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Frequency of HBV among Hepatocellular Carcinoma Patients in Khartoum State, Sudan

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Abstract

Hepatitis B is global health problem and it is a potentially life-threatening infectious disease caused by the Hepatitis B Virus (HBV) which affects the liver and may cause hepatocellular carcinoma. The objective of this study was to determine the frequency of HBV among hepatocellular carcinoma patients. The study was conducted during the period between January and April 2015.

A total of seventy venous blood specimens (n=70) were collected from patients with hepatocellular carcinoma. The patients were hospitalized in Soba University Hospital, Ibrahim Malik Teaching Hospital, Ibn Sina Specialized Hospital and Antalya Diagnostic Center, in Khartoum State. The samples were collected from 37 male and 33 female and all patients were over 60 years of age. A 5 ml blood sample was collected from each patient. Serum was obtained by centrifugation at 3000 rpm for 5 min. The sera were examined for the presence of HBsAg using Enzyme Linked Immuno Sorbent Assay (ELISA).

The results showed that out of 70 blood samples investigated, 18 (26%) were positive for HBsAg. The rest 52 (74%) were negative. Among the positive blood samples, 11 (61%) were obtained from males and 7 (39%) from females. Out of 37 males examined 11 (30%) were positive for HBsAg, while the rest 26 (70%) were negative: furthermore out of 33 females examined 7 (21%) were positive for HBsAg while the rest 26 (79%) were negative.

The study concluded that the HBV infection in hepatocellular carcinoma patients is not so high. The level of infection is higher in males than in the females. Further studies with a large number of samples and more advanced technique are required to validate the results of the present study.

Keywords: HBV; Hepatocellular carcinoma; ELISA

Introduction

Hepatitis is the inflammation of the liver cell and it can be caused by different insulting agents among which Hepatitis B Virus (HBV) is common. HBV infection is an infectious disease and it can be acute or chronic. The disease often has no symptoms during the initial infection. But, some patients develop a rapid onset of disease with vomiting, feeling tired, yellow skin, abdominal pain, and dark urine. In most cases, these symptoms last during a few weeks. Of those who get infected around the time of birth, 90% subsequently develop chronic hepatitis B infection, in contrast, only less than 10% from those whom above 5 years at the time of infection develop chronic disease. Most of those with chronic infection have no symptoms; however, liver cirrhosis and hepatocellular carcinoma may eventually develop. About 15% to 25% of those who get these complications upon chronic infection will be dead [1].

Around a third of the world community has been infected at some point in their lives among which 240 million to 350 million will have been developed chronic infections. Each year over 750,000 people die of hepatitis B infection. Nowadays the infection is only common in East Asia and sub-Saharan Africa where 5% to 10% of adults have a chronic disease. Rates in Europe and North America are less than 1% [1].

HBV is a prototype member of the family Hepadnaviridae with a genome size of ~3,200 base pairs: the virus is hepatotropic and non-cytopathic. The HBV genome contains a partially double–stranded, relaxed-circular DNA (RC–DNA) genome, constituting a complete non–coding strand (negative strand) and an incomplete coding strand (positive strand), which replicates by reverse transcription *via* an RNA intermediate [2].

There are several modes of transmission of HBV infection; these include exposure to infectious blood or body fluids. In areas where the infection is common the most frequent method by which Hepatitis B is acquired is vertical transmission at the time of birth or from contact with other people during childhood, while in other areas where the infection is uncommon, the intravenous drug use and sexual intercourse are the most frequent routes of infection. In addition to that, there are other risk factors including working in healthcare, blood transfusions, and dialysis. The virus cannot be transmitted by holding hands, sharing eating, kissing, coughing, sneezing or breastfeeding [1].

Hepatocellular carcinoma (HCC) is now the seventh most common cancer in men and the ninth in women, with an estimated worldwide incidence of 0.25–1.2 million new cases per year. The coastal areas of Mainland China including Hong Kong are high-risk areas with more than 25 cases per 100,000 populations per year [3].

The mechanism of HCC induced when a mutation in the hepatocyte machinery occur and this will cause the cell to replicate at a higher rate and/or results in the cell avoiding apoptosis. Causes of HCC include chronic infections with hepatitis B and/or C, repeated consumption of large amounts of ethanol. Besides, cirrhosis is commonly caused by alcoholism, chronic hepatitis B and chronic hepatitis C. Certain Aspergillus species of fungus produce aflatoxin which builds up in the liver and leads to hepatocellular carcinoma. The combination between aflatoxin and other liver insulting agents like HBV and HCV may lead to relatively high rates of hepatocellular carcinoma in these regions this occurs especially in certain world area like China [4].

Traditional screening regimes for the detection of HCC have included measuring serum alpha–fetoprotein (AFP) levels and performing liver ultrasounds, used together in order to improve screening accuracy, as their individual sensitivity and specificity are relatively low particularly among people with cirrhosis (4).

Epidemiological observations clearly indicated that age, male sex, alcohol abuse, hepatitis B virus, and hepatitis C virus (HCV) and liver cirrhosis are the most important risk factors for developing HCC. Among all the related etiologic agents, HBV infection has the strongest association with HCC. It has been observed that there is close correlation between the geographic distribution of HBsAg carriers and occurrence of HCC. In endemic areas such as China where the HBsAg carrier rate is more than 10%, HCC presents an incidence of up to 150 cases per 100,000 per year. On the contrary, in no endemic areas like the United States where the HBsAg carrier rate is less than 1%, HCC presents an incidence of fewer than 4 cases per 100,000 per year. A prospective study from Taiwan showed that the relative risk of HCC among HBsAg-positive men as compared with HBsAg- negative men was 98 [3].

Although most HCCs arise in a cirrhotic liver, in HBV-related HCC tumour can frequently develop on top of chronic active hepatitis. Hepatocyte necrosis secondary to chronic HBV infection triggers an inflammatory response with the synthesis of various cytokines. Some of them, such as tumour necrosis factor, may stimulate liver-cell proliferation during which DNA mutations and chromosomal rearrangements may be produced. Furthermore, extensive fibrosis disrupts the normal lobular structure and potentially leads to a further loss of control over cell growth [3].

Hepatitis B Virus infection remains a major health problem causing considerable morbidity and mortality. The World Health Organization (WHO) estimates that more than one-third of the world population has been in contact with the virus, resulting in >350 million HBV chronic carriers, with >18% of them living in Africa. Sudan is classified among the African countries with high HBV endemicity, with infection rate ranging from 6.8% in central Sudan to 26% in southern Sudan [5,6].

Moreover, HBV is one of the most important etiological factors for HCC in humans. It can induce HCC directly by activating cellular oncogenes or indirectly through chronic liver injury, which facilitates mutation. Screening of HBV among patients with HCC and detection of the association between them may play role in placing a program to prevent hepatitis B infection which may subsequently lead to HCC.

Materials and Methods

This study was cross-sectional–It was conducted in three hospitals and centers in Khartoum State. These were Soba University Hospital, Ibrahim Malik Teaching Hospital, Ibn Sina Hospital and Antalya Diagnostic Center. The practical part of this study was done in the Research Laboratory, Sudan University of Science and Technology (SUST), during the period of January to July 2015. A total of seventy blood samples (n=70) were obtained from patients with hepatocellular carcinoma confirmed by histological techniques and CT scan and ultrasound, both males and females were included.

Specimens collection and processing

A volume of 5 ml blood was collected from each patient through venepuncture technique then displaced into a plain container. Each blood sample was centrifuged at 3000 g for 5 min, and then serum was gently collected into Eppendorf tube and stored at –20°C until the serological analysis.

Specimens analysis

The samples were analysed for the presence of HBsAg by a commercially available enzyme-linked immune-sorbent assay "HBsAg ELISA" kit (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT4I IQS United Kingdom). The assays were performed following the instructions of the manufacturer. Positive and negative controls were included in each assay. According to the information included in the kit's insert, the immunoassay used has a specificity of 99.94%.

Procedure of ELISA test

All reagents and specimens were settled to reach room temperature, 20 ul of specimen diluents was added to each well except the blank then 100 ul of a positive control; negative control and specimen were added to their respective wells. The plate was covered with plate cover and incubated for 60 minutes at 37°C. At the end of incubation period, 50 ul of HRP-conjugate was added to each well except the blank; the plate was covered and incubated for 30 minutes at 37°C. By the end of the incubation period, each well was washed 5 times with diluted wash buffer [7]. Finally, 50 ul of chromogen A and chromogen B solutions were added to each well including blank, then the plate was incubated at 37°C for 15 minutes and stop solution was added [8].

Quality control and calculation of the results

Reagent, standard, and control were checked for storage, stability, and preparation before starting work. Each micro plate was considered separately when the results were calculated and interrelated; the results were calculated by relating each specimen absorbance (A) to the cut off (c.o.) of the plate.

Cut off value was calculated through the equation of (C.O.)=NC × 2.1 (NC is mean of the three negative controls).

The OD value of the blank was less than 0.080 at 450 nm.

The OD value of the positive control was more than 0.80 at 450 nm.

The OD value of the negative control was less than 0.1 at 450 nm.

Interpretation of results

Positive more than cut-off value.

Negative less than cut-off value.

A method used for data collection

Data were collected by using administrated questionnaire including the gender and age.

Data analysis

The data that collected from questionnaire and laboratory results were analysed by SPSS version 15 computerized programs.

Results

A total of seventy venous blood specimens (n=70) were collected from patients with hepatocellular carcinoma in four hospitals and center in Khartoum State. The samples were collected from Soba University Hospital 8 (11%), Ibrahim Malik Teaching Hospital, Ibn Sina specialized Hospital 21 (31%) and Antalya Diagnostic Center 5 (7%) (Table 1).

Hospital	Patients	
	No.	%
Soba University Hospital	8	11
Ibrahim Malik Teaching Hospital	36	51
Ibn Sina Hospital	21	31
Antalya Diagnostic Center	5	7
Total	70	100

Table 1: Distribution of patients according to the hospital.

The seventy samples were involved 37 (52%) male and 33 (48%) female (Table 2).

Gender	Patients	
	No.	%
Males	37	52
Females	33	48
Total	70	100

Table 2: Distribution of patients according to the gender.

All specimens were examined for the presence of HBsAg using ELISA Kit. The result showed that out of 70 blood samples investigated, 18 (26%) were positive for HBsAg. The rest 52 (74%) were negative (Table 3).

Results	Samples	
	No.	%
Positive	18	26
Negative	52	74
Total	70	100

Table 3: Frequency of HBsAg among hepatocellular carcinoma patients.

From the positive blood samples, 11 (61%) were males, and 7 (39%) were females (Table 4).

Samples		Gender
%	No.	
61	11	Males (no: 36)
39	7	Females (no:33)
100	18	Total

Table 4: Frequency of HBsAg according to the gender.

Out of 37 male samples examined 11 (30%) positive for HBsAg, while the rest 26 (70%) were negative. Furthermore out of 33 females examined 7 (21%) positive for HBsAg while the rest were negative 26 (79%) (Table 5).

%	No.	Gender	Result
30	11	Male	Positive (n:18)
21	7	Female	
70	26	Male	Negative (n:52)
79	26	Female	

Table 5: Frequency of HBsAg in each gender separately.

Discussion

Since its discovery about 45 years ago, Hepatitis B Virus (HBV) has been studied extensively as a causative agent of hepatocellular carcinoma. Chronic infection with HBV was noted to be associated with the development of hepatocellular carcinoma. The present study was aimed to detect the prevalence of HBV among hepatocellular carcinoma patients in Khartoum State, so we can describe to which extend this virus contributed to causes hepatocellular carcinoma because there is a lot of other causative agents which result in liver diseases and subsequently cancer formation. Out of 70 blood samples investigated, only 18 (26%) were positive. This result is similar to that obtained in Japan by Suga et al., who reported that 27% of Japanese patients with hepatocellular carcinoma were positive for HBV. But our finding disagrees with that reported in Taiwan by Chang et al., who reported that over 80% of hepatocellular carcinoma patients were positive for HBV, and the rate of seropositivity for HBV was nearly 100% in children. This difference may be due to the high endemnicity of HBV infection in Taiwan.

In the present study, the prevalence of HBV was higher in males than in females (61% *vs.* 39%). This may be explained by the occupational exposure of male to the infection. This high prevalence in males is agreed with that carried out by Le et al., who reported that HBV prevalence among hepatocellular carcinoma patients was 7.9% and 16.8% for females and males respectively.

It is worth mentioning that Hepatocellular carcinoma is caused by a different causative agent other than HBV infection. So, it is important to do further studies and include other factors (e.g. Alcohol consumption, cigar rate smoking HCV infection and schistosomiasis) to determine the contribution of each factor and the most injurious

agent for the liver. So, to treat and reduce the incidence of insulting agents and subsequently reducing the incidence of HCC in Sudan.

Conclusion

We concluded that there is a moderate prevalence of HBV infection in patients hospitalized with hepatocellular carcinoma. The level of infection is higher in males than in females. Further studies with a large number of samples and more advanced technique are required to validate the results of the present study.

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