



Evaluation of Platelet Indices in Young Male Patients with Hepatitis C

Hepatit C Enfeksiyonu Olan Genç Erkek Hastalarda Platelet Parametrelerinin Değerlendirilmesi

HCV Infection and Platelet Indices

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Özet

Amaç: Hepatit C virüs (HCV) enfeksiyonu olan hastalarda enflamasyon ve fibrozisin şiddetine bağlı olarak platelet sayısı ve boyutlarının değişebileceği gerçeği doğrultusunda, HCV enfeksiyonu olan erkek hastalarda sağlıklı kontrol grubuna kıyasla platelet parametrelerinin ve platelet-lenfosit oranı (PLR) seviyelerini ve önemini araştırmayı amaçladık. **Gereç ve Yöntem:** 01 Ocak 2012 ve 01 Haziran 2015 tarihleri arasında Enfeksiyon Hastalıkları polikliniğine HCV enfeksiyonu nedeniyle müraacaat eden hastaların dosyaları geriye dönük olarak değerlendirildi. Karaciğer biyopsisi olmuş 21 erkek hasta ile cinsiyet ve yaş uyumlu 22 sağlıklı kontrol çalışmaya dahil edildi. İstatistiksel analizler SPSS 15.0 programı ile yapıldı. **Bulgular:** Hasta grubu (ortalama yaş=24.62±5.84 yıl ve yaş aralığı=21-41 yıl) ve kontrol grubu (ortalama yaş=24.59±5.21 yıl ve yaş aralığı=21-41 yıl) arasında yaş değişkeni açısından anlamlı farklılık yoktu. Hastaların %23.8'inde fibrozis skoru 1, %61.9'unda fibrozis skoru 2 ve sadece %14.3'ünde fibrozis skoru 3 olarak belirlendi. Kontrol grubuna göre hasta grubunda, lenfosit sayıları, platelet sayıları, PLR, plateletcrit ve platelet hacmi dağılım genişliği düşük ve ortalama platelet hacmi (MPV) daha yüksek olsa da, iki grup arasında istatistiksel olarak anlamlı farklılık yoktu. Ayrıca, platelet sayıları, platelet parametreleri ve PLR değerleri açısından fibrozis skoruna ve histolojik aktivite indeksi skoruna göre hasta grupları arasında da anlamlı farklılık yoktu. **Tartışma:** Çalışmamız sonucunda HCV enfeksiyonuna bağlı minimal ve orta derecede fibrozisi olan genç yetişkin erkek hastalarda, platelet sayıları, platelet parametreleri ve PLR değerlerinde anlamlı olmayan minimal bir etkilenme tespit edildi. Bununla birlikte, platelet sayılarının daha düşük beklendiği ilerlemiş fibrozisi veya sirozu olan hastalarda, platelet parametrelerinin önemi artabilir.

Anahtar Kelimeler

Hepatit C Virüsü; Fibrozis; Platelet Parametreleri; Ortalama Platelet Hacmi; Platelet-Lenfosit Oranı

Abstract

Aim: In light of the fact that platelet counts and sizes or lymphocyte counts may be altered in patients with hepatitis C virus (HCV) depending upon the severity of inflammation or fibrosis, we aimed to investigate the values and the roles of platelet indices and platelet-to-lymphocyte ratio (PLR) in male patients with HCV infection compared with healthy controls. **Material and Method:** Patients with HCV infection who applied to an Infectious Diseases clinic between 01 January 2012 and 01 June 2015 were evaluated retrospectively. Overall, 21 male HCV patients who underwent percutaneous liver biopsy and 22 gender-and age- matched healthy controls were included in the study. Statistical analyses were performed by SPSS 15.0. **Results:** There was no significant difference in age variable between the patients group with an age range of 21-41 years [22 (Iq=4), 24.62±5.84 years] and the control group with an age range of 21-41 years [22.5 (Iq=5), 24.59±5.21 years]. The distribution of fibrosis scores among HCV patients was 23.8% fibrosis score 1, 61.9% fibrosis score 2, and only 14.3% fibrosis score 3. Lymphocyte counts, platelet counts, PLR, platelet volume, distribution width, and platelet-crit values were lower, and mean platelet volume (MPV) values were higher, in the patients group compared with controls, but there was no statistical difference between the two groups. There was also no significant difference in platelet counts, platelet indices, or PLR values between groups according to the fibrosis scores or according to the histological activity index (HAI) score. **Discussion:** In our study, there were minimally but not significantly affected platelet counts, platelet indices, and PLR values in young adult male patients with minimal or moderate fibrosis induced by HCV infection. However, in patients with advanced liver fibrosis or cirrhosis whose platelet counts may be lower, platelet indices may increase in importance.

Keywords

Hepatit C Virus; Fibrosis; Platelet Indices; Mean Platelet Volume; Platelet-To-Lymphocyte Ratio

DOI: 10.4328/JCAM.4537

Received: 05.04.2016 Accepted: 25.04.2016 Printed: 01.09.2016

J Clin Anal Med 2016;7(5): 706-10

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Introduction

Chronic hepatitis C virus (HCV), a leading cause of cirrhosis and hepatocellular carcinoma, is a global health problem with 80-185 million infected individuals worldwide, although the treatment of HCV has shown significant progress with the development of direct-acting antivirals [1]. Percutaneous liver biopsy is the gold standard for detection of liver fibrosis, which is important in predicting prognosis and selecting treatment options in patients with viral hepatitis [2]. However, because liver biopsy is an invasive method and may cause severe complications, there are attempts to find non-invasive predictive models to substitute for liver biopsy; these include elastography, Forns' index, FIB-4 index (fibrosis index based on four factors), non-invasive hepatitis C related cirrhosis early detection (NIHCED) score, aspartate aminotransferase-to-platelet ratio index (APRI), and platelet indices [3-7].

Platelets also have an important role in the inflammatory response, apart from their function in haemostasis. Their counts or sizes may change in parallel with the severity of infections, including primary viral hepatitis [3-11]. Furthermore, fibrosis severity in patients with hepatitis is another factor influencing platelet counts and indices [3-7].

Aside from total platelet count, indices of platelets such as mean platelet volume (MPV), platelet volume distribution width (PDW), and plateletcrit (PCT) can easily be calculated from routine complete blood count (CBC). MPV measures the average size of the platelets and indicates platelet activation. MPV levels have been described as a diagnostic or prognostic predictor not only for patients with prothrombotic conditions including obesity, hypercholesterolemia, diabetes, hypertension, smoking, and arterial stiffness, but also for those with proinflammatory conditions including rheumatic diseases or infectious disorders such as sepsis, infective endocarditis, pneumonia, or viral hepatitis [3-11]. Also, decreased MPV levels in human immunodeficiency virus (HIV) patients compared with controls have been shown to be correlated with PCT, PDW, and viral load [12]. PDW, an indicator of the variation in platelet size, has also been found significantly higher in non-survivor sepsis patients and has been defined as an independent variable determining the severity of fibrosis [5, 8, 9]. PCT, a reliable parameter of platelet biomass but also the most neglected CBC parameter in clinical practice, is influenced by platelet numbers as well as platelet sizes [8, 12, 13]. PCT has been reported to be negatively correlated with parasitema in patients with malaria caused by *Plasmodium vivax* [13]. Abnormal platelet indices have also been found to be associated with more severe illness and higher risk of death in intensive care unit patients [14].

Not only platelet counts, but also lymphocyte counts can be affected by chronic inflammatory diseases, infectious diseases, malignancies and myeloproliferative disorders [15-17]. Therefore, the platelet-to-lymphocyte ratio (PLR) may be altered by these disorders. PLR has been shown to be associated with inflammation and inflammation severity and is also described as an independent predictor of some infectious diseases such as infective endocarditis [15-17].

In this study, in light of the fact that platelet counts and sizes or lymphocyte counts may be altered in patients with HCV infection depending upon the severity of inflammation or fibrosis, we

aimed to investigate the values and the roles of platelet indices and PLR in male patients with HCV infection compared with healthy controls.

Material and Method

For this study, ethics committee approval was obtained from the Gülhane Military Medical Academy Haydarpaşa Teaching Hospital Non-invasive Clinical Research Ethics Committee. Patients with HCV infection who applied to the Kasımpasa Military Hospital Infectious Diseases clinic between 01 January 2012 and 01 June 2015 were evaluated retrospectively. Male HCV patients who underwent percutaneous liver biopsy were included in the study. Patients with hepatitis B (HBV) or HIV coinfection or with comorbidities such as chronic inflammatory diseases, malignancies, corticosteroid use, myeloproliferative disorders, and other any infectious diseases were excluded. Gender- and age-matched healthy individuals with no known disease and normal physical examination from whom blood samples had been obtained between the same dates were enrolled in the control group.

In the patients group, the data including patients' age, platelet counts, lymphocyte counts, values of MPV, PDW, PCT, and PLR, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, HCV ribonucleic acid (RNA) levels, fibrosis score, and histological activity index (HAI) score were recorded. Also age, platelet counts, lymphocyte counts, and values of MPV, PDW, PCT, and PLR of healthy controls were noted. In the patients group, CBC was performed with samples that had been taken about one week before liver biopsy. Fibrosis score and HAI score were evaluated by means of modified histological activity index.

Statistical analyses were performed by SPSS 15.0 (SPSS Inc., Chicago, IL, USA). For determining the distribution of continuous variables, the Shapiro-Wilk test was used. All continuous variables were summarized as mean±standard deviation and median [interquartile range (Iq)]. The Mann-Whitney U test or Student's t-test were applied to compare continuous variables. Correlations between the variables were examined using Pearson's test or Spearman's correlation test, depending on the normality of the data distribution. Also, the differences in the variables were analyzed using analysis of variance (ANOVA) or the Kruskal-Wallis tests. p values <0.05 were considered to be statistically significant for all analyses.

Results

Overall, 21 male patients who underwent liver biopsy due to HCV infection without any comorbidities were enrolled in the patients group and 22 gender- and age-matched healthy individuals were included as the control group. All HCV patients were treatment naive. The age variable was not distributed normally. There was no significant difference ($p=0.800$) in the age variable between the patients group, with an age range of 21-41 years [22 (Iq=4), 24.62±5.84 years], and the control group with an age range of 21-41 years [22.5 (Iq=5), 24.59±5.21 years].

While platelet count, PLR, and MPV variables were distributed normally, lymphocyte count, PCT, and PDW variables were not distributed normally. Results of these parameters are summa-

rized in Table 1. Lymphocyte counts, platelet counts, PLR, PCT, and PDW values were lower, and MPV values were higher in the patients group compared with controls, but there was no statistical difference between the two groups (respectively, $p=0.551$, $p=0.562$, $p=0.656$, $p=0.117$, $p=0.362$ and $p=0.433$).

Patients were divided into 3 groups according to their fibrosis score. There were 5 patients with fibrosis score 1 (23.8%), 13 patients with fibrosis score 2 (61.9%), and only 3 patients with fibrosis score 3 (14.3%). The results of platelet counts, MPV, PDW, PCT, and PLR according to the fibrosis score in the patients group are summarized in Table 2. There was no significant difference in platelet counts, MPV, PDW, PCT, and PLR variables between the groups according to the fibrosis scores, whereas there were declining trends in platelet counts, MPV, and PLR levels when the fibrosis score increased.

Patients were divided into two groups according to their HAI score. Patients whose HAI score was equal or lower than 6 ($n=14$, 66.7%) were in the first group and patients whose HAI score were higher than 6 ($n=7$, 33.3%) were in the second. Results of platelet counts, MPV, PDW, PCT, and PLR according to the HAI score in the patients group are summarized in Table 3. All parameters were lower in patients with higher HAI score, but there was not any significant difference between groups.

The mean AST value was 57.57 ± 28.71 U/l with a range of 23-124 U/l. There was no correlation between AST-platelet counts ($r=0.226$, $p=0.324$), AST-PLR ($r=0.056$, $p=0.811$), AST-MPV ($r=0.155$, $p=0.502$), AST-PCT ($r=0.341$, $p=0.130$), and AST-PDW

($r=-0.050$, $p=0.829$).

The mean ALT value was 97.14 ± 71.70 U/l with a range of 23-285 U/l and median (Iq) of 66 (118). Also, we found no correlation between ALT and platelet counts, PLR, MPV, PCT, or PDW (respectively, $r=0.274$ and $p=0.229$, $r=0.232$ and $p=0.311$, $r=-0.105$ and $p=0.652$, $r=0.236$ and $p=0.303$, $r=0.017$ and $p=0.942$).

The mean HCV RNA level was 6.12 ± 0.26 log₁₀ IU/ml with a range of 2.76-7.28 log₁₀ IU/ml and median (Iq) of 6.56 (1.57) log₁₀ IU/ml. There was also no correlation between HCV RNA-platelet counts ($r=0.144$, $p=0.533$), HCV RNA-PLR ($r=-0.065$, $p=0.780$), HCV RNA-MPV ($r=-0.101$, $p=0.664$), HCV RNA-PCT ($r=0.091$, $p=0.695$), and HCV RNA-PDW ($r=-0.246$, $p=0.283$).

Discussion

Platelets have an important role in the inflammatory response in addition to their function in haemostasis. Platelet numbers and sizes may be affected depending upon the severity of inflammation in patients with infection and also the severity of fibrosis in patients with viral hepatitis such as HBV or HCV [3-7]. Accordingly, we investigated the values and the roles of platelet numbers, platelet indices, and PLR in young adult male patients with HCV infection compared with healthy controls.

The median age was 22 (Iq=4) years and the mean age was 24.62 ± 5.84 years in the patients group with an age range of 21-41 years. The distribution of fibrosis scores was 23.8% fibrosis score 1, 61.9% fibrosis score 2, and only 14.3% fibrosis score 3. Also, 14 patients (66.7%) had an HAI score equal to or lower than six and 7 patients (33.3%) had an HAI score higher than six. We think that these low scores, particularly fibrosis scores, may result from the fact that the patients were young adults and so their exposure to the virus has been of short duration.

In our study, platelet counts and also PLR values were lower in the patients group compared with in controls, but there was no statistical difference between the two groups. Due to immune mechanisms including platelet-specific antibodies or auto-immunity, HCV-mediated bone marrow suppression, hypersplenism, inadequate production of trombopoietin, endothelial dysfunction, and drugs used in therapy or fibrosis, thrombocytopenia can

Table 1. Results of platelet counts, lymphocyte counts, MPV, PDW, PCT, and PLR.

Variables	Patients Group		Control Group		P value
	Mean \pm standard deviation	Median (interquartile range)	Mean \pm standard deviation	Median (interquartile range)	
Lymphocyte ¹ ($10^3/mm^3$)	2.25 \pm 0.48	2.20 (0.48)	2.34 \pm 0.69	2.30 (0.76)	0.551 ³
Platelet ² ($10^3/mm^3$)	261.90 \pm 59.103	255 (68)	272.05 \pm 54.512	263.00 (74)	0.562 ⁴
PLR ²	120.15 \pm 35.68	114.21 (40.40)	125.31 \pm 39.52	127.06 (61.30)	0.656 ⁴
MPV ² (fl)	7.95 \pm 1.25	7.80 (2.15)	7.72 \pm 0.50	7.80 (0.50)	0.433 ⁴
PCT ¹ (%)	0.206 \pm 0.055	0.182 (0.070)	0.224 \pm 0.0506	0.210 (0.060)	0.117 ³
PDW ¹ (%)	27.36 \pm 16.34	17.40 (31.70)	31.56 \pm 16.19	39.80 (30.50)	0.362 ³

¹not distributed normally by Shapiro-Wilk test

²distributed normally by Shapiro-Wilk test

³by Mann-Whitney U test

⁴by Student's t-test

Table 2. Results of platelet counts, MPV, PDW, PCT, and PLR according to the fibrosis score in patients group.

Fibrosis score	Number (%)	Platelet count ($10^3/mm^3$)			MPV (fl)			PLR			PCT (%)			PDW (%)		
		M \pm Sd	Md (Iq)	p value	M \pm Sd	Md (Iq)	p value	M \pm Sd	Md (Iq)	p value	M \pm Sd	Md (Iq)	p value	M \pm Sd	Md (Iq)	p value
		1	5 (23.8%)	264.2 \pm 92.7	264 (162)		8.04 \pm 1.25	8.50 (2.45)		124.3 \pm 32.6	127.1 (54.1)		0.214 \pm 0.087	0.175 (0.163)		27.98 \pm 17.99
2	13 (61.9%)	263.9 \pm 51.8	250 (64)	0.931 ¹	8.01 \pm 1.37	7.80 (2.15)	0.857 ¹	121.7 \pm 39.3	113.33 (47.91)	0.778 ¹	0.209 \pm 0.049	0.212 (0.070)	0.827 ²	28.16 \pm 16.25	17.40 (28.50)	0.568 ²
3	3 (14.3%)	249.3 \pm 36.8	255 (-)		7.50 \pm 0.98	7.00 (-)		106.2 \pm 31.0	110.86 (-)		0.185 \pm 0.011	0.180 (-)		22.86 \pm 20.11	13.10 (-)	

M \pm Sd; Mean \pm standard deviation

Md (Iq); Median (interquartile range)

¹ by analysis of variance (ANOVA) test

² by Kruskal -Wallis test

Table 3. Results of platelet counts, MPV, PDW, PCT, and PLR according to the HAI score in patients group.

HAI score	Number (%)	Platelet count (10 ³ /mm ³)			MPV (fl)			PLR			PCT (%)			PDW (%)		
		M ±Sd	Md (Iq)	p value	M ±Sd	Md (Iq)	p value	M ±Sd	Md (Iq)	p value	M ±Sd	Md (Iq)	p value	M ±Sd	Md (Iq)	p value
≤ 6	14 (66.7%)	271.86 ±67.20	265 (98)	0.193 ¹	8.00 ±1.39	7.70 (2.38)	0.781 ¹	121.41 ±39.26	116.62 (49.26)	0.809 ¹	0.216 ±0.063	0.197 (0.099)	0.478 ²	28.86 ±17.29	17.70 (34.25)	0.247 ²
> 6	7 (33.3%)	242.00 ±34.14	241 (68)		7.85 ±1.00	7.80 (1.70)		117.62 ±29.89	113.33 (32.20)		0.187 ±0.030	0.180 (0.060)		24.35 ±15.05	14.80 (24.70)	

M±Sd; Mean±standard deviation

Md (Iq); Median (interquartile range)

¹ by Student's t-test² by Mann-Whitney U test

be seen with 24% prevalence in patients with HCV infection [18]. Indeed, hypersplenism, inadequate production of thrombopoietin, and endothelial dysfunction are associated with advanced fibrosis [18]. So, it may be that because our patients had minimal or moderate fibrosis, there was no significant difference in platelet counts between patients and controls. Furthermore, there are trials suggesting different results regarding platelet numbers in patients with HBV or HCV infection [3-7, 19-23].

We found lower PCT and PDW values and higher MPV values in the patients group compared with the controls. However, there was no statistical difference between the two groups. Under normal circumstances, high MPV levels in destructive thrombocytopenia and low MPV levels in hypoproliferative thrombocytopenia, high PDW values due to swelling, destruction, and immaturity, and PCT values are expected to be non-linearly correlated with platelet counts [8, 9, 24]. PDW has been considered to be a more reliable marker for distinguishing hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia [24]. In the literature, there are studies showing higher MPV values in patients with HCV or HBV infection, no significant difference in MPV values in acute HBV patients or inactive HBV carriers, decreased MPV levels in HIV patients, and lower PCT values and higher PDW values in patients with HBV infection compared with those in controls [6, 7, 12, 20-23]. We consider that platelet indices in our patients may not be significantly affected, in parallel with minimal thrombocytopenia.

Among HCV patients in this study, there was no significant difference in platelet counts, MPV, PDW, PCT, and PLR variables between groups according to the fibrosis scores or according to the HAI score, whereas there were declining trends in platelet counts, MPV, and PLR levels when the fibrosis score increased, and all parameters were lower in patients with a higher HAI score. There are also debates about the predictive value of platelet indices. Although MPV has been shown to be positively correlated with the model for end-stage liver disease (MELD) score, not only high MPV values but also low MPV values have been reported to be independent predictors for cirrhosis or in determining the severity of fibrosis in different trials [3-7]. Also, in a study investigating MPV levels in patients with HBV or HCV infection, no reliable results in HBV patients have been reported [3]. In contrast, in patients with HCV infection, MPV has been described as a useful marker in predicting advanced liver damage [3]. In one study, PDW was determined to be an independent variable determining the severity of fibrosis in HBV patients,

while another study including HBV patients showed that there was no significant difference in PDW levels between the groups according to the fibrosis scores [4, 5].

In our study, no correlation between serum AST, ALT, or HCV RNA levels and platelet indices was found in young adult male patients with HCV infection. In the literature, decreased MPV levels in HIV patients have been shown to be correlated with PCT, PDW, and viral load [12]. Also, platelet counts and PCT have been reported to be inversely correlated with HBV deoxyribonucleic acid (DNA) levels [25]. In another study, there was no difference in MPV values between patients with normal ALT levels and patients with high ALT levels or between patients with HCV RNA negativity and patients with HCV RNA positivity [23]. There are several limitations in the present study, the most important of which is the retrospective design. In addition, the number of study patients was limited. One of the reasons for this limitation was the difficulty in finding HCV patients who underwent liver biopsy. The fact that the study was a single-center study may be another drawback and another cause of the limited number of study patients. Furthermore, the patients included in the study were young adults and male because our hospital is a military hospital and our patients are usually young adult males. This may result in short exposure duration to the virus and therefore resulting in low fibrosis scores.

Consequently, it is a fact that thrombocytopenia can be seen in patients with HCV infection, there is a relation between severity of fibrosis and platelet counts, and platelet indices may be altered due to thrombocytopenia [18, 24]. The question remains whether platelet counts, platelet indices, or PLR in patients with minimal or moderate fibrosis may differ significantly from those in healthy individuals. In our study, there were minimally but not significantly affected platelet counts, platelet indices, and PLR values in young adult male patients with minimal or moderate fibrosis induced by HCV infection. However, in patients with advanced liver fibrosis or cirrhosis whose platelet counts may be lower, platelet indices may increase in importance. There are several attempts to find non-invasive predictive models to substitute for liver biopsy. Although there are debates about the value of platelet indices in predicting or determining the severity of fibrosis and there are several disorders affecting platelet indices as a confounding factor, platelet indices, especially in a scoring system, may be a cost-effective and useful marker for evaluating the fibrosis degree in patients with viral hepatitis. However, in order to highlight the role and importance of platelet indices in patients with viral hepatitis, randomized

large-scale studies are required. We are planning to investigate different scoring systems containing platelet indices and evaluating the fibrosis degree in patients with viral hepatitis in a large-scale, multi-center study.

Competing interests

The authors declare that they have no competing interests.

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How to cite this article:

Afyon M, Artuk C, Yenilmez E, Şimşek B, Kaya A. Evaluation of Platelet Incides in Young Male Patients with Hepatitis C. *J Clin Anal Med* 2016;7(5): 706-10.