

Hepatitis B Virus, Hepatitis C Virus Markers and Serum Alanine Amino-Transferase (ALT) Levels, in a Young Adult Population of Sylhet District

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Summary:

Objective: To find out the seroprevalence of the hepatitis B and C viruses in a young adult population along with estimation of aminotransferase levels. And so as to increase the awareness and augment preventive measures against these viral hepatitis related morbidity.

Methods: A total of 2611 apparently healthy young adults were screened from January 2010 to December 2010 at a Medical Checkup center of Sylhet for hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti HCV), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels.

Results: Out of 2611 subjects 2536(97.1%) were male and 75(2.9%) were female with comparable mean ages (29.08 vs. 30.6 years, P.056). A total of 77(3.0%) men were HBsAg positive and only four (0.16%) men were positive for anti

HCV. Women were all negative for HBsAg or anti HCV. None had co-infection with HBV and HCV. Mean ALT and AST levels of study population were 31.85 I.U./L and 26.18 I.U./L respectively and were not found to vary with age and sex. Mean ALT levels were more in HBsAg positive cases (42.03 I.U./L vs. 31.5 I.U./L, P.000) and HCV infected cases (49 I.U./L vs. 31.51 I.U./L, P.000) than non-infected persons.

Conclusion: Hepatitis B infection is of intermediate endemicity among young adults while hepatitis C virus infection is low among this group. Mean amino transferase levels were higher in asymptomatic infected persons in comparison to non-infected person. We suggest the need for revision of upper limit of normal for ALT in our population for early detection and treatment of liver diseases.

Key Words: Hepatitis B virus, hepatitis C virus, alanine amino-transferase, young adults.

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Introduction:

Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are global challenges. Globally 350 million people are suffering from chronic (lifelong) infections and more than one million people die each year from liver cirrhosis and liver cancer¹. In the Middle East and Indian sub-continent, HBV infection is of intermediate endemicity with chronic HBV carriage rate of 2-5% among general population¹. In Bangladesh, there is paucity of information on the prevalence of HBV infections. According to a recent report hepatitis

B surface antigen (HBsAg) positivity was 5.5% among the general population of a semi-urban area near Dhaka city². The reported prevalence of HBsAg in selected population of Bangladesh ranges between 2-3.5%³⁻⁵. The prevalence is much higher in high risk group⁶⁻⁹.

According to World Health Organization (WHO), 130-170 million persons are chronically infected worldwide with hepatitis C virus (HCV).¹⁰ Approximately 10% to 20% of chronic HCV infection cases, will progress to cirrhosis and hepatocellular carcinoma.¹⁰ The highest prevalence rates are reported from developing poor countries from Africa and Asia. Estimates of HCV prevalence in Southeast Asian countries are 2.0% to 3.8%^{11, 12} for the general population, 12.5% for patients with chronic liver disease¹³, and more than 90% for injecting drug users¹⁴. Currently, there is limited information on the HCV prevalence and risk factors in the general population of Bangladesh. The reported seroprevalence of HCV among people of an impoverished area of Bangladesh¹⁵, rural area,¹⁶ healthy blood donors³ and drug addicts¹⁷ were 0.2%, 0.6%, 0.25% and 15.0% respectively.

Serum alanine aminotransferase (ALT) concentration is the most widely used sensitive and reliable marker of

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liver diseases. Several population-based studies have found slightly increased ALT levels within the current normal range to be closely related to comorbidities and mortality.¹⁸⁻²⁰ Current upper limit of normal (ULN) for ALT level were set, on average, ranging from 30 U/L to 50 U/L over the past 10 years. Such thresholds, however, vary tremendously among hospitals, research centers and geographic locations. The normal range of serum ALT for any laboratory test is the mean value plus two standard deviations in a supposedly healthy reference population, and the upper limit of normal (ULN) is established statistically as the value at the 97.5th percentile.²¹ Currently, several studies have been reevaluated the ULN ALT in different countries by involving different age groups. And the recommended ULN ALT was 30 U/L for men and 19 U/L for women respectively.^{18, 22-24} Based on the previous results it is found that, recent ULN ALT greatly increase the number of asymptomatic patients with abnormal ALT values, and would identify more patients with nonalcoholic fatty liver disease (NAFLD) and clinically mild HBV/HCV infection.

We have no data regarding the upper limit of normal for ALT in our population. Most of the laboratories follow the manufacturer's recommendation (40 - 65 I.U./L) for a particular analyzer to establish ULN ALT without healthy volunteer testing. It is evident that HBV and HCV infections are rapidly spreading in developing countries due to the lack of health education, poverty, illiteracy and lack or cost of proper vaccination. As many chronically infected individuals remain asymptomatic, and thus undetected for many years, we planned this serological study to determine the seroprevalence of HBsAg and anti-HCV and to estimate corresponding serum ALT levels among a group of apparently healthy people coming for medical checkup for the purpose of jobs in the Middle East. We hoped that the findings might guide eventually the development, adaptation, and evaluation of management strategies.

Methodology

Hepatitis C virus (HCV) and hepatitis B virus (HBV) seroprevalence study was undertaken among 2611 subjects coming for medical checkup in one of three reference centers of Sylhet district. All were young adults applying for job visas to different Middle East countries. Data were collected by the trained staff from the medical center over a period of 10 months, September 2011 to June 2002 and were recorded in the registry

book of the medical centre. Physical examination and laboratory investigations including markers for several infectious diseases and drugs of abuse were carried out as required by the countries recruiting the workers. Consents were taken for blood tests Immediately following the interview, a 10-mL aliquot of blood was drawn from each participant to test for hepatitis B surface antigen (HBsAg), anti-HCV and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels.

Data analysis

Data were analyzed using the SPSS version 20.0 statistical program. Prevalence estimates of cases positive for HBsAg and anti-HCV were stratified by demographic characteristics. A χ^2 test was used for comparisons between proportions, while Students t test was used for comparisons of means, with α set at the 5% level.

Ethics

Participation was voluntary and informed consent for interviewing and blood tests was obtained. Participants who tested positive for HBsAg or anti-HCV were offered posttest counseling by specialists. The study protocol was approved by the Ethics Committee of Northeast Medical College of Sylhet, Bangladesh.

Result:

Out of 2614 participants, 3 cases were excluded for very high level (> 1000 I.U./L) of SGPT and SGOT level. One of them had bilirubin level 7.30 mg/dl. Viral markers were negative in all three subjects.

Among the remaining 2611 subjects, 2536(97.1%) were male and 75(2.9%) were female with comparable mean ages (29.08 ± 6.767 vs. 30.6 ± 7.600 , $P = .056$). Out of this study population, 77(3.0%) male were HBsAg positive while none of the women were HBsAg positive. Study population mostly belonged to 21-40 years group ($n=2351$, 90.0%) and HBsAg positivity among this group were 2.85% ($n=67$) (table 2). Only four men (0.16%) were positive for anti HCV and women were all negative for anti HCV. None had co-infection with HBV and HCV.

Mean ALT and AST levels of study population were 31.85 I.U./L (range, 17-226 I.U./L) and 26.18 I.U./L (range, 14- 211 I.U./L) respectively and mean values were 8.15 unit and 13.82 unit lower than upper level of normal values (40 I.U./L). No significant difference was noted in these enzyme levels in males and females (table 1). No significant difference was noted in ALT and AST

levels in more than 40 years and less than 40 years group (table 2). Mean bilirubin level of study population was $0.667 \pm .258$ mg/dl (range .10-7.30mg/dl). No significant difference was noted in the mean bilirubin level in HBV and HCV infected persons than non-infected persons (table 3 and 4)

ALT levels more than 40units/L was found in 107 cases; among them 78 cases (3.1%) were both HBSAg and anti HCV negative while 26 cases (33.8%) were HBsAg positive (P 0.000) and 3(75.0%) were anti HCV positive. ALT level more than 30 I.U. was found in 77.4% (n=55) of HBsAg positive males while In anti HCV positive males

all 4 had ALT more than 30 I.U./L. In female ALT level >19 was found in 98.7%(n=74) and in 96.0%(n=72) ALT was less than 40 I.U./L. Similarly AST levels more than 40 I.U./L was found in 2.3 % (n =58) non-infected subjects and in 22.0%(n=17) of HBsAg positive cases(P 0.000).

Mean ALT and AST levels were significantly more in in HBsAg positive cases than HBsAg negative cases (table3). These two levels were also significantly more in anti HCV positive cases than anti HCV negative cases (table 4). The ALT and AST values in HBsAg and anti HCV negative subjects (n=2530) at 97.5th percentile were 49 I.U./L and 40I.U./L respectively.

Table-I

<i>Liver function test profile according to sex</i>			
	Male N=2536	Female N=75	P-value
HBsAg positive	77(3.0%)	0	.171
Anti HCV positive	4(0.16%)	0	NS
Mean ALT	31.9±12.4 I.U./L	29.4±7.2 I.U./L	0.073
Mean AST	26.2±10.4 I.U./L	24.1±5.9 I.U./L	.077
Mean bilirubin	.67±.23 mg/dl	.58±.15 mg/dl	0.001

Table-II

<i>Liver function tests according to age category</i>		
Age category		P-value
HBsAg positive		
<20 years(n=48)	1 (2.08%)	0.482
21-40 years(n=2351)	67 (2.85%)	
>40 years(n=212)	9 (4.3%)	
Anti HCV positive		
<20 years(n=48)	0	0.453
21-40 years(n=2351)	3 (.13%)	
>40 years(n=212)	1 (.47%)	
Mean ALT level		
<20 years(n=48)	29.3±7.7 I.U./L	0.331
21-40 years(n=2351)	31.9±12.5 I.U./L	
>40 years(n=212)	31.7±10.3 I.U./L	
Mean AST level		
<20 years(n=48)	23.96±6.8 I.U./L	0.248
21-40 years(n=2351)	26.3±10.5 I.U./L	
>40 years(n=212)	25.7±8.1 I.U./L	
Mean bilirubin level		
<20 years(n=48)	.78±.44mg/dl	0.007
21-40 years(n=2351)	.67±.26mg/dl	
>40 years(n=212)	.65±.19mg/dl	

Table-III

<i>Mean bilirubin, mean ALT and mean AST levels in HBsAg positive subjects</i>			
	HBsAg positive N=77	HBsAg & Anti HCV negative N=2530	P-value
Mean Bilirubin (mg/dl)	.72±.25	.67±.26	.084
Mean ALT(I.U./L)	42.0±17.3	31.5±11.9	.000
Mean AST(I.U./L)	34.3± 13.96	25.9±10.0	.000

Table-IV

<i>Mean bilirubin, mean ALT and mean AST levels in Anti HCV positive subjects</i>			
	Anti HCV positive N=4	HBsAg & Anti HCV negative N=2530	P-value
Mean Bilirubin (mg/dl)	.53±.05	.67±.26	.271
Mean ALT(I.U./L)	49.0±14.9	31.5±11.9	.000
Mean AST(I.U./L)	40.0±11.78	25.9±10.05	.000

Discussion:

HBsAg is the most reliable biological biomarker of chronic HBV infection, and the antibody against hepatitis B core antigen (anti-HBc) is an important marker for surveying the burden of HBV infection for identifying both past and current HBV infection.²⁵ Thirty years ago Islam et al. in 1984, reported a seroprevalence 7.8% of HBsAg among apparently healthy workers applied for jobs in foreign countries.²⁶ Thirty years later we found a seroprevalence of 2.95%, which corresponds to the findings of Rumi et al,²⁷ who reported a prevalence of 4.4% among healthy workers.

The HBsAg prevalence of 2.95% among our study population corresponds to the range of 2-7% reported by previous studies from the selected population: 2% among healthy blood donors³, 3% among healthy adults and children⁴ and 3.5% among pregnant women.⁵ The prevalence also corresponds to reports among high-risk group: 8.0% among drug addicts,⁸ 5.9% among truck drivers and helpers.²⁸ Lack of proper health care, lower socioeconomic status, less public awareness about the HBV transmission and lack of vaccination are proposed reason for high prevalence of HBV. High prevalence also reported in other high risk groups of Dhaka: 7.6% among women at a sexually transmitted disease (STD) clinic⁹, 8% among intravenous drug users (IDUs)⁸, 8.6%

among surgically operated patients,⁶ 9.7% among commercial sex workers(CSWs),⁷ 19% among hepatitis patients²⁹; and 47% among hepatocellular carcinoma.³⁰

The estimates of HBV exposures were far higher when anti HBc was used as marker for HBV infection among urban slum (22.6%),¹⁵ drug addicts (27.8%)¹⁷ and healthy adults and children(21.1%)⁴. Anti HBc positivity was much higher in the high-risk groups of Dhaka: 24.1% in non-IDUs and 31.8% in IDUs¹⁷; 35.2% among women at a STD clinic⁹; 48.1% among truck drivers and helpers,²⁸ 49.3% among women living near a truck stand,³¹ and 73% among CSWs.⁷ The apparently low prevalence of HBV infection in the present study may be due to use of single marker or may reflect the true low prevalence among non-vulnerable population.

The 0.15% prevalence of anti-HCV observed in our study population is lower than that reported from high-risk groups of Dhaka: 0.8% among truck drivers and helpers,²⁸ 0.9% among women at a STD clinic,⁹ 1.6% among women living near a truck stand,³¹; 5.8% in non-IDUs and 24.8% in IDUs,¹⁷ and 13% among hepatitis patients.²⁹ The report corresponds to the findings of Ashraf et al. where seroprevalence among population of an impoverished area were .2%.¹⁵ Our results of Ant HCV prevalence was much lower than other South Asian countries.^{11, 12} As HCV infection is rare, and as a

consequence co-infection with HBV and HCV is much rarer in our subjects. In the context of the absence of an HCV vaccine and the prohibitive cost of HCV treatment for the vast majority of the world's HCV-infected population, identification and elimination of risk factors for HCV remains the only option for reduction of HCV-related disease burden for most developing economies.

ALT is a good indicator of health and meets most of the accepted criteria for a screening test.³² Current upper limit of normal (ULN) for ALT levels (30-50 I.U./L) were set based on the studies conducted prior to the introduction of hepatitis C virus (HCV) testing and prior to the development of concept of nonalcoholic fatty liver disease (NAFLD). For a number of reasons researchers support the lowering the ULN to achieve appropriate screening and medical attention for patients with borderline ALT levels. First, screening using the past range of normal serum ALT values might underestimate the prevalence of CLD. Second, considering the natural courses of NAFLD or chronic HBV/HCV infections, disease progression with significant degrees of necroinflammatory activity and fibrosis of liver might occur in patients with persistently normal ALT levels.²⁷⁻³¹ According to current guidelines, antiviral therapy should be initiated with evidence of viral replication in all patients with serum ALT levels more than twice the ULN and in selected patients with serum ALT levels one to two times the ULN.^{27, 35} Adjustment of the ULN by defining borderline ALT levels as abnormal would allow more vigorous surveillance and earlier initiation of treatment. Third, Consistent with this finding, borderline rise of ALT values are early indicators of co-morbidities associated with lifestyle and with liver injury due to hepatic steatosis.³⁷

In the present series mean ALT level was 31.93 I.U./L (range 17-226 I.U./L). It was not found to vary significantly with age (P 0.331) and sex (P .073). Mean ALT level in HBsAg positive subjects was significantly more than in HBsAg negative subjects (P .000) (table 3). When ULN ALT was considered as 40 I.U./L only 33% HBsAg positive subjects were found to have raised ALT, but when upper level was lowered to 30 I.U./L, 71.4% HBsAg positive subjects were found to have abnormal ALT values. In case of hepatitis C infection this percentages raised to 100% from 75.0%. From our study results it is apparent that with currently adopted ALT values a good number of HBV and HCV infected

patients remained outside the scope of antiviral treatment and these patients may inadvertently exposed to many hepatotoxic medications.

We cannot comment on healthy ULN ALT as other diseases like NAFLD and metabolic disorders were not excluded by ultrasonography/MRI, liver biopsy and other relevant tests. But when HBV, HCV infection cases were excluded, at 97.5th percentile ALT level was 49 I.U./L. this level is higher than recently recommended ULN ALT.²²⁻²⁴

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, or polymerase chain reaction (PCR) to detect HCV infected individuals. All the above limitations are mainly due to study cost constraints, mostly related to laboratory tests. The third limitation is that the familial clustering effects of hepatitis viruses, and risk factors for acquisition of viral hepatitis were not assessed. The fourth limitation is that the study was conducted in a selected population of Sylhet district, and may not reflect all of Bangladesh. A final limitation is the relatively short observation window, which may have missed important secular trends in the background prevalence of both the hepatitis B and C viruses.

Conclusions:

The results of our study indicate an intermediate level of endemicity of HBV infection as 2.95% our valuable young adult population were HBsAg positive. However, we observed a much lower prevalence of HCV infections in the same community. We hope that this endemic presence of HBV virus, would make awareness among our health care providers and policy makers in designing and implementing effective preventive programmes. The findings also highlighted the need for prevention and control of HBV and HCV infections in Bangladesh by implementing universal hepatitis B vaccination and creating public awareness to prevent viral transmission. We suggest the need for revision of Upper Limit of Normal for ALT in our population for early detection and initiation of treatment of liver diseases.

Long-term population-based surveillance studies, with extended HBV serology, are needed for more accurate assessment of the hepatitis B and hepatitis C disease burden in our country, the impact of vaccination, and to guide prioritization of limited health care resources. Further studies are also required to confirm the familial clustering effect, for exploring transmission dynamics and to identify risk factors for viral hepatitis.

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