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Epidemiological study of active Hepatitis B and C viruses' infection among patients attended in tertiary care hospital in Dhaka City, Bangladesh

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ABSTRACT

Viral hepatitis is a major global public health problem and both hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are rapidly spreading in the developing countries including Bangladesh. A molecular characteristic-based epidemiological study was conducted to identify the Molecular Characterization of Hepatitis B and C Viruses in Dhaka City, Bangladesh among all age groups, gender and marital status and to identify the possible risk factors for acquiring the infections. Informed consent was taken from every individual being tested and approval was obtained from institutional ethical review committee. Using the Enzyme-Linked Immunosorbent Assay for 1855 blood samples, our study clearly indicated a high prevalence of active HBV and HCV as 8.0% and 3.0% respectively in general public of Savar, Dhaka. Both HBV and HCV prevalence varied significantly in different age groups with respect to gender and marital status. In case of HBV, it was least prevalent for individuals whose age was above 60 years. Contrary in case of HCV, it was least prevalent for individuals whose age was below 11 years and above 60 years. However, middle aged populations, especially 31–40 and 21–30 year individuals were observed at higher risk of hepatitis B and C ailments with 11.28% active HBV prevalence and 5.77% active HCV prevalence with respect to gender, respectively. The findings and further studies of genotype distribution might guide eventually the development, adaptation and evaluation of prevention strategies.

INTRODUCTION

Viral hepatitis is a serious public health problem affecting billions of people globally. Caused mainly by hepatitis viruses A, B, C, D and E, and rarely by cytomegalovirus (CMV), Epstein-Barr virus (EBV) and fungal infections, the spectrum of hepatitis range from sub-clinical to milder and life threatening illness including acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Zuckerman, 1997; Khan *et al.*,2000). It is evident that hepatitis virus infections are rapidly spreading in developing countries including Bangladesh due to the lack of health education, poverty, illiteracy and lack of hepatitis vaccination. There is also lack of information on the prevalence of hepatitis infection among the general population and nearly all previous studies were conducted in selected group of people (Sattar, 1996; Bogaerts *et al.*,2001; Gibney *et al.*,2001). Hepatitis B is one of the most common infectious diseases globally. Globally two billion people are infected with HBV, and 350 millions of them have chronic (lifelong) infections, who are at high risk of death from liver cirrhosis and liver cancer that kill more than one million people globally each year (Hepatitis B, 2008). It has been estimated that there are 350 million chronic hepatitis B virus (HBV) carriers worldwide. The prevalence of chronic HBV infection varies geographically, from high (>8%), intermediate (2-7%) to low (<2%) prevalence. In Bangladesh, there is paucity of information on the prevalence of HBV infections among general population and majority of the previous studies were conducted in selected group of people with higher risk factors such as blood donors, drug addicts, commercial sex workers (CSWs) or hospitalised patients (Islam *et al.*,1984; Sattar and Islam, 1996; Rumi *et al.*,1998; Mostafa *et al.*,1989; Ahmad *et al.*,1991).

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HBsAg is the most reliable biological biomarker of HBV infection. Hepatitis C is an infectious liver disease of humans and chimpanzees and is caused by the HCV (Chen and Morgan, 2006). The infection is often asymptomatic especially in its early stages but once established, it can progress to advanced liver diseases such as liver fibrosis and ultimately cirrhosis. These liver diseases can further lead to other complications such as liver failure and liver cancer (Villano *et al.*,1999). In 2004, the World Health Organization (WHO) reported that annual deaths all over the world due to liver cancer and cirrhosis caused by HCV were about 308,000 and 785,000, respectively and about 200 million people, the 3.3% of the world's population, are infected with HCV. Moreover, around 3 to 4 million individuals are diagnosed as new cases every year 9 Ray, 2002). In Bangladesh, it needs to study the Epidemiological and Molecular characterization of active hepatitis viruses' infection.

As many chronically infected individuals remain asymptomatic, and thus undetected for many years and majority of the previous studies in Bangladesh examined only the prevalence of HBsAg (Khan *et al.*,2000; Islam *et al.*,1984; Sattar and Islam, 1996; Rumi *et al.*,1998; Mostafa *et al.*,1989; Sabin *et al.*,2003; Laskar *et al.*,1997) and most of them were conducted in selected group of people with higher risk factors, we decided to identify the Molecular Characterization of Hepatitis B and C Viruses in Dhaka City, Bangladesh among all age groups, gender and marital status and to identify the possible risk factors for acquiring the infections. We hoped that the findings might guide eventually the development, adaptation, and evaluation of prevention strategies.

METHODOLOGY

Study site

The study was conducted in the Immunology laboratory in Enam Medical College and Hospital over a period of two year extending from July 2011 to June 2013.

Collection of blood samples

In this study, 1855 blood samples were collected randomly from individuals visiting the clinical (immunology) laboratory of Enam Medical College and Hospital at Savar between 2011 and 2013. As the study was designed to represent the general features of molecular characterization and genotype distribution of hepatitis viruses, the samples were collected randomly from individuals who visited laboratories for any purpose such as some clinical test, sample submission, report collection or blood screen etc. Samples were collected from both genders in accordance of marital status having ages ranging from 1 to 90 years. Informed consent was taken from every individual being tested and approval was obtained from institutional ethical review committee. History of individuals was recorded in the form of questionnaires. To get sufficient number of sample size to reach the ultimate goals of the research, we considered the tertiary care hospital named Enam Medical College and Hospital, savar, Dhaka, as the population suitable for this study.

Study procedure

The trained research technicians explained the purpose and objective of the study and obtained written informed consent from study participants, or parents for eligible children. They administered a pre-tested questionnaire to the participants at a mutually agreed date at the clinic to collect their socio-demographic characteristics including age, gender, years of education, socio-economic status and marital status by using a structured questionnaire. HBsAg and Anti-HCV are the most reliable biological biomarker of HBV and HCV infections respectively.

About 3 ml blood sample was drawn per patient and centrifuged within 30 minutes at 3000 rpm for 5 minutes. Serum was separated and stored at -20ºC until assayed. Tests were carried out by using an Automated Immunology Analyzer, Beckman Access 2 Coulter, USA. For diagnosis of HBV infection, HBsAg was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Manufacturer: Autobio, England). For diagnosis of HCV infection, anti-HCV antibody was detected using a third-generation ELISA kit (Manufacturer: DiaSorin S. A., Italy). The laboratory test results were kept confidential and we shared the results with the participants. Infected individuals were provided with appropriate information on the prevention of spread of these infections to others, and referred them to the nearest public health care facilities.

Serological tests

We only performed HBsAg and anti-HCV to reduce the laboratory test costs.

HBsAg Dignosis

HBsAg is the serologic hallmark of HBV infection. It appears in serum 1-10 weeks after an acute exposure to HBV, prior to the onset of hepatitic symptoms. Persistence of HBsAg for more than six months implies chronic infection. The sensitivity of HBsAg is 100%, the specificity is 99.5%, and the limit of detection is 0.05 PEI units/ml.

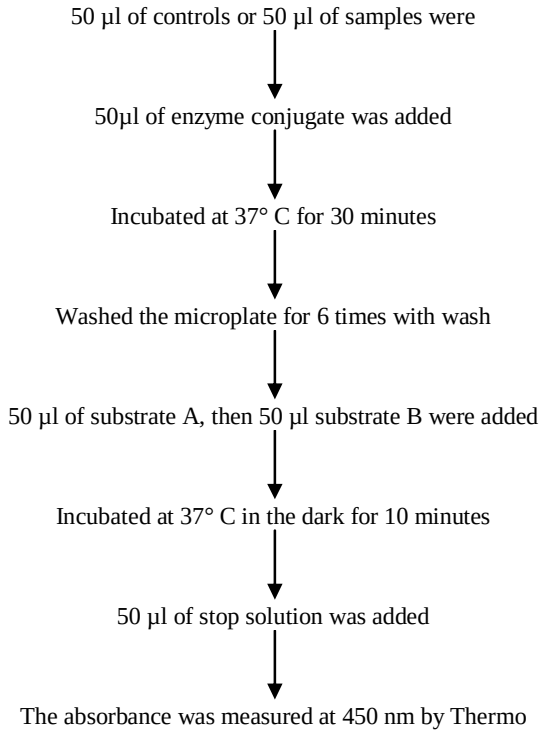
If the infection is self-limited, HBsAg disappears in most patients before the serum hepatitis B surface antibody (anti-HBs) can be detected. Persistence of HBsAg for more than six months implies chronic infection.

Measurement Principle

This assay is based upon the one step sandwich method. Sample, Anti-HBs coated microplate and enzyme-labeled Anti-HBs are combined. During the incubation, HBsAg present in the sample is allowed to react simultaneously with the two antibodies, resulting in the HBsAg being sandwiched between the solid phase and enzyme linked antibodies.

After washing, a complex is generated between the solid phase, the HBsAg within the sample and antibody in enzyme conjugate by immunological reactions. Substrate A and substrate B are then added and catalyzed by this complex, resulting in a chromogenic reaction which is measured as absorbance. The color

intensity is proportional to the amount of HBsAg in the sample (Dienstag, 2008; Knowels *et al.*,1980; Rehermann *et al.*,1996; gust, 1996; Toukan, 1990).



PROCEDURAL FLOW CHART

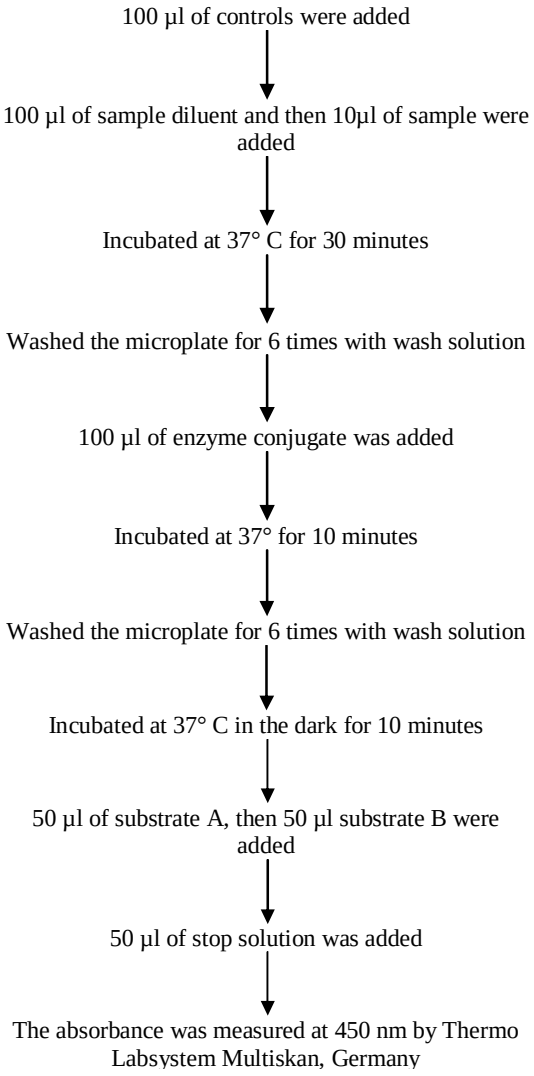
Anti-HCV Diagnosis

Although effective treatments are available to clear HCV infection from the body, most persons with HCV do not know they are infected (Robin *et al.*,2011 and Southern *et al.*,2011). The discovery of HCV in 1989 led to the development of an antibody diagnostic assay (anti-HCV) based on viral recombinant peptides. Detection of anti-HCV indicates present or previous HCV infection but cannot discriminate acute from chronic or resolved HCV infection.

Measurement Principle

This assay is based upon the two-step indirect method. In the first step, sample and recombinant HCV coated microwells are combined. During the incubation, the Anti-HCV present in sample binds to the antigen coated on the wells. After the washing, in the second step, enzyme conjugate is added to the reaction mixture. During the incubation, the Anti-HCV present in the sample reacts with mouse Anti-human IgG within enzyme conjugate. Then a complex is generated between the solid phase, the Anti-HCV within the sample and the mouse Anti-human IgG in the enzyme conjugate by immunological reactions. After a second washing, substrate A and substrate B are then added and catalyzed by this complex, resulting in a chromogenic reaction which is measured as absorbance. The color intensity is proportional to the amount of Anti-HCV in the sample (Kuo *et al.*,1989; Esteban *et al.*,1990; Zeldis *et al.*,1992; Esteban *et al.*,1989; Houghton *et al.*,1991).

Measurement procedure



PROCEDURAL FLOW CHART

Limitations of the procedures

If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Patients routinely exposed to animas or animal serum products can be prone to this interference and anomalous values may be observed.

Statistical Analysis

All data were recorded systemically in a performed data collection form and statistical analyses were performed by using SPSS for windows version 11.5. Descriptive statistics of socio-demographic variables and other characteristics of the sampled population were computed. The analysis was carried out at three levels of descriptive, bivariate and test of hypothesis specifically chi-square test. The chi-square test implies that logistic regression analysis is required further to take the decision about the phenomenon more appropriately.

RESULTS

Statistical differences have been observed considering the age group criterion. Table 1.1 shows the prevalence of active hepatitis B virus in general patient of Tertiary hospital at Savar with respect of gender. According to this table it is clear that age group 21-30 contains highest number of hepatitis B virus infected male and female thereby its prevalence rate is high among all of the age groups. Consequently people of age group 21-30 are most prone to be affected by HBV.

Table. 1.1: Prevalence of active hepatitis B virus in general patient of Tertiary Hospital at Savar with respect to gender.

Age Interval	Male +/-	Female +/-	Prevalence Rate (%)
1-10	2/21	1/9	9.10
11-20	12/52	7/189	7.28
21-30	**25/90**	**5/145**	**11.28**
31-40	31/135	15/415	7.71
41-50	6/52	4/82	6.94
51-60	5/40	3/35	9.64
61-70	1/20	1/21	4.55
71-80	0/9	0/12	0
81-90	0/7	0/3	0
Total	82/426	36/911	
	Male positive patient	Female positive patient	
Average age (yr)	31.11	33	
Standard Error (yr)	1.29	2.27	
95% CI	(28.59 , 32.77)	(28.56 , 37.44)	

The table 1.1 also implies that the age of Hepatitis B infected male patient is 31.11 yr on an average and its standard error is 1.29 yr while female patient is 33 yr and 2.27 yr. If we take sample repeatedly from the population and construct confidence interval for each sample in such case it can be said that 95 times out 100 times the age of Hepatitis B infected male patient will lie between 28.59 yr to 32.77 yr and at the same time the age of infected female patient will lie between 28.56 yr to 37.44 yr. Consequently, the male people ages between 28.59 yr to 32.77 yr while the female people ages between 28.56 yr to 37.44 yr are most prone to hepatitis B virus in the study region.

Figure 1.1 shows the bar diagram for the Number of HBV reactive male/female in different age groups using Table 1.1. It is seen that the highest number of HBV positive male and female lies in the age group 21-30.

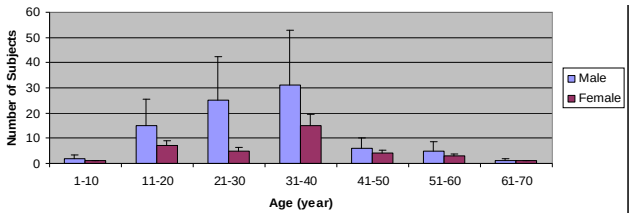


Fig. 1.1: Number of HBV positive samples in male/female population in different age groups.

Table 1.2 shows the prevalence of active hepatitis B virus in general patient of tertiary hospital at Savar with respect of marital status. According to this table it is clear that age group 31-40 contains highest number of hepatitis B reactive married individuals (both male and female) while age group 21-30 contains highest number of hepatitis B reactive single (both male and female) individuals. In addition, the prevalence rate of age group 31-40 is high among all of the age groups. Consequently people of age group 31-40 are most prone to be affected by HBV with respect to marital status. Odds ratio column implies that for age groups 31-40, 41-50, and 51-60, exposure (here marital status) is positively related to the HBV positive ness which means that the probability of becoming HBV reactive for both married male and female is higher than for both single male and female while for age groups 11-20, and 21-30, exposure is negatively related to the HBV positive ness which means that the probability of becoming HBV positive for both married male and female is lower than for both single male and female. For rest of the age groups the probability of becoming HBV positive for both male and female whether they are single or married is zero.

Table. 1.2: Prevalence of active hepatitis B virus in general patient of Tertiary Hospital at Savar with respect to marital status.

Age Interval	Single +/-	Married +/-	Probabilities		Odds ratio	Prevalence Rate (%)
			Single	Married		
1-10	3/30	0/0	0.1	0		9.090
11-20	15/184	4/57	0.062	0.016	0.254	7.307
21-30	25/207	21/343	**0.045**	0.038	0.833	7.7181
31-40	3/32	27/203	0.012	**0.114**	10.038	**11.320**
41-50	2/18	8/116	0.014	0.059	4.190	6.944
51-60	2/8	6/67	0.026	0.08	3.173	9.638
61-70	0/10	2/31	0	0		4.651
71-80	0/7	0/14	0	0		0
81-90	0/2	0/8	0	0		0
Total	48/494	70/843				

Figure 1.2 shows the bar diagram for the number of HBV positive samples in married/single population in different age groups. It is seen that the number of married HBV reactive individuals (both male and female) is much higher than single HBV reactive individuals (both male and female) for all age groups except 1-10, 11-20 and 21-30.

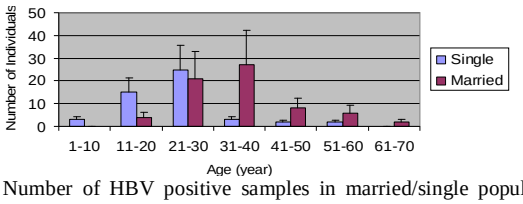


Fig. 1.2: Number of HBV positive samples in married/single population in different age groups.

Table. 1.3. Overall summary of HBV patients.

			Marital Status		Total
			Single	Married	
Reactive	GENDER	Male	38	44	82
		Female	11	25	36
	Total		49	69	118
Non reactive	GENDER	Male	176	250	426
		Female	322	589	911
	Total		498	839	1337

Table 1.3 shows the overall summary of HBV tested patients. Here, the number of married HBV reactive patients for both male and female is higher than the number of single HBV reactive patients.

Figure 1.3 shows the number of HBV reactive samples in male/female and married/single population using Table 1.3. Here, it is seen from the following figure that the number of married HBV reactive individuals (both male and female) is higher than individuals who are single.

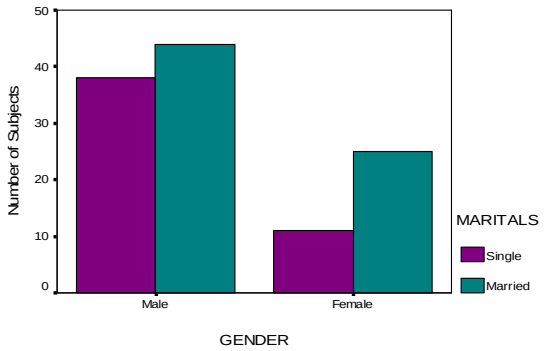


Fig. 1.3: Number of HBV positive samples in male/female and married/single population

Statistical differences have been observed considering the age group criterion. Table 2.1 shows the prevalence of active hepatitis C virus in general patient of Tertiary hospital at Savar with respect of gender. According to this table it is clear that age group 21-30 contains highest number of hepatitis C virus infected male and female thereby the prevalence rate for age group 21-30 is high among all of the age groups. Consequently people of age group 21-30 are most prone to be affected by HCV with respect to gender.

Table. 2.1: Prevalence of active hepatitis C virus in general patient of Tertiary Hospital at Savar with respect to Gender.

Age Interval	Male +/-	Female +/-	Prevalence Rate (%)
1-10	0/4	0/4	0
11-20	0/25	1/35	1.64
21-30	**2/37**	**4/61**	**5.77**
31-40	1/30	1/43	2.67
41-50	0/20	1/31	1.92
51-60	2/25	0/19	4.35
61-70	0/11	0/15	0
71-80	0/8	0/11	0
81-90	0/6	0/3	0
Total	5/166	7/222	

	Male positive patient	Female positive patient
Average age (yr)	39.5	28.36
Standard Error (yr)	6.78	3.60
95% CI	(26.21 , 52.79)	(21.31 , 35.40)

The table 2.1 also explains that the age of Hepatitis C infected male patient is 28.36 yr on an average and its standard error is 6.78 yr while female patient is 33 yr and 3.60 yr. If we take sample repeatedly from the population and construct confidence interval for each sample in such case it can be said that 95 times out 100 times the age of Hepatitis C infected male patient will lie between 26.21 yr to 52.79 yr and at the same time the age of infected female patient will lie between 21.31 yr to 35.40 yr. Consequently, the male people ages between 26.21 yr to 52.79 yr while the female people ages between 21.31 yr to 3540 yr are most prone to hepatitis C virus in the study region.

Figure 2.1 shows the bar diagram for the Number of HCV reactive male/female in different age groups. It is seen that the highest number of HCV reactive male and female lies in the age group 21-30.

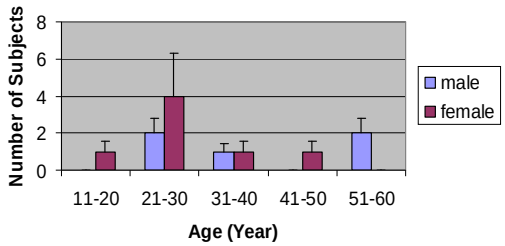


Fig. 2.1: Number of HCV positive samples in male/female population in different age groups.

Table 2.2 shows the prevalence of active hepatitis C virus in general patient of Tertiary hospital at Savar with respect of marital status. According to this table it is clear that age group 21-30 contains highest number of hepatitis C positive married and single individuals (both male and female) thereby the prevalence rate of age group 21-30 is high among all of the age groups. Consequently people of age group 21-30 are most prone to be affected by HCV with respect to marital status.

Table. 2.2: Prevalence of active hepatitis C virus in general patient of Tertiary Hospital in Savar with respect to marital status.

Age Interval	Married +/-	Single +/-	Prevalence Rate (%)
1-10	0/0	0/8	0
11-20	0/35	1/25	1.64
21-30	**4/51**	**2/47**	**5.77**
31-40	0/31	1/42	1.33
41-50	0/20	1/31	1.92
51-60	0/10	2/34	4.35
61-70	0/11	0/15	0
71-80	0/7	0/12	0
81-90	0/2	0/7	0
Total	4/167	8/221	

Figure 2.2 shows the bar diagram for the number of HCV reactive samples in married/single population in different age groups. It is seen that the number of married HCV reactive individuals (both male and female) is much higher than single HCV reactive individuals (both male and female) for the age group of 21-30 while there is no married HCV reactive individuals for rest of the age groups.

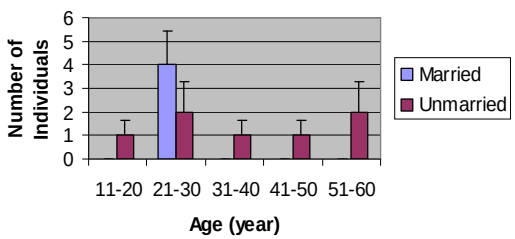


Fig. 2.2: Number of HCV positive samples in married/single population in different age groups.

Table 2.3 shows the overall summary of HCV tested patients. Here, the number of unmarried HCV reactive patients for both male and female is higher than the number of single HCV reactive patients

Table. 2.3: HCV summary.

			Marital Status		Total
			married	unmarried	
Reactive	GENDER	male	1	4	5
		female	3	4	7
	Total		4	8	12
Non Reactive	GENDER	male	67	99	166
		female	100	122	222
	Total		167	221	388

Figure 2.3 shows the number of HCV positive samples in male/female and married/single population using Table 2.3. Here, it is seen from the following figure that the number of unmarried HCV positive individuals (both male and female) is higher than individuals who are married.

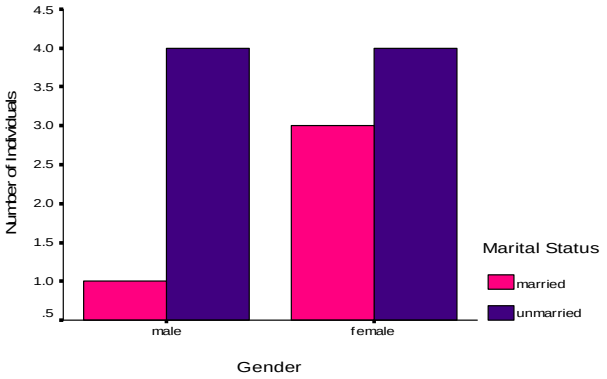


Fig. 2.3: Number of HCV positive samples in male/female and married/single population.

Test of Independence

The entire test procedure is as follows:

$H_{0:}$ There is no association between two categorical variables

$H_{1:}$ There is association between two categorical variables

The test is conducted at 5% level of risk. Under H_0 the test statistic is given by

$$\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} \sim \chi^2_{(r-1)(c-1)}$$

Where O_i is the observed frequency in the ith cell,

E_i is the expected frequency in the ith cell,

r is the number of rows, and

c is the number of columns.

Here in case of our problem at 5% level of risk, the critical value is $\chi^2_{.025,1} = 5.02$ (for 2×2 contingency table). Table 3.1 shows the values of Chi-square test statistic calculated using 1456 HBV tested individuals and 400 HCV tested individuals along with decision.

Table. 3.1: Test of Independence Using Pearson Chi-Square Statistic.

	Variable	Pearson Chi-square	Decision
HBV	Gender	69.38	H_0 is rejected
Presence	Marital Status	1.44	H_0 is not rejected
HCV	Gender	0.006	H_0 is not rejected
Presence	Marital Status	0.448	H_0 is not rejected

This table shows that only the presence of HBV is associated with gender. Consequently a binary logistic regression model can be fitted using HBV presence and gender.

There was an evidence in favor of the alternate hypothesis i.e., the presence of HBV is associated with gender provided by Chi-square test in terms of sample individuals. This implies that further research is need here. One can apply logistic regression approach to compute probabilities as well as odds ratio.

DISCUSSIONS

Although prevalence studies are not always easily undertaken in the developing countries such as Bangladesh due to high cost, we made efforts to prospectively estimate the prevalence of HBV and HCV infections among a population living at savar, Dhaka.

The results of our study suggest a high HBV exposure among our study population. The HBsAg prevalence of 8% among our study population is very close within the range of 2-7%, reported by previous studies from selective population of Dhaka (Zaki *et al.*,2003) and gradually increases. However, a recent report showed 5.5% HBsAg positivity among the general population living in Savar, a semi-urban area on the outskirts of Dhaka (Mahtab *et al.*,2009). The higher rates among our study population could be attributed to the general lack of proper health care because of deprived socio-economic status (monthly household income of US $50) and less public health awareness about the transmission of HBV infection as well as the lack of hepatitis B vaccination in the community.

The 3% prevalence of anti-HCV observed in our study population is higher than that reported from high-risk groups of Dhaka: 0.8% among truck drivers and helpers (Gibney *et al.*,2001); 0.9% among women at a STD clinic (Bogaerts, *et al.*,2001); 1.6% among women living near a truck stand (Gibney *et al.*,2001) and lower than that reported from high-risk groups of Dhaka: 5.8% in non-IDUs and 24.8% in IDUs (Shirin *et al.*,2000); and 13% among hepatitis patients (Khan *et al.*,2000).

Both HBV and HCV prevalence among the study population varied in different age groups with respect to gender and marital status. In 2004, the Government of Bangladesh and UNICEF have introduced the hepatitis B vaccine into the Expanded Programme on Immunization (EPI) against six infectious diseases. The successful continuation of the programme is expected to reduce chronic HBV infections in the next

generations. Since 90% of the HBV infected older children and adults successfully clear the infection and do not become chronic carriers, the prevalence of HBsAg alone might not describe the total burden of HBV infections. Therefore, estimation of the prevalence of anti-HBc, in addition to the estimation of the prevalence of HBsAg which is the most reliable biological biomarker of HBV infection, is much more informative about indicator of HBV disease burden among the population. Detection of anti-HCV indicates present or previous HCV infection but cannot discriminate acute from chronic or resolved HCV infection *(Hepatobiliary Pancreat Dis Int*, 2008) which clearly indicates the requirement of further studies.

CONSTRAINTS

There are some limitations of our study. First, we did not perform some diagnostic tests for HBV, e.g. anti-HBc IgM, the presence of which indicates acute infection; and anti-HBs that differentiates susceptible persons from those immune persons, which can be due either to natural infection or hepatitis B vaccination. Second, we did not perform some diagnostic tests for HCV, e. g. recombinant immunoblot assay (RIBA) to confirm HCV exposure. All the above limitations are mainly due to study cost constraints, mostly related to laboratory tests. A final limitation is that the study was conducted in a clinical laboratory based single population at Savar, and may not reflect all of Bangladesh, although the literature we have cited suggests that it should.

CONCLUSIONS

Using the Enzyme-Linked Immunosorbent Assay (ELISA) for 1855 blood samples, the overall prevalence of active HBV and HCV was estimated as 8.0% and 3% respectively in general public of Savar, Dhaka. Significant differences in male and female as well as in married and single were observed in different age groups. That means both HBV and HCV prevalence varied in different age groups with respect to gender and marital status. In case of HBV, it was least prevalent for individuals whose age was above 60 years. Contrary in case of HCV, it was least prevalent for individuals whose age was below 11 years and above 60 years. However, middle aged populations, especially 31–40 and 21–30 year individuals were observed at higher risk of hepatitis B and C ailments with 11.28% active HBV prevalence and 5.77% active HCV prevalence with respect to gender, respectively. This report will provide the active HBV and HCV prevalence data for further meta-analysis, which can be helpful to health policy makers to devise strategies for the control of hepatitis B and C disease in Bangladesh.

Future more long-term population-based surveillance studies and follow-up measures are required to confirm the family clustering effect of hepatitis B and C virus infection and to identify their common risk factors for acquiring hepatitis viruses infection in Bangladesh. This would be very helpful to initiate and implement national epidemic preparedness for HBV and HCV infections and improve the impact of vaccination, and to guide prioritization of scarce health care resources.

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