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# Diagnostic Significance and Association of Reticulin Fibrosis in Benign Hematologic Disorders

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## ABSTRACT

Reticulin fibrosis is a feature of benign illnesses. Reticulin staining is used to identify benign hematological abnormalities in bone marrow, with trichrome staining being the most appropriate procedure for histological examinations. **Objective:** To assess the association of reticulin fibrosis to benign hematological disorders. **Methods:** Patients with benign hematologic illnesses such as iron deficiency anemia, megaloblastic anemia, aplastic anemia, and immune thrombocytopenic purpura at department of hematology, Sheikh Zayed Medical Complex, Lahore were included. The sample size was 96 cases, with 24 cases for each disorder. Bone marrow samples were taken from the anterior iliac spine of patients diagnosed with benign hematologic diseases. The reticulin fibers were graded using the Thiele grading scale. **Results:** The gender distribution was significant, with ITP and IDA being higher in females, whereas MA was more prevalent in men. The age distribution was almost the same, with ITP the lowest mean age was 40.5 years, while the highest mean age was 46.7 years in cases with aplastic anemia. Reticulin stain results showed significant differences among the four groups, with all cases in MA, IDA, and AA having grade-0 results. **Conclusions:** The reticulin stain can distinguish between ITP and other hematological illnesses, as well as grade reticulosis in bone marrow biopsies, making it a helpful tool for detecting benign hematological disorders.

## INTRODUCTION

The hematopoietic system consists of organs and tissues where hematopoietic cells grow, mature, and remove. The extracellular matrix primarily is made of calcium phosphate and mineral salts, and cells make up the bone marrow. Bony cells consist of osteoblasts, osteoclasts, and osteocytes [1]. Both organic and inorganic elements, such as calcium and phosphorus, magnesium, and carbonate, make up the bone marrow matrix. Calcium and phosphorus occupy bony space in the form of hydroxyapatite crystals, along with collagen fibers. Collagen, reticular, and amorphous ground material constitute the organic part of the bone marrow matrix [2]. Whereas reticular cells provide a framework to support hematopoietic cells, sinusoidal endothelial cells serve as specific barriers for extravascular and vascular areas [3]. Reticulin fibrosis is a feature of several benign illnesses, such as megaloblastic, iron deficiency, and folate deficiency anemias, which are classified as acquired disorders. Disorders such as hemoglobinopathies, thalassemia, sickle cell anemia, autoimmune hemolytic anemia, and bleeding disorders can manifest as reticulosis [4]. Throughout the world, Iron Deficiency Anemia (IDA) is a prevalent kind of nutritional anemia. Diagnosis of IDA involves many physiological and biochemical features of red cells, including blood ferritin levels, MCV, serum iron level, transferrin saturation, and hypochromic features [5-7]. Retic cells, the youngest form of erythrocytes, are used


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to project iron status. Hemoglobin content in reticulocytes is crucial for diagnosing IDA. Immature cells typically have two dots of reticulin fibers, while a greater number of reticulin dots are seen in more immature forms [8]. According to a new study, reticulocyte hemoglobin equivalent is crucial for IDA diagnosis and may serve as a useful biomarker for diagnosing iron deficiency and IDA [9]. Megaloblastic Anemia (MA) is a condition characterized by the absence of essential micronutrients like vitamin B12 and folate, which affect the maturation process of erythrocytes in the bone marrow. This results in the formation of large RBCs with an asynchrony between cytoplasm and nucleus, which during the processes of maturation and proliferation interacts with DNA synthesis [10]. All myeloid cell lines cause the bone marrow to develop into hypercellular, with erythroid components taking precedence. These erythroid blasts show oval-shaped, massive features with a lacy nucleus and immaturity, which are regarded as suspicious to folate and/or vitamin B12 insufficiency [11]. Ovoid red blood cells with an MCV greater than 115 are indicative of a nutritional shortage, which is thought to be caused by low folic acid and vitamin B12 levels in MA [12, 13]. Usually, megaloblastic bone marrow is related to anemia because of inefficient erythropoiesis [14]. Aplastic anemia is a rare bleeding disorder where the lymphatic system lacks cell lines, leading to uncontrolled bleeding and infections. The autoimmune disorder attacking bone marrow stem cells is the main cause, but other factors like chemotherapy, radiation treatments, exposure to toxins, viral infections, pregnancy, and certain drugs can also affect cell line production [15]. Diagnosis involves physical examination, baseline investigation, radiograms, and bone marrow analysis. The interpretation of a bone marrow biopsy is dependent upon cellularity, myeloid series proportions, megakaryocytic and erythrocyte cell lines, bone constituents, and reticulin [16]. Immune thrombocytopenia, is an autoimmune bleeding disorder, caused by antibodies against platelet antigens, leading to the destruction of platelets [17]. The main reason for ITP is the binding of glycoproteins to IgG antibodies. ITP can happen for no apparent reason or as a result of comorbid illnesses like autoimmune diseases, whereas autoimmune disorders can develop as a result of genetic predisposition and environmental triggers. Numerous microorganisms, such as bacteria, viruses, and parasites, are linked to autoimmune diseases [18]. Although the pathophysiology of ITP is not well understood, it is thought to be a dysregulation of the immune response, with T-cells controlling plasma cells during antibody-mediated destruction, which is triggered by B-cell autoreactive clones [19]. They produce IgG auto-antibodies, which attach to platelet glycoproteins on their surface and transport them to the reticuloendothelial system for lysis. Another independent multidirectional mode causing ITP is impaired platelet formation [20, 21]. Regarding the staining process, the hematoxylin and eosin (H and E) staining process is a standard procedure used in all histopathology labs worldwide. On the other hand, reticulin fibers, which branch out from fine textiles, can be difficult to see in H and E preparations. The most appropriate procedure for histological examinations of bone marrow is pre-sensitization with potassium permanganate (PH of 9.0), which allows for the accurate visualization of these fibers [22]. Moreover, regarding the cellularity of bone marrow, the state depends on underlying conditions and is determined by bone marrow biopsy and aspirate, which are utilized for the definitive detection of pancytopenia and other hematological problems [22, 23]. Reticulin stain analysis must be done in accordance with recognised guidelines in order to meet the goal of our study. With respect to reticulin fibrosis, this is detected by trichrome staining [24].

Thus the goal of the current study was to use reticulin stains to identify different benign hematological abnormalities in bone marrow, as they vary by geography and ethnicity. This study must be a significant contribution to the body of literature as it will also offer enough details about the applicability of this method in Pakistan.

## METHODS

The cross-sectional study included patients of IDA, MA, iron transfusion polymorphism (ITP), and AA from Sheikh Zayed Medical Complex Lahore from 20 July 2022 to 20 December 2022. The research was approved by the Institutional Review and Research Advisory Board (IRB ID: SZMC/IRB/Internal/87-B/2022) of Sheikh Zayed Postgraduate Institute. The patients' consent was obtained before to the study. Adopting a conservative approach, we estimated a sample size of 96 patients (24 instances for each disease category) using a 95% confidence level and a 10% margin of error. We also included in the predicted incidence of fibrosis Grade-1 (22%). A convenient sampling strategy was employed. There were a total of 144 patients in the research, with 24 instances each representing four distinct hematological disorders: Iron Deficiency Anemia (IDA), Megaloblastic Anemia (MA), Aplastic Anemia (AA), and iron transfusion polymorphism (ITP). The inclusion criteria included male and female adults, who were patients with iron deficiency anemia, megaloblastic anemia, oraplasticanemia. Patients with malignant hematologic disorders and individuals undergoing chemotherapy for any malignant disease were excluded. Using a laboratory sample collection approach, the study gathered data on 96 patients with benign hematologic illnesses (IDA, MA, ITP, and AA). Using a trephine Jamshidi needle, bone marrow samples were taken from the anterior iliac spine of individuals who had been diagnosed with benign hematologic diseases. The


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biopsies were processed in the histology department after being fixed in 10% neutral-buffered formalin. Every slide was stained with reticulin, and then deparafinization, oxidation, bleaching, ferric chloride treatment, and drying were carried out. Using low, medium, and high power, the dyed slides were examined under a microscope. Numerous reticulin fiber-related observations were noted, including their quantity, density, thickness, and ratio to normal haemopoietic tissues. The reticulin fibers were graded using the Thiele grading scale, with grades ranging from 0 to 3 (there is an enormous network of reticulin fibers that are densely and diffusely increased, and there is also localized and severe osteosclerosis). The study assessed the prognostic value of reticulin fibrosis grades in benign hematological disorders. The data were analyzed by using SPSS version 25.0. Quantitative variables were provided as mean and standard deviation, whilst qualitative variables showed up as frequency and percentage.Chi-square tests were used to assess the association of hematological disorders among four group to reticulin grades, where p-value below 0.05 was considered significant.

## RESULTS

There were 43 (44.8%) males and 53 (55.2%) females among all cases(figure 1).

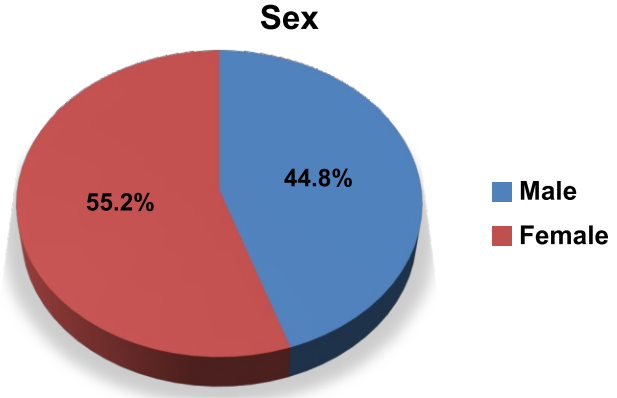


**Figure 1:** Gender Distribution of all Cases

Among the four categories, there was a statistically significant difference in the distribution of genders (p = 0.008). Males were more likely to have the MA, but females had much greater rates of IDA and ITP. Aplastic anemia patients were evenly distributed by gender(Table 1).

**Table 1:** Gender distribution of cases for four different hematological disorders(n=96)

| Disorder | Gender | | Chi-Square | p-Value |
|---|---|---|---|---|
| | Male Frequency (%) | Female Frequency (%) | | |
| Iron Deficiency Anemia | 8 (33.3%) | 16 (66.7%) | 11.92 | 0.008 |
| Megaloblastic Anemia | 17 (70.8%) | 7 (29.2%) | | |
| Aplastic Anemia | 12 (50%) | 12 (50%) | | |
| ITP | 6 (25%) | 18 (25%) | | |

All four categories had similar case age distributions. Everyone in the groups is around the same age. Despite the fact that Aplastic anemia had a higher average age of 46.7 years and ITP the lowest at 40.5 years, the difference was not statistically significant (p = 0.707)(Table 2).

**Table 2:** Age Distribution of Cases with Four Different Disorders

| Disorder | Age Mean ± SD |
|---|---|
| Iron Deficiency Anemia | 42.3 ± 16.5 |
| Megaloblastic Anemia | 43.6 ± 19.3 |
| Aplastic Anemia | 46.7 ± 19.8 |
| ITP | 40.5 ± 19.3 |

The results of the reticulin stain were collected for four groups, and it was noted that there were substantial differences in the findings among the four groups (p-value <0.001). A total of 12 cases (50.0% of the total) in ITP had a grade of 0, 11 cases (45.8% of the total) had a grade of 1, and 1 case (4.2%) had a grade of 2. All cases in IDA, MA, and AA had a grade of 0 (Table 3).

**Table 3:** Diagnostic Finding on Reticulin Stain for Benign Hematological Disorders

| Disorder | Bone Marrow Biopsy Finding on Reticulin Stain | | | | |
|---|---|---|---|---|---|
| | Grade-0 N (%) | Grade-1 N (%) | Grade-2 N (%) | Likelihood Ratio | p-Value |
| Iron Deficiency Anemia | 24 (100%) | 0 (0%) | 0 (0%) | 39.07 | <0.001 |
| Megaloblastic Anemia | 24 (100%) | 0 (0%) | 0 (0%) | | |
| Aplastic Anemia | 24 (100%) | 0 (0%) | 0 (0%) | | |
| ITP | 12 (50%) | 11 (45.8%) | 1 (4.2%) | | |

## DISCUSSION

Megaloblastic anemia was more common in males and iron deficiency anemia and immune thrombocytopenia in women, according to our study's results, which showed a statistically significant difference in gender distribution across the four groups (p-value = 0.008). The present study's most important discovery included an examination of four hematological illnesses and the predictive usefulness of reticulin fibrosis grades. With a p-value less than 0.001, the findings of the reticulin stain were substantially different across the four groups. Twelve patients (or 50%) had grade-0 reticulin stain in ITP, eleven cases (or 45.8%) had grade-1, and one case (or 4.2% of the total) had grade-2. Not a single instance in IDA, MA, or AA had a grade. In contrast, 61.8% of patients with more than 5% sideroblasts were determined to have IDA in a different research that employed reticulin stain in a bone marrow inquiry. Nevertheless, class 0, 1, 2, and 3 were detected in 14.54%, 63.63%, 14.54%, and 7.27% of the patients, respectively [26]. Eighteen percent of the patients in this research had high-grade alterations, whereas the remaining patients had low-grade changes. This study also showed a correlation between anemia and higher lactate dehydrogenase levels and high-grade alterations with ITP. More intriguingly, the IPSS distinct risk categories and the correlation between the RCO score and mortality were found to be significant (p=0.013). The Ing-rank test demonstrated that the probability of survival could be


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distinguished between high and low-grade patients. The current research's findings are equivalent to those of this study with regard to ITP, but not with regard to MA, AA, or IDA. The same criteria and measures employed in this investigation were also utilized by Ern *et al.*, to distinguish between bone marrow fibrosis and non-fibrosis in thrombocytopenia patients, and no statistically significant difference was found in any of the parameters [24-26]. When interpreting the results, it is important to keep in mind that our study had several limitations. Firstly, the sample size was small, so our findings may not be applicable to a larger population. Secondly, we couldn't establish causal relationships between the variables because our study was cross-sectional. Lastly, we didn't control for potential confounding factors like lifestyle and genetics that could influence the development of hematologic disorders.

## CONCLUSIONS

Reticulin stain is useful for diagnosing ITP since it can distinguish ITP from other hematological illnesses and detect reticulosis grades in bone marrow biopsies, according to the study's findings. Prospects of the current study include the correlation between the reticulin score and mortality, the classification of risk groups based on IPSS, and the chance of survival concerning the reticulin score.

## Authors Contribution

Conceptualization: YS
Methodology: RC, UW, YS, AH
Formal analysis: QAA
Writing, review and editing: AH, UW, RC, SH

All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflict of interest.

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