Usefulness of acute phase proteins for monitoring development of hepatocellular carcinoma in hepatitis B virus carriers

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Summary

Background: **Serum levels of acute phase proteins (APP) have been used to diagnose and follow up treatment of liver diseases. This study was carried out to determine the usefulness of APP to predict development of hepatocellular carcinoma (HCC) among Hepatitis B virus (HBV) carriers.**

Study design: **In a prospective study, serum haptoglobin, transferrin and alpha 2 macroglobulin levels of 55 subjects consisting of 20 HBV carriers, 18 HBV-positive HCC patients and 17 controls were determined using single radial immunodiffusion.**

Result: **The mean levels of haptoglobin were 141.75mg/dl±133.76, 97.11mg/dl±92.62, 161.59mg/dl±146.86 for HBV carriers, HBV-positive HCC and controls respectively. The mean transferrin levels for HBV carriers, HBV-positive HCC and controls were 166.4mg/dl±88.31, 140.0mg/dl±68.73 and 270.35mg/dl±122.79 respectively while similar values for alpha 2 macroglobulin were 195.4mg/dl±93.86, 189.83mg/dl±77.19 and 127.53mg/dl±43.29.**

No significant difference in the mean serum haptoglobin levels of HBV carriers and HBV-positive HCC (p=0.526), HBV carriers and controls (p= 0.883) and HBV-positive HCC and controls (p=0.295).

The difference between the mean serum transferrin levels was insignificant between HBV carriers and HBV-positive HCC, p= 0.671, but was significant between HBV carriers, and HBV-positive HCC compared with controls, (p=0.005 and 0.000 respectively).

No significant difference in alpha 2 macroglobulin between HBV carriers and HBV-positive HCC, (p=0.972), but the differences were significant between HBV carriers, and HBV-positive HCC and controls, (p=0.024 and 0.048 respectively).

Conclusion: **Haptoglobin, alpha 2 macroglobulin and transferrin lack predictive value for development of HCC in HBV carriers. Reduced transferrin and increased alpha 2 macroglobulin in HBV carriers might suggest active liver disease.**

Key-words: ***Usefulness, Acute phase proteins, Hepatitis B carriers, Hepatocellular carcinoma.***

Résumé

Introduction: On avait utilisé les niveaux du sérum de la phase de la proteine aigue (ppA) pour faire le diagnostic et le traitement post hospitalier des maladies de culin foie. Cette étude a été éffectuée afin de décider l'utilité de PPA pour prévoir le développement du carcinome hépatocellulaire (CHC) chezs les porteurs de virus B Hépatite (VBH)

Plan d'étude: A travers une étude en perspective, sérum d'haptoglobine, transferrine les niveaux du macroglobuline alpha 2 de 55 sujets composés de 20 porteurs de VBH, 18 VBH - positif patients atteints de CHC et 17 contrôles étaient décidé avec l'utilisation de immunodiffusion radiale seule.

Resultats: Les niveaux moyen d'haptoglobine étaient 141,75 mg/dl± 133.76, 97, 11mg/dl±92,62, 161,59mg/dl±146,86 pour des porteurs de VBH, VBH-positif CHC et des contôles respectivement.

Les niveaux moyen du transferrine pour des porteurs des VBH, CHC VBH-positif et des contrôles étaient 166,4mg/dl±88,31, 140.0mg/dl±68,73 et 270,35mg/dl±122,79 respectivement tandis que les valuers semblables pour macroglobuline 2 alpha étaient 195,4mg/dl±93,86, 189,83mg/dl± 77.19 et 127,53mg/dl±43,29. Il n'y avait aucune différence dans le moyen des miveaux d'haptoglobine sérum des porteurs de VBH et CHC VBH positif (p=0,526), porteurs de VBH et contrôles (P=0,883) et CHC VBH-positif et contrôles (P=0,295).

La différence entre le moyen des niveaux du serum de transferrine était important entre les porteurs de VBH et CHC VBH positif, P -0,671, mais était important entre les porteurs de VBH, CHC VBH positif par rapport aux contrôles 0=0,005 et 0,000 respectivement).

Il n'y avait aucune différence sensible dans la macroglobuline alpha 2 entre les porteurs VBH et VBH positif CHH, (P=0,972), mais les différence étaient importantes entre les porteurs VBH, et VBH-positif HCC et contrôles, (p=0,024 et 0,048 respectivement).

Conclusion: La macroglobuline alpha 2, la Haptoglobine et la transferrine manque la valuer prédictive pour un développement en HCC chez les porteurs de VBH. La transferrine baissée et la macroglobuline alpha 2 augumentée chez des porteurs de VBH pourraient suggérer la maladie du culin foie active.

Introduction

Hepatitis B Virus (HBV) is a major aetiologic agent of hepatocellular carcinoma (HCC) in Asia and Africa [1,2]. Low incidence of 0.01 – 0.1% have been reported in North

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Table 1 Acute Phase Proteins (x ± SD) in Hepatitis B Virus carriers, Hepatocellular carcinoma and controls.

	Number	Haptoglobin mg/dl	Transferrin mg/dl	Alpha2 macroglobulin-mg/dl
Hepatitis B carriers	20	141.75 ± 133.76 (15-488)	166.4 ± 88.31 (57-332)	195.4 ± 93.86 (85-340)
Hepatitis B positive-HCC.	18	97.11 ± 92.62 (15-244)	140±68.73 (57-332)	189.83 ± 77.19 (85-340)
Controls	17	161.59 ± 146.86 (31-488)	270.35 ± 122.79 (89-554)	127.53 ± 43.29 (60-70)
p- values[a]		0.526 (ns)	0.671 (ns)	0.972 (ns)
p- values[b]		0.883 (ns)	0.005 (s)	0.024 (s)
p-values[c]		0.295 (ns)	0.000 (s)	0.048 (s)

(Range of value in parentheses)

a = HBV carriers compared with HCC.
b = HBV carriers compared with controls.
c = HCC compared with controls.
ns= Not significant
s = significant

America and Western Europe.

HCC has a poor response to therapy and portends a grave prognosis, with mortality approaching 100% in 6 months, therefore, early diagnosis at a presymptomatic stage is an urgent priority among HBV carriers.

Several diagnostic and screening tests have been employed for HCC, but the ultimate test remains elusive as diagnosis is usually made at advanced stage of the tumour when little could be offered by way of therapy[3]. The levels of serum acute phase proteins (APP) are often altered in specific disease states, thereby serving as markers of disease, which may be inflammatory, degenerative, malignant or genetic.

C-reactive protein (CRP), was recently shown to be elevated in malignant fibrous histiocytoma (MFH) as well as melanoma. C-reactive protein has also been found to be raised in the serum as liver disease progresses from benign to malignant.[4] It is however rated to be a poor marker for HCC except when levels are very high in cirrhotic patients[5]. It is imperative, therefore to continue to search for other useful APP that will afford early diagnosis of HCC in at risk groups and ultimately reduce the burden of the disease in HBV endemic zones.

The objective of this study is to determine the usefulness of haptoglobin, transferrin and alpha-2 macroglobulin in predicting the development of HCC among HBV carriers.

Materials and methods

A total of 55 adult subjects aged 18 years and above at the University College Hospital (UCH), Ibadan, were enrolled for the study over a period of 6 months. The subjects were 20 Hepatitis B virus carriers,18 HBsAg-positive Hepatocellular carcinoma patients, and 17 apparently healthy controls.

The inclusion criteria for HBV carriers were absence of clinical features of acute or chronic liver disease, abnormal liver chemistry, previous jaundice and presence of HBsAg in the serum.

Inclusion criteria for HCC were positive hepatitis B surface antigen (HBsAg), hepatomegaly suggestive of HCC, histological diagnosis compatible with HCC, ultrasonographic finding suggestive of HCC or raised serum alpha fetoprotein (>400ng/ml). Excluded from the study were patients with sepsis or in coma and pregnant women.

The controls were apparently normal adults who were pre-screened and found to be HBsAg negative and with no history or clinical features of liver disease.

Hepatitis B surface antigen was detected using the 3rd generation enzyme linked immunosorbent assay (ELISA) method[6]. The ELISA method for HBsAg determination utilizes enzyme immunoassay technique in which monoclonal antibodies bind the antigen (HBsAg) in the presence of horseradish peroxidase in wells. Ninety six-well microtiter plate was coated with 100 ul of HBsAg (1:200 with PBS, pH 7.2). The free space was blocked with 100ul of PBS-1%BSA. The wells were washed 3 times with PBS – 0.1% Tween 20. 50ul of diluted human serum (1:200) was added. This was followed by addition of HBsAg, horseradish peroxidase, O-phenylediamine and H_2O_2. The reaction was stopped with 4N H_2SO_4. This was incubated, after which the optical density was read at 492nm with ELISA reader.

Sera of all subjects were analyzed for haptoglobin, transferrin and alpha-2 macroglobulin with anti-transferrin, anti-alpha-2 macroglobulin and anti-haptoglobin (Boehring, Germany) using single radial immunodiffusion technique as modified by Salimonu et al 1978[7]. The single radial immunodiffusion involved the use of 3% noble agar mixed with appropriate antisera (anti-haptoglobin, anti-transferrin and anti-alpha-2-macroglobulin) on immunoplate in which wells of equal diameter were dug; standard and samples were then applied to the wells and subsequently incubated in a humid box at room temperature. The ring diameters of

precipitin were read and concentration was determined from curve of the standard.

SPSS version 11.0 was used for statistical analysis of mean, standard deviation and significance. Level of statistical significance was specified at $p < 0.05$

Results

The age of the subjects among HBV carriers was 20-36 yrs, with a mean of 27.2±4.7; HBV-positive HCC and normal controls were 18-75 yrs (48.2±16.1) and 20-40 yrs (28.5±5) respectively. There was no significant difference in the age of HBV carriers and controls but the age of HCC patients is significantly higher than that of HBV carriers and controls. Among the HBV carriers, haptoglobin levels ranged between 15mg/dl-488mg/dl (141.75±133.76mg/dl). Transferrin level ranged between 57mg/dl-332mg/dl (166.4±88.31mg/dl), while alpha 2 macroglobulin ranged between 85mg/dl - 340mg/dl (195.4±93.86mg/dl). (Table 1). There was no significant difference in the mean serum haptoglobin levels between carriers and hepatitis B positive HCC patients,(p=0.526), but both had lower mean values than in controls,(p=0.883 and 0.295 respectively). The mean serum transferrin level was significantly lower in HBV carriers and HBV-positive HCC compared with controls, (p=0.005 and p=0.000 respectively) but no significant difference between HBV carriers and HBV-positive HCC (p=0.671). The mean alpha 2-macroglobulin levels were not significantly different between HBV carriers and HBV-positive HCC (p=0.972) but significant difference was observed between HBV-positive HCC compared with controls (p=0.048), (Table 1). Similarly, there was a statistically significant difference between HBV carriers and controls (p= 0.024).

Discussion

Our study showed that carriers of HBV are relatively younger than the patients with HCC suggesting that HBV infection is acquired earlier in life. This is in agreement with previous findings in developing countries where the common mode of transmission is horizontal from child to child with HCC developing later in life[8]. The patients with HCC are relatively older in age than the subjects who are carriers of HBV and the normal controls. Since age may affect the serum levels of certain biomolecules, it may be assumed that this would affect the serum levels of the APP in this study. A previous study of complements and APP during aging among Nigerians has shown a lack of significant alteration in the serum levels of acute phase proteins with age[9]. It is well known that levels of serum haptoglobin tend to increase during acute phase reaction as a result of increased synthesis by the liver during inflammatory process[10]. The mean level of haptoglobin in HBV carriers is within the normal limits and is similar to the level in HBV-positive HCC. The serum level of haptoglobin in controls did not show a different trend when compared with HBV carriers and HBsAg-positive HCC. There was no statistically significant difference between HBV carriers and HBsAg-positive HCC, HBV carriers and controls and HBsAg-positive HCC and controls, though the mean levels were lower in HBV carriers and HCC patients than in the normal control group. These findings suggest that serum haptoglobin levels are not significantly different in the three groups and therefore not discriminatory in HBV carriers and HCC patients. This is contrary to the findings of Arekuul[11], who observed low levels of haptoglobin in patients with infectious hepatitis and carcinoma of the liver when compared with normal controls. In spite of the lack of significant difference, it is noted that the mean serum level of haptoglobin in HCC is relatively lower than those of HBV carriers and normal controls, this may be due to the reported expression of haptoglobin receptor in HCC[12] leading to internalisation of haemoglobin-haptoglobin complex which may serve to reduce serum level of haptoglobin in HCC.

The mean serum level of transferrin was statistically significantly lower in HBV carriers and HCC patients compared with normal controls, there was however, no statistically significant difference in the mean levels between HBV carriers and HBsAg-positive HCC, though HBV carriers had slightly higher levels of transferrin. The relatively lower levels of transferrin in patients with HCC may be due to the excessive expression of transferrin receptors by HCC[13]. The excessive expression of transferrin receptors by HCC has been reported to lead to sequestration of transferrin in the HCC cells on the one hand and microheterogeneity of transferrin in HCC on the other. The microheterogeneity makes it impossible to measure a subfraction of the transferrin in the serum of HCC patients[14]. It is therefore clear from this study that serum transferrin has no discriminatory power to differentiate between HBV carriers and HBV positive HCC patients, but a reduced transferrin level in HBV patients should call for further evaluation as this might suggest development of active liver disease, possibly predicting HCC

The mean serum level of alpha 2 macroglobulin among HBV carriers and HCC subjects were found to be significantly higher than for normal controls. This is similar to the finding of Meliconi[15], which showed increased levels of alpha 2 macroglobulin in patients with liver cancer compared with benign liver disease. Higher levels of alpha 2 macroglobulin were also found by Shiota[4] in HCC patients compared with patients with chronic hepatitis and liver cirrhosis.

It is concluded that serum haptoglobin, alpha 2 macroglobulin as well as transferrin levels lack discriminatory power to differentiate HBV carriers and HBsAg positive HCC. They are therefore, not recommended for monitoring of HBV carriers to detect early development of HCC. However, a reduced transferrin and increased alpha 2 macroglobulin in HBV carriers might suggest development of active liver disease requiring further investigations. We however suggest a larger study to validate our findings in this study

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