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Using Various Serological Markers to Characterize Hepatitis B - Infected Patients Who Visit Clinical Analysis Laboratory, KNUST

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Abstract

Ghana is not an exception from developing countries suffering from hepatitis B infection. Research has shown that various communities have different prevalent rate as result of life style and socioeconomic levels. This study investigated the prevalence of the infection, possible risk factors, determined liver enzymes (AST and ALT) of infected respondents and serologically characterized the infected respondents in KNUST community. In a total of 85 respondents, 8 of them tested positive to the HBsAg indicating 9.41% prevalence. Males have higher prevalence (13.16%) than females (6.38%). Ages from 20-29 recorded the highest prevalence followed by 30-39 and 50-59. The mean values obtained for AST in U/l was (40.33±13.60) and ALT in U/l was (25.17±5.70). The mean values indicate normal health for liver cells. Three of the respondents reported of having a family history of HB virus, two claimed they had been blood-transfused and one was involved in sharing of devices. Therefore, age, sex, history of blood transfusion, use of shared items and blood contacts have much influence and could be major contributing factors to HBV infection. Serological evidence indicates that positive respondents to HBsAg fall within the inactive chronic hepatitis B carrier phase and so it can be concluded that the chronic hepatitis B-infected patients visiting the Clinical Analysis Laboratory are in the inactive phase.

Keywords: Hepatitis B; Prevalence; Serology; Liver Enzymes; Inactive Phase

1. Introduction

One of the commonest diseases that have attracted the attention of both scientists and the world is hepatitis B. Viral hepatitis means an inflammation of the liver caused by different types of viruses; hepatitis A, B, C, D, and E [1].

Among these, only a few are of global significance, with hepatitis B being a serious infectious disease of the liver affecting millions of people throughout the world [2].

Hepatitis B virus is a common pathogenic infection, which causes acute (infection persist up to six months) and chronic hepatitis where disease last more than six months (the chronic hepatitis occur in phases which include; immune tolerance phase, positive HBeAg chronic hepatitis, inactive HBsAg carrier phase and HBeAg negative chronic phase), liver cirrhosis, and hepatocellular carcinoma. Liver cirrhosis is an end stage chronic liver disease. Crawford [3] showed that 10% to 33% of those who develop persistent infection end up with chronic hepatitis of which 20% to 50% may develop liver cirrhosis.

The epidemic nature of hepatitis B has become a serious public health problem. Weiss and McMichael's data of infectious diseases [4] reveal that chronic hepatitis B is among the leading causes of preventable death worldwide. Recently, according to the World Health Organization (2004), it is estimated that more than 2 billion people globally have been infected with the hepatitis B virus [5].

According to Lavanchy [6], there is a wide variation of hepatitis B prevalence in the world, ranging from 0.1% to 20% in different parts of the world. Studies show that hepatitis B surface antigen (HBsAg) prevalence of more than 8% in a community is considered "high", 2-7% are "intermediate" and below 2% as "low". Amidu *et al.* [7] reported that prevalence in sub-Saharan Africa ranges from 3-22% in blood donors.

An abnormality in the laboratory test of aspartate transaminase (AST) and alanine transaminase (liver enzymes) is an indicator for signs of liver diseases [8]. Serology and measurement of aspartate transaminase (AST) and alanine transaminase (ALT) has been long term diagnostic process for chronic hepatitis B virus infection [9].

Hepatitis B has been a major health concern worldwide posing threat to life, more especially in developing countries, due to poor living conditions, educational level, financial burden, poor hospital facilities and lack of vaccination. Ghana known to be part of the high prevalent zones as works done by Foli and Swaniker [10] shows sero-prevalence rate of 6.7% to 10% among blood donors and Amidu *et al.* [11] showed sero-prevalence rate of 8.68% among blood donors in three suburbs of Kumasi. This research seeks to determine the phase of disease growth among infected persons living in KNUST community.

2. Methodology

The study was cross-sectional, carried out at the Clinical Analysis Laboratory of the Department of Biochemistry and Biotechnology-KNUST which took place between the months of February to April 2014. A study population of 85 were obtained for the study. Personal data was collected from patients through a questionnaire which sought information concerning age, gender, blood transfusion, shared items, body tattoo, blood contact and sexual history. Blood samples (5 ml each) of those who consented were taken after the study was clearly and concisely explained.

HBV screening was performed first for all samples for qualitative determination of surface antigen (HBsAg), using a rapid chromatographic immunoassay. Respondents who showed positive results to surface antigen had a profile test to qualitatively determine, HBsAb, HBeAg, HBeAb, HBcAg using plasma from patient's sample. Test kits were obtained from **ABON™** (Biopharm (Hangzhou) Co., Ltd, China). Test was carried out based on manufacturer's manual. Few drops of sample (plasma) was applied on test strip and allowed to react by capillary action to flow through the bound surface for the immunological reaction to occur.

Depending on the type of antigen or antibody to be detected in sample, the appearance of two bands in the test and control region kits implies a positive result as in the case of HBsAg, HBsAb, and HBeAg and a single band in the control region indicate negative and invalid for a band in only the test region. For HBeAb and HBcAg, a band in the control region indicates positive and two bands for test and control region indicating negative while only a band in the test region indicates invalid.

Further analysis was carried out on patients who showed positive results to hepatitis B surface antigen (HBsAg) to quantitatively determine liver enzymes concentration in patients' blood sample which is a reflection of disease progression. Reagents were obtained from **Fortress Diagnostics Limited (**Unit 2C Antrim Technology Park, Antrim, BT41 1QS UK**)** for quantitative determination of ALT and AST in blood of patients. Blood samples collected were centrifuged to obtain plasma and using the manufacturer's protocol, test was carried out and spectrophotometric measurement taken to quantitatively determine ALT and AST concentration.

Data was analyzed using Statistical Package for Social Sciences (SPSS V.17) using the t-test to determine the means of AST and ALT.

3. Results

Out of the 85 subjects who were involved in the study, 77 of the subjects tested negative whilst 8 of the subjects tested positive to hepatitis B, which constitute 9.41% prevalence (Table 1).

Gender	Frequency	No. of HBsAg positive	HBsAg positive (%)
Male	38	5	13.16
Female	47	3	6.38
Total	85	8	9.41

Table 1: HBsAg status by sex of respondents

Patients who were positive to HBsAg also had their HBsAb and HBeAg to be negative and their HBeAb and HBcAb were positive as depicted in Table 2.

Patient's code	HBsAg	HBsAb	HBeAg	HBeAb	HBcAb
21	+	-	-	+	+
25	+	-	-	+	+
43	+	-	-	+	+
77	+	-	-	+	+
78	+	-	-	+	+
79	+	-	-	+	+
82	+	-	-	+	+
85	+	-	-	+	+

+ = Positive; - = Negative

Table 2: HB virological profile of HBsAg positive respondents

AST and ALT values for patients-77 and 78 were not recorded as samples were discarded before the test was carried out. However, the AST values obtained for the remaining patients ranged from 14 to 81U/l with patient-79 and patient-43 recording the minimum and maximum AST values respectively. Contrarily, the values for ALT activity ranged from 11 to 46 U/l with patient-82 and patient-25 recording the minimum and maximum values respectively (Table 3).

Patient's code	AST U/l	ALT U/l
21	19	21
25	65	46
43	81	39
79	14	17
82	17	11
85	26	17

Table 3: Liver enzyme activities for positive HBsAg

The HBsAg positive modal age group of present study was 20-29 years, followed by 30-39 years and 50-59 years (Table 4).

Age group (years)	Frequency	Number of HBsAg positive
0-19	4	0
20-29	47	5
30-39	20	2
40-49	3	0
50-59	4	1
60-69	3	0
70-79	4	0
Total	85	8

Table 4: HBsAg status among age groups

The possible risk factors for hepatitis B virus transmission include blood transfusion, shared items, blood contact, multiple partners and tattoo. Out of the 8 patients who tested positive, 18% admitted undergoing blood transfusion, 9% had multiple sexual partners, 28% share items such as sponge with others and 45% have had blood contact. None had tattoos on their any part of the body (Figure 1).

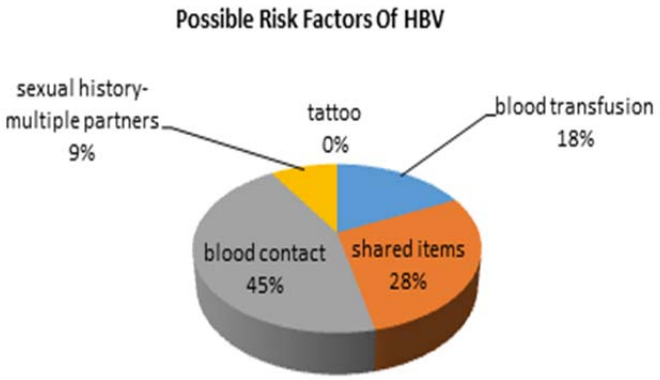

Figure 1: Possible risk factors associated with HBV transmission

Table 5 shows the knowledge of the respondents on HBV infection. Most of the respondents responded yes, as to the hearing of HBV infection, but in relation to family history, symptoms and mode of transmission, a polar response was obtained. Approximately 94.1% had no idea of family history whilst 74.1% were ignorant on the symptoms of the disease. However, about 67.1% had an idea on the various mode of transmission.

Factor	Number of subjects	Have an idea (%)	Have no idea (%)
Family history	5	5.9	94.1
Mode of transmission	57	67.1	32.9
Symptoms	22	25.9	74.1

Table 5: Knowledge on HBV of respondents

Demographics of mean liver enzymes activities in both sexes showed a higher mean values of ALT and AST in males than females as seen in Table 6.

Gender (mean values)	AST U/l	ALT U/l
Male	49.75±19.24	30.75±6.98
Female	21.50±4.50	14.00±3.00
Total	40.33±13.60	25.17±5.70

AST = Aspartate Transaminase; ALT = Alanine Transaminase

Table 6: Mean values of ALT and AST in subjects by gender

4. Discussion

In the West African sub-region, sero-prevalence levels ranging from 3%-22% has been reported among blood donors [12]. Foli and Swaniker [10], showed a sero-prevalence of HBV to be between 6.7 and 10% among blood donors in Ghana. The present study determined the sero-prevalence of HBsAg, possible risk factors, AST and ALT levels of HBsAg positive patients and to serologically characterize hepatitis B-infected patients by disease progression.

Out of the 85 subjects studied, 8 of them tested positive to HBsAg, constituting 9.41% prevalence. Of the 38 male subjects, 5 males tested positive, giving a prevalence of 13.16%. Three females were positive, giving a prevalence of 6.38%. Results from this study showed HBsAg positivity prevalence being 2.06 times more among males than the females. Available reports show that men are more likely to test positive for HBsAg than women [13]. This trend is not dependent on both the prevailing risk factors and the level of endemicity in any randomly sampled population. Some studies suggest that plasma clearance rate for HBsAg in males is slower, compared to females and this might be responsible for this ratio [14].

The prevalence of HBsAg positivity of 9.41% indicates a high prevalence rate. Work done by Amidu and colleagues [11], in three densely populated communities in Kumasi-Ghana showed overall seroprevalence of 8.68%. With respect to the three communities, different prevalence rates were observed. HBsAg prevalence levels in the suburbs of Kumasi were 6.78% for Garrison, 9.02% for Aboabo and 10.0% for Tafo. This result is a reflection of lifestyle and socioeconomic differences among the various communities. The prevalence rate obtained in their research is lower, compared to the 12.64% prevalence rate of HBsAg in the northern Ghana [7]. This result poses a threat to the community and country and needs immediate attention since the prevalence obtained in this research is high compared to the prevalence rate for Garrison and Aboabo where the research was conducted about two years earlier which is an indicator that disease awareness is very poor and people are not going for vaccination as vaccines are expensive and it also shows that preventive methods are not applied by communities to eradicate spread of hepatitis B virus infection.

The study also sought to find out the possible risk factors or attitudes that might lead to one being exposed to hepatitis B virus. From WHO [15], possible factors of hepatitis include; blood transfusion, blood contact or blood products, shared items, multiple sexual partners and tattoo. From the study some of the common devices shared included; blade, toothbrush, towel, sponge. Out of the eight HBsAg positive respondents, two of them reported to have blood transfusion and one, sharing of devices like sponge. The remaining did not fall under any of the aforementioned risk factors.

The study also looked at the respondent's knowledge of HBV infection, to which 98.8% said they were aware of. This means the knowledge of the respondents was very high.

From Table 2, serology revealed that all the 8 respondents who tested positive to hepatitis B virus fall within the inactive HBsAg carrier phase. This is characterised by the patients remaining negative for HBeAg and positive for anti-HBe antibody. Seroconversion is usually accompanied by stabilization of hepatitis, characterized by normalization of ALT levels and decreases in HBV DNA to low (<1000 copies/mL) [16]. This phase is also characterised by normal ALT levels. Alanine transaminase (ALT) and AST are the most two reliable markers of hepatocellular injury or necrosis. ALT is thought to be more specific for hepatic injury because it is present in the cytosol of the liver and in low concentrations elsewhere [17].

The overall mean values for AST of 40.33±13.60 and ALT of 25.17±5.70 fall within the normal ranges, which indicates a healthy liver of infected respondents. The mean AST value was higher because it can also be found in other organs and not highly concentrated in the liver.

5. Conclusion

The prevalence of HBV infection in this study was 9.41%, so the KNUST community falls within the high prevalence zone. The HBsAg positive respondents had normal liver function based on normal levels of liver enzymes, AST and ALT. Though AST values were higher, the increase is not clinically significant to cause liver injury but may be a reflection of damage to other organs. Blood transfusion and shared items are possible causes of HBV infection. Males have higher prevalence (13.16%) than females (6.38%). Ages from 20-29 years recorded the highest prevalence. Serological markers also categorized infected patients to fall within the inactive chronic hepatitis B carrier phase, and this support the normal transaminase levels.

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Conflict of Interest

No conflict of interest declared by authors.

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