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ORIGINAL RESEARCH ARTICLE

ASSOCIATION OF LIVER FUNCTION TEST PARAMETERS WITH HEMATOLOGICAL INDICES IN SEROPOSITIVE HEPATITIS B PATIENTS

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ABSTRACT

Background: Inflammation is increasingly being implicated for progression of Chronic Hepatitis B. Besides the usual liver function test (LFT) parameters, systemic inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have generated significant interest that could be useful in stratifying the severity and prognosis of liver disease. The aim of this study was to compare LFT parameters and hematological ratios (NLR and PLR) between seropositive hepatitis B patients and healthy controls and explore associations between them.

Methods: This cross-sectional study included 64 Hepatitis B surface antigen (HBsAg) positive patients and 62 apparently healthy seronegative individuals visiting Chitwan Medical College between March to October 2019. Laboratory data included standard LFT panel and hematological parameters including NLR and PLR. Data was analyzed using SPSS software, version 20. P<0.05 was considered to be statistically significant.

Results: The two groups (seropositive and seronegative group) did not differ by age and gender. LFT parameters except for total protein were higher in the seropositive group. Both median NLR and PLR were considerably lower in the seropositive group compared to the seronegative group (2.99 and 102.32 vs. 3.67 and 126.15 respectively). However, NLR showed a considerable overlap between the groups, indicating a high variability. No significant correlation was observed between these hematological indices with other LFT parameters.

Conclusions: NLR and PLR are easily obtainable, cost-effective parameters that are inversely related with Hepatitis B infection. They could supplement routine LFT parameters in characterization of the phase and severity of chronic Hepatitis B infection.

INTRODUCTION

Hepatitis, a general term referring to inflammation of the liver, may result from both infectious and non-infectious causes. Hepatitis B (Hep B), one of the commonest infectious causes, has been a global problem because of its' multiple transmission methods and propensity to progress into chronic hepatitis, which may manifest as hepatic cirrhosis and hepatocellular carcinoma (HCC).[1] Globally, the burden of hepatitis B and its related complications is significant. An early nationwide study in Nepal reported an average HBsAg carrier rate of 0.9%.[2] Liver function test (LFT) parameters include measurement of serum levels of bilirubin, hepatic enzymes (e.g. AST, ALT) and protein, among many others. These tests are used not only for routine screening of patients with suspected liver disease[3] but also for severity assessment and prognostication by being components of prognostic scores such as Child-Pugh or Model for end-stage liver disease (MELD) scores.

Inflammation is increasingly being recognized to play a key role in the progression of liver disease; therefore, systemic inflammatory markers such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) could be useful in stratifying the severity and prognosis of liver disease.[4,5] These hematological indices are easy to obtain and relatively inexpensive; and therefore, have important clinical utlity. However, the relationship between these ratios and LFT parameters, particularly in HBsAg positive patients has been scantily explored.

The objective of this study was to compare LFT parameters and hematological ratios (NLR and PLR) between seropositive hepatitis B patients and healthy controls and explore associations between them.

METHODS

This cross-sectional, comparative study included 64 Hepatitis B surface antigen (HBsAg) positive hepatitis B patients and 64 age- and gender-matched apparently healthy seronegative individuals visiting Chitwan Medical College Teaching Hospital (CMCTH), Bharatpur, Chitwan. The study was conducted from March 2019 to October 2019. Cases overlapping with the symptoms of Hepatitis B (that were seronegative) and cases with conditions that influence NLR such as chronic inflammatory diseases, cardiac disease, diabetes , renal and/or hepatic failure, metabolic syndrome were excluded from

the study. Ethical approval was taken from CMC-Institutional Review Committee [Ref No. CMC-IRC/076/077-037].

Demographic and laboratory data were collected. About 5ml of venous blood sample was collected from the patients using aseptic technique into a clot activator tube (Yellow capped vial). The serum sample was then separated and utilized for analysis of different parameters. Liver function test (LFT) parameters included serum bilirubin total and direct, Alanine Transaminase (ALT) and Aspartate Transaminase (AST), Alkaline Phosphatase (ALP), Albumin and Total Protein (TP). The hematological parameters included hemoglobin, total leucocyte count, differential leucocyte count, RBC count, Platelets count, RBC indices (MCV, MCH, and MCHC), platelet-to-lymphocyte ratio (PLR) and Neutrophil-to-lymphocyte ratio (NLR). NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count; PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. The LFT parameters were measured by standard colorimetric assays using DIMENSION Clinical Chemistry System, SIEMENS. HBsAg was measured using semi-quantitative Enzyme Immunoassay (EIA) method, with s/c (signal per cut-off) values ≥ 0.2 categorized as "positive" result. Data was analyzed using SPSS software, version 20. Continuous variables were expressed as mean ± SD [for normally distributed variables] or median (min – max) [for variables with skewed distribution]. Categorical variables were expressed as frequency (percentage). The comparison of mean or median values of variables between seropositive cases and healthy controls was done by independent t-test or Mann-Whitney U test respectively. The differences in the categorical variables between the groups was analyzed by Chi-Squared test. Spearman's rank correlation coefficient was used to explore correlation between the variables. P<0.05 was considered to be statistically significant.

RESULTS

A total of 64 seropositive cases and 62 seronegative controls were included in the study. The demographic characteristics and different liver function test (LFT) parameters are shown in Table 1. The age and gender distribution were comparable between the two groups. Considering liver function test (LFT) parameters, as expected, total and direct bilirubin, ALT and AST were higher in seropositive group, but did not reach statistical significance. The median serum total protein was lower in the seropositive group (Table 1).

Table 1: Demographic characteristics and LFT parameters of cases and controls, expressed as mean ± SD or median (minimum - maximum) as appropriate

Variables	Seropositive cases (n = 64)	Seronegative controls (n = 62)	p-value
Age (years)	47.9 ± 18.9	46.4 ± 18.2	0.45
Sex			
Male, n (%)	44 (68.7)	34 (54.8)	0.07
Female, n (%)	20 (31.3)	30 (45.2)	
HBsAg	2.06 (0.72 – 2.92)	0.15 (0.09 – 0.19)	<0.001#
Total bilirubin (mg/dL)	1.2 (0.4 – 34.2)	0.8 (0.3 – 27.7)	0.13
Direct bilirubin (mg/dL)	0.3 (0.1 – 22.0)	0.2 (0.1 – 10.8)	0.34
ALT (IU/L)	44 (11 – 2080)	34 (10 – 276)	0.04*
AST (IU/L)	45 (10 – 1413)	32 (12 – 984)	0.04*
Total protein (g/dL)	6.6 (2.2 – 8.3)	6.9 (4.2 – 8.7)	0.06

Abbreviations: HBsAg, Hepatitis B surface antigen; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase

* statistically significant (p <0.05), # statistically significant (p <0.001)

Table 2: Hematological parameters (including NLR and PLR) of cases and controls, expressed as median (minimum - maximum)

Variables	Seropositive cases (n = 64)	Seronegative controls (n = 62)	p-value
Hb (g/dL)	12.3 (7.0 – 17.0)	12.6 (5.8 – 17.1)	0.62
MCV (fL)	85.4 (59.5 – 108.4)	84.0 (66.4 – 103.8)	0.49
MCH (pg)	28.8 (19.0 – 35.4)	29.2 (21.7 – 36.9)	0.75
MCHC (g/dL)	33.7 (23.0 – 70.3)	34.2 (30.3 – 37.7)	0.01*
TC (per mm^3)	7650 (2000 – 23010)	8195 (2900 – 26300)	0.31
NLR	2.99 (0.57 – 35.88)	3.67 (1.18 – 31.60)	0.12
PLR	102.32 (24.80 – 454.90)	126.15 (6.43 – 621.96)	0.03*

Abbreviations: Hb, Hemoglobin; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; TC, Total WBC count; NLR, Neutrophil Lymphocyte ratio; PLR, Platelet Lymphocyte ratio

* statistically significant (p <0.05)

Considering the hematological parameters, only median mean corpuscular hemoglobin concentration (MCHC) was significantly lower (p = 0.01) in the seropositive group. Both NLR and PLR were lower in the seropositive group compared to the seronegative group (Table 2).

Error bars for NLR showed a considerable overlap between cases and controls whereas it was not pronounced for PLR (Fig-

ure 1).

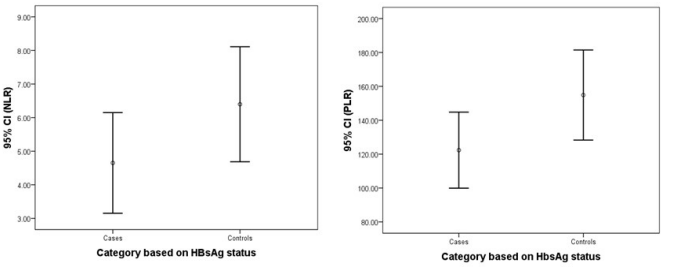

Figure 1: Error bars for NLR and PLR (representing mean and 95% CI) between cases and controls

Among seropositive patients (n = 64), HBsAg levels did not demonstrate any significant correlation with NLR or PLR. NLR had a significant positive correlation with PLR (Spearman's rho=0.53, p=0.02 respectively). No significant correlation was observed between the hematological indices (NLR and PLR) with other LFT parameters (total bilirubin, direct bilirubin, ALT and AST).

DISCUSSION

This study compared LFT and hematological parameters between seropositive (HBsAg positive) and seronegative individuals and explored associations between these parameters. Our study observed a higher prevalence of hepatitis B seropositivity in males (68.7%), which has been observed in studies elsewhere.[6,7] As expected, elevated transaminase (AST and ALT) activities (that reflect cellular injury by hepatotropic viruses) and lower albumin levels were found in seropositive patients compared to healthy controls, corroborating with findings described by other authors.[6,8] Transaminases, however lack necessary sensitivity to predict inflammation in Chronic Hepatitis B (CHB) patients, particularly active carriers and therefore do not provide additional diagnostic value.[9]

There is growing amount of evidence that complete blood count and the derived ratios: NLR and PLR could supplement/substitute LFT parameters in hepatitis B virus (HBV)-related disease. Our study reported a lower NLR and PLR in seropositive cases as observed in several other studies[6, 8,10] reflecting the effect of the viral infection on the concentration of platelets, neutrophils and lymphocytes. A study by Abdullah SM et al. in participants undergoing premarital screening noted a significantly lower NLR and PLR in patients with diagnosed HBV or HCV infection.[6] In patients infected with HBV, a low-grade immune response is responsible for persistence of HBV leading to development of CHB.[11] The pivotal role of lymphocytes in inflammatory pathways leading to development of liver fibrosis in CHB have been well elucidated.[12-14] Accordingly, several studies have demonstrated a significant negative correlation between NLR and fibrosis scores as well as histological activity index (HAI), the commonly used histological indication for treatment of CHB.[8,15] Similarly, in a prospective study including inactive hepatitis B carriers (that constitute the largest proportion of CHB), a significant negative correlation was found between NLR and degree of liver fibrosis.[10] A study by Kekilli et al. also reported significantly lower NLR in CHB patients with advanced fibrosis compared to patients with no/minimal fibrosis.[16] Studies have suggested variable cutoffs for NLR for the identification of advanced fibrosis in CHB, with optimal values ranging from ≤ 1.9 - 1.6.[10,16]

PLR has also been used as a marker for prediction of inflammation, disease severity and prognosis of various diseases, including systemic lupus erythematosus (SLE) and cardiovascular diseases.[17,18] A study by Zhao et al. noted a significant positive correlation between PLR and serum HBeAg and HBV DNA in CHB, suggesting PLR as a simple marker that reflects degree of active virus replication.[9] A decreased PLR in CHB could be attributed to increased lymphocytic activity and decreased thrombopoietin production in the liver.[19] PLR could have another very important clinical implication; Meng et al. concluded that PLR monitoring could predict success during treatment of chronic hepatitis C, with an upward trend predicting a good virological response.[20]

NLR and PLR are readily available, non-invasive, inexpensive options that could have massive clinical utility in assessment of severity of liver damage and fibrosis due to variety of conditions, including CHB. Besides their poor correlation with other LFT parameters as observed in our study and other literature,[10] the major problem with hematological indices is the differences in the methods of measurement, analyzers used and quality control implementation between laboratories, leading to high variability in the results. Moreover, there is no established universal reference range for these ratios, and therefore individual reference values for local use need to be established.[21]

Our study is limited by a small sample size and single-center study design. Some of the clinical information, particularly phase of the hepatitis B infection was missing. Similarly, the association between the hematological indices and severity of liver fibrosis could not be evaluated because of the lack of liver biopsy or other non-invasive tests information. Studies with prospective design are needed to verify the predictive ability of these parameters for severity of liver disease and fibrosis.

CONCLUSION

Hematological indices (NLR and PLR) were found to be lower in seropositive Hepatitis B patients. They could be simple and cost-effective investigations that provide additional information for characterization of the phase of chronic Hepatitis B infection beyond that given by routine LFT parameters.

CONFLICT OF INTEREST: None

FINANCIAL DISCLOSURE: None

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