Vol. 32, No. 5, May 1986

)RIGINAL ARTICLES

Hepatitis B_eAg in Chronic Asymptomatic Hepatitis B Surface Antigen Carriers and in Primary Hepatocellular Carcinoma Patients

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ABSTRACT

total of 246 HR_s Ag – positive subjects were sted for the presence of Hepatitis B_e antigen, nd total anti-delta IgG. Of the 246, 174 were IB_sAg – positive asymptomatic chronic carriers nd 72 were primary hepatocellular carcinoma atients attending the two teaching hospitals, i.e. arirenyatwa and Harare Central Hospital.

All sera were examined by the enzyme-linked mmunosorbent assay (ELISA), and results were ad spectrophotometrically at a wavelength of 92 nm· 82 (33,3%) were HB_eAg – positive. Of e 246,56 (39,4%) males and 26 (25%) females ad HB_eAg present in the serum. However, the revalence of HB_eAg from only the chronic symptomatic HB_sAg – positive carriers in males nd females was 17 out of 103 (16,5%) and 12 ut of 71 (16,9%), respectively—thus with an verall prevalence of 31 (17,8%) from the symptomatic group. Consequently, the prevalence HB_eAg from the primary hepatocellular rcinoma (PHC) group was 32 of 72 (44,4%). he rate of positives from the males and females the PHC group was 46,2% and 42,4% respecvely. Total anti-delta IgG was not detected in e 246 examined sera.

INTRODUCTION

epatitis B surface antigen (HB_sAg) is intimately sociated with viral heptatitis type B. It exists in e form of 20 nm spheres, 20 nm diameter laments and on the surface of 42 nm Dane rticles.

Hepatitis B_e Antigen (HB_eAg), however, occurs a molecular entity separate from HB_sAg, lthough it, too, may exist on the surface of Dane particles. The e-antigen is potentially usable as a prognostic marker of the chronicity of hepatitis B virus (HBV) infection,[1] and would give information concerning the infectivity of HB_sAg – positive individuals.

Although HB_sAg can be present in non-infectious or mildly infectious blood, the additional finding of HB_eAg in serum apparently indicates the presence of circulating infectious HBV particles.[2] Blood positive for HB_eAg has accordingly been found to transmit hepatitis B in a mother-infant series and in exposure studies to a HB_eAg, but positive for the corresponding antibody, anti-HB_e.[3,4]

Previous studies on HB_sAg in Zimbabwe showed a high prevalence (13,7%)[5] of positive results obtained from the healthy population. This study, however, was undertaken to determine the proportion of HB_eAg positivity, in sera from both asymptomatic BH_sAg chronic carriers and primary hepatocellular carcinoma patients. In this study, 'chronic asymptomatic carriers' is used to refer to those healthy individuals who are carriers of HB_sAg and do not show any clinical symptoms.

METHODS AND MATERIALS

The study population consisted of 104 females and 142 males. Thus the total number examined was 246. Of the 104 females, 71 were HB_sAg – positive asymptomatic carriers whereas 33 were primary hepatocellular carcinoma patients who were positive for HB_sAg. Among the 142 males, 103 were HB_sAg – positive asymptomatic carriers and 39 were patients suffering from primary hepatocellular carcinoma and at the same time positve for HB_sAg.

The age range of the HB_sAg – positive asymptomatic carriers was from 11 to 60 years with a mean of 24 years. The 0–10 age group was not included in the study because of problems that the investigator faced in obtaining specimens (5–8 ml of blood). But the ages of the primary hepatocellular carcinoma subjects were not available, although all of the subjects were adults.

Samples from the HB_sAg – positive asymptomatic chronic carriers were collected from Gweru, Masvingo, Harare, Kariba and Gokwe areas. However, sera from confirmed cases of hepatocellular carcinoma were collected only from patients attending Parirenyatwa and Harare Central Hospitals.

Procedures

Samples of 5 ml venous blood were collected into 10 ml clotted test tubes and were immediately

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cepted: 17 July, 1986

transported to the laboratory for serum separation. Separated sera were transferred into 3 ml vials and stored at – 20°C if not examined on the same day.

For serological detection of HB_sAg, sera were tested by the Ortho Enzyme-linked immunosorbent assay following the manufacturers' procedures with few modifications to suit our laboratory's conditions. Briefly, 100 ul of each test specimen was added into separate wells of the anti-HB_s coated microplate. Similarly 100 ul of both the negative and positive controls were added into four and two wells respectively, and plates were incubated at 37°C in a water bath for two hours. After incubation the contents of each well were removed and plates were washed four times with distilled water following which 100 ul of the enzyme conjugate was added to each well and plates incubated for two hours in similar conditions as above. After two hours incubation plates were washed as above and 200 ul substrate solution which was prepared by adding 4 0 – phenylenediamine 2 HCL (OPD) to 12 ml deionized water in a clean plastic container. Following complete disolution of OPD tables 5 ul of 30% hydrogen peroxide was added and the substrate used within 30 minutes. Finally, plates were incubated at room temperature in the dark for 20 minutes. The reaction was then stopped by adding one drop (50 ul) of 2,5 N HCL to each well. Results were read visually by taking all the wells with yellow colour change as positive, and these were recorded.

Only sera positive for HB_sAg were stored at – 20°C and later shipped to the Centre for Diseases Control in the USA, where they were examined for the presence of both HB_eAg and anti-Delta IgG. This portion of the work was undertaken by the investigator with some assistance from Dr Howard Fields, a WHO consultant in the Hepatitis Branch at CDC.

Commercial kits for the detection of HB_eAg and anti-delta IgG from Abbot Diagnostic Laboratories were used. Results for both HB_eAg and anti-delta IgG were read spectrophotometrically using the Abbot Quantum 11^{Tm} at a wavelength of 492 nm.

Patients queried for primary hepatocellular carcinomas were screened for the presence of PHC by detecting the rise of alpha-fetoprotein. Only patients with 100 ng/ml were considered positive for primary hepatocellular carcinomas.

RESULTS

Of the 246 examined sera, 82 (33,3%) were positive for HB_eAg. 56 (39,4%) of the males and 26 (25%) of the females had HB_eAg in their sera. However, the prevalence of the HB_eAg in chronic asymptomatic hepatitis B surface antigen carriers in males and females were 17 (16,5%) and 12 (16,9%) respectively, but the overall prevalence in this group was 31 (17,8%).

Consequently, the relationship between age-group and percentage distribution of hepatitis B_e antigen marker in the healthy chronic asymptomatic HB_eAg – positive carriers is as shown in Table I. The frequency of HB_eAg – reactivity decreases with increasing age.

Because of the unavailability of information on age from the primary hepatocellular carcinoma patients, this group, therefore, was not included in Table I, but its prevalence was found to be 32 (44,4%) out of the 72 tested subjects. The positivity rate in males and females from this group was 18 of 39 (46,2%) and 14 of 33 (42,4%) respectively.

Interestingly, total anti-delta IgG was not detected in all the subjects examined, i.e. in all the 246 HB_sAg – positive carriers.

DISCUSSION

Hepatitis B_e antigen, associated with HBV was originally described by Magnius and Espmark,[6] and has not been fully characterised, although it exists primarily in a soluble, non-particulate form in contrast to HB_sAg and HB_eAg. The e-antigen is usually found in sera that are reactive for HB_sAg. HB_eAg has a variable distribution throughout the world. In Taiwanese,[7] in blood donors from Paris,[8] in Indochinese refugee blood donors,[9] the prevalence of HB_eAg was 32%, 33,7% and 54,8% respectively. However, from this study the prevalence of 17,8% was drastically lower than the forementioned studies from similar subjects, although higher than studies from Senegalese,[10] American Red Cross blood donors in Bethseda Maryland[11] or in Japanese[2] which indicated a prevalence of 6%, 7,4% and 6,2% respectively.

It was clearly indicated in the study that the presence of the HB_emarkers was correlated with age and the findings were compatible with other similar studies.[12,13,14] Thus the prevalence of the HB_eAg decreased with increasing age. Therefore, findings in this study suggest that HB_eAg - positivity in HB_sAg carriers is of limited duration.

Although there is no established difference in the acquisition of HBV in relationship to sex under conditions of equal exposure, it has been shown that males are probably more prone to become asymptomatic chronic carriers of HB_sAg,[5,15] thus increasing the chance of detecting the antigen.

This study showed a statistical significa

TABLE I—*Relationship between age group and percentage distribution of HB_e Ag marker in chronic HB_s Ag carriers*

Age Group	No. exam.			No. (%) Positive		
	F	M	Total	F	M	Total (%) Positive
11–15	5	13	18	1 (20)	4 (30,8)	5 (27,8)
16–20	25	20	46	5 (19,2)	6 (30)	11 (23,9)
21–25	12	9	21	2 (16,7)	4 (44,4)	8 (38,1)
26–30	7	25	32	1 (14,3)	1 (4)	2 (6,3)
31–35	9	8	17	2 (22,2)	0 (0)	2 (11,8)
36–40	3	8	11	1 (33,3)	2 (25)	3 (27,3)
41–45	4	6	10	0 (0)	0 (0)	0 (0)
46–50	2	7	9	0 (0)	0 (0)	0 (0)
51–55	2	5	7	0 (0)	0 (0)	0 (0)
56–60	1	2	3	0 (0)	0 (0)	0 (0)
Total	71	103	174	12 (16,9)	17 (16,5)	31 (17,8)

difference $p \leq 0{,}001$ in prevalence of HB_eAg with regards to sex. It was, however, comparable to similar studies in Japan,[2] but studies somewhere[8] showed a significant difference (29% and 46,6% positivity rate) as per male versus female groups.

In the primary hepatocellular carcinoma patients the HB_e – positivity rate was significantly higher $p \leq 0{,}01$, than that of the asymptomatic carriers. It was also shown that HB_sAg – positive carrier males showing a higher prevalence of that antigen showed a statistical significant difference $p \leq 0{,}001$ of the e – antigen as compared to females. But results of males from the two groups, i.e. the asymptomatic carriers and the PHC subjects when analysed together, indicated a higher prevalence, 39,4% in males to 25% in females.

The fact that the HB_eAg was more common in the PHC subjects could probably be as a result of these subjects having been currently infected with HBV. As a result, investigations are underway to determine the correlation between HBV markers and PHC in patients attending Parirenyatwa and Harare Central Hospitals.

Anti-delta is normally dectectable in on-going or recent infections with HBV. Consequently, the delta-antigen can exist as a co-infection or superinfection. Although no anti-delta antibodies were detected in this study, this may not suggest a total absence of the delta viral antigen because several factors could have contributed to negative results. Detectability of anti-delta antibody highly depends onthe high concentration or circulating antibodies in sera, which could be during current infection. Studies consisting of patients with active viral hepatitis are underway in order to establish an acceptable status of anti-delta in Zimbabwe.

In conclusion the study revealed that the prevalence of HB_eAg in HB_sAg – positive asymptomatic carriers was lower compared to that found in the Far East, but reasonably similar to several studies conducted in the West. Secondly, the positive rate of HB_eAg from PHC was higher than that of the asymptomatic carriers. However, there was no significant difference in the positive rate between males and females, but a frequency of HB_e reactivity was shown to be decreasing with increasing age.

ACKNOWLEDGEMENTS

The author would like to thank Mrs C. Zhakata for her technical assistance. Above all I thank the Secretary of the Ministry of Health for permission to carry out this study. Final thanks are extended to Mrs M. Masterman for secretarial assistance.

This study was supported by the Research Board of the University of Zimbabwe.

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