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Vitamins (A, B_1 and C) status of Nigerians with *Trypanosoma brucei gambiense*-infection

[*1]Isaac, C, [1]Nmorsi, O.P.G., [1]Igbinosa, I.B and [2]Umukoro, D.O

[1]Tropical Disease Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria
[2]Family Medicine Department, Delta State University, Abraka, Nigeria

***Corresponding author:** cle212000@yahoo.com

Abstract

Vitamins A, B_1 and C profiles were evaluated among 35 *Trypanosoma brucei gambiense* seropositive volunteers. Of the 35 seropositives, 9 were weakly positive, 12 had moderate positivity and strongly positive infection occurred among 14 of them. Parasites were detected in the cerebrospinal fluid (CSF) of 4 volunteers with strong positive titre (≥1:32). In the blood/serum, the prevalence of the *T. b. gambiense* were 4 for weakly positive, 5 for moderately positive and 7 for strongly positive volunteers. The mean vitamin A concentrations between the control subjects (7.77±6.99 nmol/L) and the seropositive volunteers were statistically significant ($p<0.05$). Vitamin B_1 levels were significantly depressed in the strongly positives (8.26±1.85 nmol/L) and the moderately positives (16.7±2.66 nmol/L) when compared with the control subjects (38.26±1.58 nmol/L) ($\chi^2=23.49$, $p<0.05$ and $\chi^2=12.14$, $p<0.05$), respectively. The differences in vitamin C concentrations in the control subjects and the infected volunteers were not statistically significant at $\chi^2=0.58$, $p>0.05$; $\chi^2=3.36$, $p>0.05$ and $\chi^2=0.94$, $p>0.05$ for weakly, moderately and strongly positive, respectively. We therefore conclude that the depressed vitamins A and B_1 concentrations may be implicated in the pathogenesis of *T.b. gambiense* infection.

Keywords: Vitamin A, Vitamin B_1, Vitamin C, *Trypanosoma brucei gambiense*, human African trypanosomiaasis (HAT), Seropositivity, Nigeria.

Introduction

Trypanosoma brucei gambiense, the causative agent of human African trypanosomiasis (HAT) in West Africa, multiplies extracellularly in the blood stream, lymph and interstitial fluids of their host. This disease threatens millions of people in 36 countries of sub-Saharan Africa with between 50,000 and 70,000 new cases per year. There are two distinct stages during the course of sleeping sickness pathogenesis. The first or early stage of the disease, known as the hemolymphatic phase is defined by the restriction of the trypanosomes to the blood and lymph system. The second or late stage of the disease, often described as the neurological/ meningoencephalitic phase is characterised by the presence of the parasites in the cerebrospinal fluid (CSF) (1). Vitamins have been associated with the pathogenesis of trypanosomiasis infection (2, 3, 4). Vitamin A which is a lipid soluble antioxidant has been reported to be depleted in liver store of *T. brucei*-infected rat (5). In contrast, it has been documented that *Trypanosoma* thrived more in vitamin-A deficient mice compared to the control host (6). The morbidity of trypanosomiasis was ameliorated after the administration of vitamin A and C combination (2). Thiamine (vitamin B_1) is essential for cerebral metabolism of glucose (7). It has been advanced that the cultivated form of *T. brucei* in the presence of vitamin B_1 showed no uptake of vitamin B_1. Temporary growth stimulatory properties of *T. cruzi* were induced by serum and blood cell thiamine chloride (8). *T. lewisi*-infected mouse when fed with thiamine-deficient diets showed a change in the enzymes responsible for thiamine-dependent glucose metabolism (7). Vitamin C administration in *T. congolense* infected rabbits had been reported to be involved in the interference with metabolism or cellular division of trypanosome parasite with little or no significant improvement in disease conditions (3). Increased systemic oxidative stress in *T. b. gambiense* and *T. b. brucei* infected rats led to a decrease in tissue ascorbic acid concentration in *T. b. brucei* infected rat and increased susceptibility of red blood cells to oxidative haemolysis in *T. b. gambiense* infected rat (9). It has been observed in rats that *Trypanosoma* infection decreased the concentration of vitamin C in spleen and adrenals and increased the proportion of vitamin C in the oxidised dehydroascorbic acid form (4). In spite of the roles of vitamins in the pathogenesis of trypanosomiasis, information on the profile of vitamins in trypanosomiasis infected humans is yet to be documented for our locality. This study therefore evaluate the vitamin A, B_1 and C status of volunteers infected with *T. b. gambiense infection* in Abraka, an endemic focus in Nigeria.

Materials and methods

This investigation was carried out in Umeghe, Urhouka and Ugonu communities in Abraka, Ethiope East Local Government Area of Delta State, Nigeria. Abraka is located between latitude 5˚N-6˚N and longitude 5˚.4'E with population of over 17,000. The vegetation cover ranges from the mangrove thick forest to mixed rain forest and grass lands. River Ethiope runs through Umeghe and Urhouka communities where most of the inhabitants visit for various domestic and recreational purposes. The people in our studied communities are predominantly farmers and fishermen, while some are civil servants. Ethical

Table 1. Number of seropositives and volunteers with parasites detected in blood/serum and CSF

Seropositive	Number of positives	No. of volunteers with parasite detected in blood/serum	No. of volunteers with parasite detected in CSF
Weakly positive	9	4	0
Moderately positive	12	5	0
Strongly positive	14	7	4
Total	35	16	4

Table 2. Vitamin A status among trypanosomiasis infected and non-infected human volunteers

	Seropositives			Control
	Weakly positive	Moderately positive	Strongly positive	
Number examined	9	12	14	10
Mean (nmol/L)	6.95±7.78	5.23±3.06	4.5±1.38	17.77±6.99
χ^2	6.58	8.85	9.98	

permission was obtained from both the Delta State Ministry of Health, Asaba, Delta State and Eku Baptist Hospital, Eku, Delta State, Nigeria. Prior to the commencement of this study, the communities were enlightened on the nature, objectives and benefits of the investigation. Thereafter, informed consents were sought and obtained from 474 volunteers who were subsequently recruited for this study. Majority of the seropositive volunteers were observed to suffer from malaise, anaemia, headache, pyrexia, weight loss and weakness following the medical history and physical examinations carried out on the volunteers. Also the individuals with parasite positive CSF suffered a level of mental disorder and sleep abnormality. Thirty five volunteers infected with *T. b. gambiense* out of the entire 44 seropositive individuals in our studied locality and 10 control subjects from the same population participated in the evaluation of vitamins profiles. Finger pricked blood was ¾ filled in heparinised capillary tube. A drop of the card agglutination test for trypanosomiasis (CATT) reconstituted reagent was added to a drop of blood on a plasticized surface. Agglutination of the mix was noted as positive. Venous blood was collected from the 35 seropositives. Sera obtained were used to categorize the level of infection using the CATT reagent by double serial dilution as: weakly positive (1:2-1:4), moderately positive (1:8-1:16) and strongly positive (≥1:32). The presence of parasites was demonstrated using various techniques namely blood films (thin, thick and wet) and concentration methods such as microhaematocrit centrifugation, buffy coat and *in vivo* inoculation of the cerebrospinal fluid (CSF) into albino rats (10). Thereafter, samples were microscopically examined for the presence of parasite. Also, CSF was collected using standard procedures by lumbar puncture. The CSF was examined microscopically for the presence of parasite. Venous blood from seropositives was subjected to the determination of vitamins A, B_1 and C concentrations using the method described by (11). Data obtained from our investigation were subjected to statistical analysis, namely, Chi-square and analysis of variance (ANOVA) using InStat and Microsoft Excel packages.

Results

A total of 35 seropositves were reported as 9 (weakly positive), 12 (moderately positive) and 14 (strongly positive). The number of volunteers with parasite detected in (blood/serum and CSF) was: weakly positive (4 and 0), moderately positive (5 and 0) and strongly positive (7 and 4), respectively (table 1). Table 2 shows vitamin A status among trypanosomiasis infected and non-infected human volunteers. The mean differences between the control subjects (7.77±6.99 nmol/L) with the weakly positives (6.95±7.78 nmol/L), the moderately positives (5.23±3.06 nmol/L) and the strongly positives (4.5±1.38 nmol/L) were statistically significant at χ^2 =6.58, $p<0.05$; χ^2=8.84, $p<0.05$ and χ^2=9.98, $p<0.05$, respectively. Statistical comparison among mean vitamin A concentrations for the different levels of infection showed no significant difference (F = 0.198, $p>0.05$). Table 3 shows vitamin B_1 status among trypanosomiasis infected and non-infected human volunteers. The mean vitamin B_1 differences between control subjects (38.26±1.58 nmol/L) with strongly positives (8.26± 1.85 nmol/L) and moderately positives (16.7±2.66 nmol/L) were statistically significant at χ^2=23.49, $p<0.05$ and χ^2=12.14, $p<0.05$, respectively. The difference between control subjects and the weakly positives (33.26±1.11 nmol/L) at (χ^2 = 0.65, $p>0.05$) was not statistically significant. Mean difference in vitamin B_1 concentrations among infected individuals was significant (F = 206, $p<0.001$). The mean differences between the concentrations of vitamin C in the weakly positives (75.52±54.13 nmol/L), the moderately positives (84.4±74.89 nmol/L) and the strongly positives (77.23±32.86 nmol/L) when compared with control subjects (69.14±27.82 nmol/L) were not statistically significant (χ^2=0.58, $p>0.05$; χ^2=3.36, $p>0.05$ and χ^2=0.94, $p>0.05$), respectively (Table 4). Among the positives, the difference in mean vitamin C levels was not significant (F = 0.034, $p>0.05$).

Discussion

Vitamin A in this investigation was depleted in infected human subjects when compared with control subjects. This presentation could be ascribed to the mechanism of pathogenesis of the parasite which is in part due to the generation of free radicals and superoxides during trypanosomal infection arising from cellular injury (12, 13). Here, it is presumed that vitamin A, an antioxidant, scavenges for oxidative species generated by *T. brucei* in an attempt to ameliorate disease conditions arising from cellular damage (2). This is in line with the report of (2) who investigated the role of vitamin A and C in amelioration of anaemia and organ damage in *T. b. brucei*-infected rat. As a

Table 3. Vitamin B_1 status among trypanosomiasis infected and non-infected human volunteers

	Seropositives			
	Weakly positive	Moderately positive	Strongly positive	Control
Number examined	9	12	14	15
Mean (nmol/L)	33.26±1.11	16.7±2.66	8.26±1.85	38.26±1.58
χ^2	0.65	12.14	23.49	

Table 4. Vitamin C status among trypanosomiasis infected and non-infected human volunteers

Title	Seropositives						
Levels of infection	Weakly positive		Moderately positive		Strongly positive		Control
	CATT positive	Parasite positive	CATT positive	Parasite positive	CATT positive	Parasite positive	CATT negative
Number	9	4	12	5	12	7	10
Mean (nmol/L)	75.52±54.13		84.4±74.89		77.23±30.86		69.14±27.82
χ^2	0.58		3.36		0.94		

consequence, endogenous antioxidant reserves in the blood are depleted in the process (9, 14). We therefore suggest that vitamin A maybe implicated in the disease pathogenesis especially among the strongly positive group in an attempt to ameliorate disease conditions. It was observed that *T.b gambiense*-infected individuals had depressed levels of vitamin B_1 (thiamine) than the control volunteers. This depression was profound among the strongly seropositive volunteers. This depression could be attributed to the fact that the trypanosome parasite is actively involved in uptake of thiamine for their metabolism. This is contrary to the findings of (15) where it was documented that *T. b. brucei* do not change its growth behaviour when placed *in vitro* in the presence of thiamine. This was linked to the inability of uptake of thiamine and structural thiamine analogues by the trypanosomes which were connected to the absence of thiamine-biosynthetic genes in the genome of *T. b. brucei* (15). The depressed thiamine concentration could result in insufficient glucose production in the brain, especially in patients with the second stage of infection, which probably explains the weakness and deteriorating mental conditions observed among some volunteers at the second stage of the infection. This study revealed that trypanosomiasis disease does not have any impact on vitamin C status in *T.b. gambiense*-infected volunteers as there was no difference with the control subjects. This finding contradicts investigation in some animal models studied (16, 9). We can deduce that the vitamin C present in the infected humans was not utilized by trypanosomes and so was not depleted when compared with the control subjects. This assertion can be supported by the fact that trypanosomes have been known to synthesize vitamin C using L-galactono-r-lactone and arabiono-r-lactone (17), an ability lacking in man as a result of absence of gulonolactone (18), which probably explains the presence of significant levels of ascorbate in trypanosomes (19). Conclusively, the depressed vitamins A and B_1 concentrations in the volunteers infected with human Africa trypanosomiasis indicate that these vitamins may be implicated in the pathogenesis of the disease.

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