ORIGINAL ARTICLE

Intestinal Helminthiasis and its Impact on Haematological Parameters of Patients with Endemic Burkitt’s Lymphoma in Northern Nigeria: Clinical Implications on Cancer Chemotherapy

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ABSTRACT

\textbf{Background:} Endemic Burkitt’s Lymphoma (eBL) and intestinal helminthiasis are common morbidities in Nigeria and share similar epidemiology with respect to age, tropical climate, underdevelopment and poverty. Hence, we predicted that eBL patients with helminthiasis will have a higher risk of anaemia than their counterparts without helminthiasis.

\textbf{Objective:} To study the prevalence of intestinal helminthiasis among eBL patients, and determine relative risk of anaemia in eBL patients with and without helminthiasis before commencing cancer chemotherapy.

\textbf{Methodology:} Retrospective analysis of haematological parameters and stool microscopy data of eBL patients diagnosed in an aggregate period of 36 years at different time intervals between 1995-2013 in five northern Nigerian tertiary hospitals.

\textbf{Result:} Out of 312 eBL patients, 233(74.7\%) had helminthiasis. Soil transmitted helminths were predominant. Compared to their counterparts without helminthiasis, eBL patients with helminthiasis had higher mean eosinophil count (0.48 vs. 0.3x10^9/l, p=0.01), higher mean platelet count (405 vs. 236x10^9/l, p=0.007), lower mean corpuscular volume (77.2 vs. 83.3fl, p=0.02) and higher prevalence of anaemia (65.2\% vs. 13.9\%, p=0.008). Patients with helminthiasis have high relative risk of anaemia (RR=3.4, CI\textsubscript{95\%}: 2.3-4.5, p=0.006).

\textbf{Conclusion:} Prevalence of helminthiasis in eBL patients is high and strongly associated with microcytic anaemia. Helminthiasis and anaemia are undesirable in eBL patients because cancer chemotherapy would potentially depress marrow and immunity, disseminate helminthiasis, aggravate anaemia and increase risk of transfusion. Hence, there is need for mandatory pre-chemotherapy stool screening for helminthiasis among eBL patients. Infected patients should be de-wormed before commencing chemotherapy, while nutritional and iron supplementation should be offered to anaemic patients.

\textbf{Key words:} Gastrointestinal Parasites, Prevalence, Malignancy, Iron Deficiency, Anaemia
INTRODUCTION

African endemic Burkitt’s lymphoma (eBL) is a high grade B-cell non-Hodgkin’s lymphoma that is geographically and climatically associated with low altitude, high annual temperatures and rainfall, and elevated atmospheric humidity. These geographic and climatic factors favour the reproductive rates and survival of *Anopheles* mosquito, which ensures high transmission rate of *Plasmodium falciparum* malaria. Hence, in malaria endemic countries, the spatial distribution and risk of eBL was found to strongly correlate with regional intensity of malaria transmission and concurrent infections with multiple *P. falciparum* genotypes. Conversely, the inheritance of certain genetic polymorphisms such as the sickle cell trait and blood group-O that protect against severe malaria have been associated with lower risks of eBL.

Moreover, eBL is also associated with low socioeconomic status and poverty, both of which are common in malaria endemic countries of the tropics. High prevalence of Epstein-Barr virus (EBV) infection acts in concert with malaria in the pathogenesis of eBL. Therefore, co-infection with *P. falciparum* and EBV is a major risk factor for eBL, which is one of the most prevalent paediatric cancers in the malaria holoendemic zones of equatorial Africa.

Recurrent and chronic immune stimulation by *P. falciparum* malaria is thought to cause immunosuppression by depressing EBV-specific T-cell immunity, which causes a deregulated proliferation of EBV infected B cells. Furthermore, it has been demonstrated that haemozoin, the pigment produced by the metabolic effect of *P. falciparum* on haemoglobin, was partly responsible for inducing a deregulated expression of the DNA-mutating and double-strand-breaking enzyme referred to as ‘activation-induced cytidine deaminase’ in infected B-cells. Consequently, the resultant deregulated B-cells develop genomic instability and have greater risks of rearrangement of the c-myc oncogene, which is subsequently activated by translocation to the proximity of a high transcription gene such as the IgH gene: t(8;14) or the kappa IgL gene: t(2;8) or the lambda IgL gene: t(8;22). Rearrangement and activation of c-myc oncogene together with EBV-induced perturbation of apoptosis within infected B cells cause clonal malignant transformation and eventually culminate in the development of eBL.

On the one hand, eBL is among the most common childhood cancers in Nigeria where the prevalence ranges from 18% to as high as 65.0% of all childhood malignant tumours; and it mainly affects children of low socioeconomic class that are between the ages of 4 and 7 years with a male to female sex ratio of about 2:1. On the other hand, intestinal helminthiasis is also very common in Nigeria, wherein it also mainly affects children and the prevalence may reach as high as 60% in the poorer, less hygienic and socio-economically deprived rural communities. It is therefore obvious that the epidemiology of eBL suggests strong correlation with childhood, poverty, malnutrition, tropical environment, underdevelopment and poor sanitation, all of which also strongly correlate with high risk of intestinal helminthiasis.
Based on this epidemiological ‘convergence’ between eBL and intestinal helminthiasis, we predicted that a remarkably high proportion of patients with eBL would have co-morbid intestinal helminthiasis, which would negatively impact their haematological parameters and adversely affect their prognosis. Moreover, it is an established fact that intestinal helminthiasis is a major cause of malabsorption, gastro-intestinal haemorrhage and anaemia in the tropics. Thus, we hypothesize that eBL patients with co-morbid intestinal helminthiasis would be at higher risk of anaemia than their counterparts without helminthiasis.

Intestinal helminthic infections in eBL patients have not been previously studied and reported. If our prediction and hypothesis are correct, the prevalence of intestinal helminthiasis would be considerably high in our patients with eBL, and eBL patients with co-morbid intestinal helminthiasis will have higher prevalence and relative risk of anaemia as compared with their counterparts without co-morbid intestinal helminthiasis. To test our prediction and hypothesis, we studied the prevalence and pattern of intestinal helminthiasis among eBL patients and determined the relative risk of anaemia in eBL patients with intestinal helminthiasis as seen at the time of diagnosis of eBL in some hospitals in northern Nigeria; and the clinical implications of our findings were discussed within the context of cancer chemotherapy, immunosuppression, blood transfusion and tumor prognosis.

METHODOLOGY

Study Description

This is a retrospective study of data accrued from eBL patients (at the time of diagnosis before the commencement of cytotoxic chemotherapy) that were investigated at different time periods between 1995-2013 in five northern Nigerian tertiary hospitals, including University of Maiduguri Teaching Hospital Maiduguri, North-East Nigeria (1995-2007); State Specialist Hospital Maiduguri, North-East Nigeria (1995-2007); Federal Medical Centre Birnin Kudu, North-West Nigeria (2004-2008); Murta Muhammad Specialist Hospital Kano, North-West Nigeria (2008-2009); and Rasheed Shekoni Specialist Hospital Dutse, North-West Nigeria (2011-2013).

Patients, Diagnoses and Selection
The patients studied in this report were confirmed cases of eBL that were diagnosed on the basis of the presence of classical morphological features as revealed by needle aspiration cytology (lymphoblasts with cytoplasmic basophilia and vacuolations) or tissue histology (lymphoblasts admixed with macrophages in a starry sky pattern) of clinically suspected tumors. However, due to local limitations of diagnostic facilities, immuno-phenotyping and cytogenetic analyses were not performed on these patients. Patients with clinical features of eBL but lacked cytological or histological confirmations were excluded. Patients that lacked pre-chemotherapy parasitological stool test or blood count results in their medical records were also excluded. Also, patients with co-morbid HIV infection and/or urinary schistosomiasis were also excluded.
Ethics and Data Retrieval
Demographic and clinical laboratory data including age, sex, and the results of haematological parameters and parasitological analysis of stool as determined at the time of diagnosis were retrospectively retrieved from the clinical records of patients. This study was conducted with the approval of local institutional ethics committees of the five hospitals within which the research was conducted.

Demography, Clinical Tumour Stages, Haematological Parameters and Stool analysis for intestinal helminthes
The age, sex and clinical tumour stages (stages A to D) of each patient with eBL as documented at time of diagnosis were noted and recorded. Haematological parameters (haematocrit, haemoglobin, red cell indices, leucocyte cell count and differentials, and platelet counts) were determined from venous blood using automatic blood analyzers. Stool samples collected in formalin-saline were investigated in the hospitals’ microbiology laboratories using a direct and iodine preparations or formal ether concentration techniques for identification of helminths ova or larvae.13

Haematological Parameters: Cut-off Values and Definitions
In accordance with the age of patients in this report, anaemia refers to Hb<11g/dl; eosinophilia refers to eosinophil count>0.5×10⁹/l; and thrombocytosis refers to platelet count>400×10⁹/l.14 In this study, normal ranges of red cell indices were taken as MCV: 76-96fl, MCH: 27-32 pg and MCHC: 30-35 g/dl; and microcytosis refers to red cell indices that are less than the lower limit of the normal ranges.14

Calculations and Statistical Analysis
Values of haematological parameters were expressed as means and standard deviations, while tumour stages, prevalence of intestinal helminths and anaemia were expressed as percentages. Values of studied parameters were compared between eBL patients with and without intestinal helminthiasis, using the t-test for mean values and the χ²-test for percentages, with p-values of less than 0.05 taken as significant. The relative risk of anaemia among eBL patients with intestinal helminthiasis was determined by logistic regression based on Poisson regression analysis with adjustments for age, sex and tumour stages. Value of relative risk (RR) was considered statistically significant if the lower limit of 95% confidence interval range (CI₉₅%) was greater than 1.0, with a p-value of less than 0.05. Statistical analyses were performed with SPSS software (IBM SPSS Statistics, version 19.0).

RESULTS
The age range of patients studied in this report was 3-10 years. Out of the 312 eBL patients studied, male: female proportion was 199: 113 (M:F); 92(29.5%) patients had localized stage-A jaw tumours, while 220(70.5%) patients had advanced tumours in stages B, C or D; and 233(74.7%) patients were infected with intestinal helminths, while 79(25.3%) patients were uninfected. The frequencies of individual intestinal helminths are shown in Table 1. Among the 220 patients with advanced diseases, the number and frequencies of metastatic sites (that were found in addition to primary jaw tumours) include orbit/central nervous system 133(60.5%), kidneys 46(20.9%), abdominal
lymph nodes 43(19.5%), liver 21(9.5%), and ovaries 5(2.3%).

Table 1. Pattern and frequency of parasites among 233 eBL patients with intestinal helminthiasis

<table>
<thead>
<tr>
<th>Parasites</th>
<th>Number of Patients</th>
<th>Infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascaris lumbricoides</td>
<td>139</td>
<td>(59.7)</td>
</tr>
<tr>
<td>Hook worm spp</td>
<td>122</td>
<td>(52.4)</td>
</tr>
<tr>
<td>Trichuris trichuria</td>
<td>104</td>
<td>(44.6)</td>
</tr>
<tr>
<td>Strongyloides stercoralis</td>
<td>22</td>
<td>(9.4)</td>
</tr>
<tr>
<td>Taenia spp</td>
<td>15</td>
<td>(6.4)</td>
</tr>
<tr>
<td>Hymenolepis nana</td>
<td>11</td>
<td>(4.7)</td>
</tr>
<tr>
<td>Enterobius vermicularis</td>
<td>10</td>
<td>(4.3)</td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td>2</td>
<td>(0.9)</td>
</tr>
</tbody>
</table>

The soil transmitted helminths including Ascaris lumbricoides (59.7%), Hook worm spp (52.4%), Trichuris trichuria (44.6%), and Strongyloides stercoralis (9.4%) were the most frequently found parasites, followed by Taenia spp (6.4%), and to lesser extent, Hymenolepis nana (4.7%), Enterobius vermicularis (4.3%) and Schistosoma mansoni (0.9%). Out of the 233 patients with helminthiasis, 71 (30.5%) had poly-parasitism (infection by two or more helminths). Comparative analysis between patients with and without helminthiasis is shown in Table 2.

The two patient categories did not differ with respect to age, proportion of male gender, tumour stage, total leucocyte, neutrophil, lymphocyte, monocyte and basophil counts. However, in comparison with patients without helminthiasis, patients with helminthiasis had higher prevalence of anaemia (65.2% vs. 13.9%, $p=0.008$); lower mean Hb (9.3 vs. 11.2g/dl, $p=0.01$); higher prevalence of microcytosis (65.2% vs. 6.3%, $p=0.007$); lower mean MCV [mean corpuscular volume] (77.2 vs. 83.3fl, $p=0.02$), MCH [mean corpuscular haemoglobin] (25.3 vs. 27.1pg, $p=0.01$), and MCHC [mean corpuscular haemoglobin concentration] (30.1 vs. 32.4g/dl, $p=0.03$); higher mean eosinophil count (0.48 vs. 0.3x10⁹/l, $p=0.01$); and higher mean platelet count (405 vs. 236x10⁹/l, $p=0.007$).

The prevalence of eosinophilia and thrombocytosis among patients with helminthiasis were 53.6% and 52.8% respectively. None of the patients without helminthiasis had eosinophilia or thrombocytosis. By regression analysis adjusting for age, sex and tumour stages, eBL patients with intestinal helminthiasis had significantly elevated relative risk of anaemia ($RR=3.4$, CI₉₅%: 2.3-4.5, $p=0.006$).
Table 2. Demography, tumour stages, and haematological profiles of 312 eBL patients with and without intestinal helminthiasis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>With Helminthiasis (n=233)</th>
<th>Without Helminthiasis (n=79)</th>
<th>P-Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)Mean ± SD</td>
<td>6.2± 2.1</td>
<td>5.8± 1.7</td>
<td>P=0.07</td>
</tr>
<tr>
<td>No. of male patients(%)</td>
<td>147(63.1)</td>
<td>52(65.8)</td>
<td>P=0.08</td>
</tr>
<tr>
<td>No. of patients with stage-A tumours(%)</td>
<td>70 (30.0)</td>
<td>22(28.0)</td>
<td>P=0.09</td>
</tr>
<tr>
<td>No. of patients with anaemia (%)</td>
<td>152(65.2)</td>
<td>11(13.9)</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Hb (g/dl)Mean ± SD</td>
<td>9.3 ± 1.0</td>
<td>11.2 ± 1.1</td>
<td>P=0.01</td>
</tr>
<tr>
<td>MCV (fl)Mean ± SD</td>
<td>77.2 ± 4</td>
<td>83.3 ± 3</td>
<td>P=0.02</td>
</tr>
<tr>
<td>MCH (pg)Mean ± SD</td>
<td>25.3 ± 1.2</td>
<td>28.1 ± 1.1</td>
<td>P=0.01</td>
</tr>
<tr>
<td>MCHC (g/dl)Mean ± SD</td>
<td>29.1 ± 1.1</td>
<td>32.4 ± 1.2</td>
<td>P=0.03</td>
</tr>
<tr>
<td>No. of patients with microcytosis (%)</td>
<td>152(65.2)</td>
<td>5(6.3)</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Total leucocyte count(x10⁹/l) Mean ± SD</td>
<td>8.62 ± 1.5</td>
<td>8.4 ± 1.3</td>
<td>P=0.07</td>
</tr>
<tr>
<td>Neutrophil count(x10⁹/l) Mean ± SD</td>
<td>2.44 ± 0.7</td>
<td>2.56 ± 0.33</td>
<td>P=0.06</td>
</tr>
<tr>
<td>Lymphocyte count(x10⁹/l) Mean ± SD</td>
<td>5.2 ± 0.26</td>
<td>5.1 ± 0.25</td>
<td>P=0.08</td>
</tr>
<tr>
<td>Monocyte count(x10⁹/l) Mean ± SD</td>
<td>0.55 ± 0.11</td>
<td>0.5 ± 0.12</td>
<td>P=0.09</td>
</tr>
<tr>
<td>Eosinophil count(x10⁹/l) Mean ± SD</td>
<td>0.48 ± 0.07</td>
<td>0.3 ± 0.06</td>
<td>P=0.01</td>
</tr>
<tr>
<td>No. of patients with eosinophilia (%)</td>
<td>25(53.6)</td>
<td>0(0)</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Basophil count(x10⁹/l) Mean ± SD</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>P=0.06</td>
</tr>
<tr>
<td>Platelet count(x10⁹/l) Mean ± SD</td>
<td>405 ± 28</td>
<td>236 ± 31</td>
<td>P=0.01</td>
</tr>
<tr>
<td>No. of patients with thrombocytosis (%)</td>
<td>123(52.8%)</td>
<td>0(0)</td>
<td>P=0.01</td>
</tr>
</tbody>
</table>

DISCUSSION

Although eBL is an important tumour in Nigeria, the national epidemiology of the tumour appears to be changing overtime with significant regional variations across the country. On the one hand, some studies suggest that the national incidence of eBL in Nigeria is gradually declining as a result of improvement in living conditions and better control of malaria.11,15 On the other hand, the results of some studies suggest that the incidence of eBL in Nigeria may be relatively lower in the northern than in the southern part of the country.16 The combined effects of the aforementioned national and regional epidemiological changes were most probably responsible for the relatively small volume of data accrued in this study, which was incidentally conducted in the northern part of Nigeria.11,15,16

Despite these epidemiological trends, the demographic profiles of the patients captured in this study revealed the classical pattern for eBL, which is characterized by preponderance of young children with predominance of the male sex as previously reported.6,11 Moreover, this study has revealed a very high prevalence (74.7%) of intestinal helminthiasis among eBL patients, about one third of whom were infected by more than one parasite. This high prevalence is consistent with our research prediction that the frequency of intestinal helminthiasis would be considerably high in patients with eBL since the two morbidities (eBL and helminthiasis) share common epidemiology.
(epidemiological convergence) thereby affecting young children within the context of poverty, tropical environment, underdevelopment and poor sanitation.

Eosinophilia is an important host immune response against parasitic infections. Although eosinophilia is usually more prevalent and more intense during the acute migratory phases of intestinal helminths infections, nonetheless eosinophilia is also to a lesser extent found in persons with chronic intestinal helminthiasis. Hence, the finding of eosinophilia in only 53.6% of our patients with intestinal helminthiasis is consistent with chronic disease as none of the patients studied in this report had clinical features of acute or migratory infection at the time of diagnostic investigations for eBL.

Only about 13.9% of eBL patients without helminthiasis had anaemia, which may be due to non-helminthic causes such as poverty, poor diet and cancer-associated anaemia of chronic disease and inflammation. The overall occurrence of localized stage-A tumours in less than 30% of our patients denotes late presentation (a common problem in African patients), which predisposes to cancer-associated anaemia. However, this study has shown that eBL with intestinal helminthiasis had significantly higher prevalence of anaemia (65.2%) with an elevated relative risk of anaemia of 3.4, which suggests that eBL patients with helminthiasis were about three and a half times more likely to develop anaemia than their counterparts without helminthiasis. This finding is consistent with our research hypothesis that eBL patients with co-morbid intestinal helminthiasis would be at higher risk of anaemia than their counterparts without helminthiasis.

Although serum ferritin was not studied in this report, the concurrence of lower red cell indices, high prevalence of microcytosis, higher mean platelet count and high prevalence of thrombocytosis among our eBL patients with intestinal helminthiasis suggested that iron deficiency was a significant cause of the anaemia seen in that category of patients. This is in keeping with the well-established fact that intestinal helminths cause iron deficiency through various forms of mucosal injury and inflammation, leading to malabsorption and chronic and/or acute gastrointestinal blood loss.

The pattern of helminthiasis found in this study was dominated by Ascaris lumbricoides, hook worm, Trichuris trichiura and Strongyloides stercoralis, which are classified as soil-transmitted helminths that are globally ubiquitous among poor populations of the underdeveloped countries wherein they cause iron deficiency. Soil transmitted helminths cause gastrointestinal mucosal injury, malabsorption, and chronic blood loss. The soil transmitted helminths are known to cause several types of gastrointestinal haemorrhage ranging from occult haemorrhage, to melaena, haematochezia and haematemesis that culminate in iron deficiency. The hook worms are particularly notorious for causing iron deficiency, and the haemorrhagic potentials of hook worm species have been extensively studied by previous researchers. While the average host blood loss due to Necator americanus is about
Despite their immunosuppressive effects, cytotoxic drugs and corticosteroids are important components of many chemotherapy regimens for the optimal treatment of eBL.\textsuperscript{38,39,41} Hence, chemotherapy for eBL patients with co-morbid intestinal helminthiasis is a risk factor for dissemination of infections. Moreover, marrow suppressive side effect of cancer chemotherapy can aggravate pre-existing anaemia in patients with helminthiasis thereby increasing the risk of blood transfusion. Blood transfusion is associated with further depression of cellular immunity, which is associated with rapid cancer progression resulting in poor prognosis and shorter survival among cancer patients.\textsuperscript{42}

The high prevalence of intestinal helminthiasis and anaemia among eBL patients in this study clearly underscores the need for routine and mandatory stool screening for helminthiasis among all newly diagnosed cases of eBL followed by anti-helminthic treatment of infected patients before the commencement of cytotoxic and steroidal chemotherapy for the lymphoma. Moreover, animal protein-rich diet and iron therapy should be offered to anaemic patients to boost their red cell mass and minimize the risk of blood transfusion during the course of cancer chemotherapy.

CONCLUSION

The prevalence of intestinal helminthiasis in eBL patients is very high and is strongly associated with microcytic hypochromic anaemia suggestive of iron deficiency. High prevalence of intestinal helminthiasis and anaemia are undesirable in eBL patients because they are destined to receive cancer chemotherapy. Despite their immunosuppressive effects, cytotoxic drugs and corticosteroids are important components of many chemotherapy regimens for the optimal treatment of eBL.\textsuperscript{38,39,41} Hence, chemotherapy for eBL patients with co-morbid intestinal helminthiasis is a risk factor for dissemination of infections. Moreover, marrow suppressive side effect of cancer chemotherapy can aggravate pre-existing anaemia in patients with helminthiasis thereby increasing the risk of blood transfusion. Blood transfusion is associated with further depression of cellular immunity, which is associated with rapid cancer progression resulting in poor prognosis and shorter survival among cancer patients.\textsuperscript{42}

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chemotherapy, which would potentially suppress marrow and immunity, cause dissemination of helminthiasis, aggravate anaemia and increase the risk blood transfusion, all of which would negatively affect prognosis and survival of affected patients. There is therefore the need for routine and mandatory stool screening for helminthiasis for all newly diagnosed cases of eBL, followed by anti-helminthic treatment of infected patients before the commencement of cancer chemotherapy. Also, dietary and iron therapy should be appropriately offered to anaemic patients to boost their red cell mass and minimize the risk of blood transfusion during the course of chemotherapy.

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