AFRICAN JOURNAL OF MEDICINE
and medical sciences

VOLUME 21, NUMBER 2, DECEMBER 1992

EDITOR: B.O. ONADEKO
ASSISTANT EDITORS:
B.O. OSOTIMEHIN and A.O. UWAIFO

SPECTRUM BOOKS LIMITED
Ibadan • Owerri • Kaduna • Lagos

ISSN 1116–4077

Afr. J. Med. med. Sci. (1992) 21, 35-38

Immune complex levels and HBs-antigenaemia in healthy Nigerians and patients with liver diseases

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Summary

Circulating immune complexes (CIC) were precipitated and assayed in the blood of 19 adult patients with liver diseases and 39 healthy adult Nigerians. The presence of hepatitis-Bs antigen (HBs-Ag) was also investigated in both the sera and CIC of both study groups. CIC levels were not significantly different in the three different liver diseases studied (acute hepatitis, chronic hepatitis and liver cirrhosis). Higher mean CIC levels which were not correlated with the presence of HBs-Ag, were found in the blood of patients, as compared to blood donors. While HBs- Ag was detected in 42.1% of patient's sera, only 12.8% of blood donor sera had detectable antigen. However, 42.1% of CIC from patients had the antigen, while 53.8% of CIC from blood donors also contained the antigen. It is suggested that the high frequency of HBs-Ag in the precipitated CIC of healthy subjects could account for the occurrence of some post-transfusion hepatitis-B infections.

Résumé

Des complexes circulants, à l'abri de la contagion (CIC), étaient precipités et titrés du sang de 19 adultes malades ayant des maladies du foie et du sang des 39 adultes nigérians en bonne santé. La présence de l'antigène hépatite – Bs (HBs-Ag) était aussi examinée dans le sérum aussi que dans les CIC des deux groupes sous examination. Les niveaux de CIC n'étaient pas d'une difference significative en les trois différentes maladies du foie etudiées (l'hépatite acuité, l'hépatite chronique et la cirrhose du foie). Les plus hauts milieux des niveaux de CIC qui ne sont pas en corrélation avec la présence de l'HBs-Ag s'étaient trouvés dans le sang des malades, en comparaison aux donneurs du sang. Pendant que l'HBs-Ag était aperçu dans 42.1% du sérum des donneurs du sang. Néanmoins, 42.1% de CIC des malades avaient l'antigène aussi que 53.8% de CIC des donneurs du sang. C'est qui est suggéré est que la haute fréquence de l'HBs- Ag dans le CIC précipité des sujets en bonne santé, peut justifier l'occurence d'infections post-transfusions de l'hépatite – B.

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Introduction

Hepatitis-B surface antigen (HBs-Ag) occurs in the sera and circulating immune complexes (CIC) of patients with hepatitis-B virus (HBV) infections[1, 2]. CIC from the blood of asymptomatic carriers have also been found to contain the antigen[1 – 4].

HBs-Ag-bearing CIC have been implicated in the pathology of some hepatitis-associated disease conditions. While some investigators suggested that such CIC may be responsible for the hepatic damage found in hepatitis infections, others found no such correlation[1, 5]. The CIC have also been implicated in the pathogenesis of certain extra-hepatic manifestations of HBV infections, such as polyarteritis nodosa, serum sickness-like symptoms and glomerulonephritis[6 – 10].

This study was undertaken to determine the frequency of HBs-Ag in the sera and precipitated CIC of healthy Nigerians and patients with liver diseases. A high frequency in the CIC of healthy subjects, might have significant implications in the rate of transmission of post-transfusion hepatitis.

Materials and Methods

The investigations were carried out on 19 patients aged 13 — 60 years, who had liver disease. There were 10 cases of acute hepatitis, 5 of chronic hepatitis and 4 of cirrhosis. However, none of the patients had any extra-hepatic manifestations of hepatitis. Sera from 39 apparently healthy adult blood donors aged 23 – 39 years, were also studied.

CIC in the sera were detected and quantitated by the polyethyleneglycol 6000 (PEG-6000) precipitation and spectrophotometric method of Haskova *et al* [11]. Precipitated immune complexes were recovered by centrifugation at 5,000 rpm for 15 mins. at 4°C. Sedimented immune complexes were then redissolved in 0.2ml of normal saline for examination for the presence of HBs-Ag.

Serum samples and redissolved immune complex solutions were examined for the presence of HBs-Ag by the enzyme-linked immunosorbent assay (ELISA) technique.

Results

Serum HBs-Ag was detected in 8 (42.1%) patients and 5 (12.8%) blood donors. Also, 8 (42.1%) patients and 21 (53.8%) blood donors had HBs-Ag in the precipitated immune complexes. Only 3 patients and 1 blood donor had antigen in both their sera and immune complexes.

The presence of HBs-Ag in the sera or CIC had no correlation with the levels of CIC. Patients with HBsAg-positive or HBsAg-negative sera had significantly higher mean levels of CIC than the corresponding groups of blood donors (Table 1). Similarly, Table 2 shows that patients with HBsAg-positive or HBsAg-negative CIC had mean CIC levels that were significantly higher than the mean levels for the corresponding control groups.

Patients with antigen-positive sera had a higher mean level of CIC (53.32 ± 22.05mg/100ml) than patients with antigen-negative sera (48.14 ± 24mg/100ml). The difference was however not significant. Similarly, the mean CIC level was slightly higher in patients with antigen-containing CIC than in patients with antigen-free CIC (55.11 ± 22.69mg/100ml and 46.84 ± 19.28mg/100ml respectively).

In blood donors, the mean CIC levels did not differ in subjects with antigen-negative CIC and those with antigen-positive CIC (30.66 ± 13.48mg/100ml and 29.82 ± 11.43mg/100ml). However, the group of blood donors whose sera were antigen-free had a significantly higher mean level of CIC (30.92 ± 12.93mg/100ml) than the group whose sera had antigen (25.35 ± 3.28mg/100ml) (t = 2.1018, P < 0.01).

Table 1: CIC mean levels in HBsAg-positive and HBsAg-negative sera.

	Sera containing HBsAg		Sera containing no HBsAg	
	Mean (± 1s.d.) CIC mg/100ml		Mean (± 1s.d.) CIC mg/100ml	
Patients (n = 19)	53.32 ± 22.05	(8)*	48.14 ± 20.24	(11)*
Normals (n = 39)	25.35 ± 3.28	(5)*	30.92 ± 12.93	(34)
Patients vs Normals	t=5.5138	P < 0.001	t=2.6533	P < 0.01

CIC – circulating immune complexes
* Number of subjects in parentheses

Table 2: CIC mean levels in subjects with HBsAg-positive and HBsAg-negative immune complexes.

	CIC containing HBsAg		CIC containing no HBsAg	
	Mean (± 1s.d.) CIC mg/100ml		Mean (± 1s.d.) CIC mg/100ml	
Patients (n = 19)	53.32 ± 22.05	(8)*	48.14 ± 20.24	(11)*
Normals (n = 39)	25.35 ± 3.28	(5)*	30.92 ± 12.93	(34)*
Patients vs Normals	t=5.5138	P < 0.001	t=2.6533	P < 0.01

CIC – circulating immune complexes
* Number of subjects in parentheses

The three patients whose sera and CIC contained antigen, had a mean CIC level of 59.97 ± 2.50mg/100ml, while the corresponding blood donor sample had a level of 24.10mg/100ml. However, the mean CIC level in this group of patients was not significantly higher than the means in patients with antigen- positive sera and those with antigen-positive CIC (53.32 ± 22.05; 55.11 ± 22.69mg/100ml).

Cirrhotic patients had the highest mean CIC concentration of 135.69mg/100ml because one patient had an extremely high level of 327.96mg/100ml. This abnormally high level of CIC recorded for this one patient may either be a true reflection of the patient's pathologic state or may have resulted from laboratory contamination of the serum sample. Patients with acute hepatitis had a mean level of 58.29mg/100ml, while those with chronic hepatitis had a mean of 41.18mg/100ml. However, there were no significant differences in the mean levels amongst the three patient groups (Table 3).

Table 3: Mean (± s.d) CIC mg/100ml in the patient groups

	Cirrhosis	*Acute hepatitis*	*Chronic hepatitis*
Mean	135.69	58.29	41.18
S.D	111.13	41.20	14.92
n	4	10	5

The occurrence of the antigen in either the sera or immune complexes could not be correlated with the disease type and therefore the degree of liver damage.

Discussion

HBs-Ag occurs in the sera and CIC of hepatitis-B patients and some asymptomatic carriers of the HBV[1 - 4]. HBs- Ag-CIC have also been incriminated in the hepatic damage that occurs in HBV infections [2, 4]. In the tropics, the antigen is found in 6 – 10% of all blood samples from apparently healthy individuals and has often been associated with glomerulonephritis [12 – 14].

In this study, HBs-Ag has also been found in the sera and CIC of a significant percentage of patients with hepatitis and liver cirrhosis. And in agreement with earlier findings, 12.8% of apparently healthy blood donor sera had the antigenaemia. However, a higher percentage (53.8%) of blood donors in comparison to the patient group, had detectable antigen in their CIC. While an explanation for this is not immediately clear, it is possible that most of the patients studied suffered from non-B viral hepatitis. Further studies are thus required to confirm this. However, the implication of the high frequency of antigenaemia in the CIC of healthy subjects is that, it could explain the occurrence of some HBV infections that follow blood transfusions, despite routine pre-transfusion screening of blood samples. While some earlier workers had reported 5% and 18% HBs antigenaemia in the CIC of asymptomatic HBsAg carriers [3, 4], there is no report on the prevalence of HBs antigenaemia in the CIC of apparently healthy individuals.

Expectedly, patients with hepatic diseases, irrespective of the presence or absence of HBs-Ag in the sera or CIC, had higher mean levels of CIC when compared with blood donors. Furthermore, patients whose sera and CIC were antigen-positive had slightly but insignificantly higher mean levels of CIC than those patients whose sera and CIC were antigen-free. The simultaneous presence of antigenaemia in both the sera and immune complexes of some patients had no apparent significance. There were also no significant differences in CIC mean levels in the three types of liver diseases studied. The latter finding supports the observation of Prince and Trapo [5] that there may be no correlation between the levels of CIC and the degree of liver damage.

Blood donors with antigen-negative sera had significantly higher mean levels of CIC than those with antigen-positive sera. The statistical difference is probably due to the comparatively smaller number of subjects with antigen-positive sera. However, there is also the possibility that the precipitated immune complexes may not all be specific for HBs-Ag.

Conversely, blood donors with antigen-negative CIC and those with antigen-positive CIC had similar mean levels of circulating immune complexes. This observation may indicate that the immune complexes are not potentially pathogenic. They could be triggered to become pathogenic if super-imposing infections occur.

While corroborative studies are necessary to confirm the present findings, we do suggest the assay of HBsAg in CIC from blood donors if possible, so that the frequency of HBV infections can be further reduced.

Acknowledgements

The authors gratefully acknowledge the expert technical assistance of Mrs. J.O. Adedugbe and the secretarial services of Mrs. G.A. Okere.

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(Accepted 24 October, 1990)