

**Research Article****Acute and Chronic Effects of *Trypanosoma Brucei Brucei* Experimental Infection on Bone Marrow and Peripheral Blood Cells in Wistar Rats**Yusuf O.S.<sup>1</sup>, Oseni B.S.<sup>2</sup>, Olayanju A.O.<sup>1</sup>, Hassan M.A.<sup>1</sup>, Ademosun A.A.<sup>1</sup> and Akele R.Y.<sup>3</sup>.<sup>1</sup>Department of Haematology and Blood Transfusion, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria<sup>2</sup>Department of Biomedical Sciences, Ladoke Akintola University of Technology, Osogbo Campus, Osun State.<sup>3</sup>Medical Centre, Joseph Ayo Babalola, Ikeji Arakeji, Osun State.**\*Corresponding author**

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**Abstract:** Peripheral and bone marrow cell changes due to *Trypanosoma brucei brucei* infection were studied in forty (40) adult male wistar rats weighing  $200 \pm 50$ g. Thirty (30) rats were infected intraperitoneally with *Trypanosoma brucei* while ten (10) uninfected rats served as control. At the acute stage of 6 days post infection (dpi) and chronic stage of 15 dpi, blood samples were obtained. Haematological investigations on the samples included Packed Cell Volume (PCV), Haemoglobin Concentration (Hb), Red Blood Cell Count (RBC), White Blood Cell Count (WBC), Platelet Counts, Reticulocyte Counts, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and Myeloid-Erythroid (M:E) ratio estimation of bone marrow cells. At the acute stage, there was significant ( $P < 0.05$ ) decrease in PCV, Hb, RBC and Platelet count while WBC and Reticulocyte Count increased when compared to control. During the chronic stage, PCV, Hb, MCV and platelet count decreased ( $P < 0.05$ ) while increases were recorded for WBC, RBC and reticulocyte counts compared to control values. Myeloid erythroid (M:E) ratio changed from 3:1 to 3:3 but bone marrow cells were hypochromic, with increased erythroid cell population and giant macrophages. It was observed that hypochromic microcytic anaemia, thrombocytopenia, leucocytosis (as a result of lymphocytosis and eosinophilia) and reticulocytosis are the most important haematological changes associated with infection of *Trypanosoma brucei brucei* in rats.

**Keywords:** *Trypanosoma brucei brucei*, bone marrow, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH)

**INTRODUCTION**

Human African Trypanosomiasis, is a parasitic disease transmitted by tsetse fly (*Glossina* Genus), caused by a protozoan parasite belonging to the genus *Trypanosoma*[1]. Trypanosome species affecting man and his domestic animals have been subdivided into two groups, the haematinic group (*Trypanosoma congolense*, *Trypanosoma vivax*) which remains in the plasma and the tissue invading group (*T. brucei*, *T. evansi*, *T. gambiense*, *T. rodesiense* and *T. equiperdum*) which is found extravascularly and intravascularly in man and rats [2-4].

African trypanosomes that belong to the *T. brucei* species cause Human African Trypanosomiasis (HAT) and Nagana, a wasting disease of animals [5]. In humans, the first stage of the disease, known as haemolymphatic phase is characterised by bouts of fever, headaches, joint pains and itching. The second stage (the neurological phase) begins when the parasite crosses the blood-brain barrier and invades the central nervous system resulting in clinical signs and symptoms such as confusion, sensory disturbances and poor coordination. Disturbance of the sleep cycle, which gives the disease its name, is an important feature of the second stage of the disease[1].

Trypanosomes are known to invade the immune response of their mammalian host. In order to evade the humoral immune system and host antibodies, they possess variable surface glycoprotein (VSG) coat on the cell surface, which undergoes constant variation and is responsible for the vast diversity of the parasite. African trypanosomiasis caused by *Trypanosoma brucei* species is fatal in both humans and animals and cannot be combated by vaccination because of extensive parasite antigenic variation [6].

Blood cells are important indicators of health status and they are invaluable in diagnosis, treatment and prognosis for many diseases [7]. Trypanosomiasis is associated with a rapid decline in red blood cell (RBC) counts, haemoglobin (Hb) concentration and packed cell volume (PCV) and pallor in mucous membranes in the infected hosts, confirming that anaemia is a critical feature in the pathogenesis of African trypanosomiasis [8]. Several other studies have been carried out on the haematology of trypanosomiasis [9-11]. But there is dearth 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 [8]. Trypanosome-infected animals experience intermittent episodes of pancytopenia including anaemia, leucopenia and thrombocytopenia. Considering the multiple blood cell lineages affected by the disease, a defect in the bone marrow, site of origin of those cells, might be a cause. Wells *et al.*, [12] had reported that transmission electron microscopy confirmed that intact trypanosomes were not observed in the sinusoids and haemopoietic compartment of the bone marrow. It is unclear if a qualitative or quantitative disturbance of the bone marrow cells can result from trypanosome infection and the mechanisms that may produce such effects.

The present study was carried out to compare the haemopoietic progenitor precursor cells of the bone marrow and their products in peripheral circulation during *T. brucei brucei* infection in rats.

## MATERIALS AND METHODS

### Animals

Fourty (40) male albino rats of the Wistar strain weighing between  $200 \pm 50$ g were used for this study. The animals were obtained from the Small Animal House of Ladoke Akintola University of Technology, Ogbomosho (LAUTECH), and then, separated randomly into eight cages of five rats each (two cages per group) where they were kept for four weeks before the start of the experiment. The animals were housed under standard conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $55 \pm 15\%$ ) and 12 hour light (7.00 am - 7.00 pm). The cages were constantly cleaned in order to prevent the animals from contracting disease. They were fed with standard commercial rat pellets (Ladokun Feeds Limited, Nigeria) and allowed water ad libitum. The Animal Care Committee of Biomedical Science Animal House, LAUTECH, Osogbo approved the experimental protocols.

### Grouping

The animals were divided into three groups as follows:

- Group One: Animals were treated with normal saline. They were the overall control group.
- Group Two: Animals were infected intraperitoneally with *T. brucei brucei* bled and sacrificed at 6 days post infection.
- Group Three: Animals were infected intraperitoneally with *T. brucei brucei* bled and sacrificed at 15 days post infection.

### Source of organism

*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.* [13].

### 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  $200 \pm 50$ g. The suspension – as described by Ekanem *et al.* [14] contained 3 or 4 trypanosomes per view at x100 magnification.

### Experimental Design

The test animals were infected by intraperitoneal injection with trypanosomes diluted 1:8 in one ml of Phosphate buffer saline while the control remain uninfected. Before and during the course of infection, a daily clinical evaluation was carried out. The animals were anaesthetized using chloroform at 6 dpi and 15dpi for the acute stage and chronic stage respectively. Blood samples were drawn into EDTA tubes from the tail vein for Full Blood Count including Reticulocyte Count and Platelet Count after which the animals were sacrificed. The sternal bone was broken into pieces and the marrow was made into Squash preparation and meandering smear on glass slides. Data was analysed using the Statsview® statistical program. Significance of differences was determined by ANOVA and t-test using Statview for Windows Version 5.0.1 (SAS Institute Inc, 1995-1998, Cary, NC).  $P < 0.05$  was considered significant.

## RESULTS

Haematocrit, RBC and Hb concentration in both test groups were significantly ( $P < 0.05$ ) reduced compared to control (Figure 1). Values obtained in the acute stage of infection were lesser ( $P < 0.05$ ) than the chronic stage. The MCH and MCHC were higher ( $P < 0.05$ ) than control values, but the difference between a higher value at the acute stage of infection compared to the chronic stage was not statistically significant (Figure 2). MCV was increased in contrast to the other absolute values. The increase progressed in the acute and chronic stage and was statistically different ( $P < 0.05$ ) from control and between both groups (Figure 2).

Decreases in white cell count and platelets (Figure 3) were similar to those of erythrocyte. The changes were statistically significant ( $P < 0.05$ ) but only the Platelets count showed a statistically different value when the chronic and acute stage were compared.

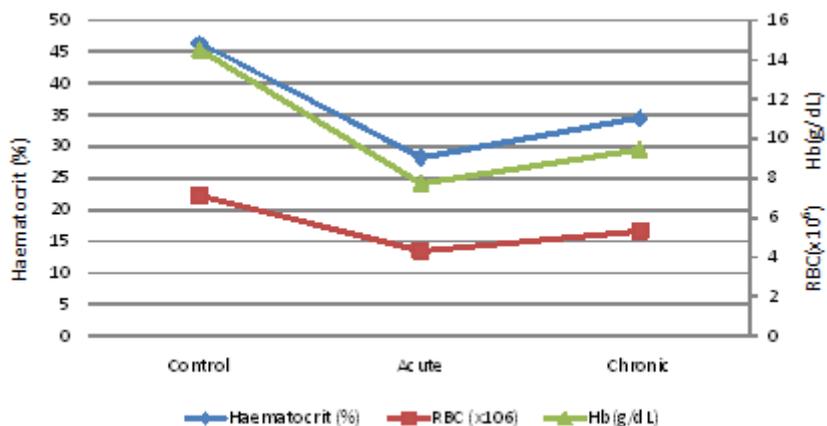


Figure 1: Haematocrit, RBC and Hb values of Control and animals infected with *Trypanosoma brucei brucei* at Chronic and Acute stages

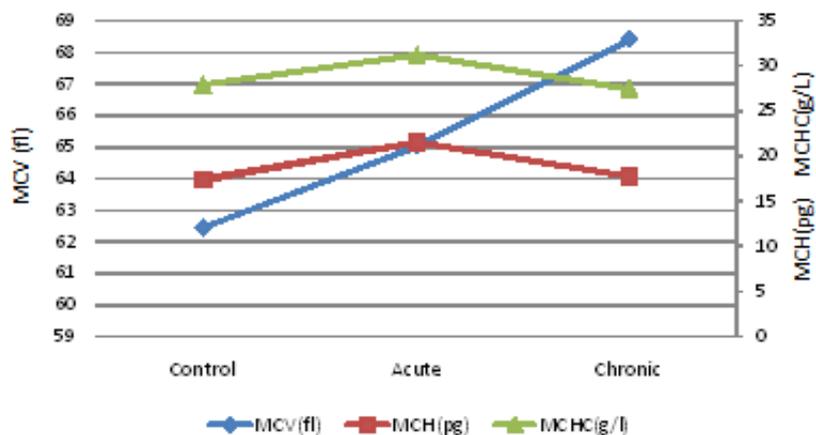


Figure 2: MCV, MCH and MCHC values of Control and animals infected with *Trypanosoma brucei brucei* at Chronic and Acute stages

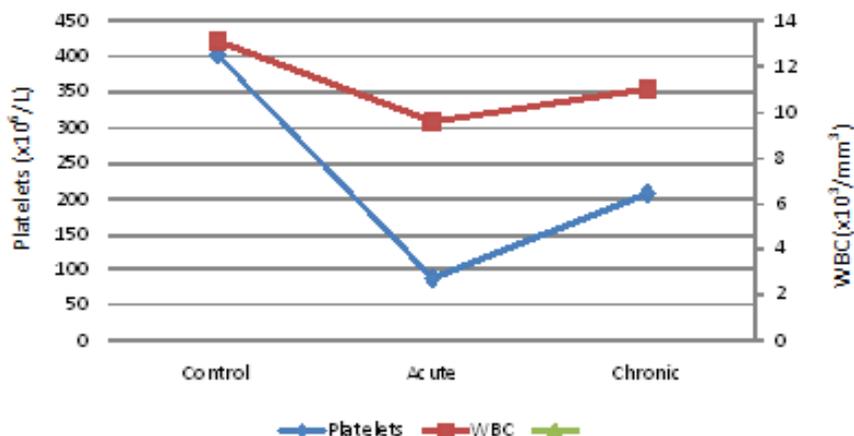
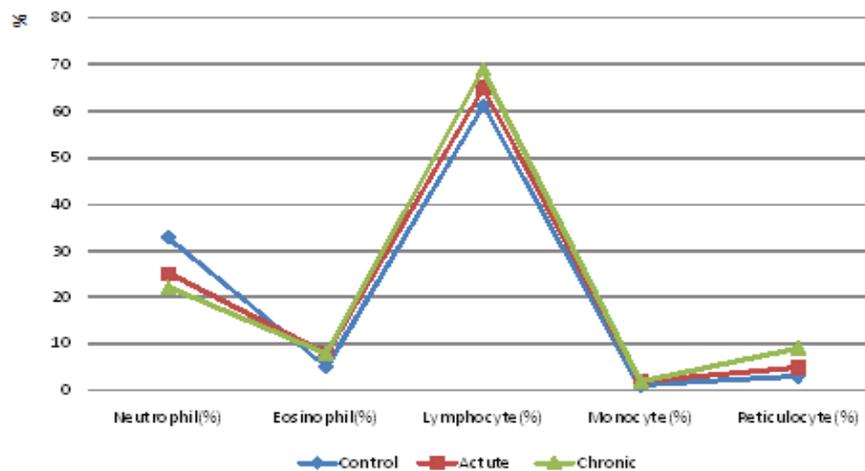


Figure 3: Platelet count and WBC of Control and animals infected with *Trypanosoma brucei brucei* at Chronic and Acute stages



**Figure 4: DLC and Reticulocyte count of Control and animals infected with *Trypanosoma brucei brucei* at Chronic and Acute stages**

### Blood Films

At the acute stage red cells showed slight to moderate hypochromasia and microcytosis. Platelet and red cells decreased in number and there was increase in lymphocytes from 61-69% and eosinophil from 5-8%. Chronic infection caused hypochromasia and microcytosis with few nucleated cells undergoing megaloblastic maturation. Reticulocyte counts was raised (3-9%) in all stages of the infection.

### Bone Marrow Films

At the acute stage bone marrow film showed normal Myeloid – Erythroid (M:E) ratio with abundant distribution of fat cells. Few immature red cells and adequate number of platelets seen. At 7 days post infection (dpi) M:E ratio increased with abundant mononuclear phagocytic cells with few showing erythrophagocytosis. Many atypical mature and immature lymphocytes with few megakaryocytes seen.

At chronic stage, bone marrow films showed hypercellularity with the erythroid line population markedly predominant.

Erythroblastic island was markedly reduced with few nucleated red cells surrounding nurse cell, very scanty mature red cells with many red cells undergoing mitotic division with megaloblastic maturation. There were numerous macrophages with ingested red cells (erythrophagocytosis) and macrophages of epitheloid series. Numerous giant macrophages present. Few granulomatous reaction seen with and without trypanosome parasites.

In the white cell series numerous promyelocytes and myelocytes present with reduced population of lymphoid series which were predominantly small lymphocytes. Few megakaryocytes present.

### DISCUSSION

In the present study, there was significant fall in haematocrit, haemoglobin concentration, red blood cell count and platelet count compared to control depicting hypochromic anaemia and thrombocytopenia which are principal manifestations in acute trypanosomiasis (Figure 1).

Haematocrit and haemoglobin concentration were significantly reduced during the chronic stage of infection, but there was no statistically significant difference in the red blood cell count due to compensatory increase in erythropoiesis (Figure 1).

Therefore, anaemia occurred in all stages of the infection and was due to intravascular haemolysis and phagocytosis [15]. Oxidative damage as a result of reduced glutathione on the membrane of the red cells may also be a contributing factor. Igbokwe and Nwosu [16] 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.

The anaemia in both stages was microcytic and hypochromic, increases noted in MCV and MCH are typically haemolytic anaemias. MCHC increased slightly at the acute stage of infection and fell below control values during the chronic stage of infection. MCHC is a more reliable predictor in the classification of anaemia. The pattern of the MCHC suggests that the qualitative bone marrow output was more during the acute stage of infection and became reduced in the chronic stage (Figure 2). This suggests that the compensatory increase in erythropoiesis was ineffective at the acute stage and more so during the chronic stage of infection.

There was marked erythroid hyperplasia in response to anaemia, evident by a M:E ratio of 3:3 as against the normal 3:1 in the bone marrow. Macrophages were a prominent feature in the bone marrow film. The maturation of cells in the erythrocyte series is closely linked to the activity of macrophages (transformed monocytes), which phagocytose nuclei expelled from normoblasts and iron from senescent erythrocytes, and pass these cell components on to developing erythrocytes.

The report of Shaw *et al.*, [9] suggested that the presence of macrophages in the bone marrow may result from their entry through the sinusoidal epithelium into the haemopoietic compartment. In a more recent study, Wells *et al.*, 2005 had noted that transmission electron microscopy confirmed that intact trypanosomes were not observed in the sinusoids and haemopoietic compartment of the bone marrow. It is possible that the trypanosome enter the marrow compartment, but are rapidly phagocytosed by the macrophages.

The total white cell count increase (leukocytosis) observed in this study (Figure 3) was a physiological increase as a consequence of Lymphocytosis and eosinophilia (Figure 4). These cells are required for immunological response against the invading parasite and modulation of allergic inflammatory response respectively [17]. The report of John *et al.* [18], a similar study using vervet monkeys is consistent with this finding.

Thrombocytopenia in the infected rats was as a result of hypertrophy of the reticuloendothelial system. Splenomegally observed in the sacrificed rats suggests platelets and other cells were trapped in the spleen and destroyed, resulting in a decrease count in the peripheral blood.

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