NORMOCYTIC ANAEMIA AND ASSOCIATED DISEASES IN KENYATTA NATIONAL HOSPITAL

BY

DR ODUOR N. JOHANSEN (MBChB)

A DISSERTATION SUBMITTED IN PART-FULFILMENT FOR THE DEGREE OF MASTERS OF MEDICINE IN PATHOLOGY (M.MED.PATHOLOGY) UNIVERSITY OF NAIROBI.

Declaration

I hereby solemnly declare that the work contained in this dissertation is my original data and has not, to the best of my knowledge been presented at any other institution of higher learning.

Signed..... **Date**3/11/08

Dr Johansen N. Oduor. Postgraduate Student in Masters of Medicine in Pathology
Department of Human Pathology. College of Health Sciences, School of Medicine
University of Nairobi

Supervisors

Dr F.K. Abdallah, Department of Human Pathology, College of health sciences, School of Medicine, University of Nairobi

Signed.....Abdallah..... **Date**3-11-08 **Dr F.K. Abdallah**

Prof O.W. Mwanda Department of Human Pathology, College of Health Sciences. School of Medicine, University of Nairobi

Signed..... **Date**4/11/08 **Prof O.W. Mwanda**

Dedication

This dissertation is dedicated to my late grandmother, Selina Obuom who passed away during the period I was collecting data, and who taught me the value of hard work.

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I wish to extend my sincere debt of gratitude to all those who have contributed directly or indirectly in enabling me to successfully complete this dissertation. To my supervisors Prof O.W .Mwanda and Dr F.K. Abdallah who played an incalculable role in guiding, encouraging and helping me to successfully put this work together.

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LIST OF ABBREVIATIONS

Ab	Antibody
ACD	Anaemia Of Chronic Disease
AIDS	Acquired Immune Deficiency Syndrome
α- IFN	Alpha Interferon
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
APP	Acute Phase Proteins
ARF	Acute Renal Failure
BFU-E	Burst Forming unit Erythroid
BM	Bone Marrow
BMI	Body Mass index
β-IFN	Beta Interferon
CD	Cluster Of Differentiation
CFU-E	Colony Forming Unit Erythroid
CHF	Congestive Heart Failure
CRF	Chronic Renal Failure
CXR	Chest X-ray
Epo	Erythropoietin
ECG	Echocardiograph
EDTA	Ethylene Diamine Tetra Acetic acid
ESR	Erythrocyte Sedimentation Rate
Fe	Iron
Fl	Femtolitres
G-CSF	Granulocyte Colony Stimulating Factor
GGT	Gamma Glutamyl Transferase
Hb	Hemoglobin
HIV	Human immunodeficiency Virus
IL-1	Interleukin -1
IL-3	Interleukin-3
IL-6	Interleukin-6

KNH	Kenyatta National Hospital
LDH	Lactose Dehydrogenase
LFTs	Liver Function Tests
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell volume
NA	Normocytic Anaemia
PBF	Peripheral Blood Film
PCV	Packed Cell Volume
RA	Rheumatoid Arthritis
RBC	Red Blood Cell
RES	Reticuloendothelial System
RF	Renal Failure
R-HuEPO	Recombinant Human Erythropoietin
SPSS	Statistical Package for Social Sciences
TIBC	Total Iron Binding Capacity
TNF	Tumor Necrotic Factor
TRAIL	Tumor Necrotic Factor- related Apoptosis Inducing Ligand

LIST OF APPENDICES:

1. ABSTRACT

Background: Normocytic anemia is probably the most frequently encountered type of anemia in clinical practice because it is usually associated with several diseases. In spite of this no study has been conducted in the Kenyatta National Hospital (KNH) to find out not only its status but also the associated diseases. Therefore this study was set to provide information on the normocytic anaemia and also the associated diseases in the KNH.

Objective: To determine the normocytic anaemia and associated diseases in KNH.

Study design: This was a cross-sectional descriptive study.

Setting: Hematology laboratory and wards at the KNH.

Methods: The recruitment of the cases involved daily perusal of all haemograms request forms and reports in the haematology laboratory and tracking the patients to the wards. All the request forms with Haemoglobin levels less than 11.5g/dl were selected. The selected forms were then scrutinized and the ones with normocytic anaemia (MCV 76-96fl) were set aside for further evaluation. The patients' address as stipulated in the forms enabled tracing the patients in the wards. Once identified, each case was then informed about the study upon which informed consent was obtained and singed. This was followed by clinical evaluation of the patient including history and physical examination. Other clinical data were retrieved from the patient's file. Blood was then drawn from the patient for the required laboratory tests. The blood samples was put in the bottle with Ethylene Diamine Tetra Acetic Acid (EDTA) and transported to the laboratory. Haematological test were performed as requested and noted in the investigation form The tests included making of the blood films for peripheral blood smear (PBF), which was stained using Romanowsky stain, and performing erythrocyte sedimentation rate (ESR) and Complete blood count (CBC) using cell dyne 3200.The PBF, ESR and haemograms print outs were further examined for the study details by the principal investigator and reviewed by the supervisors. All the data were entered in a proforma questionnaire data sheets.

Outcome measures: For every case of the age above 13 years, the following were sought and documented ; age in years , sex, residence (province), occupation, the diagnosis, Hb levels, MCV, total WBC count, WBC differential count (neutrophil ,lymphocyte , monocyte, eosinophil, basophils) platelet count and peripheral blood film, ESR and HIV status .

Results: A total of 637cases were evaluated out of whom 62 %(395) had normocytic anaemia. Overall the age characteristics were as follows; range 13-89years, mode age 32 years and median 37 years. Female and males constituted 63% (n-250) and 37% (n-145) respectively .Of the provinces Nairobi and neighboring provinces of Central and Eastern constituted the major proportions. The results also showed that the ten disease associated with normocytic anaemia were, infections (40.4%) mainly HIV infection, malignancies (20.5%) ,genitourinary 6.6%), cardiovascular (6.3%), central nervous system (5.8%), endocrine (5.6%), gastrointestinal tract (4.6%), unclassified(4.1%), haematological (3.8%), and connective tissue (2.3%). Of those with infections diseases the majority had HIV infection as shown by sero -positive tests by ELISA which is the KNH current testing technique. The level of anaemia in the majority the studied cases were mild to moderate while the WBC counts were within the normal range in most of the patients. The white cells differential count; Neutrophil, lymphocyte, eosinophil and monocytes were found to be within the normal ranges. However in those who were HIV seropositive, most had low lymphocyte count. Total platelet count was within the normal range in most of the patients. However, the ESR was raised in 98.7% of the cases this is consistent with the majority being infections among whom HIV the major component. The occupation of the majority of the study cases was in the transport industry the matatu industry however the major portion of the females in the study were housewives.

Conclusions:

This study showed that of the anaemic patients aged above 13 years old in the KNH; normocytic anaemia constituted 62% and is the commonest type of anaemia. The anaemia in the majority was of mild to moderate level. Females were more than males and the overall mean age was 37 and mode 32 years. The main occupation of the study cases were; housewives, and the unemployed while those employed were in the transport industry matatu and teachers being the majority and minority respectively.

Associated diseases in a descending order were; infections mainly HIV infection, malignancies, renal, cardiovascular, central nervous system, endocrine, gastrointestinal tract, haematological and connective tissue diseases

Other laboratory features documented were Human Immunodeficiency Virus infection, low lymphocyte count and raised erythrocyte sedimentation.

Recommendations: Upon these results and the limitations of the study the following are the some of the recommendations. That in patients of the age range 13 to 89 years at the KNH with normocytic anaemias, the management should consider first the following disease conditions; infections , neoplasms, renal , cardiovascular , central nervous , endocrine , gastrointestinal , hematological , and connective tissue .

That evaluating for the actual cause of the associated diseases is the recommended approach to correcting the anaemia.

Studies are desirable in the pathophysiology of the normocytic anaemia in the KNH.

2. INTRODUCTION

Normocytic anemia (NA) is characterized by reduced hemoglobin, normal mean cell volume (MCV), mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC) and reduced serum iron. The MCV is usually between 76 and 96fl 1. This type of anaemia (NA) has been also referred to by various terminologies such as anemia of chronic disease when it is due to an underlying inflammation, neoplasm or longstanding infection.[1-3] It is probably the most common type of anemia encountered in clinical practice and is most commonly found in patients with chronic diseases, inflammatory conditions, malignancies and immune diseases. [2-4] These conditions include diseases like chronic renal failure, congestive cardiac diseases, infections which include HIV and tuberculosis, diabetes mellitus and autoimmune diseases including rheumatoid arthritis. [4-10] It appears gradually in the course of a disease and tends to improve with effective management of the underlying disease.

A number of different mechanisms contribute to the development of anemia in these conditions including diversion of iron traffic, a diminished erythropoiesis, a blunted response to erythropoietin, erythrophagocytosis or bone marrow invasion by tumor cells or pathogens.[11,12 13]

The clinical presentation varies and reflects the underlying condition complications and only rarely due to the anemia. These may include fatigue, dizziness, fainting, headache, dyspnoea on exertion, palpitations etc. In addition, the patient may present with symptoms of the underlying disease.[14, 15]

Evaluation of the patient begins with clinical history, which may help in pointing towards the underlying disease. Signs include: palor, jaundice, oedema, wasting, and others, which depend on the underlying condition.[15]

Laboratory findings include reduced haemoglobin levels of mild to moderate, and sometimes relatively severe anemia, but normal mean cell volume (MCV). Other laboratory findings depend on the initial diagnosis and reflect the causes of normocytic anaemia.[16, 17] Therapy includes treatment of underlying disease, blood transfusion to correct heamoglobin (Hb) when low and symptomatic, and use of recombinant human erythropoietin (r- HuEPO). However, response to r-HuEPO is sometimes low .[18, 19]

3. LITERATURE REVIEW

Normocytic anaemia occurs commonly in many illnesses particularly chronic ones. It is characterized by reduced hemoglobin, reduced serum iron and total iron binding capacity, but normal MCV, usually between 76 and 96fl.[2, 10, 20] In different populations, anaemia can be defined differently, however the functional definition of WHO is generally accepted.[21, 22,23,24,25] The lower values of Hb used in Africa for defining anaemia is due to the fact that there is usually a high prevalence of comobid conditions including malnutrition and parasitic infections.[26]

In our set up, anecdotal observation shows that in many hospitals; haemoglobin levels of less than 10g/dl are taken as defining anaemia. Many adult patients presenting in our hospitals with haemoglobin of≥ 10g/dl are usually assumed to be having normal haemoglobin and hence are never treated for anaemia.

Aetiology

The causes of NA are varied but are believed to include any of the following: a decreased production of normal-sized red blood cells (e.g., anemia of chronic disease, aplastic anemia); an increased destruction or loss of red blood cells (e.g., haemolysis; either intra or extra vascular, post hemorrhagic anemia); an uncompensated increase in plasma volume (e.g., pregnancy, fluid overload); or a mixture of conditions producing microcytic and macrocytic anemias.[20, 27]

Epidemiology

Initially NA was described in association with chronic disease as anaemia of chronic disease (ACD) which on the other hand was first described in association with infections. It later became apparent that ACD could be induced experimentally by infection or sterile inflammation. In fact it has been shown in most hospitalized patients with active infection, inflammatory conditions or malignancy and the most common presentation was found in association with the chronic disease (ACD). [20-28] A study performed on women with anemia between 20 – 89 years of age showed that 60 -76% had normocytic anaemia.[29] ACD is therefore one of the most common causes of anemia. In another observation 52% of patients who had been hospitalized over a 2 month period had anemia but were not iron deficient, showing any features of haemolysis, or suffering from an established hematological malignancy but met laboratory criteria for ACD.[1,2,28] Also normocytic normochromic anemia has been found diagnosed in up to 27% of outpatients with

rheumatoid arthritis (RA). [30-32] while in patients with chronic renal failure (CRF), 64% have been shown to have normocytic anemia according to a study in Indonesia. [33] Another study done in USA on a spectrum of diseases associated with ACD, the most common underlying disease was renal insufficiency at 31%. [28]

In the developing world, multiple co-morbid conditions such as malnutrition, parasitoses and haemoglobinopathies contribute to the aggravation of anemia observed in patients with end stage renal disease as found out in a study conducted in Tunisia.[34]

In HIV (human immunodeficiency syndrome) patients referred to the department of pathology, Golwilkar Labs, India for CD4 and CD8 count, 73% had normocytic anaemia.[35] Most hospitalized patients with ACD do have active infections, inflammatory conditions or malignancy. Normocytic anemia is also found in 84% of patients with pulmonary tuberculosis.[36] In patients with disseminated tuberculosis, a study found that 65% had normocytic anaemia.[37]

A study in Egypt found that 62.5% of patients with *Fasciola hepatica* had normocytic anaemia[38]

In a study to determine the prevalence of hospital acquired anaemia, it was found out that 65.3% of patients developed anemia during hospital stay. Out of these, the most common was ACD, which was seen in 57.4% of patients.[39]

The blood picture of patients who survived from an intensive care unit also showed that ACD was the most common anemia, at the time of being discharged.[40]

Pathophysiology of normocytic anemia

Anaemia can develop due to processes that do not physically invade the bone marrow or markedly accelerate the destruction of erythrocytes although the specific pathogenesis is not well understood in many instances. However a variety of pathophysiological processes are thought to be involved and these include, cytokines, defects in erythropoietin, changes in iron kinetics, mild alteration of red cells life span and crowding out of marrow environment.[20,27]

Inflammatory cytokines

Inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrotic factor alpha (TNF-α), alpha interferon(α-IFN), beta interferon (β- IFN) and gamma interferon (γ- IFN),

are thought to play a major role of inhibiting burst forming units erythroid (BFU-E) and colony forming units erythroid (CFU-E).[41]

These are usually generated in chronic illnesses and lead to hepatic secretion of acute phase proteins (APPs).

In 37 pediatric patients infected with both HIV and *Mycobacterium avium intracellulare*, TNF was markedly elevated and there was an association between TNF and anaemia.[42] INF-α is noted to be elevated in those with AIDS.[43]

Competition for Epo receptors by IFN and IL-1may possibly lead to erythropoietin (Epo) resistance.[38] Depressed response to Epo is seen more in chronic renal failure (CRF).[45]

IL-1 and TNF-α also inhibit hepatic and renal production of Epo, further contributing to the development of normocytic anemia.[46]

Inflammatory cytokines are found to be directly correlated with the level and degree of anemia and are often elevated in rheumatoid arthritis and other chronic inflammatory diseases.[4, 41, 47]

Erythropoietin

Studies have shown that in ACD, there is a reduced level of Epo and this occurs frequently in malignancies, infections and inflammations.

Several experimental, studies have shown increases in haematocrit in patients with RA, HIV, and patients with malignancies receiving chemotherapy who have been put on r-HuEPO.[48] Erythropoietin has also been used to raise haemoglobin levels in patients with chronic renal failure.[48] Therapeutic value of r-HuEPO is also increasing in elderly patients with chronic anaemia.[46]

Changes of iron kinetics

Another theory for the formation of normocytic anemia in chronic illnesses is that inflammation in chronic illness causes changes in the dynamics of recirculation with iron being retained in reticuloendothelial system (RES)- reticuloendothelial block; rather than being released for the development of RBC in bone marrow.[48]

Decreased transferin receptors both in the serum and on erythroblasts have been advanced as one of the defects in Fe^{++} turnover in ACD.[13, 50]

Others have suggested that increases in the release of lactoferrin from neutrophils or, in the synthesis of apoferritin, leads to a pool of iron trapped in storage form, unavailable for haemoglobin (Hb) synthesis. [41]

There is also reduced intestinal absorption of ferrous iron in ACD. This is because elevated levels of inflammatory cytokines in chronic inflammation stimulate secretion of hepcidin, which causes reduced intestinal absorption of Fe^{++} as well as reducing the release of Fe^{++} from monocytes and macrophages. [52-55]

Reduced red blood cell lifespan

The lifespan of red blood cells in this form of anemia is also reduced.[20] it is usually mild, at a level that shall be compensated by a normal bone marrow.

This mild decrease however, in combination with the reduction of Hb precursors cannot account for the extent of anemia found in most patients. It is hence probable that other compensatory factors are not functioning in ACD which may include direct haematopoiesis inhibition and relative Epo deficiency. [56]

Renal diseases

Anaemia develops almost invariably in chronic renal failure with significantly impaired renal function; not commonly, patients with chronic renal failure first seek medical advice because of symptoms due to anemia. In acute renal failure, the same mechanisms operate, but hypervolaemia due to plasma volume expansion may be an additional factor causing haemodilution, and *microangiopathy* is usually more common than in chronic renal failure.[57]

In acute and chronic renal failure, the anemia is usually normocytic but may be microcytic. In renal failure, anemia occurs in part because uremic metabolites decrease the lifespan of circulating red blood cells and reduce erythropoiesis

Anemia secondary to uremia is characterized by inappropriately low erythropoietin levels, in contrast to the normal or high levels that occur with most other causes of anemia. To further confuse the presentation, serum iron levels and the percentage of iron saturation are often low, apparently because of negative acute-phase reactions. [57, 58] Furthermore, the serum creatinine level and the degree of anemia may not correlate well.[59]

In patients with terminal renal failure, it was found that the cause of anemia was multifactorial as not all these patients responded to Epo therapy. In these patients, there was an increased activity of lysosomal and proteolytic enzymes. These enzymes split sialic acid off from the Epo molecule leading to the accumulation of inactive Epo in peripheral blood.[60]

Endocrine diseases

Endocrine deficiency states, including hypothyroidism, adrenal or pituitary insufficiency, and hypogonadism, may cause secondary bone marrow failure because of reduced stimulation of erythropoietin secretion, hence these present as normocytic anaemias.[27]

Hypothyroidism

The anemia is due to adaptation to decreased basal metabolism. Thyroid hormone, acts directly or indirectly through erythropoietin to stimulate the growth of erythroid colonies (BFU-E, CFU-E) .[61, 62]

Anaemia develops in about one-third to one-half of patients with hypothyroidism and tends to occur more frequently and to be more severe in patients with severe myxoedema, although the anemia does not necessarily parallel the severity of the other clinical features of the myxoedema in a majority of cases.[61]

Hyperthyroidism

The haemoglobin level and red cell indices are usually normal in hyperthyroidism. Anaemia is uncommon in hyperthyroidism and if present is similar to the one found in hypothyroidism most often in patients in whom the disorders are of unusual severity of prolonged duration.[62]

Hypopituitarism

Mild to moderate anemia develops in most cases of hypopituitarism. Haemoglobin levels usually range from 8 to 11 g/dl, although lower values may occur. The anemia is normocytic. [61]

Addison's disease

The total red cell mass in the body is usually slightly reduced in Addison's disease, and mild normocytic anemia is common. [44, 47, 61]

Infections:

Many infective conditions are associated with some degree of anemia. These can be chronic or occasionally acute infections. The infections are those associated with significant inflammatory features. In addition to causing infection in its own right infections can also blunt the response of other types of anemia. The most common infections causing anemia are those associated with chronic inflammation of female reproductive system, urinary tract, lung, osteomyelitis bacterial endocarditis, tuberculosis, typhoid and abscesses at other sites. Human Immune Deficiency Virus (HIV) currently is associated with many of these sites infections.[32, 35, 36, 41-42]

In HIV-infected patients, red cells are mostly normocytic and normochromic. The primary defect is the failure of the bone marrow to produce the normal number of erythrocytes (hypo plastic anemia). Some of the postulated mechanisms include direct toxic effect of HIV on hematopoietic stem cells and the bone marrow microenvironment, abnormal expression of inhibitory cytokines, relative deficiency of erythropoietin and defective iron metabolism and reutilization. Recent studies suggest that there may be a direct cytotoxic effect of HIV on hematopoietic stem cells as well as on the stromal microenvironment of the bone marrow. This leads to decreased production of G-CSF and IL-3 resulting in defective erythropoiesis. [36, 42, 63]

In malaria, anaemia is due to release of inflammatory cytokines, including IL-1 and IL-6 .[64]

In Kala-azar, peripheral blood changes in uncomplicated cases are that of pancytopenia with the RBC morphology being normocytic.[65]

Gastrointestinal diseases

Several conditions and primary abdominal illnesses accompanied by, diarrhea, constipation, persistent nausea and vomiting, pain, and hepatosplenomegaly are associated with some degree of anaemia.[66, 67]

Liver Disease

Anaemia is common in chronic liver disease, and occurs in about two –thirds of patients with cirrhosis. It is usually moderate in degree, but occasionally is severe. The clinical and haematological picture depends on the dominant factor or factors responsible. Sometimes the cause of the anaemia is multifactorial.[68]

Protein malnutrition

An adequate dietary supply of protein containing essential amino acids is necessary for formation of Hb in sufficient amounts to maintain a normal level of haemoglobin in the peripheral blood. Demands for the synthesis of haemoglobin have a high priority for the amino acid pool and take precedence over serum and tissue protein formation in states of protein malnutrition.[69, 70]

Scurvy

Anaemia is common, but not invariable, in scurvy. In adults, the MCV is usually normal or slightly increased.[71]

Collagen diseases

Rheumatoid arthritis

Anaemia develops in many patients with active rheumatoid arthritis and it is usually mild to moderately severe, Hb levels 9-11g/dl. The majority of cases males and females vary with somewhat higher values in males. The severity of disease also seem to influence the level of Hb and severe disease showing lower levels.[30]

Systemic lupus erythematous (S L E)

Hb levels almost invariably develop at some stage in this disorder. Anaemia occurs in more than half of patients with SLE. Hb levels of between 9 and 11g /dl are the most common. More severe disease and complicated forms however show with lower Hb levels.[72, 73]

Polyarteritis nodosa

Moderate to severe anaemia is commonly found in Polyarteritis nodosa. A moderate leucocytosis is found as well in this condition. [74]

Scleroderma

Anaemia occurs in about 30% of cases and most often appears to be related to the degree of activity of the disorder.[75]

Dermatomyositis

Mild to moderate anaemia is common; the white cell count is usually normal but occasionally elevated. [76]

Malignancies

Anaemia is a common accompaniment of malignancy. In many patients, it is absent at the time of diagnosis but develops in the course of the illness. It is particularly common with malignancy of the alimentary tract. The type of anaemia depends on the dominant underlying mechanism or mechanisms.[77, 78]

Of the wide variety of haematological abnormalities associated with cancer, anaemia is by far the commonest. Anaemia is almost invariable with metastatic disease, but also arises frequently in localized cancer. Indeed, it is rare for any malignant disease to run its natural course without haematological complications.

The anaemia of malignancy has many causes, including haemorrhage, deficiency states, haemolysis, haemodilution, leuco-erythroblastosis and may be further complicated by chemotherapy. It may sometimes be the presenting feature, though the primary cause more often dominates the clinical picture. The red cells are normochromic and normocytic, though they may be hypochromic and microcytic, particularly if there is bleeding. [78, 79]

Other diseases

Other unusual causes of normocytic anaemia include chronic lead poisoning and inflammatory disorders which can present as pancytopenia with the anaemia being normocytic. [80, 81]

Clinical features of note in normocytic anaemia.

The patient with anaemia secondary to chronic disease will virtually always be symptomatic of the underlying disease. Therefore, the important points to note in history and examination include the following; in the history, the following may be pointing, rate of onset of symptoms and signs, weight loss, anorexia, fever, pain, diarrhea, constipation, nausea, vomiting, medical, drug, social and family history. On examination, the salient features include palor, jaundice, lymphadenopathy, finger clubbing, and oedema

Systemic review shows;

Skin: rash, dyspigmentation.

Abdomen: Hepatosplenomegaly, masses, tenderness.

Cardiac: Tachycardia, haemic murmurs, increased jugular venous pressure.

Respiratory: dyspnoea, tachypnoea, reduced breath sounds.

Genitourinary system: abdominal tenderness, oedema, discharges

Central Nervous system: focal neurological signs.

Musculoskeletal system: joint, bone tenderness, swelling.

Others; Goiter, lymphoedema,

Investigation of normocytic anaemia

Blood Examination

Red cell morphology: Normocytic **to Marked anisopoikilocytosis.**

White cell count and differential: A normal or slightly increased white cell count is seldom present. Abnormal neutrophils with bilobed Pelger- Huet nuclei or deficient granulation

Reticulocyte count: The Reticulocytes count is normal or reduced the normal range is usually 40-140x10^9/l (1-3%)

Serum iron low (normal range 12-24 µmol/l in males and 9-23 µmol/l in females), TIBC low (normal range 54-72 µmol/l in males and 55-81 µmol/l in females)

Renal function tests; Urea, creatinine and electrolytes: performed in case one suspects renal causes of the anemia.

Liver function tests: In case of chronic liver disease as a cause of the normocytic anaemia, or in patients presenting with jaundice.

Radiological; chest X Rays, CT scans, MRI- in case of malignancies, chest infections, brain abscesses

Special investigations and other investigations ***(when clinically indicated)***: urinalysis, blood cultures, Coombs tests, Mantoux tests, Barium meal, enema or endoscopy (gastrointestinal bleeding)

Tests of thyroid function in hypo- or hyperthyroidism.

4. RATIONALE AND RELEVANCE OF THIS STUDY

Anecdotal observations suggest that normocytic anaemia is one of the commonest types of anaemia and is associated with many illnesses. However in KNH the prevalence of this type of anaemia and the commonest associated diseases are not documented. These are also against a background coupled with serious blood transfusion problems and a large pool of haematological disorders. Providing information and documenting features including disease conditions associated with NA would enhance the management and also avoid the use of blood as a means of alleviating anaemia in some of the cases of chronic disorders.

Furthermore, despite the fact that the anecdotal observations show that the prevalence may be high among many chronic conditions, it has received little attention among the Kenyan health care professionals who need to recognize that approaches to its treatment significantly impacts in the overall management of many chronic diseases frequently found associated with this form of anaemia. This is relevant in as much as anaemia per se reduces one's quality of life and can increase morbidity and mortality

To our knowledge, no study has attempted to link specific disease conditions to normocytic normochromic anaemia (NNA) in Kenya or in the KNH.

While there are aspects of Normocytic Anaemia documented in the literature, this study will bring out the following aspect in KNH patients; age, sex, geographical (province) origin and social dimensions also the proportion each of the common disease conditions associated with it.

It is against this background that this study's results is going to set stage for further studies perhaps elsewhere as well in Kenya to gain even more insight into the normocytic normochromic anaemia and associated diseases.

In addition, the compelling rationale for this study is on the basis of management of normocytic anaemia in the background of some diseases. It is prudent to correct the underlying disease condition rather than the anaemia. However in a setting where the likely cause is not established, this may be costly. Another form of management component is blood transfusion. In established causes or when the anaemia is severe, this could be a straightforward option. However, it is prudent to avoid unnecessary blood transfusion. This would require a basis on which to make decision on and the results of this study will provide such some information to these effects.

It is documented that upfront treatment with iron is harmful except in rare cases where iron deficiency is evidenced by the absence of stainable iron in the bone marrow preparation. Iron treatment, either oral or parenteral will only worsen reticuloendothelial iron overload and will not improve the Hb level. The results of the study will provide some rational bases on the majority of instances.

The usual trend taken in most cases with anaemia is to do a thorough search for associated diseases contributing to the anaemia. This can be ameliorated by results of a study like this that aims at increasing the index of suspicion by providing the commonest associated disease conditions and documenting the initial investigations to be performed.

As NA is a multifactorial disease and is seen in many clinical settings, this study aims at highlighting the commonest association thereby assisting in the differential diagnosis and improving in the overall management of the cases. In this multiplicity of disease associations with normocytic anaemia it is possible that some conditions occur more commonly or in company with others. Therefore this study may assist in ranking and sorting out the commonest associations.

Euphemistically and the snapshot of this study will lead to the following:

1. To bring to light the status of normocytic anaemia in KNH patients. This will aid in rational use of haematinics and improve the overall cost of patients' management.
2. Documentation of level NA and associated factors will raise the index of suspicion regarding the condition likely to be involved, hence leading to improved overall patient management.
3. Reducing the number of steps involved in the evaluation of the disease conditions accompanied by NA will impact positively not only on the patient but to the institution as well perhaps reducing the psychological problem of unsettled diagnosis in a patient.
4. Aid in hastening the investigation of anaemia in KNH. This is because it will have pointed to the most appropriate blood picture that needs to be considered and in so doing, will develop investigation algorithms for normocytic anaemia and in developing the basis of management of normocytic anaemia.
5. This study may also add to the existing literature on the haematological profile of the identified ten commonest disease conditions that the study may bring up.

5. STUDY HYPOTHESIS AND OBJECTIVES.

Hypothesis

Null hypothesis: Normocytic anaemia is not the most common type of anaemia in the ten commonest chronic disease conditions in KNH.

OBJECTIVES

General objective

To determine the normocytic anaemia and the associated diseases in KNH.

Specific objectives

1. To document the demographic characteristics of patients presenting with normocytic anaemia
2. To determine the complete blood count profile of patients with normocytic anaemia in the study cases.
3. To determine the proportion of normocytic anaemia in the anaemic cases, in the patients investigated at haematology laboratory in KNH.
4. To document the ten most common diseases associated with normocytic anaemia.

6. MATERIALS AND METHODS

Study design

This was a cross- sectional descriptive study conducted from September 2007.

Study area

Hematology laboratory and wards at the KNH.

Study population: Patients presenting with anaemia and classified haematologicaly as normocytic anaemia during the study period at the KNH laboratory

Inclusion criteria

- Patients aged above 13 years, both male and female.
- Patients whose baseline haematology profile show; Hb levels below 11.5g/dl, MCV within the normal reference range of 76fl to 96fl and PBFs reveal a normocytic RBC picture.
- Patients who consent to the study.

Exclusion criteria

- Patients who are not assigned a disease condition or specific diagnosis and can not be traced in the wards.
- Patients who are too sick to consent and whose relatives cannot be traced to consent for them.
- Patients with anaemia of chronic illness and low MCV.
- Pregnancy.

Identification and recruitment of study cases

Daily perusal of all haemograms reports was performed in the haematology laboratory and those showing anemia i.e. with Hb below 11.5g/dl were selected. Only those with normocytic anaemia (MCV 76-96fL) were evaluated further, whereas those with other types of anaemia were noted. Other information obtained from the requests of patients with normocytic anaemia included the ward address of the patient, in- patient number, laboratory number, name, age, sex, the complete blood count results and other haematological requests such as ESR and reticulocyte counts. The patients were then traced back to the ward, where the principle investigator proceeded. The patients were then informed about the study after which an informed consent was then sought. Clinical information of the patients was then obtained by history taking, physical examination and perusal of the patients medical records all these with the view to noting the disease condition, diagnosis and other results of the evaluatory investigations so far performed. These were then filled in a preformed questionnaire. Blood was then taken from the patients for the required tests including, full haemograms, PBF and ESR. Information regarding other investigations that have been performed on the patient was then obtained from the patient's file, as per indications. A research assistant was also involved, whose role included identification of the patients, location of the patient's files and helping in the transportation of the samples to the laboratory.

Procedure

Clinical assessment through clinical information, history, physical examination, investigations and perusal of the care records in the wards for the required details which were noted and transferred to the proforma questionnaire.

For the haematological test, venous blood was collected using a G 21 needle. Blood was then put in appropriate bottle with EDTA (Ethylene Diamine Tetra Acetic Acid) after which it was transported to the laboratory. Haematological tests were done within 4 hours of specimen collection those not done were stored in a refrigerator at 4^0C, (and processed the following day). This included making of blood smears for PBF, which was stained in the laboratory through the use of Romanowsky stain, and performing CBC using a cell counter. All the samples for this study were performed on Cell-dyne 3200.ESR was done on the day of collection using the Westergren method.

Laboratory

Examination of the haemograms, PBF and ESR was performed by the principal investigator and reviewed by supervisors. Examination of the haemograms involved interpretation of the results from the Cell-dyne machine print-out. The PBF examination involved blood films stained by May-Grünwald Giemsa stain. The blood film was examined systematically, and started with macroscopic observation of the film for staining quality. It was then examined under lower magnification(x 10 and X20 objectives to get the area of ideal thickness assess for rouleaux formation, distribution of white blood cells and for platelet aggregations. The film was then examined at x40 objective to assess the size and shapes of red blood cells, and perform differential counts of WBC and platelet counts. Finer cellular details were then examined at x 100 oil objectives. All these features were recorded and reviewed by study supervisors. The performance of other laboratory tests, including non hematological tests depended on the patient's diagnosis and physical examination and was used to assist in the filling of the questionnaire.

Reporting and diagnosis

The results were reported by the use of standard reporting methods, including RBC features like size, shape, abnormal forms, rouleaux formation and inclusion bodies of present; WBC features, including presence of neutropenia, and differential counts; and platelets, including adequacy and presence of giant and abnormal forms. These were then counterchecked by the study supervisors

Quality assurance

Prevention of pre-analytical errors

a) Specimens were collected in blood collection bottles containing EDTA preservative.
b) Proper patient identification: including positive identification of the patient, labeling of the specimen bottle with the patient's name and in- patient number, and then confirming that the same is repeated on the request form.
c) Ensuring that the specimen is analyzed in the laboratory within the required time.
d) Storage of any sample that could not be analyzed on time at 4^0C then analyzing it the following day.

Prevention of analytical errors

- Proper calibration of automated machines for the study
- Use of control samples for the study.
- Use of clean slides to make blood smears and ensuring that the smears are well made and immediate labeling of the slides after making smears.
- Performing manual differential white blood count and platelet counts.
- Counterchecking of the peripheral blood film by the study supervisors.

Sample size calculation

Primary outcome variable =presence of normocytic anaemia

Best guess of expected percentage (proportion) = 50% (0.05)

Desired width of 95% confidence interval

(Fisher & Van belle) formula for sample size calculation

The formula for the estimation of a single proportion was as follows at 80% power.

$$\frac{n = Z^2p(1-p)}{\partial^2}$$

Where;

n= required sample size

p= expected proportion

∂=The required precision (0.05)

Z= the value corresponding to 95% confidence interval

Substituting the above formula we get:

$$N = \frac{(1.96)^2 x\ 50\ \%(1-50\%)}{0.05^2} = 384.16$$

≈ 385 respondents

Consenting

An informed consent was obtained from the patients included in the study. For those below 18yr or those too ill to consent, consent was obtained from their parents/guardians.

Questionnaire administering

A questionnaire was administered and filled appropriately. This was done by filling in the patients data, the results of the haematological tests and non haematological as per indications.

Data analysis plan

Data was pooled, screened, and entered on data analysis proforma sheet and subsequently entered into the SPSS version 12- computer software system. All statistical analyses were carried out using the same system. Means and standard deviations was then calculated and statistical significance between different groups determined by Mann-Whitney test. Association between age, sex, social factors and disease conditions was evaluated using the Chi-Square Pearson test. A *p*- value of <0.05 was considered significant.

The total number of patients presenting with anaemia and the number of cases with NA in the period during the study provided the denominator and numerator respectively from which the total number, the proportion with normocytic anaemia was calculated.

Results were then presented in graphs, charts, percentages and narrative comparisons and discussed.

7 ETHICAL ISSUES

- Clearance was sought from the KNH Scientific and Ethical Review Committee and the study then commence upon approval.
- Consent was sought from the patients or the parent/guardian for patients too ill to give consent or those below 18 years of age.
- Patients did not pay for any charges connected to this study
- Usual care and evaluation procedures were facilitated for the study patients and results of investigations were then communicated to the usual health care providers to facilitate improved care of the patients.
- Those who declined to consent were not discriminated against with respect to provision of the medical attention.

8 STUDY LIMITATIONS

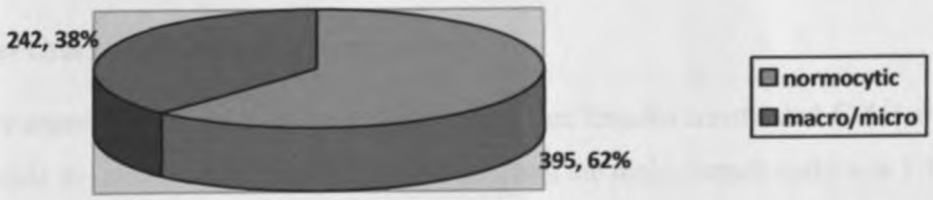
During selection, all the MCV below 76fl and above 96 fl were assumed to be microcytic and macrocytic anaemia respectively yet it is acknowledged that some could be associated with anaemia of chronic disease. However, since iron studies were not done, in was considered appropriate to leave out this category of patients.

9 . RESULTS

During the period, 637 patients were assessed. Out of these 395 patients (62%) were found to have normocytic anaemia, while 242 patients had macrocytic and microcytic anaemia. This classification was based on the blood picture consisting of haemogram results and morphological assessment on the blood film as per study criteria.

On cell counter (cell dyne 3200), all had Hb below 11.5g/dl and MCV as follows: microcytic <76fl, normocytic 76-96fl and macrocytic > 96fl. These were verified by peripheral blood film and red blood cell count based on size, shape and central palor. The results were as presented in figure 1.

Figure 1:Distribution of the types of anaemia in study cases



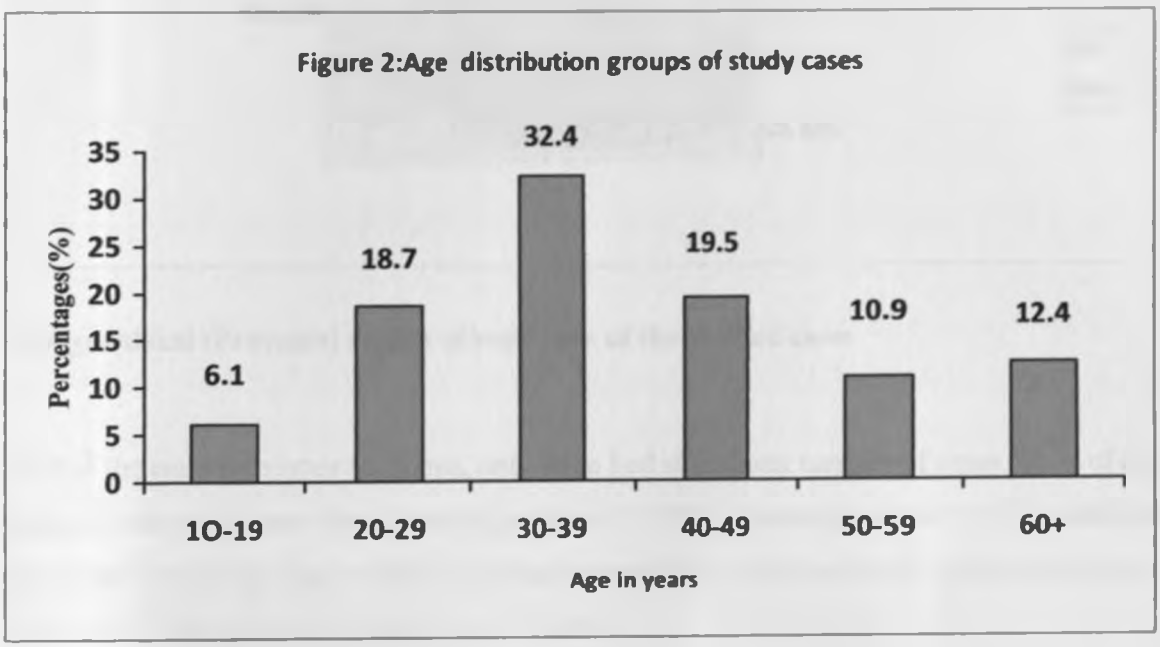
Demographic characteristics of patients with normocytic anaemia in KNH

Of the 395(62%), the following were the results for the demographic profiles.

Age characteristics of study cases

Only those who were 13years old and above were studied and the results showed the oldest recorded case was 89years old. The overall age pattern was as follows; range 13 to 89 years,

mean age of 39.62 and a standard deviation of 15.16. The median was 37 years. The mode age group was the 30-39 age (32.4%), while the least age group was 10-19 years (6.1%) The profile is shown in the figure 2.



Gender characteristics of the study cases

The gender aspects of the studied cases documented that females constituted 63 %(n=250) of the subjects while the males constituted 37 %(n=145), and the male: female ratio was 1:1.7

Gender distribution is as shown in the figure 3.

Figure 3:Gender distribution of patients with normocytic anaemia.

Geographical (Province) region of residence of the studied cases

Out of the eight provinces of Kenya, only three had significant number of cases. Most of the patient in the study came from Nairobi province (51.9%) , Central province (21.3%) and Eastern province(12.9%).The least number of patients came from Coast and North Eastern provinces (1% each). This is shown in figure 4 and Table 1.

Figure 4:Distribution of patients with normocytic anaemia by the province of residence

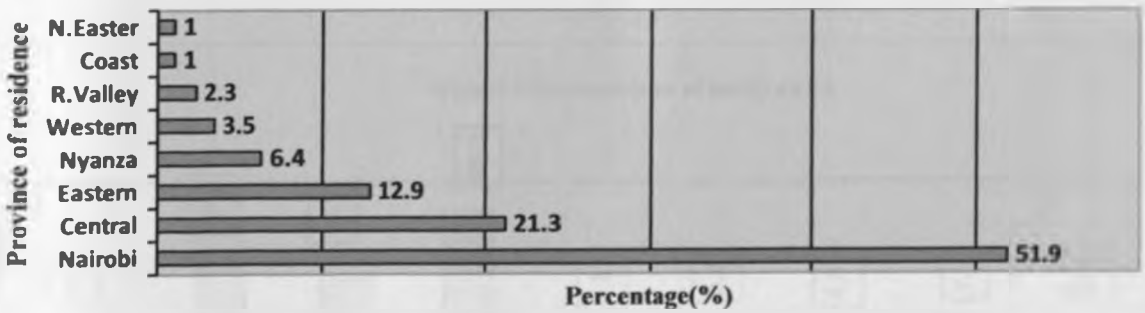


Table 1: Province of residence and disease conditions

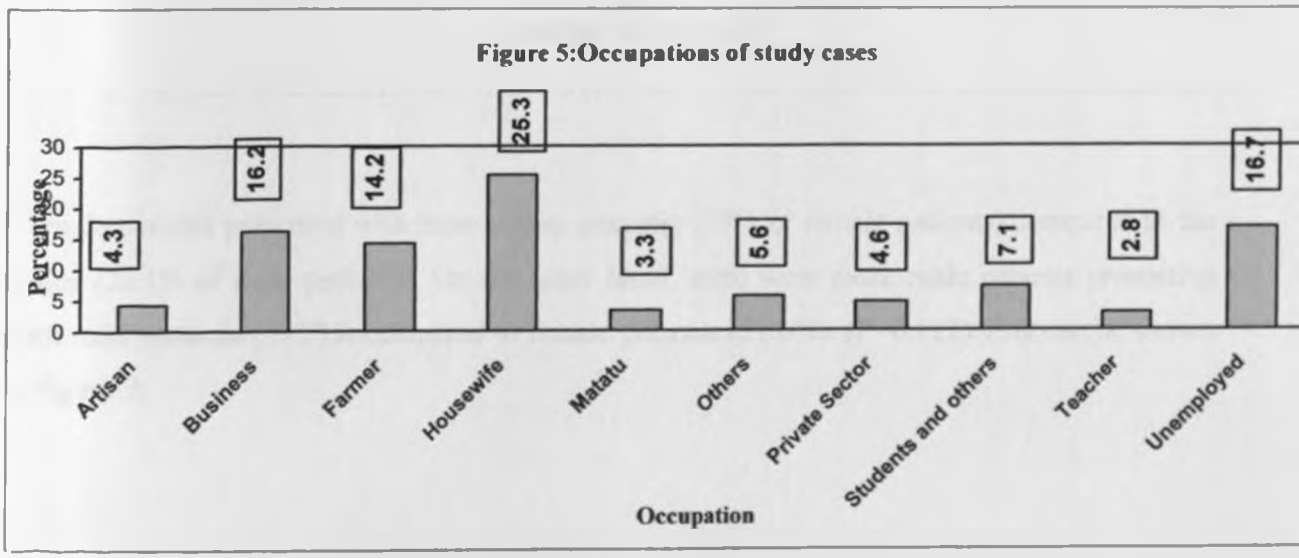
	PROVINCES								
	Central	Coast	Eastern	North Eastern	Nairobi	Nyanza	Rift valley	Western	Total
TOTAL	83	4	50	4	205	24	11	14	395

These data show that of the 205 patients from Nairobi 44.5 %(n=92) of these patients came from the parts of Nairobi classified as slum areas. These areas were as follows: Huruma (n=17); Mathare (n=16); Kibera (n=17); Kariobangi (n=15) and Dandora (n=27). Patients from the more middle class estates of Nairobi were the least and presented 1.95% of the total number of patients from Nairobi (n=4). These estates were Langata (n=2) and south C (n=2).There was no patient from the upper class estates in Nairobi during the study period.

Occupation of patients in the study.

The following occupations were documented; housewives (25.3%) unemployed (16.7%) business (16.2%) farmers (14.2%), teachers (2.8%). Teachers accounted for the least percentage of patients (2.8%). Figure 5 illustrates the findings.

Figure 5:Occupations of study cases



Complete blood count profile of the study cases.

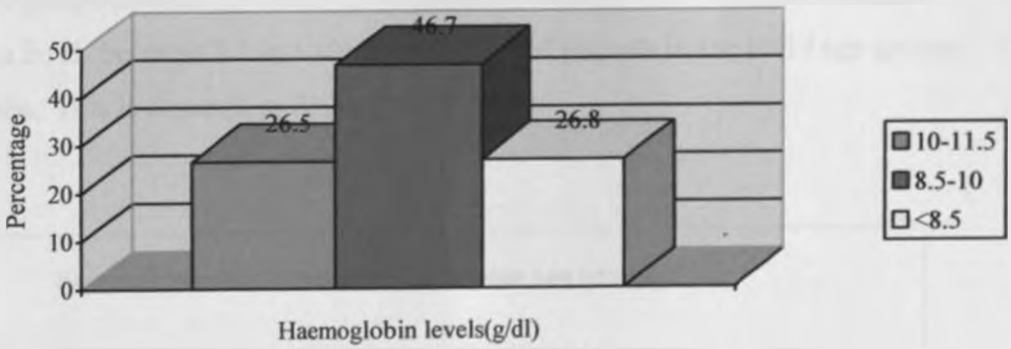
The complete blood picture with regards to the study consisted of cell count print out showing Hb, RBC, PCV, WBC (total and differentials- neutrophils, lymphocytes, monocytes, eosinophils and basophils) and platelets. The results showed the following as stipulated in the charts.

Focus was further put in correlating Hb and WBC with other patients' parameters as outlined below.

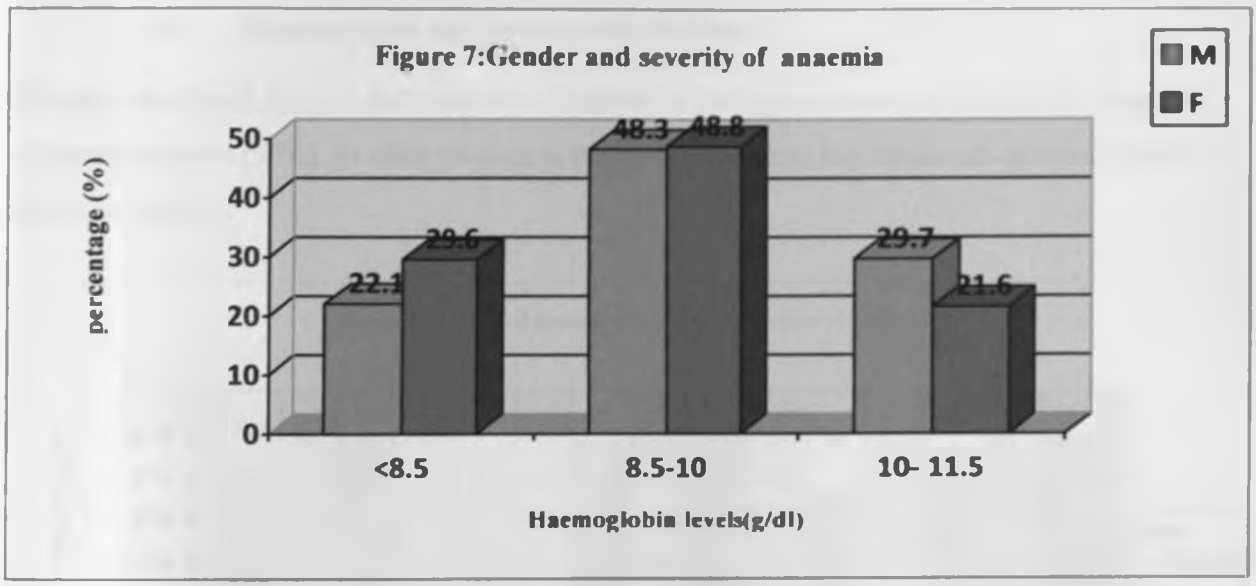
A Haemoglobin level

Mild to moderate anaemia was found in 73.2% of patients with normocytic anaemia. Severe anaemia was found in 26.8% of the patients. This is as shown in figure 6.

Figure 6 Haemoglobin levels of the study cases

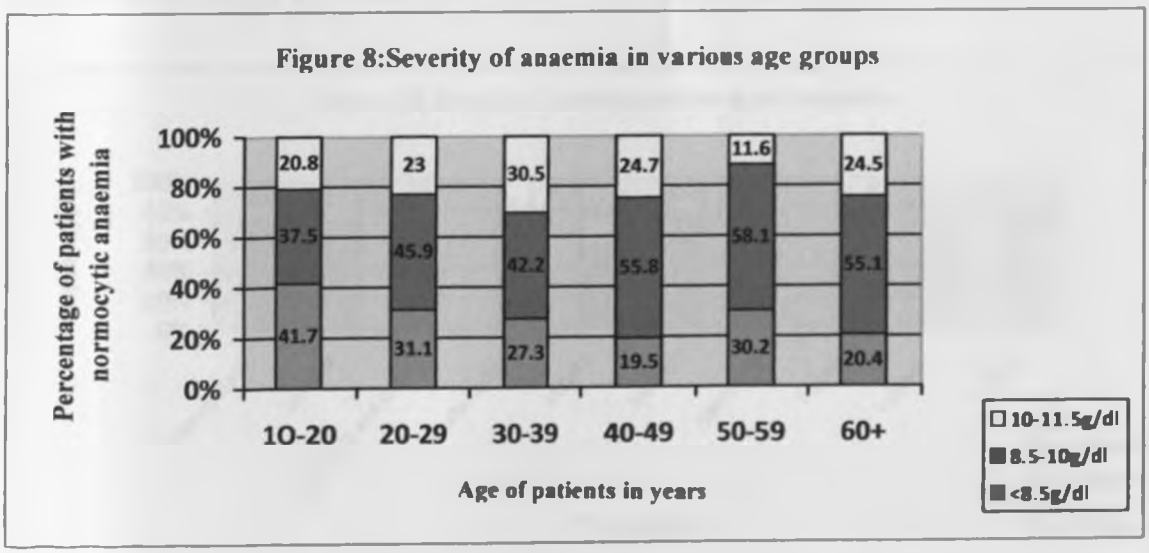


Female patients presented with more severe anaemia (29% of female patients) compared to the males (22.1% of male patients). On the other hand, there were more male patients presenting with mild anaemia (29.7%) compared to female patients (21.6%). (P=0.122).This can be shown in figure 7.



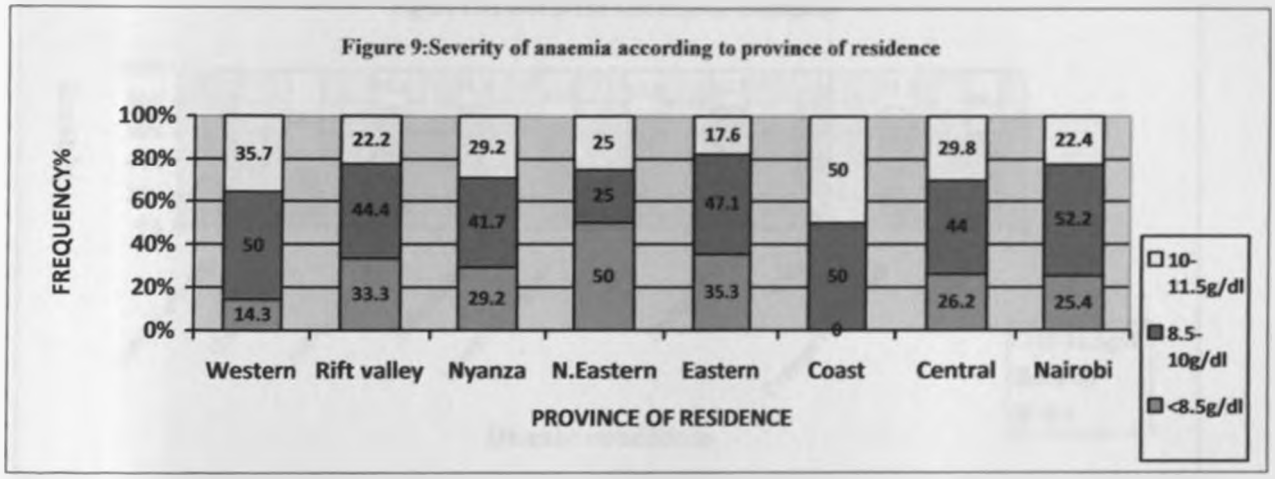
i) Haemoglobin and age

In most of the age groups studied, the majority of patients presented with moderate anaemia, with haemoglobin levels between 8.5 and 10g/dl. Majority of patients in the 10-19 age groups had severe anaemia. This is depicted in figure 8 (p=0.183).



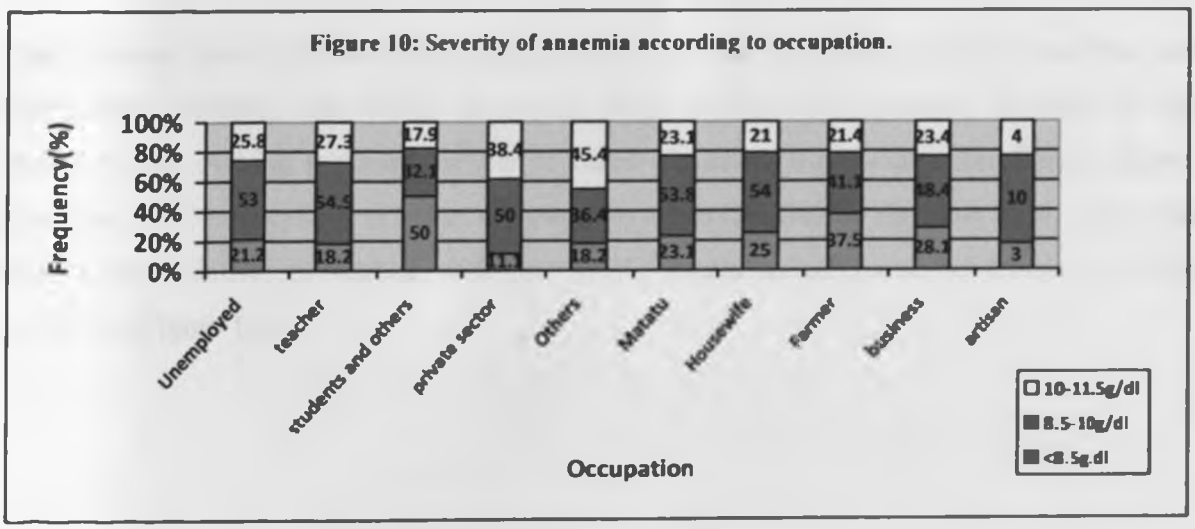
ii) Haemoglobin and province of residence

Patients from North Eastern province were found to be having more severe anaemia as compared to other provinces (50%). In other provinces, majority of patients had moderate anaemia. This is shown in figure 9.



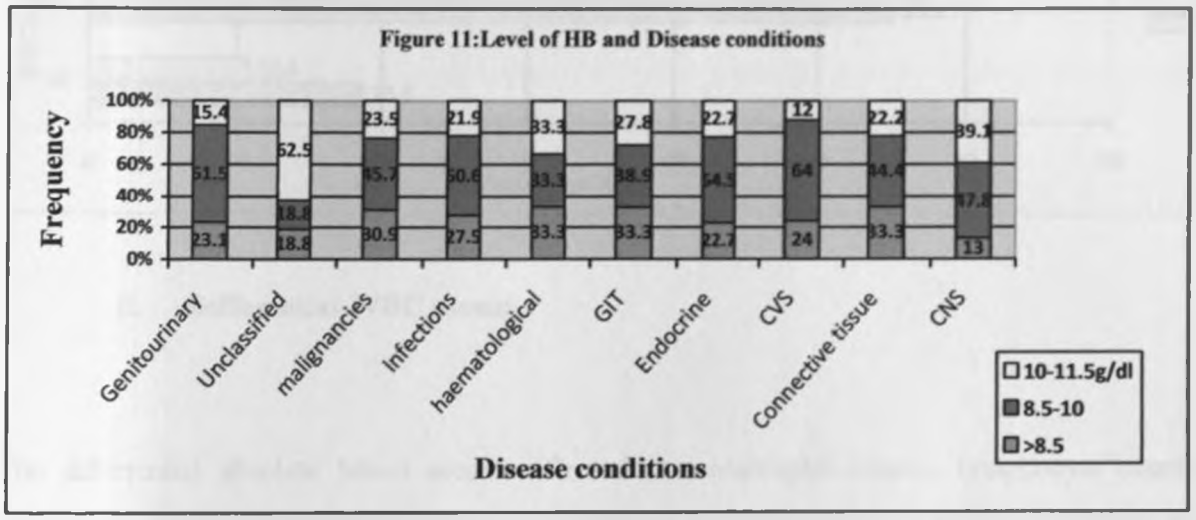
iii) Haemoglobin and occupation

The level of Hb in most of the occupation categories was found to be of moderate anaemia. (p-0.3). However among the students, in was severe in 50%. (Figure 10)



iv) Haemoglobin and disease conditions

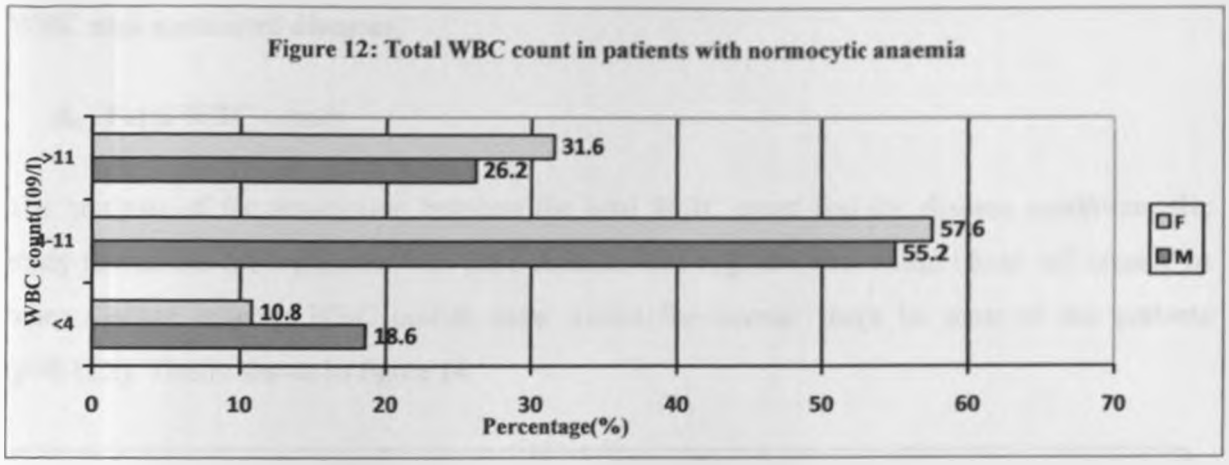
Majority of patients across all the disease categories had moderate anaemia as shown in figure 11 below (p=0.845).



B White blood cell counts

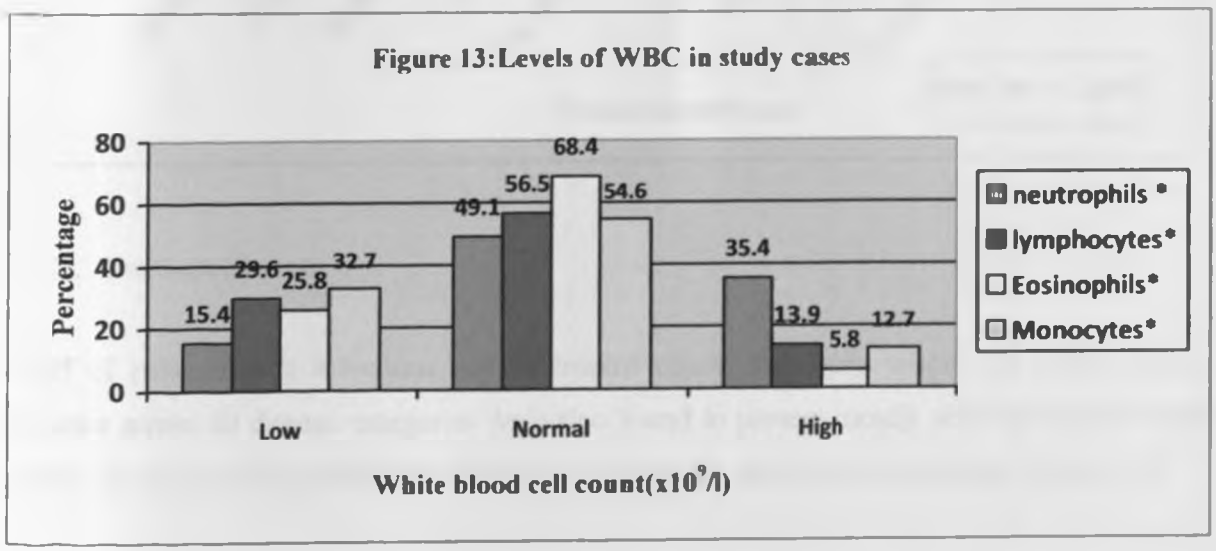
i. Total white blood cells count.

The reference level for Total white counts was 4-11x10^9/l. The study results showed that total WBC count evaluated was within the normal range in most of the patients. (Females 57.6%; Males 55.2%). Among the total number of cases evaluated, there were more female patients presenting with leucocytosis (31.6%), compared to males (26.2%).On the other hand, there were more males (18.6%) presenting with low WBC counts as compared to females (10.8%), (p=0.078) (Figure 12).



ii. Differential WBC counts

The differential absolute blood counts reflected that neutrophil counts, lymphocyte counts, eosinophil counts and monocyte counts of all patients with normocytic anaemia were within the normal range in most of the patients as shown in figure 13.



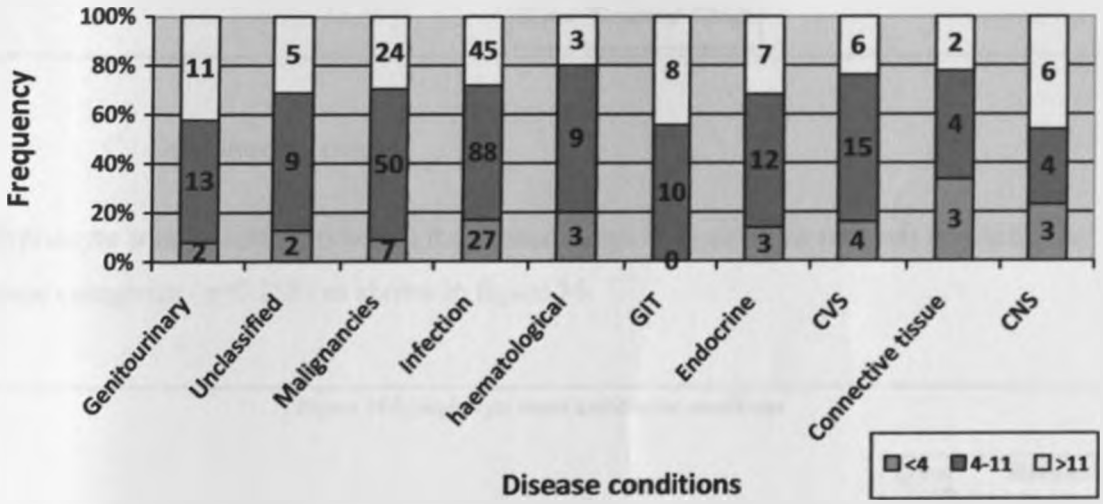
*Reference levels are in the appendix VII

WBC and associated diseases.

A. Total WBC counts

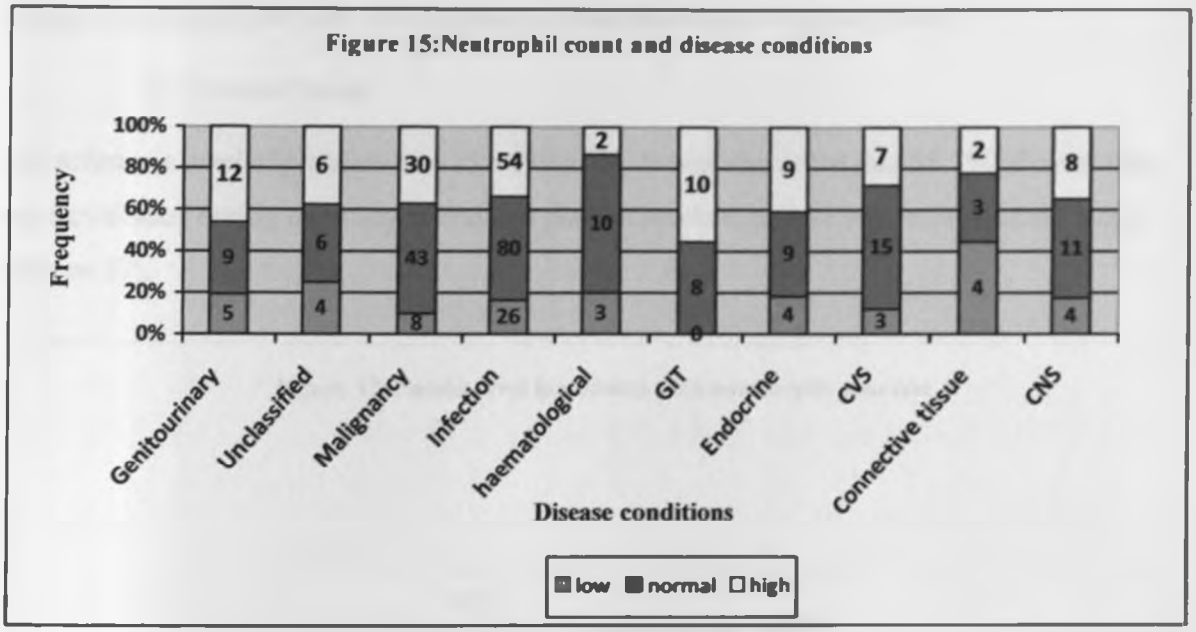
The analysis of the association between the total WBC count and the disease conditions, the study found that most patients with CNS diseases had high absolute white blood cell counts. In other disease criteria; WBC counts were within the normal range in most of the patients (p=0.122). This is shown in figure 14.

Figure 14: WBC count and disease conditions



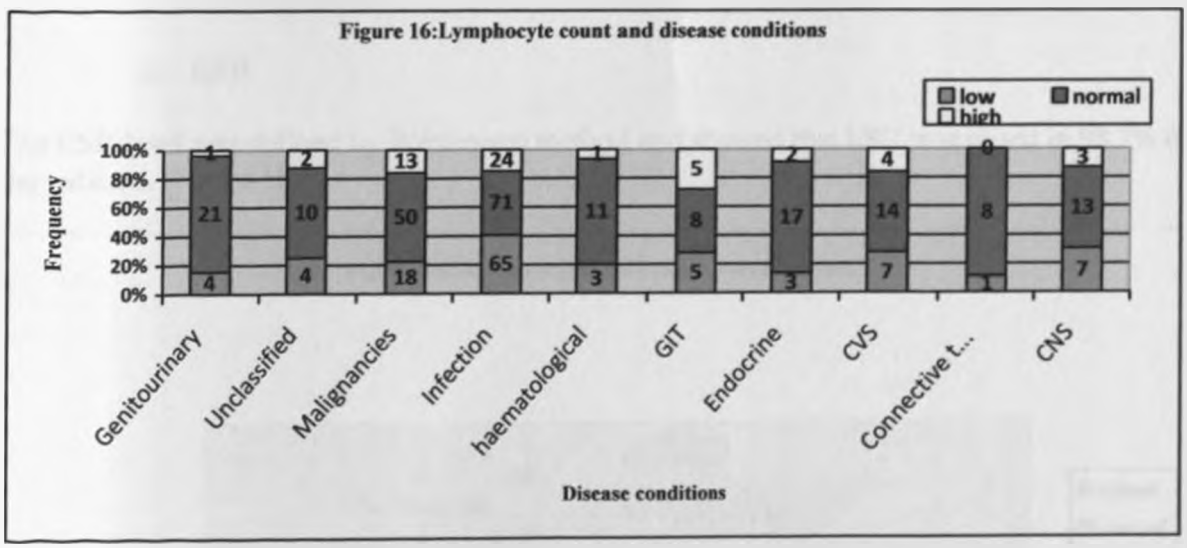
B. Neutrophil counts

Half of patients with infections had neutrophil counts that were within the normal range. Patients across all disease categories were also found to present mostly with neutrophil counts within the normal range (P=0.734). This was however not statistically significant. (Figure 15)



C. Lymphocyte counts

Lymphocyte counts were also within the normal range in most of the patients across all the disease categories (p=0.258) as shown in figure 16.

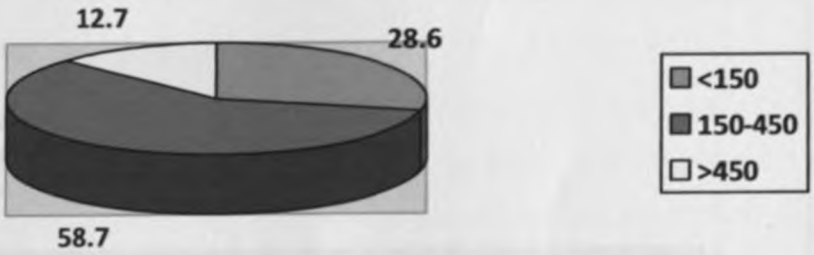


Platelet levels and ESR were further analyzed and the results were as follows

D. **Platelet levels**

The reference level of platelets was 150-450x10^9/l. It was also noted that 58.7% of cases that were evaluated during the study period had platelet levels that were within the normal range. (Figure 17).

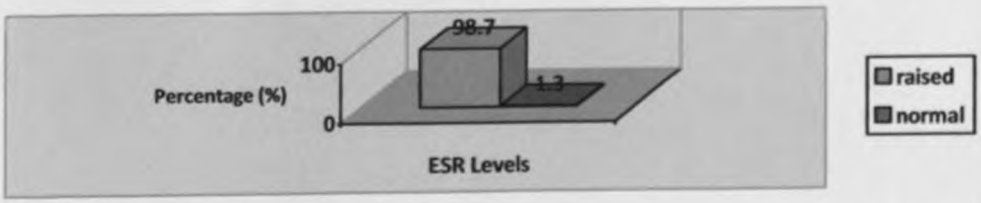
Figure 17:Platelet level in patients with normocytic anaemia



E. **ESR**

The ESR level was defined by Westergren method and showed that ESR was raised in 98.7% of the patients. (Figure 18)

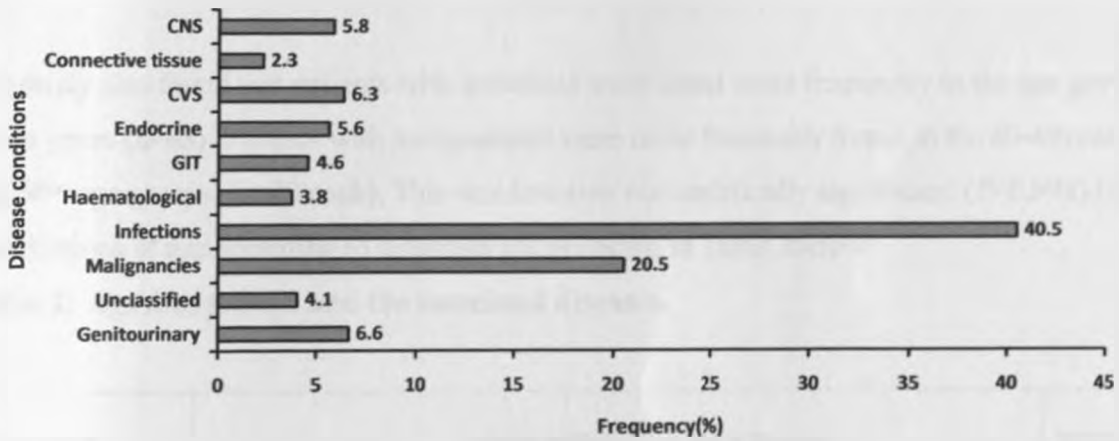
Figure 18:ESR levels in patients with normocytic anaemia



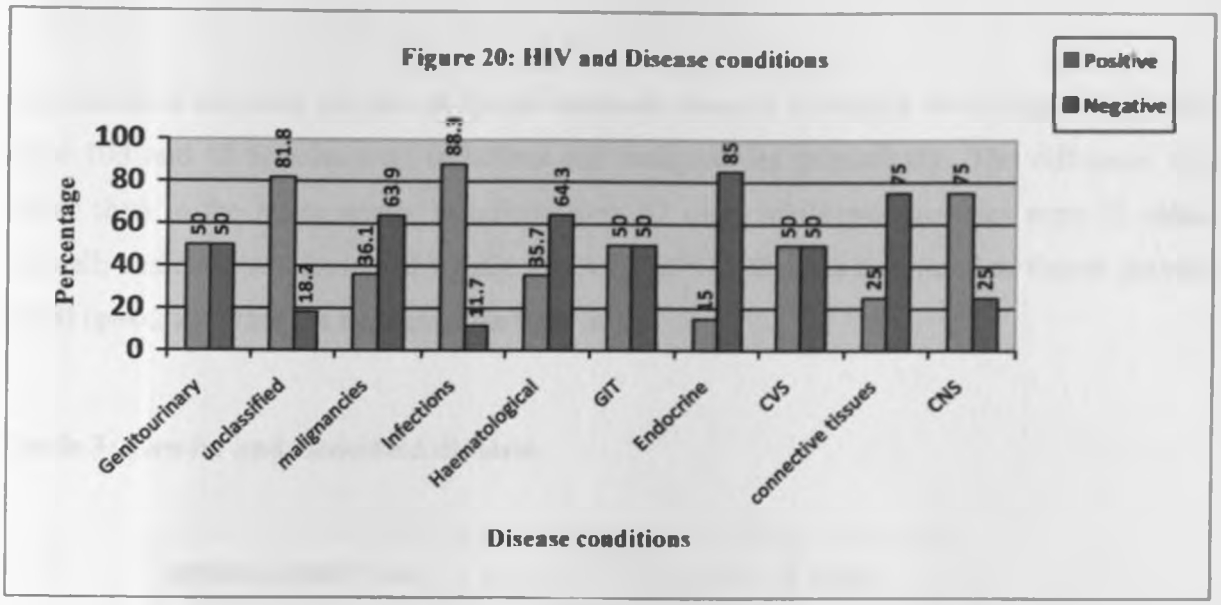
Associated diseases with normocytic anaemia

In addition this study evaluated for associated diseases in those with normocytic anaemia. This was done by perusing and seeking out for the patient's clinical details. This included the impression or diagnosis as stipulated on the patient's request form and corroborating the information by clinical evaluation of the patient and looking at the file and other records available at the ward. The results showed that the most common disease conditions in patients with normocytic anaemia during the study period were infectious diseases (40.5%), of which majority of them had HIV infection. The complete picture is presented in figure 19.

Figure 19:The ten associated diseases documented in the study cases



Of the patients with infections, 88.3% were also HIV positive. (On the other hand, 85% of patients with endocrine diseases were HIV negative). The P value was <0.005, which was statistically significant. (Figure 20).



The study also found that patients with infections were found more frequently in the age group 30-39 years (n=63). Patients with malignancies were more frequently found in the 40-49years and 60+ age groups. (n=20 each). This was however not statistically significant. (P=0.998).Other distributions of age according to diagnosis are as shown in Table 2below

Table 2: Age distribution and the associated diseases.

DISEASE CONDITION	AGE GROUPS IN YEARS						TOTAL
	10-19	20-29	30-39	40-49	50-59	60+	
CNS	1	4	8	4	4	2	23
Connective tissue	0	3	2	1	2	1	9
CVS	4	4	4	5	3	5	25
Endocrine	0	1	4	9	4	4	22
GIT	1	5	5	3	0	4	18
Haematological	2	5	5	3	0	0	15
Infections	11	38	63	27	12	9	160
Malignancy	4	7	15	20	15	20	81
Unclassified	1	4	7	3	1	0	16
Renal	0	3	10	4	3	6	26
TOTAL	24	74	123	79	443	51	395

Also noted in the study was that of the two common diseases; infections and malignancies, there were 103 and 48 females with infections and malignancies respectively. The difference was wider than in the males where infections were 57 cases while malignancies were 33 cases. Overall, male patients presented mostly with GIT (n=10) diseases compared to female patients (n=8) (p=0.213). This can be seen in the table 3.

Table 3: Gender and associated diseases.

DISEASE CONDITIONS	GENDER		TOTAL
	FEMALE	MALE	
CNS	16	7	23
CONNECTIVE TISS	6	3	9
CVS	14	11	25
DIABETES	17	5	22
GIT	8	10	18
HAEMATOLOGICAL	13	2	15
INFECTION	103	57	160
MALIGNANCIES	48	33	81
UNCLASSIFIED	10	6	16
RENAL	15	11	26
TOTAL	250	145	395

Of the associated diseases, infections were more frequently encountered in the housewife (n=37) category of patients followed by business category of occupation (n=32). On the other hand, larger number of unemployed presented with CNS diseases (n=7) (p=0.642). This is shown in table 4.

Table 4: Occupation and disease conditions

EASE IDITIONS	OCCUPATION										TOTAL
	Artisan	Business	Farmer	Housewife	Matatu	Others	Private sector	Student and others	Teacher	Unempl	
NS	0	3	3	5	0	2	3	0	0	7	23
onnective tiss	0	1	1	3	0	0	0	1	0	3	9
VS	1	2	5	6	0	2	2	2	2	3	25
ndocrine	0	3	7	7	0	1	1	0	0	3	22
IT	2	4	3	2	0	0	2	2	0	3	18
aematological	0	0	2	6	1	0	0	2	0	4	15
fection	10	32	15	37	6	12	7	15	6	20	160
alignancies	2	10	14	22	5	4	2	4	1	17	81
nclassified	1	6	1	4	0	1	0	2	0	1	16
enal	1	3	5	8	1	0	1	0	2	5	26
TAL	17	64	56	100	13	22	18	28	11	66	395

The results show that Nairobi province had the highest percentage of the total number of patients presenting with infections; n=104(65%) and malignancies; n=37(45.6%). Central province had the highest number of patients presenting with haematological diseases; n=7(46.6%). This was however not statistically significant (p=0.176).Other distributions of diagnosis according to the province of origin are as shown in table 5.

Table 5: Province of residence and disease conditions

DISEASE CONDITIONS	PROVINCES								TOTAL
	Central	Coast	Eastern	North Eastern	Nairobi	Nyanza	Rift valley	Western	
CNS	4	0	5	1	10	1	1	1	23
Connective tissue	1	1	3	0	3	0	0	1	9
CVS	6	1	3	0	12	1	1	1	25
Endocrine	7	0	5	0	9	1	0	0	22
GIT	6	0	1	0	9	1	0	1	18
Haematol	7	1	1	0	5	1	0	0	15
Infections	20	0	15	1	104	7	7	6	160
Malignancies	24	0	10	1	37	8	0	1	81
Unclassified	4	0	4	0	6	1	0	1	16
Renal	4	1	3	1	10	3	2	2	26
TOTAL	83	4	50	4	205	24	11	14	395

10. DISCUSSION

General discussion

This study has clearly documented that normocytic anaemia is the most common type of anaemia as during the study period, normocytic anaemia constituted 62% of all the cases that were classified as anaemia at the KNH. These findings appear consistent with those of Cash et al [28]. it also correlate well with a study conducted on women 20-89years of age in USA, which showed 60-76% had normocytic anaemia[29]

Furthermore, 26.5% had mild while 46.7% had moderate anaemia. The consistency is partially due to the facts that the diseases associated with the anaemia were all chronic, usually established to cause anaemia of chronic disease. The later is initially normocytic normochromic however, as the disease progresses, anaemia exhibits with microcytic hypochromic picture.

Indeed as in other studies, this study demonstrated that the most common disease associated with normocytic anaemia in patients admitted in KNH was infectious diseases (40.5%), with a larger number of these testing positive for HIV infection (88.3%). Neoplasms (20.5%), genitourinary diseases (6.6%), cardiovascular diseases (6.3%), central nervous system diseases (5.8%), endocrine diseases (5.5%) haematological diseases (3.8%) and connective tissue diseases (2.3%) were also associated with normocytic normochromic anaemia. This can be compared to a study in USA among patients with ACD. It was found that 36% had some sort of infection, including cellulitis, urinary tract infections, TB, liver abscess and endocarditis Renal insufficiency was diagnosed in 16%.Six percent had inflammatory disease: SLE, scleroderma, unspecified polyarthritis. However there were some differences as in the studies from the west 24% diseases including alcoholic liver disease, cardiovascular diseases and endocrine diseases[28]. In a similar study in Australia, the most common disease condition associated with normocytic anaemia was infections (51%).[60] In a study performed on 100 patients with chronic diseases at Jacquelyn McClure Lupus Center, Atlanta, Georgia USA, infections (seen in 43 patients) was the most common diagnoses. Other diagnoses included: malignancy (16), rheumatologic disease (30), other inflammatory diseases (seven), renal disease (25), and miscellaneous problems (38).[82]

Most of the patients with normocytic anaemia were females, representing 63% of the study subjects, with the mode age of 30-39 years. Occupation of the majority of these patients was housewives (25%), followed by the unemployed (16%). Teachers were the least in the occupations category (2.8%). The majority of patients were residents of Nairobi, followed by Central and eastern Provinces.

In all the categories of diseases, the white blood cell counts were within the normal range. Over half of the patients had total white blood cell counts within the normal range (p-0.122). Absolute white blood cells counts also showed similar picture, with neutrophil counts, (49.1%; p-0.734) lymphocyte counts (56.6%; p-0.258), eosinophil counts (68.4%; p-0.012) and monocyte counts (54.5%; P-0.06) being within the normal range in most patients in all of the diseases which were studied.

Platelet counts were also within the normal range in most of the patients with normocytic anemia. ESR was raised in most of the patients.

The shortcoming of this study was inability to perform iron studies in the bone marrow of these patients. This is because some of the patients with anaemia of chronic illness and whose peripheral blood is showing normocytic anaemia may actually be having iron deficiency anaemia and hence may require iron therapy as part of treatment.

The demographic characteristics of patients presenting with normocytic anaemia

1. **Age -sex distribution**

The age of patients admitted with normocytic anaemia during the study period ranged between 13 and 89 years. The mean age was 39.62 with a standard deviation of 15.160. The majority of patients were between 30-39 years of age. The least number of patients were those who were below 20 years of age.

Females accounted for 63% of the subjects while males. M: F ratio was 1:1.7. In all the age groups studied, most of them had moderate anaemia. However, the patients in the age group below 20 year of age presented with severe anaemia. In a study done in Australia in patients with anaemia of chronic illness, there were 49% female subjects while males were 51%.[60] The large number of female patients in the study may be accounted for by the fact that they account for the

largest number of patients who seeks medical treatment. They also accounted for a higher percentage of patients with HIV (63%).

2. Province of residence in relation to normocytic anaemia

Most patients with normocytic anaemia who were admitted with chronic diseases at KNH during the study period resided in Nairobi and they accounted for almost half (41%) of the patients. Out of 205 patients from Nairobi, 44.5% came from the slum areas of Nairobi including Kibera, Mathare, Kariobangi, Huruma and Dandora. This was followed by Central province (21.3%), Eastern province (12.9%), Nyanza province (6.4%), Western province (3.5%), Rift Valley province (2.3%) then finally coast and North Eastern provinces, where 1% of the patients in the study came from.

The study also found that most of patients with infections and neoplasms were from Nairobi. The largest number of patients with haematological diseases came from central province.

The large number of patients from Nairobi is because KNH is located in Nairobi, hence the proximity of the hospital to the area of residence of these patients. Even though it is a referral Hospital, it serves the whole of Nairobi, with its population of about 3 million people. Most of these people live in poverty as reflected by the relatively high percentage of patients from the slum areas, and can only afford services KNH, which is a government hospital.

Both Central and Eastern provinces are closer to Nairobi, compared to other provinces. As such, the number of patients admitted to the KNH during the study period followed those from Nairobi.

Being a referral hospital, patients also came from other provinces. Some of these were referred with chronic diseases, which were also complicated with anaemia, which was observed to be moderate in most of the patients (p-0.456), with an exception of North Eastern Province, where most patients came with more severe anaemia (50%). However the number of patients from North Eastern was small (n-4) and this could account for this high percentage. The high number

of patients from this province with severe anaemia may also be due to the fact that the province is far from KNH and once referred; the patients may take a long time to reach the hospital.

3. Occupation of the study subjects in relation to normocytic anaemia

Housewives accounted for the largest percentage of patients that were recruited into the study with normocytic anaemia, representing 25% of the study subjects. They were followed by the unemployed (16. %). Teachers were the least category of the occupation as they represented 2.8% of patients with normocytic anaemia during the study period. As noted here, most of the patients in the study were unemployed (both the housewives and unemployed categories).They were also living in the slum areas. As such most of them seek treatment from clinics in the slums, most of which are run by non qualified medical staff. This hence leads to chronicity of their disease and hence by the time they are referred to KNH, they are already anaemic from the inflammatory conditions accompanying their chronic diseases.

As for the low number of the teachers encountered during the study, they are more educated and hence seek medical attention earlier and probably from the qualified medical staff. Many of them also are able to afford better treatment in more established private hospitals, hence their low number at the KNH during the study period.

It was observed that most of the patients across the occupational criteria with normocytic had moderate anaemia. However, 50% of students had severe anaemia (p-0.3). This was however not statistically significant.

Complete blood cell profile of patients with normocytic anaemia in the study cases

1. White blood cells(total and absolute)

The white blood count of most of the patients in the study was within the normal range. For females, 57.6% of the patients had white blood cell counts within the normal range (p-0.122).

This finding was also seen in the neutrophil count (49.1%); lymphocyte count (56.5%); eosinophil count (68.4%) and monocyte count (54.6%) which showed that in the majority of the patients, these counts were within the normal range.

It is usual to associate inflammatory conditions with raised levels of neutrophils. The probable cause of the observations in this study is that the infections were in the majority of the cases and these were associated with HIV. It is established that in HIV infections while other white cell parameters may be slightly increase, the lymphocytes are usually decrease. This would in overall bring the total count to within reference range.

No direct relationship was found between white blood cell counts and occupation or region of residence, nor between the later and neutrophil, eosinophil, and monocyte count. However, among the patients working in the matatu sector, 61.5% had low lymphocyte count (p-0.790), though this was not statistically significant.

2. Platelet count

The majority (58.7%) of patients with normocytic anaemia had platelet level within the normal range (p -0.136) though this was also not statistically significant.

Erythrocyte Sedimentation Rate

Erythrocyte sedimentation rate (ESR) in 98.7% of patients in the study was elevated.ESR levels are usually raised in chronic inflammatory conditions.

In general using the results obtained from haematological parameters of this study, it is possible to suggest some differential diagnosis and also to assist in disease monitoring of normocytic anaemia patients. The elevated ESR in the HIV cases and the level of haemoglobin being in the mild and moderate appear consistent for this type of anaemia and associated diseases. The other useful parameter that may assist in differential diagnosis is total WBC count which was elevated in most patients with CNS diseases. In addition the study has verified that normocytic anaemia is indeed the commonest type and that there are associated diseases thus providing data which has been lacking in KNH..

The 10 most common diseases associated with normocytic anaemia

Infectious diseases were the most common disease cluster associated with normocytic anaemia and accounted for 40.5% of patients admitted with normocytic anaemia who were recruited into the study. This can be compared by studies in other parts of the world. A study performed on patients with anaemia of chronic diseases in Australia sowed that 51% of the patients had infections[60] In another study in USA, 36% had some sort of infection[28]. The greater majority of patients who were in the study were from the medical wards. Most of these patients were diagnosed to be having chest infections, mainly pulmonary tuberculosis this was followed by meningitis, then pneumonia and gastroenteritis in that order. This can explain the larger percentage of patients in the study with infectious conditions. Majority of these patients (88.3%) also had HIV infection, further contributing to the high number of patients in this category. Most of these patients also had moderate anaemia, total and absolute WBC counts within the normal range. Also noted was that most patients presenting with infections were females, were housewives, resided in Nairobi and were in the group of 30-39 years cluster.

Malignancies accounted for 20.5% of patients admitted to Kenyatta National Hospital during the study period with normocytic anaemia second to infections. There were more female patients presenting with neoplastic diseases. Most of these neoplasms were in the female genitary system, majority of which was cancer of the cervix. This was followed by gastrointestinal tract malignancies, then leukaemias. It was also noted in the study that most of the patients presenting with neoplastic conditions were in the 40-49years and >59 years age groups. In a study done on anaemia in cancer patients in Germany appearing in the *European Journal of Clinical Investigations*, the most common anaemia was anaemia of chronic disease. Most of the patients in the study had ovarian carcinoma[83].

Genitourinary diseases were found in 6.6% of patients with normocytic anaemia. Most of these had either acute renal failure or chronic renal failure, with most of the patients having the later diagnosis. The occurrence and severity of anaemia in patients with renal diseases usually depend on the duration of illness. A study performed on patients presenting with anaemia of chronic diseases in USA found out that 16% of them had renal insufficiency[28]. The difference in

frequency of occurrence of renal disease between the two studies could be due to the fact that in the USA study, the investigator looked at the prevalence of ACD in renal patients as opposed to this study which renal diseases were taken as part of the ten commonest diseases associated with normocytic anaemia.

Patents presenting with cardiovascular diseases were 6.3% of study subjects. Most of these patients had hypertension, valvular heart diseases and congestive cardiac failure. Anaemia is usually not very common in hypertensive patients and if present, may be associated with co-morbid conditions such as renal failure or cardiac diseases. Anaemia is prevalent and adverse co morbidity in congestive heart failure, but little is known about its origins. Studies have suggested that not only is anaemia more common in congestive heart failure than could be accounted for by age and other demographic characteristics, but that its presence is associated with greater symptoms, exercise intolerance, and an amplified risk of mortality.[84-87]

Central nervous system diseases accounted for 5.8% of the patients with normocytic anaemia during the study period. Most of these patients were diagnosed with encephalopathy. Others had epilepsy and trauma. There was also a patient who presented with ascending paralysis.

Patients in the endocrine disease category represented 5.5% of patients. All of these patients had the diagnosis of diabetes mellitus. No other endocrine disease was diagnosed during the study period. In a study performed in Australia, 14% of patients with diabetes mellitus had anaemia of chronic illness. The likelihood of a diabetic having anaemia was also increased by presence of renal disease.[81]

Patients presenting with gastrointestinal tract diseases accounted for 4.6% of patients with normocytic anaemia. Most of these patients had liver diseases, the most common being liver cirrhosis. There were also other gastrointestinal conditions which included upper gastrointestinal bleeding and gastritis.GIT diseases were also noted to be more common in male patients.
Patients with upper gastrointestinal bleeding mostly present with iron deficiency anaemia, which in the peripheral blood film is seen as microcytic hypochromic anemia. However, during the initial stages of iron deficiency anaemia, the peripheral blood picture may by normocytic

normochromic. This explains the occurrence of normocytic anaemia in the patients in this study with upper gastrointestinal bleeding.

Haematological diseases were found in 3.8% of patients with normocytic anaemia. Most of these patients were females and had deep venous thrombosis. There were also some who were diagnosed with idiopathic thrombocytopenic purpura. These are chronic inflammatory conditions which lead to anaemia due to increased production of cytokines which plays a role in the pathogenesis of anaemia of chronic diseases. There were more patients presenting with haematological conditions coming from central province in comparison with other provinces.

Patients with connective tissue disorders accounted for the least number of patients with normocytic anaemia and accounted for 2.3% of the total number of patients encountered during the study period. Most of these patients were elderly and had arthritis.

There were other disease conditions which were clustered together as non- classified and they accounted for 6.1% of patients with normocytic anaemia.

10 CONCLUSIONS

a. Normocytic anaemia is the most common type of anaemia in the studied cases at the KNH.

b. The age range was between the 13 and 89 years, mean of 37 years and mode age of 32 years. Also that the females accounted for 63% of the patients.

c. The occupation of the study cases were; housewives (25%) followed by unemployed (15%). The least occupation category of patients with normocytic anaemia at the KNH is teachers (2.8%).

d. The geographical areas (province) of residence were; Nairobi followed by Central Province then Eastern provinces.

e. Total white blood counts of patients with normocytic anaemia are within the normal range in most of the patients. While elevated ESR (98.7%) was documented in the majority of cases.

f. The study showed that the associated diseases at the KNH include infections (most of these patients also have HIV infection), malignancies, renal diseases, central nervous system diseases, endocrinopathies particularly diabetes mellitus, gastrointestinal tract diseases, haematological diseases and connective tissue diseases, in a descending order.

11 RECOMMENDATIONS

In patients presenting at the KNH with normocytic anaemias, the management should consider first the following disease conditions; infections , malignancies, renal , cardiovascular , central nervous , endocrine , gastrointestinal , hematological , and connective tissue .

Furthermore it is recommended that in these patient anaemia is mild to moderate and may not require immediate and urgent direct attention. Evaluating for the actual cause of the disease condition associated with the anaemia is the recommended approach to correcting the anaemia.

Since some aspects of assessment of these patients was not performed, it is recommended that studies be undertaken in these patients to enable understand the pathophysiology of the normocytic anaemia in the KNH

12 REFERENCES

1. Ward, H.P, Kurnick, J.E .Pisarczk, M.J. Serum level of erythropoietin in anemias associated with chronic infection, malignancy and primary hematopoietic disease. J .Clin Invest.1971. 50, 691-707.
2. Lee G.R. The anaemia of chronic disease. *Semin. Hematol.* 1983; **20** 61.
3. Cartwright, G.E. & Lee G.R. (1971).The anaemia of chronic disorders. *Brit. J. Haematol.* **21**:147
4. Caro J., Brown S., Miller O. et al. Erythropoietin levels in uremic and anephric patients. J. *Lab. Clin. Med.* 1979; **93:** 449
5. Mansell M., Grimes A.J. Red cell and white cell abnormalities in chronic renal failure. *Brit. J. Haemat.* 1979; 42: 169.
6. Corr W.P, Kyle R.A., Boivie E.J.W. Haematologic changes in tuberculosis. *Am. J. Med. Sci.* 1964;**248**: 709
7. Glasser, RM., Walker RI., Heron J.C. The significance of haematologic abnormalities in patients with tuberculosis. *Arch. Int. Med.* 1970; **125**: 691.
8. Bentley C.P., Williams P. Serum ferritin concentrations as an index of iron storage in rheumatoid arthritis. *J. Clin. Path* 1974; **27**:786.
9. Donkas MA. HIV-associated anaemia. *Med Clin. N. Amer.* 1992; **76**: 699-709
10. Dinant HJ, de Maat C: erythropoiesis and red cell lifespan in normal subjects and in patients with anaemia of active rheumatoid arthritis.*Br j Haematol.* 1978;**39**:437
11. Freireich EJ, Miller A, Emerson C.P and Ross JF: the effect of inflammation on the utilization of erythrocyte and transferin bound radio- iron for red cell production. *Blood.* 1957;**12**:972,
12. Chernow B, Wallner SF: is the anaemia of chronic disorders normocytic normochromic? *Mil Med* .1978;**143**:345,
13. Feelders RA, Vreugdenhil G, van Dijk J et al: decreased affinity and number of transferin receptors on erythroblast in the anaemia of rheumatoid arthritis. *Am J Haematol* .1993;**43**:200,
14. Balaban E.P, Sheehan R.G. Demian SE et al: evaluation of bone marrow iron stores in anaemia associated with chronic diseases: a comparative study of serum and red cell feritin. *Am J Haematol.* 1993 ;**42**:177,
15. Vreugdenhil G, Wognum AW, van Eijk Hg, Swaak AJ: Anaemia in rheumatoid arthritis; the role of iron, vitamin B12, and folic acid deficiency, and erythropoietin responsiveness. *Ann Rheum Dis.* 1990; **49**: 93
16. Sears DA: anaemia of chronic diseases. *Med Clin North Am.* 1992;**76**:567,
17. Christensen DJ: differentiation of iron deficiency and the anaemia of chronic diseases. *J Fam Pract.* 1985;**20**:35,
18. Flemming D, wood RJ: Plasma transferrin receptors help to predict iron deficiency in anaemia of chronic disease. *Nutr rev.* 1995;**53**:167,
19. Ludwing H, Sundal E. Pecherstrofer M et al: Recombinant human erythropoietin for correction of cancer associated anaemia with and without concomitant cytotoxic chemotherapy. *Cancer.* 1995;**76**:2319
20. A Victor Hoffbrand, S. Mitchell Lewis and Edward G.D. Tuddenham. The regulation of haemopoietic cell production. In Postgraduate hematology, 4th Eds. Nydia G Brill and T. Michael Dexter. Arnold, London. 2001; 1-11.

21. Cosby C, Holzemer WL, Henry SB, Portillo CJ: Haematological complications and quality of life in hospitalized AIDS patients. *AIDS Patient Care STDS.* 2000;**14**;269-279
22. Patton LL. Haematologic abnormalities among HIV- infected patients; associations of significance for dentistry. *Oral Surg Med Oral Pathol Radiol Endod.* 1999; **88**:561-567.
23. Keller LJ, Hausdorff JM, Kyne L, Wei JY. Is age a negative prognostic indicator in HIV infection or AIDS? *Aging (Milano)* 1999; **11**:35-38.
24. Raal FJ, Wypkema EM, Bayens RD. Haematological abnormalities in the acquired immunodeficiency syndrome. *S. Afr med J.*1988;**74**:157-160
25. Zon Li, Arkin C, Groopman JE. Haematologic manifestations of the Human immune deficiency virus (HIV). *Br J Haematol.*1987; **66**:251-256.
26. Beuter E and West C. Haematologic differences between African –Americans and whites: The role of iron deficiency and β thalassemia on haemoglobin levels and mean corpuscular volume.*Blood.*2005 **106:** 740-745.
27. John R. Brill and Dennis J Baumgardner. Normocytic anaemia. *Am Fam Physician* .2000; **62**: *Issue 10.*
28. Cash J.M, Sears D.A. The anaemia of chronic disease: Spectrum of associated diseases in series of unselected hospitalized patients. *Am J Med* .1989; **87**(6): 638-44
29. Miller W.M. Anaemia in women ages 20 –89yrs; Rationale and tools for differential diagnosis. *Clin Ther.* 1993; **15**: 192-203.
30. Bear A.N, Dessypris E.N, Krantz S.D. The pathogenesis of anaemia in Rheumatoid arthritis; A clinical and laboratory analysis. *Semin Arthritis Rheum* .1990; **19**:209.
31. Cartwright G, Wintrobe M. Anaemia of chronic infection. *Advanced Intern Med.* 1952; **5**: 165.
32. Lee G.R. Anaemia of chronic disease. *Semin Haematology.* 1983; **20**:61.
33. Suega K, Balata M, Dharmayudha T.G, Lukman J.S, Suwitra K; Profile of anaemia in chronic renal failure patients: Comparison between pre- dialyzed and dialyzed patients at a division of nephrology, department of internal medicine, Sanglah hospital Deupasar, Bali Indonesia. *Acta med Indones.* 2005; **37**:190-4
34. He'di Ben, Maiz, Ezzedine Abderrahim and Kanni Zonaghi. Anaemia and end stage renal disease in the developing world. *Artificial organs.* 2002; **26**:1525-1594.
35. Patwardhan M.S, Golwikar A.S, Abhyanker J.R, Atre M.C. Haematological profile of HIV positive patients. *Indian J Pathol Microbiol.* 2002; **45**: 147-50.
36. Singh K.J, Ahuwalia G, Sherma G, Sherma S.K, Saxener. Significance of Haematological manifestations in patients with tuberculosis. *J Assoc Physicians, India* .2001 ; **49**:788,790-4
37. A.K. Chakrabarti, A.K.Dutta, B. Dasgupta, Dhima Ganguli and A.G. Ghosal. Haematological changes in disseminated tuberculosis; *Ind J Tub.* 1995; **42**: 168.
38. El Shazly A.M, El Naha H.A. Human fascioliasis and anaemia in Dakahlia Governorate, Egypt. *J Egypt Soc Parasitol* .2005; **35**: 421 –32.
39. Wong P, Intragumtornchai T. Hospital acquired anaemia. *J Med Assoc Thai.* 2006 ; **89**: 63-7
40. Walsh TS, Saleh EE, Lee RJ, McClelland DB. The prevalence and characteristics of anaemia at discharge home after ICU care. *Intensive care med* .2006; June 2; [Epub ahead of print]
41. Birgens H.S. Anaemia of chronic diseases; *Ugeskr Laeger.* 1994; **156**: 3163-7.

42. Ellaurie M, Rubenstein A: Elevated TNF-α in association with severe anaemia in HIV and *mycobacteria Avium intracellulare* infection. *Pediatric haematology* .1995; **12**:221.
43. Swindells S, Baldwin T, and Kelly C. Regulation and characterization of IF-α present in patients advanced HIV-1 disease –*J interferon cytokines*.
44. Bertero MT, Caligaris –Cappio F. Anaemia of chronic disorders in systemic autoimmune diseases. *Haematologica.* 1997; **82**:375.
45. J. Pinerich, J, Petersen. Erythropoietin therapy in patients with chronic renal failure. *West J Med* .1992; **157**: 154-157.
46. Joosten E. Strategies of the lab diagnosis of some common causes of anaemia in elderly patients. *Gerontology*.2004; **50**: 49-56.
47. Maury CP. Anaemia in rheumatoid arthritis: role of cytokines. *Scand J Rheumatol* 1989; **18**:3–5
48. Krantz S.B. Erythropoietin and the anaemia of chronic disease. *Nephrol Dial Transplant.* 1995; **10(suppl 2):**10-7
49. Ferguson BJ, Skinkne BS, Simpson K.M et al. Serum transferin receptor distinguishes anaemia of chronic disease from iron deficiency anaemia. *J Lab Clinical Med* .1982; **119**:385.
50. Konijin A.M, Hershko C. Feritin synthesis in inflammation. Pathogenesis of impaired Iron release. *British J of haematology* 1997; **31**:7.
51. Van Snick JL, Masson PL, Heremans JF. The involvement of lactoferrin hyposideremia of acute inflammation. *J Exp Med* .1974; **140**: 1068.
52. Grantz T. The role of Hepcidin in iron sequestration during infections and in the pathogenesis of anaemia of chronic disease. *Isr Med Assoc J* .2004; **4**:1043-1045.
53. Nemeth E, Rivera S, Gabayan V. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of iron regulating hormone hepcidin. *J Clin Invest.* 2004; **113**:1271-1276.
54. Dallalio G, Law E, Means RJ Jr. Hepcidin inhibits in vitro colony formation at reduced erythropoietin concentration. *Blood* .2006; **167**: 2702
55. Hamy P. Ward, John E. Kurnick, and Michael J. Pisarczyk. Erythropoietin in Anemia of Chronic Disorders. *The Journal of Clinical Investigation.*1971; **50**.
56. Abramson SD, Abramson N. Common uncommon anaemia. *American Family Physician.* 1999;**59**:851-8
57. Popovic M. Anaemia in chronic renal insufficiency-Case report. *Med Pregl.* 1999; **52**: 279-81.
58. Spivac JL. The blood in systemic disorders. *Lancet.* 2000; **355**: 1707-12
59. Schnall SF, Berliner N, Duffy TP, Benz EF Jr. Approach to the adult and child with anaemia. In: Hoffman et al, *Haematology basic principles and practice, 3rd Edition New York; Churchill Livingstone, 2000:367-82*
60. Igor Theur, Verena Mattle, Markus Seifert, Mariagabriella Mariani, Christian Marth, and Günter Weiss. Dysregulated monocyte iron homoeostasis and erythropoietin formation in patients with ACD. *Blood.*2006; **107**:4142-4148.
61. Antonijevic N, Nesovic M, TrBojevic B, Milosevic R. Anaemia in hypothyroidism. *Med Pregl.* 1999; **52**: 136-40.
62. Fein H.G. Rivlin R.S. Anaemia in thyroid diseases. *Med Clin North Am.* 1975; **59**: 1133-45.

63. M. Haseeb-Ul Hassan, Moinuddin. Hematological manifestations in HIV infected persons. *Pak J Med sci* .2003; 19: 315.
64. Ian A Clark, Alison C Budd, Lisa M Alleva and William B Cowden. Human malarial disease: a consequence of inflammatory cytokine release. *Malaria Journal* .2006; **5:**85.
65. George E, Catwright MC. Studies on the pancytopenia of Kala-azar. *Blood*. **3**: 249-275.
66. Cooper R. A Diloy –Puray, M., Lando, p. et al (1972). An analysis of lipoproteins, bile acids and red cell membranes associated with target cells and spur cells in patients with liver disease. *J Clin Invest*. **51**,3182
67. Hines J.D.(1969) Reversible megaloblastic and sideroblastic marrow abnormalities in alcoholic patients . *Brit J Haemat*. **16**,87
68. Sherlock, S. (1985) Diseases of the liver and biliary system 7th Ed., Blackwell scientific publications, Oxford.
69. Adams, E.G. (1970) Anaemia associated with protein deficiency, *Semin. Hemat*. 7,55
70. Goldberg A. (1963) .The anaemia of scurvy. *Quart. J. Med* **32**, 51.
71. Cox, E.V, Meynell. M.J., Northam, N.E. *et al*. (1967) the anaemia of scurvy. *Am J Med*. **42**, 220
72. Budman, D.R. & Steinberg, A.D. (1977) Haematologic aspect of systemic lupus erythromatosus. *Ann. Int Med*. **86**,220
73. Harvey, A.M., Schulman, L.E., Tumultry, P.A. *et al*.(1954) Systemic lupus erythromatosus: review of the literature and clinical analysis of 138 cases. *Medicine*, **33**,292.
74. Miller, H.G. & Daley, R. (1946) clinical aspects of polyarteritis nodosa.*Quart.J.Med*.**14**, 255.
75. Westerman, M.P., Martinez, R.C., Medsger, T.A. et al. (1968) Anaemia and scleroderma. Frequency, causes and marrow findings. *Arch. Int Med*. **122**,39
76. Dacie, J.V. &Lewis, S.M. (1984) *Practical Haematology*, 6th Ed, Churchill Livingstone, London.
77. Garrett, T.J., Gee, T.S., Lieberman, P.H. et al (1976).The role of bone marrow aspiration and biopsy in detecting marrow involvement by non haematologic malignancies. *Cancer*, **38**,2401
78. Hansen HH, Muggia, FM & Selawry, OS (1971) Bone marrow examination in 100 consecutive patients with bronchogenic carcinoma. *Lancet*, **ii**, 443
79. Molitarno A.R, Spivak JL; anaemia of cancer. Haematol Oncol Clin North Am 10:345,1996

80. Jessica L VanArsdale. Lead poisoning from a toy necklace. *Pediatric*. 2004; **144:**1096-1099
81. Agrawal V, Sachater A, Lehls, Basu S. Unusual haematological alterations in rheumatoid arthritis. *Journal of Post graduate Medicine*. 2004; **50**: 60-61.
82. Lluberas-Acosta G, Schumacher HR Jr, Markedly elevated erythrocyte sedimentation rates: consideration of clinical implications in a hospital population.*Br J Clin Pract* .1996 Apr-May;**50**(3):138-42
83. C. Bokemeyer, K. Oechsle and J-T. Hartmann. Anaemia in cancer patients: Pathophysiology, incidence and treatment. *European Journal of Clinical Investigations*.2005;**35**(suppl.3),26-31

84. Sharma R, Francis DP, Pitt B, Poole-Wilson PA, Coats AJ, and Anker SD. Haemoglobin predicts survival in patients with chronic heart failure: a substudy of the ELITE II trial. *Eur Heart J* .2004; **25**: 1021–1028.
85. Kalra PR, Bolger AP, Francis DP, Genth-Zotz S, Sharma R, Ponikowski PP,Poole-Wilson PA, Coats AJ, Anker SD. Effect of anemia on exercise tolerance in chronic heart failure in men. *Am J Cardiol* 2003; **91**:888–891.
86. Okonko DO, Anker SD. Anemia in chronic heart failure: pathogenetic mechanisms. *J Card Fail* 2004; **10**:S5–S9.
87. Merlin C Thomas, Richard J Mc Isaac, Con Tsalamandris, Lynda Molyneaux, Inna Goubina, Greg Fulcher, Dennis Yue and George Jerums. Anaemia in Patients with Type 1 Diabetes. *The Journal of Clinical Endocrinology and Metabolism* 89**(9)**:4359-4363.

14. APPENDICES

APPENDIX 1

PROFORMA QUESTIONARE

Date of study | | | |

Date of report | | | |

Study Number | | | |

Lab. Number | | | | |

Inpatient Number (IP) Number | | | | | | |

Patient information

Name.....

Address.....

Occupation.....

Province of residence (6months) .....Actual residence: I Rural .....

II Urban .....

Sex……

Age……

Ward……

Requesting Doctor ……

Diagnosis / clinical impression…

CLINICAL FINDINGS

General condition

Good ☐

Fair ☐

Poor ☐

Pallor ☐

Jaundice ☐

Cyanosis ☐

Adenopathy ☐

Oedema ☐

Finger clubbing ☐

Vital signs

Blood pressure ☐

Pulse rate ☐

Respiratory rate ☐

Temperature ☐

Central nervous system

Infections ☐

Neoplasia ☐

Others ☐

Respiratory system

Infections ☐

Neoplasia ☐

Allergic ☐

Others ☐

Cardiovascular system

Hypertension ☐

Cardiac ☐

Others ☐

Hepatobiliary system

☐

Infections

Inflammatory ☐

Cirrhosis ☐

Neoplasms ☐

Others ☐

Urogenital system

Kidneys

Infections ☐

ARF ☐

CRF ☐

Neoplasms ☐

Others ☐

Genital

Female

Infections ☐

Neoplasms ☐

Breast

Infections ☐

Neoplasms ☐

Others ☐

Male

Infections ☐

Inflammations ☐

Neoplasms ☐

Others ☐

Endocrine system

Diabetes mellitus ☐

Hyperthyroidism ☐

Others ☐

Skin

Inflammatory ☐

Infective ☐

Malignancies ☐

Allergies ☐

Others ☐

Musculoskeletal

Arthritis ☐

Bone

Infections ☐

Neoplasms ☐

Others ☐

Diagnosis / clinical impression _____

Laboratory findings

a) Haematological profile

Hb ☐☐ g/dl

RBC ☐☐☐ $X10^{12}/l$

WBC ☐☐☐ $x10^{9}/l$

Differentials

Neutrophils ☐☐ %

Lymphocytes ☐☐ %

Eosinophils ☐☐ %

Monocytes ☐☐ %

Basophils ☐☐ %

RDW ☐☐ %

RBC ☐☐ $x10^{12}$

PCV ☐☐ %

MCV ☐☐ fl

MCH ☐☐ pg

MCHC ☐☐ g/dl

Reticulocytes count…

Coombs test……

PBF report……

ESR……

b) **Liver function tests**.

Total proteins……..

Albumin .....

ALT.....

AST.....

GGT.....

ALP.....

Total Bilirubin.....

Direct Bilirubin.....

c) Renal function tests

Sodium....

Potassium...

Chloride .....

Urea.....

Creatinine.....

Urinalysis...

d) Others

Uric acid levels....

Stool.....

CXR.....

LDH .....

Bone Marrow aspiration (the Overall comment).....

Bone marrow stainable iron.....Present.....Absent.....

HIV test.....Method

Laboratory diagnosis.....

APPENDIX II

13.1 CONSENT/ EXPLAINATION FORM

NORMOCYTIC ANAEMIA AND ITS ASSOCIATICIATED DISEASES IN KNH

My name is Dr Oduor J and I am carrying out a study to know the diseases associated with the type of anaemia (anaemia is reduced haemoglobin/blood level) you are suffering from. The blood tests already performed on a sample of your blood show that you have anaemia the type which is associated with perhaps another disease the doctors are already treating you for. It is important that the two conditions be fully understood so as to direct the treatment to the cause/s. There is no risk that you will be exposed to as a result of the study and there is no cost you will to incur as a result of participating in the study. The study will benefit you and will be done free of charge, the results will be communicated to your doctor who will then give you the feedback as well as decide on any adjustment to the treatment. The results will also assist the doctors in deciding on how to deal with the disease like the one you are having in future. Your decision is entirely voluntary and even if you decide not to participate your treatment will not be withheld. I am request for your permission to use your medical record and blood samples for the study.

Since the participation in this study is voluntary and one is allowed to withdraw at any time and this will not interfere with management of your illness by your doctor. I .....do consent to participate in this study being carried out by Dr Oduor J. I have asked questions and every thing has been explained well by him.

Date.....

Signature of patient or relative .....

If you have any concerns about this study you can contact the chairperson of the ethical and research committee at KNH. You may also contact my supervisors

Dr F.K. Abdallah Tel: 2721815

Department of hematology

University of Nairobi

Prof O.W. Mwanda Tel: 2721815

Department of hematology

University of Nairobi

My contact as the principal investigator is 0722884592

APPENDIX III

Preparation and staining procedure for blood films

- Blood films were made on clean glass slides.
- Blood used was from EDTA- anticoagulated blood.
- A small drop of blood was placed in the centerline of a slide about 1 cm from one end.
- A spreader will be placed in front of the drop at an angle of about 30^0 to the slide and moved back to make contact with the drop.
- With steady movement of the hand the drop of blood was spread along the slide, with the spreader not lifted off until the last trace of blood has been spread out.
- The film was labeled immediately after spreading, on the frosted edge of the slide.
- The films were then allowed to dry in air.
- The films were then fixed by the use of methyl alcohol or ethyl alcohol (absolute alcohol) for 5-10 minutes.
- The films were then transferred into a jar containing May- Grünwald stain freshly diluted with an equal amount of buffered water, and allowed to stain for 15 minutes
- Without washing, the slides were transferred into a jar containing Giemsa stain freshly diluted with 9 volumes of buffered water, pH 6.8 and allowed to stain for 10-15 minutes
- Then the slides were transferred onto a jar containing buffered water at Ph 6.8 and washed in three to four changes of water and then allowed to stand in water (for 2-5mins) for differentiation to take place.
- Once differentiation was complete, the slides were put upright to dry.

APPENDIX IV

The table below shows some of the causes of normocytic anaemia

Table 1: Causes of Normocytic Anemias

1. **Increased red blood cell loss or destruction**
   - Acute blood loss
   - Hypersplenism
   - Hemolytic disorders
     i. Congenital conditions
        - Hemoglobinopathies
          a. Homozygous sickle cell disease (hemoglobin SS disease)
          b. Heterozygous sickle hemoglobin C disease (hemoglobin SC disease)
        - Membranopathies
          a. Hereditary spherocytosis
          b. Hereditary elliptocytosis
        - Enzymopathies
          a. Glucose-6-phosphate dehydrogenase deficiency
          b. Pyruvate kinase deficiency

     ii. Acquired conditions
        - Mechanical hemolysis
        - Macro vascular disorders
        - Microangiopathic disorders
          - Disseminated intravascular coagulopathy
          - Hemolytic-uremic syndrome
          - Thrombotic thrombocytopenic purpura
        - Autoimmune hemolytic anemias
          - Warm-reactive anemias
          - Cold-reactive anemias
          - Drug-induced anemias
        - Paroxysmal nocturnal hemoglobinuria

2. **Decreased red blood cell production**
   - Primary causes
     - Marrow hypoplasia or aplasia
     - Myelopathies
     - Myeloproliferative diseases
     - Pure red blood cell aplasia
   - Secondary causes
     - Chronic renal failure
     - Liver disease
     - Endocrine deficiency states
     - Anemia of chronic disease
     - Sideroblastic anemias

3. **Overexpansion of plasma volume**

1. Pregnancy
a) Over hydration

APPENDIX V

TIMETABLE

Activity	2	3	4	5	6	7	8	9	10	11	12
Proposal writing	■	■									
Ethical clearance			■	■							
Data collection, entry, editing					■	■	■				
Data analysis								■	■		
Report writing										■	
Report submission											■

APPENDIX VI

BUDGET

Activity	**Amount (Ksh)**
Ethical committee fee	500
Secretarial work	10000
Research assistant / data collection	20000
Reagents and other materials	50000
Data entry and analysis	40000
Book writing and binding	20000
Subtotal	**140500**
Contingencies (15%)	21075
Grand total	**161575**

APPENDIX VII

Normal reference levels

Haemoglobin >11.5g/dl

MCV 76-96 fl

WBC count

Total WBC count 4-11x10^9/l

Differential counts

Neutrophils	2.0-8.0 x10^9/l (50-80%)
Lymphocytes	1-5x10^9/l (25-50%)
Monocytes	0.1 -1.0 x10^9/l (2-10%)
Eosinophils	0.02-0.5 x10^9/l (1-6%)
Basophils	0.02.0.1 x10^9/l (0.1-1%)

Platelets 150-450 x10^9/l

ESR levels

Males ≤10mm/hr

Females ≤12mm/hr

APPENDIX VIII

ETHICAL REVIEW LETTER

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ 01/4749

20th September 2007

Dr. Oduor N. Johansen
Dept. of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Oduor

RESEARCH PROPOSAL: "NORMOCYTIC ANAEMIA AND ASSOCIATED DISEASES IN KENYATTA NATIONAL HOSPITAL" (P236/8/10/2006)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your revised research proposal for the period 20th September 2007 – 19th September 2008

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication

Yours sincerely

DR. L. MUCHIRI
AG. SECRETARY, KNH-ERC

c.c. Prof. K M Bhatt, Charperson, KNH-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept. of Human Pathology, UON
Supervisors: Dr F K Abdallah, Dept. of Human Pathology, UON
Prof W O Mwanda, Dept. of Human Pathology, UON