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Immature Platelet Fraction in Patients with Chronic Liver Disease, A Marker for Evaluating Cirrhotic Changes

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ABSTRACT

Objective: To evaluate the role of Immature platelet fraction in patients with chronic liver disease, a marker for evaluating cirrhotic changes.

Methodology: This case control study was conducted at Department of Pathology, Aziz Fatima Medical and Dental College, Faisalabad, from June 2020 to December 2020. A total of 126 participants were included in the study consisting of 63 patients with chronic liver disease in group A and 63 participants without any known disease in group B as control. Ethylene diamine tetraacetic acid was used to collect the blood sample for IPF measurement and was maintained till analysis on room temperature. Ten repeated analyses, immediately and after 24 hours were done for reproducibility of IPF%.

Results: The mean age of liver disease patients was 52.35 ± 13.64 years and in the control group, the mean age was 51.62 ± 11.27 years. There was no significant (p-value > 0.05) difference between both groups based on age and gender. The hemoglobin level and red cell count was found to be significantly (p-value < 0.05) reduced in the cases group. While white blood cells count was comparable in both groups. The mean platelet count was significantly (p-value < 0.05) less in cases group (163.5 ± 90.4 vs 233.4 ± 54.5 (x10*3/µl). The mean value of immature platelet fraction (IPF%) was significantly (p-value < 0.05) raised in cases group (5.62 ± 2.92 vs 3.06 ± 1.87). The multivariate discriminant analysis (MDA) score showed a significant (p-value < 0.05) association with chronic hepatitis as compared to other liver related diseases.

Conclusions: In chronic liver disease patients, there is an inverse relationship between platelet count and IPF% with decreased platelet count and increased IPF%. The proposed MDA function can be used to identify the cirrhotic changes in liver disease patients.

Keywords: Liver disease, Liver Cirrhosis, Multivariate Discriminant Analysis (MDA) score.

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Introduction

Thrombocytopenia is very common in chronic liver disease patients, which complicates the medical management of these patients. There is a great diversity in the prevalence of thrombocytopenia depending upon cut-off values and patient population. In different studies, it ranges from 6% in non-cirrhotic patients to 70% in patients with liver cirrhosis.[1] The severity of the liver disease is associated with both prevalence and level of thrombocytopenia as well as prognosis of the patients. Platelets have a critical role in normal hemostasis and coagulopathy due to thrombocytopenia is predisposed among chronic liver disease.[2]

There are several causes of thrombocytopenia, which is defined as a platelet count less than 150 x 10*9/l in peripheral blood. Mainly there are two possible mechanisms either decrease in production or increase in destruction. The identification of causing mechanism is crucial for proper management. The thrombopoiesis can be identified by immature platelet fraction (IPF%), because of rise in percentage with adequate megakaryocytic activity and high turnover, in contrast, to decrease in percentage and low megakaryocytic activity with low turnover of the platelet.[3,4]

Sysmex XN analyzer is used for the measurement of immature platelet fraction, which evaluate young or immature platelets in peripheral blood in proportion to total platelet count (IPF%). Their lifetime is less than 24 hours. The IPF have ideal reference ranges varies from 1.6-7.1% in adults, and 1.0-6.8% in children.[5]

Sysmex automated blood cell analyzers are used to develop immature platelet fraction related parameters like immature platelet fraction count, immature platelet fraction percentage and highly fluorescent mature platelet fraction percentage (H-IPF%).[6] Thrombocytopenia due to increased platelet destruction can be identified based on an increase in IPF#, and IPF% which can be measured by Sysmex XE series and is considered to be effective in the differential diagnosis of thrombocytopenia.[7]

Thrombocytopenia is a frequently associated complication of advanced liver disease and its incidence further increases in liver cirrhosis patients, ranging from 77% - 85%. Thrombocytopenia among patients of chronic liver disease enhances the risk of bleeding requiring repeated platelet transfusions, increased ambulatory visits and inpatient hospital stays in comparison to patients without thrombocytopenia.[8]

This increased risk of bleeding in chronic liver disease patients due to thrombocytopenia is highly influenced by the severity of the liver disease. Many medical procedures like surgery or liver biopsy are affected because of this risk of bleeding among chronic liver disease patients. So, the management and prognosis of liver disease patients become compromised, showing a vital need of non-invasive techniques for differential diagnosis of thrombocytopenia and other conditions. In this context, immature or reticulated platelets have been reported to be useful in such situations. A lower fraction of immature platelets has been reported among patients of liver cirrhosis and thrombocytopenia patients in comparison to other liver disease patients.[9] The immature platelet fraction can be further helpful in the assessment of the stage of chronic liver disease. Many other factors are associated with the poor prognosis of patients with chronic liver disease or liver cirrhosis. These factors include the development of complications such as variceal bleed, ascites, hepatorenal syndrome, and spontaneous bacterial peritonitis etc.[10] The severity of liver disease can be assessed by platelet numbers. Cirrhotic thrombocytopenia is mainly associated with inadequate production of thrombopoietin and decrease the production of platelets, but the underlying mechanism is not yet fully cleared. This present study was planned to assess the role of immature platelet fraction for differential diagnosis of liver cirrhosis and other chronic liver diseases. So, that a rapid and non-invasive method for differential diagnosis of liver diseases could be identified in our setup.

Methodology

This Case Control study was conducted in the Department of Pathology, Aziz Fatima Medical and Dental College, Faisalabad. The study was completed in a period from June 2020 to December 2020. Approval of the study was taken from the hospital ethics committee. The patients of both genders, having age from 18 to 65 years presenting with liver dysfunction, diagnosed with the liver disease based on full history, clinical examination, and examinations needed for accurate diagnoses, such as serological markers, abdominal ultrasonography, and/or computed tomography with or without liver biopsy were selected for cases group with Non-Probability consecutive sampling technique. The healthy controls without any liver disease were enrolled for the control group. The patients presenting with severe anemia, history of PCI or coronary artery bypass grafting, renal disease, malignancy and prior blood transfusions were excluded from the study.

A total of 126 participants were included in the study consisting of 63 patients with chronic liver disease in group A and 63 participants without any known disease in group B as control. The sample size was calculated by WHO sample size calculator with the help of 5% level of significance, 80 % Power of test, 5.5 population standard deviation, mean value of IPF% in patients of liver disease 5.77, and in participants without liver disease 3.02.[11]

All the participants selected for the study were described about the study protocol and the researcher took informed written consent. Confidentiality regarding their medical

and non-medical details were maintained. Information on demographic characteristics including age, gender, and comorbidities like diabetes mellitus, hypertension, etc were recorded. Peripheral blood samples were taken from all the patients and healthy controls for IPF parameter calculations.

The IPF master program in combination with XE-2100 multiparameter automatic hematology analyzer was used to measure the immature platelet fraction. Two fluorescent dyes one of polymethine and other of oxazinedye in RET-SEARCH (II) reagent penetrate cells and stain DNA/RNA. Then mature and immature platelets were identified on the basis of difference in RNA content. Ethylene diamine tetraacetic acid was used to collect the blood sample for IPF measurement and was maintained till analysis on room temperature. Ten repeated analyses immediately and after 24 hours of blood sample collection were done for reproducibility of IPF% after collection of a blood sample from a healthy individual. Various other hematological parameters including platelet numbers were recorded at the same time. The platelet count was measured based on impedance measurement on XE-2100.

All the collected data was entered and analyzed with SPSS v. 25. Descriptive statistics like mean ± SD were calculated for quantitative variables and frequency with percentages were presented for qualitative variables. Independent sample t-test and chi-square tests were used to compare means and proportions between both groups, respectively. P-value ≤ 0.05 was taken significantly. Multivariate discriminant analysis was performed using the Mahalanobis distance (MD2) with SPSS software. The equation for the MDA function was established according to the method described by Mahalanobis (1936): Score = platelet count (10^{*09}/l) x 0.021791 + IPF% x 0.026576 – 3.7791

Results

In this case control study a total of 126 participants were enrolled, consisting of 63 cases of liver disease and 63 control without any liver disease. The mean age of liver disease patients was 52.35 ± 13.64 years and in control group the mean age was 51.62 ± 11.27 years. There was male dominance in both case and control groups. In cases group 33 (52.38%) were males and in the control group 40 (63.49%) were male participants. There was no statistically significant (p-value > 0.05) difference between both groups based on age and gender. The comparison of both groups based on different blood parameters showed that the hemoglobin level was found significantly (p-value < 0.05) reduced (11.29 ± 2.63 g/dl) in cases of liver disease as compared to (12.78 ± 1.78 g/dl) healthy controls. In cases with liver disease the red cell count was found to be significantly (p-value < 0.05) less (4.29 ± 0.67, ($x10^6$/µl)) as compared to (5.12 ± 0.58, ($x10^6$/µl)) healthy controls. The mean value of white blood cells in patients of liver disease was noted to be 8.92 ± 4.73 (10^3/µl) which is not different from the mean value of the healthy control group having mean value of 8.34 ± 1.62 (10^3/µl) with an insignificant P-value > 0.05 as elaborated in Table I.

Table 1: Demographic Characteristics of the participants both groups

Characteristics	Cases (n=63)	Controls (n=63)	P-value
Age of participants			
Mean ± SD	52.35 ± 13.64	51.62 ± 11.27	0.744
Gender of the participants			
Male	33 (52.38%)	40 (63.49%)	0.206
Female	30 (47.62%)	23 (36.51%)	
Hemoglobin level (g/dl)			
Mean ± SD	11.29 ± 2.63	12.78 ± 1.78	0.000
Red Cell Count ($x10^6$/µl)			
Mean ± SD	4.29 ± 0.67	5.12 ± 0.58	0.000
White Blood Cells (10^3/µl)			
Mean ± SD	8.92 ± 4.73	8.34 ± 1.62	0.359

The mean platelet count was significantly (p-value < 0.05) less among patients of liver disease in cases group having a mean value of 163.5 ± 90.4 ($x10^{*3}$/µl) as compared to healthy control with mean value of 233.4 ± 54.5 ($x10^{*3}$/µl). The proportion of the patients having a platelet count less than 150 $x10^{*3}$/µl was significantly (p-value < 0.05) very high among patients of liver disease (44.44%) in cases group in comparison to (1.58%) in healthy control group. The mean value of immature platelet fraction (IPF%) was found significantly (p-value < 0.05) raised with a mean value of (5.62 ± 2.92) as compared to healthy controls having mean value of (3.06 ± 1.87) as shown in Table II.

There was a total of 31 patients of chronic hepatitis, 22 patients of liver cirrhosis, and 10 patients of other liver diseases in our study sample. The multivariate discriminant analysis (MDA) score showed a significant (p-value < 0.05) association with chronic hepatis as compared to other liver related diseases with a cutoff value of "0". The majority of the patients having MDA score of > 0, had chronic hepatitis, and only 9.10% of the

patients having liver cirrhosis had MDA > 0 as elaborated in Table III.

Table II: Distribution and Comparison of Total platelet count and IPF between both groups

Parameters	Cases (n=63)	Controls (n=63)	P-value
Total Platelet Count (x10*3/µl)			
Mean ± SD	163.5 ± 90.4	233.4 ± 54.5	0.000
Total Platelet Count (x10*3/µl)			
< 150	28 (44.44%)	1 (1.58%)	0.000
150-200	23 (36.51%)	27 (42.86%)	
> 200	12 (19.05%)	35 (55.56%)	
IPF %			
Mean ± SD	5.62 ± 2.92	3.06 ± 1.87	0.000

Table III: Multivariate Discriminant Analysis of scores of chronic hepatitis and liver cirrhosis patients

	Chronic Hepatitis	Liver Cirrhosis	Other liver disease	P-value
MDA > 0	20 (64.52%)	2 (9.10%)	1 (10 %)	0.000
MDA < 0	11 (35.48%)	20 (90.9%)	9 (90%)	
Total	31	22	10	

Discussion

One of the most common complications of chronic liver disease patients is thrombocytopenia. There is variation in the prevalence of this complication and is directly proportional to the severity of the liver disease. Thus, increasing severity of liver disease, enhances the risk of thrombocytopenia and severe liver disease shows some degree of thrombocytopenia. It is believed that 15% to 30% of the patients of chronic liver disease have some degree of thrombocytopenia.[12]

The newly released thrombocytes are termed as immature platelets or reticulated platelets. These are identified due to their large size and high RNA cytoplasm concentration. The percentage of immature circulative platelets to the total number of platelets is known as immature platelet fraction (IPF). The analytical standardization of hematological parameters have contributed a lot toward the better results. New automated devices allow a better exploration of its contribution toward thrombocytopenia. Several studies have confirmed that IPF can be utilized to differentiate different causes of thrombocytopenia.[13]

There are no specific laboratory or clinical parameters which can differentiate the different thrombocytopenias like immune thrombocytopenia, aplastic anemia, or hematologic malignancy. This scarcity causes delay in adequate treatment of the patients. Thus, it become necessary for the physicians to consider bone marrow examination in a significant number of patients, which is an invasive procedure and should be avoided as much as possible due to other associated risks like pain and bleeding. Since bone marrow procedure is invasive and painful for the patient as well so both physician and patients are reluctant to perform bone marrow examination. Then other noninvasive procedures like immature platelet fraction (IPF%) is proposed to use for differential diagnosis instead of bone marrow examination.[14,15]

In this present study, the hemoglobin level was found significantly (p-value < 0.05) reduced (11.29 ± 2.63 g/dl) in cases of liver disease as compared to (12.78 ± 1.78 g/dl) healthy controls. In cases with liver disease the red cell count was found to be significantly (p-value < 0.05) less (4.29 ± 0.67, (x106/ul)) as compared to (5.12 ± 0.58, (x106/ul)) healthy controls. The mean value of white blood cells in patients of liver disease and healthy control group was comparable. These results are in accordance with literature that shows that chronic liver diseases are frequently associated with hematological abnormalities. Anemia of diverse etiology occurs in about 75% of patients with chronic liver disease.[16] A major cause of anemia associated with chronic liver disease is hemorrhage, especially into the gastrointestinal tract. Patients with the severe hepatocellular disease develop defects of blood coagulation as a consequence of endothelial dysfunction, thrombocytopenia, deficiencies of coagulation factors and various associated disorders.[17]

The etiology of thrombocytopenia in chronic liver disease patients has been postulated to be associated with some factors including the destruction of immune mediated platelets, decrease in the synthesis of TPO, hypersplenism and viral effects on megakaryocytes and platelets. In the current study IPF% was increased above the upper limit of normal in the patients of liver diseases (5.62 ± 2.92 %) and was significantly higher than IPF% in normal control subjects (3.06 ± 1.87, P < 0.001). Similar results were found in different studies like a study by Zucker ML, el al found a significantly increased level of IPF%.[18] It is evident from these findings that destruction or sequestration of peripheral platelets is a main mechanism to cause thrombocytopenia among the patients of chronic liver disease. The decrease in compensation of peripheral platelet consumption is most

likely the contributory mechanism for thrombocytopenia in liver disease. It can be concluded that main associated factors with thrombocytopenia in chronic hepatitis C are also the factors generally linked with liver disease.

Liver biopsy is considered as gold standard for staging fibrosis in chronic liver diseases including hepatitis, which is an invasive technique and with lot of recent advancements in noninvasive techniques no technique was available for this purpose. Due to associated morbidity with invasive procedures greater efforts should be dedicated for the development of noninvasive technique.[19] In this present study it was tried to discriminate patients with and without significant fibrosis on the basis of IPF%. It was found that MDA function has very high efficacy in the identification of considerable fibrosis, and it was noted that it correctly classified 90% of the patients with liver cirrhosis. The cutoff values for this efficacy was set at MDA = 0. Similar findings for this function have been found in other studies like the study by Nomura T, et al found that about 95% of patients were correctly identified with MDA function.[11,20]

Conclusion

In chronic liver disease patients, there is an inverse relationship between platelet count and IPF% with decreased platelet count and increased IPF%. The proposed MDA function based on platelet count and IPF%, can be used to identify the cirrhotic changes in liver disease patients. This MDA function can be a better alternate to invasive evaluation. So, the measurement of IPF can be very helpful for differential diagnosis of liver diseases based on MDA function.

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