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SERUM FERRITIN AND HCV INFECTION IN NIGERIAN PATIENTS WITH PRIMARY LIVER CELL CARCINOMA

[1]Ola, S.O., [2]Odaibo, G.N

Departments of [1]Medicine and [2]Virology, College of Medicine, University of Ibadan, University College Hospital, Ibadan; Nigeria.

Correspondence:- Dr S.O. Ola, Email-address: soola@comui.edu.ng

Abstract

A prospective study aimed at determining the relationship between hepatitis C virus (HCV) infection and serum ferritin in Nigerian patients with primary liver cell carcinoma (PLCC) was carried out at the University College Hospital (UCH), Ibadan; Nigeria. The study involved 42 adult Nigerians made up of 14 healthy subjects as controls and 14 patients each with PLCC and liver cirrhosis (LC) who consented to participate in the study. The subjects were controlled for age and sex. The diagnosis of the diseases was made from relevant clinical features, ultrasonography and histology of liver biopsy specimen. Blood specimen collected from the subjects were analysed for ferritin by radio-immuno assay using Amersham Kits, hepatitis B virus (HBV) infection using HBsAg detection and anti-HCV by ELISA (Sanofi Pasteur, France). The study protocol was approved by the Joint UI/UCH Ethical Review Board. Data obtained was analysed with the SSPS software at a level of significance of $p<0.05$. Serum ferritin >700ng/ml was detected only in 50% and 14% of the patients with PLCC and LC respectively with specificity of 93% as well as negative (78%) and positive (79%) predictive values. Serum anti-HCV and HBsAg were present in 14% and 71% of patients with PLCC respectively ($p<0.005$). Similarly, 29% and 14% of the patients and the Controls respectively were sero-positive for anti HCV while serum HBsAg was detected in equal proportions of the patients with LC (50%) and the Controls (43%). There was correlation between elevated serum ferritin and HBsAg (X^2 with Yates correction = 5.04, $p= 0.025$) but none with serum anti-HCV.

In conclusion, the study shows that serum ferritin level ≥ 700ng/ml is indicative of PLCC among Nigerians especially in the presence of HBV infection but may not be useful when there is associated HCV infection.

Introduction

Hepatitis C virus (HCV) infection occurs globally(1). It presents more with asymptomatic course resulting in chronic hepatitis and later progressing to primary liver cell carcinoma (PLCC). Although, PLCC has been associated more with hepatitis B virus infection in Nigeria(2), may assume increasing trends once against HBV becomes efficient as in ies(3,4). Hence, HCV infection and its sequellae **remains a burden** since there is presently no vaccine against the virus. Efforts directed at treatment of PLCC have been met with poor success because of the late presentation of the patients at hospitals(5). Early diagnosis of the disease is therefore necessary. To ensure this, various tumour markers have been shown to be useful in the diagnosis of PLCC among different populations(6,7). Specifically, serum ferritin has been documented to be a useful marker in diagnosis of PLCC among Nigerians(8). The significance of serum ferritin in the presence of HCV infection remains unknown. This study was designed to determine the level of serum ferritin in Nigerian patients with primary liver cell

carcinoma (PLCC) especially when there is associated hepatitis C virus infection.

Materials and Methods

Forty-two adult Nigerian subjects were recruited into the study after obtaining informed consent. The subjects comprised 14 apparently healthy adults as control (group I) and 14 patients each with liver cirrhosis-LC (group II) and PLCC (group III). They were sex and age matched. There was no ingestion of multivitamin medication, obvious blood loss or blood transfusion in any of the subject within 12 months prior their inclusion in the study. In addition, no subject was anaemic at entry into the study. Relevant clinical features, ultrasonography and histology of liver biopsy specimen were utilised to make the diagnosis of both LC and PLCC. Blood specimens were collected from the subjects for packed cell volume (PCV) estimation, sero-analysis of ferritin, liver function tests as well as the serology of HBV and HCV infections. The assay of serum ferritin was carried out by radio-immuno assay using Amersham Kits (Ferritin RIA Kit, IM 1051). The serum HBsAg (HBV infection) and anti-HCV were determined by ELISA. Liver function tests were measured in all patients by routine laboratory methods.

The study was carried out after obtaining clearance from Joint UI/UCH Ethical Review Board. The SPSS statistical package was utilized for data entries and analysis on a micro-computer. The Kruskal Wallis statistics, a non parametric equivalent of the analysis of variance technique and the Mann Whitney U test, a non parametric equivalent of the student's t-test were used to compare the means of values of continuous variables. The Chi-square test and Fisher's exact test were used to determine the statistical significance of the association between two categorical variables. The validity of the diagnostic value of ferritin for PLCC was also examined by calculating the sensitivity, specificity, positive and negative predictive values where the histological findings were the gold standard. All statistical analysis were carried out at 5% probability level.

Results

The forty-two 42 subjects studied were made up of controls (14) and patients with PLCC (14) and LC (14). They were 48 ± 14, 49 ± 15,and 55 ± 12 years of age respectively. Each group had male:female ratio of 11:3. The Child Pugh stages of the patients in both PLCC and LC were similar (4 and 10 patients in each group were scored A and B respectively). The PCV in the subjects were 43±4%, 38±9% and 33±7% for the control subjects and the patients with PLCC and LC respectively. The PCV among the control subjects was significantly higher than the value for either the patients with PLCC ($P<0.05$) or LC ($p<0.001$). Serum ferritin >700ng/ml was detected in 50% of the patients with PLCC (specificity, negative and positive predictive values were 93%, 78%, and 79% respectively, Table I) compared to only 14% of the patients with LC and none of the Controls who had values serum ferritin above the cut-off level, $p<0.05$ each. Serum anti HCV and HBsAg were present in 14% and 71% of patients with PLCC respectively ($p<0.005$, Table II). Similarly, serum anti-HCV was present in 29% and 14% of the patients with LC and controls respectively while serum HBsAg was detected in equal proportions of the patients with LC (50%) and the Controls (43%). Elevated serum ferritin correlated with serum HBsAg (X^2 with Yates correction = 5.04, $p= 0.025$) but not with serum anti-HCV. Among all the subjects studied, 2 patients each with PLCC and LC had co-infection of HBV and HCV with the former patients also having elevated serum ferritin levels.

Table I. The validity of serum ferritin in diagnosing PLCC among adult Nigerian patients at different cut-off points.

			Validity(%)			
Ferritin (ug/L)	positive	negative	Sen.	Spec.	PPV	NPV
≥ 700	7	2	50	93	78	79
< 700	7	26				
≥ 600	7	6	50	78	54	76
< 600	7	22				
≥ 800	6	2	43	93	75	76
< 800	8	26				

% - percentage Sen. - Sensitivity Spec. - Specificity
PPV - Positive Predictive Value NPV – Negative Predictive Value
PLCC – Primary Liver Cell carcinoma

Table II The association between serum ferritin, HBV and HCV in adult Nigerian patients with PLCC, LC and controls

	Serum ferritin ≥700ug/L				
			PHCC	LC	Control
Number of subjects	Total (42)		14	14	14
	≥700ng/L	<700ng/L			
@HBsAg+	7	16	6	1	-
HBsAg-	2	17	1	1	-
*Anti-HCV+	2	6	1	1	-
Anti-HCV-	7	27	6	1	-

@ X^2 with Yates correction = 5.04, p=0.025 for Ferritin & HBsAg in PLCC *P=0.64 for ferritin & HCV in PLCC
PLCC – Primary liver cell carcinoma, LC - Liver Cirrhosis
HBV - Hepatitis B Virus HCV - Hepatitis C Virus
HbsAg - Hepatitis B surface Antigen

Discussion

Elevated serum ferritin level has been reported to be diagnostic of PLCC in different populations including Nigerians(6,8). This study has taken into consideration the role of blood loss, infectious anemia as well as ingestion of iron tablets on serum ferritin level hence the packed cell volume of the subjects studied are not unexpected. The serum ferritin level of ≥700ug/L as diagnostic level for PLCC is a better discriminatory level for the tumour from liver cirrhosis than the value of 400ug/L earlier reported among Nigerian(8). High serum ferrittin levels in our patients with PLCC might be due to the production of ferritin by the tumour rather than to liver necrosis(9). Although, at serum ferritin level of ≥700ug/L, two of our patients with LC were shown to have elevated levels of serum ferritin which may be suggestive of occult PLCC. This shows the limitation of histological diagnosis in absence of screening with a tumour marker. It is however comparable to the report among Europeans (10).

In view of the vary proportions of our patients with PLCC having HBV and HCV infections, both viruses could have contributed to the aetiology of the disease. Despite the use of ELISA for assay of HBsAg in our study, the correlation observed between elevated level of serum ferritin corroborates previous reports where heamaglution method had been utilized for the detection of serum HBsAg(8). This also shows that serum level of ferritin ≥ 700ng/ml is diagnostic of PLCC especially in the presence of HBV infection. It suggests that serum level of ferritin in patients with PLCC may be influenced by HBV infection since the virus gets intercalated with the hepatocellular DNA in aetio-pathogenesis of the tumour. In view of the diverse aetiological factors for PLCC, there is the need to define the effects of the other causal factors like HCV on the expression of the markers that may aid diagnosis of the tumour(11). In spite of the worldwide prevalence of HCV infection and its relationship to PLCC, the association between serum ferritin and HCV infection has hitherto remained unknown among Nigerians. However, our study has shown that there is absence of correlation between elevated serum levels of ferritin and HCV infection in Nigerian patients with PLCC. This observation might not be unconnected with the predominantly chronic clinical course and pathology of HCV infection(12). The presence of elevated level of serum ferritin among the two patients who had combined infection of HBV and HCV could be due to the effects of HBV. However, further study involving a larger sample size will be necessary to elucidate this observation.

In conclusion, this study shows that serum ferritin level ≥ 700ng/ml is diagnostic of PLCC among Nigerians. In addition, serum ferritin may not be useful in screening for PLCC among Nigerians with HCV infection but the converse holds for those infected with HBV.

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