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COMPARITIVE EVALUATION OF HAEMATOLOGICAL PROFILE IN PATIENTS WITH ALCOHOLIC LIVER DISEASE AND NON ALCOHOLIC FATTY LIVER DISEASE WITH SPECIAL EMPHASIS ON PLATELET INDICES

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ABSTRACT The study was conducted as a randomized , cross sectional analysis . It consisted of total 150 subjects divided into 3 groups Group A(alcoholics), Group B(non alcoholics) and Group C(controls), each group having 50 subjects.The samples were assayed in complete blood count differential mode in the sysmex XN1000 haematology analyserWe compared RBC indices and platelet indices between the 3 group. Hb, MCV and RDW were statistically significant in Group A and Group B than in Group C, more so in Group A than in Group C. Platelet count and plate crit were statistically lower in Group A and Group B than in Group C, more so in Group A than in Group C. We concluded that along with RBC indices, platelet indices can be used for the non invasive assessment of degree of fibrosis in ALD and NAFLD patients thus decreasing the need of liver biopsy for the same.

KEY WORDS : Alcoholic liver disease, Non Alcoholic fatty liver disease, RBC indices, Platelet indices

INTRODUCTION

Hemostasis and hepatology are closely related and cirrhosis of liver show a marked decrease in the liver synthesis of coagulation factors, leading to prolongation of prothrombin time. Equally important are the physiological processes of clot formation and the defects in the primary hemostasis in patients with liver disease.Blood platelets initiate the hemostatic process by interacting with the damaged vessel wall. These form a hemostatic plug within seconds after injury. The secondary hemostasis starts simultaneously but enrolls slower and strengthens the plug by cross linked fibrin within minutes(22).

Thrombocytopenia is a marked feature of chronic liver disease and cirrhosis. This thrombocytopenia is attributed to platelet sequestration in the spleen, increased platelet breakdown and decreased platelet production plays a more important role. Besides the reduction in number, other studies suggest functional platelet defects. This platelet dysfunction is probably both intrinsic to the platelets and secondary to soluble plasma factors. It reflects not only a decrease in aggregability, but also an activation of the intrinsic inhibitory pathways. The net effect, finally, is a decreased platelet function in the various types of chronic liver diseases and cirrhosis(22)

Thrombopoietin (TPO) is the most important growth factor in the regulation of megakaryocyte development and platelet production. As the liver is the major site of TPO production it is reasonable to expect a decreased plasma level and indeed TPO mRNA levels in the liver were slightly decreased in cirrhosis(22).

Platelet indices are the first hematologic indices to be affected in cirrhosis. The platelet indices namely platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and platecrit are affected in cirrhosis(17).

MPV is the measure of average size of platelets in circulation, PDW is an index reflecting the heterogeneity of platelets and platecrit is a measure of total platelet mass. The etiopathogenesis of platelet abnormalities in cirrhosis include portal hypertension-induced splenic sequestration, alterations in thrombopoietin, bone marrow suppression mediated by toxins, consumptive coagulopathy due to low-grade disseminated intravascular coagulation, acquired intravascular coagulation, fibrinolysis(17)

Cirrhosis of liver is the final common end point of many chronic liver diseases and thrombocytopenia is a well known feature of cirrhosis. It leads to both quantitative and qualitative changes in the platelets(22). The etiopathogenesis of platelet abnormalities in cirrhosis include portal hypertension-induced splenic sequestration, alterations in thrombopoietin, bone marrow suppression mediated by toxins, consumptive coagulopathy due to low-grade disseminated intravascular coagulation, acquired intravascular coagulation and fibrinolysis (17).

In the present study with the above background the two conditions leading to cirrhosis - ALD and NAFLD,have been considered and compared. Alcoholic beverages have been associated with human civilization since the prehistoric times. Although the bad effects on health are multidimensional, yet it is estimated that 3.5% of the global burden of the disease is attributable to alcohol.According to WHO estimates 26% men and 4% women consume alcohol(7).

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and liver transplantation in western countries. Increasing incidence of NAFLD has been well documented from Asian countries like Japan and China. Diabetes mellitus (DM), obesity, hyperinsulinemia are predisposing factors for NAFLD(18). There is increase in incidence of DM, obesity and insulin resistance in India in last two decades. Hence it is logical to expect increase in incidence of NAFLD in India(1).

Although liver biopsy is the gold standard for the diagnosis of both these clinical conditions, ethical considerations as well as inherent risks associated with the procedure limit its widespread acceptance. Radiological imaging with Ultrasonography, Computed Tomography or MRI have used either singly or in combination have an adequate threshold for the detection of fatty infiltration of liver. Besides, each of these modalities has its own pitfall. Hence there is a search for a surrogate marker for these conditions. Examination of peripheral blood usually provides an excellent clue of NAFLD / NASH and in subjects with chronic liver disease needs evaluation. The combined platelet indices in the form of platelet crit(PCT),mean platelet volume(MPV).and platelet distribution width(PDW) are now widely available and the combined interpretation of these parameters would be useful in the differential diagnosis of thrombocytopenia resulting from ALD / NAFLD(5).

The study assumes greater significance due to the fact that the prevalence of both ALD and NAFLD is higher in the state and a search for a marker which is noninvasive and easy to perform is the need of the hour.

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MATERIALS AND METHODS

The study was conducted in the Department of Medicine of Shri Guru Ram Rai Institute of Medicine and Health Sciences Patel Nagar Dehradun.

It is a cross sectional study in which total 150 patients were included 50 of ALD, 50 of NFALD and 50 controls. The samples were assayed in complete blood count differential mode in the sysmex XN1000 haematology analyser

These subjects would be screened for chronic liver disease which would be based on

1. Detailed clinical history
2. Thorough clinical examination
3. Liver function tests
4. Ultrasonography

A definitive diagnosis of ALD or NAFLD would be established based on the above criteria.

INCLUSION CRITERIA

1) 18 to 65 years
2) Drinking of alcohol less than 20 grams per day for NAFLD
3) More than 80 grams per day for 5 years ALD.

EXCLUSION CRITERIA

1) Age more than 65 years
2) H/o hepatotoxic drugs or
3) herbal supplements
4) Viral hepatitis

RESULTS

In table 1 one way ANOVA TEST has been applied to compare the mean RBC indices amongst Group A(Alcoholics) , Group B(Non alcoholic fatty liver disease) and Group C(Controls). MCV , MCH , RDW were significant when Group A and Group B were compared. Hb was not significant when Group A and Group B were compared.Hb, MCV,MCH and RDW were significant when compared in Group A(Alcoholics) and Group C(Controls). Hb was significant when Group B (Non alcoholic fatty liver disease) and Group C(Controls) were compared .MCV, MCH, RDW were not significant when Group B (Non alcoholic fatty liver disease) and Group C (Controls) were compared .

In table 2 one way ANOVA TEST has been applied to compare the mean platelet indices amongst Group A(Alcoholics) and Group B(Non alcoholic fatty liver disease) and Group C(Controls).Plate crit and platelet count were significant when compared in Group A(Alcoholics) and Group B(Non alcoholic fatty liver disease). MPV, PDW and P-LCR were not significant when compared in Group A(Alcoholics) and Group B(Non alcoholic fatty liver disease).Plate crit, Platelet count were significant when Group A(Alcoholics) and Group C(Controls) were compared. MPV, PDW and P-LCR were not significant when Group A(Alcoholics) and Group C(Controls) were compared. MPV,PDW and P-LCR were significant when Group B(Non alcoholic fatty liver disease) and Group C(Controls) were compared. Plate crit and platelet count were not significant when Group B(Non alcoholic fatty liver disease) and Group C(Controls) were compared.

Table No: 1- ***Comparison of mean RBC indices among three groups***

Indices	Alcoholics (Group A)	Non-alcoholics (Group B)	p Value	Alcoholics (Group A)	Controls (Group C)	p value*	Non-alcoholics (Group B)	Controls (Group C)	p value*
	Mean± SD	Mean± SD		Mean± SD	Mean ± SD		Mean± SD	Mean ± SD	
HB	9.15 ± 2.96	10.42 ± 3.01	0.08	9.15 ± 2.96	11.79 ± 2.58	0.01#	10.42 ± 3.01	11.79 ± 2.58	0.05#
MCV	95.01 ± 9.26	87.49 ± 10.44	0.001#	95.01 ± 9.26	85.58 ± 7.55	0.001#	87.49 ± 10.44	85.58 ± 7.55	0.89
MCH	32.15 ± 4.23	29.49 ± 4.51	0.004#	32.15 ± 4.23	28.59 ± 3.27	0.001#	29.49 ± 4.51	28.59 ± 3.27	0.81
RDW	18.4 ± 4.24	15.17 ± 2.74	0.001#	18.4 ± 4.24	14.55 ± 2.06	0.001#	15.17 ± 2.74	14.55 ± 2.06	0.97

Table No: 2- ***Comparison of Mean Platelet Indices Among Three Groups.***

Indices	Alcoholics (Group A)	Non-alcoholics (Group B)	p value*	Alcoholics (Group A)	Controls (Group C)	p value*	Non-alcoholics (Group B)	Controls (Group C)	p value*
	Mean± SD	Mean± SD		Mean± SD	Mean± SD		Mean± SD	Mean± SD	
MPV	11.68 ± 1.25	12.07 ± 1.43	0.44	11.68 ± 1.25	11.23 ± 1.37	0.30	12.07 ± 1.43	11.23 ± 1.37	0.007#
PDW	14.79 ± 3.64	15.36 ± 3.41	1.00	14.79 ± 3.64	13.68 ± 3.32	0.33	15.36 ± 3.41	13.68 ± 3.32	0.05#
LCR	37.1 ± 8.53	40.24 ± 11.18	0.39	37.1 ± 8.53	34.6 ± 3.11	0.70	40.24 ± 11.18	34.6 ± 3.11	0.02#
PLT crit	0.15 ± 0.12	0.23 ± 0.11	0.005#	0.15 ± 0.12	0.23 ± 0.12	0.001#	0.23 ± 0.11	0.23 ± 0.12	1.00
PLT count	1.23 ± 0.97	1.88 ± 1.02	0.01#	1.23 ± 0.97	2.27 ± 1.27	0.001#	1.88 ± 1.02	2.27 ± 1.27	0.24

DISCUSSION

In Group B the mean Hb concentration , MCV, MCH and RDW appeared normal. These parameters did not show a significant variation as compared to controls. For that Group A and Group B were compared for the haematological profile and they were matched for the mean Hb concentration. There was no significant difference in the mean Hb concentration implying indirectly that number of other factors might be responsible for variation in other parameters and also chronic blood loss which may alter MCH and MCV.MCV estimates the average erythrocyte volume and serves as an indicator of macrocytois. The results show significantly increased MCV in ALD as compared to NAFLD, p value< .001. Similar results were obtained by Das SK et al (2011), Edward Ret al (2002), , Savage D et al (1986) and M Shigeo et al(2001)(6,8,19,13). The mean MCV, although with in normal range in the two groups was significantly higher in alcoholics as compared to non alcoholics. It is important to highlight that liver changes are more rapid in alcohol mediated liver injury as compared to NAFLD and that, Group A has a mixed population of different grades of fatty liver disease. Since the sonographic categorization has not been compared separately in the two groups the MCV reflects relatively more severe form of maturation arrest of RBC. Thus increased MCV can be used as a marker for detecting alcohol abuse. It reflects the severity of the underlying liver disease and the amount of recent alcohol intake. Odula et al (2205) also had similar observations(21). They had also observed that these haematological changes were more marked in heavy drinkers as compared to occasional drinkers or less frequent drinkers. MCV responds slowly to abstinence, as a RBC survives for 120 days after it has been released into circulation(21,14). Its normalization may require 2 to 4 months (21). This observation further emphasizes toxic effect of alcohol on red cell morphology and its precursors.

The mean MCH did not show a similar trend of MCV unlike the study of Heidmann E et al in which 32% of alcoholics had an increase in MCH. The possible reason for its non concordance is the fact that Group A is a various degrees of alcoholic liver disease and hence may not be translated linearly, however this trend could be more appreciated if this group would have been sonograhically categorized. These results are also in concordance with observations made by Das SK et al, Qamar et al, Elanchezhian et al and Chauhan N et al(6,2,9,4).

The mean RDW also showed a statistically significant variation compared to non alcoholics as mentioned earlier, acute alcohol intoxication is associated with changes in the mean MCV, as

compared to non alcoholics. There is also a high prevalence of variceal and non variceal bleed(alcoholic gastropathy) which results in relative microcytosis. Further the nutritional deficiencies of Vitamin B12 and Folic acid in alcoholics lead to macrocytosis and hence increase in RDW. Similar observation were made by Fang L et al(2016) and Shigeo M et al(2001), but S millic et al(2011) did not find any between ALD and NAFLD severity in accordance with a Child Pugh grade (12,13,14). The possible reason is that the Child Pugh represents a set of variables, to determine the severity and mortality risk of disease. Better results would be yielded if RDW was correlated as an independent predictor of disease outcome.

On comparing the various platelet indices of Group A and Group B, the platelet count and plate crit were found to be lower in Group A(Alcoholics) than Group B(Non Acoholics) p value <0.005(Table number 9) and these values were also low p <0.005 in Alcoholics as compared to controls.

Thrombocytopenia is the most common and first haematological index abnormality to develop in cirrhosis. Changes in platelet parameters accompany the progression of various form of liver disease. Thrombopoietin(TPO) is a hormone produced mainly by the hepatocytes which promote platelet production by the marrow. TPO is directly correlated to degree of liver fibrosis and impairment of hepatocyte function. Both of which contribute to ALD and NAFLD, but to varying degree. Since alcohol induced hepatocyte is more rapid these changes are relatively more robust in ALD as compared NAFLD. Peripheral platelet count can possibly reflect the severity of liver injury and may also serve as a marker of the liver injury, rather than differentiating between ALD and NAFLD(to differentiate etiologically). Further plate crit was found to be significantly lower in alcoholics compared to non alcoholics and also controls. Similar results were obtained by Mukker P et al(2016) Tamara et al (2017) and Afagh G et al(2015)(17,15,10). Multiple factors contribute to this and include splenic platelet sequestration, immune mediated destruction, bone marrow suppression by toxins and reduced level of activity of growth factor(TPO). TPO is mainly dependent on hepatic function and it stimulates the differentiation of megakaryocytes to functional platelets. Serum TPO levels correlate inversely with degree of severity of fibrosis and Child Pugh class.

The development of portal hypertension and ascites predispose to sepsis, which may further result in the decrease survival of circulating platelets and increased removal of platelet from circulation due to phagocytosis by the macrophages. In our study the subject with sepsis have not been exclude. However the PDW in the 2 Groups remained equal and did not change significantly with the controls as well, signifying that the sepsis has not contributed much for the changes in the platelet indices. Further plate crit can be used as a marker for assessing the risk of bleeding in cirrhosis.

Our results were not consistent with the previous studies by Tamara et al(2017), Ozhan et al(2010), Shin WY et al(2011) and Mehmet et al(2013) which showed a statistically significant rise in MPV associated with NAFLD(15,16,20,3).

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