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# Sero-prevalence of Hepatitis B and C Virus from rural areas of northern Punjab (Sargodha District), Pakistan

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**Abstract.** Pakistan is endemic for hepatitis B virus (HBV) and hepatitis C virus (HCV) infections with 10 and 11 million infections, respectively. The epidemiological studies of these virus showed that the information is only from few cities of the country and is relevant to high risk groups. It is of great importance to have an idea about the prevalence of infectious agents in general population to help in identification of hot spot for infections. Identification of hot spots will help in disease management for future. As the there is no report form district Sargodha (Punjab Province) so this study was designed to analyze the prevalence of HBV and HCV in general population. Blood samples of 2373 randomly selected individuals from six different tehsils were collected and were analyzed for HBV and HCV sero-positivity. An overall prevalence of both HBV and HCV in district Sargodha was 28.10% (667/2373). HCV prevalence was (20.01%) and HBV seropositivity was (8.09%). Males were more infected than females, and a significant difference was found in positive cases between male (58.77%) and female (41.22%). The most common routes of transmission of hepatitis virus in present study were shaving assisted by barbers in male patients 143 (21.43%), non sterile or used needles & syringes 127 (19.04%), dental surgical procedures 88 (13.19%), and sharing razors in males 49 (7.34%). In female patients a significant factor is labor and child birth process. Most cases of hepatitis were seen in mesons, farmers and house wives. The prevalence of HBV and HCV in general population of district Sargodha is very high. The study will help for better management of disease to contain the disease spread. The study highlighted that District Sargodha is endemic for these viral infections and it is highly warranted to carry out more studies to get better idea about the infection spread. Community education campaigns are also highly warranted to general population as well as high risk population to control future disease spread.

## INTRODUCTION

Infections caused by hepatitis B and C virus are among leading public health dilemmas, having wide clinical spectrum and natural history of viral infection varies between asymptomatic infections, symptomatic acute infections to chronic infections leading to cirrhosis and hepatocellular carcinomas (Duddempudi & Bernstein, 2014; Villar *et al.*, 2014). According to World Health Organization (WHO) prevalence of HBV and HCV is approximately 240 million and 185 million respectively across the globe (World Health Organization, 2014).

A comprehensive population based epidemiological survey data on the prevalence of HBV and HCV is lacking from Pakistan. Only a few reports based on convenient sampling methods and patients attending hospital OPDs are available. Most of the studies targeted a specific population type like Hemodialysis patients, injection drug users, jail prisoners, thalasemic patients or co-infections with HIV, high risk populations and children etc (Reviewed in Umer and Iqbal, 2016; Afzal MS., 2016). Changing global epidemiology of the HBV and HCV infection requires the comprehensive annual reports of viral prevalence from all parts of the world to estimate the morbidity and mortality as well as future disease burden (Mohd *et al*., 2013; Ditah *et al*., 2014; Esteban *et al*., 2008). One such study was conducted by Pakistan Medical Research Council in year 2007-2008 to estimate the prevalence of HBV and HCV in Pakistan. It was estimated that the prevalence of HBsAg was 2.5% and for anti HCV it was 4.8%. Collectively the prevalence of both infections was 7.6%. Moreover the estimated chronic carriers for both the infections were 13 million. Most significant contributory factor in disease acquisition was the reuse of syringes through therapeutic injections. More than 30% of population had 10 injections/person/year. The prevalence of HBV and HCV was not uniform in all districts. There was a high prevalence of HBV and HCV in 30 districts from all provinces (PMDC, 2009; WHO, 2015). The comprehensive data in high risk population is not available with reference to HBV and HCV prevalence. It is reported that the prevalence of HCV in female sex workers and eunuchs is between 4.3-38% with an average of 19%. Likewise another high risk population is multiple transfused patients suffering from hemophilia, thalassemia and other disorders where HBV prevalence was 5-8.4% (average 7.8%) and HCV prevalence was alarmingly high ranged between 25-60% (on an average 47.2%). Another high risk studied population was chronic dialysis patients with an average 14.6% (12.4-16.6%) HBsAg seropositivity, the values for HCVAb was very high 38% (23.7-68%). A relatively high risk was observed in healthcare workers 0-10.3% HBV (weighed average 6%) and HCVAb 4-5.9% with weighted average 5.5%. Pregnant females showed higher risks of HCV as compared to HBV, weighted average 5.3% and 2.9% respectively (Ali *et al*., 2009).

In Pakistan HCV disease burden is increasing because of previously infected Hepatitis cases and newly reported cases. While partial control of HBV infection is the consequence of availability of an efficacious vaccine particularly in geographical areas with high prevalence. In Pakistan the HBV vaccine became the part of National Expanded Program on Immunization (NEPI) in year 2002 (Mangrio *et al*., 2008; Omer *et al*., 2009; Siddiqi *et al*., 2007). As there is no information about the prevalence of HBV and HCV from District Sargodha; therefore this study was designed not only to collect information about the prevalence of these viruses but to identify the risk factors for these infections.

## MATERIALS AND METHODS

*Study area and population*

The aforesaid study site for hepatitis strains project was rural sites from district Sargodha (N 32° 5' 1", E 72° 40' 16"), in Punjab province, Pakistan. Sargodha district has a population size of 26, 65,979 individuals according to 1998 census report. Administratively it has been divided into six tehsils, viz Sargodha (542761 individuals) (N 31° 50' 11", E 72° 32' 51"), Bhalwal (population of 1056600 individuals) (N 32° 15' 59", E 72° 54' 00"), Kot Moman (50,000 individuals) (N 32° 11' 01", E 73° 01' 35"), Sahiwal (235,600 persons) (N 31° 58' 26", E 72° 19' 56"), Shahpur (274,000 individuals) (N 32° 16' 15", E 72° 28' 23"), Sillanwali (255,000) (N 31° 49' 09", E 72° 32' 18") (Figure 1). A total of 2373 male and female patients aged 11 to 60 and above were the study participants. All of the study participants were selected randomly from the above mentioned six tehsils of district Sargodha for screening of hepatitis B and C viruses.

*Sample size calculation*
Sample size was calculated using following formula

Risk: 3% HBV
10% for HCV

Sample size calculation formula

$$n = \frac{(z_{a/2})^2\, p(1-p)}{E^2}$$

z = qnorm (.975)
p = 0.5
d = 0.05
z^2*p*(1-p)/d^2

*Door to door Survey*
Random sampling was carried out from six tehsils of the district Sargodha. A brief questionnaire was designed regarding the demographic and other information of the participants. Informed written consent was obtained from each participant. The study was approved by Departmental ethical committee, Zoology Department, University of Sargodha,Sargodha, Pakistan For female cases additional questions involved the number of children, mode of birth etc as other risk factors. Monthly income of the household was noted in order to calculate the poverty index (Ditah *et al*., 2014) and to find any possible correlation between low socioeconomic status and disease positivity. Moreover it was emphasized that educational years would have an impact in disease management and can its occurrence be related to educational level of the individual. As we assume and suppose that a well educated person having knowledge about the disease can better handle it if he/she acquires such infection. Moreover educated person has a better understanding about the mode of spread of blood borne viruses and therefore can minimize the risk of disease acquisition.

*Laboratory Analysis*
From sampled blood, the serum was drawn for further analysis. Qualitative determination of HBsAg was made using third generation ELISA kit (Diapro). For determining seropositivity of HCV a third-generation enzyme-linked immunosorbent assay (ELISA) kit (Diapro) was used for the detection of anti HCV antibody according to manufacturer protocol.All the samples were retested for the validation of results and if in the retest values were less than the cutoff values the samples were considered negative for HBV and HCV.

*Statistical analyses*
Data from the epidemiological survey of Sargodha district, Punjab, Pakistan was used to describe the current epidemiology of HCV infection in this area of Pakistan. All analyses were performed using IBM Statistical Package for Social Sciences (SPSS) version 20 (IBM SPSS Statistics Publisher's). Prevalence of HCV was presented by a variety of demographic factors. Data fromsurvey were combined to evaluate trends and riskfactors associated with HCV infection. Only individuals 11 years and older were included in the risk factor analysis. Correlation of disease positivity with age groups and genders was calculated using the Chi-square Test. p values<0.05 were considered significant in the model.

*Standard deviation of prevalence*
Standard deviation of prevalence was calculated using following formula. It's calculated as sqr[p(1-p)/n] (Where P is prevalence and n is that population's size).

## RESULTS

**Characteristics' of studied cohort**
A total of 2373 representatives of general population, from Sargodha Punjab, participated in the current survey conducted between January to December 2014. Participants belonged to all six tehsils of Sargodha district (Figure 1). Male study participants were 1321 (55.66%) while 1052 (44.33%) were the females.

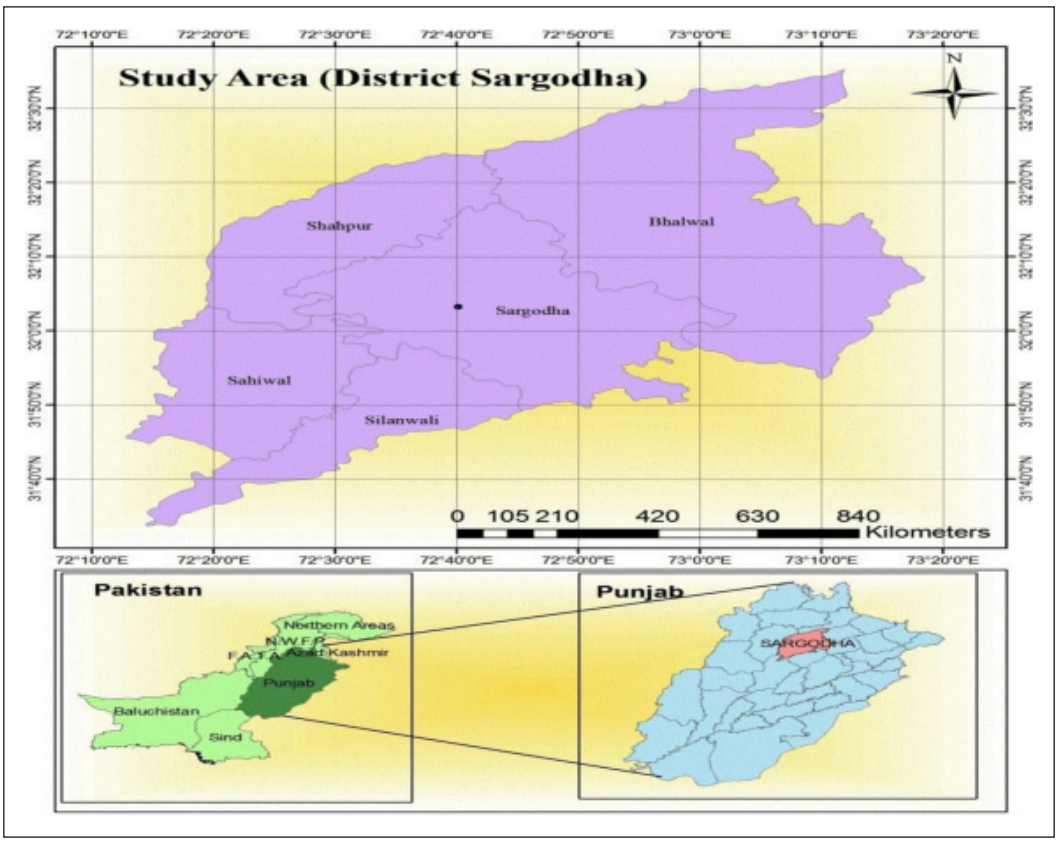


Figure 1. Map showing study area of Sargodha district, Punjab, Pakistan.

**Prevalence of HBV and HCV in studied Cohort from Sargodha district as to 2014**
Screening of blood revealed that 192 (8.0%) were positive for HBV and 475 (20%) were HCV ELISA positive.

**Prevalence of HBV and HCV Co-infections in studied Cohort from Sargodha district as to 2014**
There were 30 cases co-infected with both hepatic viruses at the same time (i.e., HBV/HCV Co-infection). It constituted 1.3% of the studied population. Among co-infected patients 26 (86.66%) were males and only four (13.33%) were females (Table 1).

Table 1. Seroprevalence of HBV and HCV Coinfections in district Sargodha (n=2373), Pakistan

| Age | Infected # (%) | |
|---|---|---|
| | Males | Females |
| 11-20 | 2 (0.084%) | 0 (0%) |
| 21-30 | 3 (0.126%) | 0 (0%) |
| 31-40 | 15 (0.632%) | 4 (0.17%) |
| 41-50 | 4 (0.17%) | 0 (0%) |
| 51-60 | 1 (0.042%) | 0 (0%) |
| 61 ≥ | 1 (0.042%) | 0 (0%) |
| Total | 26 | 4 |

**Tehsil wise prevalence of HBV and HCV infections**
Out of 667 positive patients, 198 (29.68%) patients were from Bhalwal, 131 (19.64%) were from Sahiwal, 119 (17.84%) were from Sillanwali, 77 (11.54%) were from Sargodha, 70 (10.49%) were from Kotmoman, and 72 (10.79%) were from Shahpur. The statistical analysis showed the significant difference of positive cases among tehsils ($p < 0.05$). Out of 667 patients, 475 (71.21%) patients

were positive (+ve) for HCV and 192 (28.78%) patients were positive (+ve) for HBV. Among 475 hepatitis C positive, 156 (32.84%) patients were from Bhalwal, 69 (14.52%) were from Sahiwal, 85 (17.89%) were from Sillanwali, 59 (12.42%) were from Sargodha, 51 (10.73%) were from Kotmoman, and 55 (11.57%) were from Shahpur. The statistical analysis showed no significant difference of HCV positive cases among six tehsils ($p > 0.05$). Among 192 hepatitis B positive, 42 (21.87%) patients were from Bhalwal, 62 (32.29%) were from Sahiwal, 34 (17.70%) were from Sillanwali, 18 (9.37%) were from Sargodha, 19 (9.89%) were from Kotmoman, and 17 (8.85%) were from Shahpur. The statistical analysis revealed a significant difference of HBV positive cases among six tehsils ($p < 0.05$).

**HCV and HBV infections in association with different age groups**

Prevalence of HBV and HCV infection was maximum in the age range of 41-50 years, 342 patients (51.27%) (Figure 2). The statistical analysis showed the significant difference in age groups and hepatitis positive cases ($p < 0.05$). Overall 110 (16.49%) patients have hepatitis victims in their family members. The statistical analysis showed the difference in patients have family members victim and hepatitis positive cases ($p < 0.05$).

**Modes of HBV and HCV Transmission in studied subjects**

The most common routes of transmission of HCV & HBV in present study for males were shaving assisted by barbers 143 (21.43%) and sharing razors 49 (7.34%). Non-sterile or used needles & syringes 127 (19.04%), dental surgical procedures 88 (13.19%) were mutual risk factors while important risk factor with reference to females was child birth. The other routes of viral transmission are also summarized in Figure 3.

**Occupational details of the HBV and HCV Cases**

In present study 174 (26.08%) of the positive cases were laborers, 147 (22.03%) were farmers, 119 (17.84%) were house wives, 60 (8.99%) were physicians and dentists, 53 (7.94%) were surgeon & theater staff, 34 (5.09%) were Government servant, 29 (4.34%) were nurses & midwives, 23 (3.44%) were drivers, 17 (2.54%) were students and 11 (1.64%) was miscellaneous group.

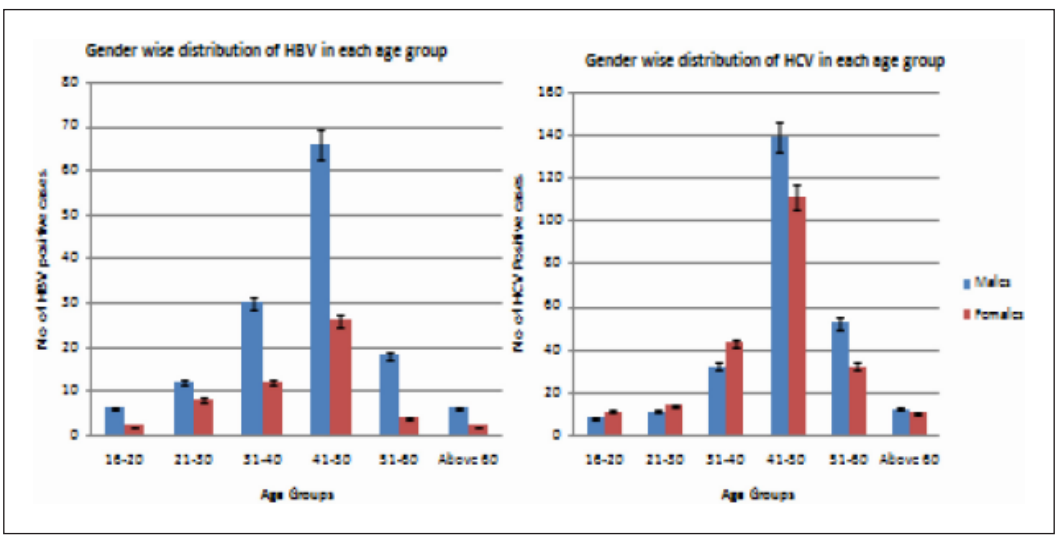


Figure 2. Age wise distribution of HCV and HBV in males and females.

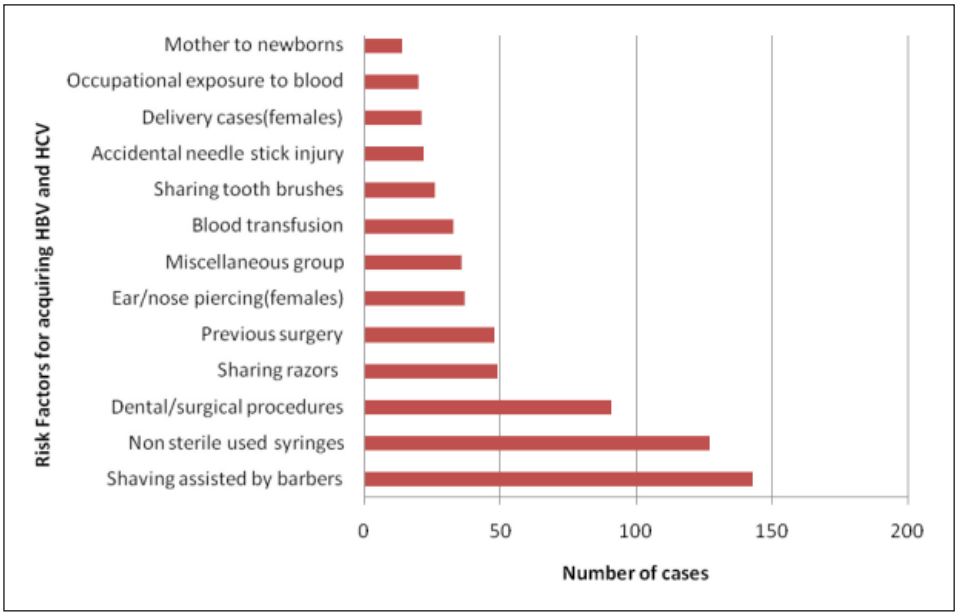


Figure 3. Routes of transmission and Risk factors of Hepatitis B and C collectively of 667 positive patients in district Sargodha Pakistan.

## DISCUSSION

Pakistan is world's sixth most populous country with estimated population in year 2015 over 191.71 million, with low living standards, educational levels and poor health conditions (Pakistan Economic Survey, 2015). The united nation's human development index out of total 174 countries ranks Pakistan at 134$^{th}$ position. The prevalence of HBV and HCV in general Pakistani population is 3% and 5% respectively. It is estimated that prevalence of chronic carriers in HBV in high risk groups varies between 6-12%. The figures are much higher for HCV i.e., upto 66%. (Babanejad *et al.*, 2016; Umer and Iqbal, 2015). Because of lack of international standard health care facilities, awareness in general public and implementation of basic health standards (sterilization, screening, disinfection) for procedures like dental, surgical, transfusion of blood and its products, syringes and needle reuse, injection drug abusing, tattooing, visiting quacks, shaving from barbers using the same shaving kits and towels, the prevalence incidences and disease burden is increasing (Afzal *et al.*, 2013; Afzal *et al.*, 2014; Raza *et al.*, 2015; Afzal *et al.*, 2016).

The present study was designed to estimate the seroprevalence of HBV and HCV in general population of Sargodha District Punjab, Pakistan. Primary observation of the current study was consistently high prevalence rate of HBV and HCV in studied population. According to the results of the study, Sargodha District is a hot spot for these infectious viruses. There are also difficult to treat cases of co-infection of HBV and HCV. As previous findings about HBV and HCV prevalence in general population is very limited and is from few metropolitan cities. HBV prevalence in general population is ranged from 2-5% while prevalence of HCV was higher i.e. 3-24% (Afzal *et al.*, 2016; Babanejad *et al.*, 2016). The results of the current study are in line with previous findings about the prevalence of HCV but the infection rate of HBV is higher as compared with previous data from the country. The higher sero-prevalence of HBV is alarming and might showing the lack of vaccination against HBV in local population of the district. The knowledge about the infection rate is always important to design the prevention strategies to contain the future spread. The overall prevalence of HBV and HCV is quite high in general population of the District which

showed the immediate need of counter strategies; not only for treatment of the infected individuals but also to limit the disease spread.

Current study demonstrates that the most affected age group in hepatitis B and C was 41-50 years (1968-1976 birth cohort) followed by 31-40 (1977-1986) and 51-60 years (1958-1967 birth cohort). One of the obvious reasons might be the acquisition of disease prior to the development of efficient screening methods for blood and blood products (Umar *et al*., 2010). Other identified possible risk factors for viral spread in general population are shaving assisted by barbers, Non-sterile or used needles & syringes, dental surgical procedures. The results of the study are in accordance with previous findings that barber assisted shaving (male participants) followed by unsafe therapeutic injections and dental procedures are the major risk factors across the country (Abbas *et al*., 2015; Ali *et al*., 2009; Bari *et al*., 2001; Khattak *et al*., 2008; Quddus *et al*., 2006; Raja & Janjua, 2008). Community education campaigns are highly warranted regarding safe use of barber's equipment, syringes, surgical procedures not only for general population but also for health care providers.

The results of current study highlight the future challenges for health care step-up of Pakistan. Most of the infected individuals are in age group 30-50 Years, it is an anticipation that with growing age of this cohort HBV and HCV related complications i.e., liver cirrhosis, end stage liver disease and hepatocellular carcinomas (HCC) will increase as we suspect these cases to acquire ailment several decades ago (Afzal *et al*., 2016). Availability of an efficient vaccine for HBV has markedly reduced the HBV prevalence and individuals between 11-30 years are comparatively less effected age cohorts, although hyper-endemicity of disease (HBV) and vertical transmission still pose threat to this age cohort. While in case of HCV the in-availability of a successful viral vaccine has resulted in a persistently high viral prevalence in Pakistani population. Only available strategy for HCV is control and that requires the in time diagnosis and efficient treatment using pangenomic HCV regimens (Afdhal *et al*., 2014; Jacobson *et al*., 2011; Lawitz *et al*., 2013; Pawlotsky *et al*., 2015; Sulkowski *et al*., 2014). Awareness of the HBV and HCV risk factors is crucial for resource allocation in preventive measures. Through electronic and print media Public health division is creating awareness in Pakistani population regarding these viral infections but still a lot of more efforts are required for an efficient control of these infections.

## CONCLUSION

Current study described the epidemiology of HBV and HCV in general population of Sargodha District, Punjab, Pakistan. Exact figures of prevalence and incidence are missing from Pakistan but it is estimated that the prevalence of HBV and HCV is very high in certain areas of the country. Most of the infections are attributed to 1961-1973 birth cohorts. High prevalence of both the infections will increase the disease burden in terms of morbidity, hepatic and extra-hepatic complications and mortality in future. There is a need to use efficient treatment strategies for the control of these viral infections. A comprehensive screening of whole population is required to get the complete picture of disease incidence and prevalence. Public awareness is the key to control and prevent the future infections.

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