Epidemiological Survey of the Prevalence of HCV and HBV in the Periphery of Lahore City

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: https://doi.org/10.32350/BSR.0301.03

Abstract

Both hepatitis C and hepatitis B are part of the major health-related issues all over the globe. Both of these are viral infections that can be disseminated via sexual contact, blood or by the use of blood products, and through methods of intra-familial transmission. Collection of blood samples of factory workers was done aseptically at KBK electronics. Overall, 272 samples of blood were taken of a variety of individuals and economically accessible ELISA (i.e. enzyme linked Immunosorbent Assay) was used to screen the individuals for Hepatitis B surface Antigen (HBsAg) and also for anti- HCV antibodies. The screening test was done as directed by the instructions of the manufacturer. Names, gender and age group date of each person was collected. Overall, 271 examinees were inspected to check the the presence of HCV and/or HBV. The ages of all the examinees ranged from a minimum of 19 years to a maximum of 50 years of age. The complete overall pervasiveness of both HCV and as well as HBV determined as 18.4% (50/271).

In contrast to HBV, HCV has a much higher ubiquity i.e. 16.17% (44/271). On the other hand, that of HBV was only 2.2% (6/271). The individuals revealed a higher percentage of positive results in hepatitis C. HBV occurrence in Lahore is much more than its neighboring areas such as Gujrat with only 0.8% prevalence, Sheikhupura with 1.6%, Narowal with 2.1%, and Hafiz Abad with 2.2% prevalence (PMRC, 2007 to 2009). The results from this study and previous literature highlight the pressing need to also conduct such kinds of experiments in even more populations because by doing so, the areas with a stronger occurrence rate of HCV and HBV can be specified and pointed out and then various methods for treating and even precluding both of these infections, can be implemented effectively.

Keywords: Hepatitis C, ELISA, Hepatitis B, Enzyme linked Immunosorbent Assay, Higher prevalence.

Introduction

Both hepatitis C and hepatitis B are part of the major health-related issues all over the globe [19]. Both of these are viral infections that can be disseminated via sexual contact, blood or by the use of blood products, and even through methods of intra-familial transmission [20]. As claimed by the World Health Organization (WHO) approximately two billion individuals have been exposed to this virus, out of which 240 million are long lasting carries [24]. Both the viral infections of hepatitis B and as well as hepatitis C and are the main world problem. About 240 million individuals are currently afflicted by the chronic form of hepatitis B virus. Moreover, approximately 130 to around 150 million people are infected chronically by the hepatitis C virus. Globally, an estimate of 240 million human beings is currently infected by the hepatitis B virus in a chronic manner and an approximate number of 130 to 150 million individuals by hepatitis C virus [24,25]. Approximately 1.45 million deaths occur due to HCV and HBV, which is 96% part of all hepatitis mortality rate [26]. Globally, the chronic infections of hepatitis and as well as HIV make up a part of the ten most extremely fatal diseases [1]. Pakistan counts as part of the most beset countries that have been affected by HCV and HBV. As compared to the other countries in the same region i.e. Myanmar, India, Iran, Afghanistan and Nepal, Pakistan is the country having much higher seropositivity and pathogenicity of the hepatitis C virus [2]. In order to observe the pervasiveness of hepatitis C and hepatitis B viruses, many studies were carried out, in Pakistan, at

various different locations [3, 4,5]. However, there are still a limited amount of studies based on population, regarding the occurrence of hepatitis virus, in certain areas of Pakistan [4].

This is due to the fact that epidemiological experiments that are related to the occurrence of hepatitis C and hepatitis B virus have been limited to only the patients that have been admitted into the hospital [6, 7]. Pakistan Medical Research Council carried out a survey throughout the entire country in a period of 10 months i.e. starting in July of 2007 and finishing in May of 2008. That survey showed that the pervasiveness of HBV is 2.5% and that of HCV is 5% within the country's common population. In Pakistan, it was shown by a gender-focused study that the prevalence of HBV is slightly higher in males than in females but the prevalence of hepatitis C has no such difference in both genders [5]. There is 3% pervasiveness of HCV all over Pakistan (the range was 0.5 to 31.9%), as specified by another small-scale analysis, highlighting its notable disparity of occurrence in different regions of Pakistan [3]. On a provincial level, the ubiquity of HBV was quite a lot in Baluchistan (i.e. 4.3%) whereas in the province of Sindh it was 2.5%, in Punjab it was 2.4% and in Khyber Pakhtunkhwa it was 1.3%. On the other hand, the occurrence of hepatitis C virus was the most in the province of Punjab i.e. 6.7%, then in Sindh it was 5.0%, in Baluchistan it was lesser i.e. 1.5% and it was the lowest in Khyber Pakhtunkhwa i.e. 1.1 % [7]. Generally, the pervasiveness of HBsAg was 2.4% in the province of Punjab and was 6.7% for hepatitis C virus. In some districts of Punjab i.e. Okara, Attock, Vehari, Mianwali, Jhang, Gujranwala, Rahim Yar Khan and MandiBhauddin, comparatively high amount of pervasiveness of hepatitis C and hepatitis B were observed. The occurrence of HBV was specified to be 2.9% in the Gujranwala district in Punjab with an estimate of 112,000 individuals infected by HBV. Furthermore, pervasiveness is 6.3% for HCV as approximately 243,000 people are infected by it [5]. In the Gujranwala district, 23.8% ubiquity of HCV has been specified [8]. There is an inadequate amount of population-level detail and information regarding HBV and HCV occurrence within Gujranwala. Seemingly, students come in contact with many HCV and HBV risk factors which causes them to be at a greater risk of becoming infected by them. For the student population at college level, there is not much information regarding the pervasiveness of HBV and HCV. Currently in district Lahore of Punjab, this study was conducted. Within the province of Punjab. Lahore is the first industrial district that is densely populated. The purpose of this study is to examine the prevalence of HCV and HBV specifically within this particular population group. This evaluation aimed to estimate the pervasiveness of Hepatitis B surface Antigen (HBsAg) and

also of anti-HCV antibodies among factory workers reside in periphery of Lahore city. The factory is KBK Electronics Pvt Ltd Lahore, 31-KM Ferozpur road, Lahore.

Materials and Methods

Collection of blood samples was done aseptically through factory workers at KBK electronics after approval of ethical committee, Central Park Teaching Hospital, Lahore with standard operating procedures. A sterilized gel vacutainer was used to collect samples from KBK Electronics Pvt Ltd. located at 31-KM Ferozpur road, Lahore. The samples were immediately brought to laboratory at Central Park Teaching Hospital, Lahore. Overall, 272 samples of blood were taken from a variety of individuals and economically accessible ELISA (i.e. enzyme linked Immunosorbent Assay) was used to screen the individuals for Hepatitis B surface Antigen (HBsAg) and anti- HCV antibodies [21]. The screening test was done as directed by the instructions of the manufacturer. Name, age, sex date of each person was collected.

Enzyme-Linked Immunosorbent Assay

The ELISA that was utilized in order to measure the concentration of IgG anti-capsular antibody, was modified by the procedure specified by Plikaytis *et al.* (23). This modified form of ELISA was prepared particularly to be used as a model for analysis for this current study and it is not proposed to be a standard form of ELISA for general use in all Hep B and C antibody concentration determinations. For the preparation of each reagent, highly pure water present in each one of the laboratories was utilized. In order to specify the optimal concentration of the polysaccharide coating on each polysaccharide, block titration was performed in a 0.01M solution of PBS (sodium phosphate buffered saline), having pH range 7.1 to 7.2, in all the laboratories, for each one of the polysaccharides. Moreover, block titrations of the conjugate enzyme and standard reference serum (89-SF) were performed in each one of the laboratories. Dilution of the samples of serum to 1:50 final to starting ratio was advisable. A 0.01 M solution of PBS-0.05% polysorbate 20 containing immunoassay-grade enzyme (EIA) 1% BSA (Bovine Serum Albumin) was utilized for each one of the dilutions for 89-SF, quality control sera, serum samples, conjugate enzyme and also for polysaccharides of cell wall (C-Ps). Neutralization of quality control sera, 89-SF (standard

reference serum) and all samples of serum was performed by utilizing C-Ps in a pure and non-diluted serum at a 500 mg/ml concluding concentration. This was done at a room temperature for a minimum duration of 30 minutes. Then all the samples of serum and 89-SF were diluted in a sequential manner after their neutralization was completed. Two, three- or six-fold sequential dilution was the commonly endorsed stratagem.

The concord ELISA comprised of a 100 ml of Hep B and C antigen polysaccharide coating at 37°C on each one of the wells of Nunc-Immuno plates. These plates that were coated were completely utilized in a period of one month and they were kept at a temperature of 4°C for storage. A 0.01 M solution of phosphate-buffered saline–0.1% Polysorbate 20 (having pH range 7.4 to 7.6) was used for the purpose of washing those coated plates for 5 times. An addition of the samples of serum (50 ml per well), sequentially diluted standard reference serum (89-SF) and C-Ps duplicated neutralized antibodies, was done. Intramural incorporation of wells related to quality-control and background (wells comprising of all the reagents but excluding the samples of serum) was done in all the evaluation tests. Cultivation of the diluted samples was done at room temperature for a time period of two hours. Then washing of the coated plates was done using the same method that has been mentioned earlier above. Then afterwards, horseradish peroxidase-labeled (HRP) conjugate enzyme (HP6043; Hybridoma Reagent Laboratory, Baltimore, Md.) solution was taken and 50 ml of this solution was appropriately diluted and incorporated into every well. Incubation or cultivation of this enzyme conjugate was done at room temperature for a minimum time period of two hours. Then, 3,39,5, 59-tetramethylbenzidine was taken as a substrate and was incorporated within every well, based on the directions of the manufacturer. Lastly, at room temperature, incubation of the coated plates was done and then the termination of the reaction of the enzyme occurred. The readings for the optical density of every well were taken and were recorded as 450 nm.

Results

Overall, 271 examinees participated in this study and each one of them was inspected for the presence of HCV and / or HBV. The ages of all the examinees ranged from a minimum of 19 years to a maximum of 50 years of age. The complete and through pervasiveness of both HCV and as well as HBV was determined as 18.4% (50/271). In contrast to HBV, HCV actually has a much higher ubiquity i.e. it has a total frequency of 16.17% (44/271) On the other hand that of HBV was

only 2.2% (6/271). All determinations were found in the male population (100%). Moreover, one test subject had been simultaneously infected by both types of hepatitis viruses i.e. HBV and also HCV. However, on an individual level, HCV pervasiveness (44/271) amongst subjects was more common in comparison to hepatitis-B (6/271) (Table 1).

The below graphs are showing that there is much more prevalence of HCV among subjects in contrast with hepatitis-B. The HCV infections pervasiveness is 16.2% and that of HBV infections is 2.2%.

Two predominant age groups affected by hepatitis B were 29-33 years and 44-48 years as opposed to the case of hepatitis C in which the predominantly effected age group was 29-33 years. These results were observed in the male population (Figure 2).

By comparing the blood group and age wise data of both hepatitis B and C individuals it was revealed that anti HCV was more predominant in B positive and O positive blood groups and HBsAg was predominant in A-positive blood group (Figure 3). According to the age wise distribution, HCV along with hepatitis B was comparatively highly prevalent amongst the age group from 29-33 years. As an epidemiologist we think that more attention should be given to the aforesaid blood and age groups. Furthermore, the male population is affected as they are spending their lives in working areas like industries and factories.

Discussion

According to different studies high pervasiveness of HCV and HBV is present within the provinces of Sindh and Punjab in Pakistan [27–28, 25]. An approximate of 3% of the entire population of the Globe (which is roughly equal to 180 million individuals) is afflicted by HCV (Hepatitis C Virus), according to an estimate by WHO (World Health Organization). Each year the number of infected individuals reaches up to approximately 3 million to 4 million people and 70% of them later on suffer from hepatitis in its chronic form [22]. In about 50% to 75% of the cases relevant to liver cancer, HCV is the main causative agent [9]. The infection of HBV is also a health issue worldwide and the number of people infected by it is around two million people globally [10, 11]. Furthermore, the chronic form of hepatitis B has infected an estimate of 400 million individuals around the globe [12]. Within the overall population of Pakistan, the prevalence of HCV infections

and HBV infections was 4.9% and 2.5% respectively, as outlined by a nationwide study that was organized by Pakistan Medical Research Council [5].

This current experiment's main aim was to analyze the pervasiveness of infections caused by both HCV and HBV infection within factory workers who were screened after the appearance of sign and symptoms. They revealed a high percentage of positive results for Hepatitis-C. The reason for this high percentage was the appearance of sign and symptoms and then they were screened for hepatitis- C. Prevalence of HBV was 2.4% in the province of Punjab ranging from 0.7%-5.7% depending upon particular areas of the province. This highlights the fact that some areas have a higher prevalence or concentration of the same infection compared to the other areas of the same province. In Gujranwala, the pervasiveness of HBV was specified as 2.9% which shows that roughly 112,000 people are suffering as a result of this virus and its infection. HBV occurrence in Lahore is much more than its neighboring areas such as Gujrat having only 0.8% prevalence, Sheikhupura having 1.6%, Narowal with 2.1%, Sialkot with 2.2% and Hafiz Abad with 2.2% prevalence [5]. In the province of Punjab in Pakistan, the prevalence rate of HCV ranges from 1.9%-13.1% in different areas and overall, in Punjab, the frequency of HCV is 6.7%. Prevalence of hepatitis C and hepatitis B among the students at college level was observed to be notably lesser (less than 3.0%) than the seropositivity rate of 30% that was observed in the paid donors and the seropositivity rate of 7% observed in the blood donors of both the replacement or family groups of donors (Khan *et al.,*). It was previously outlined that the seroprevalence rate of HBV was 5.96% and that of HCV was 0.07% in an analysis of a total of 1426 individuals who donated blood in Liaqatpur [13]. Bangash *et al., (*2009) analyzed that amongst the healthy donors of blood in the Kurram Agency, Northern Area, Pakistan, about 5% were tested positive for HBsAg and they also found that for the anti-HCV antibody, 1.1% of the same donors were tested positive [14]. The overall frequency of HCV and HBV occurrence was determined as 12.99% through a different analysis [15]. In the present study KBK Electronics Pvt Ltd. located at 31-KM Ferozpur road, Lahore revealed only 16.2% positive cases of HCV. 2.2% cases of HBV. Hence, the pervasiveness of HCV was comparatively much higher in examined subjects (16.2%). Identical results were also observed through other evaluations conducted within Pakistan at areas in which the occurrence rate of the hepatitis virus among the males was predominant [16, 17, 18]. Khan *et al*., previously delineated a much larger frequency of hepatitis C, in comparison with hepatitis B, in males [13]. The cause for this more frequent occurrence of the viruses among males could be due to reason

that males in Pakistan have comparatively more liberation for public level motility. This leads to them being generally more subjected to social life and as well as working conditions like factories so consequently they are liable to getting infected by the viruses and can be more easily exposed to a high amount of risk factors for the infection. The results from this study and previous literature highlight the pressing need to also conduct such kinds of evaluations in even more populations because by doing so, the areas with a stronger occurrence rate of HCV and HBV can be specified and pointed out and then various methods for treating and even precluding both of these infections, can be implemented effectively.

Acknowledgment

All the authors are very grateful to the HEC (Higher Education Commission) of Pakistan for to its aid and support.

Conflict of Interest:

Authors declare no conflict of interest

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Table 1: Pervasiveness pertaining to both HCV and HBV among individuals at KBK Electronics Pvt. Ltd. Lahore.

Prevalence of Hepatitis			
	Total Subjects	**Positive Cases**	**Prevalence (%)**
Male	271	50	18.4
Prevalence of Hepatitis B			
	Total Subjects	**Positive Cases**	**Prevalence (%)**
Male	271	06	2.2
Prevalence of Hepatitis C			
	Total Subjects	**Positive Cases**	**Prevalence (%)**
Male	271	44	16.2

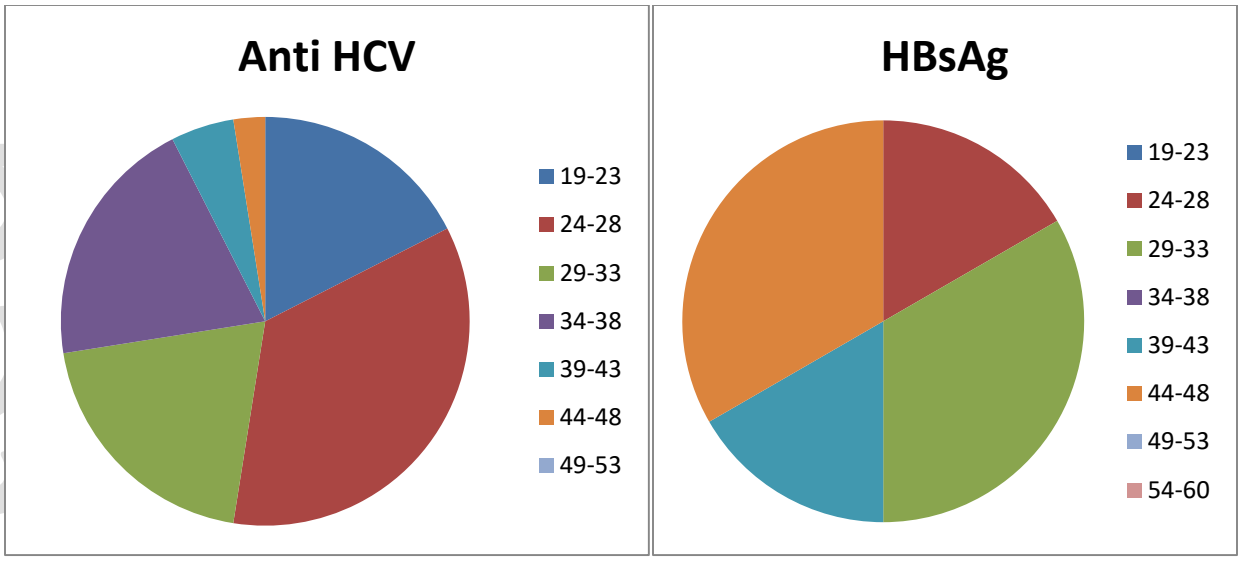

Figure 2: Age wise distribution of hepatitis B & C data in males

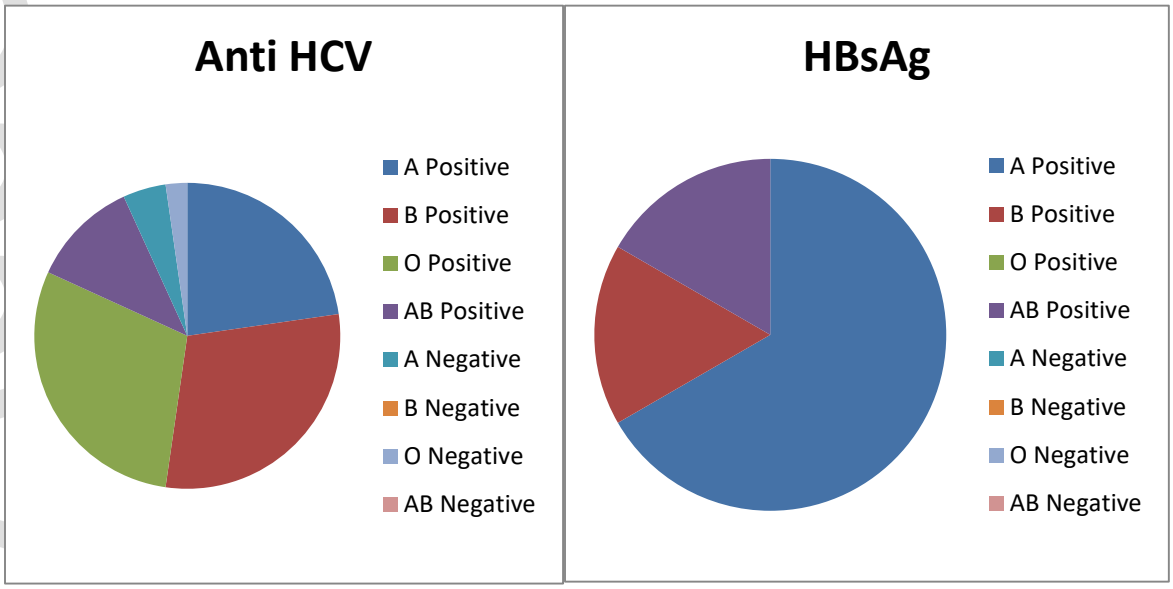

Figure 3: Distribution of hepatitis B & C positive cases in different blood groups