International Scholars Journals

African Journal of Parasitology Research ISSN 2343-6549 Vol. 6 (7), pp. 001-004, July, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Trace metal profiles of Nigerians with *Trypanosoma brucei gambiense*-infection

C. Isaac[1], O. P. G. Nmorsi[1]*, I. B. Igbinosa[1], D. O. Umukoro[2], C. I. Ikaraoha[3] and N. C. D. Ukwandu[4]

[1]Tropical Disease Research Unit, Department of Zoology, Ambrose Alli University, Ekpoma, Nigeria.
[2]Family Medicine Department, Delta State University, Abraka, Nigeria.
[3]Department of Chemical Pathology, Igbenidion University, Okada, Nigeria.
[4]Department of Medical Microbiology, Ambrose Alli University, Ekpoma, Nigeria.

Accepted 09 May, 2019

We investigated the profile of some trace metals in human African trypanosomiasis (HAT) infected individuals in three Abraka communities of Delta State, Nigeria. Thirty five seropositives were categorized as weakly positive (n = 9), moderately positive (n = 12) and strongly positive (n = 14). The iron concentrations for weakly, moderately and strongly positives when compared with control subjects were statistically significant ($p < 0.05$). Elevated levels of zinc were observed for moderately positive and strongly positive ($p < 0.05$). Significant difference was observed in the level of copper in both the moderately and strongly positives when compared with their non- infected volunteers ($p < 0.05$). The differences in selenium levels between weakly positive, moderately positive and strongly positive with control subjects were not statistically significant ($p > 0.05$). The elevation of iron, zinc and copper concentrations observed among the *Trypanosoma brucei gambiense*- infected volunteers indicate the alteration of the profile of these micronutrients in infected volunteers, thereby implicating them in the pathogenesis of human African trypanosomiasis.

Key words: Human African trypanosomiasis, metals, volunteers.

INTRODUCTION

Human African trypanosomiasis (HAT) is a neglected tropical disease with an estimated clinical transmission of about 300,000 to 500, 000 cases yearly. HAT presents two stages of infection as hematolymphatic stage characterized by parasite presence in the blood/lymph fluid and the meningoenecephalitic stage which presents the parasite in the cerebrospinal fluid (CSF) (Buscher and Lejo, 2004). Altered metal levels have been associated with *Trypanosoma* infection with its pathogenic effects (Da Silva et al., 2009; Mwangi et al., 1995). Iron an essential nutrient is involved in cellular processes (Lieu et al., 2001) and has been implicated in the course of trypanosomiasis infection. For example, the magnitudes of decrease in iron concentrations correlated closely with the level and duration of the parasitaemia in rabbits with its associated pathological consequences (Mwangi et al., 1995). Similarly, serum iron concentrations regardless of The diet regimen showed higher levels in non-infected than infected rats (Lee et al., 1977). In contrast, an increase in serum iron levels in untreated infected rats than treated infected rats has been documented (Ekanem et al., 2009).

In an investigation, decrease in zinc levels coincided with the onset of *T. brucei gambiense* in peripheral blood of rabbit (Mwangia et al., 1995). Zinc-deficient animals showed three times the number of trypanosomes as that of the complete and pair-fed mice (Lee et al., 1983). A report suggested that cattle zinc profile could be responsible for either susceptibility or resistance to *Trypanosoma* infection (Traoré-Leroux et al., 1985). Copper supplementation suppressed the effects of *Trypanosoma* infection in rabbit (Omole and Onawunmi, 1979). Also, the effect of the addition of copper to the diet of infected rat raised natural resistance of the rat to *Trypanosoma* infection (David, 1934). In another report, copper concentrations were increased in *Trypanosoma*-infected cat than the controls (Da Silva et al., 2009).
Patients with trypanosomiasis infection have demonstrated

*Corresponding author. E-mail: nmorsiopg@yahoo.com.

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

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

Table 2. Iron status among HAT infected and non-infected human volunteers.

Levels of infection	Seropositives						
	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	14	7	10
Mean (g/L)	0.012 ± 0.0024		0.013 ± 0.00017		0.018 ± 0.0055		0.0086 ± 0.0018
	3.517		5.88		5.335		

reduced selenium level with higher mortality rate when compared with the uninfected control (de Sousa et al., 2002). In contrast, parasite load was not different for selenium fed and selenium deficient *Trypanosoma*-infected mice (de Sousa et al., 2002). Also, a report has it that selenium deficient and control mice post inoculation after a while ensured parasite clearance (Ongele et al., 2002).

In view of varying reports of the impact of trypanosomiasis on trace metals and the lack of information on trace metals status in HAT infected individuals in our locality, we investigated the profile of some trace metals (iron, zinc, copper and selenium) in relation to *Trypanosoma brucei gambiense* infection in Abraka, an endemic focus of HAT in Nigeria.

MATERIALS AND METHODS

This study was carried out in three agrarian villages in Abraka, namely, Umeghe, Urhouka and Ugonu communities in Abraka, Delta State, Nigeria. These communities lie between latitude 5˚47’-6˚15N and longitude 5˚.42’-6˚E with population of over 5,000. 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 major economic activities in these villages are farming. We obtained ethical permission from Delta State Ministry of Health, Asaba, Delta State, Nigeria and Eku Baptist Hospital, Eku. The villagers were assembled in their town halls where they were enlightened on the nature, objectives and benefits of the investigation. Informed consents were sought and 474 consenting volunteers were subsequently recruited for this study. Thirty five *T. brucei gambiense* seropositives and 10 control subjects within the same population were further recruited for this evaluation. We obtained medical history and physical examination from these volunteers. We documented their age, weight and those with overt diseases such as measles, HIV, sickle cell anaemia were eliminated from the investigation using standard procedures. 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 to 1:4), moderately positive (1:8 to 1:16) and strongly positive (1:32) according to the manufacturer’s instruction (Intituut voor Tropische Geneeskunde, Antwerpen, Belgium). The presence of parasite was detected using various techniques like blood films (thin, thick and wet) and concentration methods such as microhaematocrit centrifugation and buffy coat. Blood were collected from the seropositves and then analyzed as described by Welz (1985), and the levels of the trace metals determined using Atomic Absorption Spectrometer. Data obtained were subjected to statistical analysis, namely, Welch t-test and analysis of variance (ANOVA) using Instat and Microsoft Excel packages.

RESULTS

A total of 35 seropositve subjects were identified with 9 (weakly positive), 12 (moderately positive) and 14 (strongly positive). The number of volunteers with parasite detected in blood/serum and CSF were: weakly positive (4, 0), moderately positive (5, 0) and strongly positive (7, 4), respectively (Table 1). Table 2 shows the iron profile of HAT infected volunteers. The iron concentrations for categories of weakly (0.012 ± 0.0024 µg/L), moderately (0.017 ± 0.0017 g/L) and strongly positive volunteers (0.018 ± 0.0055) when compared with the control (0.0086 ± 0.0018) were statistically significant (t=3.517, $p<0.05$; t= 5.88, $p<0.5$, t = 5.33, $p<0.01$. Among

Table 3. Zinc status among HAT infected and non-infected human volunteers.

Levels of infection	Seropositives						
	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	14	7	10
Mean (g/L)	0.0015 ± 0.00055		0.0025 ± 0.00055		0.0042 ± 0.0015		0.00013 ± 0.00039
t-values	0.92		2.804		5.56		

the categories of positives, the difference in the iron level was not significant ($F = 3.42$, $p > 0.05$). Table 3 shows the zinc status of the different categories of seropositives. Elevated levels of zinc was observed for weakly positive (0.0015 ± 0.00055 g/L), moderately positive (0.0025 ± 0.0013 g/L) and strongly positive volunteers (0.042 ± 0.0016 µg/L). The zinc levels for weakly positive was not significantly elevated when compared with the control subjects (0.0013 ± 0.00039 g/L) ($t = 0.92$, $p > 0.05$). Comparing the levels of zinc among categories of seropositive individuals, there was no significant difference ($F = 3.22$, $p > 0.05$).

The profile of copper for categories of seropositives is presented in Table 4. Significant difference was observed in the level of copper in both the moderately (0.0012 ± 0.00076 µg/L) and strongly positives (0.0012 ± 0.00046 µg/L) when compared with their non-infected counterparts (0.00057 ± 0.00048 µg/L) ($t = 16.46$, $p < 0.05$; $t = 22.4$, $p < 0.05$). Copper levels were not significantly elevated for weakly positive individuals (0.00072 ± 0.00025 µg/L) ($t = 0.089$, $p > 0.05$). Differences in copper levels among seropositive groups was not significant ($F = 1.13$, $p > 0.05$) . Table 4 shows the profile of selenium in HAT positive volunteers. The differences between weakly positive (0.00057 ± 0.00012 µg/L), moderately positive (0.00046 ± 0.00014 µg/L) and strongly positive patients (0.00053 ± 0.00012 µg/L) with control subjects (0.00055 ± 0.00014 µg/L) were not statistically significant at $t = 0.332$, $p > 0.05$; $t = 1.50$, $p > 0.05$; $t = 0.375$, $p > 0.05$, respectively. Among the categories of positives, the differences in the selenium levels were not significant ($F = 0.29$, $p > 0.05$).

DISCUSSION

We reported higher iron levels in *T. brucei gambiense* infection than the control subjects. Also, most positives were presented with anaemia. This corroborates the report of (Stijemans et al., 2008) who reported increase in tranferrin, ferroportin and ceruloplasmin in trypanosome infection resulting in increased ferritin which skewed towards iron sequestration whose process was suggested to hamper iron export. Also, the increased iron concentration in trypanosome infected individuals has been reported to be injurious because of its possible catalysis of the formation of hydroxyl in which initial reaction with protein, lipids and nucleic acid, forms a cross link. This process has been documented to lead to destruction of red blood cells (Bacon and Britton, 1990). African trypanosomiasis is accompanied by a severe drop in packed cell volume and red blood cell counts due to erythrocyte destruction leading to increased iron levels in peripheral blood (Akol et al., 1986; Shoyinka and Uzoukwu, 1986).

Anaemia has been associated with increased iron concentration (Singh and Mistra, 1986; Ekanem et al., 2009). Our report indicates an increase in zinc levels in HAT volunteers compared with the control subjects. This supports earlier report (Traoré-Leroux et al., 1985) of elevated zinc levels in cattle sensitive to trypanosomiasis than the resistant ones. Elevated zinc levels depressed the stimulation of T- cells by trypanosomes *in vitro* and have been reported to inhibit antigen presentation (Traoré-Leroux et al., 1985). Also, a report has it that zinc supplementation led to an effective host immune response by up-modulating the host's immune response, thus contributing to a reduction of blood parasites and the harmful pathogenic effects (Brazo et al., 2008). We suggest that the increased zinc levels in HAT positive individuals is an indication that zinc may be implicated in the disease pathogenesis in an attempt to ensure parasite clearance and ameliorate disease conditions. Our data show higher levels of copper in HAT volunteers than the control subjects. This increase however failed to confer protection from HAT infection as proposed by Omole and Onawunmi (1979) who reported that supplemental copper fed *T. brucei-gambiense* infected rabbit conferred protection and brought about improved pathological conditions. We therefore suggest that the increase in copper in infected individuals may play a role in the pathogenesis of *T. brucei gambiense* infection instead of the protection observed in animal model.

This investigation revealed no significant difference in the selenium levels of HAT infected volunteers and the non-infected subjects. We suggest that selenium is not directly implicated in the pathogenesis of HAT disease in our locality. In line with this, it has been documented that selenoprotein P, a transporter of selenium could be truncated during trypanosome infection through trypanosome elicited immunologic response (Dreher et al., 1997).

Table 4. Copper status among HAT infected and non-infected human volunteers.

Levels of infection	Seropositives						
	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	14	7	10
Mean (g/L)	0.00072 ± 0.00025		0.0012 ± 0.00076		0.0012 ± 0.00046		0.00057 ± 0.00048
t-values	0.089		16.46		22.4		

Table 5. Selenium profile among HAT infected and non-infected human volunteers.

Levels of infection	Seropositives						
	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	14	7	10
Mean (g/L)	0.00057 ± 0.00012		0.00046 ± 0.00014		0.000532 ± 0.0012		0.00055 ± 0.00014
t-values	0.33		1.50		0.37		

Hence, ensuring the maintenance of similar concentrations of selenium in HAT infected and control subjects. The elevated levels of iron, zinc and copper found among human African trypanosomiasis positives may implicate these trace metals in HAT pathogenesis among volunteers investigated in our locality.

REFERENCES

Akol GWO, Authie E, Pinder M, Moloo SK, Roelonts GE, Murray M (1986). Susceptibility and immune response of zebu and Taurine cattle of West Africa with *Trypanosoma congolense* transmitted by *Glossina morsitans centralis.* Vet. Immunol. Immunopath., 11: 361-373.

Bacon BR, Britton RS (1990). The pathology of hepatic iron overload: a free radical mediated process? Hepatology, 11: 127-137.

Brazo V, Caetano LC, Del Vecchio F, Toldo M, Naiara MPA, Caetano L, Clovis PJ (2008). Zinc supplementation increases resistance to experimental infection by *Trypanosoma cruzi.* Vet. Parasitol., 154(1-2): 32-37.

Bernhard W (1985). Atomic absorption spectrometry. Second edition.VCH Publishers USA and Canada, 303 N.W., 12th Avenue, Deerfield.

Buscher P, Lejon V (2004). Diagnosis of human trypanosomiasis. In: the trypanosomiases. ed Maudlin, I. Holmes, P.H & Miles, A.A. CABI Publishing.

Da Silva SA, Hoehne L, Tonin AA, Zanette AR, Woo P (2009). Trypanosoma evansi: levels of copper, iron, Zinc in the bloodstream of infected rats. Exp. Parasitol., 123(1): 35-38.

David PMP (1934). The protective action of copper against Trypanosoma equiperdum infection in Albino rats. J. Exp. Med., 60: 541-546.

De Souza PA, De Oliveira MG, Neve J, Vanderpas J, Pirmez C, De Castro LS, Jorge-Araujo ST, Rivera TM (2002). Trypanosoma cruzi: host selenium deficiency leads to higher mortality but similar parasitaemia in mice. Exp. Parasitol., 101(4): 193-199.

Dreher I, Jakob T, Kohrle J (1997). Cloning and characterization of the human selenoprotein P. promoter: Response of selenoprotein P expression to cytokines in liver cells. J. Biol. Chem., 272: 29364-29371.

Ekanem TJ, Johnson TO, Balogun AE, (2009). Serum iron and nitric oxide production in *Trypanosoma brucei* infected rats treated with Tetracycline. Biokemistri, 21(1): 41-51.

Lee CM, George GI, Aboke-Cole G (1977). Iron metabolism in *Trypanosoma lewisi* infection: serum iron and serum iron binding capacity. Parasitol. Res., 53(1): 1-6.

Lee CM, Humphrey PA, Aboko-Cole FG (1983). Interaction of nutrition and infection: effect of zinc deficiency on resistance to *Trypanosoma musculi*. Int. J. Biochem., 15 (6): 541-547

Lieu PT, Heiskala M, Peterson PA, Yang Y (2001). The roles of iron in health and diseases. Mol. Aspects Med., 22: 1-87.

Mwangi SM, McOdimba F, Logan-Henfrey L (1995). The effect of *Trypanosoma* brucei brucei infection on rabbit plasma iron and zinc concentrations. Acta Trop., 59(4): 283-291.

Omole JA, Omowunmi OA (1979). Effect of copper on growth and serum constituents of immunized and non-immunized rabbits infected with *Trypanosoma brucei.* Ann. Parasitol. Hum. Comp., 54(5): 495-508.

Ongele EA, Ashrat M, Nesbitt RA, Humphrey PA, Lee CM (2002). Effects of selenium deficiency in the development of trypanosomes and hummoral immune responses in mice infected with *Trypanosoma musculi*. Parasitol. Res., 88(6): 540-545.

Shoyinka SVO, Uzoukwu M (1986). The comparative effect of cortisol and voltaren on anaemia and temperature changes in experimental Trypanosoma brucei brucei infection in rats. J. Comp. Path., 96: 277-284.

Singh BP, Mistra SK (1986). Haematological changes in *Trypanosoma evansi* infection in calves. Ind. J. Vet. Med., 6: 108-109.

Stijlemans B, Vankrunkelsvena A, Brysa L, Mageza S, De Baetseliera P (2008). Role of iron homeostasis in trypanosomiasis-associated anaemia. Immunol., 213(9-10): 823-835.

Traoré-Leroux T, Famoux F, Roelants GE (1985). Correlation of serum zinc levels with resistance of cattle to trypanosomiasis. Acta Trop., 42(1): 39-44.