count at Diagnosis (Mean ± Standard Deviation)			
Platelet Count	8.55 10^3/µL ± 5,32		
White Blood Count	7.42 10^3/ µL ± 2,63		
Hemoglobin Count	12.9 g/dL ± 2,04		
Neutrophil Count	4.82 10^3/ µL ± 2,13		
Viral Markers			
		n	**%**
Viral Marker N/A		2	3,8
Anti-Hbs-Ag	Negative	35	66
	Positive	16	30.2
HbsAg	Negative	48	90.6
	Positive	3	5.7
Anti-HCV	Negative	51	96.2
	Positive	0	0
Anti-HIV	Negative	51	96.2
	Positive	0	0
Autoimmune Markers			
Anti-nuclear Antibody	Negative/N/A	40	75.5
	Positive	13	24.5
Other Immune Markers	Negative/N/A	52	98.1
	Positive	1	1.9

* Multiple options are marked.

The treatment methods applied to the participants were defined as first, second, third, and fourth-line therapy. Accordingly, 1 mg/kg steroid treatment was given to all patients in the first line treatment. In the second line, 16 patients received 1mg/kg steroid, 5 patients received immunosuppressive treatment (including azathioprine), 3 patients received rituximab, and one patient underwent splenectomy. In addition, seven patients received intravenous immune gloubulin rescue therapy (Four patients taking corticosteroid, two patients taking azathiopurine, one patient taking rituximab). In the third line, 1mg/kg steroid was applied to 5 patients, rituximab to 2 patients, immunosuppressive treatment to 1 patient, eltrombopag to 2 patients, and splenectomy to 1 patient. In the fourth line, 3 patients received eltrombopag **(Table 2)**. The response rate of the participants to the first line treatment was 90.6%, and the mean response time was 3.94 ±(3.72) days.

Response rate to the second line treatment was 84%, mean response time was 3±(1.73) days. Response rates according to treatment modality were 87.5% in patients receiving steroid treatment, 67.7% receiving rituximab treatment, and 80% receiving immunosuppressive therapy.

Table 2: Applied Treatment Methods

		N	%
First Line	Corticosteroid	53	100
	Total	53	100
Second Line	Corticosteroid	16	64
	Rituximab	3	12
	Immunosuppressive	5	20
	Splenectomy	1	4
	Total	25	100
Third Line	Corticosteroid	5	45.4
	Rituximab	2	18.2
	Eltrombopag	2	18.2
	Immunosuppressive	1	9.1
	Splenectomy	1	9.1
	Total	11	100
Fourth Line	Eltrombopag	3	100
	Total	3	100

The response rate to the third line treatment was 72.7%, and the mean response time was 9.33±(4.5) days. In the 3rd line, one patient who underwent splenectomy, 4 of 5 patients who received steroid, 2 patients who received rituximab, and 1 of 2 patients who received eltrombopag had responded to treatment. There was no response in one patient who received immunosuppressive therapy. In patients with relapse, the mean time to transition to the second treatment after response to the first treatment was 209.9 days, and the mean time to transition to the third treatment after response to the second treatment was 35.4 days.

Evaluation of significant correlation between bone marrow reticular fiber grade and total blood count at diagnosis, response times to the first, second- and third-line treatment, post-treatment platelet counts and time between two treatment lines revealed no significant correlation **(Table 3)**.

Table 3: Investigation of Reticular Fiber Grade and Correlation of Some Values with Age

	Mean Value	Correlation with Reticular Fiber Grade P	Correlation with Age P
Platelet Count at Diagnosis	8.55±(5.32)	0.755	**0.028**[c]
White Blood Cell Count at Diagnosis	7.42±(2.63)	0.227	0.170
Hemoglobin Count at Diagnosis	12.90±(2,04)	0.435	**0.014**[b]
Neutrophil Count at Diagnosis	4.82±(2,13)	0.581	**0.021**[b]
Response Time to First Line Treatment	3.94[a]	0.811	0.364
Response Time o Second Line Treatment	3.0[a]	0.989	0.267
Response Time to Third Line Treatment	9.33[a]	0.713	0.827
Platelet Counts in First Response	28.50±(25.5)	0.616	0.661
Platelet Counts in Second Response	42.38±(19)	0.101	0.921
Platelet Counts in Third Response	47.5±(32.7)	0.920	0.870
Time Interval between Loss of Response and Second Line Treatment	209.9	0.954	0.111
Time Interval between Loss of Response and Third Line Treatment	35.4	0.482	0.352

a: day b: negative correlation c: positive correlation

There was a significant and positive correlation between platelet count at diagnosis and age ($p<0.05$). In addition, there was a statistically significant and negative correlation between white blood and neutrophil counts at diagnosis and age ($p<0.05$) (Table 3). Then, we evaluated whether the patients' response to the first- and second-line treatment and the response time to the first- and second-line treatment were related to various variables such as age, gender, presence of comorbidity and antinuclear antibody positivity and response rate and time. However, no statistically significant correlation was found between these variables **(Table 4)**.

Table 4: Analysis of Time of Treatment Response and Response Status According to Some Variables

		Response to First Line		Response to Second Line		Response Time to First Line Treatment	Response Time to Second Line Treatment
		Available(%)	None(%)	Available(%)	None (%)	Mean*	Mean*
Age	<65	86	14	82.4	17.6	3.16	3.38
	≥65	100	0	87.5	12.5	5.50	2.38
		p=0.152**		p=0.618**		p=0.078	p=0.201
Gender	Male	80	20	100	0	3,81	2.55
	Female	97	3	71.4	28.6	4.00	3.50
		p=0.061**		p=0.079**		p=0.416	p=0.213
Comorbidities	None	90.6	9.4	87.5	12.5	4.55	3.23
	Available	90.5	9.5	77.8	22.2	3	2.63
		p=0.667**		p=0.458**		p=0.415	p=0.651
Anti-nuclear antibody	Negative/None	87.5	12.5	84.2	15.8	3.80	3.20
	Positive	100	0	83.3	16.7	4.31	2.50
		p=0.229**		p=0.694**		p=0.991	p=0.418

*day **Fisher's exact test

No significant correlation was observed between the degree of fibrosis and the patient's age ($p=0.4$). The patients with grade 1 fibrosis or more and those without fibrosis were compared by taking the cut-off level at 65 and 75 years of age and there was no significant difference between the age groups in terms of fibrosis ($p=0.2$ and $p=0.3$ respectively). There was no statistically significant correlation between the gender of the patients and the reticulin fiber grade ($p=0.4$). Also, the reticular fiber grade of subset of patients, who received only corticosteroids as second line treatment, were not significantly different when compared to the other treatment groups ($p=0.2$).

DISCUSSION

Platelet-related autoantibody production can be seen in 60-70% of patients with ITP; these antibodies also inhibit platelet production from megakaryocytes (16, 17). It has been reported that some platelet autoantibodies may also cause apoptosis of platelets (18). Although the underlying causes are not fully understood, myelofibrosis can also be seen in autoantibody-related diseases. Autoimmune myelofibrosis can be detected in systemic lupus erythematosus (SLE), rheumatoid arthritis, autoimmune hemolytic anemia and ITP (19). In SLE, autoantibodies, immune complexes, and cytokines, es-

pecially transforming growth factor-β (TGF-β) along with TGF-β, platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), IL-2, IL-8, IL-17 and interferon-γ (IFN-γ) have been shown to be effective in fibrogenesis and those cytokines were blamed in the etiopathogenesis of autoimmune myelofibrosis (19-22). Depending on these factors, collagen is produced by fibroblast activation in the bone marrow (22). In addition, disturbances in collagen breakdown due to decreased collagenase activity may also contribute to fibrosis (23). In studies conducted with chronic ITP patients, among those cytokines related to fibrosis, only elevated levels of IFN-γ and IL-17 were detected; TGF-β levels were found to be similar to control patients (24). Based on these findings, the increase in reticular fiber degree that was seen in patients with ITP seemed to be related to a different pathway, independent of TGF-β and driven by IL-17 and IFN-γ. For this reason, studies evaluating the cytokine pathways responsible for fibrotic and non-fibrotic patients with ITP are clearly needed. The clinics are also quite different from ITP; pancytopenia and splenomegaly are observed in autoimmune myelofibrosis, but none of these entities can be seen besides thrombocytopenia in ITP. Similar to ITP, response to steroid therapy is quite good; generally, complete recovery can be seen regarding to cytopenia and spleen size (20).

Autoimmune myelofibrosis related to ITP has not been thoroughly investigated. There were very few trials evaluating the bone marrow fibrosis in ITP,; therefore, a clear opinion on this subject could not be reached (12 - 14).

Those studies highlighted that the minority of patients had mild increase in bone marrow reticulin content and this had not been related to response to therapy and prognosis. However, in our study, we found that 79.3% of patients had marrow reticulin content grade 1 or more. Ettrup et al. (13), had found only 39% of patients had grade 1 or more marrow fibrosis and, Rizvi et al. (12) found an even lower percentage of patients (29.1%) were in this group. Our results clearly showed that, bone marrow fibrosis in ITP might have been more prevalent than previously thought and this is the first study to demonstrate this finding. In this study, we evaluated whether the bone marrow fibrosis had any impact on therapeutic response and prognosis of the patients with newly diagnosed ITP. We could not find a significant correlation between bone marrow fibrosis and mean duration of remission, platelet levels after therapy and time for response to treatment lines. Corticosteroid therapy is the treatment choice of both ITP and autoimmune myelofibrosis at first line and provides a dramatic improvement in clinical and laboratory findings in patients with autoimmune myelofibrosis. In our study, three of seven relapsed patients with reticular fiber grade 2 or more received corticosteroid therapy and did not need additional therapy until the end of their follow-up (for at least more than twelve month). Bone marrow biopsy was not performed again after remission or relapse after first line treatment in most of our patients; therefore, we could not evaluate whether corticosteroid therapy induced a decrease in bone marrow reticular fiber or relapse was associated with an increase in reticular fiber grade. These three patients were above 65 years old, therefore, we also evaluated whether age over 65 had a correlation with bone marrow fibrosis but there was no significant correlation between age and fiber grade. Also, there was no significant difference between reticular fiber of second line steroid patients and other groups. Therefore, based on our findings, we could not suggest that bone marrow fibrosis may be associated with autoimmunity, or that steroid therapy is associated with remission in patients with high fiber grade, but we believe that this hypothesis can be tested in larger prospective studies. Aging can be related to low haemoglobin and leukocyte counts (25), and we also showed that there was a negative correlation between age and haemoglobin-leukocyte and neutrophil levels of the patients. However, it is also interesting to note that no other studies dealing with ITP and fibrosis could demonstrate this finding. A negative correlation was found between leukocyte counts and testosterone and sex hormone binding globulin levels before and testosterone was also found to significantly and negatively affect platelet reactivity (26, 27). We can speculate that, en-

docrinologic and/or immunologic changes in elderly population adversely affect relapse and treatment need on ITP, but studies are needed to verify our results and test this hypothesis. Fibrosis in ITP could also be related to thrombopoietin receptor agonist therapy, but it was found to be very infrequent (7, 11, 28). We had two patients receiving Eltrombopag but there was no clinical need to evaluate these two patients about increased reticular fiber grade, so no bone marrow biopsy was performed during their follow-up. That's why, we could not evaluate the relationship between fibrosis and Eltrombopag in this study. The main weakness of the study was its retrospective design. If, after steroid therapy and before relapse, had biopsy was taken and the reticular fiber level had been examined, the relationship between fibrosis and remission and relapse could have been revealed more clearly. Apart from this, if approximately forty-one patients with whom we could reach reticular fiber grades but without follow-up data could be included in the study, it would have been possible to study with a larger patient group. However, the main strengths of the study were it presented new information to the literature due to few studies on the subject, had a higher patient population than other studies and the degree of reticular fiber was found higher than other studies.

In conclusion, there was a significant and positive correlation between platelet count at diagnosis and age and significant negative correlation between white blood and neutrophil count at diagnosis and age. We found a higher grade of fibrosis in our study population with ITP for the first time. The American Society of Hematology guidelines did not offer most of the newly diagnosed patients with ITP if there were not additional abnormalities to evaluate (2) but, according to data from this study, every patient with a diagnosis of ITP might need evaluation with a bone marrow biopsy at first. ITP might be related to autoimmune fibrosis and prospective studies with follow-up bone marrow biopsies are needed to validate this hypothesis.

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