Prevalence of HBV Infection Among the Healthy Nepalese Males: A Serological Survey

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Sera from 478 healthy Nepalese male population, inhabiting various districts of five development regions of Nepal were collected from October 1996 to March 1997 and examined for the presence of HBsAg by third generation ELISA and Latex agglutination test in the laboratory of Central Department of Microbiology, Tribhuvan University, Kirtipur. The surface antigen was detected by ELISA in 4.0% (19/478) of subjects studied. However, different results were obtained in Latex agglutination test. The correlation between the results obtained from these two different tests is statistically significant (χ²=85.11, P<0.05). The results obtained from ELISA showed the probability of Nepal to fall in WHO category of intermediate endemicity zone for hepatitis B infection. The percent positivity of HBsAg was found to increase steadily from Eastern (2%) to Far Western (6.2%) development regions. Among the districts studied, Kailali district gave characteristic high prevalence followed by Rukum and Kaski. Other, districts representing for the prevalence are Sankhuwasabha, Jhapa, Ramechhap, Sarlahi, Dhanusa, Baglung, Gulmi, Palpa and Dang. None of the samples represented from Kathmandu valley were positive for HBsAg.

The age groups of 16-20 years and 36-40 years were found to be associated with the hepatitis B infection. Furthermore, various percent prevalence of the infection were encountered from 16 to 40 years subjects and a single case was also observed from 63 years old man. All the samples recorded positive for HBsAg from ELISA were assayed for the amount of ALT as a liver function test. Where, 15.8% (3/19) of the infected subjects had impaired liver function, hence it showed that, the subjects with high ALT to be the possible carrier of the HBV. Conversely, the remaining 84.2% (16/19) of the total HBsAg positive subjects were concluded to have the infection either at incubation or at prodrome period. J Epidemiol, 2000; 10: 410-413

ELISA, HBsAg, HBV, Nepal, Prevalence

INTRODUCTION

Nepal is one of the developing countries of south Asia, having comparatively poor health status than many other developed countries of the world. Due to lack of adequate knowledge in health and hygiene, people of this country are usually victimised by many types of infections, specially by communicable diseases, which can be prevented with prior knowledge of sanitation. It is therefore not surprising that, most of the infectious diseases like AIDS, Viral Hepatitis, etc. which are threatening globally nowadays, are also common in our country. Unfortunately, such complications are often remain undetected or misinterpreted if detected. In case of viral hepatitis for instance, huge number of cases reported in rural areas are unknowingly misdiagnose as a case of common jaundice with reporting dark urine and yellow skin. As a result, such patients might suffered from unrecoverable complications and even die. Therefore, this present research on HBV infection was conducted to focus its prevalence and also to determine the high risk age group of the country.

As we know, the viral hepatitis has become an important cause of morbidity and mortality in human community (population). It is also estimated that, the number of people die of AIDS per year will die of the Viral Hepatitis in a day. Among

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six different types of Viral Hepatitis, (i.e. A, B, C, D, E and G) HBV infection is the most common one that transmits via blood and blood products containing the virus itself or its antigens. Its prevalence rate is found to be very high in South East Asia and Western Pacific regions, where the commonest route of transmission was determined to be by parenteral route and through blood and blood products containing the HBV or its antigens.

HBV infection may be an acute or chronic depending upon the period of exposure to the virus and the immune response of the host. An adequate immune response of the host will lead to the production of antibodies (Anti-HB antibodies) that tends to clean the infection and produce life long immunity. But, an inadequate immune response allows the virus to replicate continuously. If such viral proliferation maintains for about six months or more the infection usually persists indefinitely and the infected individual becomes an asymptomatic carrier of the HBV. Once an individual becomes a carrier, there is no way to get ride from the infection.

METHODS

Study Area and Subjects

All of the development regions representing the country as a whole were taken as study area for this research and the blood sample were drawn from the subjects mostly at zonal hospitals and some at private pathology laboratory of respective regions. The subjects of this research were healthy Nepalese males. The reason for selecting such specific subjects was to accommodate large sample size of specific type and also in viewing the point that males are most commonly exposed to the infections because of their various mode of occupational characteristics.

Though this research was aimed to study randomly on 100 samples from different districts of each development region, due to certain unavoidable circumstances like limit budged, time and long distance to travel; only 97 and 81 samples were collected from Mid Western and Far Western regions. Therefore, the total sample size investigated in this research became 478 from 59 districts of five development regions. The age range of the subjects was 13 to 71 with an average age of 22 years.

Collection of Samples

Samples of about 5 ml of venous blood was drawn and dispersed in labeled test-tubes to centrifuged at 2000 rpm for 10 min. In this way serum was separated and transferred to clean 5 ml injection vials of respective numbers. Thus collected sera were stored at -20°C and taken to research laboratory of Central Department of Microbiology, Tribhuvan University, Kirtipur within 3 days of collection. During transportation, all the sera samples kept at deep-freeze were directly transferred into an ice box labeled as "High Risk Samples" and transported safely. After reaching to the research laboratory, processing of the samples started on the same day.

Procedure

All the reagents were brought at room temperature and the sera were tested serologically by commercially available Latex Agglutination Test kit and Enzyme Linked Immuno Sorbent Assay (ELISA) kit for detection of Hepatitis B Surface Antigen (HBsAg) by overnight incubation procedure. Again the sera found positive for HBsAg were assay for serum alanine aminotransferase (ALT) enzyme as liver function test. The methodologies for Latex agglutination, ELISA and ALT estimation were exactly followed as directed by their manufacturers - Monozyme India Ltd.; Bioelisa, Barcelona, Spain and Span Diagnostics Ltd. India respectively. The bioelisa kit used in this research is qualified as 3rd generation ELISA for HBsAg detection, which has the sensitivity of 0.125 HBsAg unit per ml for "ad" and "ay" subtypes in overnight incubation procedure.

In latex agglutination test, all the sera giving agglutination within 5 min of test were taken as positive for HBsAg and no agglutination within this period were considered as negative for HBsAg. However, all the sera, though become negative from latex test, also tested by ELISA for final result. Simultaneously, the specificity of latex agglutination test was also studied with respect to ELISA.

For interpretation of result in ELISA, absorbance of the wells testing for HBsAg, alongwith the positive and negative controls were measured from ELISA reader at 450 nm.

Finally, the amount of ALT present per ml of serum in all the samples detected as positive for HBsAg by ELISA were estimated by extrapolating their corresponding absorbance determined by spectrophotometer at 505 nm on a standard reference curve.

RESULTS

Out of 478 healthy Nepalese males tested, 4.0% (19/478) were carriers of HBsAg. The prevalence of HBsAg positive was found somewhat uniformly distributed throughout the country. However, relatively high rate of prevalence was found towards west side of the country. On the basis of development regions, the percent prevalence of HBsAg was found steadily increasing from Eastern to Far Western regions, with slightly increased rate in Western region.

Far Western region showed the highest prevalence rate of 6.2%; followed by Mid Western region 4.1%, Western region of 4%, Central region with 3% and least at Eastern region of 2% (Table 1).

In Eastern region, each of the two positive cases were detected from Sankhuwasabha and Jhapa districts. Similarly, in case of Central region the districts giving positive cases are Dhanusa, Ramechhap and Sarlahi; in Western region the districts are Rukum and Dang; and in Far Western region the dis-
trict is Kailali.

Regarding the age, a wide range of people from 13 to 71 years were taken for the study (Table 2). Among which 21 to 25 years of age group represents 24.5% and 26 to 30 years of age group represents 25.1%. Therefore, more than half of the total population studied belong to young adults of productive age. Prevalence of HBsAg was found in all age groups from 16 to 40 years. In addition to this, one positive case, out of two subjects tested of age group 61 - 65 years, was also detected.

From ALT estimation, only 15.3% (3/19) of subjects infected with HB virus were found to have damaged liver. And, rest of the sera recorded positive for HBsAg by ELISA had normal range of enzyme ALT, showing normal physiological condition of their liver.

A comparative serological results obtained from Latex agglutination and ELISA indicate that, out of 478 sera tested for HBsAg 1.67% (8/478) were detected as positive and 94% (450/478) were detected as negative from both of the methods used (Table 3). However, latex agglutination test gave additional 9 positive cases which were detected as negative from ELISA. Whereas, other 19 positive cases detected from ELISA were observed negative from Latex.

**DISCUSSION**

At present, the prevalence and better preventive measures of HBV infections have been studying continuously in most parts of the world, which show an average prevalence rate of
HBsAg carrier as 11.9 - 20.2%. However, the prevalence rate found in the current study was found in between 2.0 - 6.2% in different regions of the country; with an average rate of 4.0%. Therefore, as classified by WHO, Nepal seems to belong to the category of intermediate endemic zone for HBV infection.

From the results, it is clear that, Eastern and the Far Western regions are the least and the most prevalent areas of the country, where the prevalence rate were 2.0% and 6.2% respectively. The prevalence of HBsAg in other developing regions are 3.0%, 5.0% and 4.1% in Central, Western and Mid Western regions respectively. Therefore, the current research shows the HBsAg positivity rate is gradually increasing from the east to the west of the country, with an exception of slightly diverse rate in Mid West. The result also clears that, the HBsAg prevalence rate is variable according to the geographical areas. Such variations in prevalence rate of HBsAg from place to place of a country was also observed in other countries of Asia. In the neighbour country India also, significantly more HBsAg positive cases were observed in southern area in comparison with north and west.

About the age distribution of the prevalence of HBsAg, the productive age group was found as the most susceptible for the infection. Although the pediatric age group (below 10), which was observed as the most susceptible age group in many reports, were not included in the current study.

Excluding the one positivity case observed out of two cases in age group 61 - 65 years, a characteristically high percent prevalence was resulted in age group of 16 - 20 years. This is in accordance with the similar study conducted in Zimbabwe, where the highest prevalence was detected in the same age group.

From the 21 years onward, the prevalence rate was found to increase with age. In fact the age group of 61-65 showed a very high prevalence of 50%, which could be the result of small number of subjects examined in this age class, hence can be omitted theoretically for relative expression. Conversely, the high percent positivity of HBsAg is found in 16-20 years group, which is decreased to the lowest rate at 20-25 years and rise slowly peaking in 36-40 years group. The factors which govern such increased prevalence rate in accordance to the age is not clearly understood. Nevertheless, this pattern of increasing prevalence in HBsAg positivity from 20-25 years onward is also in agreement with the result obtained in Singapore.

The detection of HBsAg in serum alone can not distinguish acute from chronic state of hepatitis B infection. Because, HBsAg may be detected either at incubation period, acute infection or chronic carrier state of the infections. Indeed, the sera which were positive for HBsAg along with the damaged condition of the liver may suggest the climax period of the infection or the persistent carrier state of the infection, provided there is no other possibilities for liver damage. It is also true that, in case of healthy individuals, no sign and symptoms will appear and occurrence of climax condition of the infection is not possible. Therefore, the result obtained from the estimation of ALT in HBsAg positive cases about 16% of the studied samples seem to have carrier state of the infection. Nevertheless, the remaining 84% of the total HBsAg positive cases may represent either for the infection at incubation period or at the prodromal period of the infection or as the persistent carrier.

Since the ELISA used in this study is comparatively much more sensitive (can detect less than 1 ng HBsAg per ml of serum) and accurate (99.93%) than the Latex agglutination test, all the results determined from ELISA were taken as the final result. However, from the statistical analysis association between the results obtained from both of the serological methods is highly significant ($\chi^2 = 85.11$, $p < 0.05$).

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