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Hepatitis B Virus and 2 β-Microglobulin Concentration in Individuals Living with Human Immune Deficiency Virus in South West Nigeria

ORIGINAL

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Abstract

Hepatitis B virus (HBV) is a major health concern with people leaving with HIV/AIDS. However, not much have associated HBV with increase in 2β-microglobulin (2MG) concentration in people living with HIV/AIDS in sub-saharan Africa though is a tumor marker. Blood sample (n=435) were collected from individuals attending various HIV screening centres in South west Nigeria after the exclusion of individuals testing positive to tuberculosis, Hepatitis C virus (HCV), Human papilloma virus (HPV), (all tested by ELISA technique) typhoid and malaria. Of the 435 individuals selected for the study, 110 were positive for HIV alone, 217 for HIV and HBV while 108 were mono-infected with HBV. All individuals were tested for 2MG using the quantitative ELISA technique. There was a significant increase in 2MG concentration among those infected with HIV and HBV as opposed to the low levels in mono-infected individuals when compared to the normal concentration of 2MG in healthy individuals.

Keywords

2 Beta Microglobulin; Hepatitis B; Hiv; Tumour, Markers.

Introduction

Hepatitis B virus (HBV) have been reported to affect over 350 million people worldwide with hepatitis B surface antigen (HBsAg) associated with chronic infection. Hepatitis B virus infection is one of the major health concern for people living with HIV/AIDS. And anti HBs seroconversion associated with best outcome in patients with HBV infection [1-3]. However, not much has associated HBV infection to 2-β-microglobulin concentration in serum recently especially in sub-saharan Africa. 2-β-Microglobulin is a 11.7KDa nonglycosyla-

ted protein comprising of 99 amino acids [4]. It is one of the major histocompatibility complex class molecules on the cell surfaces of all nucleated cells. It interacts and stabilizes the tertiary structure of the major histocompatibility complex class 1 alpha-chain [5]. This protein is present in nearly all tissues of the body with exception of the red blood cells [6]. It is a member of the HLA where it is expressed in certain diseases conditions such as tumor in elevated concentration in the blood and detected in serum. WANCHU *et al*. [7] reported an elevated β-2-microglobulin in HIV patients infected with tuberculosis unlike those with HIV alone. GARCÍA-GARCÍA *et al*. [8] reported that β-2-microglobulin (2βM) level could be reduced in HCV infected individuals undergoing antiretroviral treatmentwhich means that a reduction in HCV infection must have led to the decrease in the 2MG concentration.

Co-infection of HBV with HIV are a public health concern with increasing incidence, their impact in the population and similarity in their epidemiological characteristics as well as similar mode of transmission [9-11]. HBV infection is considered opportunistic in HIV infected individuals due to a reduced immunity which makes HIV infected individuals becoming immune compromised thereby progressing the HBV infection to chronic hepatitis [9, 12-14]. HBV is usually more prevalent in HIV infected individuals than the general population [9, 15]. HBV infections could be seen as chronic, acute, persistence or occult with different HBV markers detected by serological means. Though in advanced countries the use of molecular method of diagnosis is being used to detect HBV DNA of this is not done in the routine clinical laboratory in Nigeria. In some individuals, the DNA may be detected without the HBsAg which could be seen as occult infection [16]. Such individuals included those with HBV risk factors, hepatic carcinoma, chronic HBV carriers, HBV-HCV co-infection, immunosuppressed and those with increase in hepatic enzymes and proteins like 2-β-microglobulin (cryptogenic cirrhosis). HBV/HIV coinfection have been reported to be as high as 10 to 20% in countries where HBV is either endemic or intermediate to high HBV cases. HBV and HIV coinfection leads to a higher morbidity and mortality rate as compared to HIV or HBV monoinfection [17]. MARIUKU *et al*. [17] reported a prevalence of HBV-HIV coinfection of 10.3% in Nairobi. Seroprevalence of HBV and HCV in Nasarawa state Nigeria have been reported among HIV positive individuals and 13.5% were positive to HBV [18]. In cuba, MARITE *et al*. [9] reported a 30.4% prevalence among HIV positive individual.

Markers that have been used to measure the progression to hepatocellular carcinoma in HBV included AST, ALT and bilirubin levels however, 2-β-microglobulin levels in serum might well measure more specific the progression to hepatocellular carcinoma since it is a tumor marker.

This cross sectional study aims to report the prevalence of HBV in HIV positive individuals as well as their progression to hepatocellular carcinoma using the 2-β-microglobulin concentration as a factor as well as monitor the levels of 2βM concentration in those receiving ART. This is the first study to assay 2βM in HBV-HIV coinfection in Nigeria and one of the few in sub-Saharan Africa.

Materials and Methods

Patients and Control

Blood samples (n=435) were collected from individuals visiting different HIV screening centres across South West Nigeria around April to August 2012. All the participants provided written consent. The 435 subjects included 110 HIV positive individuals, 217 HIV and Hepatitis B virus (HBV) positive subjects and HBV positive (108) individuals. All the participants were screened for human papilloma virus, Hepatitis B and D viruses, tuberculosis, typhoid and malaria fever, observation for any sign of diarrhea by a clinician and anyone positive to any of these

organisms/viruses or symptoms were excluded from the study. The HBV and HIV positive participants (mono-infected) alone served as control.

Blood Processing

Aseptically collected blood into sterile vacutaniers was immediately centrifuged and sera separated from whole blood and then stored in a -20oC refrigerator before use. The Enzyme Linked Immunosrbent Assay (ELISA) was used to analyze all the sera. The sera were brought out of the refrigerator and allowed to attain room temperature. ELISA kits for HIV IgM and IgG, HPV IgM and IgG, tuberculosis ELISA, HCV ELISA, typhoid and malaria were used to screen for the exclusion criteria in the first instant. All kits were allowed to attain room temperature and test carried out as directed by the manufacturer (WKEA medical supplies, China). Absorbance was measured in a microplate reader (Thermomax, Molecular devices, USA) and cut-off calculated as directed by the manufacturer of the kits. The cut-off value is the value of the absorbance from where an absorbance may be regarded as positive and as well as negative. Any value of absorbance below the cut-off is regarded as negative while those above the cut-off are regarded as positive. Hepatitis B surface antigen (HBSAg) in HIV positive individuals was also screened for using the ELISA technique with Kits from Wkea Medical Supplies (China). Absorbance was measured with microplate reader (Thermomax, Molecular Devices, USA) at 450 optical density. Cut off was also calculated as directed by the kit manufacturer. Both cases and controls were screened for HBsAG. Monoinfected individuals were also identified. 2βM was screened for by using the quantitative ELISA technique, sera and ELISA kits were allowed to come to room temperature and the test carried out as described by the manufacturer. Optical density values were read at 450nm. Graphs and regression analysis of absorbance were carried out using the online myassays software.

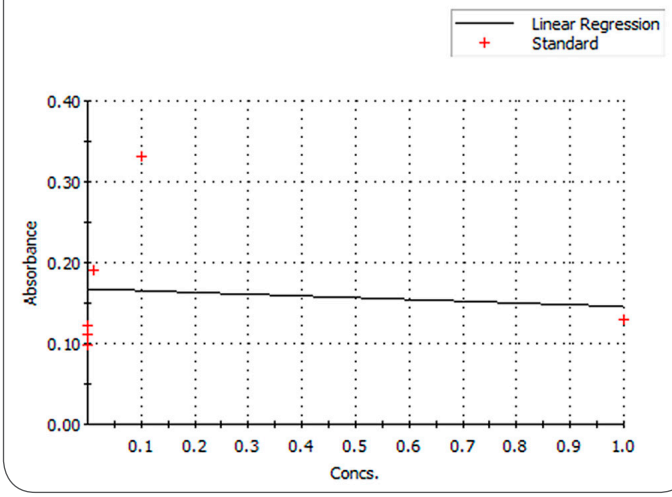
Results and Discussion

Of the 110 monoinfected HIV positive individuals (mean = 12.222) 39 (35.45%) were males while 71 (64.55%) were females. HIV+HBV with total number of participants as 217 (mean = 24.111) had 86 (39.63%) males and 131 (60.37%) females, the 108 (mean = 12) participants with HBV alone positive 41 (37.96%) were males while 67 (62.04%) were females **(Table 1)**.There was no significant difference between the number of males and females participants though they were not matched. Sex and age of participants did not correlate with 2βM levels which support an earlier report by GARCIA-GARCIA *et al*. [8]. The absorbance of each of the samples increased as concentration of the 2βM increased in each of the samples **(Figure 1)**. This study reveals the increase in 2βM levels in serum of HIV patients infected with HBV as compared to those infected with HIV and HBV alone respectively which has lower levels of 2βM. The normal 2βM levels in healthy individuals which served as baseli-

Table 1. Descriptive statistics and characteristics of participants.

Characteristics	HIV	HIV+HBV	HBV	F	p
Number of Subjects	110	217	108	2.861	0.079
Number of Males	39	86	41		
Number of Females	71	131	67		
Mean	12.222	24.111	12		
Median	10	21	10		
Standard Deviation	8.800	20.833	9.927		
Conf. Interval (95%)	6.764	16.052	7.128		
Standard Error	2.933	6.961	3.091		
Variance	87.714	404.982	90.411		
Correlation	0.850	0.491	0.841		
Regression analysis and Analysis of variance: R=0.887, SE: 3.268, Sig. of F=0.0230					

Figure 1: Linear regression of 2βM concentration in HIV and HBV positive samples their and absorbance.



ne for this study is 2.6-4.5µg/ml (CI=95%) (WKEA Medical Supplies, China). There have been increasing reports that some infections in HIV patients may lead to an elevated concentration of 2βM levels and some have implicated such increase with the development of hepatitis in HIV patients and also in HIV negative individuals [19, 20, 21, 9]. Though there have been reports of elevated 2βM in HIV patients [22, 23], 2βM in HIV patients gets higher with infections like HBV. These reports have it that 2βM also correlate well with CD4 cell count and as such a good maker for monitoring disease progression in HIV infection especially those that could lead to tumour. However, recent reports using 2βM as a marker to HBV infection in HIV patients and development to carcinoma are sparse. In a study, YEGANE *et al.* [21] reported an elevated level of 2βM while monitoring chronic HBV which explains why this study included both HIV monoinfected patients and HBV monoinfected patients as controls in this study. The normal levels of 2βM was observed in 32 (29.1%) individuals monoinfected with HIV while 45 (40.91%) had high levels and the rest (30%) had very high levels. This observation agrees with ELEFSINIOTIS *et al.* [5] which reports that there was an increase in 2βM during immune activation in the early phase of HIV infection before seroconversion and most of the individuals with high and very high levels of 2βM are those in the early phase of infection. The 2βM levels were in the normal range in 37 (17.1%) individuals co-infected with HIV and HBV and 63 (57.3%) had high levels while the rest 25.6% had very high levels. The increase in the number of individuals with very high level of 2βM in HIV/HBV coinfected individuals as against HIV monoinfection is as a result of the involvement of HBV which may have elevated the immune activation more than that of moninfection with HIV. Individuals monoinfected with HBV had 15 (13.89%) individuals with normal levels of 2βM while 63 (58.33%) had high levels and the rest (27.78%) had very high levels of 2βM. The χ^2 (χ^2 = 69.89; p-value = <0.0000001) revealed no significant relationship between the 3 variables and hence χ^2 was rejected **(Table 2)**. This means that more HIV/HBV coinfected patients are in

Table 2. The number of individuals with different concentration of 2βM in all participants.

2βM conc.	HIV	HIV+HBV	HBV	Total
0-2	10 (66.70) (9.10)	0 (0) (0)	5 (33.30) (4.60)	15
3-5	22 (64.70) 9 (20)	2 (5.90) (0.90)	10 (29.40) (9.30)	34
6-7	20 (26%) (18.20)	35 (45.50) (16.10)	22 (28.60) (20.40)	77
8-10	25 (21.70) (22.70)	61 (53) (28.10)	29 (25.20) (26.90)	115
11-13	10 (15.40) (9.10)	43 (66.20) (19.80)	12 (18.50) (11.10)	65
14-16	15 (21.70) (13.60)	36 (52.20) (16.60)	18 (26.10) (16.70)	69
17-19	5 (15.20) (4.50)	21 (63.60) (9.70)	7 (21.20) (6.50)	33
20-22	2 (11.80) (1.80)	11 (64.70) (5.10)	4 (23.50) (3.70)	17
23-25	1 (10) (0.90)	8 (80) (3.70)	1 (10) (0.90)	10
Total	110 (25.30)	217 (49.90)	108 (24.80	435
				^{x} = 69.89; p-value = <0.0000001

the very high level of 2βM and also in the high level as defined by this study. This observation supports a report by GARCIA-GARCIA *et al*. (2003) which reported an elevated levels of 2βM in HIV/HCV coinfected patients. The odds of having high concentration of 2βM in HIV+HBV infected individuals when both controls were added together **(Table 3)** was lesser than when the controls were separated **(Table 4** and **5)**. This confirms that the immune activation in HIV/HBV patients was more intense with regards to the concentration of 2βM as 2βM is a product of immune activation and has also helped in transferring the virus to the hepatocytes with likely progression to carcinoma. It has been reported that as part of HLA class I complex, 2βM is responsible in the transport of viral antigens to the hepatocyte surfaces [21]. MARIANO *et al*. (2000) reported that an increase in concentration of 2βM in HCV infected individuals correlated with hepatocellular carcinoma, a report this study agrees with and suggests that HIV/HBV co-infected individuals with high levels of 2βM are at a high risk of developing carcinoma.

Table 3. Odds of concentration of co-infection of HIV and HBV using HIV moninfection and HBV monoinfection together as control.

2βM conc.	HBV+HIV	HIV and HBV Controls	Total	Odds
0	0	15	15	0
3	2	32	34	0.06
6	35	42	77	0.83
9	61	54	115	1.13
12	43	22	65	1.95
15	36	33	69	1.09
18	21	12	33	1.75
21	11	6	17	1.83
24	8	2	10	4
Total	217	218	435	
Extended Mantel-Haenszel chi square for linear trend=33.64				
p-value(1 degree of freedom)=<0.0000001				

Table 4. Odds of concentration of co-infection of HIV and HBV using only HIV moninfection toas control.

2βM conc.	HBV+HIV	HIV only (Control)	Total	Odds
0	0	10	10	0
3	2	22	24	0.09
6	35	20	55	1.75
9	61	25	86	2.44
12	43	10	53	4.3
15	36	15	51	2.4
18	21	5	26	4.2
21	11	2	13	5.5
24	8	1	9	8
Total	217	110	327	
Extended Mantel-Haenszel chi square for linear trend=36.21				
p-value(1 degree of freedom)=<0.0000001				

Table 5. Odds of concentration of co-infection of HIV and HBV using HBV monoinfection as control

2βM conc.	HBV+HIV	HBV only (Control)	Total	Odds
0	0	5	5	0
3	2	10	12	0.2
6	35	22	57	1.59
9	61	29	90	2.1
12	43	12	55	3.58
15	36	18	54	2
18	21	7	28	3
21	11	4	15	2.75
24	8	1	9	8
Total	217	108	325	
Extended Mantel-Haenszel chi square for linear trend=12.96				
p-value(1 degree of freedom)= 0.0003238				

Conclusion

This study suggests that HIV/HBV patients are at higher risk of developing hepatocellular carcinoma than those monoinfected using 2MG as a marker and this study is the first to report the use of 2βM as a marker of HBV hepatocellular carcinoma in Nigeria. Hence in co-infection with HBV efforts must be made to monitor development to hepatocellular carcinoma using more specific markers like 2βM.

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