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Cover Page Footnote

We are highly thankful to CDA hospital for their cooperation.

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SEROPREVALENCE, BIOCHEMICAL INVESTIGATION AND RISK FACTOR ASSESSMENT FOR HBV & HCV INFECTION IN HOSPITAL BASED PATIENTS OF ISLAMABAD, PAKISTAN

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ABSTRACT

Viral hepatitis poses a serious threat to mankind. Hepatitis B and C Virus are blood borne pathogens that affect millions of individually globally. This study was conducted on a hospital-based population in Islamabad, Pakistan over a period of 4 months, utilizing ELISA as the diagnostic technique which suggested a higher seroprevalence rate for both HBV and HCV i.e. 2.07% and 8.24% respectively. A correlational analysis of the biochemical parameters of these individuals with HBV and HCV infection was carried out and the results indicated a positive correlation of HBV with Alkaline Phosphatase (ALP), HCV with Total Bilirubin (TBil) and both the viruses with Alanine Aminotransferase (ALT). Furthermore, the risk factors in relation to these viral infections were explored upon which our data suggested that surgery, blood transfusion and contact with contaminated instruments at the barber for haircut/shaving and jewelers for piercings were the major risk factors responsible for aiding the contraction of the viral disease by patients in the hospital. These high percentages of the viral infection among the population require proper management and prevention techniques to minimize the number of casualties and further cases to provide a healthier surrounding for the people to live in.

Keywords: HBV, HCV, seroprevalence, biochemical investigation, risk factor**.**

INTRODUCTION

Hepatitis is a global health problem. In 2017, 1.3 million people died with viral hepatitis (Roth et al., 2018). Liver inflammation is a lethal hepatic abnormality caused due to either viral or non-viral factors that tends to progress towards cirrhosis and hepatocellular carcinoma (HCC) (Karim et al., 2015; Zhu et al., 2008). Hepatitis B & C Viruses are blood borne pathogens belonging to family Hepadnaviridae and Flaviviradae, containing dsDNA with genome of 3.2 kb and ssRNA with genome of 9.6 kb respectively (Seeger and Mason, 2000; Torres et al., 2013; Catanese et al., 2013). Both HBV and HCV show great tropism towards hepatocytes. Globally, the chronic infection rate observed for HBV and HCV is 257 million and 150 million respectively with a mortality rate of 720,000 was observed owing to cirrhosis and 470,000 due to hepatocellular carcinoma collectively caused by HBV and HCV (Alam et al., 2007; Schweitzer et al., 2015; World Health Organization, 2017).

Despite the availability of immunogenic, anti-viral and effective vaccines (for HBV), statistics show that 350-400 million people are carriers of HBV. Appropriate clinical manifestation is required to distinguish between HBV infection from other hepatitis causing viruses (World Health Organization, 2017; Datta et al., 2012). Different molecular and routinely performed serological assays like PCR, qPCR, Competitive PCR, ICT and ELISA are used for the quantification of HBV and HCV (Aguiar et al., 2014; Pawlotsky et al., 2002; Lok and McMahon 2007; Rahman et al., 2008).

Along with molecular and serological methods different biochemical

markers are involved to determine hepatic function in case of hepatitis. Among these marker ALT, AST, ALP, albumin and bilirubin are considered vital for the assessment of hepatic function in the blood of infected individual (Chen et al., 2014; Pattullo, 2015; Sharif et al.,2016; Locasciulli et al., 1993; El-Kady et al., 2017). It was noted that in chronic liver infection, the level of ALT increased due to parenchymal hepatic cell damage and excessive leakage of the enzyme into blood stream was observed. In patients with untreated chronic infection, the increase of ALT levels up to 400UI/L was observed (Olut et al., 2007; Mohamadnejad et al., 2003; Khan et al., 2016). Another important biochemical marker involved in metabolism and transportation of metabolites across the membranes is ALP. Studies show that the cholestatic liver diseases cause more release of this enzyme and increase serum ALP level in chronic hepatitis (Pattullo, 2015; Sleisenger et al., 1998; Whitehead et al., 1999). The level of these biochemical markers on individual basis or in combination help to determine the hepatic function in viral hepatic diseases.

It has been observed through different studies that multiple risk factors are involved in the viral transmission. Different factors like mother's age, number of pregnancies, repeated injection, addiction and history of blood transfusion could be involved in vertical transmission of hepatitis B & C from mother to infant (Mehnaz et al., 2002; Fauteux-Daniel et al., 2017). Risk factors which are the main source of hepatitis transmission include blood transfusion/transmission, dialysis, reuse of needles for ear and nose piercing, reuse of syringes, tattooing, shaving/cuts from barbers and the usage of unsterilized dental and surgical instruments (Idrees et al., 2011; Bukhari et al., 1999; Waheed et al., 2009). Intravenous drug abuse is also considered to be one of the most pivotal risk factors for causing Hepatitis B or C infection (Kazi et al., 2010; Fayyaz et al., 2006; Chen and Morgan, 2006).

Pakistan is a developing country with low investments on health It has been estimated that approximately 32% of the Pakistani population is exposed to HBV with 2-4% chronic HBV prevalence rate and 10% of the population suffers from HCV infection with approximately 4.5-8.2% seroprevalence in adult population (Khan et al., 2011; Waheed et al., 2018; Iqbal et al., 2014; Umer and Iqbal 2016).

There is a strong need to do research on hepatitis B and C in Pakistan. The purpose of the present study was to find the seroprevalence of HBV and HCV infections among the population of twin cities, the risk factors associated with these viral infections and to find the correlation between Hepatitis B and C Sero-positivity with commonly measured liver biochemical markers.

MATERIALS AND METHOD

Sample Collection and Processing

A total of 1977 blood samples were collected from Capital Development Authority (CDA) Hospital Islamabad from June-October 2018. Blood (3-5 ml) was collected via venipuncture technique and stored in vacutainer tubes with clot activator to produce serum upon centrifugation. The samples were centrifuged right after collection at 5000 rpm for 5 min to obtain serum which was then separated using filter tips. Each serum sample was divided into two parts each for both serological and biochemical testing.

Serological Testing

The serum samples were qualitatively tested against HBsAg (Hepatitis B surface antigen) and Anti-HCV antibodies through rapid diagnostic test i.e. Immunochromatographic test (ICT) followed by ELISA-based confirmation automated Vitros®Eciq Immunodiagnostic Analyzer Biochemical Analysis. For

quantification of liver specific enzymes, centrifuged serum samples of HBV and HCV screened patients were subjected to Liver function tests (LFTs) and Renal function tests (RFTs). Biochemical analysis was performed in Selectra XL analyzer.

Data Analysis

To find an association between HBV and HCV serological positivity with liver enzyme abnormalities, Pearson`s correlation test was run, where the (*p*-value) less than 0.05 with confidence interval of 95% was considered statistically significant.

RESULTS

Seroprevalence of HBV and HCV Based on ELISA

Serum from the blood samples of 1977 patients was initially screened by ICT method followed by confirmation with enzyme-linked immunosorbent assay (ELISA) for the detection of HBsAg and anti-HCV antibodies. The results revealed that 40/1977 (2.07%) patients tested positive for Hepatitis B Virus and 163/1977 (8.24%) of the patients tested positive for Hepatitis C Virus in their serum (Figure 1).

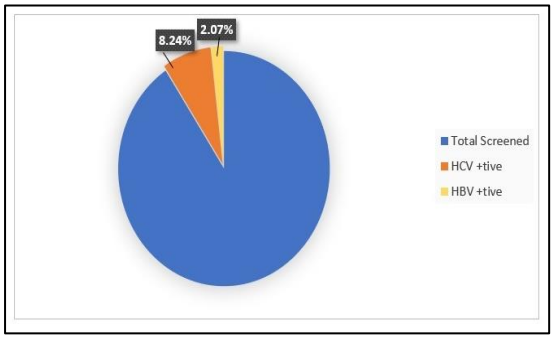


Figure 1: Seroprevalence of HBV and HCV among hospital-based patients.

Gender-Wise Distribution of HBV and HCV Infection

Out of the 1977 patients subjected to screening for HBV and HCV, 50.32% of the patients were male and 49.67% were female. Whereas the patients screened for HCV were 45.22% male and 54.77% female. The data suggests that 2.7% of the males and 1.3% of the females were positive for HBV infection. Whereas 7.24% males and 9.04% females were positive for anti-HCV antibodies in their serum (Figure 2).

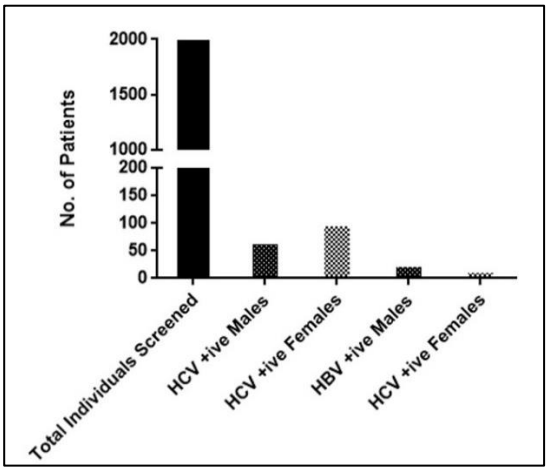


Figure 2: Gender based Seroprevalence of HBV and HCV.

Age-Wise Distribution of HBV and HCV Infection

The patients who tested positive for HBV or HCV in their serum were divided into seven age groups. The minimum age group was kept at <20 years and the maximum was kept at >70 years. The rate of infection was seen to be the highest among the individuals from the age group 61-70 years. Based on this data, a range of age-wise frequency distribution has been shown in Figure 3.

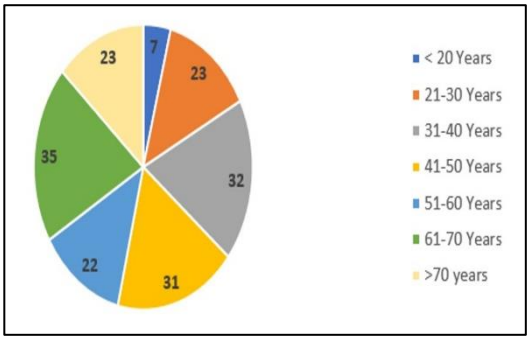


Figure 3: Age-wise distribution of HBV and HCV infected patients among hospital-based patients.

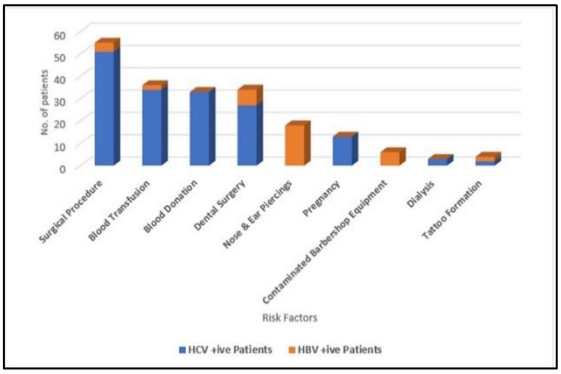
Correlational Analysis of HBV and HCV Groups

Using Pearson Product Moment Correlation, the relationship of the HBV and HCV positive and negative participants with the variables under study was analyzed. Results of the correlational analysis suggested that HBsAg sero-postivity was significantly associated with abnormal levels of Hepatic enzymes (Alanine Aminotransferase) ALT and (Alkaline Phosphatase) ALP ($p<0.05$) whereas HCV sero-positivity showed a significant and positive correlation with the increased levels of the hepatic enzymes ALT and Total Bilirubin ($p<0.05$) (Table 1).

Risk Factor Analysis

In the study sample, the patients who tested positive for HBV and HCV in their serum showed multiple risk factors responsible for rendering them more susceptible to infection. Except for a few, most of the risk factors for contracting HBV and HCV were the same, for both the viruses are blood-borne. The risk factors included general surgery, dental surgery, blood transfusion and donation, pregnancy, tattoo formation, nose and ear piercing, cuts from barber shop equipment etc. The highest prevailing risk factor among the Hepatitis B Virus infected individuals in the sample was nose and ear piercing whereas among the Hepatitis C Virus infected individuals was general surgery (Figure 4).

Fig.4: Risk factors observed among the positive patients of HBV and HCV.



DISCUSSION

Prevalence

As per the findings of this study, the seroprevalence of Hepatitis B Virus among the patients who visited the hospital facility of Rawalpindi-Islamabad for treatment or diagnosis was calculated to be 2.02% and in case of Hepatitis C Virus, it was calculated to be 8.24%. Numerous studies have been conducted worldwide to observe the prevalence of hepatic viruses to resolve the epidemiological concerns. Our results were supported by the study conducted by Haider et al. (2017) which showed that the prevalence of HBsAg+ among their sample was 2.14%. The research conducted by Muzaffar et al. (2016) indicated the prevalence of HCV to be 5.31% among the population of the twin cities and other additional studies carried out in different regions of the country showed various results such as Lahore (4.94%) (Anwar et al., 2013) and Mansehra (7.0%) (Ali et al., 2010). The difference between the prevalence of HCV in the current study and other respective studies mentioned above may have occurred since data collection and sample screening in this study was done at a hospital facility with people visiting either for treatment or diagnosis.

It was seen that in case of HBV infection, the percentage of male cases was

higher than female cases which is supported by the findings of other studies (Naz et al., 2002; Khan et al., 2011**).** A possible cause for the larger percentage of males being infected is their frequent visits to the barber shop and direct contact with contaminated instruments used for grooming. The number of females who tested positive for HCV infection was higher than males because the patients from the gynae ward paid more frequent visits to the hospital facility where the data collection was carried out.

Considering the age-wise distribution of viral hepatitis cases caused by Hepatitis B and C in the current study, the maximum number of cases were seen in the age group (61-70 years). This finding was supported by the survey conducted by (Ali et al., 2008) which showed that the maximum number of infected individuals were above 60 years of age. The saturation of cases in this age group can be since old age brings along consequences such as weakened immune system and it can render the individual more susceptible to contraction of the viral disease.

Biochemical Analysis

In the current study, the correlational analysis of viral hepatitis with different biochemical parameters which included ALT, ALP, Total Bilirubin and creatinine was carried out. A positive and significant correlation was observed between both HBV and HCV with ALT, where ($p<.05$), similar results were obtained by other studies (Mastoi et al., 2010; Wahib et al., 2006), where ALT levels increased significantly among the HCV infected patients. Also, elevation in serum ALT levels was seen among patients suffering from chronic hepatitis B infection (Liaw et al., 2005). The requirement of ALT for the synthesis reactions in the liver are the cause for its quantity to be around 3000 times higher than the serum and this becomes the reason for ALT to be a marker for liver damage. When hepatocellular injury or death is concerned, ALT seeps out from the damaged cells in to the serum and ultimately, it is detected upon testing (Kim et al., 2008).

Hepatitis B Virus showed a positive and significant correlation with ALP and Hepatitis C Virus with Total Bilirubin, where ($p<.05$). These findings were corroborated by the research carried out by Abulude et al. (2017), the results of which showed elevated ALP levels among their sample i.e. HBV positive patients. High levels of ALP can be seen due to blockage along the biliary tract (biliary obstruction) or pressure build up in the liver due to scarring which can, but do not necessarily indicate a viral infection. A study was carried out to investigate similar parameters and elevated levels of Total Bilirubin were seen among 35% of their study sample which was infected by HCV (Wahib et al., 2006). The results of another study by (Ijaz et al., 2011) showed elevated levels of bilirubin among patients infected with Hepatitis C Virus (Genotype 4) as well. Hyperbilirubinemia is directly related to viral infection and the extent to which histological injury of hepatocytes has occurred (Thapa et al., 2007). Liver being the detoxifying organ of the body clears away the bilirubin produced upon breakdown of RBCs and due to scarring, it is unable to carry out this function and thus bilirubin levels increase within the serum (Limdi and Hyde, 2003).

Risk Factor Analysis

In the current study, the highest number of HBsAg seropositive patients in case of females were those had their ears or nose pierced and came in contact with contaminated instruments where as in case of males were the patients who paid frequent visits to the barbershops using un-hygienic grooming instruments. Similar findings were observed by other studies (Yousfani et al., 2006; Asia et al., 2008) where piercings were the main risk factor for HBV transmission amongst females and frequent trips to barber shops using

contaminated instruments in males and children.

The risk factors which were seen to be highly prevalent among the HCV sero-positive patients in the current study were general surgery, blood donation & transfusion where the individuals had direct exposure to contaminated blood products or surgical instruments. A previous research corroborates the findings of this study and reported the population of Pakistan to be at a very high risk of developing an HCV infection through blood and blood-borne products due to unsafe medical procedures, lack of awareness, weak commitment to a safe environment, and illiteracy (Janjua et al., 2005).

In this study, 7.97% of the pregnant female patients tested positive for the virus in their bloodstream. This finding was corroborated by the study conducted by Arshad and Ashfaq (2017) who observed that pregnant females contributed to 4.65% of their sample infected with the virus. Previous studies have indicated injectable drug users (IDUs) to be at the highest risk of developing Viral Hepatitis (Kuo et al., 2006; Arshad and Ashfaq, 2017). However, the sample under study for this research didn't have any IDUs due to the setting where the sample collection was done i.e. the out-patients department (OPD) at the Capital Development Authority (CDA) Hospital, Islamabad.

CONCLUSION

The serological prevalence of Hepatitis B and C virus among the population of Rawalpindi/Islamabad who visited the hospital for treatment or diagnosis was high, which is an alarming situation for the residents of the region. The age groups of the infected patients ranged from 15 – 70 years. The individuals who were at a high risk of acquiring the infection included people who had undergone surgical procedures, blood transfusions, gotten nose and ear piercings, pregnant women and patients undergoing dialysis. Awareness campaigns to educate people about the transmission of viral hepatitis and to warn them regarding the threat it poses to people's lives and the lives of those living among them must be organized. Measures to eliminate the spread of this disease must be taken. The biochemical markers of Hepatitis B and C Virus in correlation with the infection yielded results which showed a positive and significant correlation among three of the markers. This aspect of the current study needs to be further investigated for this field needs further probing to provide the required information for treatment and better understanding.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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