A PROFILE OF ACQUIRED CAUSES OF CHILDHOOD ANAEMIA IN GENERAL PAEDIATRIC WARDS AT MUHIMBILI NATIONAL HOSPITAL DAR ES SALAAM, TANZANIA

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By

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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Medicine (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned have read and hereby recommended for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: **"A Profile of Acquired Causes of Childhood Anaemia in General Paediatric Wards at MNH, Dar es Salaam, Tanzania"** in partial fulfillment of the requirements for the degree of Master of Medicine (Haematology and Blood Transfusion) of Muhimbili University of Health and Allied Sciences

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Date .....29th October, 2010.....

DECLARATION AND COPYRIGHT

I, Alex S. Magesa, declare that to the best of my knowledge this dissertation is my own original work and has not been presented and will not be presented to any other University for a similar or any other degree award.

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Date ..... 22 November, 2010 .....

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DEDICATION

"To my Wife Bwiro Joseph, parents and my sons Anthony and Shawn for their love and encouragement"

ABSTRACT

Background: Anaemia is a growing public health problem and greatly determines the prognosis of children in the paediatric wards. There is paucity of data describing its magnitude, haematological values and acquired causes among patients admitted in paediatric wards. Anaemia is the major cause of morbidity and mortality in paediatric age yet the magnitude of acquired causes of anaemia have been inadequately studied in Tanzania.

Objectives: The study was aimed at determining the profile of acquired causes of childhood anaemia in general paediatric wards at Muhimbili National Hospital (MNH) in Dar es Salaam.

Study design: This was a descriptive cross -sectional study. **Study setting**: This was conducted at MNH in general paediatric wards from 20th August, 2009 to15th December, 2009; and **Subjects**: A total of 315 patients, aged1- 84 months, consecutively admitted were recruited in the study.

Methods: After informed verbal consent from the guardian or parent was obtained, information on demographic and clinical characteristics was collected from the parent or guardian. Physical examination and laboratory tests on blood ; stool samples for hookworm screening; blood slides for malaria parasites; Human Immunodeficiency Virus (HIV) screening; blood peripheral smears; active serum vitamin B_{12}; serum folate; serum transferrin; and serum iron were done on all subjects. Additional information was taken from medical files. The prevalence of anemia was determined as a percentage of all paediatric patients recruited during the time of data collection.
All information was recorded using questionnaires and analysis was done using SPSS version 13.0. A p value of < 0.05 was considered statistically significant.

Results: Fatigue, headache, pallor, jaundice, hepatomegaly and splenomegaly were shown to be the most common presenting clinical feature in patients with anaemia ($p < 0.05$). The status of anaemia (Hb < 11 g/dl) was 80.3 %.

The proportion of malaria was7.9%, HIV seropositve was 10.2% and hookworm was1.0% of all admissions. There was an increased risk of anaemia in patients with

HIV seropositve and or malaria although this was not statistically significant (OR > 1.0, $p > 0.05$).

Iron deficiency was the most common micronutrient deficiency occurring in 42% of all admissions and 41 - 46% among the anaemic children; around one third of non anaemic patients had iron deficiency ($p = 0.05$). The proportion of folate deficiency was 8.6% and vitamin B_{12} deficiency 3.4% of all recruited patients ($p > 0.05$).

Conclusions: Status of anaemia was 80.3%. Iron deficiency was common but HIV seropositive, malaria, hookworm, folate and vitamin B_{12} deficiencies were not prominent. It was noted that one third of the non anaemic paediatric patients have iron deficiency as initial stages of developing anaemia.

Recommendations: Iron studies should be performed in microcytic anaemia and causes of iron deficiency investigated. Iron food fortification programs should be developed and implemented. Further studies should be done to determine serum B_{12} and serum and red cell folate levels in children on a large scale in order to resolve the controversy of these micronutrient deficiencies in children.

Study limitation: Since this was a cross sectional hospital based study, bias could not be avoided in determining the profile of acquired causes of childhood anaemia.

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LIST OF ABBREVIATIONS

CPL= Central pathology laboratory

EDTA= Ethyl diamine tetra acetic acid

FBC= Full blood count

Hb=Haemoglobin

HCT= Haematocrit

HPLC= High performance liquid chromatography

IDA= Iron deficiency anaemia

LYM = Lymphocytes

MCH=Mean corpuscular haemoglobin

MCHC=Mean corpuscular haemoglobin concentration

MCV=Mean corpuscular volume

MNH = Muhimbili National Hospital

MONO = Monocytes

MOHSW=Ministry of Health and Social Welfare

NA=Not Applicable

PLT = Platelets

RA= Research Assistant

RBC= Red blood cells

WBC= White blood cells

Definition of Terms

Anemia was defined as a hemoglobin level of less than 11g/dl. It was graded as follows (based on WHO classification) (1)

Grade 1 anemia (mild): Hemoglobin level of 9.5 - 10.9 g/dl

Grade 2 anemia (moderate): Hemoglobin level of 8.0 - 9.4g/dl

Grade 3 anemia (severe): Hemoglobin level of 6.5 - 7.9g/dl

Grade 4 anemia (life-threatening): Hemoglobin level of < 6.5g/dl

Definition of anaemia by age specific categories for anaemia (2)

1 - 2 months Hb < 10.7g/dl

2 - 3 months Hb < 9.0g/dl

3 - 6 months Hb < 9.5 g/dl

6 - 84 months Hb < 11g/dl

Clinical chemistry

Serum Transferrin (age < 14 yrs) using manufacturer's ARCHITECT Package insert instructions reference, ARCHITECT 1EO4 - 20 Transferrin Reagent kit

- Male 1.86 - 3.88 g/l (normal range)
- Female 1.80 - 3.91 g/l (normal range)

Serum Iron using manufacturer's Package insert instructions reference, ARCHITECT c8000 system, IRON 7D68 - 20 and 7D68 – 30

- 4.4 - 27.9 umol/l (normal range)

Serum Folate using manufacturer's AxSYM Package insert instructions reference, AxSYM Folate Reagent Pack (Dual Pack), (7K46 - 20)

- < 3 ng/ml (folate deficiency)

Serum Active Vitamin B_{12} using manufacturer's AxSYM Package insert instructions reference, AxSYM Active vitaminB12 Reagent Pack (1P43 - 20)

- 8.9 - 123 pmol/l (normal range)

1. INTRODUCTION

Worldwide anaemia is a public health problem. Globally it is estimated that about 2 billion people suffer from anaemia(3-5) Anaemia is predominant in the developing countries harboring 90% of the anemia cases (1). Women and children under-five years of age are the most affected (1, 6).

In Tanzania, severe anemia is a public health problem as it is one of the leading causes of morbidity and mortality in most of the hospitals. Prevalence of anemia according to the 2004/2005 Tanzania national prevalence of anaemia in under-fives is 71.8 %. (7) All age groups and both sexes are affected suggesting that several factors may be involved as causes of anaemia (3,4). Anaemia features in the top ten diseases in many hospitals all over the country (3,4) . In Tanzania, like many other sub-Sahara African countries, there is a high prevalence of many tropical and congenital diseases that are associated with anaemia.

In malaria endemic areas anaemia is usually a silent condition that nevertheless places a considerable burden on health services.

Due to the epidemic of Human Immunodeficiency Virus (HIV) in sub-Saharan countries, Tanzania being one of them, the prevalence of anemia is said to increase. It is estimated at 63% to 95% making anaemia more common than thrombocytopenia or leucopenia (8).

A large proportion of hospitalized children at MNH are due to HIV/AIDS associated illnesses. According to the 2003-2004 report of the department of paediatrics at MNH, a total of 4,275 children were admitted of which 6.9% of the admissions were due to HIV/AIDS associated illnesses (9).

Hookworm anaemia is common in Tanzania and may account for 10% of all patients admitted with severe anaemia at MNH (3, 4). A variety of haemoglobinopathies common in the tropics occur. In Dar es Salaam, approximately 17% of children entering hospital for any reason are carriers of a sickle cell gene (10).

Food fortification programs are being implemented in the developed countries. Micro-nutrients, e.g. iron, folate and vitamin B12 are added to food articles to reduce the morbidity caused by anaemia (11)

In the third world countries, anaemia is caused by multiple factors. Iron deficiency is the major cause of anaemia among the under fives (12). Cases of severe anaemia often require hospitalization and blood transfusions.

In the previous studies at MNH, folate and vitamin B_{12} were not determined and the tests were not available. It is therefore, important to have current baseline data on the magnitude of the prevalence and acquired causes of anaemia in children in order to decide about the level of investment in management and prevention of anaemia in our setting where resources are limited.

2. BACK GROUND

Anaemia is a condition of having less than the normal number of red blood cells or less than the normal quantity of hemoglobin in the blood. The oxygen-carrying capacity of the blood is, therefore, decreased. WHO defines anaemia as HB less than 11g/dl.

Pathogenesis and Classification of anaemia

Haematopoiesis is the process by which the formed elements of the blood are produced. The process is regulated through a series of steps beginning with the pluripotent hematopoietic stem cells. Stem cells are capable of producing red cells, all classes of granulocytes, monocytes, platelets, and the cells of the immune system. Commitment of the stem cell to the specific cell lineages appears not to be regulated by known exogenous growth factors or cytokines. Rather, stem cells develop into differentiated cell types through incompletely defined molecular events that are intrinsic to the stem cell itself. Following lineage commitment (i.e. differentiation), hematopoietic progenitor and precursor cells come increasingly under the regulatory influence of growth factors and hormones. For red cell production, erythropoietin (EPO) is the regulatory hormone. EPO is required for the maintenance of committed erythroid progenitor cells that, in the absence of the hormone, undergo programmed cell death (apoptosis) (13).

The earliest recognizable erythroid cell in the marrow is the normoblast. The normoblast usually undergoes four cell divisions to produce 16 daughter cells, which mature, extrude their nuclei, and become reticulocytes. With each cell division the daughter cells become smaller than their parents and progressively change in hue from blue to red as haemoglobin content increases and ribosome nucleic acid (RNA) content decreases. In patients with anaemia due to haemolysis or acute blood loss, there is an increase in erythropoietin and consequent increase in the number of dividing cells.

Reticulocytes are released which are larger (macrocytic) and bluer (polychromatophilic) than mature red blood cells. If anemia is severe enough, stimulation of red cells may be sufficient for red cells to enter the peripheral circulation before they extrude their nuclei giving a peripheral blood picture of nucleated red blood cells (NRBCs). Anaemia produced by different causes may lead to different morphologic findings. Deficiencies will produce recognizable morphologic changes in the mature red cells. (5)

Anaemia can be classified according to the associated changes in the red cell size (14, 16). Morphological classification of anemia is based on mean corpuscular volume (MCV) and haemoglobin concentration see appendix VI for paediatrict normal ranges. Note; The use of MCV alone without film morphology could not differentiate between microspherocytes, seen in severe burns and microcytic hypochomic cells of iron deficiency and thalassemia hence require at least blood film.

Hypochromic microcytic anaemia (MCV < 74 fl, MCH < 23 pg and MCHC < 28 g/dl)(2) suggests IDA, anaemia of chronic disease, thalassaemia or sideroblstic anaemia(16).

Normochromic macrocytic anaemia (MCV > 108 fl, MCH 23 -36 pg, MCHC 28 - 36 g/dl) (2) suggests vitamin B_{12} or folate deficiency. Vitamin B_{12} deficiency is estimated to affect 10% – 15% of people over the age of 60 yrs, and the laboratory diagnosis is usually based on low serum vitamin B_{12} levels or elevated serum methylmalonic acid and homocysteine levels (17) . Vitamin B_{12} and folate deficiencies are rarely seen in under-fives (18).

Normochromic normocytic anaemia (MCV = 74 -108 fl) (2) suggests acute blood loss, anaemia of chronic illness, haemolysis or bone marrow failure syndromes, e.g. myelodysplastic syndrome and aplastic anaemia.

Anaemia can also be classified based on the cause of anaemia (14-16). There are those conditions which are associated with decreased or ineffective marrow production, such as lack of iron, vitamin B_{12} and folate; bone marrow failure syndromes (aplastic anaemia, myelodysplastic syndrome) and infiltration of bone marrow by abnormal cells (primary or secondary) and fibrosis. Other conditions are associated with increased destruction of cells by hemolysis, hypersplenism or excess blood loss.

Clinical presentation of anemia

Fatigue and weakness (tiredness, lassitude, easy fatigability and generalized muscular weakness) are the most common and often the earliest symptoms of anaemia) (19). Pallor is the most prominent characteristic sign. It may be seen in the skin, nail beds, mucous membranes and conjunctivae. Clinical manifestations in the cardiovascular system result from the effect of hypoxia on the myocardium, pre-existing heart disease and the high cardiac output state. Less common symptoms include faintness, effort intolerance, pounding in the ears and effort angina. These are general presenting feature and could be a result of or due to complication of anaemia.

Anaemia is a serious and common problem among young children in Sub-Saharan Africa. In a study done to evaluate the ability of health workers to use clinical findings to identify children with anaemia, health care workers examined a total of 1104 children less than 5 years of age at two hospital-based outpatient clinics in rural Malawi. Blood samples were taken to determine haemoglobin concentration. Pallor of the conjunctiva, tongue, palm or nail beds was 66% sensitive and 68% specific in distinguishing children with moderate anaemia (haemoglobin concentration, 5 - 8 g/dl) and 93% sensitive and 57% specific in distinguishing those with severe anaemia (haemoglobin concentration < 5 g/dl). Even without laboratory support, which is often

unavailable in rural Africa, clinical findings can identify the majority of children with anaemia (20).

Investigations

Full blood count

This is a test that gives information about the cells in a patient's blood. A complete blood count will normally include RBC indices, WBC count, platelet count and a blood film.

Red cell values:

Red blood cell count - The number of red blood cells is given as an absolute number per liter.$4.7 \pm 0.6 \times 10^{12}$ / l (21)

Hemoglobin concentration is the amount of hemoglobin in the blood expressed in grams per deciliter.12.6 ± 1.5 g/dl (21)

Hematocrit or packed cell volume (PCV) is the fraction of whole blood volume that consists of red blood cells. 0.35 ± 0.05 l/ l (21)

Red blood cell indices:

Mean corpuscular volume (MCV) is the average volume of the red blood cells measured in femtolitres. Anemia is classified as normocytic, microcytic or macrocytic based on whether this value is normal, below or above the expected range (74 -108 fl).

Mean corpuscular hemoglobin (MCH) is the average amount of hemoglobin per red blood cell expressed in picograms. Anaemia is classified as normochromic or hypochromic based on whether this index is normal or low (23 -36 pg).

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin in the red cells. Anaemia is classified as normochromic, hypochromic or hyperchomic based on whether this index is normal or outside the expected range (28 -36 g/dl).

Red blood cell distribution width (RDW) is a measure of the variation in size of the RBC population (21, 22).

Peripheral blood smear

A peripheral blood smear or film is a microscope slide preparation made from a drop of blood that allows the cells to be examined microscopically. A microscopic inspection of a peripheral blood film will give information about the morphology of normal and abnormal blood cells, a thing that an automated blood analyzer would not give. It will confirm the authenticity of the blood cell counts especially the quantity of the platelet count. A blood film is also valuable in detecting blood parasites such as malaria, trypanosomes and microfilaria and other abnormal cellular inclusions, e.g. Auer rods which are pathognomonic for acute myeloid leukaemia.

Other investigations in anaemia may include:

- Bone marrow examination
- Reticulocyte count
- HB Electrophoresis and HPLC
- Coombs test: Direct and indirect antiglobulin tests
- Iron studies: serum iron, transferrin% saturation, TIBC, serum ferritin, transferrin receptor and Perls Prussian blue stain of bone marrow
- Vitamin B12 and folate assays
- Liver function tests
- Renal function tests
- Lactate dehydrogenase (LDH)
- Infection screens: HIV, malaria and hookworm

Choice of Investigations

The following investigations were done in order to address the specific objectives of this dissertation: FBC and blood film; serum transferrin; serum folate and serum vitamin B12; BS for malaria parasites; HIV screening; and stool examination for parasites. Other relevant investigations could not be done due to lack of time and funds.

3. LITERATURE RIVIEW

Magnitude of anaemia

Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children (1) . The global prevalence of anaemia for the general population is 24.8% and it is estimated that 1620 million people are affected by anaemia. In preschool-age children, the anaemia prevalence is 47.4%, affecting 293 million children globally, the highest prevalence being in Africa (67.6%)(1) . For pregnant women the prevalence is slightly lower; however, its distribution by region follows the same trend as the one observed for preschool-age children. Additionally, the global prevalence of anaemia in school-age children is 25.4%, in men 12.7% and in the elderly 23.9 % (1). Most of the studies done showed a prevalence of 30 - 50% in child bearing age women and 50% in under five years children(3, 4) .

In a community-based cross-sectional study carried out in rural areas of West Bengal State during 2002 – 2003, a total of 437 pre-school children were studied. The majority (81%) of the rural children of West Bengal was anaemic; the prevalence was higher among children aged 1 – 3 years (91%) as compared to those aged between 4 – 5-years (74.6%) (23).

Prevalence of anemia according to the 2004/2005 Tanzania national prevalence of anaemia in under-fives is 71.8 %. (7)

Malaria and anaemia

Malaria is a major threat to public health in Africa, and remains the leading cause of death in children under 5 years in this region (24).

A study done at the Eldoret District Hospital in Western Kenya over an 18 months period, there were 4,720 paediatric admissions. The most frequent 20 diseases were identified and their respective case fatality rates calculated. Malaria was the most common cause for admission (33.0%) and the fourth most common cause of death with a case fatality rate of 2.2%. The overall mortality rate on the paediatric wards was 8.2% with 64.9% of the deaths occurring within the first 24 hours of hospitalization. Three-fourths (75.0%) of all admissions were due to four diseases: malaria, pneumonia, gastroenteritis and measles (25).

A prospective case-controlled study was conducted for children aged 2 - 59 months admitted to a district hospital in southern Tanzania. There were 216 cases of severe anaemia [packed cell volume (PCV) < 25%] and 234 age-matched controls (PCV ≥ 25%). Most cases 120 (55.6%) were < 1 year of age. Anaemia was significantly associated with the educational level of parents, type of accommodation, health-seeking behaviour, the child's nutritional status and recent and current medical history. Of these, the most important factor was *Plasmodium falciparum* parasitaemia (OR 4.3, 95% CI 2.9 - 6.5, P < 0.001)(26).

Anaemia and HIV/AIDS

In a cross-sectional study done in Lagos among HIV-1-infected children, haematological disturbances included bone marrow abnormalities and peripheral cytopenias. All three major cell lineages were depressed. Sixty eight children with confirmed HIV infection were studied. Anaemia (Hb < 10.0 g/dl) was present in 77.9%, severe anaemia (Hb < 6.0 g/dl) in 5.9%, moderate anaemia (Hb 6.0 - 7.0 g/dl) in 32.3% and mild anaemia (Hb 8.0 - 9.9 g/dl) in 39.7%. The mean haemoglobin concentration decreased as the disease progressed ($p < 0.05$); 6% had leucopenia,

17.5% had neutropenia and 2.5% (one case) had thrombocytopenia. It was concluded that anaemia was the most common haematological abnormality in HIV patients. The severity of the peripheral cytopenia was related to the disease burden (12).
In a prospective cohort study among 847 under fives done in Kampala Uganda in 2007 to determine the effect of HIV on morbidity and mortality in children with severe malarial anaemia, the prevalence on HIV was found to be 9.2%. It was concluded that HIV infected children with malarial anaemia suffered higher all-cause mortality and malaria-related mortality than HIV-uninfected children (27).

In a study done in Tanzania, among 10,037 subjects aged 15 years and over, of males, 29.8% were anaemic (Hb < 13.0 g/dl) in the most prosperous rural area, 47.9% in the city, and 73.5% and 79.9% in the two poorer rural areas. In the latter areas 25.1% and 26.3% had Hb values of < 11 g/dl, respectively. Equivalent values for females were 25.8% were anaemic (Hb < 12 g/dl) in the most prosperous rural area, 62.6% in the city and 80.5% and 80.1% in the two poorer rural areas. In the latter areas 16.9% and 26.8% had Hb values of < 10 g/dl. (28).

Anaemia and Hookworm

Hookworms infect approximately 1 billion people worldwide. Prevalence rates range from 10% to 20% in relatively dry, unsanitary areas such as Iran and parts of Pakistan to greater than 80% in rural, unsanitary conditions in the wet, humid tropics (29). Tanzania is in the tropics and is poor country; sanitary condition for most of the people is also poor, hence at high risk of hookworm infestation.

A study done among 525 school children in Pemba Island, Tanzania, compared the degree of anaemia and iron deficiency associated with the two hookworm species at the individual and community levels. In the 492 children with hookworm positive faecal samples, haemoglobin and ferritin concentrations decreased with increasing proportions of *A. duodenale*. Among children with only *N. americanus* larvae, the prevalence of anaemia was 60.5 % and that of ferritin (ferritin < 12 microg/ l) was

33.1%, while in children with ≥ 50% *A. duodenale* larvae, the respective prevalence were 80.6% and 58.9%. It was concluded that the species of hookworm being transmitted in a community influences the burden of iron deficiency anaemia in the community and should be considered in prioritizing and planning programmes for hookworm and anaemia control(30).

Iron deficiency anaemia

In a 5 - 14 years age group, the common causes of iron deficiency anaemia was worm infestation and dietary factors(31). However, in Kenya a hospital-based study showed that the main causes of anaemia in paediatrics were haemolytic anaemia due to malaria and sickle cell anaemia 30.2%. Iron deficiency of nutritional origin accounted for 26.2% and hookworm infestation played a significant role in aggravating iron deficiency anaemia(31).

A study done in patients with anaemia in Dar es Salaam in 1962, iron deficiency was found to be the commonest cause of anaemia accounting for 50% of cases in children and adults. Eighteen patients were children, 17(94.4%) of them were iron deficient but they were not categorized into age groups (32).

In a study in 1966, nineteen out of sixty patients with anaemia had bone marrow examination and 94% (18 out of 19 patients) had iron deficiency (33). The prevalence of iron deficiency anaemia in school children(5 - 14yrs) was found to be 40.9% in Lindi district, Tanzania 1998 (34).

Anaemia associated with folate deficiency and vitamin B_{12} deficiency

Vitamin B_{12} deficiency is estimated to affect 10% – 15% of people over the age of 60 yrs, and the laboratory diagnosis is usually based on low serum vitamin B_{12} levels or elevated serum methylmalonic acid and homocysteine levels (17) . In a case control study done in Malawi among 381 preschool children with severe anaemia (Hb < 5g/dl)

it was found out that folate and iron deficiencies are not prominently associated with severe anaemia in under-fives. The prevalence of vitamin B_{12} deficiency (< 13.6 ng/dl) was found to be 30.4% among the cases (18).

4. STATEMENT OF THE PROBLEM

Anaemia is reported as a significant global public health issue. It is a common finding among patients admitted to paediatric wards due to various disorders. Anaemia is a known risk factor for long-term and even permanent impairment in mental and motor development in children (35).Severe anaemia is associated with increased mortality among patients admitted to paediatric wards with other diseases such as malaria, HIV, cancer, renal failure and infections (36).

Studies on the magnitude and acquired causes of anaemia among children in Tanzania are sparse and limited. Most of such studies have been done outside Tanzania, only a few in Africa and far less than in the West or in Asia. Few published work on the prevalence and causes of anaemia among patients admitted to paediatric wards at MNH have been done based on red cell morphology, full blood count, bone marrow, and serum Iron studies. Most of these studies were done more than ten years ago and due to the change in population and economic status with time, there is a need to be reviewed. In the previous studies at MNH, folate and vitamin B_{12} were not determined as the test were not available and were thought as not contributing significantly to anaemia in children.

It is therefore, important to have baseline data on the proportion and the profile of acquired causes of anaemia in children in order to decide about the level of investment in management and prevention of anaemia in our setting where resources are limited.

5. RATIONALE OF STUDY

Anaemia is still a public health problem in Africa and especially Sub Sahara Africa. The reason for doing this study is to establish baseline data on the profile of acquire causes of anaemia among patients admitted to paediatric wards at MNH. The baseline data will improve standard of care in children with anaemia by setting priorities and focusing on the main causes of anaemia in children in Tanzania.

6. RESEARCH QUESTIONS

1. To what level is anaemia prevalent among children at MNH general paediatric wards in Dar es Salaam?
2. To what level are Vitamin B_{12}, folate, and iron deficiency contributing to the level of anaemia in admitted children at MNH general paediatric wards?
3. To what extent is HIV seropositive, malaria, hookworm associated with anaemia among children admitted in general paediatric wards MNH?

7. STUDY OBJECTIVES

- **Objectives**

To determine the profile of acquired causes of childhood anaemia in general paediatric wards at MNH.

- **Specific Objectives**

1. To determine the profile of anemia among patients admitted in paediatric wards at MNH
2. To determine the proportion of anaemia associated with infections (HIV, malaria, hookworm) among patients admitted in general paediatric wards at MNH
3. To determine the proportion of micronutrient deficiency (iron, B_{12} and folate) among patients admitted in general paediatric wards at MNH

8. MATERIALS AND METHODS

Study design

This was a descriptive cross-sectional hospital based study.

Study setting

Muhimbili National Hospital, this is the biggest public hospital in the country and the only national hospital. It receives patients from all over the country.
Patients aged 1 - 84 months with medical conditions are been admitted in paediatric wards. On average 2 - 7 patients are being admitted each day.

The link between MNH and the municipal hospitals

In Dar es Salaam there are three municipal hospitals which are Mwananyamala, Temeke and Amana. It is from these municipal hospitals the majority of patients are referred to MNH. Some patients come from up country hospitals through a referral system. Hence MNH patients represent not only the Dar es Salaam population but Tanzania in general.

Patients are referred to MNH because of several reasons e.g. burns, bleeding, trauma. Some of these reasons include further investigations, better treatment options and expertise. The referred patients may have symptoms and signs altered by intervention.

The Pathology Laboratories

The central pathology laboratory (CPL) is the biggest laboratory at MNH. It consists of five laboratory sections, namely parasitology; microbiology and immunology; histopathology; haematology and blood transfusion; and clinical chemistry. Most of the tests from MNH and the nearby hospitals are done in CPL. It is open for 24 hours and the staff (technologist) work in shifts of 8hrs.
Most of the results take a few hours to be obtained exception those which involve histopathology samples, cultures or special tests.

Study population

A total of 315 recruited patients admitted to the general paediatric wards between 20th of August, 2009 and 15th of December, 2009 and fulfilled the enrollment criteria were included in this study.

Recruitment criteria

Inclusion

1. Consecutive patients admitted in the general paediatric wards during week-days and whose parents or guardians freely gave consent to participate in the study.
2. Children age 1 - 84 months

Exclusion

1. Children whose parents or guardians refused to participate in the study.
2. Children readmitted due to the same problem within the study period.
3. Children who received blood transfusion 48 hrs before blood sample collection.

Sample size calculation

The minimum required sample size was calculated from the following statistical formula for sample size:

$N = Z^2 P (1 - P)/d^2$, whereby

N = minimum sample size;

Z = standard normal deviate corresponding to two sided specified significant levels; this is 1.96 (at 95% confident interval);

P = prevalence of anemia (According to the 2004/2005 Tanzania National Prevalence of Anaemia in Under-fives P is percent) (7)

D = margin of error taken as 5% in this study

Putting P = 71.8 % and d = 5%, the minimum calculated sample size was N = 311. The recruited sample size was 315 patients.

Sampling procedure

Paediatric patients consecutively admitted to the general paediatric wards during the time of data collection constituted the sampling frame. Parents or guardians were invited as they were admitted, those patients who came late in the day were seen on the next day and informed consent was obtained. Those who met the inclusion criteria were recruited. The process of recruitment continued until the eligible sample size was obtained.

Recruitment of research assistants and the roles of the principal investigator

One research assistant and one experienced laboratory technician were recruited for the process of data collection in the wards and laboratory, respectively. The training process was conducted and continued during the period of data collection. The emphasis was on understanding the objective of the study and the contents of the questions to be asked to the study subjects. The technologist was responsible for making sure samples were properly processed and stored in the lab at the required temperature, to insuring proper results were obtained and filed or registered.

The principal investigator was responsible for the data collection process; counseling; filling the questionnaires and the case report book; reporting to the supervisor or to the attending doctor on clinical cases that needed haematological intervention during the interview; review of questionnaires daily and making sure samples reached the lab on time.

Intern doctors were asked to assist in patient identification and sample collection in order to avoid unnecessary multiple blood puncturing of the patients.

The roles of the principal investigator were to train the research assistant and the laboratory technologist; to collect and process data entry; to take samples and make sure samples reached the laboratory in time; to review the filled questionnaires daily

and to clean, interpret haematological data while in the wards and to discuss the management of haematological cases with the attending paediatricians.

Data collection procedure

The principle investigator obtained informed verbal consent from the parents or guardians, administered the questionnaire and collected blood samples from the patients with the help of the trained intern doctors from the respective wards. The samples were properly labeled and sent to the laboratory. The questionnaires were checked daily at the laboratory to ensure complete and proper filling of information by the study team.

Filling the questionnaire

A structured questionnaire with a serial identification number (Appendices I and II) was used by the principal investigator or assistant to obtain patient's information from the parents or guardians, the case file and the laboratory results.

The information filled in the questionnaire included the child's name, his or her file number, age, sex, level of education and occupation of the guardian or parent, co-morbid condition seen, history of blood transfusion, numbers of blood transfusion received, treatment offered by the attending clinician and discharge diagnosis.

Clinical assessment

The physical examination included taking the clinical history, anthropometric measurements, and detection of pallor, jaundice, fever, lymphadenopathy, hepatomegaly and splenomegaly. This was done by the principal researcher. The information was captured in the structured questionnaire.

Specimen collection

Venipuncture from the cephalic, medial or femoral vein was done aseptically on all patients and a total of 3 ml of blood was drawn. The site of venipuncture depended on the accessibility of the vein. Two ml were put into a serum separator bottle and 1 ml

into an EDTA tube by the researcher assistant or intern and the samples were sent to clinical chemistry and haematology laboratories, respectively.
Stool samples were collected in special containers and were sent to parasitology laboratory for routine examination. Thick blood smears for malaria parasites were prepare and sent to the parasitology laboratory. Results from parasitology laboratory were sent back to the wards and they were easily obtained from the register book in parasitology laboratory and in the patients' files.

Laboratory procedures

Haematological tests

Specimens for Full Blood count were fully analyzed using a Cell Dyne 37000 automatic machine. Processing of this test was done with the assistance of the lab technicians within 12 hours of sample collection. In the laboratory, the FBC and the peripheral blood smears were done within 6 hours of sample arrival. The blood films were stained using Leishman's staining method and reported by the principal investigator under the supervision of a haematologist.

Clinical chemistry and immunological tests

The samples sent to the clinical chemistry laboratory were analyzed using Architect c8000 System and Axsym System. The samples were identified by their unique serum separator bottles from the general paediatric wards against a list of the daily admissions given to the technician.

The laboratory technician on call centrifuged the samples at 3000 rpm for 10 min within 6hrs of arrival of the samples. The serum was stored in a deep freezer at minus 24°C in the haematology laboratory. The serum samples were then run in batches after the reagents arrived from Abbott, in the clinical chemistry department (CPL). Serum iron, serum transferrin, active vitamin B_{12} (holotranscobalamin) and serum folate

assays were done on all patients' samples with the exception of 25 samples which were thrown away accidentally during a water shortage incidence.

Serum Vitamin B_{12} assay

The method followed carefully the AxSYM Package insert instructions reference, AxSYM Active vitaminB12 Reagent Pack (1P43 - 20.

Vitamin B_{12} (cobalamin) in serum is bound to two proteins, transcobalamin (TC) and haptocorrin (HC). The transcobalamin-vitamin B_{12} complex is called holotranscobalamin (Holo - TC). Holo – TC contains the biologically available cobalamin because only Holo - TC promotes the uptake of its cobalamin by all cells via specific receptors. Assay of active vitamin B_{12} is based on micro-particle enzyme immunoassay (MEIA) technology. The results were measured in pmol/ L. normal ranges for children were 8.9 – 123 pmol/ l.

Serum Folate assay

The method followed carefully the AxSYM Package insert instructions reference, AxSYM Folate Reagent Pack (Dual Pack), (7K46 - 20)

Folate are a class of vitamin compounds related to pteroylglutamic acid (PGA) which serves as cofactors in enzymatic transfer of single carbon units in variety of metabolic pathways. Folate assay is based on ion capture technology. The results were measured in ng/ml. Normal ranges for children were (5-20) but would be considered deficient when < 3ng/ml.

Serum transferrin assay

The method followed carefully the ARCHITECT Package insert instructions reference, ARCHITECT 1EO4 - 20 Transferrin Reagent kit

Transferrin is a beta globulin synthesized primarily in the liver which is the principal protein responsible for iron transport. Transferrin is responsible for 50 - 70% of TIBC

of serum. Indications for transferrin assay include: screening for nutritional status; differential diagnosis of microcytic hypochromic anaemia; and monitoring response to the treatment of iron deficiency anaemia. The serum transferrin assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to transferritin is added to the sample. These were measured in g/ L and normal ranges were 1.8 – 3.91 g/ l.

Serum iron assay

The method followed carefully the ARCHITECT c8000 system. Package inserts instructions reference, ARCHITECT c8000 system, IRON 7D68 - 20 and 7D68 - 30

Serum iron has been determined by several spectrophotometric methods. The serum iron assay utilizes an acidic media to release ferric iron from transferrin. The ferric iron is converted to the ferrous form by the action of hydroxylamine hydrochloride. The released ferrous iron reacts with FERENE to produce a colored iron-FERENE complex which is read by the machines. These were measured in umol/l and normal ranges were 4.4 – 27.9 umol/l.

HIV screening

HIV testing was done in the blood bank section in CPL using Bioline for HIV test. All positive samples were confirmed by a second different test called Determine for HIV and no discordant results were seen. Samples from children of less than 18 months of age were sent to the department of microbiology for HIV - PCR testing to exclude maternal IgG anti-HIV antibodies which cross the placenta.

Routine stool examination

Stool samples were processed as routine. A Kato technique by filter method was used to examine stool for worms, cysts or ova. Absence of any of the above was reported as nothing abnormal detected (NAD).

Blood slide for malaria

Thick blood slides for malaria parasites were made and reported by an experienced technician. Patients were labeled as positive for malaria test if malaria parasite were seen on the blood slide and as negative if no malaria parasites were seen.

Case report forms

The collected patients' information and laboratory results were recorded in the case report forms and then entered into the database (SPSS version 13.0) by the principal investigator and research assistant and then verified

Data Management

Data were analyzed using the SPSS version 13.0. Descriptive statistics were computed and comparison of categorical variables was done using chi^2 for tables with cells that have numbers more than 5 and Fishers exact test for tables with cells that have numbers less than 5. Comparison of means and medians was done using the Student's t -test and Mann –Whitney test, respectively. A p value of ≤ 0.05 was considered statistically significant.

Outcome measures

As outcome measures, anaemia was dichotomized as non anaemic and anaemic; active vitamin B_{12}, serum iron, serum transferrin as low, normal or high; and folate as low or normal; malaria, HIV and stool examination for parasites as positive and negative. The outcome measurements and categorization were defined as described below.

9. ETHICAL CONSIDERATION

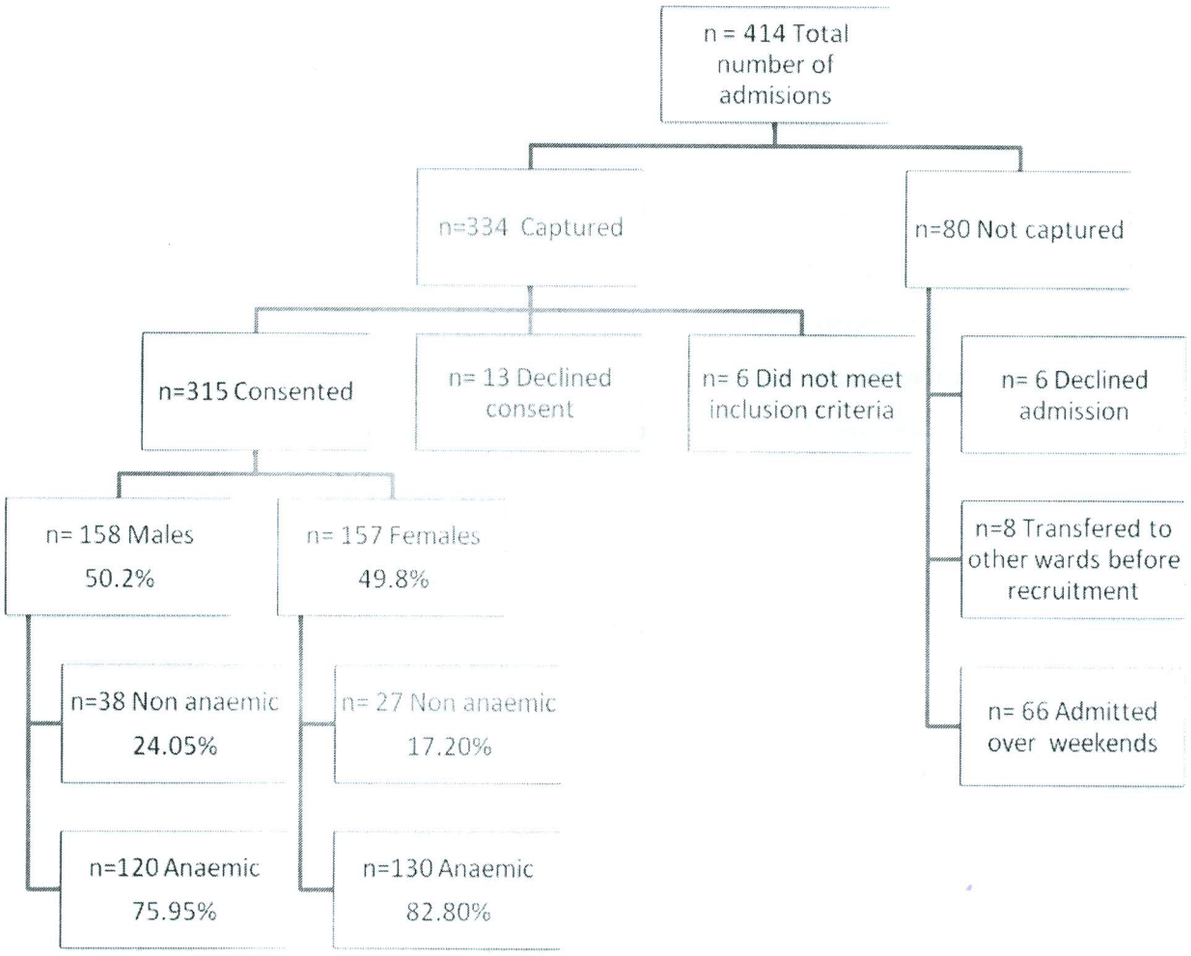
Ethical approval to conduct the study was obtained from Muhimbili University of Health and Allied Sciences ethical committee and the permission to perform the study at MNH was obtained from the MNH authority.

Informed verbal consent was requested from all subjects' parents or guardians. The principal investigator and the research assistant described the purpose, advantages, risks or discomfort of the study to the parents or guardians. Aseptic techniques were used to avoid infections or contaminations .All these issues were clarified to the guardians or parents before the consent for inclusion into the study was obtained. Any other requested additional information was provided to parents or guardians by the study personnel.

10. RESULTS

Figure 1 represents the flow of patients as they were recruited and analyzed. A total of 414 children were admitted into the general paediatric wards of MNH during the study time of data collection from 20th August, 2009 to 15th December, 2009. However, only 334 parents or guardians were approached and invited to participate in the study. Therefore, 80 children were not captured due to several reasons, e.g. they were admitted over the weekends; were transferred to other specialized paediatric wards; or declined admission after registration. The majority (n = 315) of parents or guardians provided informed consent for participation; hence the response rate for eligible study participants was 94.3%. All 315 (100.0%) of the eligible participants had samples collected for laboratory processing and data analysis.

Figure 1: Flow chart of patients as they were recruited and analyzed



Demographic characteristics of the study population

Table 1 summaries the demographic characteristics of the 315 participants by their haemoglobin status dichotomized as non anaemic or anaemic. The age of the participants ranged from one month to 84 months (median age = 24 months; mean age = 31.2 ± 26.7 months). A total of 158 (50.2%) of the participants were males and the remainders were females. A total of 165 (52.6%) of the participants were Muslims and 149 (47.5%) were Christians. A large proportion of the parents or guardians of the children were housewives (43.5%) and self-employed (29.8%), respectively; minorities of the study population were business-persons (10.5%), civil servants (9.8%), and peasants (6.3%), respectively. Two thirds (66.7%) of the parent or guardians had only primary education, nearly a quarter (22.9%) had secondary education; and a small number had either no formal education (6.7%) or tertiary education (3.8%). The associations between anaemia with the demographic factors were not found to be statistically significant (p-values > 0.05).

Table 1 Profile of anaemia by demographic characteristics N=315

Characteristic	Anaemia status (Hb < 11g/dl)		Total N	P-Value
	Non anaemic n (%)	Anaemic n (%)		
All	62 (19.7)	253 (80.3)	315	
Age in months				
1 - 23	30 (48.4)	124 (49.0)	154 (48.9)	0.451
24 - 59	25 (40.3)	86 (34.0)	111 (35.2)	
60 - 84	7 (11.3)	43 (17.0)	50 (15.9)	
Median age = 24.0				
Sex				
Male	38 (61.3)	120 (47.4)	158 (52.2)	0.065
Female	27 (38.7)	130 (52.6)	157 (49.8)	
Religion *				
Christian	26 (41.9)	123 (48.8)	149 (47.5)	0.395
Muslim	36 (58.1)	129 (51.2)	165 (52.3)	
Parent/Guardian occupation				
Farmer	4 (65.0)	16 (6.3)	20 (6.7)	0.962
Businessperson	5 (8.1)	28 (11.1)	33 (10.5)	
Civil servant	7 (11.3)	24 (9.5)	31 (9.8)	
Self employed	19 (30.6)	75 (29.6)	94 (29.8)	
Others/H.wife	27 (43.5)	110 (43.5)	137 (43.5)	
Level of education of parent/guardian*				
No formal	2 (3.3)	19 (7.5)	21 (6.7)	0.439
Primary	41 (67.2)	168 (66.4)	209 (66.6)	
Secondary	14 (23.0)	58 (22.9)	72 (22.9)	
Tertiary	4 (6.6)	8 (3.2)	12 (3.8)	

* One missing not filled in the questionnaire

Profile of anaemia

Table 2 summarizes the profile of anaemia among 315 participants by the WHO definition and age specific groups.

Automated full blood picture to determine the profile of childhood anaemia at MNH was successfully done on 315 samples. The results showed that 253 (80.3%) of the study population had anaemia by WHO (HB < 11g/dl). However, when anaemia was defined for each age specific group, the overall profile of anaemia dropped slightly to 79.4%. A large proportion of anaemic children fell into the age groups of 6 - 84 months (84.0%), and a minority into the age groups 1 - 2 months (6.4%) and 3 - 6 months (7.6%). The age group of 2 - 3 months had the lowest proportion (1.2%) of the anaemic children. These differences in the distribution of anaemia among the four specific age groups were found to be statistically significant (p = 0.0001).

Table 2 Association between anaemia and age specific groups N = 315

Characteristic	**Anaemia status**		**Total**	**P-Value**
	Non anaemic n (%)	**Anaemic** n (%)		
WHO definition (Hb < 11g/dl)	62 (19.7)	253 (80.3)	315	
Age group in months and cutoffs for anaemia				
1 - 2 (Hb < 10.7g/dl)	7 (10.8)	16 (6.4)	23 (7.3)	0.0001
2 - 3 (Hb < 9.0g/dl)	7 (10.8)	3 (1.2)	10 (3.2)	
3 - 6 (Hb < 9.5g/dl)	11 (16.9)	19 (7.6)	30 (9.5)	
6 - 84 (Hb < 11g/dl)	40 (61.5)	212 (84.0)	252 (80.0)	
All	65 (20.6)	250 (79.4)	315	

Table (2.1) The trend of tests for anaemia by age specific groups

variable	OR	chi2	P > chi2	95%CI
Age in months				
1 - 2	1			
2 - 3	0.187	4.33	0.0374	0.03 - 1.1
3 - 6	0.755	0.22	0.638	.23 - 2.4
6 - 84	2.318	3.14	0.076	.89 - 6.04

Test of homogeneity (equal odds): chi2 (3) = 24.35, Pr > chi2 = 0.0000

Score test for trend of odds: chi2 (1) = 12.06, Pr > chi2 = 0.0005

This table 2.1 shows heterogeneity of age variation with anaemia which is highly statistically significant $P < 0.0001$. It also shows that there is a trend of odds which shows that anaemia increase with age.

Distribution of severity of anaemia by age and sex

Appendix VII summarizes the grades of anaemia of 315 participants into mild, moderate severe and life threatening anaemia by age and sex.

About one quarter (24.8%) of the children had grade four anaemia; nearly another quarter (23.8%) had grade two anaemia; the lowest proportion (13.3%) of the study population was found in grade three; and about a fifth of the study population were not anaemic.

Severity of anaemia in the grade 4 category increased with age. It was also observed that the female gender was associated more with anaemia of grade four than the males.

These values were shown to be statistically significant ($p \leq 0.05$).

Association between anaemia and infections (HIV, Malaria and Hookworm) among children admitted at MNH

Table 3 summarizes the association between infections and anaemia status dichotomized as non anaemic or anaemic.

The proportion of malaria, HIV and hookworm were 7.9%, 10.2% and 1% respectively.

The results show that malaria, HIV and hookworm infection had no statistically significant association with anaemia (p = 0.796, p = 0.645 and p = 0.483) respectively. However, there was an increased, non significant trend of association of anaemia and HIV (OR = 1.36, 95% CI = 0.49 - 4.73) and malaria (OR = 1.31, 95% CI = 0.42 - 5.46). The number (3) of children exposed to hookworm infestation was too small to give statistically significant results.

Table 3 Distribution of anaemia by infections N = 315

Infection	Anaemia status (WHO)		Total N	OR (95% CI)
	Non anaemic n (%)	Anaemic n (%)		
All	62 (20.6)	253 (79.4)	315	
Malaria				
Negative	58 (93.5)	232 (91.7)	290 (92.1)	1.31 (0.42 - 5.46)
Positive	4 (6.5)	21 (8.3)	25 (7.9)	
HIV				
Negative	57 (91.9)	226 (89.3)	283 (89.8)	1.36 (0.49 - 4.73)
Positive	5 (8.1)	27 (10.7)	32 (10.2)	
Hookworms				
Negative	61 (98.4)	251 (99.2)	312 (99.0)	0.49 (0.02 -29.14)
Positive	1 (1.6)	2 (0.8)	3 (1.0)	

The proportion of micronutrient (Iron, Vitamin B_{12} and Folate) deficiencies in children with anaemia

Table 4 summarizes the proportion of micronutrient deficiency in children with anaemia dichotomized into non anaemic and anaemic of the 290 patient tested.

The study found out that of the 290 tested for micronutrient deficiency; the commonest micronutrient deficiency was iron deficiency with an average prevalence of about 42% and of the anaemic group 41 - 46.2 % had iron deficiency; serum vitamin B_{12} and folate deficiency are present but with a very low prevalence 10 (3.4%) and 25 (8.6%) respectively and among the anaemic patients 21 (9%) had folate deficiency anaemia and 9 (3.8%) had vitamin B_{12} deficiency anaemia. When the results were tested for significance, they were not statistically significant $p > 0.05$.

Table 4 Distribution of anaemia by micronutrient deficiencies N = 290

Micronutrient	**Anaemia status(WHO)**		**Total**	**P-**
	Non anaemic n (%)	**Anaemic n (%)**	**N**	**Value**
All N = 290	56 (20.3)	234 (79.7)	290	
Serum iron				
Low	21 (37.5)	98 (41.9)	119 (41.0)	0.829
Normal	34 (60.7)	130 (55.6)	164 (56.6)	
High	1 (1.8)	6 (2.6)	7 (2.4)	
Serum transferrin				
Low	17 (30.4)	108 (46.2)	125 (43.1)	0.062
Normal	39 (69.6)	125 (53.4)	164 (56.6)	
High	0 (0.0)	1 (0.4)	1 (0.3)	
Serum Folate				
Low	4 (7.1)	21 (9.0)	25 (8.6)	0.796
Normal	52 (92.9)	213 (91.0)	265 (91.4)	
Active Vitamin B_{12}				
Low	1 (1.8)	9 (3.8)	10 (3.4)	0.118
Normal	43 (76.8)	199 (85.0)	242 (83.4)	
High	12 (21.4)	26 (11.1)	38 (13.1)	

The distribution of anaemia according to the common presenting clinical symptoms and signs of patients admitted in the MNH general paediatric wards

Table 5 shows the common presenting clinical features of 315 participants by their anaemic status dichotomized as non anaemic and anaemic. It was noted that the commonest symptoms were fatigue 101 (56.1%) patients, breathlessness 77 (45.3%) patients and headache 19 (19.4%) patients. The least common symptoms were palpitation 12 (12.1%) patients, dizziness 7 (7.2%) patients, tinnitus 2 (2%) patients dimness of vision 2 (2%) patients, insomnia 2 (2%) patients, and paraesthesia 3 (3.1%) patients

On statistical analysis, fatigue (60.8%) and headache (24.1%) were found to be associated with anaemia ($p = 0.01$ and 0.02) respectively. Breathlessness was not a statistically significant clinical symptom in prediction of anaemia ($p = 0.116$)

The commonest signs were pallor 147 (58.1%), fever 147 (58.1%), jaundice 61 (24.1%), splenomegaly32 (12.6%), and hepatomegaly 27 (10.7%). The least common presenting sign were tachycardia 21 (8.3%), systolic murmur 22 (8.7%), oedema 5 (2.0%) and bleeding 3 (1.2%).

When the clinical signs were statistically analyzed, the following clinical signs were found to be associated with the presence of anaemia: pallor ($p = 0.0001$), jaundice ($p = 0.001$), splenomegaly ($p = 0.005$) and hepatomegaly ($p = 0.015$).

Table 5 Distribution of anaemia by clinical presentation

Clinical presentation	Anaemia status		Total (%)	p-Value
	Non anaemic n (%)	Anaemic n (%)		
Fatigue	11 (34.4)	90 (60.8)	101(56.1)	0.01
Breathlessness	10 (32.3)	67 (48.2)	77 (45.3)	0.116
Palpitation	0 (0.0)	12 (15.2)	12 (12.1)	0.118
Dizziness	0 (0.0)	7 (9.0)	7 (7.2)	0.339
Headache	0 (0.0)	19 (24.1)	19 (19.4)	0.02
Pallor skin	8 (12.9)	147(58.1)	155 (49.2)	0.0001
Jaundice	4 (6.5)	61 (24.1)	65 (20.6)	0.001
Tachycardia	4 (6.5)	21 (8.3)	25 (7.9)	0.796
Systolic murmur	3 (4.8)	22 (8.7)	25 (7.9)	0.435
Edema	0 (0.0)	5 (2.0)	5 (1.6)	0.587
Fever	29 (46.8)	147 (58.1)	176 (55.9)	0.192
Splenomegaly	1 (1.6)	32 (12.6)	33 (10.5)	0.009
Hepatomegaly	1 (1.6)	27 (10.7)	28 (8.9)	0.023
Lymphadenopathy	1 (1.6)	14 (5.5)	15 (4.8)	0.319
Bleeding	0 (0.0)	3 (1.2)	3 (1.0)	1.0

Mean haematological values

Table 6 summarizes the distribution of the mean haematological values against sex and age specific groups dichotomized as non anaemic and anaemic groups among paediatric patients admitted at MNH. The Hb means ± SD of the non-anaemic and anaemic children were 12.7 ± 2.3 and 7.6 ± 2.2, respectively. The MCV means ± SD of the non-anaemic and anaemic children were 78.7 ± 18.8 and 75.3 ± 11.5, respectively. The WBC means ± SD of the non-anaemic and anaemic children were 8.8 ± 4.7 and 13.7 ± 11.6, respectively. The Platelet count means ± SD of the non-anaemic and anaemic children were 341.1 ± 157.7 and 398.2 ± 240.4, respectively. These findings show that the anaemic children on average had severe anaemia, a slightly lower MCV, elevated WBC and platelet counts when compared to the non-anaemic children. When the two sexes were compared, there were no important differences in Hb concentration and MCV levels both in the non anaemic and the anaemic groups. However, male children had higher WBC count than their female counterparts. When the age-specific categories were compared among the non anaemic group, there was an initial downward trend followed by an upward trend, with increasing age, in Hb concentration with the lowest level of 10.7 ± 1.2 g/dl at the age group of 2-3 months. The lowest MCV of 76.9 ± 4.9 fl was found in the age category of 3-6 months in the non anaemic group. Among the non anaemic children, the lowest WBC count of 7.2 ± 2.0 x 10^9/ l and the highest WBC count of 11.7 ± 6.9 x 10^9/ l were found at 2 – 3 and 3 – 6 month age groups, respectively. There were no important differences in the platelet counts among the non anaemic children or the anaemic group. However, the anaemic children had higher platelet counts when compared to the non anaemic children.

Table 6 Distribution of mean haematological values by age, sex and status of anaemia

Character	Haematological values (Mean ± SD)			
	Hb(g/dl)	MCV (fl)	WBC(k/ul)	PLT(k/ul)
All (315)				
Non anaemic n = 65	12.7 ± 2.3	78.7 ± 8.8	8.8 ± 4.7	341.1 ± 157.7
Anaemic n = 250	7.6 ± 2.2	75.3 ± 11.5	13.6 ± 11.6	398.2 ± 240.4
Total n = 315	8.7 ± 3.1	76.0 ± 11.0	12.7 ± 10.7	386.4 ± 226.8
Male				
Non anaemic n = 38	12.8 ± 2.3	78.4 ± 9.0	9.2 ± 5.0	331.3 ± 175.3
Anaemic n = 120	7.4 ± 2.2	74.2 ± 11.3	15.3 ± 14.5	423.1 ± 256.2
Total1 n = 58	8.7 ± 3.2	75.2 ± 10.9	13.8 ±13.1	401.0 ± 241.9
Female				
Non anaemic n = 27	12.5 ± 2.3	79.2 ± 8.7	8.2 ± 4.3	354.8 ± 131.2
Anaemic n = 130	7.8 ± 2.3	76.4 ± 11.6	12.2 ± 7.7	375.2 ± 223.5
Total n = 157	8.6 ± 2.9	76.8 ± 11.2	11.5 ± 7.4	371.7 ± 210.3
Age specific groups in months				
1-2				
Non anaemic n = 7	13.3 ± 2.2	78.5 ± 8.0	8.1 ± 4.0	376.0 ± 52.9
Anaemic n =16	8.2 ± 1.8	87.1 ± 15.1	7.9 ± 5.1	374.9 ± 175.3
Total n = 23	9.7±3.1	84.5±13.7	8.0±4.7	375.3±147.3
2-3				
Non anaemic n = 7	10.7 ± 1.2	81.8 ± 5.6	7.2 ± 2.0	325.1 ± 206.6
Anaemic n = 3	7.8 ± 1.8	75.5 ± 3.2	13.1 ± 6.3	422.3 ± 116.6
Total n =10	9.9 ±1.9	79.9 ± 5.7	9. 0 ± 4.4	354.3 ± 183.6
3-6				
Non anaemic n =11	11.3 ± 1.4	76.9 ± 4.9	11.7 ± 6.9	383.6 ± 198.6
Anaemic n = 19	7.6 ±1.8	69.7 ± 11.5	13.0 ± 9.1	431.9 ± 282.2
Total n = 30	9.0 ±2.4	72.3 ± 10.1	12.5 ± 8.3	414.2 ± 252.2
6-84				
Non anaemic n = 40	13.3 ± 2.3	78.7 ± 10.2	8.4 ± 4.3	326.0 ± 150.7
Anaemic n = 212	7.6 ± 2.3	74.9 ± 10.7	14.2 ± 12.1	396.6 ± 242.8
Total n = 252	8.5 ±3.1	75.5 ±10.7	13.2 ± 11.4	385.4 ± 231.9

Blood cells (RBC, WBC and PLT) morphology of paediatric patients admitted at MNH

Appendix VIII summarizes the blood cells morphology of paediatric patients (N = 315) admitted at MNH. Abnormal red blood cell shapes ($p < 0.0001$), microcytosis ($p = 0.061$), hypochromia ($p < 0.0001$), abnormal WBC ($p < 0.0001$) and normal platelet morphology ($p < 0.0001$), were common in paediatric patients with anaemia compared to those who had no anaemia.

Distribution of MCV by infections and micronutrients (Table 7)

Patients with HIV infection had a statistically significant relationship with MCV. Among the HIV positive individuals, the majority 19/32 had a low MCV (< 74 fl). The same trend 176/283 was noted among the HIV negative individuals.

Most of the children with a positive blood slide for malaria 13/25 (52%) had a normal MCV (74 -108 fl) as compared to those with negative results whose majority had low a MCV (< 74 fl). There were too few positive cases 3 /315 of hookworm to carry out statistical tests. Both malaria and hookworm infection when analyzed were statistically not significantly associated with anaemia ($p > 0.05$).

Most of the patients with low serum iron 92 (51.4%) and low serum transferrin 96(53.6%) were highly associated with a low MCV (< 74 fl) as compared to the others ($p < 0.001$). Patients with low serum folate 2 (50%) and low active vitamin B_{12} 1 (25%) clinically were more associated with macrocytosis as compared to the others, but statistically not significant ($p = 0.056$ and $p = 0.127$, respectively).

Table 7 Distribution of MCV by infections and micronutrients N = 315

Infections	Mean Corpuscular Volume(fl)			Total	P-value
	< 74 n (%)	74 - 108 n (%)	108 + n (%)	N (%)	
All (N = 315)	195 (61.9)	116 (36.8)	4 (1.3)	315 (100.0)	
HIV test					
Negative	176 (90.3)	106 (91.4)	1 (25.0)	283 (89.8)	0.005
Positive	19 (9.7)	10 (8.6)	3 (75.0)	32 (10.2)	
Hookworm					
Negative	193 (99.0)	115 (99.1)	4 (100.0)	312 (99.2)	1.0
Positive	2 (1.0)	1 (0.9)	0 (0.0)	3 (1.0)	
Negative	183 (93.8)	103 (88.8)	4 (100.0)	290 (92.1)	0.256
Positive	12 (6.2)	13 (11.2)	0 (0.0)	25 (7.9)	
Serum iron					
Low	92 (51.4)	27 (25.2)	0 (0.0)	119 (41.0)	< 0.001
Normal	83 (46.4)	77 (72.0)	4 (100.0)	164 (56.6)	
High	4 (2.2)	3 (2.8)	0 (0.0)	7 (2.4)	
Serum transferrin					
Low	96 (53.6)	29 (27.1)	0 (0.0)	125 (43.1)	< 0.001
Normal	83 (46.4)	77 (72.0)	4 (100.0)	164 (56.6)	
High	0 (0.0)	1 (0.9)	0 (0.0)	1 (0.3)	
Serum Folate					
Low	14 (7.8)	9 (8.4)	2 (50.0)	25 (8.6)	0.056
Normal	165 (92.2)	98 (91.6)	2 (50.0)	265 (91.4)	
Active Vitamin B_{12}					
Low	4 (2.3)	5 (4.7)	1 (25.0)	10 (3.4)	0.127
Normal	154 (86.0)	85 (79.4)	3 (75.0)	242 (83.4)	
High	21 (11.7)	17 (15.9)	0 (0.0)	38 (13.1)	

Morphological Pattern of anaemia

In this study three types of morphological anaemia were described (Table 7). The majority of the studied population had microcytic type of anaemia 195 (61.9%). The others were normocytic 116 (36.8%) and macrocytic 4 (1.3%).

11. DISCUSSION

The study aimed at determining the profile of acquired causes of childhood anaemia in general paediatric wards at MNH. The median age was 24 months with a range of one to 84 months. The majority of the patients (154) were under 24 months of age, hence skewed to the left. Due to the normal physiological changes of haematopoiesis which vary with age, special considerations of age groups especially for haematological indices between the ages of two and six months were considered. Normally between six months to seven years of age, these haematological parameters tend to remain constant (2, 21).

Profile of anaemia

Worldwide anaemia is a major health problem and in many African countries it is the leading cause of morbidity (1, 12, 23, 37-39). The prevalence of anaemia for the underfive children is 47.4%, affecting 293 million children globally, the highest prevalence of 67.6% being in Africa (1). The report shows variation of prevalence rates between different countries and indeed this survey showed that the highest prevalence was in sub-Saharan Africa. According to the 2004/2005 Tanzania Demographic and Health Survey (TDHS) report, the national prevalence of anaemia in under-fives is 71.8 %. The study showed a wide range of prevalence of anaemia, and it was suggested that these follow climatic patterns and are influenced by seasons. For instance, prevalence ranges varied from 26 % to 95 % (7). The prevalence of anaemia among less than 7 years children admitted to hospitals in Tanzania is scanty. A study done among 60 under-five children with severe anaemia (Hb < 7.0 g/dl) admitted at MNH in 1982 found a prevalence of 11.5%. In the same study the prevalence of anaemia (Hb < 11.0 g/dl) in the first two years of life was 75.0% (40). The current study has found a prevalence of anaemia (Hb < 11.0 g/dl) among under-five year's children to be 80.3%. When the age specific groups were taken into consideration, an almost similar prevalence of 79.4% was found. The high profile of anaemia in the current study is therefore, in support of similar previous studies and confirms that anaemia is still a major health problem at MNH.

Association of anaemia and infection

Anaemia associated with infection has multifactorial causes. Haematological disturbances associated with HIV infection include bone marrow abnormalities and peripheral cytopenias (12, 41).

In this study the overall prevalence of HIV among 315 patients admitted at MNH was found to be 10.2%. Among the anaemic group, 10.7% were HIV positive. The difference between the anaemic and non-anaemic groups was clinically but not statistically significant. ($p = 0.645$, OR = 1.36, 95% CI = 0.49 - 4.73). This finding is similar to a study done in Uganda where prevalence was found to be 9.2% (27). According to the 2003-2004 report of the department of paediatrics at MNH, a total of 4,275 children were admitted of which 6.9% of the admissions were due to HIV/AIDS associated illnesses (9). The slightly higher prevalence in the current study could be due to the small sample size or due to the fact that the majority of the patients were under two years of age and they could be still carrying maternal antibodies.

The association of malaria, HIV and anaemia has been well documented in various studies (42, 43) In all malaria-endemic countries in Africa, 25 - 40% (average 30%) of all outpatient clinic visits are due to malaria and most of the diagnosis is made clinically. In these same countries, between 20% and 50% of all hospital admissions are a consequence of malaria (36). A prevalence of malaria of 20% was noted in among under-fives in Simanjiro District, Tanzania. (44)

In the current study 25 (7.9%) children had a positive blood slide for malaria parasite and among the anaemic patients 21 (8.3%) had a positive blood slide for malaria parasites. Although there was clinically an increased risk of anaemia in those with a positive blood slide for malaria, this was not statistically significant ($p = 0.796$, OR = 1.31, 95% CI = 0.42 - 5.46). There has been a decrease in prevalence of malaria in Tanzania, since 2008; programs such as the Presidential Malaria Initiation program (PMI) continued to support Muleba District, consolidating the gains in malaria control seen during the previous years. A further drop of 55 percent in blood smears positive for malaria in patients of all ages was observed during 2008.

In total, between 2006 and 2008, the prevalence of malaria during the peak transmission period of June-July fell by 73 percent (45).

Hookworm anaemia is common in Tanzania and may account for 6% of all patients admitted with severe anaemia at MNH (3, 4) and 10% in the community (46). In this study only three out of 315 had hookworm ova or cysts. Among the anaemic patients, only two (0.8%) had hookworm infestation. This low prevalence may be due to a large proportion of the study subjects being under 24 months and therefore less exposed to hookworm infestation. However, the small numbers were not sufficient to give reliable statistical analysis for the association between hookworm infestation and anaemia.

Proportion of micronutrients and anaemia

Iron deficiency anaemia

Iron deficiency affects approximately 30% of the global population, making it the most widespread nutrient deficiency. In a study done in Côte d'Ivoire, the prevalence of iron deficiency was 41 – 63% in women and children, whereas the prevalence of IDA was 20 – 39% (47).

These results were similar to a study done previously in Dar es Salaam among children and adults which showed that iron deficiency was the commonest cause of anaemia accounting for 50% of cases (32, 35). The prevalence of iron deficiency anaemia in children aged between 5 and 14 years was found to be 33.8% in Lindi District, Tanzania in 1998 (34).

In this study, 290 children were tested for micronutrient deficiency. The overall prevalence of iron deficiency was 42% and of the anaemic group 41 - 46.2%. Iron deficiency was found in the non anaemic group to be between 30.4 - 37.5%.

These findings are in support of the previous studies that iron deficiency anaemia is a public health problem. However, these results need to be taken with caution as serum iron and transferrin vary with inflammatory and disease conditions.

Vitamin B_{12} and folate deficiencies

In previous studies, Vitamin B_{12} and folate deficiencies were considered to be very rare causes of anaemia in children, hence were not investigated (40). A case – controlled study done among 381 preschool children with severe anemia (Hb < 5.0 g/dl) and 757 preschool children without severe anemia in urban and rural settings in Malawi in 2008 did not find any cases of folate deficiency. However, 30.4% of patients with severe anaemia had Vitamin B_{12} deficiency compared to 15.6% in the control group (18). In the current study, the prevalence of serum vitamin B_{12} and folate deficiencies were found to be 10 (3.4%) and 25 (8.6%), respectively. Among the anaemic patients, 9 (3.8%) had vitamin B_{12} deficiency and 21 (9%) had folate deficiency. Therefore, there were no statistically significant differences between the non-anaemic and the anaemic groups ($p = 1.0$ and $p = 0.353$, respectively). These results concur with the observations made earlier (40). However, the results from the current study were different from the study done in Malawi (18). These differences in the results between the two studies might be due to the difference in methodology and a relatively small sample size in the current study. This controversy needs further study.

Common presenting clinical features of anaemia in paediatric patients

In this study, the common presenting symptoms among the anaemic children were fatigue 90 (60.8%) and headache 19 (24.1%). In the non-anaemic group, the frequency of fatigue was 11(34.4%) and no one complained of headache. Fatigue and headache were statistically significant in predicting the presence of anaemia ($p = 0.01$ and 0.02, respectively). The least common symptoms were palpitation, dizziness, tinnitus, insomnia and paraesthesia. Among the clinical signs, pallor, jaundice, hepatomegaly and splenomegaly were shown to predict the presence of anaemia ($p = 0.0001$, 0.001, 0.023 and 0.009, respectively). Oedema, fever, tachycardia and lymphadenopathy were least common. Very few patients presented with bleeding 3 (1.2%). The clinical features found in the current study are similar to those of other workers (19, 40).

Full blood picture of paediatric patients admitted at MNH

In this study, the Hb means (g/dl) ± SD of the non-anaemic and anaemic children were 12.7 ± 2.3 and 7.6 ± 2.2, respectively. The MCV means (fl) ± SD of the non-anaemic and anaemic children were 78.7 ± 18.8 and 75.3 ± 11.5, respectively. The WBC means (x 10^9/ l) ± SD of the non-anaemic and anaemic children were 8.8 ± 4.7 and 13.7 ± 11.6, respectively. The Platelet count means (x 10^9/ l) ± SD of the non-anaemic and anaemic children were 341.1 ± 157.7 and 398.2 ± 240.4, respectively. These findings show that the anaemic children on average had severe anaemia, a slightly lower MCV, elevated WBC and platelet counts when compared to the non-anaemic children. These findings are similar to those done by other workers (2, 21)

When the two sexes were compared, there were no important differences in Hb concentration and MCV levels both in the non anaemic and the anaemic groups. However, male children had higher WBC count than their female counterparts. These findings are similar to those done by other workers (2, 21)

When the age-specific categories were compared among the non anaemic group, there was an initial downward trend followed by an upward trend, with increasing age, in Hb concentration with the lowest level of 10.7 ± 1.2 g/dl at the age group of 2 - 3 months. The lowest MCV of 76.9 ± 4.9 fl was found in the age category of 3 - 6 months in the non anaemic group. Among the non anaemic children, the lowest WBC count of 7.2 ± 2.0 x 10^9/ l and the highest WBC count of 11.7 ± 6.9 x 10^9/ l were found at 2 – 3 and 3 – 6 month age groups, respectively. There were no important differences in the platelet counts among the non anaemic children or the anaemic group. However, the anaemic children had higher platelet counts when compared to the non anaemic children. These findings are similar to those done by other workers (2, 21). The mean haematological values in the non anaemic children were similar to those of other

workers. The lower normal WBC counts found in this study are in keeping with previous studies done in people of African descent.

Study limitations;

- This was a cross sectional hospital based study; bias could not be avoided in determining the prevalence and causes of childhood acquired anaemia.

12. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- The prevalence of anaemia among patients admitted in the general paediatric wards at MNH was high at 80.3% and severe anaemia grade 4 (Hb < 6.5 g/dl) was 24.3%.

- Iron deficiency state was the most common nutritional deficiency with an overall prevalence of 42% [low serum iron in 119 (41%) and low serum transferrin in 125 (43.1%) patients]. Of the anaemic group, 41- 46.2% were iron deficient. Interestingly, about one third of the non anaemic paediatric patients had iron deficiency.

- Folate and vitamin B12 deficiencies occurred at low frequencies of 3.8 % and 9.0 %, respectively among the anaemic children. These frequencies are controversial to previous studies and need further elucidation.

- HIV, malaria and hookworm infections occurred at low frequencies of 10.7%, 8.3% and 0.8%, respectively among the anaemic children.

- Fatigue and headache were found to be the commonest presenting symptoms in paediatric patients and were highly predictive of anaemia. Pallor; jaundice, hepatomegaly and splenomegaly were the commonest presenting signs of anaemia in this study.

Recommendations

- To perform iron studies and to determine the causes of iron deficiency anaemia in children with microcytosis, since a high proportion of children had iron deficiency anaemia.

- To develop and implement iron food fortification programs, since about one third of children were found to be in iron deficiency state.

- To determine serum B_{12} and serum and red cell folate levels in children on a large scale in order to resolve the controversy of these micronutrient deficiencies in children.

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