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ORIGINAL ARTICLES

Haematological features in children less than 12 years on cotrimoxazole prophylaxis seen in opportunistic infection clinics at Harare and Parirenyatwa teaching hospitals

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Abstract

Objective: To determine the prevalence of peripheral haematological abnormalities in children receiving cotrimoxazole prophylaxis.
Design: An outpatient hospital based cross sectional study.
Setting: The study was conducted at two tertiary peadiatric HIV clinics that offer comprehensive care to children living with HIV.
Subjects: 202 HIV infected, antiretroviral therapy naive children aged between 3 months and 12 years who were receiving cotrimoxazole prophylaxis for at least 1 month with more than 95% adherence to prophylaxis were included.
Main Outcome Measures: Haematological abnormalities on full blood count and peripheral film.
Results: The prevalence of anaemia was 62% with normocytic normochromic anaemia being the most frequent type (45%). The commonest red blood cell abnormality was rouleaux formation on the peripheral film. Monocytosis occurred in 62%, leucopaenia in 39%, eosinophilia in 34%, neutropaenia in 18% and lymphopaenia in 10% of the children.
Conclusion: This study showed a high prevalence of haematological abnormalities in HIV infected children on cotrimoxazole prophylaxis. It emphasizes the need for evaluation for anaemia and its management in children on cotrimoxazole prophylaxis.

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Introduction

Paediatric Human immunodeficiency virus (HIV) infection has been growing parallel to the adult pandemic. Sub-Saharan Africa accounts for more than two thirds of the people living with HIV in the world. Approximately 90% of children with HIV infection in the world reside in this region. Estimates show that more than 145 000 children were living with HIV in Zimbabwe in 2010[Ministry of Health and Child Welfare, AIDS and TB Unit, Unpublished data]. Maternal to child transmission of HIV accounts for more than 95% of infections in children. Opportunistic infections (OI) are an important cause of morbidity and mortality in children infected with HIV. These infectious complications are critical indicators of disease progression.

Cotrimoxazole prophylaxis has been shown to reduce morbidity and mortality in HIV infected and exposed children .The WHO recommends prophylactic dosing

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with cotrimoxazole for HIV infected and exposed children. HIV exposed infants should receive cotrimoxazole prophylaxis from 4 to 6 weeks after birth until HIV infection has been excluded and the mother has stopped breastfeeding. Any child identified as HIV infected with clinical signs or symptoms suggestive of HIV should also receive prophylaxis with cotrimoxazole. Despite the benefits of cotrimoxazole, it has its own limitations . Cotrimoxazole has been shown to cause haematological side effects when given as treatment for common infections even for periods less than two weeks . This makes it important to study the haematological features in children on long term cotrimoxazole prophylaxis. Cotrimoxazole is a combination of Trimethoprim and Sulfamethoxazole in the ratio of 1:5. Cotrimoxazole blocks synthesis of di-hydrofolic acid. However, it inhibits this process 50 000 times more in bacteria than human cells. Its spectrum of activity includes respiratory pathogens such as streptococcus pneumonia, Hemophilus influenza and pneumocystis jiroveci. It also has activity against urinary and gastrointestinal pathogens. Side effects of Cotrimoxazole include nausea, vomiting, glossitis, nephrotoxicity, glaucoma, dermatological and haematological abnormalities. Haematological side effects of TMP include megaloblastic anaemia and granulocytopaenia. Side effects of sulfamethoxazole include fever, skin rashes, crystalluria, hemolytic or aplastic anaemia, leucopenia, and thrombocytopenia. Reports have described haematological abnormalities in 72% of HIV uninfected children on intravenous trimethoprim-sulfamethoxazole short treatment course[13] and 48% of children on oral combination[14]. It is not clear how cotrimoxazole prophylaxis impacts on the haematological profile of HIV infected children given the fact there are several haematological abnormalities associated with HIV infection. These include bone marrow abnormalities and peripheral cytopaenias: anaemia, leucopaenia, lymphopaenia and thrombocytopaenia. Anaemia is the commonest haematological abnormality occurring in up to 84% of children with HIV. The mechanism of anaemia is multifactorial. Cotrimoxazole may compound the haematological effects of HIV infection.

Many children who are HIV infected or exposed are going to be exposed to cotrimoxazole. Some of the HIV exposed children are not infected but, because of limited access to early infant diagnosis for children before 18 months, they will be exposed to cotrimoxazole prophylaxis. The haematological side effects of cotrimoxazole could potentially worsen the haematological effects of HIV infection. This study provides an opportunity to describe the peripheral haematological features in children taking cotrimoxazole prophylaxis.

Materials and Methods

A hospital based cross sectional study was conducted in Harare the capital city of Zimbabwe from December 2007 to May 2008. Patients were recruited from the paediatric HIV clinics at Harare Children's Hospital and Parirenyatwa Hospital. These clinics were set up to provide comprehensive care to HIV infected children. Both hospitals are tertiary referral centers and teaching hospitals. Children were recruited consecutively to achieve a sample size of 210. Sample size was calculated using an estimated prevalence of haematological abnormalities that was determined by Asmar et al in children who were treated with oral cotrimoxazole of 48 %[14]. A desired confidence interval of 95% and a margin of error of 7% were assumed. Children between 3 months and 12 years of age who were on oral Cotrimoxazole for at least a month with more than 95 % adherence to prophylaxis, and were not receiving anti-retroviral treatment were recruited. Children who had been hospitalized in the previous 2 weeks, children who were acutely ill and needed hospitalization and children whose date of birth could not be ascertained were excluded from the study.

Demographic and clinical data were obtained through administration of a standardized questionnaire and physical examination at enrolment. Date of birth was confirmed by double checking with the child health card, registration forms at the clinics or birth certificate if available. Duration of cotrimoxazole prophylaxis was confirmed with the children's medical records to minimize recall bias. Clinical staging for HIV infection using the WHO HIV clinical staging system, measurement of weight, length for children below 2 years and height were done by a single person to minimize inter- observer variation. Height and length were measured using a height board. A hanging Salter scale model 2356S (manufactured in 2001 England) was used to measure weight in children 15kg and under. A standing Salter scale model 204948 was used to measure weight in children above 15kg. Scales were standardized every morning. Weight for height indicator was used to define malnutrition and Z scores employed to grade malnutrition. Malnutrition was defined as weight for height below Z score -2.

Laboratory diagnosis.

Two milliliters of venous blood were collected once in EDTA tubes and sent to a private laboratory within 2 hours of collection for a full blood count and peripheral film. Specimens were analyzed with Cell-Dyn 3700SL manufactured February 2005 in German and the peripheral films viewed under a light microscopy after Giemsa staining by a haematology technician under the supervision of a haematologist. Laboratory machines were calibrated every morning using control samples supplied by the manufacturer. Anaemia was defined as haemoglobin concentration 2 standard deviations below the mean for age. The rest of definitions used are shown in appendix 3.

Ethics.

Written informed consent from care givers and assent

from children above 7 years were obtained. The study protocol was approved by the Ethics committees of the local institutional boards at Harare hospital, the Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee and the Medical Research Council of Zimbabwe (MRCZ).

Statistics.
Data analysis was done using the Stata version 10.0 (Statacort, Texas 77845, USA). The prevalence of anaemia, neutropaenia, thrombocytopaenia and other haematological abnormalities detected were calculated within 95 % confidence interval. Univariate and multiple logistic regressions were used to investigate associations between the haematological abnormalities with age, sex, duration of prophylaxis with cotrimoxazole, WHO HIV clinical staging and nutritional status. A p-value of <0.05 was considered significant.

Results

A total of 210 children were recruited into the study and of these 8 were excluded from analysis because of incomplete data. The demographic characteristics of the children are shown in Table I.

Table I: Baseline characteristics of the children (n=202).

Characteristics	
Gender n (%)	
Male	88 (44)
Female	114 (56)
Age (months)	
Median (Q1; Q3)	78 (47; 105)
Height (cm)	
Median (Q1; Q3)	109 (90; 120)
Weight (kg)	
Median (Q1; Q3)	17 (12.5; 21)

Various haematological abnormalities were detected. Prevalence of anaemia was 62% and most of the anaemic children had normocytic normochromic anaemia as shown in Table II.

Table II: Types of anaemia detected in study participants.

	Proportion (%)
No anaemia	38
Nomrocytic normochronic anaemia	45
Microcytic anamemia	12
Macrocytic anaemia	5
Total	**100**

Rouleaux formation was the commonest peripheral film abnormality in 45% of the children. Other abnormalities included anisocytosis, hypochromia, anisochromia, polychromasia, poikilocytosis and target cells. A longer duration of taking cotrimoxazole prophylaxis seemed protective from anaemia (p = 0.02).

Table III: White blood cell abnormalities detected in study participants.

White cell abnormalities	n (%)
Monocystosis	126 (62)
Eosinophilia	69 (34)
Leucopaenia	39 (19)
Leucocystosis	12 (6)
Neutropaenia	36 (18)
Neutrophilia	6 (3)
Lymphopaenia	21 (10)

Older children were less likely to have monocytosis (p 0.00).Monocytosis was more prevalent in those with advanced stage of HIV infection (p 0.00). Male participants were less likely to have eosinophilia (p 0.02). Lymphopaenia occurred less in children with longer exposure to cotrimoxazole (p 0.03). Leucopaenia, leucocytosis, and neutropaenia, were not significantly associated with any of the patient characteristics.

Platelet abnormalities detected.
The prevalence of thrombocytosis was 14 % and that of thrombocytopaenia was 7 %. Severe thrombocytopaenia was detected in only 2 children. Platelet clumping was noted on the peripheral film in 2 children. Thrombocytopaenia was significantly associated with malnutrition (p 0.04). Thrombocytosis was not significantly associated with any of the participants' characteristics.

Discussion

Haematological abnormalities such as anaemia, leucopaenia and thrombocytopaenia are associated with more rapid progression to AIDS and reduced survival . Findings in this study showed a large proportion of children had anaemia. Normocytic normochromic anaemia was the most common type of anaemia found. The commonest red cell abnormality detected was the presence of rouleaux on peripheral film. Various white cell abnormalities were detected with monocytosis being the most frequent.

The prevalence of anaemia in this study was lower than that detected in South Africa[20] of 73% in children on cotrimoxazole prophylaxis. Normocytic normochromic anaemia was the commonest type of anaemia and this is similar to the findings in South African children. This reflects anaemia of chronic illnesses. Inflammatory cytokines released during HIV infection and accompanying opportunistic infections inhibit erythropoiesis, blunt the erythropoietin

response, reduce red blood cell survival and prevent release of iron from the reticuloendothelial system resulting in anaemia of chronic illnesses. Macrocytic anaemia was present in 5% of the children. This is less than the 10% that was reported in HIV infected Zimbabwean children who were not on cotrimoxazole prophylaxis [17] despite the fact that in the current study participants were taking an antifolate drug. Causes of macrocytosis include nutritional deficiencies of folate and vitamin B12. Presence of rouleaux was the most common red blood cell disorder. Rouleaux formation occurs when plasma proteins block the negative charge on erythrocytes surface and red cells stack in long columns. This is a non-specific finding in many clinical conditions especially if the erythrocyte sedimentation rate is raised. Anisocytosis was the second commonest abnormality. It is also a non-specific feature that maybe present in any red blood cell disorder including iron deficiency anaemia and anaemia of chronic illnesses. In South Africa anisocytosis was the commonest RBC abnormality with a prevalence of 68%[20]. The children in the South African study were younger (median age 25 months) compared to those in the current study who had a median age of 78 months.

Monocytosis (62%) was the commonest white blood cell disorder followed by eosinophilia (34%). A report on haematological abnormalities prior to cotrimoxazole prophylaxis in Zimbabwe showed a monocytosis prevalence of 10% [17]. The differences between the two studies are in the definition of monocytosis and sample size. They used a much higher cut off of 1x 10^9/litre and had a smaller sample size. Monocytes are mononuclear phagocytes and have an important role in host defenses, tissue repair and remodeling. Many pathogens including viruses, bacteria, fungi and parasites can induce monocytosis. Monocytosis has also been noted to preceed neurological symptoms of HIV infection such as encephalopathy in children and may be used as markers of neuropathogenicity.

The prevalence of thrombocytosis (14%) was higher than that of thrombocytopaenia (7%). Compared to findings in the study by Adewuyi and Chitsike of thrombocytopaenia prevalence of 30%[17], this study has a much lower prevalence despite the fact that children are on cotrimoxazole which can cause thrombocytopaenia. However, reduced infections in children on cotrimoxazole prophylaxis could explain the difference. In Nigeria a prevalence of only 1% was detected, but a cut off of 100x 10^9/litre was used which would miss patients with mild thrombocytopaenia[19]. The major difference between this study and the studies done in Zimbabwe and Nigeria are that age specific definitions of the haematological abnormalities were employed. The other two studies used blanket definitions yet these parameters change with age. However, a reference range derived in America was used and this is a limitation. Doing a malaria test, reticulocyte count, bone marrow biopsy and CD4 count would have helped in characterizing the hematological abnormalities further. Children whose date of birth could not be determined were excluded from the study and this introduced sampling bias. The HIV clinics tended to look after older children. This may be because of challenges in diagnosing HIV infection in children below 18 months or that infants infected in utero and perinatally have rapidly progressive disease, therefore are more likely to die or be on antiretroviral therapy which was an exclusion criteria. One cannot conclude a causal relationship of cotrimoxazole and haematological findings because this was a cross sectional study. In addition, the back ground HIV infection is a confounding factor. The normal values used to define the various haematological abnormalities are not locally derived hence caution should be exercised. Black Africans have been shown to have lower leucocyte, neutrophil (benign ethnic neutropaenia), and platelet counts compared to Caucasians. This is more in males and children younger than five years of age. The explanation is still not clear but nutritional and genetic factors are implicated.

Conclusions

A significant number of children on cotrimoxazole prophylaxis had anaemia. The most common type of anaemia was normocytic normochromic anaemia. A longer duration of exposure to cotrimoxazole was less likely to be associated with anaemia. It is recommended that a clinical assessment for pallor be done at each clinic visit and if present a full blood count should be done in children on cotrimoxazole prophylaxis. Detected abnormalities should be treated since some cytopaenias such as anaemia are known risk factors for mortality in HIV infection.

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