

THROMBOCYTOPENIA ASSOCIATED WITH CHRONIC HEPATITIS-C INFECTION

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Abstract: Hepatitis C virus infection affects over 71 million people worldwide and predominantly leads to momentous morbidity and mortality through its predisposition to fibrosis, cirrhosis, and liver cancer. Annually, hepatitis C causes nearly 399,000 casualties globally. The main causes of death are usually cirrhosis and hepatocellular carcinoma. Along with hepatic snags, chronic HCV infection is also concomitant with numerous extra-hepatic manifestations including thrombocytopenia. Thrombocytopenia in individuals with chronic hepatitis C virus infection is a gigantic complication. The pathophysiology of thrombocytopenia is dependent on a number of factors, especially genetic factors. HCV virus also causes direct bone marrow suppression with subsequent thrombocytopenia. The present study has been conducted at the College of Allied health Professionals Faisalabad. Blood samples were collected from 146 subjects using an aseptic technique. All the individuals were screened for the presence of the hepatitis C virus by ELISA and ICT. Positive patients were further tested for platelet count. Out of 146 subjects 101 (69.17%) were male and 45 (30.82%) were female. While 48 % and 24% were positive for HCV by ICT and ELISA techniques respectively. 14 (38.8%) Patients out of 36 having HCV infection were positive for Thrombocytopenia

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INTRODUCTION

The liver is a vital organ it performs many functions which correlate with one another. The normal Human liver contains 50,000 to 100,000 lobules. Venous sinusoids are lined by two types of cells typical endothelial cells and Large Kupffer cells. The endothelium of liver sinusoids has tremendously large pores. Under these linings, there is a constricted space called the spaces of Disse. Due to these large pores in the endothelium, substances, and other components in the plasma freely move into these spaces (1). The liver has an extraordinary ability to re-establish after hepatic tissue injury and loss of tissue by hepatectomy. During the process of regeneration of the liver, hepatocytes replicate until the normal size of the liver has been achieved (2).

The different functions of the liver include detoxification, metabolism of hormones, fats, carbohydrates, and other particles, formation of bile, Storage of vitamins and iron (Ferritin), and Formation of coagulation factors. Hepatitis C

is a transmitted disease which is caused by the virus hepatitis C that mainly disturbs the liver. Throughout the infection people frequently have moderate or no signs and symptoms. Sometimes fever, dark-colored urine, pain in the abdomen, and yellow coloration of the skin occur. The virus continually exists inside the liver about 75% - 85% of those people who are infected once in their life. Chronic infection usually has no signs or symptoms. However, sometimes it causes liver infection and Sometimes cirrhosis. In somecases, those with injury and cirrhosis

develop problems such as liver failure, and cancer (3).

TRANSMISSION

HCV infection spreads mainly through blood among drug abusers with the use of the intravenous needle, poorly sterilized equipment, injuries in healthcare through needle sticks during sample draws, and in some cases during transfusions. Congenital transmission from mother to fetus is also important. Not spread through superficial contact. The diagnosis is made by doing a blood screening. The people at risk should be screened regularly (4)

DISTRIBUTION

About 130–200 million people are infected with the hepatitis C virus Worldwide. In 2013

11 million cases of hepatitis C infection have been reported. Most of the cases occur in East Asia Africa and Central Asia. Due to Liver cancer, 343,000 deaths have been reported worldwide and due to cirrhosis of the liver, about 358,000 deaths occur in the year 2013 due to hepatitis C virus infection (5).

SIGN AND SYMPTOMS

Hepatitis C virus (HCV) infection causes acute type of symptoms in 15 percent of cases. Symptoms of hepatitis include weight loss, muscle or joint pains, fatigue, nausea, and rarely acute liver failure. Many cases of mild infection are not connected to jaundice. Infection resolves instinctively in 10–50 percent of cases, which occur more commonly in those who are young (6). Chronic hepatitis C may be linked with fatigue and mild cognitive problems. Chronic infection after many years can cause cancer of the liver and cirrhosis (7). Fatty changes occur before cirrhosis develops in the liver in half of those people who are infected with HCV infection. Frequently these changes affect the liver by about 80 percent. Liver cirrhosis can cause bleeding, easy bruising, increase the

blood pressure in the veins system can also increase the fluid inside the abdomen sometimes increasing the size of veins, particularly in the esophagus and stomach (8).

STRUCTURE OF HEPATITIS C VIRUS (HCV)

The hepatitis C virus is an RNA virus that belongs to the family of Flaviviridae viruses, and the genus is Hepaciviral which is small and enveloped and single-stranded in shape when we see them under an electron microscope. There are 7 main genotypes of the Hepatitis C virus, and the genotypes are subdivided into many sub-types, and the number of sub-types relay on the genotype (9). HCV which infects the liver multiplies and increases the number of copies inside the lymphocyte (10).

ASSOCIATION WITH THROMBOCYTOPENIA

Pakistani populations have the world's hepatitis cases. Extra-hepatic complications can be commonly seen in patients with hepatitis c infection (11). One of these complications is thrombocytopenia. Different kinds of cases have been reported related to these complications. Multiple factors are involved, due to these factors there is increased sequestration in the spleen, bone marrow suppression due to hepatitis c infection and interferon treatment, and reduced production of hormone thrombopoietin cytokine regulates megakaryocyte maturation and platelet production) (12). The mechanisms involved in these kinds of abnormalities include an autoimmune reaction to platelet and destruction of platelets, portal hypertension, hypersplenism (Enlargement of the spleen) in end-stage liver disease, infections of platelets and megakaryocytes by Hepatitis C (13)

Thrombopoietin (TPO) is produced by the liver. Thrombopoietin is a glycoprotein; also

regulate megakaryopoiesis and platelet produced in the human body. Thrombopoietin levels and platelet counts are associated with liver function problems and the degree of severity of liver fibrosis in chronic hepatitis infection. Thrombocytopenia has a very adverse influence on the evaluation of this disease, primarily in the later stages, when the number of platelets may fall to less than 50,000/ μ L (14). Many cases have shown that hepatitis infection in non-cirrhotic patients is one of the main causes of thrombocytopenia. The degree of severity of thrombocytopenia might be variable very low or low. Interferon treatment is known to cause 10-50% in platelet levels (15). Thrombocytopenia can prevent diagnostic procedures such as liver biopsy, due to the risk of bleeding (16). The aim of the study will be helpful in planning further therapy and plan of action in patients with chronic liver disease infection and prevent bleeding.

LITERATURE REVIEW

Olariu et al, (2010) conduct research on "Thrombocytopenia in Chronic Hepatitis C" in which they concluded the concomitant presence of the autoimmune mechanism. produced more severe thrombocytopenia than bone marrow inhibition alone. A significant correlation was found between the degree of thrombocytopenia and the level of viral load. The same association was reported by Chia-Yen-Dai et al (11). Beyan et al, (2002) conduct research on "Severe thrombocytopenia as a presenting symptom of hepatitis C virus infection" in which they concluded that these results are significantly different as compared with age-matched controls patients, platelet-associated IgG (PIA IgG) was found to be negative. Nagamine et al reported thrombocytopenia in patients with chronic HCV infection (12). Behnava et al, (2002) conduct research on "The Prevalence of Thrombocytopenia in Patients with Chronic Hepatitis B and C" in which they concluded that significant difference in the prevalence of thrombocytopenia between groups

($P=0.007$). The prevalence of thrombocytopenia in chronic hepatitis C and B was more than in control subjects (13). Iman et al, (2009) conduct research on "Thrombocytopenia in chronic Liver disease due to hepatitis c virus" in which they concluded that Thrombocytopenia is a common and important finding in CLD due to HCV patients presenting with thrombocytopenia should be screened for HCV, especially in endemic areas for where blood and blood products are not properly screened (14).

OBJECTIVE

- To find out the incidence of thrombocytopenia in healthy donors and patients of hepatitis reporting at multi-centers of Faisalabad.
- To study the association of thrombocytopenia in the patient with HCV infection.

MATERIAL AND METHODOLOGY

The study was conducted at the college of allied health Professionals in Faisalabad. 146 individuals were recruited in the study from District Head Quarter Hospital Faisalabad.

METHODOLOGY

The following methods were used during this study to get results.

SPECIMENS

All samples were collected using an aseptic technique. The venipuncture site was selected and disinfected with a spirit swab. The needle was inserted carefully, and 2-4 ml of blood was taken. After taking the sample, the needle was removed from the patient's arm. Serum separated in a test tube after being centrifuged at 3500 rpm for 3 minutes. All the patients were screened for the presence of the hepatitis C virus and positive patients were further tested for platelet count. Enzyme-Linked Immunosorbent Assay (ELISA).

PRINCIPLE OF ELISA

The specimen is diluted and incubated with antigens which are coated at a microtiter plate. If antibodies are present in the specimen, they will combine with antigens and form an antigen antibodies complex. After washing with a washing solution, they will remove the other unbound materials and extra antibodies. After the addition of conjugate, it will combine with complex, and the addition of substrate break down by the enzyme and produces a color which is then measured on a spectrophotometer. Patients identified HCV-positive by ELISA tested for platelet count. Whole Blood Analysis by Using Automatic Hematology Analyzer SYSMEX

PRINCIPLE OF THE TEST

SYSMEX analyzer uses “fluorescence flow cytometry” to measure normal 5 (differential) parts of the blood cells, immature white blood cells (granulocytes) myelocytes, promyelocytes, metamyelocytes, and RBCs. Platelet counting was done by using the automated analyzer. The sample was sucked by the analyzer's sucker and the platelets count was observed.

PLATELET MEASUREMENT

Platelets sedate with the sheath drift “DC detection” process. By calculating a higher number of platelets this procedure suggestions raised precision. Though, when a higher number of Red Blood Cells remnants or higher Platelets are existing, precision may be less. For that sample that has less precision for those samples, the platelets-o measurement confirms larger precision than the “DC detection” process.

RESULTS

A total number of 146 subjects were enrolled in the study out of the total 146 subjects 101 (69.17%) were male and 45 (30.82%) are female as shown in table.01

Further, the male subjects who were HCV positive on the ELISA technique

are 28 and were tested for Complete Blood Count (CBC) on the SYSMEX automatic hematological analyzer and out of 28 subjects 11 (39.28%) have thrombocytopenia. On the other hand, out of 8 female subjects, 3 (25%) had thrombocytopenia as shown in table.03.

DISCUSSION

Those subjects who had chronic liverdisease showed thrombocytopenia. Of subjects with average low platelet counts, 21.42% have merely marrow reticence, 14.28% have auto-immune devastation, and most of the subjects (64.3%) revealed the occurrence of both mechanisms' involvement. A known association has been proven because of the low platelets count and age. No association was found between the sex of the patients and thrombocyte count. The varying quantity of thrombocytopenia is exaggerated due to a central or peripheral autoimmune process. The autoimmune process was present in patients who have mild thrombocytopenia. Hepatitis C virus infection may use its special effects on “Thrombogenesis” or by directly brutal influence on bone marrow, may lead to low production of megakaryocytes, or on the other hand, it may have a direct influence on the megakaryocytes, which then lower the platelets production. A study was conducted by Mohamed (et al) in Egypt and the prevalence of thrombocytopenia among patients was 24/100 (24%). The results agree with our study findings. SafiaBano et al from Pakistan reported that the prevalence of thrombocytopenia was found to be 43.3% among those with hepatitis C. In another study, Bitabehnavia et al from Iran showed that the prevalence of thrombocytopenia was 17.7% in patients with chronic active hepatitis B and 10.6% in HBV inactive carriers, 13.3% in patients with chronic hepatitis C. Wang from Taiwan Reported 10.2% thrombocytopenia prevalence among anti-HCV-positive subjects (13).

The study indicates that those subjects who have chronic hepatitis C virus infection, deprived of “Hypersplenism” or

sign of “Anti-platelet Autoantibodies”, the alpha interferon treatment is monitored and significantly raised platelet in numbers because of a significant decrease in the viral load.

CONCLUSION

This study concluded that HCV lowers the production of platelet because of bone marrow suppression. This study reveals that chronic hepatitis C infection is correlated with low platelets.

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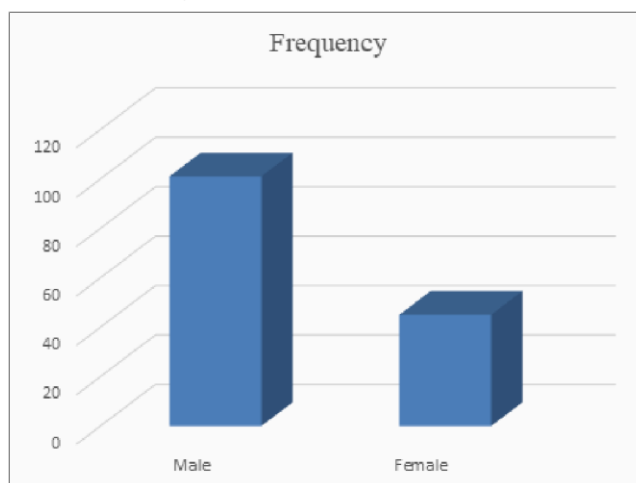
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Table.1: Gender Distribution among subjects

Gender	Frequency	Percentage
Male	101	69.17
Female	45	30.82
Total	146	

Graph.1: Gender Distribution among subjects



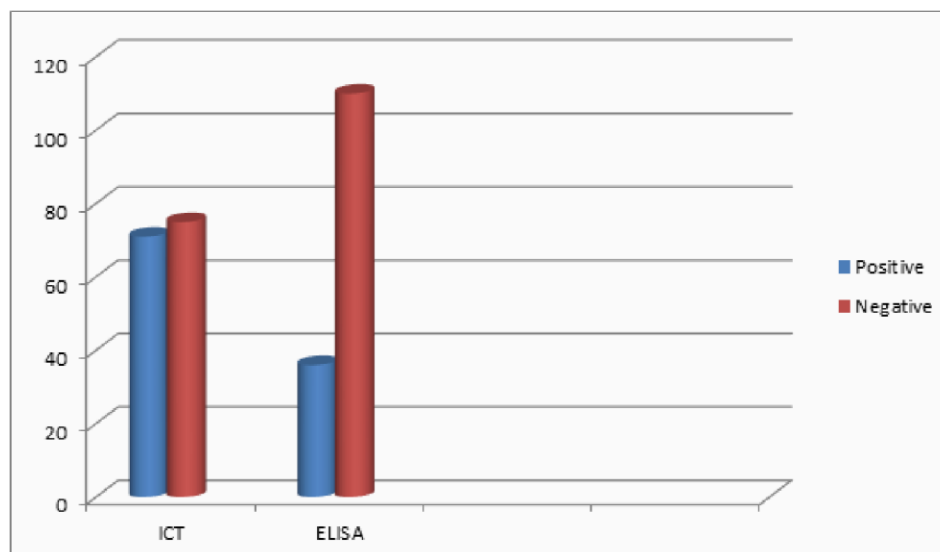
All subjects were tested by Immunochromatographic Technique (ICT). Out of 146 subjects, 71 (48%) were positive for HCV by ICT method. ELISA test was positive in 36 (24%) individuals as shown in table 02.

Table.02: ICT and Anti HCV ELISA

ICT and Anti HCV ELISA				
		Negative	Positive	Total

HCV (ICT Method)		75	71	48%
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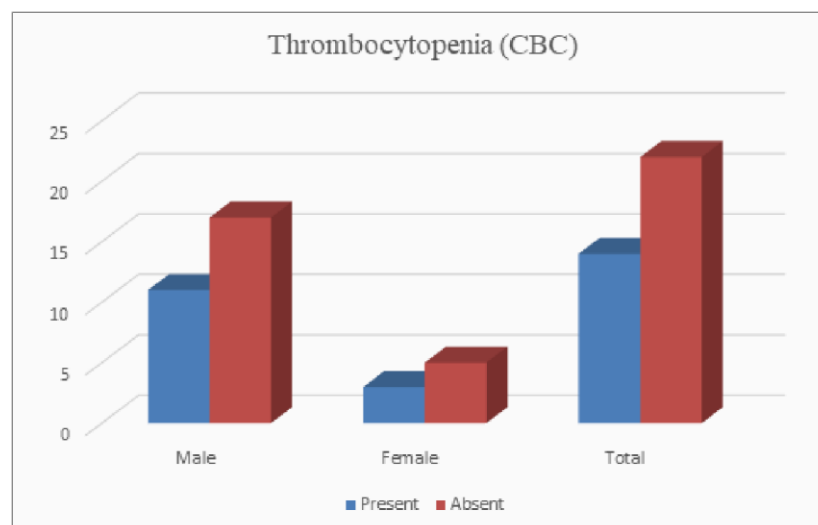
ELISA		110	36	24%
Total				



Graph. 02: Frequency of Anti HCV by ICT and ELISA Cross tabulation

Table.03: Thrombocytopenia by Complete Blood Count (CBC)

Thrombocytopenia (BC)			
Gender	Present	Absent	Total
Male	11	17	28
Female	03	05	08
Total	14	22	36



Graph. 03: Thrombocytopenia by Complete Blood Count (CBC) Cross Tabulation