



Prevalence, Blood Counts and Chemistry of Hepatitis-B and C in the Students of Bahauddin Zakariya University Multan, Pakistan

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Abstract

Hepatitis is one of the leading disease and considered as a threat to human health in the Southern Punjab of Pakistan. Therefore, many studies have been reported for the prevalence of hepatitis in the general population of different areas of the country. However, the incidence of hepatitis and its blood chemistry in the young generation at the university level in Southern Punjab is not yet reported. In this study, we chose 113 healthy student volunteers with mixed-gender (age: 18 to 30 years) from more than twenty departments of Bahauddin Zakariya University, Multan, Pakistan, with their prior consent for donating the blood for research purpose. The percentage of Hepatic B viral (HBV) is 1.77 %, and Hepatic C viral (HCV) is 3.54 % in a young and healthy generation. Our results demonstrate that hepatitis is more prevalent in males as compared to the female gender. The complete blood counts in infected volunteers were showed deviations from normal ranges of parameters. The enzymes of liver functions were also elevated in infected subjects as compared to the normal one. There is an immediate need to take preventive and effective health measures for liver infection in our youth of this region at the university level.

1. Introduction

The weak economy, illiteracy, and lack of health facilities elevate hepatitis. Viral diseases like hepatitis are of high risk so far mortality is concerned. In Pakistan, hepatitis is mainly due to unclean water, barber culture, unscreened blood transfusion, unhygienic food, and the reuse of syringes (Abbasi et al., 2014, Parveen et al., 2012, Afzal et al., 2014). According to the statements of health administration, the concerned disease is present from 4.8 to 5.8 % in the country's general population. Though millions of Pakistanis have been suffering from the fatal virus, the health department lacks its precise details of the prevalence. Hepatitis B virus (HBV) is more assimilated in the blood than HIV. Hepatitis C Virus (HCV) prevails in the body for years and that is why it is called a 'silent epidemic.' In addition, apparently, it shows no grounds while assaulting the liver, but it leads to death. The chronic infections are caused by viruses, which duplicate in

the body instead of hosts' immune pathway, commonly present in the HBV and HCV. People affected for a long time of such infections are known as carriers, and they are considered to be the sink of the virus. The disease is called epidemic, when a high ratio of carriers is present in the population (Alter, 2007, Al Kanaani et al., 2018, Arauz-Ruiz et al., 2002). Hepatitis B is more prevalent in males as compared to females. A study on children revealed that 1.8 % of children were positive for HBV, and 1.5 % were positive for HCV, which turned an overall infection rate to 3.3 %. Patients of chronic liver disease and hepatocellular carcinoma (HCC) had an incidence of 55 % and 24 % of HBV and HCV, respectively (Ali et al., 2011, Kazmi et al., 2003, Van Meer et al., 2013).

Hepatitis is a liver infection, which can be identified by different clinical tests like Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Chest X-ray, Ultrasound, and Laparoscopy.

Furthermore, the perturbation in complete blood counts and liver function tests (LFTs) also reveal the indication of hepatitis (Pawlotsky, 2002).

The incidence of hepatitis B is reported 10 to 14 % through enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) techniques in Pakistan. Daudpota and Soomro reported a high occurrence of 9.33 % & 14 % for HBV and HCV, respectively, in surgical patients above thirteen years of age (Daudpota et al., 2008). Other studies reveal that the incidence of HBV and HCV were 2.4 % and 2.1 % in children, while 2.4 % and 3.0 % in healthy adults, respectively (Shamsul et al., 2000, Ali et al., 2009)

The prevalence ratio of all the studies conducted in Pakistan varies due to the selection of objects and unauthorized laboratories. To date, there is no report on the prevalence of hepatitis B and C in the young generation of Pakistan. Therefore, we conducted a study on the incidence of hepatitis in male/female students with the age limits of 18-30 years. The volunteers were chosen from more than twenty departments of the University, taking their signatures on the consent letter and approval of the head of the departments (HODs). Our results revealed that hepatitis is more prevalent in the male gender. However, hepatitis C is two-fold more prevalent as compared to hepatitis B. We also checked the effects of hepatic infections on blood counts and liver enzymes, which were increased/decreased about healthy volunteers. In this study, our focus was to get precise and accurate results by adopting the International Organization of Standardization-9000 (ISO-9000) certified laboratory and standard operating procedures (SOPs).

2. Materials and Methods

Total 113 students (males: 61, females: 52) of age between 18 and 30 years (mean 22.5 ± 2.92) were included in the study. The students with any pre-clinical problems were excluded. The students were selected from more than 20 different departments of Bahauddin Zakaryia University Multan (5-6 students from each department). Student volunteers were approached for blood sampling after getting the official permission from chairpersons of the concerned departments. The significance of the tests in this study was first explained to each student verbally. When convinced, he/she was asked to fill the consent letter and took his/her signature on it. The skilled technician of the Medical Centre of University did Blood sampling. The volume of blood (5 ml) of each subject was taken from a vein, and the blood

volume was divided into two 4 ml vials; 1 ml of blood was transferred to No - 1 vial containing 2 μ l anti-coagulant agent ethylenediaminetetraacetic acid (EDTA) to avoid the coagulation. Subsequently, it was used for the measurement of complete blood counts (CBC). The remaining 4 ml blood was transferred into No - 2 to collect the serum by centrifuging at 2000 RPM (round per minutes) for 10 minutes. Both tubes were correctly tagged.

Complete blood counts were performed by MEK -6318J/K Haematology analyzer manufactured by NIHON KOHDEN. Hitachi Universal-II made in Japan was used for centrifugation of blood samples. All specimens were appropriately stored to avoid haemolysis and bacterial degradation. The qualitative detection of HBV and HCV was done by screening methods. The standard diagnostic (SD) kits were arranged by the biochemical laboratory of the Multan institute of nuclear medicine and radiotherapy (MINAR). The usual procedure was adopted for the qualitative analysis; 100 μ l serum was used in each kit for HBV and HCV. 2-3 μ l buffer solution was also used for the HCV kit. After 15 minutes, the results were observed. If on the kit, there was a red line other than control that was considered as positive.

The chemistry of all samples was studied by liver function tests or enzymes tests like serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and alkaline phosphatase (ALPH) by using 50 μ l, 50 μ l, and 10 μ l sera and 500 μ l reagent of each enzyme. The liver function tests were performed on microlab-300 at room temperature with the absorbance at 340 nm, and for ALPH, the absorbance was measured at 405 nm.

3. Results and Discussion

A total of 113 student volunteers (males: 61, females: 52) were studied. The mean age of these volunteers was 22.5 ± 2.92 years (range: 18-30). The subjects were divided into two groups based on gender, one for males and second for females. Both groups comprised those volunteers, who were found positive/negative for HBV or HCV. Out of 113 volunteers, 2 (1.77 %) were found positive for HBV, whereas 4 (3.54 %) were found positive for HCV infection. The comparative analysis of HBV infection in male and female student volunteers was done by the chi-square method, which showed the prevalence of these infections is statistically not different ($p = 0.50$). Whereas, the comparative analysis of HCV infection between males and females showed the prevalence is statistically different ($p = 0.05$). Student t-test and chi-square tests were used to compare the variables. Out of 61 male volunteers

(Group-1), 1 (1.64 %) were found positive for HBV, and 4 (6.55 %) were found positive for HCV infection. In group-2, one (1.92 %) out of 52 female volunteers was found positive for HBV. However, there was no HCV positive individual. The overall incidence of HBV and HCV, including males and females, was 2 (1.77 %) and 4 (3.54 %), respectively. The comparison of the prevalence of HBV in both groups by the chi-square test shows statistically the same incidence ($p = 0.50$). The comparison of prevalence of HCV in both groups by the chi-square test shows a statistically non-significant extent ($p = 0.05$) (Table-1).

Mean SGPT, SGOT and ALPH levels observed in HBV negative males were, 19.75 ± 7.48 (IU/L), 34.83 ± 11.1 (IU/l) and 235.4 ± 75.68 (IU/l) respectively, whereas SGPT, SGOT and ALPH levels observed in HBV positive males were 35 (IU/L), 72 (IU/l), and 182 (IU/l) respectively. Comparison of these values by student's t-test showed that the values of SGPT and SGOT in HBV positive males were significantly increased as compared to HBV negative males ($p = 0.0009$) and ($p = \text{less than } 0.0001$) respectively, where ALP values of HBV positive cases were significantly decreased ($p = 0.23$). Mean SGPT, SGOT, and ALPH levels observed in HCV negative in males were 19.8 ± 7.6 (IU/l), 34.2 ± 10.1 (IU/l), and 238.3 ± 72.79 (IU/l) respectively. Mean SGPT, SGOT, and ALP levels observed in positive HCV in males were 23 ± 9.309 (IU/L), 53.75 ± 22.6 (IU/l), and 181.3 ± 103.1 (IU/l) respectively. Comparison of these values by student's t-test showed that values of SGPT and SGOT in HCV positive males were significantly increased as compared to HCV negative males ($p = 0.42$) and ($p = 0.0011$) respectively, where ALP values of HCV positive cases were decreased considerably ($p = 0.14$). Mean SGPT, SGOT, and ALPH levels observed in HBV negative in females were 19.5 ± 7.56 (IU/l), 34 ± 13.1 (IU/l), and 231.9 ± 70.58 (IU/l) respectively. Mean SGPT, SGOT, and ALPH levels observed in HBV positive in for HBV were 10 (IU/l), 33 (IU/l), and 127 (IU/l), respectively. Comparison of these values by the student's t-test showed that the values of SGPT and SGOT in HCV positive males were significantly increased as compared to HCV negative males ($p = 0.058$) and ($p = 0.90$) respectively. However, ALPH values of HBV positive cases were significantly decreased ($p = 0.014$). Mean SGPT, SGOT, and ALPH levels observed in negative HCV in females were $19.35 \pm$

7.605 (IU/l), 33.96 ± 12.97 (IU/l) and 229.96 ± 71.381 (IU/l) respectively. However, there was no positive case for HCV in females. Comparison of LFTs values between HBV/HCV positive and HBV/HCV negative persons showed an increased SGPT, SGOT, and ALPH levels of positive for HBV and HCV infection. A comparison of the levels of LFT parameters showed that the mean levels of a significant proportion negative for HBV and HCV were higher. However, the mean levels in subjects positive for HBV/HCV were relatively high (Table -2). The blood counts like WBC, RBC HCT, and other parameters were also distorted in the infected students. The comparative-analysis of the student's t-test is shown in Table -3-4.

In Pakistan, viral hepatitis is a significant cause of chronic liver disease and the prevalence rate varies in different areas of country. Pakistan Institute of Medical Sciences, Islamabad conducted a study using the ELISA technique reported 3.31 % and 1.92 % incidence of HCV and HBV, respectively. In this study, the coincidence rate was high as compared to HBV. The researcher stated that vaccination and public awareness decreased this rate. In rural Sind (another state of Pakistan), the HCV occurrence was tested in visiting patients to the hospitals and found 17 % of patients were infected with HCV (Aziz et al., 2010). The objective of this project was to see the ratio of patients who were not infected by Hepatitis B, C, and the impact of infections on the enzyme chemistry and have scientific data to express the importance of fatal disease in our youth. Another study found that 4.1 % of blood donors were found positive for HCV, and, the authors claimed that positive donors had jaundice inherently, which had been shaved by barbers (Khokhar et al., 2003). The incidence of HBV in donors varies from 1.46 % (HMC, Peshawar) to 8.4 % (DHQ hospital Skardu). The prevalence rate for HBV in Southern Punjab blood donors is 4.7 %. However, the incidence rate of HCV in different studies varies from 8.86 to 0.27 % (Iqbal, 2002, Bosan et al., 2010).

Youth is the significant population of Pakistan. In our study, the prevalence rate of HBV and HCV in youth was found 1.8 % and 3.54 %, respectively. It is a very alarming situation in Pakistan because of the high proportion of viral infections. In our study, we found that the male students have higher HBV and HCV occurrence as compared to female students of Bahauddin Zakariya University Multan-Punjab, Pakistan. Our

finding shows that the prevalence is much higher than already reported. We believe that the methodology adapted previously might not be very

accurate. Our data is more reliable for this region of the country because the biochemistry laboratory of MINAR is ISO 9000 certified.

Table - 1: Incidence of HBV & HCV in BZU Students

	Number	HBV Positive	HCV Positive
Total students	113	2(1.77%)	4(3.54%)
Total males	61	1(1.64%)	4(6.56%)
Total females	52	1(1.92%)	0

Table -2: Mean (\pm SD) of SGPT, SGOT, and ALPH of BZU students

S. No	Students	SGPT	SGOT	ALPH
1	All students	19.7 \pm 7.61	34.76 \pm 12.4	232.4 \pm 73.26
2	All males	20 \pm 7.67	35.44 \pm 12	234.5 \pm 75.36
3	HBS (-) males	19.75 \pm 7.48	34.83 \pm 11.1	235.4 \pm 75.68
4	HBS (+) males	35	72	182
5	HCV(-) males	19.8 \pm 7.60	34.2 \pm 10.10	238.3 \pm 72.79
6	HCV(+) Males	23 \pm 9.31	53.75 \pm 22.6	181.3 \pm 103.10
7	All Females	19.35 \pm 7.61	33.96 \pm 13	229.9 \pm 71.38
8	HBS(-) Females	19.5 \pm 7.56	34 \pm 13.10	231.9 \pm 70.58
9	HBS(+) Females	10	33	127
10	HCV(-) Females	19.35 \pm 7.61	33.96 \pm 13	229.9 \pm 71.39
11	HCV(+) Females	-----	-----	-----

Comparisons (unpaired t-test)

All males versus All students: SGPT: $p = 0.80$, SGOT: $p = 0.73$, ALPH: $p = 0.86$

All Females versus All students: SGPT: $p = 0.78$, SGOT: $p = 0.71$, ALPH: $p = 0.84$

All males versus All Females: SGPT: $p = 0.65$, SGOT: $p = 0.53$, ALPH: $p = 0.74$

HBS (-) Males versus HBS (+) Males: SGPT: $p = 0.0009$, SGOT: $p =$ is less than .0001, ALPH: $p = 0.23$

HCV (-) Males versus HCV (+) Males: SGPT: $p = 0.42$, SGOT: $p = 0.0011$, ALPH: $p = 0.14$

HBS (-) Females versus HBS (+) Females: SGPT: $p = 0.058$, SGOT: $p = 0.90$, ALPH: $p = 0.014$

HCV (-) Females versus HCV (+) Females: No female volunteer was found positive for HCV.

Table -3: Mean (\pm SD) of White Blood Counts (WBC), Platelets (PLT), Lymphocytes (LY), Monocytes (MO), Granulocytes (GR), Eosinophils (EO)

S. No	Objects	WBC	PLT	LY	MO	GR	EO
1	All students	6.7 \pm 1.7	215 \pm 51	32 \pm 8.83	4.1 \pm 1.90	63.2 \pm 8.54	1 \pm 0.40
2	All Males	6.68 \pm 1.6	205 \pm 48	31.2 \pm 10	4.1 \pm 1.60	64.2 \pm 8.62	1 \pm 0.40
3	HBS (-) Males	6.7 \pm 1.64	205 \pm 49	30.9 \pm 9.5	4 \pm 1.60	64.6 \pm 8.32	1 \pm 0.40
4	HBS (+) Males	3.8	217	48	6	45	1
5	HCV (-) Males	6.7 \pm 1.72	206 \pm 49	31 \pm 9.7	4 \pm 2	64 \pm 8.6	1
6	HCV (+) Males	6.45 \pm 0.9	195 \pm 46	32.3 \pm 9	2.8 \pm 1	64 \pm 9.83	1
7	All Females	6.7 \pm 1.88	226 \pm 52	32.8 \pm 7.7	4.2 \pm 2.2	62 \pm 8.36	1 \pm 0.3
8	HBS (-) Females	6.64 \pm 1.9	227 \pm 52	33 \pm 7.79	4.2 \pm 2.18	61.9 \pm 8.41	0.9 \pm 0.34
9	HBS(+) Females	8.7	173	27	5	67	1
10	HCV (-) Females	6.7 \pm 1.88	226 \pm 52	32.8 \pm 7.8	4.2 \pm 2.2	62 \pm 8.36	1 \pm 0.3
11	HCV(+) Females	-----	-----	-----	-----	-----	-----

Comparisons (unpaired t-test)

All males versus All students: WBC: $p = 1.00$, PLT: $p = 0.23$, LY: $p = 0.60$, MO: $p = 0.84$, GR: $p = 0.45$, EO: $p = 0.93$

All Females versus All students: WBC: $p = 1.00$, PLT: $p = 0.19$, LY: $p = 0.54$, MO: $p = 0.84$, GR: $p = 0.40$, EO: $p = 0.9$

All males versus All Females: WBC: $p = 0.99$, PLT: $p = 0.030$, LY: $p = 0.33$, MO: $p = 0.72$, GR: $p = 0.16$, EO: $p = 0.88$

HBS (-) Males versus HBS (+) Males: WBC: $p = 0.0033$, PLT: $p = 0.67$, LY: $p = 0.0030$, MO: $p = 0.044$, GR: $p = 0.0002$, EO: $p = 0.11$

HCV (-) Males versus HCV (+) Males: WBC: $p = 0.78$, PLT: $p = 0.67$, LY: $p = 0.83$, MO: $p = 0.098$, GR: $p = 0.95$, EO: $p = 0.79$

HBS (-) Females versus HBS (+) Females: WBC: $p = 0.065$, PLT: $p = 0.083$, LY: $p = 0.20$, MO: $p = 0.52$, GR: $p = 0.30$, EO: $p = 0.85$

HCV (-) Females versus HCV (+) Females: No female volunteer was found positive for HCV.

Table -4: Mean (\pm SD) of Red Blood Cells (RBC), Hemoglobin (HGB) and Hematocrits (HCT)

S. No	Objects	RBC	HGB	HCT
1	All students	4.58 \pm 0.65	12.5 \pm 1.85	38.4 \pm 4.83
2	All Males	4.89 \pm 0.67	13.5 \pm 1.62	41 \pm 4.22
3	HBS (-) Males	4.89 \pm 0.67	13.5 \pm 1.64	41 \pm 4.25
4	HBS (+) Males	4.97	13.7	40.9
5	HCV(-) Males	4.9 \pm 0.7	13 \pm 1.6	41 \pm 4.2
6	HCV(+) Males	4.89 \pm 0.06	15.2 \pm 0.34	44.9 \pm 2.29
7	All Females	4.22 \pm 0.41	11.4 \pm 1.39	35.3 \pm 3.54
8	HBS(-)Females	4.23 \pm 0.41	11.4 \pm 1.4	35.3 \pm 3.58
9	HBS(+)Females	3.89	11.2	34.7
10	HCV(-) Females	4.22 \pm 0.41	11.4 \pm 1.39	35.3 \pm 3.54
11	HCV(+)Females	-----	-----	-----

Comparisons (unpaired t-test)

All males versus All students: RBC: $p = 0.32$, HGB: $p = 0.0006$, HCT: $p = 0.0005$

All Females versus All students: RBC: $p = 0.41$, HGB: $p =$ less than 0.0001, HCT: $p =$ less than 0.0001

All males versus All Females: RBC: $p = 0.12$, HGB: $p =$ is less than 0.0001, HCT: $p =$ less than 0.0001

HBS (-) Males versus HBS (+) Males: RBC: $p = 0.81$, HGB: $p = 0.88$, HCT: $p = 0.50$

HCV (-) Males versus HCV (+) Males: RBC: $p = 0.99$, HGB: $p = 0.034$, HCT: $p = 0.055$

HBS (-) Females versus HBS (+) Females: RBC: $p = 0.60$, HGB: $p = 0.81$, HCT: $p = 0.77$

HCV (-) Females versus HCV (+) Females: No female volunteer was found positive for HCV.

4. Conclusion

The findings of this work reveal that the incidence of HBV and HCV is in very high ratio in youth of Southern Punjab region. Our results demonstrate that hepatitis is more prevalent in male gender as compared to female gender. The percentage of Hepatic B viral (HBV) is 1.77 % and Hepatic C viral (HCV) is 3.54 % in young generation of South Punjab. The complete blood counts in infected volunteers were disturbed. The enzymes of liver functions were elevated in infected subjects as compared to normal students. Comparison of LFTs values between HBV/HCV negative persons and HBV/HCV positive persons show that the mean LFTs values of HBV/HCV

negative persons are low as compared to HBV/HCV positive persons where a very high proportion of these subjects have high LFTs values. The high LFTs values in our youth also show that our people also have liver infections other than HBV or HCV. Therefore, an effective health measures and mass screening programs should be started to prevent liver infections at national level. A proper vaccination system against viral diseases should to be regulated for healthy persons. Such types of programs should also be started at university levels immediately to prevent liver infection in Pakistani youth.

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6. Conflict of Interests

Authors declare no conflict of interests in this study.

7. Contribution of Authors

T. M Ansari and Khan .M Sajid conceived and supervised the research. Manzar Abbas communicated with the volunteers and collected the samples, properly stored and label the samples,

planned and performed the experiments. All authors have contributed in writing the paper and approved for submission.

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