Blood Cellular Haematological Changes in Patients with Visceral Leishmaniasis Attending Kimalel Sub-County Hospital, Baringo County

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ABSTRACT

Background: Visceral Leishmaniasis (VL) or Kala-azar is a chronic infectious disease caused by parasites of the Leishmania donovani complex that can cause various hematologic manifestations. Anemia is the most common hematological manifestation but the disease may also be associated with leucopenia, thrombocytopenia, and hemophagocytosis and disseminated intravascular coagulation. VL is characterized by fever, hepatomegaly, and splenomegaly and weight loss. Diagnostic as well as clinical symptomatology is non-specific in VL; the diagnostic mechanisms utilized are both invasive and time consuming. Using hematological blood cell parameter changes as a unique indicator of VL will pave way for fast Turnaround Time (TAT) and management. Objectives: to determine unique blood cellular changes that can be used to aid in early diagnosis of visceral leishmaniasis. Study subjects: seventy four participants diagnosed with visceral leishmaniasis. Materials and Methods: splenic aspirates stained with Giemsa stain were examined for detecting LD bodies. Complete blood cell count and differential counts, red blood cells examination were done. Results: There were no significant unique blood cellular changes that can be used for early diagnosis and detection of VL. Conclusions: While cellular changes are noted in VL, they may not be solely significant in the diagnosis of VL.

Keywords: Visceral leishmaniasis, unique blood cellular changes, complete blood and differential counts

1. INTRODUCTION

Leishmaniasis is a protozoan parasitic infestation, associated with three main types of disease patterns: visceral, cutaneous, post kala-azar dermal leishmaniasis (PKDL) and mucocutaneous leishmaniasis. Different manifestations are found in different forms of leishmaniasis. Visceral leishmaniasis (VL) manifests as fever, splenomegaly, hepatomegaly, lymphadenopathy and pancytopenia. The VL or Kala azar is endemic in more than 60 countries worldwide Murray, 2002[1] including Southern Europe, North Africa, Eastern Africa, the Middle East, Central and South America and the Asia subcontinent. In Kenya, the VL disease is endemic in Turkana, Baringo, Kitui, West Pokot, Machakos, Mwingi, Meru, Wajir, Mandera, Keiyo, Kajiado and Marakwet counties Tunui,2006, Fendall,1961, Ngoka et al,1978 and Siongok et al,1982 [2, 3, 4, 5]. The VL is potentially fatal if untreated, diagnosis of the disease rests on microscopic demonstration of amastigotes from the spleen, bone marrow, lymph
nodes, or by PCR. These procedures are invasive and require skilled clinicians to undertake them. Splenic aspiration carries the added risk of intraperitoneal bleeding, Sundar et al., 2002[6]. The rk39 rapid diagnostic test (RDT) and direct agglutination test (DAT) introduced in the 1990s for leishmania antibody/antigen detection respectively, in Kala-azar endemic areas of Kenya were severely constrained with limited sensitivity and specificity Chappuis et al, 2006[7].

Recent studies in VL endemic areas of Baringo and West Pokot counties of Kenya using serum for RDT kit DiaMed-IT LEISH (DiaMed AG Switzerland) and Signal KA (Span Diagnostic, India) Cunningham et al, 2012[8] showed sensitivity and specificity of 80% to 95%. Nevertheless, the cold chain requirement of the Signal KA (storage at 2-8°C) is a major obstacle for field use in most VL endemic areas. The major limitation of these serological tests is that though antibodies decrease after successful treatment they remain detectable up to several years after cure. This has led to the continuous use of the current invasive diagnostic methods. Therefore, this study was designed to help develop alternative convenient noninvasive and simple tests to replace the invasive tests for diagnosis of VL.

2. MATERIALS AND METHODS

Splenic aspirates and blood samples were collected from 74-screened suspects of VL seeking medical care at the Kimalel sub-county hospital, Baringo County. Qualified clinicians collected and prepared smears for the splenic aspirates, collected venous blood for a complete blood count and peripheral blood film preparation at the hospital's laboratory. Giemsa staining was performed on all the splenic aspirate smears using standard operating procedures (SOPs) and examined under oil immersion for Leishmania donovani (LD) bodies and assessment of cellular changes in the cohort. Complete blood counts were analyzed using a fully automated hematology analyzer after performance and evaluation of quality controls. The parameters of interest included: white blood cells with their differentials, platelets, red blood cells and their indices.

Preparation of Thin Blood Films for Differential Count

Blood in EDTA anticoagulant was mixed thoroughly, a small drop of blood (about 2-3 mm in diameter) placed on a clean slide on the bench, and a thin film prepared using a spreader. The films were labeled with the participants initials and study numbers at the frost ends of the slides.

Films were air-dried and fixed with absolute methanol then stained with 10% Giemsa stain for 10 minutes. The stain was gently rinsed using tap water and left to air dry and examined under oil immersion. Percentages were used for all counted white blood cells while platelets and RBCs were also examined for any morphological variations.

Quality control

AcT Cell Control Plus (commercial cell controls) for Abnormal Low, Normal and Abnormal High levels of cell controls were run daily when analysis was conducted. All reagents were used according to the manufacturer’s recommendations and within their expiration dates. Two smears of splenic aspirates/bone marrow and peripheral blood films for differential counts were prepared, stained with 10% Giemsa stain and examined independently by two qualified Technologists. Freshly prepared 10% Giemsa stain was used daily.

Statistical approach

ANOVA and independent samples t-test were used to test significance of the results at an alpha value of 0.05. Using the statistical package software for social scientists (SPSS) Version 20.

Ethical approval

The use of samples drawn from human subjects and all experimental protocols was reviewed and approved by both Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (Approval No. P566/09/2014).

3. RESULTS

Whole blood from 74 diagnosed positive cases of VL was analyzed for various blood cellular changes. The study targeted age groups 1 to 60 years old of all gender as shown below.

Majority of the patients seen in this cohort were males at 58.1% (n = 43) and the females at 41.9% (n = 31). The largest age group was 1-17 year accounting for 55.4% (n = 41) with males accounting for 28.4 % (n = 21) of this age group and 27 % (n = 20) for the females. This was closely followed by patients aged 18 years and above at 44.6% (n=33) and 29.7% (n=22) of the population were males above 18 years old while females were 14.9% (n=11) of the same age bracket (Table 1).
The hematological red cell analytical parameters including the ranges, mean and the SD for both categories of patients are generally low as indicated in the table except for the MCV mean of 83.43. The p-values for both gender ranged from 0.63 to 0.96.

**Table 2: t-test for red blood cells and red blood cell indices for the studied population**

<table>
<thead>
<tr>
<th>Hematological Parameter</th>
<th>MALE (N=43)</th>
<th>FEMALE (N=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>WHO Range</td>
</tr>
<tr>
<td>RBC's (x10^12/µl)</td>
<td>1.19-3.85</td>
<td>4.5-6.0</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>3.8-9.8</td>
<td>14-18</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>12-33.5</td>
<td>35-47</td>
</tr>
<tr>
<td>MCV (%)</td>
<td>56.2-103.2</td>
<td>80-99.9</td>
</tr>
<tr>
<td>MCH (P)</td>
<td>16.2-32.1</td>
<td>27-31</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>25.6-42.9</td>
<td>33-37</td>
</tr>
</tbody>
</table>

**Table 3: White blood cells and platelet changes in leishmaniasis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WHO range</th>
<th>MALE (N=43)</th>
<th>FEMALE (N=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>S.D</td>
</tr>
<tr>
<td>PLT (10^3/µl)</td>
<td>150-450</td>
<td>21-225</td>
<td>74.55</td>
</tr>
<tr>
<td>WBC (x10^3/µl)</td>
<td>4.5-10.5</td>
<td>0.6-3.3</td>
<td>1.8</td>
</tr>
<tr>
<td>NEUT</td>
<td>45-75</td>
<td>29-71</td>
<td>50</td>
</tr>
<tr>
<td>LYMPH</td>
<td>25-45</td>
<td>26-70</td>
<td>49</td>
</tr>
<tr>
<td>MONO</td>
<td>2-10</td>
<td>0-5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Neut-Neutrophils, Lymph-Lymphocytes, Mono-Monocytes

The mean total WBC count was 2.0 and 1.8 while the platelet counts were 100.3 and 74.6 for females and males respectively. The means for the differential white blood cells were: neutrophils (50, 53), lymphocytes (49, 45), monocytes (1.47, 1.97) for males and females respectively. The p-values were 0.0001 and 0.1107 for WBC and the platelets respectively. The p-value for the differentials ranged from 0.0971 to 0.3094.

### 4. DISCUSSION

The VL is associated with the following hematological changes: general reduction in WBC, RBC and platelets in infected patients. Among the WBC, differentials changes have been noted in neutrophils and eosinophils reduction, lymphocytes increase and platelets reduction Dash et al 1991[9]. Similarly, the finding in this study agrees with studies undertaken by Singh k. et al 1999[10]. However, the present findings...
contradict previous report by Agrawal et al, 2013 [11] due to variation in the study age groups. Agrawal and colleagues focused on common age group below 30 years while the present study focused on age group from 1-60 years however, the lowest age group in this study was 6 years.

The findings in this study may be associated with infiltration of the bone marrow by the parasite, which is associated with elytroid hyperplasia, increased plasma cells and intracellular parasites in mononuclear phagocytes. There is pancytopenia which occurs because of splenic sequestration of blood cells. Pancytopenia is the combined reduction in RBCs, WBCs and platelets in the peripheral blood below lower limits of normal range. It is the combination of anemia, leucopenia and thrombocytopenia. This may result from decreased production of blood cells or bone marrow suppression.

While other studies have indicated a lack of utility of hematological findings in the diagnosis VL, these findings are in tandem with previous study findings. The p-value for platelets 0.1107 and those of differentials ranged from 0.0971 to 0.3094 are not significant while the p-value for WBC 0.0001 was significant. This could be attributed to duration of the illness. However, this finding may not be relevant in aiding in diagnosis because of its non-specificity.

In this study, RBCs, Hb, and PCV values were found to be low in both male and female patients, and anemia of hypochromic, normocytic and microcytic was found in all study participants. This agrees with a study by Al Jurrayan and co-workers, 1995 [12]. There was thrombocytopenia in all the study patients which is similar with studies by Dube et al, 1995 [13] and Middib et al. 2014 who observed low platelets in the participants.

The total leukocytes (white blood cells, WBCs) were significantly reduced compared to World held organization WHO normal values given (Table 3). This is similar to studies by Al-Muhammad et al., 2004[14] who found that infected age groups had significantly lower levels of total leucocytes.

The values of lymphocytes were higher than WHO normal ranges shown in table 3 differing with other studies by, middib MM. et al. 2014[15] who found lymphocytes to be within normal range except in one patient who had low percentage.

Monocytes were found to be lower in our study in both infected age groups. The values of monocytes percentage of both age groups of VL recorded non-significant difference with differential leucocyte count percentage (neutrophils, lymphocytes and monocytes) as shown in table 3.

The most affected patients who participated in the study were the minors under 18 years and were mostly school going children (Table 1).

5. CONCLUSION

While cellular changes are noted in VL, they may not be solely significant in the diagnosis of VL.

6. RECOMMENDATION

Cellular changes should be combined with other specific diagnostic modalities in the diagnosis of VL.

REFERENCES

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