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Original Article

The relationship between serum ferritin level and fibrosis and splenomegaly in myelofibrosis

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Abstract: Introduction: Myelofibrosis (MF) is a disease in which the grade of bone marrow fibrosis increases in proportion to the degree of extramedullary hematopoiesis and splenomegaly. Associated with increased cytokines and inflammation, anemia deepens and an increase in serum ferritin levels is also expected. There are no studies addressing the relationship between ferritin and splenomegaly or fibrosis. In this study, the relationship between serum ferritin level and splenomegaly and bone marrow fibrosis was examined. Material and Method: The study was performed retrospectively in 46 MF cases diagnosed between 2012 and 2020. MF was divided into 3 separate subgroups: Primary myelofibrosis, secondary myelofibrosis and myeloproliferative neoplasms (MDS/MPN) with myelodsplastic syndrome. Results: Thirty (28.3%) of cases were PMF, 26 (56.5%) were SMF and 7 (15.2%) were MDS/MPN. There was no relation found between serum ferritin and splenomegaly in none of the cases or subgroup analysis (for PMF p: 0.564, for SMF p: 0.192, for MDS/MPN p: 0.364). There was a statistically significant relationship between serum ferritin and marrow fibrosis within the group of ages 60 years and older (p: 0.016). Discussion and Conclusion: Disruption of hematopoiesis and progressive splenomegaly causes an increase in iron stores associated with an increased need for transfusion. This causes iron-related organ toxicity and bone marrow hematopoiesis disruption, leading to an increase in morbidity. We see that a significant relationship between ferritin and fibrosis has been revealed in the group aged 60 years and older. It is an unprecedented study in the literature in terms of both examining the relationship ferritin and fibrosis or splenomegaly and its results.

Keywords: Myelofibrosis, splenomegaly, ferritin, prognosis

Introduction

Myelofibrosis (MF), which is one of the Philadelphia negative chronic myeloproliferative neoplasms, appears as a disease with bone marrow fibrosis, splenomegaly and pancytopenia [1, 2]. MF may be divided into groups such as primary myelofibrosis (PMF), secondary myelofibrosis (SMF) such as post essential thrombocythemia myelofibrosis (post-ET MF) and post polycythemia vera myelofibrosis (post PV MF) and myeloproliferative neoplasms with myelodisplastic syndrome (MDS/MPN). Organ toxicities that develop as a result of increasing deepening cytopenia and increasing erythrocyte transfusion increase mortality [1-3].

Iron accumulation in the bone marrow over time increases the reactive oxygen derivatives and has a toxic effect on hematopoiesis [4]. Disruption of hematopoiesis and progressive splenomegaly causes an increase in iron overload associated with increased need for transfusion. This causes iron-related organ toxicity and bone marrow hematopoiesis disruption, leading to an increase in morbidity [4]. Therapies that reduce the need for transfusion and lower the level of serum ferritin are used for this purpose. It also contributes to improvement in hematopoiesis by reducing the iron level [5].

Iron accumulates in different organs, causing toxicity, stimulating fibrosis and disrupting the function of many organs [6]. In one reported case, a 67-year-old male patient with PMF was reported to achieve partial transfusion independence with iron chelation therapy [7]. In another study [8], it was indicated that survival

rate increased after iron-binding therapies in patients with PMF. Currently, MF is the only curative treatment for allogeneic stem cell transplantation (ASCT) and only a few of the patients are considered suitable for the transplant. Palliative treatments are often preferred for this reason.

In some recent publications, the negative effect of iron load on long-term morbidity and mortality has been reported in transplant cases [9-11]. In one study, 68.9% of those with ASCT were found to have ferritin levels above 500 ng/ml and it was reported that complications were higher in the early post-transplant cases with high ferritin levels [11]. Additionally, splenomegaly targeted therapies are recommended in MF before ASCT [12, 13]. Bone marrow fibrosis has also been reported decreasing the regression of splenomegaly.

In patients with MF, the progression of the disease and splenomegaly also increases serum ferritin level. Literature review shows that there are no studies examining the increase of ferritin and splenomegaly or fibrosis. In this study, the goal was to investigate the relationship between all subgroups, serum ferritin level and fibrosis and splenomegaly.

Material and method

Patients

The study was carried out retrospectively in 46 cases followed up with the diagnosis of MF in the hematology clinic between 2012-2020. The information was obtained from patient files and electronic records upon obtaining patient consent.

Recorded parameters

Serum ferritin and other biochemical parameters, cytogenetic results such as JAK2 mutation, bone marrow biopsy pathology results and ultrasonography reports were recorded. The cases which meets the definitive diagnostic criteria according to the World Health Organization (WHO) 2016 Myeloproliferative Neoplasms Diagnostic Criteria were included in the study [14].

Patient subgroups

Cases diagnosed with myelofibrosis were divided into 3 subgroups as primary myelofibrosis (PMF), secondary myelofibrosis (SMF) and myeloproliferative neoplasms with myelodysplastic syndrome (MDS/MPN). Post polycythemia vera MF and post essential thrombocythemia MF were considered as SMF.

Ferritin level, spleen size and treatment

The ferritin level was considered as high above 300 ng/ml (reference range 30-300 ng/ml) [15]. In ultrasonography, the spleen vertical size was accepted as splenomegaly over 120 mm [16]. Patients who received JAK inhibitor (ruxolitinib) and iron chelation therapy for minimum of 3 months within the past year were analyzed as subgroups. Additionally, the patients were divided into two groups based on the number of transfusions they received within the last year: with minimum of 10 units and above, under 10 units or without erythrocyte suspension transfusions.

Exclusion criteria

Patients received splenic radiotherapy, had other comorbidities that may cause splenomegaly, received iron replacement therapy within the last year or had a congenital or acquired disease associated with iron deposition were not included in the study.

Statistical analysis

NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used evaluating the study data. The suitability of the quantitative data for normal distribution was tested by Shapiro-Wilk Test and graphical evaluations. Kruskal Wallis Test was used for comparisons of three or more groups that did not show normal distribution, and the Bonferroni-Dunn Test was used for binary comparisons. Spearman's Correlation Analysis was also used to evaluate the relationships between quantitative variables. Significance was evaluated at the level of $P < 0.05$. The evaluation of the correlation coefficient (r) was made according to the following criteria: Very weak (0.0-0.25); weak (0.26-0.49); moderate (0.50-0.69); strong (0.70-0.89); very strong (0.90-1.00).

Results

Descriptive data

Of the 46 patients with myelofibrosis, 52.2% (n: 24) were male and 47.8% (n: 22) were fe-

Table 1. Patients characteristics

Age	*Min-Max (Median)*	31-90 (70)
	Mean ± S.d.	66.07±13.73
	< 60	15 (32.6)
	≥ 60	31 (67.4)
Gender	Male	24 (52.2)
	Female	22 (47.8)
Subtype	PMF	13 (28.3)
	SMF	26 (56.5)
	MDS/MPN	7 (15.2)
Follow Up Period (Month)	*Min-Max (Median)*	2-22 (6)
	Mean ± S.d.	6.30±3.69
Bone Marrow Fibrosis	Grade I	16 (34.8)
	Grade II	19 (41.3)
	Grade III	11 (23.9)
Spleen Size (mm)	*Min-Max (Median)*	132-250 (177)
	Mean ± S.d.	177.57±27.64
Ferritin (ng/ml)	*Min-Max (Median)*	16-1500 (132.5)
	Mean ± S.d.	386.48±496.97
Need of Transfusion	(-)	30 (65.2)
	(+)	16 (34.8)
Drug Usage	(-)	35 (76.1)
	(+)	11 (23.9)
	Ruxolitinib	6 (54.5)
	Chelation Therapy and Ruxolitinib	5 (44.5)

PMF: Primary myelofibrosis, SMF: Secondary myelofibrosis, MDS/MPN: Myeloproliferative neoplasms with myelodsplastic syndrome.

Table 2. Relationship between spleen size and ferritin levels in all patients

		Serum Ferritin Level and Splenomegaly
All patients (n: 46)	r	0.244
	p	*0.103*
-PMF (n: 13)	r	0.176
	p	*0.564*
-SMF (n: 26)	r	0.264
	p	*0.192*
-MDS/MPN (n: 7)	r	0.408
	p	*0.364*

PMF: Primary myelofibrosis, SMF: Secondary myelofibrosis, MDS/MPN: Myeloproliferative neoplasms with myelodsplastic syndrome.

male. The median age was 66 years old, and 67.4% of the cases were 60 years old and older. 28.3% (n: 13) of the patients were diagnosed with PMF, 56.5% (n: 26) of with SMF, and 15.2% (n: 7) of with MDS/MPN. When the cases were examined based on the bone marrow fibrosis levels; 34.8% (n = 16) had grade I, 41.3% (n: 19) had grade II and 23.9% (n: 11) had grade III fibrosis. Median serum Ferritin level was 132.5 (December: 16 to 1500 ng/ml). When subdivided according to ferritin levels, 65.2% were normal, 21.8% were high but do not require treatment (between 300-1500 ng/ml), 13% were high and require treatment (1500 ng/ml and above). While erythrocyte transfusion was not being transfused in 65.2% of the cases, 34.8% were found to have 10 or more erythrocyte transfusions in the last 1 year. The percentage of patients who did not receive transfusion and did not use ruxolitinib or chelation therapy was 56.5 (n: 26) (**Table 1**).

Serum ferritin level and splenomegaly

There was no significant relationship between serum ferritin level and splenomegaly in any of the cases and in patients with PMF, SMF or MDS/MPN (P > 0.05) (**Table 2**).

There was no statistically significant relationship between serum ferritin level and splenomegaly in patients who did not receive transfusion (P > 0.05). There was also no significant relationship in the PMF and SMF subgroups. In all transfused cases and the PMF, SMF and MDS/MPN subgroups, there was no statistically significant relationship between serum ferritin and splenomegaly (P > 0.05) (**Table 3**).

Serum ferritin level and bone marrow fibrosis

While there was no significant relationship between bone marrow fibrosis and spleen size in the group with 60 years old or younger, a statistically significant difference was found between bone marrow fibrosis and splenomegaly in the group aged 60 years old and older. A significant difference was found between bone marrow fibrosis and splenomegaly and serum

Table 3. Relationship between serum ferritin level and splenomegaly in all patients without transfusion or drug usage

		Serum Ferritin Level and Splenomegaly
All Patients (n: 26)	r	0.281
	p	*0.164*
-PMF (n: 8)	r	-0.595
	p	*0.120*
-SMF (n: 18)	r	0.202
	p	*0.421*

PMF: Primary myelofibrosis, SMF: Secondary myelofibrosis.

ferritin levels in the group aged 60 years old and older which constitutes 67.4% of the cases (P: 0.016) (**Table 4**).

Discussion

Many patients diagnosed with PMF are at risk of iron overload and iron-related toxicity due to transfusion dependence. In this context, iron chelation therapies are an important treatment step in order to prevent hemosideros is related toxicity. Leitch et al. analyzed 41 patients with PMF retrospectively in their study and found that those who received iron chelation therapy had significantly better overall survival (OS) [8]. Although the effect of iron chelation therapies on disorders that require frequent transfusion such as MDS is known, the literature data for PMF is limited. In the study of Elli et al. [10]; the change in median serum ferritin levels obtained with chelation therapy was higher in hematological responders (HR) compared to non-responders (NR). Similarly, these patients were found to have a better OS and a lower incidence of leukemic transformation. These two important studies are considered as examples of limited literature data between iron overload and PMF prognosis. In addition to all this information; there was an opportunity to evaluate the relationship between iron overload and bone marrow fibrosis and splenomegaly in this study. It is possible to say that serum ferritin level is very important in terms of explaining the relationship with poor OS. In the light of the findings of this study, the increase in serum ferritin level directly increases bone marrow fibrosis and splenomegaly; therefore it can be significantly associated with poor OS in PMF.

Pardanani et al. [17] investigated the effect of serum hepcidin, ferritin and inflammatory cytokines on prognosis in PMF. In the initial diagnosis, plasma hepcidin levels of 203 patients with a diagnosis of PMF were measured and hepcidin levels were found statistically significant higher than healthy controls ($P < 0.0001$). It has been also demonstrated that high hepcidin and ferritin levels were shown to be statistically significant with following parameters such as: < 10 g/dL hemoglobin, erythrocyte transfusion requirement, > 500 mg/L serum ferritin level, higher the Dynamic International Prognostic Scoring System (DIPSS) plus score, presence of circulating blasts, age > 65 and leukocyte count $< 4 \times 10^9$. In the same study, increased serum hepcidin and ferritin levels were found to be associated with lower OS independent of DIPSS plus score and inflammatory cytokines. The most important aspect of the study is that it revealed the association of many parameters with high hepcidin and ferritin levels. Similarly, in this study, which showed a significant relationship with high ferritin levels and bone marrow fibrosis in patients 65 years old or older, it should be underlined that high ferritin levels were not significantly associated with bone marrow fibrosis in the general population.

In various studies conducted with PMF, it has been found that serum ferritin level and frequent transfusions could not clearly prove fibrosis and associated organomegaly [18-21]; however, in the same studies, the relationship between fibrosis and OS is clearly demonstrated. In the study conducted by Li et al. in 2016 [18]; bone marrow fibrosis in patients with PMF was shown as an independent risk factor for OS. In this context, and based on the results of this study, specifically in the group 60 years old or older, it shows that high serum ferritin levels can give an idea in the prediction of bone marrow fibrosis and OS without the result of bone marrow biopsy. It is possible to say that it will contribute to scoring systems developed for determination of prognosis. All of these findings of this study contribute to the literature.

This study had also limitations: Splenomegaly and fibrosis relationship, which is the expected finding, may not be demonstrated among those 60 years old or younger because of the small case subgroup and the majority is cumulative

Table 4. Relationship between bone marrow fibrosis and spleen size - serum ferritin level in two different age groups

Age Group ≥ 60	Spleen Size (mm)		Serum Ferritin Level (ng/ml)	
	Min-Max (Median)	Mean ± s.d.	Min-Max (Median)	Mean ± s.d.
Fibrosis				
Grade I (n: 7)	140-200 (176)	172.29±19.79	17-82 (32)	37.43±20.92
Grade II (n: 5)	155-210 (165)	174.00±22.19	21-42 (26)	29.40±9.21
Grade III (n: 3)	150-220 (180)	183.33±35.12	16-607 (125)	249.33±314.51
[a]*p*	*0.909*		*0.619*	
Grade I (n: 9)	132-190 (156)	159.67±20.86	16-820 (103)	201.11±268.96
Grade II (n: 14)	148-250 (165.5)	175.57±27.44	37-1500 (321.5)	588.79±548.15
Grade III (n: 8)	172-247 (204)	205.88±25.69	85-1500 (676)	821.00±596.31
[a]*p*	*0.006*		*0.016*	

in the group aged 60 years old or older. Similarly, there was no finding that the relationship between ferritin and fibrosis in the same age group could be proven.

As a result, even there was no statistically significant difference between serum ferritin level and splenomegaly in the general population in this study; a significant relationship has been demonstrated between fibrosis and ferritin in the group 60 years old and older. In this respect, ferritin is very important in showing the relationship between fibrosis. This study recommends that ferritin-fibrosis relationship can be demonstrated also in the general population with a study in a larger case group.

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We respectfully remember all of our colleagues we lost in the COVID-19 fight.

Disclosure of conflict of interest

None.

Abbreviations

MF, Myelofibrosis; PMF, Primary myelofibrosis; ET, Essential thrombocythemia; PV, Post polycythemia vera; MDS/MPN, Myeloproliferative neoplasms with myelodysplastic syndrome; ASCT, Allogeneic stem cell transplantation; JAK, Janus kinase; WHO, World Health Organisation; SMF, Secondary myelofibrosis; NCSS, Number cruncher statistical system; OS, Overall survival; HR, Hematological responder; NR, Non responder; DIPSS, Dynamic international prognostic scoring system.

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