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Association of hepatic function and antioxidant micronutrients with *Schistosoma haematobium* infection in a Nigerian population

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Abstract: *Schistosomiasis is associated with liver damage possibly due to production of free radicals, which can be alleviated by antioxidants. The relationship of hepatic function, lipid peroxidation marker and antioxidant micronutrients in S.haematobium infected and non-infected individuals were investigated.*
70 subjects positive for urinary schistosomiasis, 50 negative exposed and 30 negative unexposed were studied. Venous blood was collected into heparinized bottles and plasma separated. Antioxidant trace elements,vitamin E, beta-carotene and malondialdehyde (MDA) were assayed by standard laboratory procedures while liver enzymes activity and bilirubin were estimated using kit. All S.haematobium positive subjects were treated with oral administration of praziquantel (40mg/kg), of which 30 subjects were followed up.
Plasma selenium, manganese, vitamin E and beta-carotene were significantly increased in unexposed than in positive and negative groups ($p<0.05$). MDA was slightly increased ($p>0.05$) in S.haematobium patients compared to uninfected groups. Aspartate and alanine amino transferases (AST,ALT) were significantly increased in the infected group. Alkaline phosphatase (ALP),gamma glutamyltransferase (GGT) were decreased in the infected than other groups ($p<0.01$). Manganese was negatively correlated with ALP and GGT in the positive group. In the follow-up treated group, there were inverse associations between Zn, Mn and ALT also AST and Mn ($p<0.05$).
Hepatocellular injury but not cholestasis was associated with S.haematobium. Negative correlations exist between antioxidant micronutrients and cholestatic markers (ALP, GGT) in schistosomiasis and also with AST, ALT in follow-up treated subjects suggesting a protective effect of antioxidant micronutrients on hepatic function in S. haematobium.

Keywords*: antioxidant micronutrients, S. haematobium, liver function, malondialdehyde*

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I. Introduction

Schistosomiasis is a debilitating disease characterized by blood in stool and urine indicating *S. mansoni* and *haematobium* infections.[1] Schistosoma eggs are carried by blood into the liver sinusoids which are too small for the eggs to traverse[2] thus study reports showed abnormal liver function in S. japonicum[3] which could aggravate to chronic hepatic fibrosis and granuloma formation in *S mansoni*.[1] However, information on S. haematobium-induced liver damage is scarce. This is because the infection is mainly linked to urinary tract infections and inflammation of the bladder. [4]

Most parasitic infections promote free radical mediated oxidative stress, which can result in initiation of lipid peroxidation and increased generation of malondialdehyde in plasma and liver.[5] Inadequateintake of antioxidants such as vitamins A, E, iron and zinc predisposes to helminth infections thus antioxidants are reported to provide protection against attack by free radicals. [6,7]This study investigates plasma antioxidant micronutrients, malondialdehyde and hepatic function in S. haematobium infected and uninfected subjects and assesses the effect of Praziquantel on hepatic function and peroxidation marker in the treated, infected subjects.

II. Materials And Methods

150 subjects aged 11-25 years were enrolled in this study. They were divided into three different groups (70 positive to *S.haematobium*, 50 Negative exposed and 30 unexposed). The positive and the negative exposed subjects were recruited from Ore Community School, Osun state, Nigeria. After treatment of the positive group with praziquantel, 30 subjects were randomly selected for the follow-up study.

- Positive group: Those that are infected with *S. haematobium*, living in an endemic area.

- Exposed uninfected group (negative control): Those that are microscopically confirmed uninfected with *S. haematobium*, though living in an endemic area.
- Unexposed uninfected group (positive control): Those that are not exposed to schistosomiasis, living in a non-endemic area.
- Follow-up group: Treated positive group showing an improvement in the egg reduction rate after 3weeks of administration of a single dose (40mg/kg) of Praziquantel.

Ethical clearance and approval was obtained from Osun state Ministry of Education and LAUTECH Institutional Review Committee before collection of blood and stool samples. Informed consent was obtained from all participants.

Inclusion criteria

Individuals diagnosed with *S. haematobium* by microscopic identification of schistosoma egg(s) in urine or stool samples.

Exclusion criteria

Subjects with hepatitis B, diagnosed using HBV strip. Students using supplement that may interfere with their antioxidant micronutrients status.

Subject preparation and sample collection

5ml of venous blood without stasis was carefully collected into Lithium heparin bottle. Plasma sample was obtained after centrifugation at 2,000rpm for 5minutes within 5hours after sample collection. The plasma was then stored at -20^0C till the analysis was carried out.

Body Mass Index (BMI) was calculated using the formular; weight(in kg)/ height(in m^2)

Parasitological Examination

Kato–Katz thick smear method[8] was used in parasitological examination. Briefly, a small mound of faecal material was placed on newspaper and the small screen on top was pressed so that some of the faeces were sieved through the screen and accumulated on top and then collected using a flat-sided spatula. The sieved faeces were added to fill the hole on the template and the excess removed from the edge of the hole. The faecal material was then covered with pre-soaked cellophane strip in glycerol solution .The faecal sample was firmly pressed against the hydrophilic cellophane strip on another microscope slide such that it was evenly spread between the microscope slide and the cellophane strip. The slide was read after 30-60 minutes at ambient temperature. The urine sample was filtered to allow for recovery and enumeration of *S.haematobium* eggs in urine. [8]

Biochemical tests

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT), conjugated and total bilirubin were analyzed using kit (Randox laboratories limited, County Antrim, UK).Plasma zinc (wavelength 213.8nm), copper (324.7nm), selenium (196nm) and manganese (236nm) were determined with flame atomic absorption spectrophotometer using a direct method.[9]Column chromatography was employed in the estimation of beta carotene and vitamin E. MDA was analyzed using the method of Varshney and Kale. [10]

Statistical analysis

Data were analyzed using SPSS version 17. Results were expressed as mean ± SD. Difference between two groups was calculated using independent t-test, ANOVA was used to determine the variance among the study groups with level of significance put at 95 and 99% (0.05 and 0.01) respectively. Association between variables was done using Pearson correlation coefficient.

III. Results

Table I showed no significant variations in mean values of age, height, weight, BMI and PCV distribution in all groups ($p>0.05$).

Table I: Biophysical parameters of baseline subjects (Mean ± S.D)

Parameters	Positive (n=70)	Negative exposed (n=50)	Unexposed uninfected (n=30)	p-value
Age (years)	14.18 ± 1.59	15.20 ± 2.46	16.5 ± 2.12	0.073
Height (m)	1.53±0.10	1.52±0.12	1.46±0.09	0.064
Weight (kg)	45.18±9.39	46.25±10.55	41.33±7.48	0.807

BMI (kg/m2)	19.24 ± 2.46	19.69 ± 2.76	19.05 ± 1.76	0.560
PCV	38.25 ± 6.27	38.40 ± 7.01	39.11 ± 0.23	0.986

BMI: Body Mass Index, PCV: Packed Cell Volume

The mean values of plasma hepatic damage markers are shown in Table 2. AST and ALT activities of the positive group were higher ($p<0.05$, $p<0.01$) respectively than in other groups. ALP and GGT were significantly increased in the unexposed group compared to the positive and negative groups but not in the follow-up group. For the follow-up group, plasma ALP and GGT were higher than in other subject groups ($p=0.01$). ALT activity in the negative control was significantly increased than the unexposed and follow-up groups. There were no significant variations in total and conjugated bilirubin levels in all groups.

Table 2: Plasma hepatic function markers in all studied group (Mean ± SD)

Parameters	Positive (n=70)	Negative exposed (n=50)	Negative unexposed(n=30)	Follow-up	p-value
AST (U/l)	17.52±10.46	8.33 ± 3.55	6.57 ± 2.90	10.40 ± 3.39	0.01*
ALT (U/l)	12.07 ± 3.72	8.96 ± 3.03	6.22 ± 2.47	5.29 ± 1.60	0.01†
ALP (U/l)	15.88 ± 3.32	16.35 ± 2.10	17.5 ± 7.56	20.88 ± 5.55	0.01*
GGT (U/l)	3.72 ± 1.70	2.32 ± 0.31	5.80 ± 2.07	10.69 ± 3.76	0.01*
ConjBil	7.03 ± 14.12	8.50 ± 15.24	6.55 ± 1.10	7.52 ± 4.95	NS
Total Bil	6.48 ± 4.194	7.54 ± 3.55	8.05 ± 2.30	6.96 ± 3.90	NS

Significant at * $p<0.05$, † $p<0.01$, NS- not significant. AST:aspartate aminotransferase, ALT: alanine aminotransferase, GGT:gamma glutamyltransferase, ConjBil:conjugated bilirubin

Plasma Cu and Zn levels in the positive subjects were reduced than the values in the uninfected groups ($p<0.01$). The slight increase in MDA in the positive group compared with negative exposed and unexposed groups were not statistically significant. Copper, zinc, selenium, manganese, vitamin E and beta-carotene were all significantly increased in the unexposed group compared to the negative exposed and positive groups but not in the follow-up group. For the follow-up group, mean plasma concentration of manganese was higher than the unexposed group ($p<0.01$). (Table 3)

Table 3 : Plasma MDA and antioxidant micronutrients in all groups (Mean ± S.D)

Parameters	Positive (n=70)	Negative exposed (n=50)	Negative unexposed (n=30)	Follow-up	p-value
MDA(mmol/L)	9.26±1.71	8.21±2.65	8.36±2.62	7.35±3.42	0.30
Cu (µg/l)	176.4 ± 80.38	135.4 ± 54.40	235.8 ± 83.39	174.1 ± 20.54	0.01*
Zn (µg/l)	119.82±24.08	122.82±37.69	136.55±30.58	116.20 ± 7.49	0.03*
Se (µg/l)	0.50 ± 0.19	0.62 ± 0.31	0.87 ± 0.58	0.60±0.09	0.01*
Mn (µg/l)	71.64 ± 4.31	72.05 ± 7.01	79.34 ± 5.36	154.00±73.15	0.01*
Vit. E (µg/l)	114.64±24.09	107.16±22.52	131.85±54.47	118.54±19.56	0.12
β-caro (µg/l)	71.62±15.93	74.18 ± 6.29	143.06±86.78	132.05±14.82	0.01*

significant @*$p<0.05$; MDA: malondialdehyde, Cu: copper, Zn: Zinc, Se: Selenium, Mn: manganese, Vit.E: vitamin E, β-caro: β-carotene

From table 4, two-tailed Pearson correlation studies showed a negative association between ALP, GGT and manganese ($p<0.05$) in the positive group. Positive correlations were recorded for Se ($P<0.01$), Mn, vitamin E and Cu ($P<0.05$). No association was seen with measured parameters and MDA.

Table 4: Correlation coefficient of measured parameters in positive subjects

Parameters	Cu	Zn	Se	Mn	Vit E	MDA
ALP	0.111	0.155	0.080	-0.290*	-0.310	0.076
GGT	-0.192	-0.196	-0.115	- 0.365†	0.070	0.150
Cu	1	0.087	0.407†	0.290*	0.269*	0.082
Zn	0.087	1	0.302*	-0.124	-0.212	-0.069

Significant @*0.05 level (2-tailed), † 0.01 level (2-tailed)

Correlation coefficient of all biochemical parameters studied in the exposed infected subjects is shown in table 5. For this negative control group, weak associations were found in Mn versus β-carotene and Se against vitamin E. Positive correlations were recorded with Zn, Mn and Cu, as well as Zn, vitamin E and Se while negative associations were observed for Cu, Zn and MDA ($p<0.05, 0.01$) respectively.

Table 5: Correlation coefficient of measured parameters in negative exposed subjects

Parameters	Cu	Zn	Se	Mn	Vit E	MDA
Cu	1	0.317*	0.148	0.381*	0.007	-0.388*
Zn	0.317*	1	0.385*	0.211	0.096	- 0.690†
Se	0.148	0.385*	1	0.003	0.405†	-0.179
β-carotene	0.085	0.790	0.157	0.439†	0.042	0.128

Significant @*0.05 level (2-tailed), † 0.01 level (2-tailed)

In the follow-up group, negative associations were observed between AST and Mn, ALT and Zn, also conjugated bilirubin and ALT ($p<0.05$). ALP was positively correlated with AST (table 6).

Table 6: Correlation coefficient of measured parameters in follow-up subjects

Parameters	AST	Conj.Bil	Cu	Zn	Mn
ALT	-0.413	-0.511*	0.003	-0.479*	0.453*
ALP	0.479*	-0.099	0.177	0.189	-0.339
Cu	0.306	0.015	1	0.304	-0.227
Mn	-0.644†	-0.425	-0.227	-0.070	1

Significant @*0.05 level (2-tailed), † 0.01 level (2-tailed)

IV. Discussion

Schistosoma infection poses significant health problems and is a socioeconomic burden in most of sub-sahara Africa including Nigeria. Risk factors include poverty, unhygienic practices and overpopulation resulting from migration of individuals from an endemic to a non-endemic environment thus transmitting the infection. [11]In this case-control study of schistosomiasis infected, exposed but non-infected (negative control) subjects residing in an endemic region and unexposed uninfected (positive control) individuals residing in a non-endemic area, we investigated the association of *S. haematobium* infection on hepatic function, lipid peroxidation and antioxidant micronutrients. We did not find any significant changes in BMI and PCV in the infected and uninfected subjects suggesting that these biophysical parameters do not play a role in occurrence of infection in the community. In addition, none of the subjects were anemic.

To the best of our knowledge, this is the first work investigating the association of S.haematobium infection with liver function in this community. From the present study, we observed significant decreases in plasma values of hepatic cholestatic markers, ALP and GGT in the positive group than in controls and even after drug administration in the follow-up group, the value was still higher than that recorded in the uninfected subjects. Nevertheless, it is noteworthy that the ALP and GGT values in the follow-up group were not up to the upper limit of normal suggested by the kit manufacturers (ALP: 60-170U/L; GGT:4-28U/L). A previous report on evaluation of abnormal liver enzyme tests showed that ALP and GGT synthesis is often induced by obstructions in the intra or extra –hepatic bile ducts [12] thus suggesting that there is no biliary obstruction in the

patients studied.This was corroborated by the non-significant differences in plasma total and conjugated bilirubin among the infected and non-infected individuals ($p>0.05$).

Liver cell membranes can become permeable when damaged, allowing for escape of intracellular enzymes, ALT and AST, into the bloodstream. [12] Data from the present study showed increased plasma AST and ALT levels in S. *haematobium* infection compared to uninfected subjects. Similar results were reported from previous works on liver function in *S.japonicum* and *S.mansoni* infections. [3, 13] These workers postulated that this observation may indicate abnormal liver function in shistosomiasis resulting from damaged hepatocytes and can constitute a risk factor for more serious outcome in endemic areas.[3]In addition, at four weeks post treatment with a single dose of 40mg/kg body weight praziquantel indicated by about 50% reduction in the egg population, [14] there exists significant reduction in plasma ALT and AST levels in the follow-up subjects ($p<0.05$). It could thus be deduced that praziquantel had a reversal effect on the hepatic granulomatosis which resulted from the hepatic cell membrane and mitochondria damage.[12]

The slight increase in mean MDA value recorded in infected subjects than non-infected groups could suggest some level of lipid peroxidation in *S.haematobium*. This observation is in agreement with the result of a study on correlation of lipid peroxidation biomarker and hepatic fibrosis in schistosomiasis. Thus it could be deduced that parasite induced oxidative stress results in increased production of MDA, an inflammatory mediator in the plasma.[15]

Vitamin E and β-carotene are strong antioxidants that play a significant role in immune system operations and protect against reactive oxygen species generated in schistosomiasis. [16, 17] Data from this study showed a significant reduction in plasma antioxidant micronutrients Se, Mn, vitamin E and β-carotene in the infected subjects. Poor nutrition and micronutrient deficiencies have been implicated in infectious disease pathogenesis. [18] Moreover, people residing in rural areas with high socio-economic burden, and under-nutrition are constantly bombarded with parasitization of helminthic infection like schistosomiasis. Thus antioxidant micronutrients status in infected populations could be causative or be indicative of disease progression. [19]

The concentrations of Cu and Zn of the follow-up group were not as high as the value in the group with the infection. This could be due to the cytosolic localization of Cu/Zn-SOD which is not being affected by the schistosomicidal activity of the praziquantel drug whose effects are more pronounced on the membranes.[20] However, there was a significant increase in the manganese concentration of the follow-up group compared to the other groups.

The weak association between the hepatic cholestasis markers, ALP, GGT and manganese support the lack of evidence of cholestasis in the studied subjects indicated by the decreased plasma levels of ALP and GGT in the group positive to *S. haematobium* than the non-infected and follow-up groups. Positive correlations were however recorded among the antioxidant micronutrients analyzed. This is consistent with the report from an earlier study that showed significant linear association of β-carotene and vitamin E levels with other antioxidant substances thus suggesting a synergy in their actions. [21]

In the Follow-up group inverse associations were observed between hepatic function markers, AST, ALT and Mn after treatment with praziquantel suggesting an ameliorative effect of micronutrients on hepatic damage.[22] Micronutrients are often linked to hepatocellular injury as they are either a cofactor for enzyme(s) involved in fibrogenesis or are membrane-bound making them to be raised when there is an injury to the liver. [23, 24]

From the present study, we found an association of hepatocellular injury but not cholestasis with *S.haematobium* infection. Negative correlations exist between Mn, an antioxidant micronutrients and cholestatic markers (ALP, GGT) in infected subjects and also with AST, ALT in follow-up treated subjects suggesting a protective effect of antioxidant micronutrients on hepatic function in *S. haematobium*.

Micronutrients are not routinely estimated in most laboratories because deficiencies are not easily observed and diagnosed clinically. Thus, this calls for the comprehensive evaluation of these micronutrients (Zn, Cu, Mn, Se, Vitamin E and β-carotene) in the rural dwellers exposed to schistosoma infection for proper guide on the severity of free radical damage. One limitation of this study was the small sample population. However, it is well-reported that *S. haematobium* infection is endemic in the study area. [14] Therefore, a further study involving a larger population will bring about a more conclusive result on antioxidant micronutrients and hepatic function in the neglected tropical infection.

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