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HCV Induced Thrombocytopenia in Punjab, Pakistan

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Abstract

HCV is the main causal agent of blood transmitted hepatitis which can lead to the development of liver cirrhosis and hepatocellular carcinoma in infected persons. In patients with HCV related hepatic diseases, immune mediated responses against surface antigens can cause the destruction of platelets. Despite a high prevalence of HCV and its extrahepatic presentations, reports on platelet count in HCV infected patients are scarce. Therefore, this study aims to determine thrombocytopenia in suspected HCV patients in Lahore, Pakistan. It also aims to find out its association with other factors such as age, gender, viral load and ALT. For this cross-sectional retrospective study, blood samples were collected from 200 suspected HCV patients, including both male and female patients, who were between 20-69 years of age. Serum ALT levels were determined and HCV antibodies were detected. Thrombocytopenia was defined as the platelet count below 150,000/µL. Pearson’s correlation was used to find out the statistically significant relationship of platelet count with age, viral load and ALT. Platelet count varied between 29.00- 402.00 x 10\textsuperscript{3}/µl. The mean platelet count was 206.87 ± 83.88 x 10\textsuperscript{3}/µl. Platelet count less than 150 x 10\textsuperscript{3}/µl was considered as thrombocytopenia, whereas platelet count measuring above 150 x 10\textsuperscript{3}/µl was considered normal. It was normal in 72% patients and low in 28% patients. There was a negative correlation between age and platelet count which was statistically significant (r = -0.304, N= 200, p = 0.00012).

Keywords: thrombocytopenia, platelets, HCV, viral load, ALT

1. Introduction

HCV is the main causal agent of blood transmitted hepatitis which can lead to the development of liver cirrhosis and hepatocellular carcinoma in infected persons [1]. According to a report of World Health Organization (WHO), approximately 80 million people are living with chronic HCV [2]. HCV is a leading cause of mortality and it causes 700,000 deaths every year due to liver related diseases [3]. Its prevalence is high in middle and low-income countries and it is mostly transmitted by unsafe injections and through iatrogenic transmission [4, 5, 6].

Hepatitis C virus is basically a hepatotropic and lymphotropic virus, although it has the ability to infect other parts of the body as well [7, 8]. These extrahepatic manifestations include a range of clinical presentations in HCV infected patients such as renal diseases, diabetes mellitus –II, cardiovascular diseases, HIV, and liver fibrosis which constitute the more frequent risk factors for clinical and biological extrahepatic manifestations in HCV patients [9, 10].
HCV is also known to interact with platelets by binding on their surface in chronically infected patients [11, 12]. The virus adsorsbs with platelets via fibronectin. These infected platelet transport HCV. [13]. Studies reported the detection of HCV in platelets of chronic HCV patients [14]. The HCV immune-mediated platelet destruction may lead to thrombocytopenia, a common clinical problem caused by infectious agents [15]. Thrombocytopenia occurs when the platelet count is low (<150,000/μL) and it results in immune suppression. Pathophysiology of HCV associated thrombocytopenia is a complex and multifactorial outcome [15]. Chronic HCV infection leads to a substantial autoimmune reaction to platelets, which may result in thrombocytopenia [16, 17, 18]. Hepatic cirrhosis and portal hypertension also cause thrombocytopenia leading to splenomegaly [19]. The severity of platelet reduction is influenced by the severity of liver destruction, viral load, gender and age of patients [20]. The presence of severe thrombocytopenia is linked to end-stage liver disease, whereas moderate thrombocytopenia with a platelet count of 50,000–75,000/μL results in bleeding, particularly in patients with other comorbidities [15]. The prevalence and severity of thrombocytopenia increases in untreated HCV patients with the progression of the disease. Its clinical manifestations are visible when extensive liver cirrhosis or fibrosis occurs. The improvement in the condition of thrombocytopenia in HCV patients after interferon therapy is suggestive of its association with the former [21].

HCV is a major health issue in Pakistan where 6% of the population is infected with it [22]. Reports suggest the prevalence of extrahepatic manifestations of HCV in chronic HCV patients at about 40%. [23]. Despite a high prevalence of HCV and its extrahepatic presentations, reports on platelet count in HCV patients are scarce. Therefore, this study aims to determine thrombocytopenia in suspected HCV patients in Lahore, Pakistan. It also aims to find out its association with other factors such as age, gender, viral load and ALT.

2. Methodology

2.1. Study Design

For this cross-sectional retrospective study, a total of 200 patients (including both male and female patients) suspected of HCV, who were between 20-69 years of age and visited National Genetic Laboratory, Lahore between February 2019 and July 2019, were screened for HCV seropositivity.

2.2. Determination of ALT

Testing was based on photometric analysis and the procedure was adopted according to the manufacturer’s (Diagnostest, Paris, France) instructions. OD was taken using semi-automated clinical chemistry analyzer Microlab-300.

2.3. Anti-HCV Screening

Patients with elevated levels of ALT were further screened for HCV antibodies through ELISA. HCV antibodies were analyzed using Anti-HCV II kit (Cobas, Roche, Switzerland) based on sandwich based ELISA. Readings were measured on Cobas e411 Analyzer. The results were determined automatically by the software by associating the electrochemiluminescence signal gained from the reaction
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product of the sample with the signal of the cutoff value before y calibration.

2.4. Platelet Evaluation

Platelets were evaluated using automation Sysmex (XN1000). The working principle of this instrument is based on spectrophotometry. Thrombocytopenia was defined as the platelet count below 150,000/μL.

2.5. Statistical Analysis

Data analysis using descriptive statistics was carried out through excel and SPSS (version 21). Demographic values were shown as Mean ± SD. Pearson’s correlation was used to find out the statistically significant relationship of platelet count with age, viral load and ALT.

3. Results

Two hundred patients suffering from HCV were included in the study. Their demographic information and clinical characteristics are shown in Table 1. These 200 patients comprised 122 men (61%) and 78 women (39%). The age of patients ranged between 21-63 years with a mean age of 44.98 ± 10.62. The mean age for men was recorded as 45.48 ± 10.44, whereas for women the mean age was 44.21 ± 10.90. We further stratified the patients into four age groups to see any association between age and platelet count. The distribution of patients by age group and gender is shown in Figure 1.

Platelet count varied between 29.00-402.00 x 10^3 /μl. The mean platelet count was 206.87 ± 83.88 x 10^3 /μl. Platelet count less than 150 x 10^3 /μl (low count) was considered as thrombocytopenia and platelet count above this value was considered normal. Platelet count was normal in 72% patients and low in 28% HCV infected patients (Figure 2). In this study, platelet count was found to be lower in males (19.5%) as compared to females (8.5%) (Table 2). The highest percentage (25%) of low platelet count was documented for the male age group of 41-51 years and 24% for 52-63 years. Low platelet count was documented for 13.85% females of all ages.

There was a negative correlation between age and platelet count which was statistically significant (r = -0.304, N= 200, p = 0.00012). Chi-square statistics for gender and platelet count (low and high) were also significant (p= 0.025). The minimum viral load was 12,599 copies/ml and the maximum was 36,821,469 copies/ml. The mean viral load was 1488511.7700 ± 4662182.38. In males, the mean viral load was 2092310 ± 5864816.28, while in females it was 544109.3590 ± 801644.28. Our study reported no statistically significant association between viral load and the number of platelets. ALT measure varied from 10 IU/L to 107 IU/L. For females, ALT level was 10 IU/L - 104 IU/L and for males, it was 10 IU/L -107 IU/L. The mean ALT for the whole study population was 38.02 ± 21.38. There was a negative correlation between ALT and PLT which was statistically significant (r = -0.285, N=200, p=0.000)

4. Discussion

A variable degree of thrombocytopenia is common in chronic HCV infection. It may be caused by a peripheral autoimmune mechanism and/or a central mechanism. As the disease progresses, platelet count decreases as measured by both mechanisms [20]. This study was conducted to find out the frequency of thrombocytopenia in HCV patients in Lahore, Pakistan. For this purpose,
peripheral platelet count along with the levels of ALT and viral load was calculated for the patients of HCV. About 28% patients included in this study were suffering from HCV induced thrombocytopenia (<150 x 10^3 / μl). A previous study from Pakistan reported thrombocytopenia in 43.3% HCV patients [24]. Behnava et al. reported the prevalence of thrombocytopenia in 13.3% chronic HCV patients [25]. Another study reported a high prevalence (22.5%) of HCV in chronic acquired thrombocytopenia [26]. A study diagnosed thrombocytopenia in 368 patients of chronic HCV. These patients showed elevated titers of platelet associated immunoglobulin G [18]. Our study found a weak and negative correlation between age and platelet count. Olariu et al. reported a positive correlation between thrombocytopenia and age [20].

Although the pathophysiology of HCV induced thrombocytopenia is not fully known, yet it has been suggested that HCV virus may generate an autoimmune reaction to platelets which may in turn lead to thrombocytopenia [18]. This study found a statistically significant association between gender and PLT, unlike the findings of another study which reported no significant association between the two variables [20]. Our study did not find any statistically significant association between viral load and platelet count but other studies have reported a higher HCV RNA and low platelet count in patients of hepatitis C virus [27]. The AST/ALT ratio of chronic HCV patients in tandem with PLT has been suggested as a predictor for liver fibrosis staging [28, 29].

![Graph](image)

**Figure 1.** Graph shows the distribution of HCV patients in the study population by age and gender. total n = 200, males n = 122, females n= 78, age range = 21-63
### Table 1. Distribution of Demographic and Clinical Variables

| Variable                | Total (n = 200) | Males (n = 122) | Females (n = 78) |
|-------------------------|-----------------|-----------------|-----------------|
|                         | Mean (SD)       | Min-Max         | Mean (SD)       | Min-Max         | Mean (SD)       | Min-Max         |
| Age (years)             | 44.98(10.62)    | 21-63           | 45.48(10.44)    | 21-62           | 44.21(10.90)    | 26-63           |
| Platelets (10^3/ μl l)  | 206.87(83.88)   | 29.00-402.00    | 203.6557(81.34) | 68-402          | 211.8974(88.03) | 29-398          |
| Viral load (copies/ml)  | 1488511.7700    | 12599-36821469  | 2092310         | 23658.00-544109.3590 | 12599.00-3.68E+007 | (801644.28) | 3.70E+006       |
| ALT(IU/L)               | 38.02(21.38)    | 10-107          | 35.55(19.18)    | 10-107          | 41.88(24.06)    | 10-104          |

### Table 2. Agewise Platelet Count in HCV Patients of Study Population

| Age (Years) | Total Count (N) | Male Low (PLT<150,000 μl) | Male Normal (PLT>150,000 μl) | Female Low (PLT<150,000 μl) | Female Normal (PLT>150,000 μl) | Total Low (PLT<150,000 μl) | Total Normal (PLT>150,000 μl) |
|-------------|-----------------|---------------------------|-------------------------------|-------------------------------|-------------------------------|---------------------------|-----------------------------|
|             | n               | %                         | n                             | %                             | n                             | %                         | n                           | %                            |
| 21-30       | 18              | 5.5                       | 8                             | 44.45                         | 0                             | 0                         | 9                           | 50                            | 1                             | 5.5                          | 17                           | 94.45                        |
| 31-40       | 57              | 12.28                     | 23                            | 40.35                         | 3                             | 5.26                      | 24                          | 42.10                         | 10                            | 17.54                        | 47                           | 82.45                        |
| 41-51       | 60              | 25                        | 26                            | 43.34                         | 5                             | 8.34                      | 14                          | 23.34                         | 20                            | 33.34                        | 40                           | 66.66                        |
| 52-63       | 65              | 24.62                     | 26                            | 40                            | 9                             | 13.85                     | 14                          | 21.54                         | 25                            | 38.47                        | 40                           | 61.53                        |
| 21-63       | 200             | 19.5                      | 83                            | 41.5                          | 17                            | 8.5                       | 61                          | 30.5                          | 56                            | 28                           | 144                          | 72                           |
Figure 2. Graph shows the overall distribution of low platelet (<150 \times 10^{3}/\mu l ) and normal platelet (150 to 450 \times 10^{3}/\mu l ) counts in HCV patients. total n = 200, males n = 122, females n = 78

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