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Original Article

Liver function biomakers in malaria and hepatitis b co-infection among patients with febrile illness in kano metropolis

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ABSTRACT

Malaria and Hepatitis B Virus (HBV) infections are co-endemic throughout much of the tropical and Sub-Saharan Africa and both present major threat to public health. A study on the status of liver enzymes and serum protein in HBV and malaria co-infection was carried out on 200 patients presenting with fever to the General Outpatient Department (GOPD) of the Murtala Muhammed Specialist Hospital (MMSH) Kano using Gold Standard microscopy and rapid diagnostic test (RDT). The effect of mono and co-infection on serum protein and liver enzymes was investigated. Fifty one (25.5%) out of the total subjects studied were malaria positive. Females had higher rate of malaria infection with 18% prevalence than males with 7.5%. Age group 15-24 had the highest malaria prevalence (11%) followed by age group 25-34 with 6.5%. Thirteen (6.5%) subjects were HBV positive. Males had higher rate of infection with 4.5% prevalence than females with 2.0%. Nine individuals representing 4.5% of the total population had co-infection with higher prevalence observed among the males with 3.0%. Age groups 25-34 were observed to have high co-infection rate of 1.5% and the least prevalence was observed among the age group 15-24 with 0.5% prevalence for both males and females. Biochemical analysis carried out on all the categories of subjects shows significant difference in mean values of AST and ALT in HBV group compared to other test groups $P<0.05$. However no significant difference was observed in the value of ALP in all the groups. Statistical difference was also established in ALB values between the co-infection and malaria groups ($P=0.037$) and between malaria and control group ($P=0.022$). There is also a statistical difference in the mean value of total bilirubin among the groups $P<0.05$ and mean value of DB between HBV and control groups ($P=0.022$). These findings ware discussed in the light of biochemical profiles in Malaria, HBV infection and co-infection with the two ailments.

1. Introduction

Malaria infection still remains the most widespread protozoal infection affecting human race globally. Various attempts at reducing the rate of transmission have been widely used but still all the efforts were futile though with reducing trend in some parts of endemic areas (WHO 2013). It is of high prevalence in sub-Saharan Africa and accounts for substantial portion of visit to outpatient units of most hospital in Nigeria and other parts of the world where it is endemic (WHO, 2010). It was estimated that 3.3 billion people are affected in 106 countries and 350-500 million cases of malaria infection occurs in African countries with 2-3 million annual death (Ikekpeazu, 2010; Paulyn, 2010; WHO, 2010).

Hepatitis B infection is caused by Hepatitis B virus, a partially double stranded DNA virus of Hepadenaviridae family. More than 2 billion people are infected globally with 300-350 million people as asymptomatic carriers of the virus antigen thus prone to chronic form of the infection causing hepatocellular carcinoma and liver cirrhosis (Kakumo, 1998). It is acquired through exposure to blood and blood products, other body fluids and usage of sharps objects.

Malaria and Hepatitis B infection occur throughout the world endemic areas. Both infections represent key threat to survival of the populace in the environment. They both have periods of high activity in the liver cells and affectation of the blood cells may lead to weakened immunity of an individual thus increase chances for contracting other infections (Paulin, 2011). Effects of malaria and hepatitis B infection on liver cells lead to alterations in liver enzymes and serum protein due to hepatocelluler damage (Lin, 1991). Liver cells are unarguably infected by a hepatotropic HBV and also Plasmodium parasites for obvious reasons that it has

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some stages of its life cycle within the hepatocytes. Liver enzymes (aspartate amino transaminase, alanine amino transaminase), Alkaline phosphatase and serum proteins are diagnostic indices that determine its cellular membrane integrity and synthetic ability in liver dysfunction. Activities of liver enzymes increase in serum when there are factors responsible for cellular membrane damage. However decrease level of serum protein could be observed in the setting of severe disease. This results in decreasing synthetic ability of the liver. Invasion of the liver cells by malarial parasites can lead to organ congestion; blockade of sinusoids with associated cellular inflammation can lead to leakage of liver transaminases and reduced liver synthetic ability translating into low serum protein level (Garba, 2004; Onyesom, 2010; Kayode, 2011; Akanninwor, 2013). Acute or chronic HBV infection is associated with mild, moderate or high elevation of transaminases and can be accompanied by impaired synthetic function of the liver (Lin, 1991; WHO, 2013). Co-infection with Plasmodium parasite and HBV may or may not worsen the serum picture of transaminases and synthetic function of the liver which is determine by low serum protein and hypoalbuminaemia depending of the effect of the infectious agents on each other and their collective effect on the liver parenchyma.

MATERIAL AND METHODS

Study Area/Study Population

Before commencement of the study, ethical clearance was obtained from the Ethical committee of the Kano State Hospital Management Board. The study was conducted between July and December 2013, in (GOPD) of the MMSH in Kano. The study was carried out among patients presented with febrile illnesses. Two hundred informed and consented aged between 15-64 were recruited for the study. Exclusion criteria were patients with an established clinical condition other than malaria and/or HBV infection such as obstructive jaundice, cirrhosis, renal diseases, hypertension, diabetes mellitus, sickle cell disease, pregnancy, cancer and patients already on course of chemotherapy or who had it in the last two weeks for treatment of an earlier diagnosed illnesses were also excluded.

Collection of Blood Samples

Five milliliters of blood were obtained via venopuncture from the subjects using vercoutainer needle (Cheesbrough 2005). Two milliliters of these were placed in ethylenediethyltetra acetic acid (EDTA) bottles for parasitological and hematological analysis. The remaining 3 mls were taken into universal bottle and centrifuged at 3000rpm for 5 minutes to obtained the serum for serological detection of the HBsAg.

Parasitological examination

Malarial parasites were examined using the gold standard microscopic procedure using Giemsa staining technique on thick and thin film smear for specie determination and the level of parasiteamia. Level of parasiteamia was expressed as number of parasite/µl of blood. (Alperex 1932, Cheesbrough, 2005)

Hepatitis B serology

HBsAg were detected from the serum samples using Micropoint ELISA commercial Kits technique following the manufacturer's instructions.

Biochemical studies

Spectrometric method was used to assay aspartate and alanine Transaminases as described by Reitman and Frankel (1957). Serum protein was estimated by the Biuret method while Albumin by the Bromocresol green method (Dumas 1971). Alkaline Phosphatase was estimated using Kingamstrong method.

Statistical analysis

Results obtained were analyzed using SPSS software (version 20) for both the descriptive and inferential analysis. Results were expressed as mean and standard deviation. One way analysis of variance (ANOVA) was used to determine the level of significance between the parameters.

RESULTS

The subjects studied were 200 in number (table 1) out of which 90 (45.0%) were males and 110 (65.0%) were females.

Table 1: Distribution of the respondent based on gender and age group

	Number examined (n,%)		
	GENDER		
Age (years)	Male	Female	Total
15-24	28 (14.0)	39 (19.5)	67 (28.5)
25-34	38 (19.0)	37 (18.5)	75 (37.5)
35-44	20 (10.0)	17 (8.5)	37 (18.5)
45-54	01 (0.5)	11 (5.5)	12 (6.0)
55-64	03 (1.5)	06 (3.0)	09 (4.5)
Total	90 (45.0)	110 (55.0)	200 (100.0)

Malaria assessment

Fifty one (25.5%) out of these 200 subjects studied were positive for malaria parasite. This comprises of 15 (7.5%) males and 36 (18.0%) females. Though statistical analysis showed no significant difference (P>0.05) in infection rates between male and female, it was observed that the female population has the higher rate of infection. Among the male population positive for malaria parasite (table 2), those that have the higher rate of infection fall within the age group 25-34 with 7 (3.5%) affected followed by 6 (3.0%) observed among the age group 15-24. More so, female positive for malaria parasites within the age group 15-24 were observed to have the highest rate of infection 18 (9.0%) followed by age groups 25-34 and 45-54 each with 6 (3.0%) of the total population studied. Female patients within the age group 35-44 have 4 (2.0%) infection rates. The least malaria positivity was observed among the age groups 35-44 and 55-64 each with 2 (1.0%) in both gender respectively.

Table 2: distribution of the respondent based on malaria positivity according to sex and age

	Malaria positive (n,%)		
	GENDER		
Age (years)	Male	Female	Total
15-24	6 (3.0)	18 (9.0)	24 (11.0)
25-34	7 (3.5)	6 (3.0)	13 (6.5)
35-44	2 (1.0)	4 (2.0)	06 (3.0)
45-54	0 (0.0)	6 (3.0)	06 (3.0)
55-64	0 (0.0)	2 (1.0)	02 (1.0)
Total	15 (7.5)	36 (18.0)	51 (25.5)

Malaria parasite density is presented in Table 5. It shows that coinfection group presented with low mean parasite density than those with only malaria infection.

Table 3: Malaria parasite density in relation to infection

Infection	Mean parasite density/µl
Malaria (n=51)	1,200±2,270
Co-infection (n=9)	518.3±263.2

HBsAg serology

HBsAg serology

Thirteen persons out of 200 (13/200) subjects studied were positive for HBsAg as shown in Table 3. Higher infection was observed to be among male population with 9 (4.5%) infection rate than female patients with 4(2.0%). Higher infection rate was observed within 25-34 and 35-44 age groups each with 3(1.5%) among the male population. This is followed by 2 (1.0%) observed within 15-24 age group for both male and female population. Least infection rate 1(0.5%) was seen within the age group 55-64 among male population and within 25-34 and 35-44 age groups among female population.

Table 3: Distribution of the respondent based on HBsAg positivity according to sex and age

HBsAg positive (n, %)			
	GENDER		
Age (years)	Male	Female	Total
15-24	2 (1.0)	2 (1.0)	4 (2.0)
25-34	3 (1.5)	1 (0.5)	4 (2.0)
35-44	3 (1.5)	1 (0.5)	4 (2.0)
45-54	0 (0.0)	0 (0.0)	0 (0.0)
55-64	1 (0.5)	0 (0.0)	1 (0.5)
Total	9 (4.5)	4 (2.0)	13 (6.5)

Nine individuals (4.5%) were observed to have co-infection of malaria and Hepatitis B. Again, male population have higher co-infection rate 6 (3.0%) than their female counterparts 3 (1.5%). Male patients within the age group 25-34 were observed to have higher co-infection rate 3(1.5%). This is followed by 2(1.0%) each for both female and male patients within the age groups 35-44 and 25-34 respectively. The least co-infection rate was observed among age group 15-24 1(0.5%) for both male and female population.

Table 4: Distribution of the respondent based on co-infection according to sex and age

CO-INFECTION (n, %)			
	GENDER		
Age (years)	Male	Female	Total
15-24	1 (0.5)	1 (0.5)	2 (1.0)
25-34	3 (1.5)	2 (1.0)	5 (2.5)
35-44	2 (1.0)	0 (0.0)	2 (1.0)
45-54	0 (0.0)	0 (0.0)	0 (0.0)
55-64	0 (0.0)	0 (0.0)	0 (0.0)
Total	6 (3.0)	3 (1.5)	9 (4.5)

Results for biochemical parameters are depicted in table 5 below. It shows a high serum AST among those with HBV infection (14.6±12.2) than what was obtained among those with only malaria (11.5±6.4) and those with co-infection (11.7 ±5.7) when compared with the control group (10.7 ±5.3) . However, statistical significant difference is observed between the value in HBV group and control P=0.036. Also there is high level of serum ALT among those with HBV infection (11.5±8.7) when compared with the co-infection group (7.6±6.1), malaria group (7.2±6.0) and the control (6.5±5.2). There is statistically significant difference between HBV group and those with malaria only P=0.017 and also between those with HBV and the control P=0.003 but no significant difference with the co-infection groups P>0.05. There is high level of ALP in malaria group (152.5±79.8) and co-infected (154.3± 43.7) when compared with that obtained among those with only HBV infection (146.2±80.6) and the control (120.5±75.8) but no significant difference among the groups P>0.05. Serum total protein remains relatively normal in Malaria (5.9 ±1.6), co-infected (5.9± 1.9) and the control groups (5.9 ±1.5) and shows a slight decreased among those with HBV infection group (5.5±1.3) with no significant difference P>0.05. Serum albumin was found to be higher among the co-infection group (4.0±1.6) and the control group (4.4±1.1) when compared with HBV group (3.7±1.0) and those with malaria only (3.6±0.9) there is a statistically significant difference between those with co-infection and malaria group P=0.037 and between the control group and those with malaria only P=0.022. Total bilirubin was observed to be relatively high among those with malaria only (2.1±1.6) as compared with HBV group (1.1±1.6) the co-infected (0.7±0.3) and control groups (0.7±0.6) with statistically significant difference between the groups P<0.05. Direct bilirubin was seen to be higher among those with HBV only (0.6±1.3) than those with Malaria only and the control group (0.3±0.2) and co-infection (0.3±0.1). There is however, a significant difference between those with only HBV and the control group P=0.022.

Table 5. Mean and Standard Deviation of biochemical parameters in all tests groups and control

Parameters	Malariapositive (n=51)	HBsAgpositive (n=13)	Co-infection (n=9)	Control (n=127)
AST(iu/l)	11.5±6.4	14.6±12.2[a]	11.7±5.7	10.7±5.3[b]
ALT(iu/l)	7.2±6.0[a]	11.5±8.[7b,c]	7.6±6.1	6.5±5.2[d]
ALP(iu/l)	152.5±79.8	146.2±80.6	154.3±43.7	120.5±75.8
TP(g/dl)	5.9±1.6	5.5±1.3	5.9±1.9	5.9±1.5
ALB(g/dl)	3.6±0.9[b,c]	3.7±1.0	4.0±1.6[a]	4.4±1.1[d]
TB(g/dl)	2.1±0.2[a,e,g]	1.1±1.6[b,f]	0.7±0.3[c]	0.7±0.5[d,h]
DB(g/dl)	0.4±0.3	0.6±1.3[a]	0.3±0.1	0.3±0.2[b]

AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline Phosphatase, TP: Total protein, ALB: Albumin, TB: total bilirubin, DB: Direct bilirubin, IU/L: international unit per litre, g/dl: gram per deciliter

Values with different superscript differ significantly at p<0.05

DISCUSSION

Liver function biomarkers are important indices that help in assessment of disease severity. Several studies reveal changes in activity level of liver enzymes and protein in the serum due to Plasmodium infection and HBV infection. The present study compares changes in these parameters ALT, AST, ALP, Total

Serum protein, albumin and bilirubin among patients with febrile illnesses who were screen for malaria, HBV and those with co-infection and control group. Serum activities of AST and ALT were seen to be more significantly high in group with only HBV infection than the malarial group, co-infection and control. This may be associated with chronic HBV infection in this category of patients with its attendant complication of hepatocytes necrosis leading to membrane damage and thus increasing serum activities of specific liver enzymes (Ikekpeazu, 2010). Several researchers reported an increase in serum activities in liver dysfunction due to Plasmodium parasite infection (Akanninwor, 2013; Uzuegbu, 2010). This study also observed significant ($p<0.05$) changes in activities of AST and ALT in malaria infection when compared with the control. Serum activities of AST and ALT was seen to be high in the co-infected group but it was observed that the mean value in co-infected group is significantly lower than the values observed in those with HBV positive only. This picture may point to modulation of severity in disease with either malaria or HBV infection when the duo occurs together as reported by Andrade (2011). Though not liver specific, ALP also shows some level of increase in serum activities. Present study presents an increase in ALP activities in the serum among the co-infection and malaria positive group when compared with control. This is consistent with other findings (Uzuebgu, 2010; Onyesom, 2010; Garba, 2004,). Serum total protein and albumin, product of synthetic liver function were also assessed in this study. Previous studies reported low serum total protein and albumineamia in malaria infection (Uzeagbu, 2010; Ikekpeazu, 2010). This study revealed hypoalbuminaemia in malaria positive only, HBV infection only and also among co-infected group as compared with control which indicates affectation of synthetic function of the liver in those with malaria and HBV infection with slight reservation of liver synthetic activities among the co-infected groups. This shows that development of the diseases together do retard much deterioration of liver synthetic functions. This finding is consistent with report by Ikekpeazu (2010).Total serum protein was seen to be the same among the co-infected and that of malaria group but slightly higher than in HBV infection group. This means, in this study group, the occurrence of both infections in same individual does not increase the severity of the other and their subsequent net effect on synthetic function of the liver cells. This finding is in contrast to other studies (Ikekpeazu, 2010) who reported decrease in serum total protein among co-infected patients than those with only malaria infection. Importance of liver in bilirubin metabolism cannot be overemphasized. As such, by implication, any disease condition that affect liver integrity can lead to poor liver handling of bilirubin and its attendant complication. Malaria and HBV infection can cause red cell destruction and hepatocyte damage thereby causing accumulation of bilirubin within circulatory system and its poor handling by the liver. This study however reports high serum bilirubin level among patient with malaria than other test groups and this is in conformity with reported findings in Malaria infection by Ikepeazu (2010). People with co-infection and control group show same level of bilirubin for both total and direct bilirubin. This further stress the fact that presence of the co-infection with HBV, effects of malaria parasite is suppressed with its associated reduced severity.

CONCLUSION

The study showed that co-infection of malaria and HBV infection had no profound effect on the level of serum protein and liver enzyme activities in the serum. This indicates preservation of liver function in settings of co-infection and that presence of both pathogens in same individual is a factor for reduce severity of infection by either malaria parasite or HBV.

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