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Cytological Indices of Peripheral Blood and Bone Marrow Smears in Albino Rats infected with Trypanosoma Brucei Brucei

Bolaji David AKINBO[1], Adedeji David ATERE[2], Oladele Olatubosun ELUWOLE[3]
[1,3]Haematology Unit, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria
[2]Chemical pathology Unit, Department of Medical Laboratory Science, Achievers University, Owo, Nigeria

Abstract: Trypanosomiasis is a debilitating disease in man and animals which has adversely affected economic and social well-being of sub-Saharan Africans. Haematological parameters were determined in fifty (50) male albino rats, in which thirty five (35) were infected with Typanosoma brucei brucei and the effects of this condition was assessed on peripheral blood and bone marrow cells using cytochemical stains. Data obtained were statistically compared and P<0.05 was considered significant. Rats in infected group had significantly lower HCT, RBC, HGB and MCV levels and significantly higher WBC, MCHC, lymphocytes and mixed cells levels compared with controls. Cytochemical reactions of bone marrow cells revealed that all granulocytes, except myelocytes and basophils, were peroxidase positive. It could be concluded in this study that the infected rat subjects suffered severe haemolytic anaemia and leucocytosis observed in the infected group attributed to the immune response to trypanosome infection.

Keywords: Hematological Indices, Trypanosoma brucei brucei, bone marrow, Albino rats.

Introduction

Trypanosomiasis is a complex and debilitating disease in man and animals which has adversely affected economic and social well-being of sub-Saharan Africans (1, 2). The Human African Trapanosomiasis (HAT) is caused by two subspecies of the protozoan parasite and it is a parasitic disease transmitted by tsetse fly (Glossina Genus) (1, 3). Trypanosomiasis which is also known as sleeping sickness and nagana in man and animals respectively is caused by African trypanosomes (4, 5, 6). The trypanosome species affecting man and his domestic animals have been subdivided into two groups, the haematinic group (Trypanosoma congolense, T. vivax) which remains in the plasma and the tissue invading group (T. brucei, T. evansi, T. gambiense, T. rhodesiense and T. equiperdum) found in extra and intra vascular spaces (7, 4, 3). The invading effects of these parasites in the blood produce numerous changes in the cellular and biochemical constituents of blood (8, 9).

Trypanosoma brucei infection precipitate increased red blood cell destruction which results in anaemia as well as tissue damage like other trypanosome infections (10, 11). These changes together with the need by the host to destroy the parasite are presumably responsible for the symptoms of African sleeping sickness. Haemolymphatic phase in humans, the first stage of the disease, is characterised by clinical features like fever, headaches, joint pains, lethargy and itching. The neurological phase, the second stage, begins when the parasite crosses the blood-brain barrier and invades the central nervous system resulting in clinical features like confusion, sensory disturbances and poor coordination. This is a very crucial stage of this phase because the sleep cycle would be disturbed and this gives the disease its name. (1, 3).

In years back, several other studies have been carried out on the haematology of trypanosomiassis and intermittent episodes of pancytopaenia and sometimes, isolated anaemia, neutropaenia and thrombocytopaenia had been reported in Trypanosome-infected animals (12, 13, 3). But there is paucity of information of the effect of trypanosomes on the haemopoietic activity of the bone marrow and the characteristics of the more primitive cells. The ability of the erythropoietic system to continue to produce normal cells from the stem cell precursors in the presence of infection is quite remarkable (14, 3). Anaemia had been reported to be a critical feature in the pathogenesis of African trypanosomosis (14). Considering a rapid decline in haemamogram and pallor in mucous membranes in the infected hosts, a defect in the bone

marrow, site of origin of those cells, might be a cause. This study was carried out to demonstrate the nature of the changes in the various haemopoietic progenitor precursor cells of the bone marrow and their products in peripheral circulation, associated with the T. brucei brucei infection in Albino rats.

Materials and methods

Fifty (50) male albino rats of average weight between 85.50 ± 5.50g were obtained from the Animal laboratory Unit of Biological Sciences Department, Achievers University Owo, Ondo State, Nigeria. They were housed under standard condition and kept in clean cages with access to clean water and commercial rat pellets ad-libitum. The cages were constantly cleaned in order to prevent the animals from contracting diseases. The principles governing the use of laboratory animals as laid out by Animal Care Committee of Biological Sciences Animal House, AUO on Ethics for Medical and Scientific Research were duly observed.

Grouping

The animals were categorised into three groups as follows:

- Group A: Fifteen Animals were treated with normal saline. They constitute the control group.
- Group B: Eighteen Animals were infected intraperitoneally with T. brucei brucei bled and sacrificed at 1 week post infection(1wpi).
- Group C: Seventeen Animals were infected intraperitoneally with T. brucei brucei bled and sacrificed at 2 weeks post infection(2wpi).

Source of Parasite

Trypanosoma brucei brucei was obtained from the Nigerian Institute for Trypanosoma Research (NITR), Vom – Jos, Nigeria. The parasite was injected into uninfected rats intraperitoneally and maintained in other rats by repeated passaging. Parasitaemia was monitored by preparing a thin film of blood obtained from animal tail and viewing under light microscope at x100 magnification according to the method of Adeyemi et al., (2) and Olayanju et al., (3).

Inoculation of rats with parasite

Parasite infected blood was obtained from the tail of infected rats at high parasitaemia and used to maintain parasite suspension in 0.85% saline solution (1 in 8 dilution) which was inoculated into the peritoneal cavity of uninfected rats weighing approximately between 85.5 ± 5.5g. The suspension – as described by Adeyemi et al., (2) contained 3 or 4 trypanosomes per view at x100 magnification.

Sacrifice of the animals and tissue collection

The animals were anaesthetized using chloroform at 1wpi and 2wpi for the acute stage and chronic stage respectively. Blood samples were drawn into EDTA tubes from the tail vein and heart for haematological parameters.

Haematological studies

Blood parameters including packed cell volume (PCV), white blood cell (WBC), MCV, MCH and MCHC counts were determined using the Automated Haematologic Analyzer, Sysmex, KX-21 (Japan) as described by Olaniyi et al, (15).

Bone marrow preparation

After completion of exposure as described above, the sternal bone was broken into pieces and the marrow was made into Squash preparation and meandering smear on glass slides for cytochemistry stain techniques.

Cytochemistry

Cytochemical staining of the bone marrow and peripheral blood smears was done using special stains and microscopic examination for cellular constituents, such as DNA, glycogen, enzymes, lipid and carbohydrates according to Li and Yam (16). These stains are able to distinguish normal cell types and identify the lineage of poorly differentiated blast cells and

highly proliferating series. The DNA, peroxidase enzyme activity, lipids and glycogen in post infected rat bone marrow and peripheral blood cells were examined using the myeloperoxidase and PAS stains (16).

Statistical Analysis

ANOVA and Student's t-test were used to compare the differences between the means. Data was analyzed using SPSS version 17.0. Value of $P < 0.05$ was considered significant.

Results

The mean blood levels of HCT, RBC, HGB and MCV were significantly lower while the mean blood level of WBC, MCHC, L and M were significantly higher in combined rat subjects exposed to T. brucei compared with controls (Table 1). In Table 2 the mean blood levels of HCT, RBC, HGB and MCV were significantly lower while the mean blood level of WBC, MCHC, L and M were significantly higher in rat subjects in 1wpi compared with controls. Also in Table 2, it was observed that rat subjects in 2wpi had significantly lower mean blood level of PCV, RBC, HGB and MCV but a significantly higher mean blood level of WBC, MCHC, L and M compared with controls. However, there was no significant value when haematological parameters were compared between rat subjects of 1wpi and 2wpi (Table 2).

Table 1: Haematological parameters in rats exposed to T. brucei and controls using student t-test

	Control (n=15)	Treatment (n=35)	P-Value
PCV (%)	35.15±2.42	21.82±2.31	0.000
WBC (10^6/µL)	12.64±1.74	19.41±1.86	0.000
RBC (10^6/µL)	3.95±0.27	2.62±0.28	0.000
HBG (g/dl)	11.85±0.79	7.87±0.87	0.000
MCV (fl)	89.07±1.53	83.40±4.24	0.000
MCH (g/dl)	30.05±0.23	29.70±1.37	0.486
MCHC (pg)	33.72±0.51	36.16±1.98	0.000
N (%)	33.87±4.82	14.46±6.37	0.000
L (%)	60.93±3.39	73.71±3.97	0.000
M (%)	4.07±1.71	12.86±2.79	0.000

Significant (P<0.05)

Table 2: Haematological parameters in rats exposed to T. brucei for 1wpi, 2wpi and controls using one way analysis (ANOVA)

	Control (n=15)	1WPI (n=18)	2WPI (n=17)
PCV (%)	35.15±2.41	22.13±2.45[a]	21.49±2.18[a,b]
WBC (10^6/µL)	12.65±1.74	19.66±2.22[a]	19.14±1.43[a,b]
RBC (10^6/µL)	3.95±0.27	2.62±0.27[a]	2.62±0.31[a,b]
HBG (g/dl)	11.85±0.79	7.88±0.82[a]	7.86±0.94[a,b]
MCV (fl)	89.07±1.53	84.47±5.39[a]	82.27±2.16[a,b]
MCH (g/dl)	30.05±0.23	29.84±0.78	29.55±1.81
MCHC (pg)	33.72±0.51	35.79±2.60[a]	36.55±0.92[a,b]
N (%)	33.87±4.82	13.28±6.84[a]	15.71±5.76[a,b]
L (%)	60.93±3.39	74.89±3.63[a]	72.47±4.05[a,b]
M (%)	4.07±1.71	12.83±3.13[a]	12.88±2.47[a,b]

[a]Significant (P<0.05) when compared with the control group.
[b]Significant (P<0.05) when compared with the 1wpi rat group

Myeloperoxidase staining: Leukocyte Peroxidase activity of the bone marrow and peripheral blood smear was examined using O toluidine staining techniques. Peroxidase activities were strongly positive.

Periodic Acid Schiff (PAS) for glycogen: PAS is a technique that is used for the demonstration of glycogen in tissues. Post-infected bone marrow showed numerous megakaryocytes showing a bright red color. Strong positivity for the periodic

acid Schiff reaction indicate the presence of glycogen in these cells which can usually result from an active gluconeogenesis resulting from the constant utilization of cellular glycogen by the trypanosomes.

Discussion

The invading effects of Trypanosoma brucei in the blood had been reported to produce numerous changes in the cellular and biochemical constituents of blood (8, 9). Igbokwe and Nwosu, (17) reported that Trypanosome infection may cause anaemia as a result of massive erythrophagocytosis by an expanded and active mononuclear phagocytic system (MPS) of the host. Haematological parameters obtained in this study agree with earlier studies (18, 19, 20).
Cytochemical stains on bone marrow precursor cells also were found to corroborate the haematological findings by distinguishing normal cell types and identify the lineage of poorly differentiated blast cells and highly proliferating series. Review of literature show paucity of information on the effect of trypanosomes on the haemopoietic activity of the bone marrow and the characteristics of the more primitive cells. Post-infection bone marrow granulocytic series cells, except myeloblasts, reacted positively to PAS. Some lymphocytes and monocytes megakaryocytes and thrombocytes were PAS positive which is in agreement with the observations recorded in diabetic rats (21) and this is indicative of an active gluconeogenesis resulting from the constant utilization of cellular glycogen by the trypanosomes.

Haematological parameters show significant low levels of Hct, haemoglobin concentration and red blood cells count observed in infected groups compared to controls may be as a result of acute haemolysis due to growing infection. Significant reduction in Hct and MCV in infected rats group compared to control group is suggestive of microcytic anaemia. Similarly, decrease in the level of haemoglobin concentration was found to be statistically significant. This observation might be due to haemolytic anaemia. However, the significant decrease in haematocrit, haemoglobin and red blood cell count are in agreement with some recent reports (11, 3). This might be the reason why post-infection smears show significant higher stain uptake than observed in control smears which may be due to the haemopoietic activity of the bone marrow and the characteristics of the more primitive cells.

Previous studies have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably as a result of depletion of reduced glutathione on the surface of the red blood cell (9, 11, 3). Our result also showed significant increase of MCHC in infected group compared with control group. Similarly, in this study, almost all the cell types in the bone smears of the infected rats showed evidence of peroxidase except myeloblasts and basophils. These might be an indication that the qualitative bone marrow output was more during trypanosome brucei infection in order to compensate for haemolytic anaemia observed.

The higher counts of WBC, lymphocytes and neutrophil observed in the infected group may be attributed to the immune response to trypanosome infection (7, 10, 3). Leucocytosis which may be due to lymphocytosis and eosinophilia have been implicated in trypanosomosis and these conditions are usually as a result of wax and wear syndrome on the animal immune system caused by the ever changing variable surface glycoprotein of the infecting trypanosomes (7).

Conclusion and Recommendation

In conclusion, this study shows that infected rat subjects have severe haemolytic anaemia and immunosuppressive condition. Therefore administration of drugs that have potentials for modulating the state of anaemia and immunosuppressive conditions in trypanosome infected rats would go a very long way in managing the condition. Conclusively, prevention of infection is being advocated to avert the possible morbidity that may arise from the poor haematological features which has been linked to Trypanosomiasis.

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