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Immunoglobulin and haematological profile of Nigerians with *Trypanosoma brucei gambiense* infection

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ABSTRACT

We investigated the immunoglobulin and haematological status of 35 human African trypanosomiasis (HAT) positive subjects and 15 control volunteers in Abraka, Nigeria. For weakly positive (1866.33±5.51mg/dl) and moderately positive (1874.50±4.35mg/dl) HAT volunteers, the IgG levels were higher than the control subjects ($p<0.0001$). Also, strongly positive (3926.33±5.77mg/dl) individuals were higher in IgG levels than the control (944.66±10.51mg/dl) subjects ($p<0.0001$). Concentrations of IgM for strongly positive (485.66±3.05mg/dl) subjects were higher than the control (107.66±6.81mg/dl) individuals ($p<0.0001$). The levels of IgA between early stage (222.66±18.5mg/dl) and late stage (480.33±2.51mg/dl) were not significant ($p>0.05$). In all, mean differences in the IgA levels of seropositive HAT volunteers and control subjects were not significant ($p>0.05$).The concentrations of IgG between HAT late stage (3927±3.6mg/dl) volunteers were higher than individuals in the early stage (1871±4.3mg/dl) at $p<0.0001$. Similarly, IgM levels among volunteers were higher for late stage (480.33±2.51mg/dl) than individuals with the early stage (135.66±2.08mg/dl) of HAT ($p<0.0001$). We observed that the volunteers with late stage were severely anaemic (82 x 10^9 g/dL) while early stage subjects were mildly anaemic (108 x 10^9g/dL). Also, individuals with late stage of HAT showed normocystic hypochromic anaemia. In addition, lower counts of white blood cells (WBC) were observed for HAT late stage, an indication of the condition of leucopinae. Furthermore, mean platelet counts for late stage (154 x 10^9cells/L) and early stage (178 x 10^9cells/L) of HAT were more depressed than their control subject (205 x 10^9cells/L). Conclusively, the indices of elevated serum IgM, IgG could be biomarkers of suspicion of HAT infection. Also, leucopinae and anaemia are complications of HAT infections in our locality.

INTRODUCTION

Infection with the protozoan parasite *Trypanosoma brucei gambiense* causes human African trypanosomiasis (HAT) and is transmitted by haematophagic tsetse fly (1). This disease is responsible for an estimated 500, 000 infection cases per annum (2). Trypanosomiasis has been reported to be associated with altered Immunoglobulin profiles and haematological parameters (3,4).

Serum IgA levels were unaltered in monkeys infected with *T.b. gambiense* (5). Similarly, patients with HAT infection demonstrated variable surface glycoproteins of *T.b. gambiense* in the blood with unaltered IgA levels (6,7).However, increased IgA levels were reported in some HAT patients without anti-galactocerebroside antibody activity (8).

IgG and IgM were increased in serum of *T.b.gambiense*-infected human subjects (7) and have been implicated in the disease pathogenesis (4). Trypanosomes have been documented to induce IgG production while IgM have been suggested to be natural antibodies that were not induced but only amplified (9). Also, IgG and IgM have been proposed to form part of the parasite defence against host immune system and functions to aid survival of trypanosomes in the presence of host antibody (10). Raised IgM levels with Gambian infection and cattle infected with *T. congolense* and *T. vivax* fell to normal concentrations after treatment (11, 12).

Anaemia is a common occurrence in *T. b. gambiense* forms of sleeping sickness (13) characterized by significantly decreased packed cell and erythrocyte volume (14). Normocytic anaemia has been observed in *T. brucei*-infected mice (15) and Nigerian mongrel dogs (16) during the acute phase of *T. b. brucei* infection. Also, microcytic hypochromic has been described in vervet monkeys infected with *T. b. rhodesiense* (17). Suppression of lymphocyte proliferation in response to specific antigens has been documented (18). Severe progressive thrombocytopaenia has been reported in *T.b. rhodesiense* animal models (16,17) and human cases of sleeping sickness (19).

The IgA, IgG and IgM profiles in the serum of HAT patients have been well documented (8,7,4). However, this information is lacking in our locality and we therefore evaluted the immunoglobulin status in some volunteers infected with *T.b. gambiense* in Abraka, Nigeria. Also, we established their relationship with the haematological profile of HAT positive subjects.

MATERIALS AND METHODS

Study area

This study was carried out in Umeghe, Urhouka and Ugono 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. The people in our studied communities are predominantly farmers.

Ethical considerations

This investigation was approved by the Delta State Ministry of Health and Eku Baptist Hospital. Prior to the commencement of this investigation, community mobilization campaign was carried

out where we explained the nature, objectives and benefits of the investigation so as to obtain informed consents.

Population recruited
A total of 474 consented volunteers were screened using card agglutination test for trypanosomiasis (CATT) kit (20). Out of the 44 seropositive volunteers, 35 of them subsequently consented to participate in the present investigation.

Staging of HAT
Sera obtained from venous blood were used to categorize the level of infection by double serial dilution as: weakly positive (1:2-1:4) (n=9), moderately positive (1:8-1:16) (n=12) and strongly positive (≥1:32) (n=14) according to the manufacturer's instruction (Intituut voor Tropische Geneeskunde, Antwerpen, Belgium). Of the 35 seropositives, 16 volunteers demonstrated parasite in the blood and were further categorized into early and late stages of HAT. Volunteers categorized as early stage, demonstrated the parasite in the blood only while late stage showed the parasite both in the blood and CSF. The staging of HAT was determined using (2) criteria.

Exclusion criteria
Volunteers with overt diseases like malaria, viral hepatitis B, HIV, measles, sickle cell anaemia were excluded from this study using standard techniques.

Immunoglobulin and Haematological analysis
5ml of venous blood was collected and Immunoglobulin (IgA, IgG and IgM) profiles were determined by single radial immunodiffusion as modified by (21). Also, haematological parameters were estimated using automated heamatology analyzer (BC-2300).

Data analysis
The data obtained in this investigation were subjected to statistical analysis, namely Welch t-test, Chi-square test and Tukey-analysis of variance (ANOVA) using Instat statistical package.

RESULTS

Table 1 shows the IgA profile of seropositive HAT volunteers and their control subjects. The mean differences in IgA levels for weakly positive (230.66±16.25 mg/dL), moderately positive (231.5±22.86mg/dL) and strongly positive (234.66±18.77pg/dL) HAT volunteers compared with the control subjects (234.66±18.77mg/dL) were not statistically significant at χ^2=0.068, p>0.05; χ^2=0.038p>0.05; χ^2=0.038, p>0.05, respectively. Also, among the 3 categories of seropositive individuals, differences in IgA concentrations were not significant (F=0.97, p>0.05).

Table 2 presents the IgG profile of seropositive HAT volunteers and control subjects. For weakly positive (1866.33±5.51mg/dL) and moderately positive (1874.5±4.34mg/dL) HAT volunteers, the IgG levels were one fold higher than the control subjects (944.66mg/dL) at χ^2= 900, p<0.0001; χ^2= 916, p<0.0001, respectively. Individuals strongly positive (3926.33±5.77mg/dL) with HAT were three times higher in IgG levels than the control subjects (χ^2= 9416, p<0.0001). There was significant difference in IgG concentrations among the 3 seropositive categories (F=154,431, p<0.0001).

The mean concentrations of IgM among weakly positive (116.33±3.51mg/dL) and moderately positive (140.33±2.51mg/dL) HAT individuals were not significantly higher than the control subjects (107.66±6.88mg/dL) at χ^2= 0.75, p>0.0001; χ^2=10.17, p>0.0001, respectively. Concentration of IgM for HAT strongly positives (485.66±3.05mg/dL) was four times higher than the control individuals (χ^2= 1335, p<0.0001). The differences in the levels of IgM for the 3 categories of HAT seropositive subjects were statistically significant (F=13,727, p<0.0001) (Table 3).

Table 4 shows the 3 immunoglobulin profiles among early and late stages of HAT. The levels of IgA between early stage (222.66±18.5mg/dL) and late stage (231.33±12.05mg/dL) of HAT was not significant (t=0.92, 95% CI: -11.92 to 29.92; p>0.05). The concentrations of IgG between late stage (3927±3.6) of HAT were 2 times higher than the early stage (1871±4.3mg/dL) at t=856.05, 95% CI = 2050.8 to 2061.2; p<0.0001. Similarly, IgM levels were 3 times higher for late stage (480.33±2.51mg/dL) than the early stage (135.66±2.05mg/dL) of HAT infection (t = 273.67, 95%CI=341.64 to 347.04, p<0.0001).

Haematological status of volunteers with HAT infection and control subjects is presented in Table 5. We observed that the volunteers with late stage of HAT were severely anaemic (82 x 10^9) g/dL while early stage individuals were mildly anaemic (108 x 10^9g/dL. Also, individuals with late stage of HAT showed a combination of normocytic [Mean Corpuscular Volume (MCV =85.55fl/red cell) was below normal range (82.00-95.00fl/red cell)] and hypochromic [Mean Corpuscular Hemoglobin Concentration (MCHC=29.0fl/red cell) was below the normal range (32.0-36.0 fl/red cell)] anaemia. Normocytic anaemia was also observed for early stage HAT. In addition, lower counts of WBC were observed for HAT late stage (WBC= 2.3 x 10^9 cell/L). Similarly, reduced lymphocyte counts were reported for late stage (1.4 x 10^9 cell/L) and early stage (3.02 x 10^9 cell/L) when compared with the control subjects (3.5 x 10^9 cells/L). Also, granulocyte number for HAT late stage individuals (0.8 x 10^9cells/L) was also depressed. Furthermore, mean platelet counts for late (154 x 10^9cells/L) and early (178 x 10^9cells/L) stages of HAT were depressed compared with the control volunteers (205 x 10^9cells/L). Mean platelet volume for late stage (10.9fl/cell) and early stage (10.15 fl/cell) compared with the control subjects (10.63fl/cell) were seen to be similar.

Table 1: IgA profile of seropositive HAT volunteers and control subjects

Level of infection	Weakly positive n = 9	Moderately positive n = 12	Strongly positive n = 14	Control N = 15
Mean IgA(mg/dL	230.66 ±16.25	231.5±22.86	237.33 ±22.74	234.66±18.77
χ^2	0.068	0.038	0.038	
F-value	0.97			

Table 2: IgG profile of seropositive HAT volunteers and control subjects

Level of infection	Weakly positive n = 9	Moderately positive n = 12	Strongly positive n = 14	Control N = 15
Mean IgG (mg/dL)	1866.33 ±5.51	1874.5±4.35	3926.33 ±5.77	944.66±10.51
χ^2	900	916	9419	
F-value	154431			

Table 3: IgM profile of seropositive HAT volunteers and control subjects

Level of infection	Weakly positive n = 9	Moderately positive n = 12	Strongly positive n = 14	Control N = 15
Mean IgM (mg/dL)	116.33 ±3.51	140.33±2.51	485.66 ±3.05	107.66±6.81
χ^2	0.757	10.17	1335	
F-value	13727			

Table 4: Immunoglobulin profiles of early and late stages of volunteers with HAT

Stage of HAT	Early stage n = 12	Late stage n = 4	Mean difference
Mean IgA (mg/dL)	222.66±18.5	231.33±12.05	t = 0.92 95% CI = -11.92 to 29.92
Mean IgG (mg/dL)	1871±4.3	3927±3.6	t = 856.05 95% CI = 2050.8 to 2061.2
Mean IgM (mg/dL)	135.66±2.08	480.33±2.51	t = 273.67 95% CI= 341.64 to 347.04

Table 5: Hematological status of volunteers with HAT infection and control subjects

Haematological Parameters	Early stage n=12	Late stage n=4	Control n=15
Mean HGB (g/L)	108 x 10^9	82 x 10^9	119.33 x 10^9
Mean MCV (fl/red cells)	86.73	85.55	82.66
Mean MCHC (g Hgb/dLRBC)	28.56	29.0	27.73
Mean WBC cell/L	5.78 x 10^9	2.3 x 10^9	5.03 x 10^9
Mean Lymphocyte cell/L	3.02 x 10^9	1.4 x 10^9	3.5 x 10^9
Mean Granulocyte (cell/L)	2.34 x 10^9	0.8 x 10^9	2.05 x 10^9
Mean Platelet count (cell/L	178.5 x 10^9	154 x 10^9	205 x 10^9
Mean Platelet volume (fl/cell)	10.15	10.9	10.63

DISCUSSION

We reported similar concentration of serum IgA between HAT patients and control subjects. This result corroborates the findings of (7,5). This suggests that IgA may not be implicated in the immunopathology of HAT infection. Anti-galactocerebroside (Anti-GalC) antibodies are distributed between IgM and IgG classes and have been reported to be associated with late pathogenic conditions observed in HAT patients (8). However, increased IgA levels without anti-GalC antibody were observed in some HAT patients. We therefore suggest that the interaction between Anti-GalC antbodies with IgM and IgG classes could be responsible for the regulation of IgA in *T. b. gambiense*-infected individuals.

This data showed that the concentrations of IgG and IgM increased with severity of HAT infection. Also, anaemic conditions were severe for late stage. These findings align with several reports ((6, 7, 11, 9) and hence suggests the involvement of IgG and IgM in the immunopathology of HAT infection. Antiglobulin tests showed that antigen-anibody immune complexes may fix complements on the erythrocyte surface resulting in anaemia probably due to intravascular hemolysis or erythrophagocytosis (13, 22, 23). Furthermore, IgM has been identified in enhancing parasitaemia control and host survival (24).

Hematological parameters showed that both early and late stages of HAT suffered from normocytic hypochromic anaemia. This observation is different from the type of anaemia described in vervet monkeys infected with *T.b. rhodesiense* (17) but similar to the normocytic anaemia observed in *T. brucei*-infected mice (15) or Nigerian mongrel dogs (16). Microcytic anaemia has been associated with iron deficiency as a result of the failure of iron incorporation into red cell precursors or inefficient recovery of iron from phagocytosed red blood cells (25). We therefore hypothesize that the normocytic anaemia reported in this study may likely be related to the ability of RBC to incorporate iron. Also, leucopinae was observed in HAT late stage. This observation is disimilar with the report of (17) where late stage of trypanosomiasis infection resulted in leukocytosis. Our data also showed reduced lymphocyte and granulocyte number compared to early and HAT negative subjects.We therefore suggest that the reduced WBC at late stage compared to the control volunteers could be due to severe suppresion of lymphocyte proliferation in response to increased burden of *T.b. gambiense* infection as documented in *T. brucei*-infected mice (18). Furthermore, platelet counts were within normal range but slightly depressed in both early and late stages when compared with their control subjects. This is in contrast with the report of (19) where thrombocytopinea was reported in humans infected with *T.b. rhodesiense*. However, we suggest that the virulence nature of *T.b. gambiense* parasite may be mildly associated with the slight depletion in platelet cells of HAT infected subjects.

Conclusively, our data have shown that IgG for HAT positive subjects were 1-3 times higher than the control volunteers. Also, 2-4 fold elevation of IgM was observed for human volunteers with *T.b.gambiense* infection. In addition, normocytic hypochromic anaemia was identified among HAT positive volunteers. We therefore suggest that the elevated IgG and IgM among positive subjects may implicate these immunoglobulin classes in the immunopathology of HAT. Also, leucopinae and anaemia may be a complication of HAT infection in our locality

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