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# Evaluation of hematological parameters in inactive hepatitis B infection; neutrophil to lymphocyte ratio and mean platelet volume

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## ABSTRACT

**AIM:** It is estimated that the hepatitis B virus, a serious global public health problem, affects more than 250 million people. Herein, our objective was to quantify several hemogram measurements, like neutrophil-lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), and mean platelet volume (MPV) and to determine their relationship with inflammation in hepatitis B infection (HBV).

**MATERIAL AND METHOD:** This study included 94 patients aged 2–18 years, who were under follow-up at our hospital for hepatitis B infection between January 1st, 2005 and December 31st, 2015. These individuals served as the patient group. The control group was composed of 88 healthy children of similar age and sex.

**RESULTS:** There was no significant difference concerning neutrophil count and NLR ( $p=0.698$  and  $0.842$ , respectively). A significantly lower absolute lymphocyte count significantly and a significantly higher MPV was found in the hepatitis B-positive group than in the controls ( $p < 0.05$  for both comparisons). The groups had similar hematocrit, hemoglobin, platelet count, RBC count, and white blood cell (WBC) count ( $p=0.642$ ,  $p=0.388$ ,  $p=0.418$ ,  $p=0.366$ , and  $p=0.824$ , respectively). The two groups individually showed a positive correlation between platelet count and MPV ( $r=0.32$ ,  $p=0.01$  and  $r=0.36$ ,  $p=0.01$ , respectively). NLR and MPV were positively correlated in both groups ( $r=0.38$ ,  $p=0.01$  and  $r=0.41$ ,  $p=0.01$ , respectively).

**CONCLUSION:** In conclusion, the study groups did not exhibit any significant difference concerning NLR, PLR, and PLT/MPV levels but it found a significant intergroup difference in MPV. We believe it is of great importance to develop simpler, inexpensive, and easy-to-use noninvasive methods to determine the level of inflammation in chronic hepatitis B infection; Parameters, such as NLR, PLR, and MPV, can be simple and rapid test markers to serve these purposes.

**Keywords:** Hepatitis B, neutrophil to lymphocyte ratio, mean platelet volume.

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# Оценка гематологических показателей при неактивной инфекции гепатита В: соотношение нейтрофилов к лимфоцитам и средний объём тромбоцитов

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## АННОТАЦИЯ

**Обоснование.** Известно, что вирус гепатита В, являющийся серьёзной проблемой общественного здравоохранения во всём мире, уже поразил более 250 млн человек в мире.

**Цель** — количественно оценить такие показатели гемограммы, как соотношение нейтрофильных лимфоцитов (NLR), соотношение тромбоцитарных лимфоцитов (PLR) и средний объём тромбоцитов (MPV), и определить их связь с воспалением при инфицировании вирусом гепатита В (HBV).

**Материалы и методы.** В исследование включены 94 пациента в возрасте от 2 до 18 лет, находившиеся под наблюдением по поводу HBV в период с 1 января 2005 по 31 декабря 2015 года. Контрольную группу составили 88 здоровых детей с аналогичными половозрастными характеристиками.

**Результаты.** Не обнаружено статистически значимой разницы в отношении числа нейтрофилов и соотношения нейтрофилов / лимфоциты ( $p=0,698$  и  $p=0,842$  соответственно). В группе с HBV+ зарегистрировано значительно более низкое абсолютное число лимфоцитов и значительно более высокий MPV, чем в контрольной группе ( $p < 0,05$  для обоих сравнений). Группы имели сходные значения гематокрита, концентрации гемоглобина, числа тромбоцитов, эритроцитов и лейкоцитов ( $p=0,642$ ,  $p=0,388$ ,  $p=0,418$ ,  $p=0,366$ ,  $p=0,824$  соответственно). Обе группы по отдельности показали положительную корреляцию между числом тромбоцитов и MPV ( $r=0,32$ ;  $p=0,01$  и  $r=0,36$ ;  $p=0,01$  соответственно). NLR и MPV положительно коррелировали в обеих группах ( $r=0,38$ ;  $p=0,01$  и  $r=0,41$ ;  $p=0,01$  соответственно).

**Заключение.** Исследуемые группы не продемонстрировали каких-либо существенных различий в отношении уровней NLR, PLR, PLT / MPV, но было обнаружено значительное межгрупповое различие в показателе MPV. Мы считаем очень важным разработать недорогие и простые в использовании неинвазивные методы определения уровня воспаления при HBV. Такие параметры, как NLR, PLR и MPV могут оказаться пригодными тест-маркерами для подобных целей.

**Ключевые слова:** гепатит В; соотношение нейтрофилов и лимфоцитов; средний объём тромбоцитов.

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## INTRODUCTION

It is estimated that the hepatitis B virus, a serious global public health problem, affects more than 250 million people [1]. As a cause of chronic liver infection, this viral infection can cause liver cirrhosis and hepatocellular carcinoma over time. Although there are many invasive and noninvasive methods to detect these complications, liver biopsy remains the main method for determining the level of necroinflammation and making the diagnosis and treatment decisions [2]. However, as an invasive method, liver biopsy is not the preferred method in every case due to the risk of bleeding, the possibility of incorrect sampling, and its painful nature [3]. In this sense, noninvasive methods can come into prominence as important tools for diagnosis and follow-up of treatment outcomes. Chronic hepatitis B patients are mostly inactive hepatitis B virus carriers, which transform into liver cirrhosis and hepatocellular carcinoma, albeit to a lesser extent later [4]. In these patients, it is important to monitor the development of hepatic cirrhosis and hepatocellular cancer, and hepatic tissue sampling is still an important tool for diagnosing hepatocellular cancer. It is important to determine the level of inflammation and chronicity using noninvasive methods among inactive hepatitis B carriers. Neutrophil-lymphocyte ratio (NLR) and mean platelet volume (MPV) represent inexpensive, easy-to-access, and easy-to-interpret markers calculated from full blood count parameters that are studied in almost every healthcare facility. The particularly neutrophil-lymphocyte ratio is considered to have an ability to guide mortality prediction in liver failure complicating acute and chronic hepatitis and to determine recurrent hepatocellular carcinoma cases after liver transplant [5, 6]. Although there is a limited number of studies on this subject, NLR levels are low in liver fibrosis cases [4]. NLR can be an ancillary marker of both liver injury and malignancy potential. MPV, another hematological parameter, has the potential as a marker of inflammatory conditions along with atherosclerosis-related disorders [7, 8]. Herein, our objective was to quantify several hemogram measurements like NLR, platelet lymphocyte ratio (PLR), and MPV and to determine their relationship with inflammation in hepatitis B infection (HBV).

## MATERIALS AND METHODS

This study included 94 patients aged 2–18 years, who were under follow-up at our hospital for hepatitis B infection between January 1st, 2005 and December 31st, 2015 as the patient group. The control group was composed of 88 healthy children of similar age and sex. All patients' medical records and digital data on the hospital automation system were retrospectively reviewed. Patients who had positive hepatitis B surface antigen (HBsAg) for a minimum of six months and who were considered inactive hepatitis B carriers (hepatitis B virus e-antigen (HBeAg) negativity and normal alanine

aminotransferase (ALT) levels in two separate measurements) were enrolled. HBV DNA; glucose; ALT; gamma-glutamyl transferase; albumin; prothrombin time; activated partial thromboplastin time; and the hemogram parameters, such as white blood cell, neutrophil, lymphocyte, platelet counts, and MPV were measured. NLR was calculated by taking the neutrophil-lymphocyte ratio. Likewise, PLR and PLT/MPV ratio were similarly obtained and recorded.

Blood samples were taken into glass tubes containing a standard amount of ethylenediaminetetraacetic acid and analyzed in our hospital's central biochemistry laboratory using a regularly calibrated device (Sysmex xn-1000 sa-01).

This study complied with the criteria of the Declaration of Helsinki. We obtained the approval of the Local Ethics Committee of MHU Diyarbakır Gazi Yaşargil Training and Research Hospital (2020/697).

## Statistical Analysis

SPSS version 16.0 (software package for Windows, USA) was used to analyze all study data. Numerical variables that met parametric test assumptions were expressed in terms of mean  $\pm$  standard deviation, whereas nonparametric variables were expressed as numbers and percentages. Categorical variables were compared using the Chi-square test. Independent samples t-test was used for continuous variables with normal distribution, whereas continuous variables without normal distribution were compared with the help of the Mann-Whitney U-test. Correlations between normally distributed variables and non-normally distributed variables were tested with Pearson's correlation test and Spearman's correlation test, respectively. A *p*-value of less than 0.05 indicated statistical significance.

## RESULTS

There were 94 inactive hepatitis B carriers and 88 healthy controls (table). Patients with inactive hepatitis B infection and the healthy controls did not significantly differ in terms of age ( $11.4 \pm 3.8$  vs.  $11.2 \pm 2.1$ , respectively;  $p=0.860$ ) or sex ( $p=0.848$ ). There was no significant difference with respect to neutrophil count and neutrophil/lymphocyte ratio ( $p=0.698$ ,  $p=0.842$ , respectively), either. A significantly lower absolute lymphocyte count significantly and a significantly higher MPV was found in the hepatitis B-positive group than in the controls ( $p < 0.05$  for both comparisons). The groups had similar hematocrit, hemoglobin, platelet count, RBC count, and WBC count ( $p=0.642$ ,  $p=0.388$ ,  $p=0.418$ ,  $p=0.366$ , and  $p=0.824$ , respectively).

The two groups individually showed a positive correlation between platelet count and MPV ( $r=0.32$ ,  $p=0.01$  and  $r=0.36$ ,  $p=0.01$ , respectively). NLR and MPV were positively correlated in both groups ( $r=0.38$ ,  $p=0.01$  and  $r=0.41$ ,  $p=0.01$ , respectively).

In both groups, positive correlations existed among lymphocyte count, platelet count, and MPV ( $r=0.289$ ,  $p=0.01$  and

$r=0.177$ ,  $p=0.047$ , respectively, for the patient group and  $r=0.187$ ,  $p=0.23$  and  $r=0.257$ ,  $p=0.001$ , respectively, for the control group).

## DISCUSSION

Although hepatitis B virus (HBV) infection can be prevented by a vaccine, its global prevalence is an ongoing problem as it is a significant source of disease burden and death worldwide [9]. According to World Health Organization data, it is estimated that 780 thousand deaths occur every year due to acute and chronic hepatitis B [10]. South America, Central America, Southern and Eastern Europe, Turkey, Central Asia, and the Middle East are considered to be middle endemic regions in terms of hepatitis B prevalence [10]. Clinically, hepatitis B has acute, subacute, and chronic forms, and may manifest with hepatic cirrhosis and hepatocellular cancer in the long term [12]. Hepatitis B constitutes a major etiology of chronic hepatic inflammation. Liver inflammation resulting from an immunological reaction increases the rates of cellular proliferation, cellular death, and genetic alterations and elevates the risk of hepatocellular carcinoma risk [13]. Therefore, having a thorough knowledge of the inflammation level in patients infected with hepatitis B is important for the patient follow-up to be ensured.

Determination of the inflammation level with simple, inexpensive, and noninvasive methods is important to predict the course of inflammation, particularly its progression to cirrhosis. Recently, noninvasive methods, such as FibroScan and tissue elastography, have been increasingly used to assess structural changes in the liver tissue [2–4]. Özer

et al. stated that NLR and MPV could be used as markers of chronic inflammation in distinct disease groups, such as cardiovascular disease, cancer, and liver cirrhosis [8]. In a study on adult patients with liver failure due to hepatitis B, NLR values of 2.36 or below were associated with lower mortality risk and values of 6.12 or above with higher mortality risk [5]. A similar study by Chen et al. showed an elevated NLR level in liver failure compared with chronic hepatitis and a healthy liver. However, the control and hepatitis B groups in this study did not differ. Similarly, Çelikbilek et al. did not find any significant difference between adult hepatitis B and control groups [3, 14]. NLR levels did not significantly differ between the patient and control groups in a study by Uluca et al. on children with inactive hepatitis B carriers [15]. Similar to the previous observations, our chronic hepatitis B and control groups did not show any significant difference regarding NLR.

There are several studies on clinical decision-making in various diseases using MPV [16, 17]. Henning et al. reported that platelet activation increased in proportion to MPV increase, and some cytokines released more easily aggregated with other mediators, thereby causing various diseases [18]. In a similar study, Ekiz et al. showed significantly higher MPV levels in patients with chronic hepatitis B than in controls [19, 20]. Atakan-Erkal et al. also showed a significantly higher MPV level in hepatitis patients than in the control [21]. In line with the literature data, our study documented higher MPV values in hepatitis B patients than in controls. A lower ratio of blood lymphocytes, which are white blood cells, may indicate a higher risk of infection. Lymphocytes affect the elimination of bacteria, viruses, toxins, and cancer cells in the human

**Table.** Demographic features and laboratory values of the study groups

Features / Values	Inactive HBV carriers (n=94)	Controls (n=88)	p
Age, years	11.9±3.4	11.0±2.7	0.86
Sex, M/F	56/38	51/37	0.84
White blood cell count, 10 <sup>3</sup> /mm <sup>3</sup>	7.56±1.90	7.62±1.33	0.82
Neutrophil count, 10 <sup>3</sup> /mm <sup>3</sup>	3.77±1.71	4.09±1.46	0.69
N/LR	2.82±1.12	2.22±1.72	0.76
Lymphocyte count, 10 <sup>3</sup> /mm <sup>3</sup>	1.62±1.4	2.46±0.58	<0.05*
PLT/LR	118.88±30.14	116.84±40.68	0.88
Platelet count, 10 <sup>3</sup> /mm <sup>3</sup>	314±76	306±49	0.41
MPV, fL	7.8±1.1	7.9±1.1	<0.05*
PLT/MPV	26.28±7.24	29.42±8.42	0.52
RCB, 10 <sup>12</sup> /L	4.68±0.52	4.62±0.54	0.36
Hemoglobin, g/dL	12.24±2.12	12.10±2.08	0.38
HCT, %	41.78±5.14	41.64±5.11	0.64

**Note:** HBV — Hepatitis B virus, MPV — Mean platelet volume, N/LR — Neutrophil/Lymphocyte Ratio, PLT — Platelet count, Hb — Hemoglobin, HCT — Hematocrit, RBC — Erythrocyte count, and WBC — Leucocyte count.

body. A fall in their number may lead to weakened immunity [22]. NLR is calculated by dividing blood neutrophil count by lymphocyte count and PLR by dividing platelet count by lymphocyte count. In clinical practice these ratios are used as markers for determining the prognosis of inflammatory diseases; particularly, NLR is considered a marker of subclinical inflammation [23, 24]. Our study did not show any significant difference between the hepatitis B and control groups in terms of NLR and PLR. The state of the immune system is correlated to inflammation; a strong immune response may prevent HBV infection, whereas a weak immune response may cause an infection to progress and become chronic. In these patients, determining inflammation level is important concerning monitoring of the process.

It is known that liver biopsy is the gold standard in the treatment and follow-up of patients with chronic hepatitis B. However, biopsy is not always possible and some markers are needed to predict liver histopathology [25].

Thus, we believe that it is of great importance to develop simpler, inexpensive, and easy-to-use noninvasive methods to determine the level of chronicity; parameters, such as NLR, PLR, and MPV, could be simple and rapid test markers to serve these goals.

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## CONCLUSION

In conclusion, the study groups did not exhibit any significant difference concerning NLR, PLR, and PLT/MPV levels but it found a significant intergroup difference in MPV. The retrospective nature of the study being conducted in a single center with a small number of patients, absence of liver biopsy results, absence of a chronic active hepatitis group, and absence of demonstrating a correlation between clinical symptoms and the study parameters are the major limitations of our study. We believe that a larger perspective and multicenter studies are needed to clarify the subject.

## ADDITIONAL INFO

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