EFFECT OF CO-TRIMOXAZOLE PROPHYLAXIS ALONE, ZIDOVUDINE ALONE,AND CO-TRIMOXAZOLE PROPHYLAXIS PLUS ZIDOVUDINE ON HAEMOGLOBIN LEVEL IN TANZANIA PATIENTS ON ANTIRETROVIRAL THERAPY

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M.Pharm (Hospital And Clinical Pharmacy) Dissertation
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By

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A dissertation Submitted in Partial fulfillment of requirements for the Degree of Master in Pharmacy (Hospital and Clinical Pharmacy) of Muhimbili University of Health and Allied Sciences

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June, 2010

CERTIFICATION

The undersigned certify that, they have read and hereby recommend for examination of dissertation titled ***Effect of co-trimoxazole prophylaxis alone, zidovudine alone, and co-trimoxazole prophylaxis plus zidovudine on haemoglobin level in Tanzanian patients on antiretroviral therapy,*** in partial fulfillment of requirements for the Degree of Master of Pharmacy (Hospital and Clinical Pharmacy) of Muhimbili University of Health and allied Sciences.

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I, Evodia K. Rugalabamu, declare that this **dissertation** is my own original work and that to the best of my knowledge it has not been submitted to any other University for a degree award.

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DEDICATION

This work is dedicated to my family, who tolerated my absence at home and for their cordial moral support and encouragement.

ABBREVIATIONS/ACRONYMS

List of Abbreviations

3TC	Lamivudine
AIDS	Acquired Immune Deficiency Syndrome
ALAS	Alpha-aminolevulinic acid synthase
ANC	Absolute Neutrophil count
ART	Antiretroviral treatment
ARV	Antiretroviral
AZTMP	Zidovudine monophosphate
AZTTP	Zidovudine Triphosphate
BFU-E	Burst-forming erythroid
CD4	CD4+T-lymphocyte cell count
CFU-E	Colony growth erythroid
CFU-GM	Colony growth as determined by assay of granulocyte-macrophage
CTC	Care and treatment center
CTX	Co-trimoxazole
d4T	Stavudine
DHR	Dihydrofolate reductase
EFV	Efavirenz
FH_4	Tetrahydrofolate
HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
IQR	Inter quartile range
MCV	Mean Cell Volume
MDH	MUHAS and Dar es Salaam City in Collaboration with Harvard

ABSTRACT

Background: Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are a major global health problem and Sub-Saharan Africa is the world's most severely affected region. Prophylaxis against opportunistic infections and drug treatment directed to the virus has been associated with a decline in morbidity, mortality and improvement in the quality of life. However, use of these interventions is associated with side effects and toxicity. For example, anaemia is one of the important side effects of zidovudine. Co-trimoxazole prophylaxis is given to all patients with high risk of opportunistic infections, yet co-trimoxazole can cause anaemia. When co-trimoxazole is given together with zidovudine the likelihood of getting anaemia is higher.

In Tanzania, the new National Guidelines for the Management of HIV and AIDS (2008 3rd Edition) recommends zidovudine (AZT)-containing Highly Active Antiretroviral Therapy (HAART) as default first line regimen and co-trimoxazole (CTX) prophylaxis when T-lymphocyte cell count CD4 < 350 Cells/μL. Both drugs have possible side effects that include blood abnormalities which end up with neutropenia and anaemia. HIV Patients receiving both co-trimoxazole and zidovudine for long time are at risk of developing anaemia.

Objective: To determine the effect of zidovudine-containing HAART regimen in combination with co-trimoxazole prophylaxis on haemoglobin concentration.

Methodology: This was retrospective, observational study conducted at Mwananyamala care and treatment center (CTC) located in Kinondoni Municipality.

Study populations: The study involved 407 Patients files of HIV-infected patients who were attending HIV CTC. The study had three arms:-

- Those who were receiving none zidovudine-containing HAART regimen plus co-trimoxazole for prophylaxis
- Those who were receiving zidovudine-containing HAART regimen alone

- Those who were receiving zidovudine-containing HAART regimen plus co-trimoxazole for prophylaxis attending HIV clinic at Mwananyamala Hospital.

The following data were recorded in each of the above mentioned arms at baseline, at 4 months at 8 months and at 12 months: haemoglobin level, CD4 count, clinical stage, concurrent diseases, concomitant drugs, co-trimoxazole use history and zidovudine use history.

Results: On average, drugs had positive effect on haemoglobin concentration with all groups demonstrating an increase in mean haemoglobin over time from mild anaemia (mean haemoglobin 10.5g/dl) at baseline to non anaemic state (haemoglobin mean >10.5g/dl) during follow up across the three arms of the study. There was a tendency of more frequent significant drop (≥1g/dl) in haemoglobin concentration in the co-trimoxazole prophylaxis plus zidovudine-containing HAART regimen group (16%) compared to the other two groups (co-trimoxazole alone and zidovudine alone between 5.5%-13%). Overall the risk for significant drop in haemoglobin level was low in all three groups (< 25%) by 12 months of follow up and the overall incidence of significant haemoglobin drop was also low 0.18 per 4240 person-month (about 5 per 10,000 person-year) of follow up. The greatest incidence of significant haemoglobin drop of 0.2 per 1648 person-month was in co-trimoxazole for prophylaxis plus zidovudine containing HAART regimen.

Conclusion and Recommendation: The mean Hb was increasing in the first 8 months suggesting of no negative effect, but 18.2% of patients had significant Hb drop. This necessitates the monitoring of Hb for early detection of significant drop in Hb level. There was a significant drop (≥1g/dl) in Hb concentration in the CTX plus AZT user group compared to the other two groups. This indicates that the combination of CTX plus AZT carries risk of anaemia than the two groups therefore should better be avoided for anaemic HIV-infected patients.

However the use of co-trimoxazole for prophylaxis in patients who later on are put on a zidovudine-containing HAART regimen was associated with most significant

haemoglobin drop. This may be a pointer to potential hazardous effect. Therefore, further studies are needed which should not only assess the consequences of combining co-trimoxazole with zidovudine in prospective study design also estimating the maximum duration for which co-trimoxazole prophylaxis can be used without hazardous effect on haemoglobin concentration.

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CHAPTER ONE

1. BACKGROUND

1.1 INTRODUCTION

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are a major global health problem and Sub-Saharan Africa is the world's most severely affected region. To date, there is no cure for the disease but prophylaxis against opportunistic infections and drug treatment directed to the virus have been associated with a decline in morbidity and mortality and improvement in the quality of life (1) by inhibiting the progress of the disease (2, 3). However, use of these interventions is associated with side effects and toxicity. For example, anaemia is one of the important side effects of zidovudine (AZT)-. In addition, co-trimoxazole (CTX) prophylaxis is given to all patients with high risk of opportunistic infections. Yet co-trimoxazole can cause anaemia, and when co-trimoxazole is given together with zidovudine the likelihood of getting anaemia is expected to increase (4, 5).

In Tanzania, the current National Guidelines for the Management of HIV and AIDS (2008, 3rd Edition) recommends co-trimoxazole prophylaxis for opportunistic infections and zidovudine containing HAART as default first line regimen when CD4 <350 cells/μL. The default first line regimen include; zidovudine 300 mg/ Lamivudine (3TC) 150 mg twice daily and Efavirenz (EFV) 600 mg once daily at night (2). However, both zidovudine and co-trimoxazole have possible side effects that include blood abnormalities which end up with neutropenia and anaemia. HIV-infected Patients receiving these drugs for long time are at risk of developing anaemia(4). Anaemia is a frequent complication that occurs in HIV-infected patients and is associated with faster disease progression and mortality (6, 7).

By the end of 2007, it was estimated that a total of 33.2 million people worldwide were living with Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS (8, 9). Although there are now reports of declining trends in HIV incidence in a number of countries, presumably due to changes in behavior and

prevention programmes, the number of people with HIV has continued to rise, due to population growth, and more recently, the life-prolonging effects of antiretroviral therapy (10). Tanzania is one of the countries in sub-Saharan Africa most affected by the HIV pandemic.

It was estimated that by the end of 2005 approximately 6.5% of adults throughout the country were living with the virus. The epidemic is characterized by large differences in the prevalence and incidence of HIV-1 infections between and within the regions (11-13).

The national response to Human immunodeficiency virus and acquired immunodeficiency syndrome has shifted hypothetically from one solely based on interventions aimed at prevention to one that puts more emphasis on care and treatment as well. In October 2004 Tanzania in collaboration with development partners initiated a program offering antiretroviral treatment at no cost to persons with HIV infection under National AIDS Control Program (14). Currently, a total of 172,736 HIV-infected people have been enrolled at 152 National Aids Control Program (NACP) sites throughout Tanzania. However, the scaling up of ART provision remains a challenge. Out of the estimated 600,000 HIV-infected Tanzanians who qualify for ART, only 136,700 (22.2%) are currently on ART.

Tanzania has responded in several ways, including putting in place a series of strategic plans for treatment and preventive interventions. Newer and more potent drugs are continuously being developed and used; and knowledge of the existing drugs in terms of their efficacy, as well as short and long-term side effects is becoming clearer as we gain more experience (14).

1.2 LITERATURE REVIEW

1.2. 1 Aetiology of HIV

In Tanzania, infection with HIV is caused by HIV-1 subtype, common being A, C, D and their recombinants (15).

1. 2.2 HIV Transmission

HIV infection is acquired through sexual intercourse with an infected partner, exposure to infected blood and blood products, and from an infected mother to her unborn child, in the uterus, during delivery, or from breast milk. More than 90% of adults in Sub-Saharan Africa including Tanzania are said to acquire HIV infection from unprotected sexual intercourse with infected partners (16).

1.2. 3 HAART used in Tanzania

The introduction of HAART has brought hope to people living with HIV/AIDS and managed to convert the formally known as death sentence to simply chronic illness which is manageable. There are more than 20 approved antiretroviral drugs across six mechanistic classes, with which to design combination regimens. These six classes include the nucleoside/nucleotide reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), fusion inhibitors, CCR5 antagonists, and integrase inhibitors (11). The use of antiretroviral therapy (ART) is associated with a decline in morbidity , mortality and improvement in the quality of life (1). Antiretroviral drugs are, however, also associated with side effects and toxicity. For instance anaemia is one of the important side effects of zidovudine (17-19).

1.2. 4 Zidovudine

Zidovudine (39-azido-39-deoxythymidine or azidothymidine) is an antiretroviral drug that is used for the treatment of infections with the HIV (20, 21). Zidovudine does not kill existing human immunodeficiency virus, and it is not a cure for human immunodeficiency virus. It is in a class of drugs called nucleoside analog reverse transcriptase inhibitors (NRTI). Reverse transcriptase is the enzyme that the virus uses to make new DNA (22). Zidovudine inhibits the activity of reverse transcriptase and blocks the production of new viral DNA and the interruption of the viral DNA chain elongation, as demonstrated by *in vitro* studies (23) Zidovudine use however; had at least one downside; it is associated with high rates of anaemia (24). Zidovudine is converted within the body to its active form zidovudine triphosphate (AZTTP). This active form is similar to the compound thymidine triphosphate (figure 1.1a and 1.1b), a chemical that is required by the Human immunodeficiency virus to make new DNA. The reverse transcriptase uses AZTTP instead of thymidine triphosphate for making DNA, and it is the AZTTP that interferes with the reverse transcriptase.

Zidovudine inhibits beta-globin gene expression and has bone marrow cytotoxicity (25). Likewise, zidovudine and its catabolite 3'-amino-3'-deoxythymidine were also reported to inhibit haemoglobin synthesis and globin gene transcription in either differentiated K562 cells (26, 27)

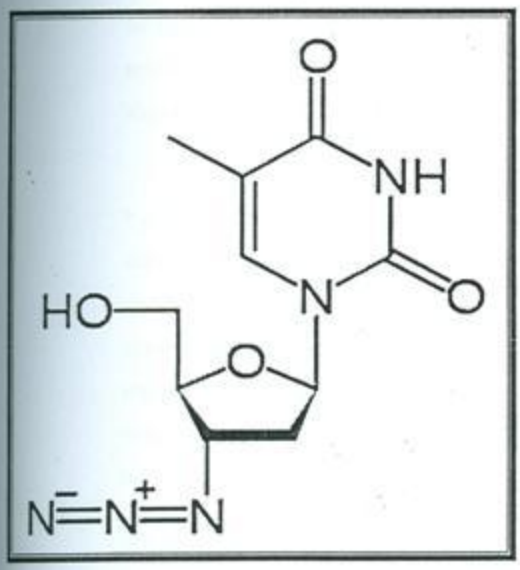


Figure 1.1: (a) Zidovudine ($C_{10}H_{13}N_5O_4$)

or

1-[(2*R*, 4S, 5S)-4-azido-5-(hydroxyl-methyl) oxolan-2-yl]-5 methyl-1,2,3,4-tetra hydro pyrimidine -2,4-dione.

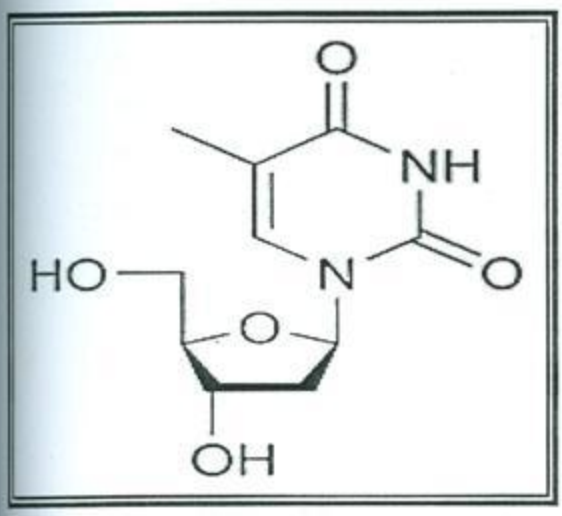


Figure1.2: (b) Thymidine

($C_{10}H_{14}N_2O_5$)

Or

Deoxyribosylthymine

In addition, the heme synthesis in butyric acid induced K562 cells was found to be inhibited after prolonged exposure to high zidovudine concentrations (26). Several other studies have shown that zidovudine inhibit heme synthesis (28, 29).

Up to 8.3% of patients who were initially put on zidovudine had to be switched to stavudine-containing HAART regimen as a result of anaemia in a study done by Torpey and his team in Ghana(1). Based on their findings Torpey and colleagues suggested that zidovudine could only be used in patients with haemoglobin level more than 8g/dl and regular monitoring of haemoglobin level to be done. Despite this recommendation they concluded that, patients put on zidovudine may require drug substitution as a result of toxicity (29).

The most severe side effect of zidovudine is bone marrow suppression, manifested by macrocytic anaemia , neutropenia and siderosis caused by inhibition of heme and globin synthesis together with a general derangement of iron supply which is seen in some patients in the study which was done D'Andrea and colleagues (18). In a study done by Lutton and his coworker on the effects of zidovudine on rat bone marrow heme metabolism and colony growth the toxicity of zidovudine on bone marrow and colony growth was illustrated (29).

In another study conducted by Cohen's team, patients who were receiving zidovudine-containing HAART regimen (12.35%) developed severe anaemia within the first 3 months of treatment, though their mean cell volume did not increase but remained within the normal range and bone marrow examination showed a pure red-cell aplasia. In some patients the anaemia resolved on discontinuation of the zidovudine and in those who were re-challenged, the anaemia recurred. This study also showed erythroid aplasia to be a major cause of anaemia (17). However, in literature data show that zidovudine-induced anaemia is macrocytic and Megaloblastic. Common side effects of zidovudine are: severe bone marrow toxicity manifested as anaemia, neutropenia, and siderosis caused by inhibition of heme and globin synthesis together with a general derangement of iron supply. In this regard, it was proven that while zidovudine and its

monophosphorylated derivative (AZTMP) were unable to chelate iron, the triphosphate form (AZTTP) displayed a significant capacity to remove iron from transferrin (18).

1.2.5 Co-trimoxazole

Co-trimoxazole a combination of two drugs that act together; (trimethoprim (fig.1.2b) and sulfamethoxazole (fig.1.2a)) is an antifolate agent with antimicrobial.

They are effective antimicrobials when used alone and each has an independent mode of action which is enhanced synergistically when the two substances are combined. Co-trimoxazole is used to prevent and treat a variety of bacterial and fungal infections; including *Pneumocystic jiroveci* pneumonia (8, 30, 31) which is an infection that is common in immunocompromised patients. The drug has been adopted as a prophylactic agent for *Pneumocystic jiroveci* pneumonia (PCP) in Human immunodeficiency virus infected individuals.

HIV-infected patients are at risk for developing *Pneumocystic jiroveci* pneumonia, which is fungal infection damaging the alveolar epithelium, and impairs gas exchange and reduces lung compliance (32). Co-trimoxazole for prophylaxis is often taken regularly at low doses (8). *Pneumocystic jiroveci* pneumonia typically develops when the CD4 T-cell count fall < 200 cells/μL and is considered an AIDS - defining illness (33). *Pneumocystic jiroveci* pneumonia was one of the leading causes of mortality among HIV-infected patients in the 1980s (7, 34) but the incidence has fallen since the introduction of highly active antiretroviral therapy (HAART) and the widespread use of *Pneumocystic jiroveci* pneumonia prophylaxis using co-trimoxazole (31, 35). *Pneumocystic jiroveci* pneumonia may also occur in patients with primary immune deficiencies, e.g. malignancy and those taking prolonged courses of high dose corticosteroids and immunosuppressive therapy after organ transplantation (36).

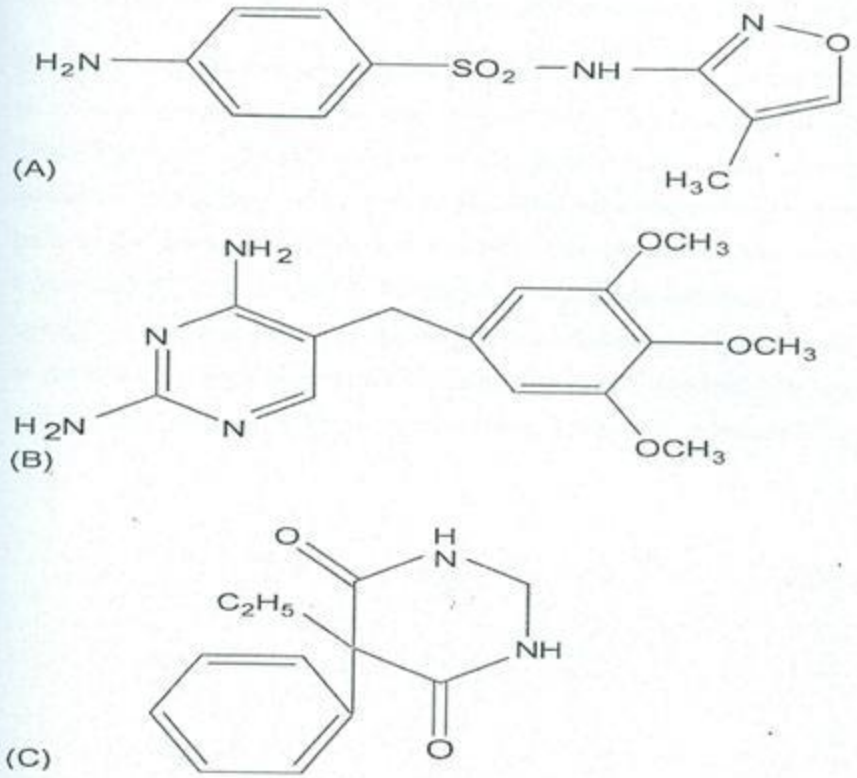


Figure 1.2:(A) Chemical structure of Sulfamethoxazole (short-acting)

Figure 1.2: (B) Chemical structure of Trimethoprim

Figure 1.2: (C) Chemical structure of the internal standard primidone.

Pneumocystic jiroveci pneumonia carries a mortality rate of 10-20% in HIV patients and approximately 40% in other immuno-compromised individuals (37, 38).

Bacteria are obligate folic acid synthesizers (i.e. they rely on the synthesis of folic acid to survive) whereas humans are able to obtain folate through dietary sources (39). Trimethoprim and sulfamethoxazole inhibit dihydrofolate reductase and dihydropteroate synthetase respectively and so provide sequential blockade of the folate synthetic pathway and prevent nucleotide synthesis (40). Trimethoprim inhibits production of folinic acid (FH_4) by binding to the enzyme dihydrofolate reductase responsible for making folic acid and blocking the enzyme from making folic acid. It inhibits the bacterial enzyme much more than the human enzyme, and, therefore, long term use of co-trimoxazole will have effect on the production of folic acid by humans (41), (Figure 1.3).

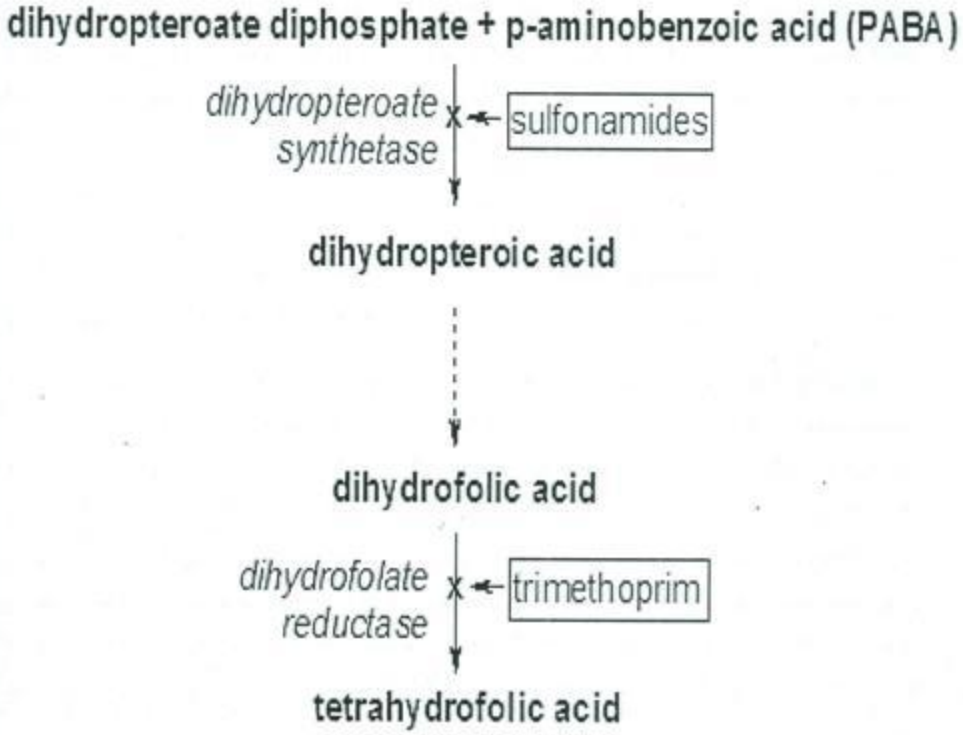


Figure1.3: Site of action of Sulfonamides and trimethoprim

The figure 1.3 shows the site of action of sulfonamides and trimethoprim in the folate biosynthesis pathway.

The two components of this combination product have a similar antimicrobial spectrum. They act synergistically because they independently inhibit different steps in the enzymatic synthesis of tetrahydrofolic acid, an essential metabolic process in susceptible bacteria. The combination of trimethoprim and sulfamethoxazole allows the use of much lower doses of individual drugs and may delay the development of resistance. Oral trimethoprim and sulfamethoxazole are readily absorbed from the gastrointestinal tract, widely distributed throughout the body and excreted by the kidneys. Trimethoprim–sulfamethoxazole is the first choice for prophylaxis, and it can be taken in double-strength form three times a week (8).However, adverse effects of trimethoprim–sulfamethoxazole may occur in as many as 50% of patients so treated, and 30% will need to change their regimen for this reason (41).

Co-trimoxazole is usually well tolerated in immune competent patients but should be avoided in those with severe hepatic or renal dysfunction .It is also best avoided during pregnancy and breast-feeding. When taken every day, it is a standard of care for persons with HIV/AIDS in USA and Europe and is increasingly being used in Africa. In HIV infection, it is highly effective for treatment of, and prophylaxis against, *Pneumocystis jiroveci* pneumonia, one of the earliest opportunistic infections to arise with increasing immune -suppression in Europe and the USA (31). Prophylaxis conducted in Africa, including Cote d'Ivoire, South Africa, Malawi, Zambia, and Uganda show reductions in mortality between 25% and 46%, and a beneficial effect of co-trimoxazole in reducing morbidity, even in areas with high bacterial resistance (31). Reduces the incidence of malaria, diarrhoea, toxoplasmosis, *Pneumocystic jiroveci* pneumonia, blood infections (septicaemia) and other illnesses affecting people living with HIV / AIDS like non typhoidal Salmonellosis (30), co-trimoxazole prophylaxis has also been found to decrease the frequency of clinic visits and hospitalizations, and improve weight (31).The beneficial effects of co-trimoxazole prophylaxis have been found to be similar in both early and advanced HIV disease (42).

Co-trimoxazole prophylaxis is neither a cure for Human immunodeficiency virus nor an anti-retroviral, but it can improve quality of life and enable people living with HIV / AIDS to live longer and healthier lives. The co-trimoxazole adverse effects are numerous and include nausea, vomiting, skin reactions, neutropaenia, thrombocytopaenia, hepatitis and cholestatic jaundice (41). The consequences of using co-trimoxazole with other haematotoxic drugs however; should be carefully assessed (41). Co-trimoxazole can cause anaemia based on the following mechanism: The folate found in food is mainly conjugated to polyglutamic acid. Enzymes found in the gut convert the polyglutamate form to monoglutamate which is readily absorbed. During absorption the folate is methylated and reduced to methyltetrahydrofolate (Methyl-FH_4) monoglutamate. This travels through the plasma and is transported into cells via a carrier specific for tetrahydrofolate (FH_4) form. Within the cell the methyl group is removed (in a reaction requiring vitamin B_{12} as shown in figure 1.4 below) and the folate is reconverted back to polyglutamate form. It has been suggested that the polyglutamate form prevents the folate leaking out of cells. The folate eventually acts as a coenzyme involved in a number of reactions including DNA and RNA synthesis. Defective DNA synthesis mainly affects cells with rapid turnover such as gastrointestinal cells and precursors of red blood cells marrow, in bone marrow, hence the sore tongue and anaemia seen in folate deficiency. During DNA synthesis the folate coenzyme is oxidized to the dihydrofolate (FH_2) form which is inactive and has to be reactivated by the enzyme dihydrofolate reductase (DHR). Dihydrofolate reductase is inhibited by trimethoprim (a content in co-trimoxazole) hence folate deficiency which could lead to anaemia (41, 43).

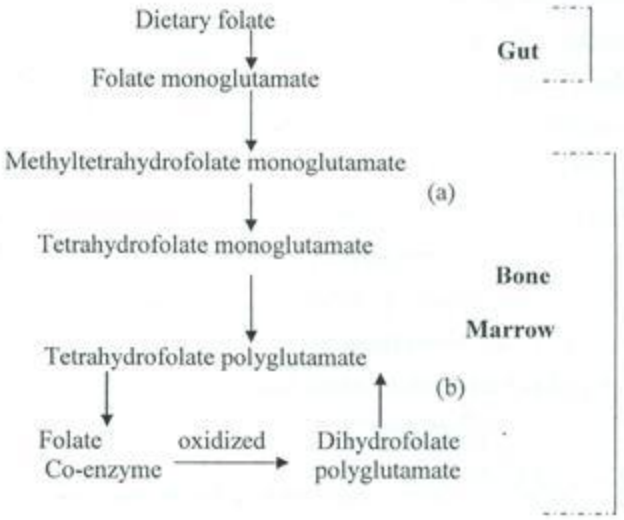


Figure1.4: The simplified pathway of folate metabolism

(a) Vitamin B_{12} is required as a coenzyme for this reaction and

(b) The enzyme dihydrofolate reductase converts inactive dihydrofolate back to the active tetrahydrofolate).

Sulfonamides (including co-trimoxazole) are also implicated in causing haemolysis in Glucose – 6 – phosphate dehydrogenase (G6PD) deficiency. Glucose – 6 – phosphate dehydrogenase is essential for the production of reduced form of phosphorylated nicotinamide-adenine dinucleotide NADPH in erythrocytes. If there is a deficiency of Glucose – 6 – phosphate dehydrogenase this decreases the production of NADPH. The NADPH is needed to keep glutathione in reduced form. The reduced glutathione maintain haemoglobin in reduced form and helps erythrocytes deal with oxidative stress. Hence, if the erythrocytes are exposed to oxidizing agent in Glucose – 6 – phosphate dehydrogenase deficiency haemoglobin becomes oxidized and forms what are known as Heinz bodies. The cell membrane is damaged and some of red cells haemolyze and others have their Heinz bodies removed by spleen to form "bite cells".

Indications for prophylactic treatment using of co-trimoxazole

Prophylactic treatment using of co-trimoxazole (2, 8, 44) should be provided for any of the following:

Adults: All HIV-infected patients, in WHO Stage 2, 3 and 4 and asymptomatic HIV disease infected individuals with CD4 count of < 350 cells/μL.

Pregnancy: All pregnant women throughout their pregnancy

Duration: If treatment with ARV is not available co-trimoxazole for adult and children who qualify but are not on ARVs, should continue for life. For those on ARV's, co-trimoxazole prophylaxis can be stopped if CD4+ count is >350 cells/μL.

1.2.6. Co-trimoxazole and zidovudine

Anaemia is one of the important side effects of both zidovudine and co-trimoxazole. Studies on haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with co-trimoxazole in Ivory Coast showed that at baseline, most patients had a normal neutrophil count and 80% of them were already receiving co-trimoxazole. An unexpectedly high rate of grade 3-4 neutropenia occurred shortly after

introduction of zidovudine. Almost all of the persistent severe neutropenia disappeared after co-trimoxazole was stopped. The study suggested that this could have been due to underlined drug interaction between the two drugs in those individuals (19). That study found that Grade 3-4 anaemia was much less frequent, but remained the first cause of zidovudine discontinuation. In HIV-infected patients, severe anaemia is most frequent with zidovudine use (7), whereas severe neutropenia is most frequent with co-trimoxazole (7). It is known that the combination of these drugs leads to a more frequent cytotoxicity than when they are used separately (4). Other studies, were reported that co-trimoxazole synergizes with zidovudine, i.e. trimethoprim decreases the renal excretion of zidovudine leading into the increase of zidovudine concentration in the human body, causing severe haematotoxicity and suppressing accessory cells-dependent immune function, the combination of both drugs may contribute to the immune deterioration in AIDS patients (4, 19).

There are a number of other studies which indicate that when co-trimoxazole and zidovudine are co administered for a long period, there will be pronounced haematological disorders than when either of the drugs is used individually (4, 45, 46).

1.2.7 Anaemia

Anaemia is a condition that occurs when there is a reduced number of red blood cells or too little concentration of haemoglobin bellow 14 g/dl in males and haemoglobin level < 12 g/dl in females (47).Anaemia is the most commonly encountered haematological abnormality in HIV-infected patients and is associated with faster disease progression and mortality . Studies have consistently shown that the prevalence of anaemia is high in the HIV-infected population, particularly among those with AIDS and is reported in 40% of untreated subjects and in 60% to 95% of treated subjects (3, 6, 7, 48). Anemia also has been associated with progression of HIV disease (3, 49).

Table 1.1: The normal ranges for haemoglobin

The normal ranges are:	
Newborns	17-22 gm/dl
Adult males	14-18 gm/dl
Adult women	12-16 gm/dl

The normal ranges for haemoglobin concentration depend on the age and the sex of the person (47).

There are several different types of anaemia, and each one has a different cause. The most common form of the condition is iron-deficiency anaemia. This is where the body lacks enough iron to keep the red blood cells functioning properly. Others are: Megaloblastic anaemia, haemolytic anaemia, aplastic anaemia and sickle-cell anaemia. Megaloblastic anaemia is characterized by many large immature and dysfunctional red blood cells because they have failed to mature properly. The disease results from inhibition of DNA synthesis in red blood cell production. It includes the type formerly known as pernicious anaemia and vitamin B_{12} deficiency anaemia. Haemolytic anaemia results from the destruction of red blood cells and may be caused by bacteria, genetic

disorders, or toxic chemicals. Aplastic anaemia is a severe anaemia in which the capacity of bone marrow cells to generate red blood cells is diminished. The condition can be congenital or it can be caused by exposure to radiation, toxic chemicals, or drugs. Sickle-cell anaemia is a life-long blood disorder characterized by red blood cells that assume an abnormal, rigid, sickle shape. Sickling decreases the cells flexibility and results in a risk of various complications. The sickling occurs because of a mutation in the hemoglobin gene.

Morphological classification of anaemia is:

1. Size (MCV-Mean Cell Volume), if size

- $94\mu m^3$ – MACROCYTIC
- $<80\ \mu m^3$ – MICROCYTIC
- $80\text{-}94\ \mu m^3$ – NORMOCYTIC

2. Haemoglobin concentration or MCHC – (Mean Cell haemoglobin concentration)

If >30% - normochromic,

<30% - hypochromic.

Table 1.1: WHO grades for anaemia are shown in table below:

Grade	Haemoglobin range
Grade1	9.5-10.5g/dl (upper limit may depend on laboratory range)
Grade2	8.0-9.4g/dl
Grade3	6.5-7.9g/dl
Grade4	<6.5g/dl

Grade 4&3-severe anaemia, grade 2-moderate.grade1-mild and >10.5 are Normal

Fatigue is perhaps the most important manifestation of mild anaemia. HIV-infected patients experience a range of haematological complications including anaemia, neutropenia, lymphopenia and thrombocytopenia. Anaemia impacts a range of dimensions of quality of life, most commonly through fatigue. The disease can be caused by a range of mechanisms including infections, dietary deficiencies and medication. Histologically, bone marrow hypoproliferation and dysplasia are most commonly seen. Both zidovudine and co-trimoxazole induce macrocytosis, however, zidovudine, has broader myelosuppressive effects both *in vitro* and *in vivo*.

The management of anaemia typically includes correction of the underlying cause(s) and anaemia is frequent complication of infection with the HIV-1 and may have multiple causes (50) . HIV infection may lead to anaemia in many different ways: in cytokine change production with subsequent effects on haematopoiesis (51, 52); decreased concentrations of erythropoietin (53); opportunistic infectious agents, such as *Mycobacterium avium* complex which suppress erythroid progenitors by a soluble factor(s) in the serum (54) and parvovirus B-19 (55); administration of chemotherapeutic agents such as zidovudine (56) ,and trimethoprim-sulfamethoxazole (57); and the autoimmune destruction of red blood cells (58). Anaemia accelerates disease progression and increases mortality among HIV-infected individuals. Few studies have characterized this problem in developing countries (5, 59-61).

1.2.8 A statement of the research problem

Although co-trimoxazole prophylaxis and ART therapy have significant benefit to HIV-infected patients, their use is associated with unwanted effects that may significantly negate the benefits. Co-trimoxazole is known to interfere with synthesis of red blood cells which may lead to anaemia. Zidovudine, a key drug in HAART, can cause anaemia through interference with the synthesis of haemoglobin as well as bone morrow suppression. Indeed clinical studies have shown that anaemia is a common complication of co-trimoxazole prophylaxis and zidovudine-containing HAART (5, 19).

According to the new national guidelines for management of HIV and AIDS in Tanzania (3rd Edition 2008), asymptomatic HIV-infected individuals with CD4 count of < 350 cells/μL who are not on HAART are recommended to receive co-trimoxazole for prophylaxis unless they have started antiretroviral therapy and their CD4 count is > 350 cells/μL (2, 11). The new default first line regimen now contains zidovudine (zidovudine, lamivudine and efavirenz). By contrast, in the previous guidelines the CD4 cell cut off to start co-trimoxazole prophylaxis was < 200 cells//μL and the default first line regimen was a stavudine-containing HAART regimen. Therefore, with the new guidelines, patients are likely to be exposed to co-trimoxazole for a longer time than previously did; and more patients are likely to be exposed to zidovudine and thus there will be increased exposure to both drugs. For this reason the implementation of the new guideline may be associated with significant increase of anaemia as a complication of prophylaxis against opportunistic infections and antiretroviral therapy. Therefore this study was conducted to assess the effect of co-trimoxazole, zidovudine or both on haemoglobin concentration over time in the setting of the new guideline in Tanzania.

The study focused on determining the effect of zidovudine-containing HAART regimen and co-trimoxazole prophylaxis on haemoglobin concentration over 12 months of follow up and to determine the risk and incidence of significant drop in haemoglobin concentration associated with the use of either drug or both. The study is providing information essential for planning enhanced care and treatment for HIV –infected patients who are going to be under ART.

The knowledge from this study will help to design a better treatment approach to be used by all healthcare providers in monitoring and management of the drugs administration,

For instance when to start, or terminate the use of co-trimoxazole prophylaxis in HIV-patients receiving zidovudine-containing HAART regimen as well as when to change the zidovudine-containing HAART regimen to none zidovudine-containing HAART regimen from ARV drug combined therapy.

1.2.9 The study questions were:

1. What are the effects of co-trimoxazole prophylaxis alone on haemoglobin concentration in patients receiving none zidovudine-containing HAART regimen.
2. What are the effects of zidovudine alone on haemoglobin concentration in patients receiving zidovudine- containing HAART regimen without co-trimoxazole for prophylaxis.
3. What are the effects of co-trimoxazole prophylaxis plus zidovudine on haemoglobin concentration in patients receiving zidovudine- containing HAART regimen and co-trimoxazole for prophylaxis.

1.2.10 Broad objectives

To determine the effect of co-trimoxazole prophylaxis, and zidovudine therapy, on haemoglobin concentration in HIV-infected patients receiving antiretroviral drugs.

1.2.11 Specific objectives

1.2.11.1. To determine the change in haemoglobin concentration over 12 months of follow up in HIV-infected patients receiving:-

- None zidovudine-containing HAART regimen and co-trimoxazole for prophylaxis,
- Zidovudine-containing HAART regimen without co-trimoxazole for prophylaxis,
- Zidovudine- containing HAART regimen plus co-trimoxazole for prophylaxis.

1.2.11.2. To determine the proportion of patients having a significant drop ($\geq$ 1 g/dl) in haemoglobin level over time in the three study patient groups.

1.2.11.3. To determine the incidence/risk of significant haemoglobin drop in the three treatment groups.

CHAPTER TWO

2. METHODOLOGY

2.1 Study design and site

This was a retrospective and observational study involving adult HIV-infected patients being started on co-trimoxazole and HAART for the first time at a CTC located at Mwananyamala Hospital.The hospital is located in Dar-Es-Salaam city. With a population of 3,000,000 (estimate from 2007 census) . Dar Es Salaam city is an administrative and business centre of Tanzania. It has three local government areas or municipal councils of Kinondoni, Temeke and Ilala each with one municipal hospital namely Mwananyamala, Temeke and Ilala respectively. Mwananyamala is a referral hospital for more than 15 primary public health facilities and about 150 private health facilities within Kinondoni Municipality. The hospital has a capacity of 240 beds and admits 100-200 patients per day and attending 1,200 - 1,500 out-patients per day. It has 383 employees of different cadres, among them 32 staff are working at the CTC which started in November 2004.To date 19,845 patients have been enrolled at the CTC. About 200-250 HIV-infected patients are seen per day and about 10 to 15 patients are new cases. The CTC operates everyday from 7.00 am to 8.00 pm except on Sundays and public holidays. The CTC provides information, counseling on transmission risk reduction (positive prevention), nutritional and family planning, adverse event management, dispensing HAART and intervention for opportunistic infection.

2.3 Study patients

During the study period (December 2007 to December 2008) a total of four thousand five hundred and sixty eight (4568) HIV-infected patients attended at CTC at Mwananyamala Hospital. Among these patients, four hundred and seven (9%) files were eligible for this study and were scrutinized as follow:-

- 226(56%) HIV-infected patients who were receiving none zidovudine-containing HAART regimen plus co-trimoxazole for prophylaxis (co-trimoxazole group),
- 19(5%) HIV-infected patients who were receiving zidovudine- containing HAART regimen without co-trimoxazole for prophylaxis (zidovudine group), and
- 162(40%) HIV-infected patients who were receiving zidovudine- containing HAART regimen plus co-trimoxazole for prophylaxis (co-trimoxazole plus zidovudine group).

2.3.1 Patients Sample size

The sample size was calculated based on the following formula.

$$n = \frac{z^2 p q}{r^2}$$

n = sample size for patients to be interviewed

p = estimated proportion of HIV patients taking cotrimoxazole = 50%

q = 100 - p

z = standard normal variety at 95% confidence interval =1.96

r = estimated error = 5%

$n = [(1.96)^2 \times 50 (100-50)] / 5^2$

n = 392

Minimum sample size 392

+10 adjusted for non-response → adjusted sample size 400

Therefore **407** patients recruited for the study.

Inclusion Criteria

The age of HIV- infected patients (> 18 years) was the basic criterion for selecting patients for this study. Patients qualifying by age led to the obtaining of three groups depending on whether HIV- infected patients who received co-trimoxazole for prophylaxis alone or HIV-infected patients who received zidovudine-containing HAART regimen plus Co-trimoxazole for prophylaxis or HIV-infected patients who received zidovudine- containing HAART regimen alone.

Exclusion criteria

HIV- infected patients aged < 18 years and those HIV-infected patients who were receiving none zidovudine-containing HAART without co-trimoxazole prophylaxis were excluded from this study.

2.4 Study procedures

The sampling frame for the study was patients' files who attended the CTC clinic during the period between December 2007 and December 2008. These files were retrieved from CTC archives sequentially. A total of four thousand five hundred and sixty eight files were found, 181 files were eligible after screening them for the use of zidovudine-containing HAART regimen. The remaining patient's records were further screened for the use of co-trimoxazole prophylaxis and a total of 226 files were selected. Therefore, a total four hundred and seven files were included and the remaining 4161 patient files were excluded from the study. The patients who were using zidovudine-containing HAART regimen were divided into those who never had co-trimoxazole prophylaxis during the 12 months of follow up 19 patients were found and those who had co-

trimoxazole prophylaxis at some point during the 12 months of follow up 162 patients were confirmed .

Demographic data was collected at the baseline while other information was collected at each visit these include the following:-

- The haemoglobin level in g/dl
- The CD4 count in cells/μL
- Clinical stage
- Weight in kg
- Nutritional status
- Pregnancy status
- Co-trimoxazole use history:-date when the patients started and stopped use co-trimoxazole (duration), daily dose, clinical stage, haemoglobin level, and CD4 count during the co-trimoxazole use.
- Zidovudine use history:-duration of zidovudine, daily dose, clinical stage, haemoglobin level, and CD4 count during the zidovudine use.
- Other ART use history: - time interval of ART use, daily dose, clinical stage, haemoglobin level, and CD4 count during the use of ART.
- Concomitant drugs history:-Name of the drug, indication, daily dose, route of administration, length of drug use.
- Concurrent diseases history: - duration and treatment.

The haemoglobin level in g/dl, the CD4 count in cells/μL, clinical stage, co-trimoxazole and zidovudine use history were used in the analysis to determine the change in haemoglobin concentration, proportion of patients having the significant drop ($\geq$ 1 g/dl) in haemoglobin level, incidence and risk for significant haemoglobin drop for the prior mentioned treatment groups over 12 months of follow up.

2.5 Statistical analysis

Collected data was checked twice for completeness and consistency, and errors or discrepancies found were corrected. Data entry was done by using Microsoft office excel 2007 and were cleaned and validated to determine coding errors by the investigator. Data analysis was done by SPSS 15.0 for windows and stata. The data analysis included: running frequency, Chi-Square test, Fisher's exact test and t-test were used to compare the data obtained and cross tabulation were done .Then data were summarized in a two way table. Those with P value < 0.05 were taken as statistically significant. Kaplan-Meier survival analysis was used to estimate the risks and incidence of significant drop in haemoglobin during 12 months of the study, as well as comparisons was made using the log-rank test.

CHAPTER THREE

3. RESULTS

3. 1 Demographic and Clinical Characteristics of Patients

The main baseline demographic and clinical characteristics of the patients for this study are summarized in tables 3.1 and 3.2.

3.1. 1 Patients selection and grouping:

Out of Four thousand five hundred and sixty eight (4568) patient's file records only four hundred and seven patient's file records (8.9%) qualified for this study. 181 patient's records (3.9%) were those using zidovudine-containing HAART regimen and 226 patient's records (5%) were those using co-trimoxazole for prophylaxis and other ARVs. The patients who were receiving zidovudine-containing HAART regimen were further divided in two subgroups; those on none co-trimoxazole prophylaxis plus zidovudine-containing HAART regimen (19 patients' records) and those on zidovudine-containing HAART plus co-trimoxazole prophylaxis (162 patients' records).

3.1. 2 Demographic data:

There were more female (71%) than male (29%) patients at the baseline and even in the three treatment groups, the difference was significant P value = 0.005. Most of patients were aged between 18-45 years, same age pattern was observed at the baseline and in the three treatment groups. The patients who were receiving zidovudine-containing HAART regimen were younger compared to the other two treatment groups. The difference between ages among treatment groups were highly significant $P < 0.001$. Most of the patients weighed between 46-60kg with no major difference in the weight among treatment groups at baseline.

3.1. 3 Haemoglobin count:

All patients included in this study had haemoglobin count available at baseline (at the month 0), mean haemoglobin was frequently increasing with time from 10.5(±2.3) at baseline to 11.7(±1.7) after 12 months. There was no significant difference in haemoglobin concentration at baseline in the three treatment groups (P value = 0.760).

At the baseline anaemia was observed in 211(52%) patients. However, the anaemic status was declining with time among the studied patients as follows:- after 4 months (38%); after 8 months (27%); and after 12 months (24%.)

According to WHO anaemia status is when haemoglobin level is less than 10.5g/dl which is further divided in four grades (grades I-IV). More patients fall into grades I and II than grade III and IV. At baseline, the highest number of anaemic patients was found among patients who were receiving non zidovudine-containing HAART regimen and other ARVs (CTX alone) (56.1 %) followed by those receiving a combination of co-trimoxazole and zidovudine (38.2%). The lowest number was among those patients who were receiving zidovudine-containing HAART regimen (5.7 %) .Te difference observed were not significant (P value = 0.537).

3.1. 4 CD4 count:

Most of the patients had CD4 count of less or equal to 200 cells//μL at baseline. The distribution was 37% in patients who were receiving zidovudine-containing HAART regimen, 83% in the co-trimoxazole prophylaxis and other ARVs group, and 75% in combination of co-trimoxazole prophylaxis plus zidovudine-containing HAART regimen group. The difference was highly significant with $P < 0.001$. Patients who were receiving zidovudine-containing HAART regimen had higher CD4 count at baseline median CD4 was 259 (174-290) cells/μL compared to other two groups: Patients who were receiving co-trimoxazole CD4 was 98 (51-98) and Patients who were receiving co-trimoxazole plus zidovudine CD4 was 137(55-204) and the difference was highly significant with p-value < 0.001.

Another observation in this study was the tendency in improvement of CD4 count level with time, after 4 months CD4 count was 161 (87-259), after 8 months CD4 count was 240 (154-342) and after 12 months CD4 count was 276 (184-377).

3.1.5 WHO Clinical stage:

The majority of the patients who qualified for this study were in the clinical stage 3. Same pattern was observed in the three treatment groups.

3.1.6 Patient follow up:

Records revealed that, with time; follow up of patients was lost due to one reason or another including being transferred to new sites or there was no information available in the file. In this study eight (2%) patients lost follow up after the first 4 months , after 8 months 25(6%) patients lost follow up. With regard to haemoglobin levels; 27 (7%) patients and 41(10%) patients had their records on haemoglobin level not available in the eighth and twelfth months respectively.

These results show that, all the three treatment groups experienced a reduction of negative hematological effects and are also, highlighting the necessity to evaluate the impact of co-trimoxazole prophylaxis before deciding the use of zidovudine-containing HAART therapy at baseline.

In this study the most anaemic patients were females, in all the three classes of anaemia at baseline. The majority of patients aged between 18-45years were more anaemic compared to others at baseline. The overall proportion of anaemic patients were 52% severe anaemia were 12%, moderate and mild were 20%, 20% respectively. At baseline the majority of anaemic patients had CD4 less or equal to 200cells/μL. The great parts of anaemic patients in this study were in clinical stage 3.The highest proportion of anaemic patients weighed between 45-60kg. (Table 3.2).

Table 3.1: Baseline demographic and clinical characteristics of study patients

Characteristics		All patients (n=407)	Treatment groups CTX (n=226)	AZT (n=19)	CTX&AZT (n=162)	P value
Gender	Female, *n* (%)	289(71)	148(65)	18(95)	123(76)	0.005
	Male, *n* (%)	118(29)	78(35)	1(5)	39(24)	
Mean Age (±SD)		38.7(9)	40(9)	33(8.6)	38(9.5)	<0.001
Age categories yrs		*n (%)*				
	18-35yrs	176(43)	81(36)	14(74)	81(50)	0.013
	36-45yrs	148(36)	92(41)	4(21)	52(52)	
	46-55yrs	57(14)	37(16)	0(0)	20(12)	
	>55yrs	26(6)	16(7)	1(5)	9(6)	
Mean Hb g/dl (±SD)		10.5(2.3)	10.5(2.5)	10(1.5)	10.6(2.2)	0.766
WHO anaemia grades,		*n (%)*				
	>10.5g/dl	195(48)	107(47)	7(37)	81(50)	0.500*
	Grade 1	83 (20)	39(17)	6(32)	38(24)	
	Grade 2	81(20)	50(22)	5(26)	26(16)	
	Grade 3	35 (9)	21(9)	1(5)	13(8)	
	Grade 4	13 (3)	9(4)	0	4(3)	
Median CD4 *(cells/μL)(IQR)*		115 (54-191)	98 (51-98)	259 (174-290)	137 (55-204)	
CD4 categories		*n(%)*				
	≤ 100	181(45)	117(52)	3(16)	61(38)	0.000*
	101-200	135(33)	71(31)	4(21)	60(37)	
	201-350	78(19)	31(14)	12(63)	35(22)	
	>350	13(3)	7(3)	0	6(4)	
WHO clinical stage,		*n* (%)				
	Stage 1	57(14)	18(8)	3(16)	36(22)	0.005
	Stage 2	64(16)	34(15)	3(16)	27(17)	
	Stage 3	276(68)	167(74)	12(63)	97(60)	
	Stage 4	10(2)	7(3)	1(5)	2(1)	
Mean weight(±SD)		54.5(12)	53(11)	60(16)	56(12)	
Weight (kg)**categories**		*n(%)*				
	25-45kg	91(22)	57(25)	4(21)	30(19)	0.046
	46-60kg	211(52)	121(54)	6(32)	84(52)	
	>60kg	105(26)	48(21)	9(19)	48(30)	

IQR: Interquartile range * Fisher's exact Test

Table 3.2: Baseline demographic and clinical characteristics of study patients by Hb status

Characte-ristics	*Total# n (%)	Normal ≥10.5g/dl n (%)	**Anemic ≤10.5g/dl n (%)	Class of anaemia Severe <8.0g/dl n (%)	Moderate 8-9.4g/dl n (%)	Mild 9.5-10.5g/dl n (%)	P value
All	407(100)	195(48)	212(52)	48(12)	81(20)	83(20)	
Gender							
Female	289 (71)	124(64)	165(78)	41(85)	59(73)	65(78)	
Male	118(29)	71(36)	47(22)	7(15)	22(27)	18(22)	
Age (y) categories	n(%)						
18-35yrs	176(43)	80(41)	96(45.3)	24(50)	31(38)	41(49)	
36-45yrs	148(36)	71(36)	71(36.3)	17(35)	34(42)	26(31)	0.782
46-55yrs	57(14)	33(17)	24(11.3)	5(10)	10(12)	9(11)	
>55yrs	26(6)	11(6)	15(7)	2(4)	6(7)	7(8)	
CD4 categories	n(%)						
1-100	181(44)	79(40.5)	102(48)	26(54)	32(40)	44(53)	
101-200	135(33)	66(34)	69(32.5)	13(27)	33(41)	23(28)	0.514
201-350	78(19)	41(21)	37(17.5)	9(19)	15(19)	13(16)	
>350	13(3)	9(5)	4(2)	0	1(1)	3(4)	
Clinical stage	n(%)						
1	57(14)	31(16)	26(12)	6(13)	8(10)	12(14)	
2	64(16)	33(17)	31(15)	8(17)	10(12)	13(16)	0.877
3	276(68)	125(64)	151(71)	34(70)	61(75)	56(68)	
4	10(2)	6(3)	4(2)	0	2(3)	2(2)	
Weight Categories	n(%)						
25-45kg	91(22)	29(15)	62(29)	18(37)	25(31)	19(23)	
46-60kg	211(52)	104(53)	107(51)	23(48)	44(54)	40(48)	
>60kg	105(26)	62(32)	43(20)	7(15)	12(15)	24(29)	

IQR: interquartile range

*Total (407) = Normal (195) + Anaemic (212)

** Anaemic (212) = severe + moderate + mild

Total# (407) = Normal (195) + severe + moderate + mild

3.2 Effects of co-trimoxazole alone

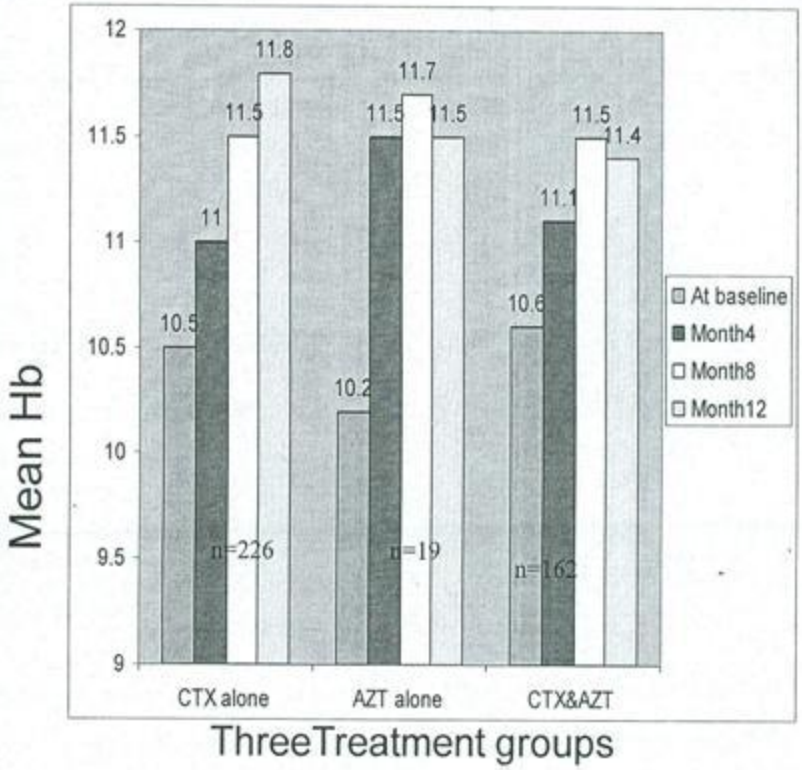
On average, the study observed that the use of co-trimoxazole plus other ART without zidovudine had positive effect of haemoglobin level demonstrating an increase in haemoglobin level over time from mild anaemia (Hb mean 10.5g/dl) at baseline to non anaemic state (Hb mean $\geq$ 11.8 g/dl) at 12 months, (table 3.3 and figure 3.1).Most of patients in this study were receiving none zidovudine-containing HAART regimen plus co-trimoxazole prophylaxis .Among patients who were receiving co-trimoxazole for prophylaxis alone anaemic patients were 88 (41%),with mean haemoglobin 11 ($\pm$2). For the period of the first 4 months among patients who were receiving co-trimoxazole prophylaxis; 20 (9%) patients dropped haemoglobin level significantly by $\geq$ 1g/dl, had mean haemoglobin 10.4($\pm$1.8). Eighty four percent had CD4 below 350 cells/μL ; 72% were in the weight group between 45 – 60 kg and 96% were aged below 45 years old. During the eighth month of the follow up co-trimoxazole users were 218 (54%) with mean haemoglobin 11.5 ($\pm$1.9). Among them anaemic patients were 27%, 29. (13.3%) patients dropped haemoglobin concentration $\geq$ 1g/dl had mean haemoglobin 10.5 ($\pm$2.5). During the period of last 4 months patients who were receiving co-trimoxazole prophylaxis were 214 (53%) with mean haemoglobin 11.8($\pm$1.8), among them anaemic patients were 47 (22%), and 27 (13%) patients dropped haemoglobin level $\geq$ 1g/dl, (table 3.5 and figure 3.1).

Table 3.3: Mean Hb (±SD) concentration of the study patients in the three treatment groups during the study period.

Treatment group	At baseline	Month4	Month8	Month 12	P-value
CTX alone(n=226)	10.5(2.5)	11(2)	11.5(2)	11.8(2)	<0.001
AZT alone(n=19)	10.2(1.5)	11.5(2)	11.7(2)	11.5(1.5)	0.003
CTX&AZT(n=162)	10.6(2.2)	11.1(2)	11.5(2)	11.4(1.7)	<0.001
Overall (n=407)	10.5(2.3)	11.1(2)	11.5(2)	11.6(1.7)	

The overall mean haemoglobin at the end of the study was 11.65 (±1.7) g/dl with an average gain of 1.14 (±2.3) g/dl. The haematological changes at the end of the last 4 months showed: - an anaemia in 24% of the cases at month 12 versus 52% at baseline. The average rate of haemoglobin concentration was 11.5 (±1.5) versus 10.2 (±1.5) g/dl at baseline in the patients who were receiving zidovudine-containing HAART regimen ($p=0.003$) and The average rate of haemoglobin concentration was 11.8(±2) versus 10.5(±2.5) g/dl at baseline in the patients who were receiving co-trimoxazole prophylaxis without zidovudine and 10.6 versus 11.4 (±1.7) g/dl at baseline in the patients who were receiving co-trimoxazole prophylaxis plus zidovudine-containing HAART regimen. Anaemia cases were decreasing among the patients in all treatment groups regardless of time and type of drugs during the study period, (Table 3.4).

Figure 3.1: Mean haemoglobin concentration of study patients, in the three treatment groups during the study period



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The mean haemoglobin level was increasing regardless of duration co-trimoxazole and zidovudine use, difference between the mean haemoglobin level were statistically significant throughout the study period with p value <0.05, as shown in table 3.3 and figure 3.1.

Table 3.4: Percentage of anaemic patients by treatment group

	Month 4 n (%)	Month 8 n (%)	Month 12 n (%)	P value
CTX	**N=216**	**N=218**	**N=214**	
Anaemic	88 (41)	59 (27)	47(22)	<0.001
Non anaemia	128(59)	159(73)	167(78)	
AZT	**N=18**	**N=18**	**N=14**	
Anaemic	6 (33)	3(17)	2(14)	0.34
Non anaemia	12(67)	15(83)	12(86)	
CTX & AZT	**N=157**	**N=144**	**N=137**	
Anaemic	61(39)	40(28)	38(28)	0.05
Non anaemia	96(61)	104(72)	99(72)	

Percentages may not add to 100 due to missing CTX1=10, CTX2=8, CTX3=12, AZT1=1, AZT2=1 and AZT3=4, CTX1&AZT1=5, CTX2&AZT2=18 and CTX3&AZT3=15.

CTX1= patients who were using cotrimoxazole during 1st 4 months (month 4)

CTX2= patients who were using cotrimoxazole during 2nd 4 months (month 8)

CTX3= patients who were using cotrimoxazole during 3rd4 months (month 12)

AZT1= patients who were using zidovudine during 1st 4 months (month 4)

AZT2= patients who were using zidovudine during 2nd4 months (month 8)

AZT3= patients who were using zidovudine during 3rd 4 months (month 12)

The number of anaemic patients appears to be decreasing with continued treatment especially in the CTX&AZT group, this is due to: - there were maybe an increased iron accumulation caused by zidovudine, a reduction of infections, suppressing viremia and thereby restoration of immune capacity.

The highest proportions of patients who were anaemic (≤10.5 g/dl) were found during the first four months in all three treatment groups, however patients who were receiving co-trimoxazole alone had highest proportion of lower haemoglobin level compared with other proportions during the first 4 months of the study the difference was highly significant $P < 0.001$, (table 3.4 & figure 3.2).The anaemic status was decreasing with time regardless of use of the medication, this may indication of the accumulation iron due to the treatments were used.

3.3 Effects of zidovudine alone

During the period of first 4 months patients who were receiving zidovudine alone were 19 (5%) with mean haemoglobin 11.2 (±1.5), among them anaemic patients were 6 (33%) and no patient on zidovudine alone dropped haemoglobin level ≥ 1g/dl. During the eighth month of the follow up patients who were receiving zidovudine alone were 18 (4.4%), among them anaemic patients were 3 (17%). During 12 month of the follow up patients who were reçeiving zidovudine alone were 14 (4%), among them anaemic patients were 2(14%) and 2(10%) had no haemoglobin level measurement. Only one (7%) patient dropped haemoglobin level to ≥1g/dl.

3.4 Effects of zidovudine plus co-trimoxazole

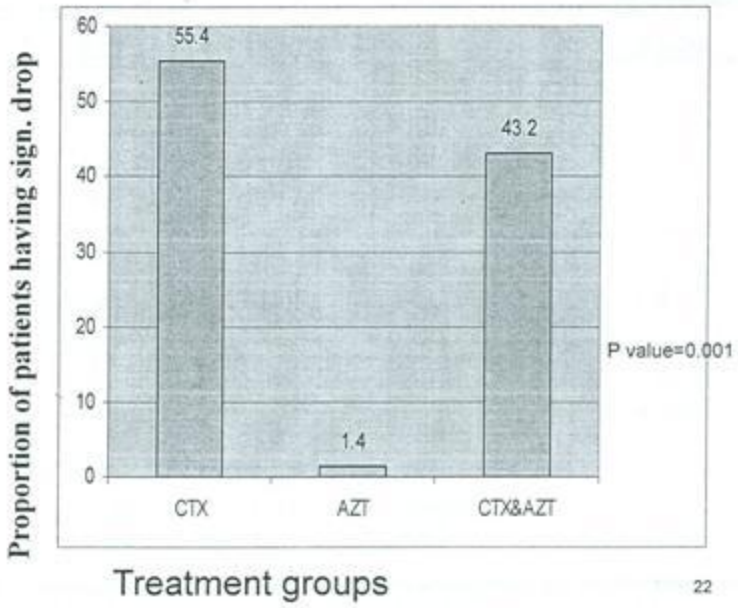
During the first 4 months the patients who received co-trimoxazole prophylaxis plus zidovudine –containing HAART regimen were 162 (40%), among them anaemic patients were 39% as shown in table 3.4. Furthermore 12 (8%) patients dropped haemoglobin level ≥ 1g/dl. During eighth month of the follow up zidovudine plus co-trimoxazole users were 144 (35.4%) patients, among them 40 (28%) patients were anaemic, furthermore 17 (12%) patients dropped haemoglobin level ≥ 1g/dl. During the 12^{th} month zidovudine plus co-trimoxazole users were 138 (33.6%) patients, among

them 38 (28%) were anaemic 15 (9%) were missing haemoglobin level. For that period 22 (15.94%) patients dropped haemoglobin level ≥ 1g/dl, their mean haemoglobin was 10.3 (±2.5).

3.5 The proportion of patients having the significant drop

It was observed that there was a tendency of more frequent significant drop (≥1g/dl) in haemoglobin concentration in the co-trimoxazole prophylaxis plus zidovudine-containing HAART regimen group (16%) compared to the other two groups (co-trimoxazole alone and zidovudine alone) between (5.5% - 13%), as shown in table 3.5.

Figure 3.2: Overall proportions of patients who dropped Hb > 1g/dl (n=74)



The highest proportion of patients having the significant drop (≥ 1 g/dl) in haemoglobin level over time was in co-trimoxazole alone group, compared to other treatment group and the difference was highly significant P<0.001,(Figure 3.2).

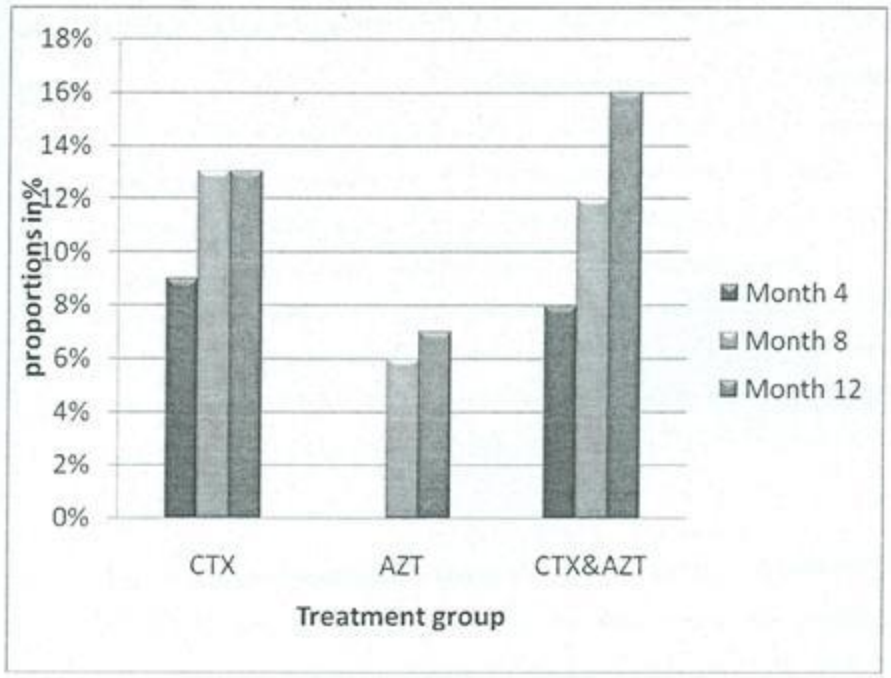
Table 3.5: Proportions of patients who dropped Hb > 1g/dl per each 4 months interval

	CTX	AZT	CTXAZT	total	X^2	p value
Month 4						
Dropped	20(9)	0	12(8)	32(8)		
No drop	196(91)	18(100)	145(92)	359(92)	2	0.37
	216	18	157	391		
Month 8						
Dropped	29(13)	1(6)	17(12)	47(12)	0.98	0.61
No drop	189(87)	17(94)	127(88)	333(82)		
	218	18	144	380		
Month 12						
Dropped	27(13)	1(7)	22(16)	50(14)		
No drop	187(87)	13(93)	116(84)	316(86)	1.3	0.52
	214	14	138	366		

Percentages may not add to 100 due to missing CTX1=10, CTX2=8, CTX3=8, AZT1=1, AZT2=1 and AZT3=4, CTX1&AZT1=5, CTX2&AZT2=18 and CTX3&AZT3=15.

Although haemoglobin level was increasing but still there were patients who dropped haemoglobin level during follow up as shown in table 3.5 and figure 3.3. The dropping tendency was in manner of gradually increase over time in each treatment group. This shows the effects of the drugs on haemoglobin level individually.

Figure 3.3: Proportions of patients who significantly dropped (Hb > 1g/dl) per each 4 successive months follow up period



The dropping tendency was gradually increasing among patients who were using cotrimoxazole alone and the combination of CTX & AZT. There was a noticeable tendency of more frequent Hb drop among patients who were using CTX & AZT. However observed differences were not statistically significant but can be medically significant as illustrated in Table 3.5 and Figure 3.3.

The greatest drop was observed in co-trimoxazole plus zidovudine group >15% by month 12, followed by co-trimoxazole alone group (>10% by month 12) and the least drop was in zidovudine alone group < 10% by 12 months of follow up.

There was an increased tendency of haemoglobin drop in co-trimoxazole plus zidovudine, as shown in table 3.5 and figure 3.2.The trend of haemoglobin level among patients who used co-trimoxazole and dropped haemoglobin level >1g/dl was slightly increasing from mean haemoglobin 10.4 (±1.8) at month four, 10.5(±2.5) at month eight and 10.6 (±2.2) at month twelve, it was proven not to be statistically significant P > 0.05 (0.93).Within patients who were receiving zidovudine plus co-trimoxazole prophylaxis in combination the trend of haemoglobin concentration was slightly decrease from mean haemoglobin of 10.5 (±1.2) at month four to 10.3 (±2.5) after month twelve but was not statistically significant P > 0.05(0.93).

3.6 The incidence of significant haemoglobin drop in the three treatment groups

The greatest incidence of significant haemoglobin drop was in co-trimoxazole for prophylaxis plus zidovudine containing HAART regimen. Over all incidence of significant haemoglobin drop was 0.18 per 4240 person-months of follow up, as shown in table 3.6.

Table 3.6: Incidence of significant drop (>1g/dl) in Hb level by exposure group

Treatment Group	n	Time at risk (person-month)	Incidence/person month of follow up
CTX	221	2388	0.016
AZT	19	204	0.005
CTXAZT	159	1648	0.2
Total	399	4240	0.18

The risk for significant drop in haemoglobin was below 25% in all groups throughout the 12 months of follow up. The overall incidence of significant haemoglobin drop was 5per 10,000 person-year of follow-up. The time at risk of dropping haemoglobin significantly by ≥1g/dl for all arms was less than 1 month and the risk for significant drop in haemoglobin was below 25% in all groups throughout the 12 months of follow up,(table 3.7).

3.6.1 The risk for significant drop in haemoglobin in the three treatment groups

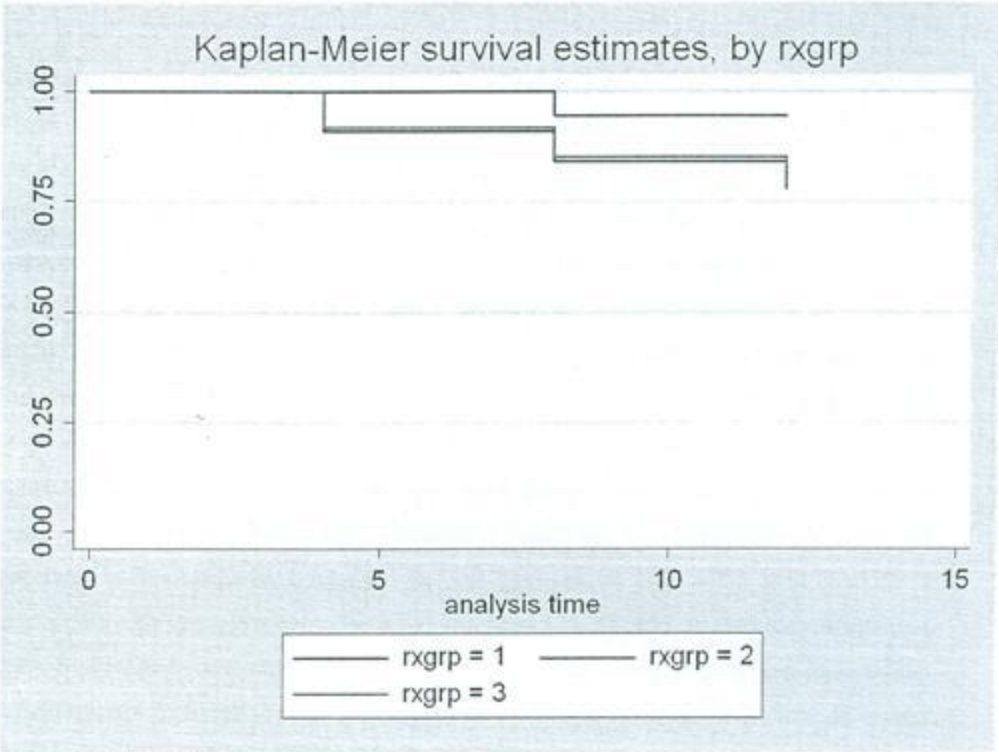
Overall the risk for significant drop in haemoglobin level was low in all three groups (< 25%) by 12 months of follow up, as shown in table 3.7 and figure 3.5. The risk for significant drop in haemoglobin by 12 months of follow up was greater in co-trimoxazole alone compared to zidovudine alone. However, the risk in co-trimoxazole plus zidovudine was greater than co-trimoxazole alone (figure 3.4).

Table 3.7: Kaplan Meier survival data

Treatment Group	n	Dropped (n/%)	No drop (n%)	Estimate time month (95%CI)
CTX alone	226	41(18)	185(82)	1.0(1.3-0.7)
AZT alone	19	1(5)	18(92)	0.7(0.7-0.0)
CTX&AZT	162	32(20)	130(80)	0. 9(1.3-0.5)
Overall	**407**	**74(18)**	**333(82)**	**1.0(1.2-0.7)**

The longest time to drop haemoglobin level by $\geq$ 1g/dl during follow up was approximately one month in patients who were using co-trimoxazole prophylaxis without Zidovudine. The patients who were receiving the combination of co-trimoxazole prophylaxis and zidovudine dropped haemoglobin level by $\geq$ 1g/dl in less than one month. The same was observed in those who were receiving zidovudine alone, although dropped haemoglobin level by $\geq$ 1g/dl slightly shorter time than the former, as shown in table 3.7.

Figure 3.4: Probability of not dropping haemoglobin level versus time interval in months



rxgrp = treatment group

rxgrp1= co-trimoxazole group (blue--3rd line)

rxgrp2= zidovudine group (red-1st line)

rxgrp3= co-trimoxazole plus zidovudine group (gree-2nd line)

The risk for significant drop in haemoglobin level by twelve months of follow up was Higher 20% in co-trimoxazole alone group compared with those patients using co-trimoxazole prophylaxis plus zidovudine 19% and zidovudine alone 1%, (figure 3.4).

CHAPTER FOUR

4. DISCUSSION

The aims of this study were to determine the effect of zidovudine alone, co-trimoxazole alone, and zidovudine plus co-trimoxazole on haemoglobin concentration; when they are used as zidovudine-containing HAART in patient not on co-trimoxazole prophylaxis, co-trimoxazole prophylaxis in patients on HAART not including zidovudine; and when used together as co-trimoxazole prophylaxis in patients on zidovudine-containing HAART. Specifically, the study also evaluated the risk for a significant drop in haemoglobin and the incidence of such drop in the three treatment exposure groups. In this study we observed that on average, the presence of zidovudine, co-trimoxazole prophylaxis, or both in a care and treatment regimen has a positive effect on haemoglobin concentration with all treatment groups demonstrating an increase in haemoglobin over time during the 12 months of follow up. This finding is also supported by other studies which have reported that the treatment of HIV/AIDS with HAART is associated with improvement in haemoglobin level (25, 62, 63). In the study which was done by Hoffman and others on HIV-infected patients using zidovudine, lamivudine, and efavirenz in South Africa an increase of haemoglobin level at 24 and 48 weeks during the use of HAART was observed (64). Similar observations were obtained by studies conducted by Semba and his team (48, 63, 65). However, contradicting results have been reported where the use of zidovudine–containing HAART had caused anaemia and bone marrow aplasia (24, 66, 67).

Our study was conducted in patients who were also taking vitamins and haematinics as a parallel study by another research group. The use of multivitamins and haematinics could probably had interfered with the trend of haemoglobin concentrations in our study patients, by improving their haemoglobin concentration such as what we observed in our study. This can partly be explained by the use of multivitamins. Thus our finding could have been a result from nutritional intervention and other care services offered by MDH. This can be supported by our observation of weight gain on every subsequent visit to CTC in all three groups of patients.

In our study, about 80% of the patients were given multivitamins of which probably helped them to improve on their vitality and appetite for food hence enhanced red blood cell production which led to rising in haemoglobin levels for most of the patients. Indeed, some studies have shown that multivitamin supplementation improves hematologic status among HIV-infected patients (68, 69).

Apart from the overall increase in haemoglobin concentration, some patients in all the three groups had a significant drop in haemoglobin concentration. Our data show that the presence of both zidovudine and co-trimoxazole prophylaxis is associated with most of these significant drops in haemoglobin, (16 % compared to 13 % co-trimoxazole prophylaxis alone and 7 % zidovudine alone). This could probably be as the result of doubling of haemototoxicity from co-trimoxazole and zidovudine as well as synergistic effects (4) which exist between co-trimoxazole and zidovudine in combined therapy (19, 45, 70). Both zidovudine and co-trimoxazole have haematological effect in their own right; zidovudine inhibits beta-globin gene expression and has bone marrow cytotoxicity (4, 40): trimethoprim, a component in co-trimoxazole, inhibits dihydrofolate reductase leading to dose dependent neutropenia and anaemia(18). In HIV/AIDS, anaemia is most frequent with zidovudine whereas severe neutropenia is most frequent with co-trimoxazole (7, 62). There is strong evidence from literature that the combination of these drugs leads to a more frequent haematotoxicity than when they are used separately (19, 46). Interestingly, our data suggest that co-trimoxazole prophylaxis is more likely associated with significant haemoglobin drop than zidovudine, and the effect observed when both drugs are present could largely be due to the effect of co-trimoxazole. However, the number of patients in the zidovudine alone group was significantly small than in the group of co-trimoxazole for prophylaxis.

Our data shows that the use of co-trimoxazole prophylaxis, zidovudine or both in care and treatment regimens is generally safe with regard to haemoglobin status. The overall risk for significant drop (>1g/dl) in haemoglobin level during 12 months of follow up was < 25%,this was observed even in all three treatment groups. To the best of our

knowledge, our study may be the first of its kind to evaluate the risk of significant drop in haemoglobin associated with the use of these drugs. However, our finding need to be confirmed in further studies as there are many other factors including other care and treatment interventions and adherence (71, 72) that may reduce the risk. Nevertheless, when we looked at the incidence of significant haemoglobin drop the finding was consistent with that of the risk of drop. The incidence was higher in co-trimoxazole alone (0.016 per 2388 person-months) compared to zidovudine alone (0.005 per 204 person-months) and greatest in co-trimoxazole plus zidovudine (0.2 per 1648 person-months). Therefore, our findings suggest that there may be a justification of carefully evaluating the role of co-trimoxazole prophylaxis in the development of severe anaemia associated with HIV/AIDS care and treatment. Our results are in agreement with those obtained from previous similar studies conducted in Uganda and Burkina-Faso (59, 73). To this end, significant drop in haemoglobin could be related to the long-term use of co-trimoxazole prophylaxis.

In addition the study observed that females were significantly more likely to have marked anaemia/low haemoglobin concentration as compared to males. This could probably be explained due to their physiological difference which exist between male and female, as women generally have on average 0.5mg less of iron than men as result of loss due to menstrual cycle, pregnancy and lactation. The average daily iron loss in a normal person is less than 1mg; however women lose an addition of between 0.6 to 1.3mg of iron daily during pregnancy or lactation (67, 74).

The number of patients who had significant drop in Hb is small (i.e. not statistically significant) it could have been a chance finding.

Limitation

In our study, we assessed any HIV-infected patients exposed to co-trimoxazole and zidovudine; we did not take into account the duration of use for these drugs, and this may lead to underestimate the true rate of haematological level of co-trimoxazole and zidovudine effect. To avoid and overcome the above limits, prospective study is needed with control group. Adherence was not taken in consideration; first we had no proof on the compliance of patients taking co-trimoxazole. It was assumed that all information recorded from the patients files were correct, fair and represented a true record of the patient; and secondly we did not measure the drug concentrations in the serum of patients; these may lead to over or underestimate the true rate and effect of zidovudine and co-trimoxazole on haematological concentration of the participating patients in our study. In addition, the involvement of our study patients in a multivitamin study could lead to rise of HB concentrations hence creating difficulty in making appropriate conclusion.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

To our understanding, this is the first study in Tanzania to determine the effect of using co-trimoxazole and zidovudine on haemoglobin concentration in HIV-infected patients receiving antiretroviral therapy. On average, drugs had positive effect on haemoglobin with all groups demonstrating an increase in mean haemoglobin over time. Nevertheless there was some tendency of more frequent significant drop (≥1g/dl) in haemoglobin concentration in the co-trimoxazole prophylaxis plus zidovudine-containing HAART regimen group compared to the other two groups. In addition the overall risk and incidence for significant drop in haemoglobin level was low.

The mean Hb was increasing in the first 8 months suggesting of no negative effect, but 18.2% of patients had significant Hb drop. This necessitates the monitoring of Hb for early detection of significant drop in Hb level. There was a significant drop (≥1g/dl) in Hb concentration in the CTX plus AZT user group compared to the other two groups. This indicates that the combination of CTX plus AZT carries risk of anaemia than the two groups therefore should better be avoided for anaemic HIV-infected patients.

However the use of co-trimoxazole for prophylaxis in patients who later on are put on a zidovudine-containing HAART regimen was associated with most significant haemoglobin drop. This may be a pointer to potential hazardous effect. Therefore, further studies are needed which should not only assess the consequences of combining co-trimoxazole with zidovudine in prospective study design also estimating the maximum duration for which co-trimoxazole prophylaxis can be used without hazardous effect on haemoglobin concentration

However studies are needed to assess the consequences of combining co-trimoxazole with zidovudine prospectively for longer time with a control group, but also to find out the actual period of time at which to stop co-trimoxazole prophylaxis in patients on antiretroviral therapy without causing significant haematological effects. Rise and fall of CD4+T is the only criteria of stopping and starting co-trimoxazole, while it is known

that one of the side effects of co-trimoxazole is haematological toxicity. One question remains; at least how long should patient use co-trimoxazole for prophylaxis of opportunistic diseases without causing significant effect on haematological concentration.

Long term use and wide-scale use of prophylactic co-trimoxazole may increase the spread of antimicrobial resistance in communities to other pathogens, notably *Plasmodium falciparum*, non-typhoidal salmonellae and *Streptococcus pneumoniae*. It would therefore be prudent to confine the use of this intervention only to those patients who will benefit most from it. Thus, further research for the optimal timing of initiation and ending of co-trimoxazole prophylaxis should be done in Tanzania. It is important for clinicians to understand the extent to which zidovudine-associated anaemia is likely to be problematic, not only because zidovudine is used in a wide array of today's regimens and is default in first line regimen in Tanzania, but also because clinicians have access to a growing number of HAART regimens that do not include zidovudine, a relevant fact when zidovudine-related toxicities are still of concern.

Conflicts of interest statement

There are no conflicts of interest concerning the findings reported in this study.

Keywords: co-trimoxazole prophylaxis, zidovudine-containing HAART regimen, anaemia, HAART, HIV/AIDS, haemoglobin concentration/level and haemoglobin drop.

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