POST SURGERY LACTATE LEVELS IN HIV NEGATIVE AND HIV POSITIVE ON HAART ORTHOPAEDIC PATIENTS AFTER TOURNIQUET USE, AT FOUR HOSPITALS IN LUSAKA, ZAMBIA

by

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DECLARATION

I, **Logizomai Epaenetus Kalela Chipasha**, declare that,

(i) This dissertation entitled *"Post surgery Lactate Levels in HIV Negative and HIV Positive on HAART Orthopaedic patients after Tourniquet use at four hospitals in Lusaka, Zambia"* represents my own work and has not been presented either in whole or in part for a diploma or degree at the University of Zambia or any other University elsewhere.

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APPROVAL

This dissertation by Dr. Logizomai Epaenetus Kalela Chipasha has been approved as partial fulfillment of the requirement for the award of the Degree of Master of Medicine in Orthopaedics and Trauma Surgery by the University of Zambia.

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ABSTRACT

Tourniquets are extensively used in Orthopaedic surgery to achieve a bloodless field at the surgical site resulting in elevated lactate levels. Use of antiretroviral drugs is also associated with elevation of lactate levels. Hyperlactatemia is associated with increased morbidity and mortality. Assessment of whether concurrent use of a tourniquet in Orthopaedic surgery in HIV positive patients on antiretroviral drugs has a dual additive effect on serum lactate levels is therefore important. However, the current literature on this subject is limited.

The principal objective of the study was to explore the effect of tourniquet use on lactate levels in HIV positive patients on HAART undergoing extremity surgery, and how it compares to those who are not negative. This was a prospective cohort study undertaken from 26 November 2015 to 26 November 2016, with a sample size of 183 participants of patients who presented for orthopaedic surgery at University Teaching Hospital, *St. John Paul II* Orthopaedic, *Beit Cure* and *Levy Mwanawasa* Teaching hospitals, with ages ranging between 18 - 67years. Consecutive series of biographic details of all eligible patients were taken and pre-operative baseline investigations done. Four lactate samples - one pre-tourniquet inflation, and three serial post tourniquet deflation venous blood lactate - were taken from fingertip or toe-tip of contralateral limb at 2min, 5min and 15min intervals and results obtained using a *Digital Lactate Plus Analyzer.*

One hundred and sixty (160) participants were recruited, with 124 being HIV negative (78 percent) and 36 being HIV positive on HAART (22 percent). There were significant statistical differences in the lactate levels of the patients before and after tourniquet use. Paired samples t-tests were conducted at a significant level of 0.05. Baseline lactate levels were higher in HIV positive patients on HAART than HIV negative patients. Post tourniquet deflation lactate levels were also elevated in both groups. HIV positive patients on HAART had consistently higher lactate levels at 2 min, 5 min and 15 min periods than HIV negative participants. The results from an independent samples t - test indicated that at 2 minutes, HIV positive on HAART participants ($M = 4.328$, $SD = 0.8397$, $N = 36$) had much higher post tourniquet deflation lactate levels than HIV negative participants ($M = 2.084$, $SD = 0.8240$, $N= 124$), $t (36) = -7.099$, $p<0.001$, two-tailed. At 5 minutes the results from the independent samples t-test revealed that HIV positive on HAART participants ($M = 3.423$, $SD = 0.6548$, $N = 36$) had much higher post tourniquet deflation lactate levels than HIV negative participants ($M = 1.655$, $SD = 0.6468$, $N =124$), $t (36) = -7.146$, $p <0.001$, two-tailed. Similarly, the results from an independent samples t-test indicated at 15 minutes revealed that HIV positive on HAART participants ($M = 2.594$, $SD = 0.8750$, $N = 36$) had much higher post tourniquet deflation lactate levels than HIV negative participants ($M = 1.294$, $SD = 0.5401$, $N =124$), $t(36) = -4.215$, $p<0.002$, two-tailed. However, these results were only significant at 2 minutes ($t=-3.279$; $df=17$; $p=0.004$) and 5 minutes ($t=0.0140$; $df=16$; $p=0.052$) but not significant at 15 minutes ($t=1.5367$; $df=15$; $p=0.780$), $t=1.999[14]$, $p=0.065$) 5 min ($t=1.116[13]$, $p=0.285$), and at 15 min ($t=1.137[12]$, $p=0.278$). No adverse events were recorded during the study.

Baseline lactate levels were higher in HIV positive on HAART. Tourniquet use induced lactate elevation post tourniquet deflation in all – both HIV negative and HIV positive on HAART patients.

The hyperlactatemia induced is higher in HIV positive patients on HAART as compared to HIV negative patients both pre-operatively and after tourniquet use in surgery. However, there is no increased risk of use of a tourniquet in orthopaedic surgery in HIV positive patients on HAART as the elevation of lactate during surgery is short lived, with lactate values returning to pre-tourniquet inflation levels.

Keywords: *Tourniquet, esmarch, Inclusion and Exclusion criteria, HAART, Hyperlactateamia, Lactic acidosis, P+S, Ischemic monomelic neuropathy, Arthrodesis, NRTI's, Hypoxia / Ischemia, Lactate Inflation / deflation*.

DEDICATION

This dissertation is dedicated to my very dear wife and friend Jenny, without whose enduring, loving support and sacrifices, this endeavor would not have happened. I sincerely salute my daughters - *Dawn* and *Nsansa,* for my absence sacrifices they put up with, while Dad was away at school many times, and they wished he were with them. I also express my heartfelt thanks to my brothers - Joscqkee, Japhet, Mwila, Mordecai and my little sisters Ruth and Mary for their concerted dedicated and uplifting support.

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LIST OF ABBREVIATIONS

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine amino transferase
ARVs	Anti-retroviral
ART	Antiretroviral therapy
AZT	Zidovudine
BCH	Beit Cure Hospital
BMI	Body Mass Index
DNA	Deoxyribonucleic acid
ddl	Didanosine
ddc	Zalcitabine
d4T	Stavudine
EFV	Efavirenz
ELL	Elevated lactate levels
FTC	Emtricitabine
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HL	Hyperlactateamia
IMN	Ischemic Monomelic Neuropathy
K Nail	Kuntscher nail
LA	Lactic acidosis
LMH	Levy Mwanawasa Hospital
mtDNA	mitochondrial DNA
NVP	Nevirapine
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
ORIF	Open Reduction and Internal Fixation
P+S	Plates and Screws
R/U	Radius/Ulna
SLL	Serum Lactate Levels
TKR	Total Knee Replacement
T/F	Tibia/Fibula
VLL	Venous Lactate Levels
TDF	Tenofovir1
UTH	University Teaching Hospital
ZDHS	Zambia Demographic & Health Survey
ZIOH	Zambian Italian Orthopaedic Hospital (St. John Paul II)
3TC	Lamivudine

CHAPTER ONE - INTRODUCTION

1.1 INTRODUCTION

The word tourniquet coined in 1718 by Jean Petit is derived from the French word ‘tourner’, meaning, to ‘turn’, and referred in its basic sense to the use of a constricting bandage to control bleeding (Kam et al, 2001). Tourniquets have been in use since early Roman times when various constricting bandages were used to control bleeding during amputations. Currently two types of tourniquets are used: an elastic rubber band called an esmarch bandage, which is applied manually with no set pressure, and a pneumatic type, which has a pressure gauge, which is used to set the pressure automatically, and the pressure can be increased or decreased similarly. Although modern tourniquets are a far cry from those rudimentary proto-types, they have the same basic function and in extremity surgery are used to create a bloodless operating field (Estebe et al, 2011). Human immunodeficiency virus (HIV) patients are among the patients where use of a tourniquet is indicated for extremity surgery.

Tourniquet use leads to elevation of lactate levels post deflation (hyperlactataemia) (Townsend et al, 1978, Haljamae et al, 1975). Normally, lactate levels range from 0.5 -1.0 mmol/ml. Hyperlactateamia (HL) is defined as mild to moderate increase in serum lactate concentration (2-5mmol/l) with normal pH values ($\leq$ 7.35) and normal bicarbonate of $\geq$ 20mmol/l (Calza et al, 2005). It presents with vague symptoms such as malaise, nausea, vomiting, abdominal pains, hepatotoxicity, tender hepatomegaly, peripheral edema and ascites (Herman et al, 2001).When severe (>5mmol/l), it progresses rapidly to cardio-myopathy, encephalopathy, peripheral neuropathy, pancytopenia, fulminant hepatic failure and cardio-pulmonary shock and is ultimately fatal (Calza et al., 2001). However, because majority of HIV/AIDS patients are also on highly active antiretroviral therapy (HAART), this confounds the problem as use of some drugs in HAART regimens can separately lead to HL (Brinkman et al.,1991). Hence, it is theorized that concomitant use of a tourniquet in a patient on HAART can have an additive effect leading to lactic acidosis (LA).
LA is defined as significantly HL> 5.0 mmol/l associated with a metabolic acidosis (pH <7.35) and bicarbonate < 20 mmol/L (Calza et al, 2005).

Currently, no studies have been done to determine whether HL occurs when a tourniquet is used on a patient on HAART and by how much.

This study aims to determine whether concomitant use of a tourniquet in a patient on HAART undergoing extremity surgery will lead to HL and how much as well as the significance of this elevation.

1.2 STATEMENT OF THE PROBLEM

According to WHO, (WHO, 2018) HIV prevalence in Zambia is 14.3 percent. Of the people living with HIV, 75 percent are on HAART. A significant number of these will need to undergo orthopaedic surgery at some point in their life that will require use of a tourniquet. Since this is the most active age group predominantly affected by orthopaedic trauma, (Singer et al., 1998), it raises the likelihood of members of this group undergoing orthopaedic surgery with the use of tourniquets.

1.3 HYPOTHESIS

Null hypothesis: The use of a tourniquet during extremity surgery in orthopaedic HIV patients on HAART does not affect their serum lactate levels significantly.

1.4 RESEARCH QUESTION

By how much do lactate levels change post tourniquet use in HIV patients on HAART undergoing extremity surgery compared to those who are HIV negative?

1.5 OBJECTIVE

1.5.1 Main Objective:

To explore the effect of tourniquet use on lactate levels in HIV positive patients on HAART undergoing extremity surgery and how it compares to those who are negative.

1.5.2 Specific objectives:

(i) To determine baseline serum lactate levels in all patients undergoing extremity surgery with use of a tourniquet.

(ii) To determine changes in serum lactate levels in HIV negative patients undergoing extremity surgery with use of a tourniquet.

(iii) To evaluate serum lactate levels changes in HIV patients on HAART undergoing extremity surgery with use of a tourniquet.

1.6 STUDY JUSTIFICATION

The high HIV prevalence in Zambia of 14.3 percent (WHO, 2018) has meant that more people are on HAART. This invariably means an increase in the number of HIV patients on HAART that require extremity surgery with use of a tourniquet. Both HAART and tourniquet use are associated with HL. In turn, HL is associated with increased morbidity and mortality in post-surgical patients. It is therefore important to carry out this study as the use of tourniquets in people on HAART potentially may elevate lactate levels to such an extent as to affect the outcome of the surgery and post-operative recovery. However, there were no studies found in public domain to show whether the use of tourniquets in HIV patients on HAART can result in significant HL with adverse surgical outcomes. It is therefore hoped that the information obtained from this study will help fill this knowledge gap that currently exists in this area. This may enlighten further research work in this and associated area that could be based on the results of this study.

1.7 ORGANISATION OF THESIS

The thesis has been organized into six primary sections forming the main text, with preliminaries preceding and the end matter concluding it.

Firstly, the Introduction presents the statement of the problem, associated research objectives and the justification. The Literature reviewed concerning tourniquets use and HL form the second. The third section shows the Research methodology employed, study setting and sampling, including ethical considerations for the sampled patients.

The Results and analysis that is linked to reviewed literature and defined methodology appear in the fourth. The fifth section discusses the different results for the various aspects affecting hyperlactatemia as outlined in the second section. The sixth chapter presents the conclusions drawn from the various investigations and the related recommendations arising therefrom. These recommendations relate to Academia, the four Hospitals that provided the study setting and source of the patients for the study and the role of the Government, through the relevant ministry tasked with managing health and human related services The study limitations are similarly highlighted based on the practical experiences encountered during the study.

CHAPTER 2 - LITERATURE REVIEW.

Normal lactic acid is a bi-product of anaerobic mechanisms in the body. It is associated with homeostatic mechanisms where tissue buffer systems are working normally and there is adequate tissue oxygenation. Lactic acidosis is defined as failure of homeostatic mechanisms to control serum lactate levels. This results in HL > 5 mmol/l, pH < 7.35, and bicarbonate concentration of < 20mmol/l (Calza et al, 2005). Two types of LA are recognized:

1. Type A, which is associated with clinical evidence of tissue hypoxia.
2. Type B, which is associated without clinical features of tissue hypoxia.

Tourniquet use results in tissue hypoxia / ischemia leading to HL (Estebe et al., 2001) which if not corrected can lead to Type A Lactic acidosis. In Type B Lactic acidosis, there is adequate tissue oxygenation with defective cell utilization of oxygen. This can occur in setting of mitochondrial toxicity e.g. due to drugs such as anti-retroviral therapy (Brinkman et al., 2001).

To better understand the relationship between venous lactate levels with tourniquet and anti-retroviral drug use, the literature review is grouped under the following subheadings:

(i) Biographic details: Age, BMI, Gender effect on lactate levels
(ii) Tourniquet effect on serum lactate levels in general
(iii) Socio-economic effects on lactate:
(iv) Occupation, alcohol consumption and smoking
(v) HAART drugs effect on lactate levels
(vi) Combined tourniquet and HAART drugs effect on lactate levels

2.1 Patient Biographic details: Age, Body Mass Index (BMI), Gender effect on lactate levels

Age results in a change in muscle properties that impair the capacity to produce energy from the glycolytic pathway (Porter et al., 1995). A study by Korhonen et al., (2005) focused on effect of age and sex on glycolytic capacity, which is responsible for lactate production.

The study found that peak lactate following a 100m - 400m sprint showed a curvilinear decline ($p<0.001$-0.05) in both male and female athletes which indicated no sex related differences. It was also noted that there were no age related differences in peak lactate before 70 years.

A number of studies have explored the relationship between BMI and elevated lactate levels. A study by Garavaglia et al., (2013) found an increased risk of elevation of serum lactate with elevated BMI in patients undergoing craniotomy. This study found that the change in lactate over 3 hours correlated with higher BMI ($p=0.010$).

2.2 Socio-economic effect on lactate levels

Low socio-economic status has long been associated with both a higher risk of trauma and increased post injury trauma burden. A study by Brattström et al (2015) of 7,382 trauma patients, found that a low level of education and income plus co-morbidity were all independent risk factors for trauma. A similar study by Abedzadeh-Kalahroudi et al., (2015) found that 49.7 percent of trauma patients received high level of hospital care. Three months post trauma, 64.2 percent of the trauma patients had some level of disability and 71.4 percent reported returning to work. However, the study also found that those with high economic status had high level of hospital care and return to work while those with a low socio-economic status had high instances of disability and death.

Studies have shown that trauma patients have higher levels of lactate when compared to non-trauma patients. Cerovic´ et al., (2003), showed that there was a relationship between trauma and serum lactate. Their study found that of the 98 severely injured patients, a total of 91 had lactate levels elevated above 2.0 mmol/l.

2.3 Alcohol and Smoking effect on Lactate levels

Studies have shown an association between smoking and lactate levels. Hui et al., (1996), conducted a study on effect of smoking acutely before exercise. The study found that there was a higher rate of lactate production during exercise among those who smoked acutely before exercise when compared with chronic smokers with pre-exercise abstinence.

A study by Kreisberg et al., (1971) carried out a study on effect of ethanol (alcohol) on lactate levels. Continuous administration of ethanol was done on eight volunteers and lactate levels noted. The study found that ethanol induced Hyperlactateamia and that this increase was mainly due to decreased lactate disposal than increased lactate production.

2.4 Tourniquet effect on Serum Lactate levels

Serum lactate is a bi-product of anaerobic respiration in red blood cells and in skeletal muscles. The normal value ranges in an un-stressed patient are between 1.0 - 1.5 mmol / l (Pisipati et al., 2013). Elevation of serum lactate can occur in cases where there is tissue ischemia leading to hypoxia. Causes of tissue hypoxia are variable and include any dysfunction at cellular level, in the circulation, or at respiratory level (Kam et al., 2001). Tourniquet use induces tissue ischemia/hypoxia leading to elevation of serum lactate levels. (Estebe et al., 2001).

In 1978, Modig et al., did a study on elderly patients undergoing total knee arthroplasty with use of a tourniquet. The study found that 3 minutes after tourniquet deflation after application for 112+/-15 min, there is associated elevation of lactate levels. The levels peaked at 2 min and remained high 30 min after deflation.

Similarly, Townsend et al., (1996), carried a study on 11 patients undergoing total knee replacement (TKR). A tourniquet was used during the surgeries and was applied for 65 -120 min. Following deflation, tourniquet - induced ischemia resulted in elevation of mean lactate levels from 0.95+/-0.06 mmol/l to 1.85 mmol +/- 0.07mmol/l. It was also found that there was a high correlation between the length of time of tourniquet application and the maximum lactate levels. Baseline levels normalized after one hour. These results are similar to what other researchers found. Kang H.J., et al., (1991), examined effects of tourniquet-induced ischemia on serum lactate levels (SLLs). They examined SLLs post tourniquet - deflation in a group of 20 patients averaging 48-70 years who were undergoing TKR under general anesthesia. Their study found that the SLLs rose from a baseline of 1.93 mmol/l to 2.45 mmol/l two minutes after tourniquet deflation and remained significantly elevated 5min after deflation (2.04 mmol/l).

2.5 HIV/AIDS Effect on serum lactate levels.

HIV infection is associated with hepatic steatosis (Lugassy et al., 2010), pancreatitis (Manfredi et al., 2008), and mitochondrial dysfunction which results in above baseline lactate levels.

This effect is seen in about 2 percent of HIV patients (Arenas-Pinto et al., 2003). However, there is insufficient literature in the public domain to show whether this effect is significant and by how much.

2.6 Effect of HAART on Serum Lactate levels

Antiretroviral drugs (ARVs) can lead to HL. ARVS associated with HL are the nucleoside/nucleotide reverse transcriptase Inhibitors (NRTIs) (John et al., 2001)). As the term suggests, these drugs work by inhibiting HIV-1 Deoxy-ribonucleic acid (DNA) reverse transcriptase enzyme. This enzyme is responsible for the replication of viral DNA. The NRTI acts a competitive inhibitor of the enzyme resulting in the termination of the replication of the viral DNA. However, the same drugs also inhibit mitochondrial DNA polymerase $_{\gamma}$. This enzyme is responsible for the replication of mitochondrial DNA (mtDNA). The inhibition results in the failure of translation of the protein subunits used in the electron transport chain resulting in turn on de-coupling of the oxidative phosphorylation. This in turn leads to mitochondrial toxicity because of accumulation of lactate from pyruvate under anaerobic states (Lugassy et al., 2010). This leads to hyperlactatemia.

Several studies have demonstrated this relationship. Arenas-Pinto et al., (2011) showed that the risk of developing HL due to NRTIs usage was 9 - 16 percent. A similar study by Brinkman (2001) showed that this risk can be between 15 - 35 percent. Some NRTIs such as Stavudine (d4T), Didanosine (ddl) and Zalcitabine (ddC) induce more mtDNA replication inhibition than others (Hocquelox et al., 2003).Their study also showed that various tissues exhibit different specificity. This was dependent on differences in cellular penetration and phosphorylation, in uptake of the different NRTI and their metabolites, and the tissue dependence on mitochondrial metabolism. Length of NRTI use also seems to determine level of HL.

The effect of NRTI on HL was also noted during the pre-HAART era. Boxwell et al., (1999) reported a research by US Food and Drug Administration on adverse drug events on patients in 1998. All the reported adverse events due to ARVs had to do with NRTIs. Out of these, 46 cases were on monotherapy, while 61 were on dual therapy. The monotherapy drug implicated was Zidovudine (ZDV) prior to 1995 and Stavudine after 1995. Gerald et al., (2000) described 14 cases of patients on NRTI over a two-year follow up. All of them developed symptomatic Hyperlactataemia with d4T being the culprit.

The first prospective HIV Cohort study investigating NRTI effect on serum lactate levels was done by John et al., (2001), in Western Australia, involving 516 participants. The study found that seven patients developed NRTI associated lactic acidosis or severe symptomatic HL. The rest had mild asymptomatic HL with d4T being implicated in most of the reported cases. The researchers also concluded that LA can develop in patients with no prior history of asymptomatic HL. A retrospective study by Coghlan et al., (2001) found that there where several cases of LA among patients who had extensive prior exposure to NRTI following a six year period. The ARVs d4T and ddl were implicated as the most offending agent. Then, in 2001, Boubaker et al., (2001), investigated prevalence and risk factors among 880 patients in a Swiss cohort study over a period of one month. It had found that, the risk factors for developing ELL were high with a d4T with or without ddl containing regimen. Other risk factors identified were duration of NRTI use, lipoatrophy, hyperlipidemia and hyperglycemia.

Hocquelox et al., (2002), examined the prevalence, risk factors and outcome of HL in a cohort of 140 HIV patients. Out of the 129 patients included in the analysis, 11 patients (8.5 percent), had HL, and all of them were on NRTIs. These were more likely than controls to receive didanosine (ddl) or stavudine (d4T) (82 percent vs. 19 percent and 82 percent vs. 48 percent respectively). Of the 3 patients who discontinued NRTIs after a 15 month follow up, serum lactate levels returned to normal. However, for the 8 who did not discontinue the NRTI use, SLL returned to normal in only 36 percent (2/8 patients).

2.7 Effect of Tourniquet use on Serum Lactate levels in patients on HAART

No research papers on effect of tourniquet use on serum lactate levels during surgery on HIV patients on HAART were found. However, as highlighted earlier, both the use of tourniquet and HAART individually in patients remarkably affects serum lactate levels.

It is therefore expected that the two when combined can have an additive effect on serum lactate levels. This knowledge gap it is hoped will be filled by this study.

2.8 Effects of serum lactate on Mortality levels

Elevated levels of serum lactate are associated with increased mortality. Khosravani et al., (2009) study showed increased fatality among ICU patients with hyperlactataemia, 20 percent compared with 5 percent for those who did not present with hyperlactateamia. Trauma patients accounted for 25 percent of these fatalities (Calza et al., 2001). A study by McNelis et al., (2001), also showed that there is a relationship between length of time it took for normalization of lactate levels post-operatively and increased mortality. A hundred percent mortality occurred in those whose lactate levels failed to normalize post operatively. The mortality was 42.5 percent for those clearing between 48 - 96 hours, 13.3 percent for lactate clearance between 24-48 hours, and finally 3.9 percent for a clearance of less than 24 hours. Higher mortality levels were recorded in those whose lactate clearance was more than 48 hours.

2.9 Serum lactate Measurements

Current research shows that point-of-care serum lactate levels can be measured in an outpatient setting by minimally trained staff to detect both symptomatic and asymptomatic hyperlactatemia in HIV/AIDS patients on HAART (Chagoma et al., 2013). A Lactate Digital Analyzer was used in the study to determine lactate levels in both HIV positive on HAART and HIV negative patients undergoing orthopaedic surgery with use of a tourniquet.

CHAPTER THREE – STUDY METHODOLOGY

We conducted a cohort study with two groups of orthopaedic patients undergoing extremity surgery with use of a tourniquet. These were:

1. HIV negative patients.
2. HIV positive patients on HAART.

3.1 Inclusion Criteria

The Inclusion criteria considered involved the following:

1. A signed informed consent form (thumb print or signature) by the patient.
2. Aged 18 years and above (18 years being age of consent).
3. HIV negative patients undergoing orthopaedic surgery with use of a tourniquet.
4. HIV positive patients on HAART undergoing extremity surgery with use of a tourniquet.

3.2 Exclusion Criteria

The Exclusion criteria considered involved the following:

1. Refusal of consent
2. Unknown HIV status in patients
3. HIV positive patients not on HAART
4. Co-morbid conditions:
    - Diabetes
    - Renal failure
    - Hyperlipidemia
    - Pregnancy
    - Patients with malignant tumors
    - Patients on chemotherapy and isoniazid.

Patients meeting the eligibility criteria above were identified and conveniently sampled. Signed informed consent was sought from all participants.

3.3 Study Setting

The study was done at four health institutions namely; University Teaching, Hospital (UTH), Levy Mwanawasa Teaching Hospital, St. John Paul II, (also known as the Zambian / Italian Orthopaedic Hospital) and Beit Cure hospital. The two teaching institutions are general public hospitals, while the other two are privately owned and managed.

3.4 Sampling

3.4.1 Size Estimation

Sample size calculation was performed using the EPI info. Statistics calculator that assumed:

(i) Confidence level 95%

(ii) 80% power

(iii) Ratio (Number of Exposed : Number Unexposed) of 1:4

(iv) Expected frequency of disease in unexposed group 10%

(v) Risk ratio 0.10

The following sample size formula was employed.

$$n = \frac{(z_1 + z_2)^2 \times 2p(1-p)}{(p_2 - p_1)^2} \times \frac{c+1}{2c}$$

Where:

Z_1	95% confidence	1.96
Z_2	90% power	1.28
	80% power : $Z_2 = 0.84$ 95% power : $Z_2 = 1.64$	
p_1	Proportion who develop disease in exposed group	0.35
p_2	Proportion who develop disease in unexposed group	0.10
p	Average of p_1 and p_2	0.225
n	Number in the exposed group	37
cn	Number in the unexposed group	146
$n + cn$	Total sample size	183

This gave a sample size of 37 for the exposed (HIV positive and on HAART) and 146 for the unexposed (those who are HIV negative) in each group with a total of 183 participants.

3.4.2 Sample size Assumptions

The assumption for the sample size estimation were got from a study by Brinkman et al., (1999), in which it was reported that lactate increased up to 35 percent if one was HIV positive and on HAART (exposure group). In another study by Cevik et al., (2013) it was found that lactate only increases up to 10 percent (Unexposed group-HIV free) for individuals who are free of HIV.

3.4.3 Sample Collection

Both types of tourniquets - an esmarch bandage and a pneumatic tourniquets were used. Point-of-care serial lactate measurements were done using a digital point of care lactate analyzer machine called *Lactate plus*. A drop of venous blood was collected from either the finger-tip or toe tip of the limb contralateral to the limb from which a tourniquet was applied. The finger-tip or toe-tip to be used was cleaned with soap and normal saline to remove any residual lactate from the skin. A lancet was used to pierce the fingertip or toe tip.

The first drop was wiped away. The second drop was introduced on a lactate strip attached to the digital lactate analyser. This then analysed the lactate amount and gave results in 13 seconds. A control solution was used on each patient to recalibrate the analyser before reusing it on the next patient. Four samples were collected from each patient: one prior to the tourniquet application and three serial measurements after tourniquet deflation at intervals of 2 min, 5 min and 15 min. Thus, both the baseline and serial post-tourniquet deflation serum lactate levels were done. The used test strips and lancets were discarded in a healthcare waste disposal bin after the results were collected.

3.5 Ethical Considerations

Participation in the study was by informed signed consent. No patient identifiers were used. All participants were noted only by age and serial numbers. The study did not interfere with the way the patients were undergoing medical and surgical care at the hospitals where they were admitted. The perceived risks to participation in the study included some minor discomforts to the digits from the micro pricks.

During participation in the study, if any biochemical derangement was noted, it was immediately brought to the attention of the attending physicians where appropriate management was recommended. Furthermore, all those that were identified to have other pathologies during the screening process, got appropriate referrals to the relevant specialties at UTH and Levy Mwanawasa hospitals.

Permission to do the study was got from the management at University Teaching, Levy Mwanawasa, St. John Paul II and Beit Cure Hospitals. Ethical approval was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZABREC).

CHAPTER FOUR - RESULTS AND ANALYSIS

4.1 Biographic characteristics of patients

There were 160 participants involved in this study. One hundred and thirty two (82.5 percent) were male and twenty-eight (17.5 percent) were female, and is outlined in Figure 4.1. The males outnumbered females by a ratio of 3.6 to 1 (i.e. 14:4). The average age of the participants was 35.45 years; with the youngest being 18 years, while the oldest was 67 years old as shown in Table 4.1.

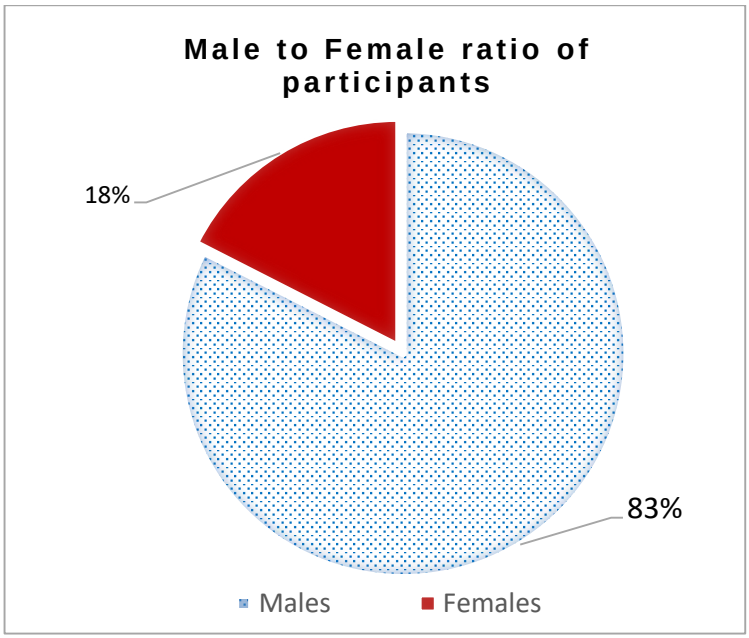


Figure 4.1: Gender distribution of participants

Table 4.1 shows that the average height of the patients was 1.69 metres while the average weight 71.10 kg. The average body mass index was 23.66 kg/m².

Table 4.1: Descriptive Statistics on height and weight of participants

	n	Minimum	Maximum	Mean	Std. Deviation
Age	1604	18	70	35.88	11.8160
Height (metres)	160	1.60	1.80	1.69	0.04104
Weight (kg)	16, 016	54.00	85.00	71.10	7.91558
BMI (kg/m²)	0	21.09	26.23	23.66	7.91558

4.2 Socio-economic history

Table **4.**2 presents findings on patients' socio-economic history regarding occupation, alcohol and smoking habits. These are graphically represented in and Figures 4.2, 4.3 and 4.4, respectively.

Table 4.2: Participants variables of Occupation, Alcohol consumption and Smoking habits

Variable	Values	Frequency	Percentage
Occupation	Manual worker	44	27.5
	Office worker	16	10
	Students	8	5
	Others	92	57.5
Alcohol consumption	Yes	90	56.2
	No	70	43.8
Alcohol Units consumed per day	One	15	16.67
	Two	15	16.67
	Three	15	16.67
	Four	22.5	25
	> more than four	22.5	25
Smoking	Yes	16	10
	No	144	90

4.2.1 Occupation

As displayed in Table 4.2 and Figure 4.2, 27.5% (n = 44) of the participants were manual workers, 10% (n=16) were office workers, 5% (n=8) were students, while 57.5% (n=92) had unspecified type of work.

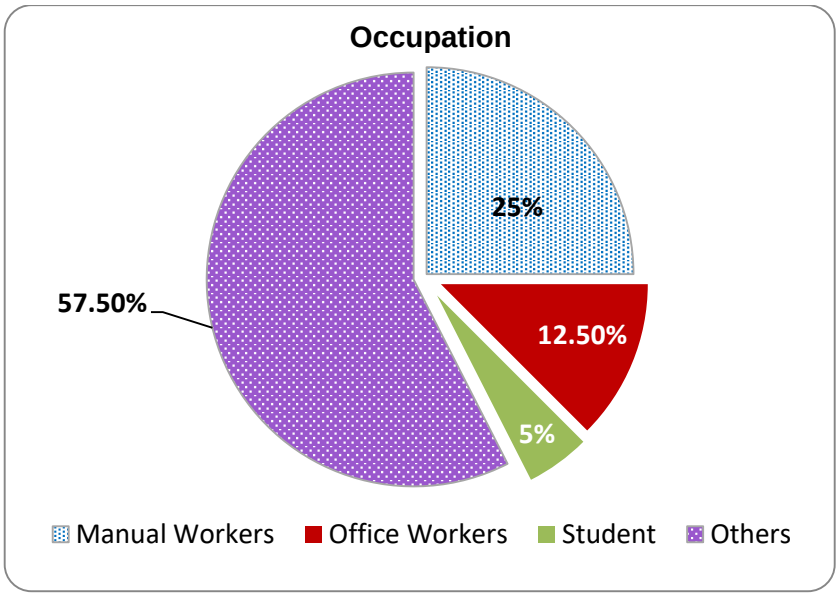


Figure 4.2: Participants Occupation distribution

4.2.2 Alcohol Consumption

As shown in Table 2 above and Figure 3 below, over half of the participants (56.2 percent) consumed alcohol.

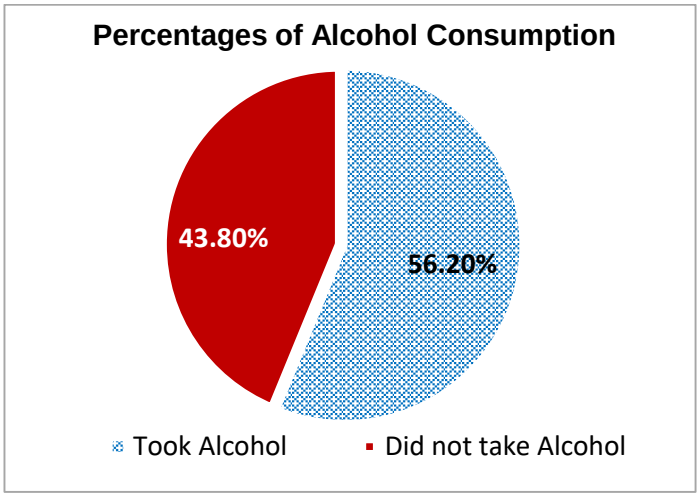


Figure 4.3: Percentages of Alcohol consumption

About 50 percent consumed at least four bottles of alcoholic beverages (beer). Table 4.3 presents the findings on Mann-Whitney U-tests conducted to establish whether there was any significant differences in the average post-operative lactate levels between patients who drank alcohol, and those who did not drink alcohol. The tests were conducted at a significant level of 0.05. The results were not significant at 2 minutes ($p>0.05$), 5 minutes ($p>0.05$), and 15 minutes ($p>0.05$).

4.2.3 Smoking

Table 4.2 and Figure 4.4 presents findings on smoking habits of participants. About 90 percent of participants did not smoke, with only 10 percent being smokers.

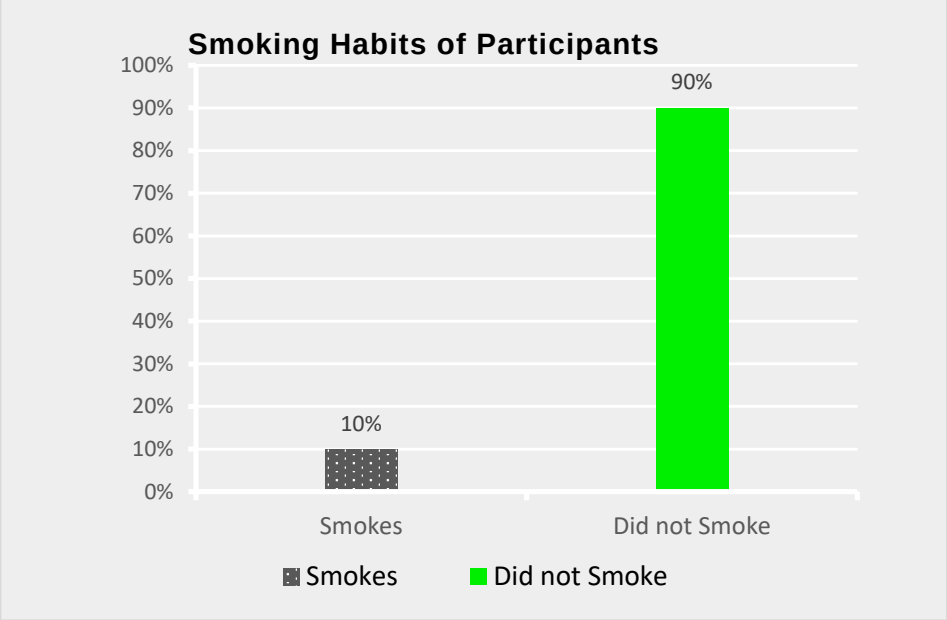


Figure 4.4: Percentage of smoking habits of participants

Table 4.3: Independent Samples T-test Summary results on Alcohol and Smoking

Variable	Post lactate levels	P values
Alcohol	2 min	0.930
	5 min	0.120
	15 min	0.617
Smoking	2 min	0.149
	5 min	0.073
	15 min	0.122

Meanwhile, Table 4.3 also presents the findings on Mann-Whitney U tests conducted to establish whether there was any significant differences in the average post lactate acid levels between patients who smoked and those who did not smoke. The tests were conducted at a significant level of 0.05. The results were not significant at 2 minutes (p=0.93), 5 minutes (p=0.12), and 15 minutes (p=0.617).

4.3 Surgical Operation Types

Figure 4.5 shows the types of operations participants had undergone. Eighty percent (128) participants had undergone plates and screws, 12.5% (20) had undergone IMN, and 7.5 percent (12) had undergone ankles and arthrodesis.

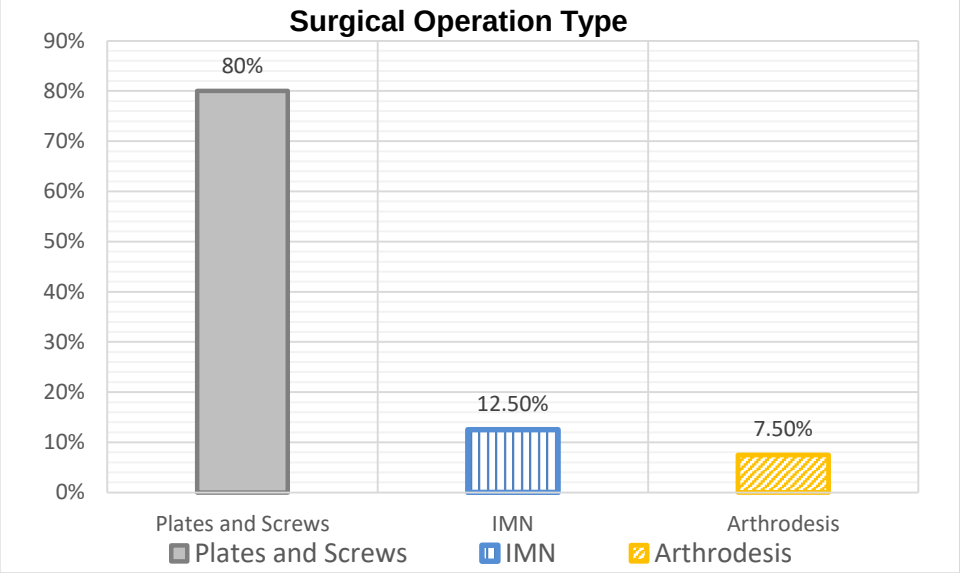


Figure 4.5: Operation types underwent by participants

4.4 Current Surgical History

The surgical history and frequency of the participants is outlined in Table 4.4. Fifteen percent of participants (n=24) had fractures 1-4 weeks old, 17.5 percent participants (n=28) , 5-8 weeks, another 17.5 percent participants, (n=28), 9-12 weeks, another 24 (15 percent), participants had fracture duration of 13-16 weeks, and the last 56 participants (35.0 percent) had fractures more than 16 weeks old.

Table 4.4: Fracture duration

Fracture duration	Frequency	%	Cumulative Percentage
1-4 weeks ago	24	15.0	15
5-8 weeks ago	28	17.5	32.5
9-12 weeks ago	28	17.5	50.0
13-16 weeks ago	24	15.0	65.0
> 16 weeks ago	56	35.0	100
Total	**160**	**100**	

As shown in Figure 4.6, most of the participants (35 percent) had fractures lasting more than 4 months. This means that most fractures operated were old fractures.

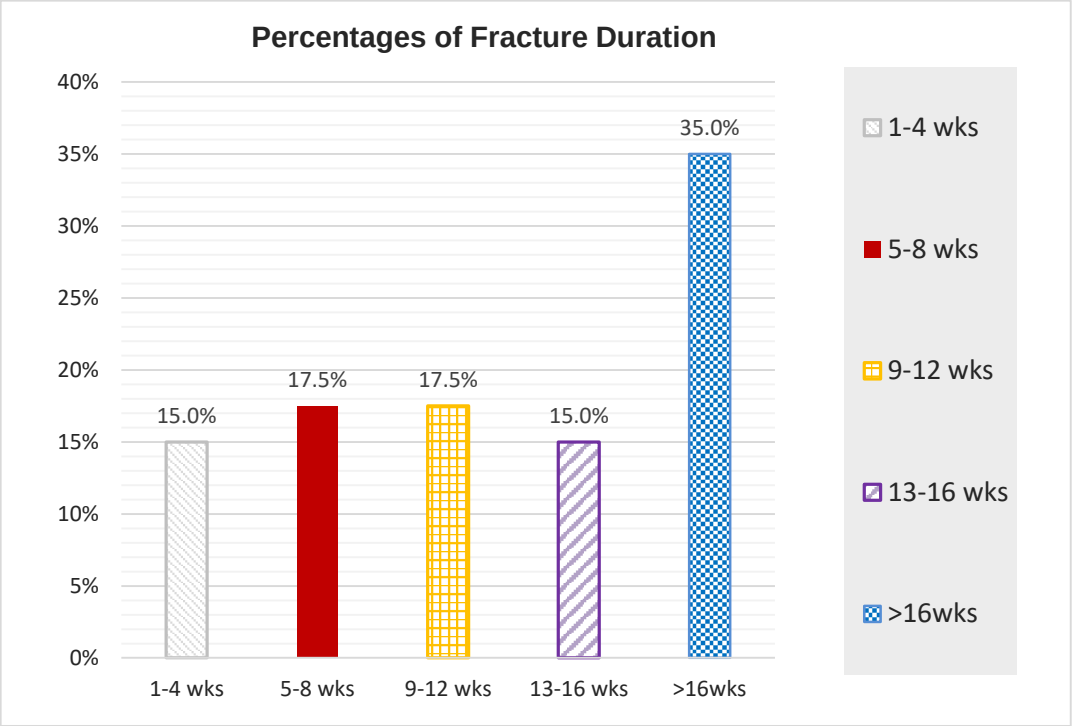


Figure 4.6: Percentages of fracture duration undergone by participants

4.5 HIV Status

Figure 4.7 presents findings on participants regarding their HIV status and other related issues. 124 of the participants (77.5 percent) reported that they were negative, while only 36 (22.5 percent) were positive.

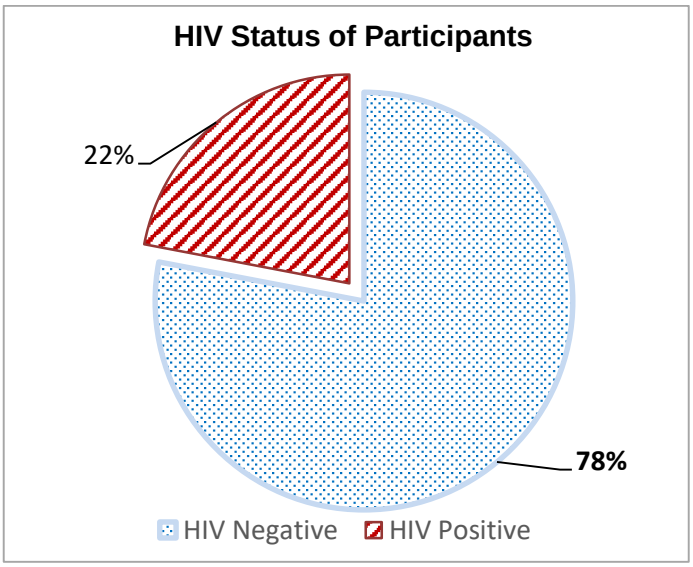


Figure 4.7: HIV status of participants

4.5.1 Duration of HIV Positive Status Knowledge

As shown in in table 4.5 and figure 4.8 below, of the 36 participants who were HIV positive, eight (25 percent) had been positive for less than 6 months, four (12.5 percent) had been positive between 12-24 months, eight (25 percent) had been positive between 24-48 months, while 16 (50 percent) had been positive for more than 48 months.

Table 4.5: Variables of HIV status, Duration of status and HAART uptake

Variable	Values	Frequency	(%)
HIV status	Negative	124	78
	Positive	36	22
Duration of HIV Positive Status Knowledge	< 6 months	8	22
	Between 6-12 months	0	0
	between 12-24 months	4	12
	between 24-48 months	8	22
	more than 48 months	16	44
HAART Uptake	Yes	36	100

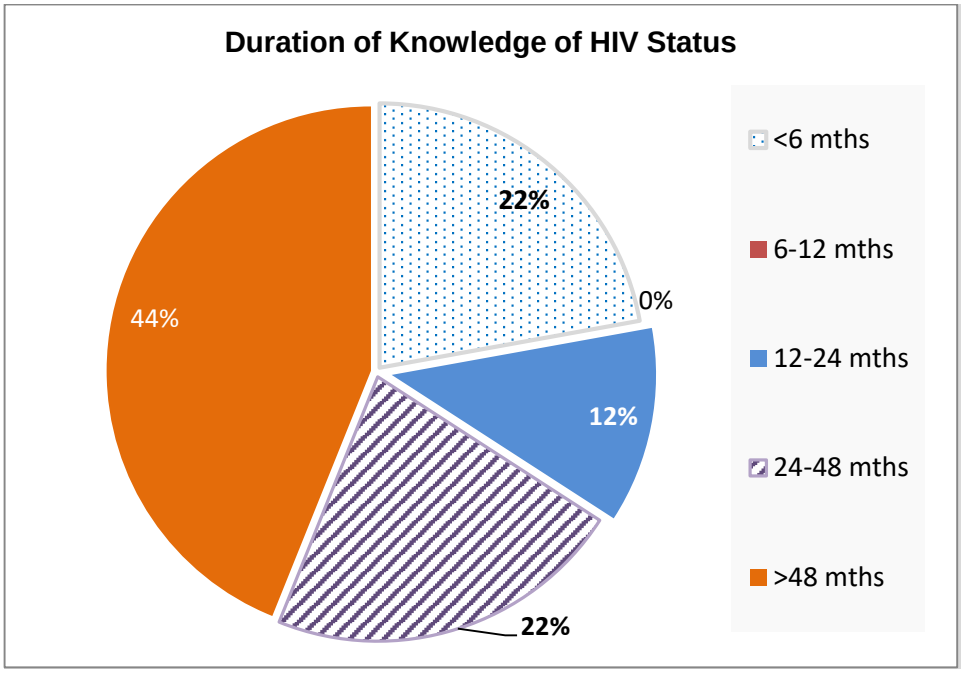


Figure 4.8: Duration of HIV Positive status knowledge of the participants

4.5.2 HAART Uptake Among HIV Positive Participants

Figure 4.9 shows that all the 36 (100%) HIV positive participants were on HAART.

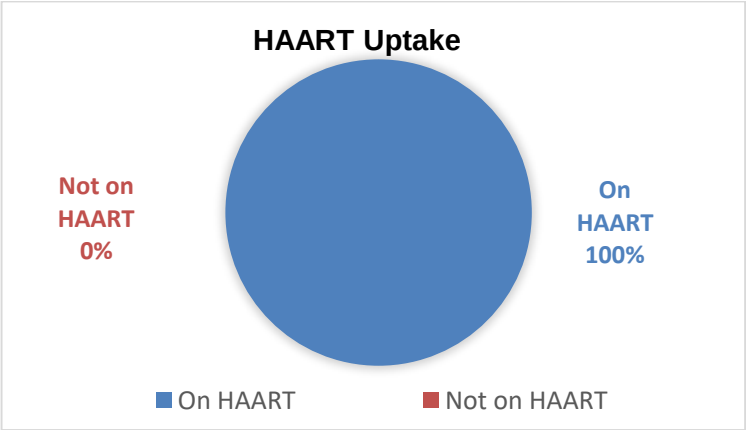


Figure 4.9: Patient HAART Uptake

4.5.3 Types of HAART Regimen

Table 4.6 and Figure 4.10 outlines the different types of HAART regimen undertaken by the HIV positive participants. As shown in Figure 4.10, eight (22 percent) of the HIV positive participants were on TDF/FTC/EFV HAART regimen while 32 (78 percent) were on TDF/3TC/EFV HAART regimen. None of the participants studied were on any other combination regimen.

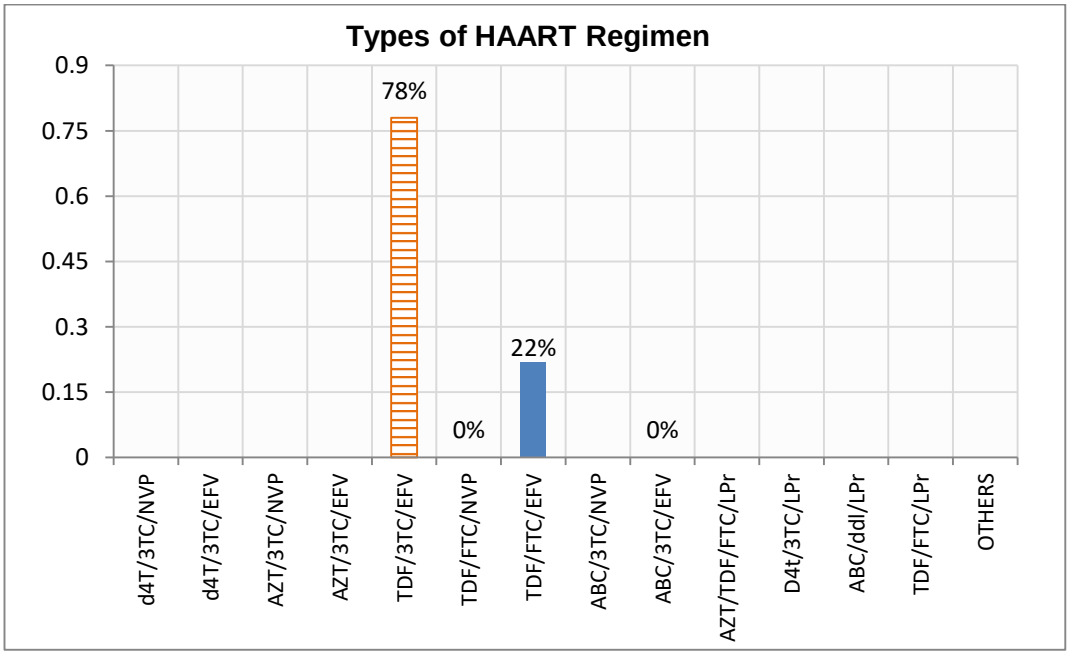


Figure 4.10: Participants HAART regimen types

Table 4.6: Types of HAART the participants were on

	HAART Regimen	Frequency	%
1	Stavudine/ Lamivudine/ Nevirapine	0	0
2	Stavudine/ Lamivudine/ Efavirenz	0	0
3	Zidovudine/ Lamivudine/ Nevirapine	0	0
4	Zidovudine/ Lamivudine/ Efavirenz	0	0
5	Tenofovir/ Lamivudine/ Efavirenz	32	78
6	Tenofovir/ Emtricitabine/ Nevirapine	0	0
7	Tenofovir/ Emtricitabine/ Efavirenz	8	22
8	Abacavir/ Lamivudine/ Nevirapine	0	0
9	Abacavir/ Lamivudine/ Efavirenz	0	0
10	Abacavir/ Zidovudine/ Tenofovir/ Emtricitabine/ Kaletra	0	0

4.5.4 Participants duration on HAART

Concerning HIV positive patients duration on HAART, eight (22 percent) of the participants had been on HAART for less than 6 months, four (11 percent) had been on ARVs for 6-12 months, another four (11 percent) for between 12-24 months. Eight (22 percent) for 24-48 months, and the last 12 (34 percent) for more than 48 months as outlined in figure 4.11.

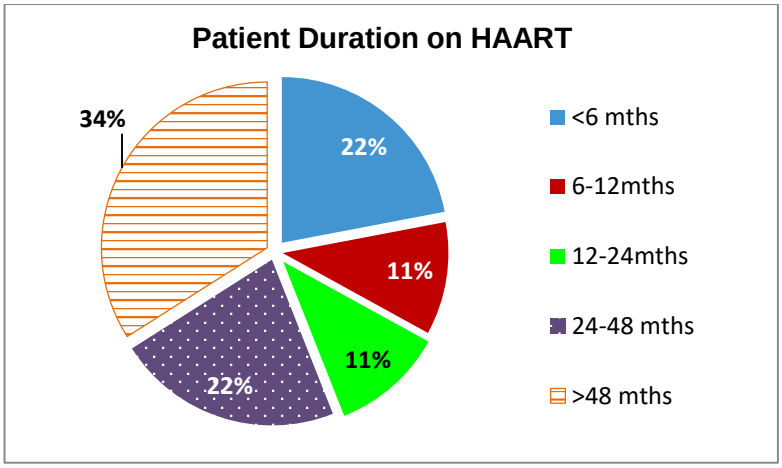


Figure 4.11: Duration of HAART Uptake by participants

In Table 4.7, a summary of the descriptive statistics on pre-operative investigations is shown. The mean pre-operative lactate was 1.39 mmol/l (SD=1.168), with a range of 0-5, the mean Hb was 13.395 g/dl (SD=1.3328), with a range of 8.5 - 15.4 g/dl; and the mean FBS was of 4.055 mmol/l (SD=0.5320), with a range of 2.1 - 5.3.mmol/l.

Table 4.7: Summary of descriptive Statistics on pre-op Lactic acid, Haemoglobin, and FBS

	N	Range	Minimum	Maximum	Mean	Std. Deviation
Pre-op lactate	160	5	0	5	1.390	1.168
Hemoglobin (g/dl)	160	8.5	8.5	15.4	13.395	1.333
FBS (mm01/l)	160	2.1	3.2	5.3	4.055	0.532

Correlation coefficient tests were conducted to establish whether there were any associations between post-op lactate levels among HIV positive participants and age, tourniquet time, BMI, Hb, and FBS at time periods of 2 minutes, 5 minutes and 15 minutes. The results are summarised below in Table 4.8. The results show that in all the circumstances there was a significant positive correlation between lactate levels and age, tourniquet time, BMI, HB, and FBS at time periods of 2 minutes, 5 minutes and 15 minutes. However, these results were not significant ($p>0.05$).

Table 4 8: Correlations between Lactate acid levels at 2 min, 5 min, and 15 min time series with tourniquet time, BMI, HB and FBS

Time (min)	Age (p)	BMI (p)	Hb (p)	FBS (p)
2	0.345	0.256	-0.011	0.101
	0.363	0.506	0.979	0.796
	36	36	9	9
5	0.384	0.386	-0.196	0.218
	0.308	0.305	0.613	0.573
	36	36	9	9
15	0.511	-0.115	0.339	-0.193
	0.159	0.768	0.372	0.618
	36	36	9	9

4.6 Post-operative Lactate before and after Tourniquet use

Figure 4.12 shows that 72 participants (45 percent) had tourniquet time ranging from 1-2 hours, and 88 participants (55 percent) had tourniquet time ranging from 2-3 hours. The average post tourniquet time was 2.05 hours.

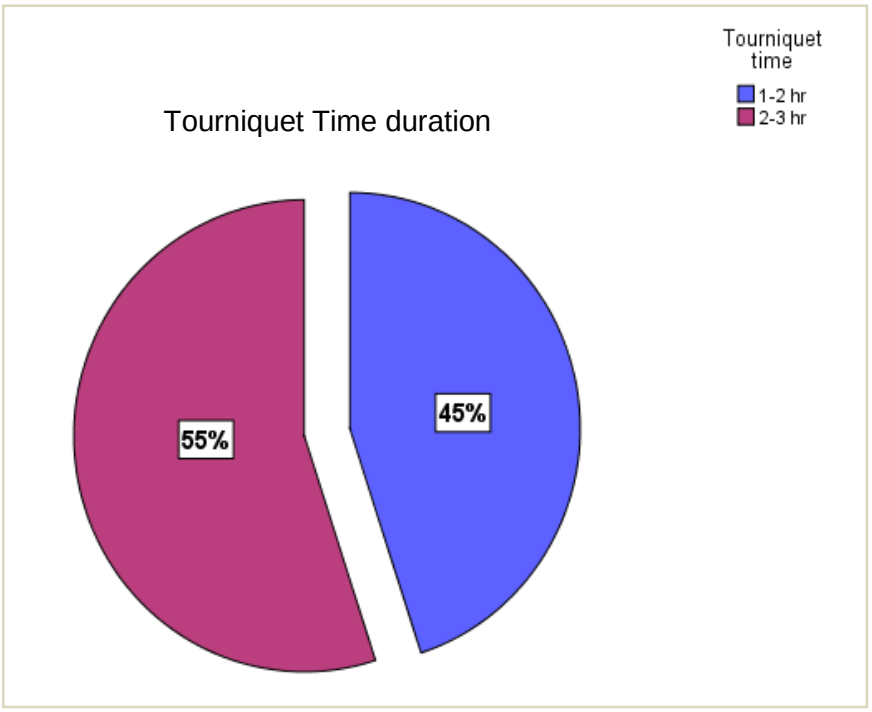


Figure 4.12: Tourniquet time duration

Further analyses were conducted to establish whether there were any significant statistical differences in the lactate acid levels of the HIV on HAART patients and tourniquet time. 45 percent of the patients (n=72) had tourniquet ranging from 1 to 2 hrs while 55 percent patients (n= 88) had tourniquet ranging from 2 to 3 hrs.). To achieve this an independent samples T-test were conducted at a significant level of 0.05. These results are presented in Table 4.9.

Table 4 9: Independent Samples t test statistics results

	Tourniquet time	N	Mean	Std. Deviation	t	df	p value
2 min	1-2 hr	72	2.540	1.3094	-0.190	38	0.850
	2-3 hr	88	2.616	1.2135			
5 min	1-2 hr	72	1.958	1.0504	-0.188	38	0.660
	2-3 hr	88	2.098	0.9484			
15 min	1-2 hr	72	1.487	0.7995	-0.556	38	0.581
	2-3 hr	88	1.635	0.8646			

The results were not significant at all the time periods, i.e. 2 minutes (t = -0.190; df = 38; p=0.850), 5 minutes (t = -0.188; df=38; p = 0.660), and at 15 minutes (t = -0.556; df=38; p=0.581). These results show that lactate levels were not affected by tourniquet time.

Further analyses were conducted to establish whether there were any significant statistical differences in the lactate levels of the patients before and after tourniquet use. To achieve this paired samples T-tests were conducted at a significant level of 0.05. The results are shown in Table 4.10.

Table 4.10 : Paired Samples Statistics results

Paired sample t-Tests		Mean	N	SD	t	df	p-value
Pair 1	Pre-op lactic acid	1.390	160	1.1680	-5.756	39	0.001
	2 min	2.582	160	1.2417			
Pair 2	Pre-op lactic acid	1.390	160	1.1680	-3.562	39	0.001
	5 min	2.035	160	0.9850			
Pair 3	Pre-op lactic acid	1.390	160	1.1680	-0.954	39	0.346
	15 min	1.569	160	0.8287			

The results were significant at 2 minutes (t = -5.756; df = 39; p=0.001), and at 5 minutes (t = -3.562; df=39; p=0.001). These results show that lactate levels were higher at 2 and 5 minutes time periods than at preoperative time period. However, the results were not significant at 15 minutes (t = - 0.954; df = 39; p=0.346).

Figure 4.13 presents descriptive statistics of serum lactate levels at post-tourniquet time series of 2 min, 5 min, and 15 min. The results show a reduction in serum lactate levels with an average of 2.582 at 2 min, 2.035 at 5 min, and 1.568 at 15 min.

Paired samples T-test results showed that there were significant differences in the serum lactate levels between 2 min and 5 min [t (40) = 10.431; df = 39; p=0.001], between 5 min and 15 min [t (40) = 7.077; df = 39; p=0.001], 2 min and 15 min [t (40) = 10.364; df = 39; p=0.001].

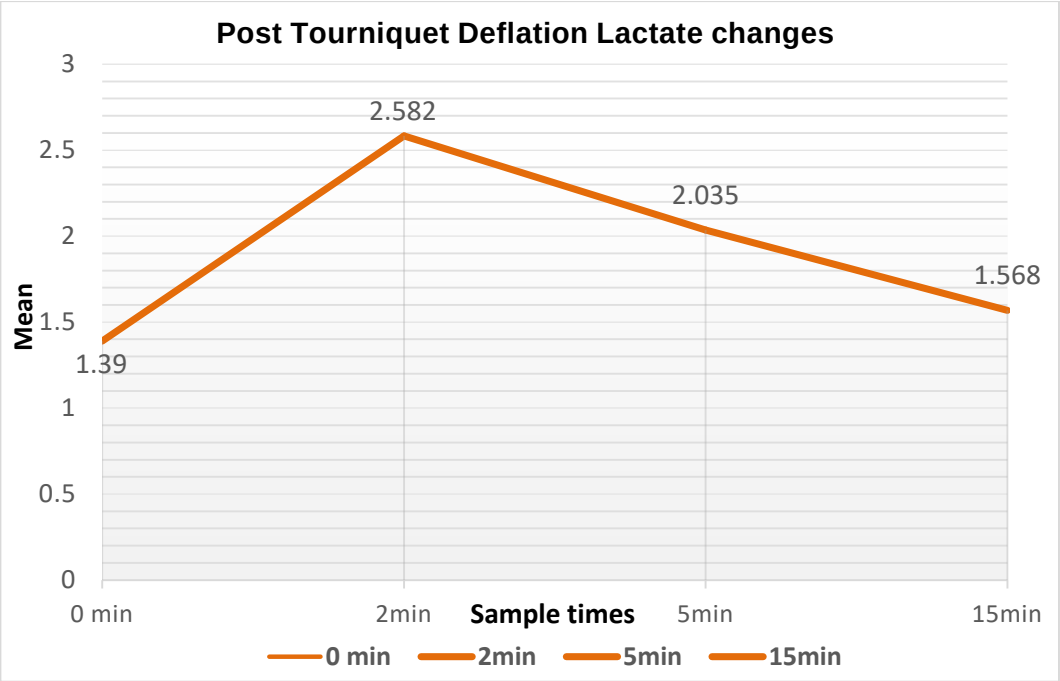


Figure 4.13: Lactate changes with time post-tourniquet deflation

4.7 Post-operative Lactate levels in relation to HAART

Figure 4.14 and 4.15 shows the distribution of post tourniquet deflation lactate levels in relation to HIV status.

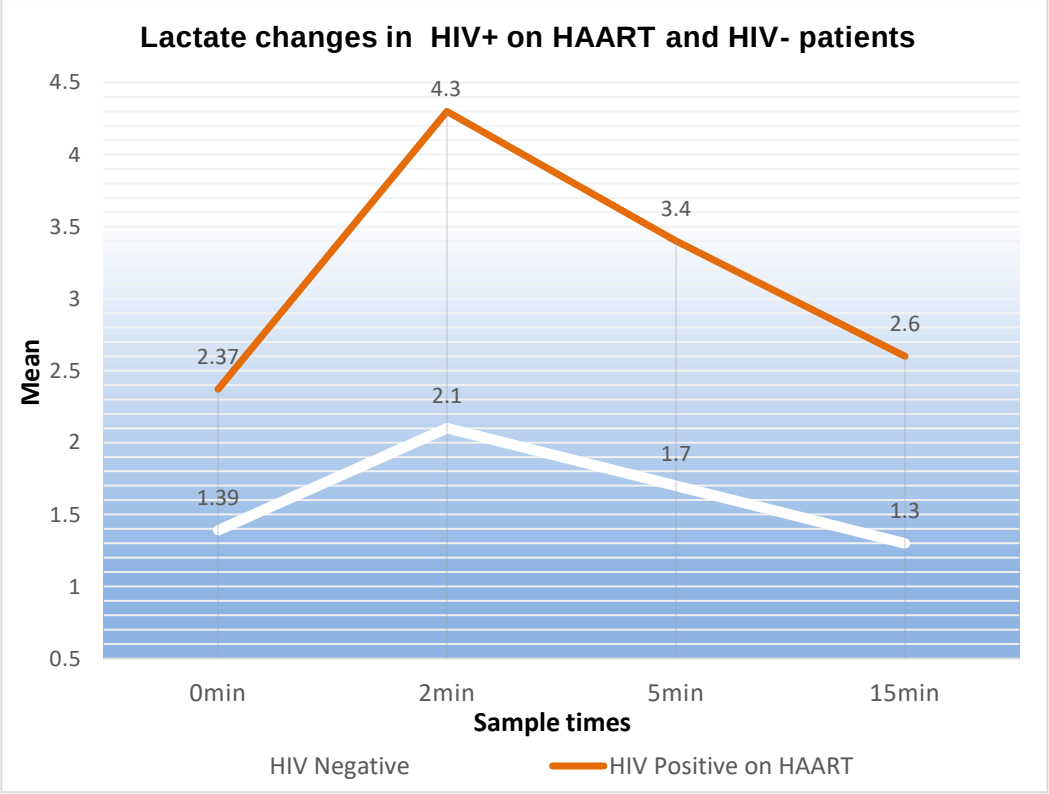


Figure 4.14: Lactate changes in both HIV positive on HAART and HIV negative participants.

The results show that post tourniquet deflation lactate levels were higher among HIV positive on HAART participants at 2, 5, and 15 minute time periods than among the HIV negative participants. The results from an independent samples T-test indicated that at 2 minutes, HIV positive on HAART participants (M = 4.328, SD = 0.8397, N = 36) had much higher post tourniquet deflation lactate levels than HIV negative participants (M = 2.084, SD =0.8240, N = 124), t (36) = -7.099, p <0.001, two-tailed.

At 5 minutes the results from the independent samples t test revealed that HIV positive on HAART participants (M = 3.423, SD = 0.6548, N = 36) had much higher post tourniquet deflation lactate levels than HIV negative participants (M = 1.655, SD = 0.6468, N =124), t (36) = -7.146, p <0.001, two-tailed.

Similarly, the results from an independent samples t test indicated that at 15 minutes revealed HIV positive on HAART participants (M =2.594, SD = 0.8750, N = 36), had much higher post tourniquet deflation lactate levels than HIV negative participants (M=1.294, SD=0.5401, N=124), t(36)=-4.215, p<0.002, two-tailed.

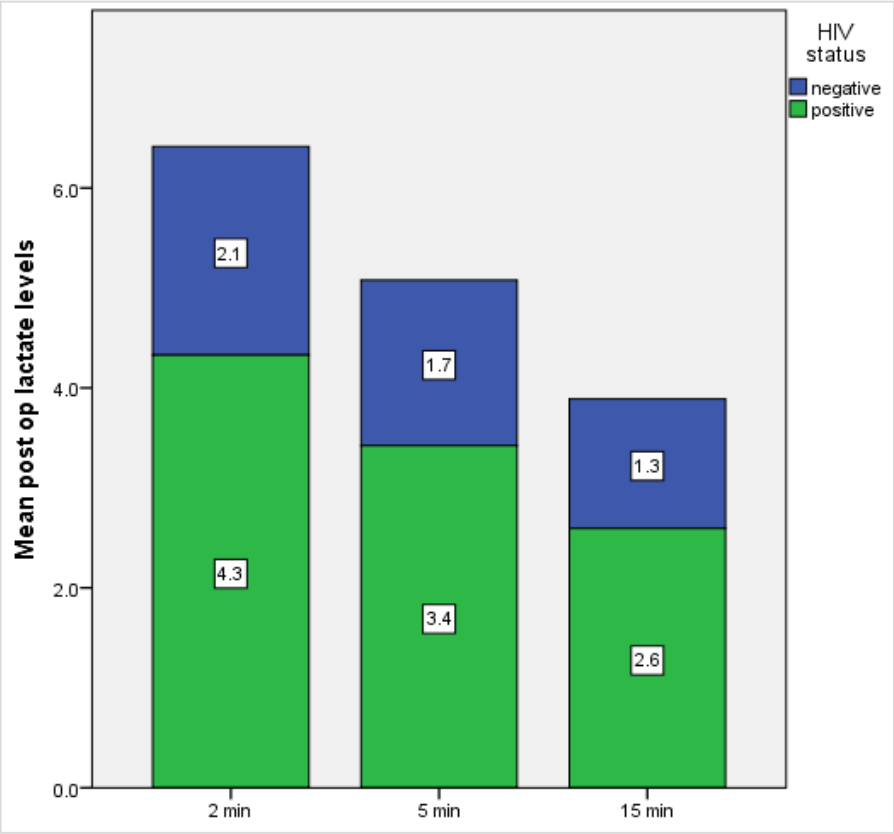


Figure 4.15 Post-op lactate acid levels in HIV Positive on HAART and HIV Negative

Further analyses were conducted to establish whether there were any significant statistical differences in the lactate levels of the patients on HAART before and after tourniquet use. To achieve this Wilcoxon Signed Ranks Test were conducted at a significant level of 0.05. Table 4.11 presents the results. The results were significant only at 2 minutes (Z = -2.023; p = 0.043) time period. These results show that lactate levels were significantly higher at 2-minute period than at the pre-operative time period. However, lactate levels were not significantly higher at 5 minutes (Z = -1.153; p = 0.249) and 15 minutes (Z = -.734; p = .463) time periods.

Table 4.11: Wilcoxon Signed Ranks Test statistics[a]

Test Statistics[a]			
	2 min – pre-op lactic acid	5 min – pre-op lactic acid	15 min – pre-op lactic acid
Z	-2.023[b]	-1.153[b]	-0.734[c]
Asymp. Sig. (2-tailed)	0.043	0.249	0.463
a - Wilcoxon Signed Ranks Test			
b - Based on negative ranks.			
c - Based on positive ranks.			

CHAPTER FIVE – DISCUSSION OF RESULTS

5.1 Demographic characteristics of participants

A total of 160 participants undergoing extremity surgery with use of a tourniquet were recruited. This was out of 2,243 orthopaedic trauma patients seen at four hospitals in the same time period at casualty. The majority of the orthopaedic participants enrolled in the study were males. This finding is similar to what Singer et al., (1998) found in a study at UTH that, the burden of trauma affected more males than females by a ratio of 2.9:1. Another study done at UTH by Seindenberg et al.,(2014), found that trauma victims presenting to Casualty department were primarily male (71.8 percent). A study in Uganda by Okello et al., (2014), found similar results with a male to female ratio of 4.1:1.

Participants averaged 35.45 years, the oldest patient was 67 years and the youngest 18 years. These figures fall within Central Statistics figures for Zambia (CSO,2018) which showed that 50.6% of Zambians are between 15-65 years. Singer et al (1998) also found that the burden of trauma in Zambia affects primarily this age group. This is because this is the most productive and hence the most mobile group. Another study by Okello et al (2014) also showed that the median age for trauma was 28.

There were no age related differences in lactate levels noted in the study ($p=0.345$ at 2 min, $p=0.384$ at 5 min, and $p=0.511$ at 15 min). This finding is similar to a study done by Korhonen et al., (2005) which showed that up to 75 years, there were no age related differences in lactate levels. This is because increased age leads to a loss of muscle mass with a resultant increase in oxidative capacity and a reduced ability to form lactate. (Porter et al., 1995). It is also thought that there is a gradual decrease in key glycolytic enzymes responsible for lactate production such as phosphofructokinase (Hunter et al., (2002), Key-Evans et al., 1992).

The BMI mean in the study of 24.4kg/m² are similar to other Southern African countries such as South Africa with average height of 1.68 meters and average weight of 70.4 kg (BMI 25.1) while Democratic Republic of Congo has similar results with height of 68 metres in male and weight of 60.4 kg (BMI of 21.8) (Worlddata.info).

BMI effect on lactate levels was not significant in the study as p levels were not significant at all times (p=0.256 at 2 min, 0.386 at 5 min and -0.115 at 15 min). The results in this study are different from those found in other studies. In Cavaglia et al., (2013), it was found that change in lactate over 3 hours in patients undergoing craniotomy correlated with BMI (p=0.010). However, this study only involved 18 participants where as in this research, 160 participants were sampled. In addition, patient selection, type of surgery and other variables were different in the two studies.

5.2 Socio-economics status

5.2.1 Occupation

More than 50 percent of the participants were involved in unspecified work. It was difficult to stratify income levels based on the parameters used. However, if this is taken as a measure of employment status, the results are slightly higher than the national unemployment rate of 41.2% (CSO 2018). This is a consequence of the closed group of participants who were involved in some form of orthopaedic trauma that needed surgery, and not representative of the society as a whole. Income as a measure of the socio-economic status as evidenced by employment status is a well-known risk factor for trauma as well as morbidity and mortality. Brattström et al., (2015) showed that there is a strong risk between socio-economic status and risk of trauma and outcome. Participants with a low socio-economic status had increased risks in life, vulnerability and poor trauma outcomes than those with a higher socio-economic status.

5.2.2 Smoking effect on Lactate levels

In this study, a smoking prevalence of 10 percent of the participants was reported. This is similar to a study done by WHO (2018), which found a smoking prevalence of 14 percent. However, an earlier study by Brathwaite et al (2014) found a smoking prevalence of 1.8 percent in Zambia. The difference in smoking prevalence between the two studies could be due to the sample sizes involved. The study by Braithwaite involved 2,093 participants while this study only involved 160 participants.

As indicated earlier, 10 percent of participants smoked. However, smoking had no effect on lactate levels both before and after tourniquet deflation as p-values were not significant at 2 min (p = 0.93), 5 min (p = 0.12), and at 15 min (p=0.617). This result differs from that by Sørensen et al., (2009), which found increased levels of lactate in the 8 participants in their study. The difference between the two studies are that in the Sørensen study, there was an acute infusion of nicotine into the participants whereas in our study the results were based on previous exposure to nicotine containing smoke. This study also had more participants than the former.

There is also a larger male preponderance in this study (100 percent) compared to the Braithwaite study where male to female ratio was (18.1:8). The Braithwaite study was based on smoking prevalence in the society whereas this study was a hospital orthopaedic trauma based study.

5.2.3 Effect of Alcohol consumption on Lactate levels

In this study, an association between alcohol consumption and lactate was noted. Fifty-six percent (n=90) took alcohol. This finding is within the expected for the country where according to ZDHS (2002) the national prevalence of alcohol consumption was 49.5 percent. In relation to the African region, this is very high as the percentage of current drinkers throughout the region stand at 32.2 percent (WHO, 2018). The number of units of alcohol consumed in the study was at least 4 pure units of alcohol. This finding is similar to what was found by WHO (2018) which found that Zambians consume on average 4 units of pure alcohol per capita per day. The same study also found this national consumption average was lower than the global average of 6.0 pure alcohol percentage. The values found in this study were lower than the world averages. However, the study findings are still higher when compared to an earlier study done by Harworth et al., (2001) which found estimated unrecorded alcohol consumption of 1.0 litre pure alcohol per capita Zambian population older than 15 for the years after 1995.

In this study, there was no significant relationship between alcohol consumption and lactate levels ($p > 0.05$). The findings are different from other studies.

In a study by Dezman et al., (2017) of 3,910 acutely injured patients, 42.8 percent of these had a positive blood alcohol. It was also noted that they had a lower average lactate clearance than the sober group (37.8 versus 47.8 percent). The lactate clearance decreased the higher the positive blood alcohol. Yang et al., (2016) also found an elevated mean lactate of 15.9 mmol/l in the 23 patients involved in their study, which aimed to determine the influence of alcohol on blood lactate levels. Of these 71.4 percent had a positive serum alcohol level. However, the *p* value was not determined hence the significance of their finding in relation to alcohol versus lactate was not elucidated.

The difference between the findings in this research and the one by Dezman et al., and Yang et al., is that in this study, all the patients were not acutely injured.

They had been on the ward in admission for some days with the majority having fractures more than 12 weeks old. None of our patients operated on had taken any alcohol 24 hours prior to surgery as they were nil per oral in preparation for the upcoming surgery.

5.3 Effect of Haemoglobin, Fasting, Blood Glucose on Lactate levels

In the study, there was no correlation between hemoglobin levels, fasting blood sugars and lactate. The findings are different from a study of Intensive Care Unit (ICU) patients by Nunci et al., (2013), which found elevated lactate (2.4 mmol/l) in patients with Hb less than 8g/dl (56%,n=33) while the lactate was less than 2.4mmol/l in patients with an Hb more than 8 g/dl (44 percent, n=26). The explanation for these differences is that, in this research study, all patients who went for surgery were fairly healthy individuals (except for the fractured limb) with Hb more than 8 g/dl and with no comorbities unlike those in the Nunci study which had other comorbities which could have contributed to the lactate changes. In addition, these patients did not undergo any orthopaedic surgery with use of a tourniquet.

With regard to association between lactate and blood glucose, a study by Adelsmay et al., (2012), found different results. A cohort study of 1,170 ICU patients found that blood glucose variability had influence on lactate (p=0.0001).

The differences in the two studies is that one involved healthy patients while the other involved acutely ill patients hence accounting for different findings.

5.4 Tourniquet effect on Lactate levels

In the study, a significant correlation between tourniquet use and lactate was found at 2 min (p=0.001) and 5 min (p=0.001) but not at 15 min (p=0.346). This finding is similar to what other studies have found. Townsend et al (1996) found in a study of 11 patients undergoing TKR, that lactate levels were elevated post tourniquet deflation (0.95+/-0.06 mmol/l to 1.85 mmol+/- 0.07mmol/l), with maximal results seen at 2 minutes. In our study, this was.2 min.

However, the baseline lactate levels returned closer to normal at 15 min than in other studies. In Townsend et al., (1996), at 30 min, baseline lactate results had not normalized by 30 min.

The explanation for this difference is that in the study, unlike in Townsend et al, the patients' age was young (35-45) with no comorbidities whereas in the latter, the patients were older.

5.5 Tourniquet Duration effect on Lactate levels

In the study, tourniquet duration averaged 2.05 hours. This result was different with other studies. In a meta-analysis study of 872 patients who underwent knee arthroplasties, Rama et al., (2007) found that tourniquet time averaged 69.3 minutes. As noted earlier, this study was a cohort study involving only 160 patients whereas the Rama study was bigger and lasted longer and it was a meta-analysis. In addition, the type of operation and the location where these two studies performed were different.

There was no correlation between the tourniquet duration time and the lactate levels in the study. However, other studies have noted different results. Townsend et al., (1996) found that higher tourniquet duration time correlated with maximal lactate levels.

5.6 HAART program

5.6.1 HAART effect on Lactate levels

In the study, HIV positive patients on HAART had higher pre-operative lactate levels than those who were HIV negative especially at 2 and 5 min ($p<0.05$). This finding is similar to what other studies have found. Ter Hosftede et al., (2003) found elevated lactate in 34 percent of HIV positive patients on HAART. In their study, 6 out of 49 HIV positive patients on HAART had lactate on the upper limit of normal without any symptoms. This is similar to the findings in the study where, despite having no obvious symptoms, all the HIV positive patients on HAART had overt elevated lactate pre-operatively (Boubaker et al., 2001). There was a 100 percent elevation in lactate in all the HIV positive patients on HAART in the study.

This was due to patients' selection. The study was a closed group of orthopaedic patients unlike in the Hosftede study, which was open to all HIV positive patients as long as they were on HAART.

Post tourniquet deflation lactate levels were elevated in all HIV positive patients on HAART. The lactate elevation effect was at present at all time periods of 2 min, 5 min ($p=0.01$) and at 15 minutes ($p=0.02$). This is expected as HAART drugs (Boubaker et al., 2001) and tourniquet use (Estebe et al., 2011) elevate lactate levels individually. What is noteworthy in this study however is that, this effect is additive. This finding is significant as no other studies were found for comparison. However, on using Wilcoxon Signed Rank Tests, it was found that though the effect of both was additive elevated lactate levels at all time periods, the elevation was only significant post tourniquet deflation at 2 min ($p<0.05$) but not at 5 min and 15 min ($p>0.05$). There were no comparative studies found to compare with this finding. Therefore, in this study, it was found that though lactate elevation is marked after tourniquet use in HIV positive patients on HAART, the long-term effect of this elevation is not significant. This could be due to the small sample size involved.

Large sample sizes are needed in order for the tests and the results to be statistically significant. There are no previous studies published to compare with regarding these findings.

5.6.2 HAART coverage

Among HIV positive patients on HAART, it was found that the HAART coverage was 100%. This compares favorably with the national policy of test and treat.

5.6.3 Types of HAART regimen

In the study, it was found that the majority of patients (78 percent) were on the TDF/3TC/EFV combination regimen, whereas the rest were on TDF/FTC/EFV This is in line with national guidelines on first line ART in Zambia (Zambia Consolidated ART Guidelines 2018).

5.7 Lactate levels versus HAART duration

In this study, no relationship between the duration of HAART and the tourniquet effect on SLL ($p>0.05$) was found. This indicates that duration of HAART may not affect serum lactate levels in surgical patients.

However, other studies have shown that the risk of developing hyperlactatemia is dependent on duration of exposure (International AID Research Group, AIDS 2007). In the case of HAART drugs such di-deoxy nucleosides, the association become less strong the longer the duration of exposure.

Additionally, other studies have shown that longer exposure to stavudine and didanosine are associated with hyperlactatemia (Carr et al, AIDS 2000). In this study, no distinction was made between the types of HAART used and the lactate levels obtained. This was due to the small numbers of the sample size involved especially the number of HIV/AIDS patients on HAART.

CHAPTER SIX – CONCLUSION, RECOMMENDATIONS AND STUDY LIMITATIONS

6.1 Conclusion

Patients admitted in the study were recruited from University Teaching, Levy Mwanawasa, St, John Paul II and Beit Cure hospitals. The patients recruited had an average age of 35 years with more males than females recruited. Majority of participants had unspecified occupations, took alcohol but there were very few smokers.

Majority of fractures operated were old fractures with plates and screws, the commonest operation done.

Patients who were HIV positive were also on HAART with many having been on HAART for at least 1 year. Commonest HAART regimen was TDF/3TC/EFV.

Pre-operative lactate levels were determined in all patient groups. Comparatively, HIV positive patients on HAART had higher pre-operative lactate levels (mean = 2.5 mmol/l) than patients who were HIV negative (mean=1.4mmol/l).

Tourniquets use elevated lactate levels in all patient groups. Comparatively, HIV positive patients on HAART had higher lactate levels post tourniquet deflation (mean=2.6mm0/l) than their HIV negative counterparts (mean =1.3 mmol/l). These elevations were more significant immediately after tourniquet deflation and remained elevated throughout the study. According to Wilcoxon Signed Rank Tests, post tourniquet deflation lactate levels were only significant at 2 min post tourniquet deflation ($p = 0.04$) but not at other time periods ($p>0.05$ at 5 min and15 min). This indicates that tourniquets can be used safely in HIV positive patients on HAART without fear of causing dramatic and dangerous elevations in lactate levels.

Post deflation lactate levels were not affected by age, sex, BMI, occupation, smoking, alcohol consumption, fracture duration, type of operation and tourniquet duration.

6.2 Recommendations

The recommendations proposed have been divided in three groups:

(i) Academia

(ii) University Teaching and Levy Mwanawasa Hospitals

(iii) Government

6.2.1 Academia

1. There is need to do a large prospective cohort study to determine whether concurrent use of tourniquets in HIV positive patients on HAART is significant. The numbers of HIV positive patients co-opted in the study were few to make any long-term conclusions.
2. There is need for more support to post-graduate students as respects research. Even though postgraduate training is a self-directed, it is difficult to formulate research topics, write proposals and conduct research without adequate support in terms of financial and other associated research and logistical support.
3. It is anticipated that the research findings in this and other studies be built on and recommendations implemented so that an expanded body of knowledge and insight on the topic is achieved, so that other researchers can build on these results.

6.2.2 The Hospitals

1. There is need for proper preparation of surgical patients. Often, results of Hb, FBS, weight, height were not available and the researcher had to follow up these at personal cost.
2. In this study setting, orthopaedic implants need to be sourced by hospital management as most patients are too poor to afford the cost of the implants. Many a time, lots of co-opted patients in the study were cancelled because patients could not afford to pay for the implants.

6.2.3 The Government

1. There is need to support the hospitals offering orthopaedic surgery with proper orthopaedic sets and implants. Orthopaedics is an expensive branch of surgery to leave the costs in underfunded hospital management to bear the costs.

Unless the central government takes up this responsibility, hospitals will have to decide between using the same resources in buying essential medicines to cater for hundreds of patients or buy one implant to cater for one study patient. This might require creating a revolving fund.

2. The Government through the Health ministry should work even more closely with the University of Zambia, other universities and related stakeholders so that the results of the research done by the postgraduate students can be harnessed to promote sustainable national development in our country, that contributes to achieving good health for all.

6.3 Study Limitations

The following limitations were experienced during the undertaking of this study.

6.3.1 Sample size

The study sample size was only partially adequate. The study findings could have been strengthened by a large sample size. This could have provided enough participants to help provide sufficient data regarding HIV positive patients on HAART concerning HAART duration and type of HAART regimen. These variables could have been analysed versus lactate changes. It could have provided enough data as to whether HAART duration could affect lactate levels. Similarly, types of HAART regimen could have been analysed against lactate change if a large number of participants (more than 36) was available. The different types of regimens could have provided more details on lactate changes with the different regimens available. In the study, only two of the possible 12 regimen types was analysed. A larger sample size would also have provided more data on the effect of hemoglobin, BMI, smoking, alcohol and tourniquet duration on SLL's.

Other studies have clearly shown an association between these variables with tourniquet elevation. Their sample sizes were larger. It is therefore hoped that a larger sample size would help show if there is any association between the aforementioned variables and the lactate levels.

6.3.2 Tourniquet Duration

The study stratified time in increments of 60 minutes. This could have contributed toward insignificant lactate changes.

Stratifying tourniquet time according to the exact tourniquet time could yield better information that will capture the micro-lactate changes and their significance. Increasing the sample size can also help to improve detection of these lactate changes.

6.3.3 Duration of study

The study duration was short (one year). To get more participants, a longer period - say five years, could have provided more participants for enrolment in the study. This would have allowed for the capture of more HIV positive patients on HAART. However, due to the postgraduate time limitations and financial outlays, this could not happen.

A longer time could also have allowed for the extrapolation of the study to include HIV positive patients not on HAART. Having this group could have helped to explore as to whether the lactate elevation noted in HIV positive patients on HAART was due to the HAART drugs alone or there was an association with the HIV disease also as literature seem to suggest.

6.3.4 Short follow-up for each patient

The follow-up time was very short for the study (15min). Although a downward trend in lactate levels was noted with increased time from tourniquet deflation, the lactate values during the period of the study did not return to normal. It would be very informative to deduce how long it takes for normalization of lactate levels against the various variables analysed. This would require more follow-up time.

6.3.5 Financial Limitations.

The study was wholly financed from personal resources with some help from Professor Jellies and LORET and other well-wishers including family and friends. The finances thus resourced barely covered the cost of the study. This necessitated the curtailing of number of lactate tests that could be done on each patient.

Each patient on average consumed 6 - 8 test strips, taking into consideration, control tests and false positives at a cost of between $US 12 - $16.

6.3.6 Lack of Orthopaedic Implants

Many patients enrolled in the study dropped off because there were no implants available for their operation. This was partly due to competing demands within hospitals for other essential medicines and requirements and the general challenging low economic status of most patients / participants. This created a high turnover of enrolled participants, making it difficult to acquire adequate numbers within the study time.

REFERENCES

Arenas-Pinto A, Grant A, Edwards S, Weller D. (2003*)* Lactic Acidosis in HIV Infected Patients: A Systematic Review of Published Cases. Journal of Sexually Transmitted Infections, 79:340–344.
Online access: http://sti.bmj.com/content/79/4/340.full.pdf+html. *(Accessed 6th April 2014*)

Braithwaite R, Addo J, Smeeth L, Lock K (2014). A systematic review of tobacco smoking prevalence in Sub-saharan Africa; 2007-2014.
Online access: htttp://www.doi:10.1371/journal.pone.0132401
(Accessed on 22nd April 2019)

Brattström O, Ericksson M, Larsson E (2015). Socio-economic factors and co-morbidity as risk factors for trauma. European Journal of Epidemiology. February 2015, Volume 30, Issue 2, pp 151-157.
Online access: htttp://www.doi.org/10.1007/s106 54-014-9969-1.
(Accessed on 17th April 2019).

Boubaker K, Flepp M, Sudre P, Furrer H, Haensel A, Hirschel B, Boggian K, Chave JP, Bernasconi E, Egger M, Opravil M, Rickenbach M, Franscioli P, Telenti A (2001). Hyperlactataemia and antiretroviral therapy: the Swiss cohort study. Clinical Infectious Diseases 33(11): 1931-1937.
Online access: http://www.cid.oxfordjournal.org/content/33/11/1931
(Accessed on 6th April 2014)

Boxwell DE., Styrt BA.,(1999*).*Lactic acidosis in patients receiving nucleoside reverse transcriptase inhibitors (NRTIs). 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco. September (Abstract 1284).
Online access: http://sti.bmj.com/content/79/4/340.full.pdf+html.
(Accessed 6th April 2014)

Brinkman K, ter Hofstede HJ,(1999*).* Mitochondrial toxicity of nucleoside analogue of reverse transcriptase inhibitors: lactic acidosis, risk factors and therapeutic options. AIDS Rev. 1: 141-148.
Online access: http://www.aidsreviews.com/files/1999_01_3_140_146.pdf
(Accessed on 5th February 2015)

Calza L, Manfredi R, Chiodo F (2005*)*. Hyperlactataemia and Lactic Acidosis in HIV Infected Patients Receiving Antiretroviral Therapy. Clinical Nutrition. Elsevier .24, 5-15.
Online access: http://www.ncbi.nlm.nih.gov/pubmed/15681087.
(Accessed 10th October 2014)

Carr A, Miller J, Law M, Cooper DA (2000). A syndrome of lipoatrophy, lactic acidemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor related lipodystrophy syndrome. AIDS 2000; 14:F25-32
Online access: http://www.dsp.dc1.ovid.com (Accessed 10th October 2014)

Chagoma N, Mallewa J, Kaunda K, Njalale N, Kampira E, Mukaka M,Heyderman R.S, van Oosterhouta J (2013). Longitudinal lactate levels from routine point-of-care monitoring in adult Malawian antiretroviral therapy patients: associations with Stavudine toxicities.Transactions of the Royal Society of Tropical Medicine and Hygiene.2013; 107: 615–619
Online access: http://www.doi.1093/trst/trt074.
(Accessed on 10th October 2014)

Estebe JP, Davies JM; Richebe P, (2011*).* The Pneumatic Tourniquet: Mechanical, Ischemia-Reperfusion and Systemic Effects. European Journal of Anaesthesiology. June 2011. Volume 28 –Issue 6-p404-411.
Online access : http://www.ncbi.nlm.nih.gov/pubmed/8734236
(Accessed on 10th October 2014).

Garavaglia M, Mak T, Cusimano MD, Rigamonti A, Crescini C, McCredy V,Romaschin A, Baker AJ, Hare GM.(2013).Body mass index as a risk factor for increased serum lactate during craniotomy.Minerva Anestesiol.2013 Oct; 79(10):1132-9.
Online access http://www.minervamedica.it
(Accessed on 21st April 2019)

Gerald Y, Maulin L, Yazdapanah Y et al (2001) Symptomatic hyperlactataemia: an emerging complication of antiretroviral therapy. AIDS 2001; 15717-23

Haljamae H and Enger M (1975). Human skeletal muscle energy metabolism during and after complete tourniquet ischemia, Ann Surgery, 182:9-14
Online access:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1343870/pdf/annsurg 0.0281-0019.pdf (Accessed on 10th October 2014).

Herman J.S., Esterbrook PJ (2001).The Metabolic Toxicities of Antiretroviral Therapy. International Journal of STDS. AIDS 2001. AIDS2 (9) 555-62(quiz 563).
Online access: http://www.std.sage pub.com/content/12/9/555.Short
(Accessed on 5th February 2015).

Hocquelox L, Albert C , Feugeas JP, Lafaurie M, Lukasiewicz E, Bagnard G, Carel O, Erlich D,Molina JM (2003). Prevalence, risk factors and outcome of hyperlactataemia in HIV-1 infected patients.HIV Medicine. 4 ,18-23.
Online access: http://www.ncbi.nlm.nih.gov/pubmed/20413022
(Accessed on 10th October 2014).

John M., Moore C.B., James I.R., Nolan D., Upton R.P., McKinnon E.J., et al., (2001).Chronic hyperlactataemia in HIV-1 infected patients taking antiretroviral therapy. AIDS, 15:717-723. Online access: http://www.ncbi.nlm.nih.gov/pubmed/11371686
(Accessed on 5th February 2015)

Kam P, Kavanaugh R. and Young F (2001). The Arterial Tourniquet: Pathophysiological Consequences and Anesthetic Implications, Anesthesia, 56: 534-545.
Online access: http://www.onlinelibrary.wiley.com/doi/10.1046/j.1365-2044.2
(Accessed on 6th April 2014).

Kang J, Han D.C, Jahong J.S, Ko O.S (1992) Blood gas and electrolyte changes after tourniquet application in total knee replacement surgery. Yonsei Medical Journal. Vol.32, No.2.
Online access: http://www.ncbi.nlm.nih.gov/pubmed/1413892
(Accessed on 12th April 2014).

Khosravani H., Shahpuri R., Stelfox T.H., Kirkpatrick A.W., Laupland K.B (2009). Occurrence and Adverse Effect in the Outcome of Hyperlactataemia in the Critically ill. Critical Care. Vol 13 No 3. Online access: http://www.ccforum/content/13/3/r90
(Accessed 10th October 2014).

Korhonen M.T, Suomine H, Mero A.(2005). Age and Sex Differences in Blood Lactate Response to Sprint Running in Elite Master Athletes. Canadian Journal of Applied Physiology. 2005 Dec; 30(6):647-65
Online access:
http://www.login.research4life.org/tacs.gr1doi_org/10.1139/h05-146
(Accessed 15th April 2019).

Lars W.A, Mackenhauer J, Roberts J, Berg K., Cocchi M., Donnino M (2013). Etiology and Therapeutic Approach to Elevated Lactate. Mayo Clinic Proccedings,2013,October,88(10);1127-1140.
Online access: http://www.doi10.1016/j.mayocp.2013.06.012
(Accessed May 20th, 2018)

Lugassy D.M., Farmer B.M., Nelson L.S.(2010).Metabolic and Hepatobiliary Side Effects of Anti-Retroviral Therapy. Emerging Med Clini N Am 28. 409-419.
Online access: http://www.ncbi.nlm.nih.gov/pubmed /20413022
(Accessed on 10th October 2014).

McNelis, J, Marrini A., Jurkiewisz D, Szamstein S., Simms. H., Ritter G., Nathan M.(2001). Prolonged lactate clearance is associated with increased mortality in the surgical intensive care unit (2001).The American Journal of Surgery. 182 pages 481-85.
Online access: http://www.ncbi.nlm.nih.gov/pubmed /1174855
(Accessed on 10th October 2014).

Modig J., Kolstad K. and Wigren A. (1978) Systemic reactions to tourniquets ischemia, Acta Anaesthesiol Scand, 22:609-14.
Online access: http://www.ncbi.nlm.nih.gov/pubmed/726866
(Accessed on 10th October 2014).

Michael Okello1, Patson Makobore, Robert Wangoda, Alex Upoki, Moses Galukande (2014), Serum lactate as a predictor of early outcomes among trauma patients in Uganda. Online access: http://www.intjem.com/content/7/1/20
(Accessed on 22nd February 2018).

Nunci LM, Ohri I, Grabocka E (2013).Comparison between hemoglobin levels and lactate blood levels as predictor of hemo-transfusion trigger. International Journal of Science and Research(IJSR). Volume 4 Issue 12, December 2015.
Online access:
http://www.ijsr.net.28d1ecea20d30ccc09331d9e06a45dba936.
(Accessed on 27th April 2019).

Pisipati R., Kurli S and Kambar C. (2013). Comparative study of prevalence of hyperlactateamia in HIV/AIDS patients receiving two antiretroviral regimen (AZT +3TC + NVP + d4T + 3TC + NVP). Journal of Pharmacy and Biological Sciences. 7 (1): 48-55

Porter M.M, Vandervoort, A.A, Lexel J (1995). Aging of human muscle: Structure, Function and Adaptability. Scan. J. Med. Sci. Sports 5:192-142.

Rama K.R.B.S., Apsingi S, Poovali S, Jetti A (2007). Timing of Tourniquet Release in Knee Arthroplasty. A Meta-Analysis of Randomized, Controlled Trials. Journal of Bone and Joint Surgery. Volume 89-A, Number 4. April 2007
Online access: http://www. doi:10.2106/JBJS.F.00497
(Accessed on 22nd October 2015)

Seindenberg P, Cerwensky K, Brown RO, Hammond E, Mofu Y, Lungu J, Mulla Y, Biemba G, Hani M (2014). Epidemiology of injuries, outcomes, and hospital resource utilization at a tertiary hospital in Lusaka, Zambia. African Journal of Emergency Medicine (2014) **4** 115-122.
Online access: http://dx.doi.org/10.1016/j.afjem.2014.01.006.
(Accessed on 25th April 2019).

Townsend H.S, Goodman S.B, Shurman D.J, Haekel H.A, Bruck-utne J.G (1996).Tourniquet release: systemic and metabolic effects. Acta Anaesthesiologica Scandinavica. 40:1234-37.
(Online access 14th October 2014)

World Health Organisation. (2018). African Health Observatory.
Online access: http://www.aho.afro.who.int>index.php.zambia
(Accessed on 23rd April 2019).

World Health Organisation (2018). Global status on alcohol and health.
Online access: http://apps.who.int>iris>97892415656.
(Accessed on 23rd April 2019).

Yang C, Chan K, Weng S (2016).Prognosis of alcohol-associated lactic acidosis in the critically ill patients: an 8 year study. *Sci. Rep.* **6** 35368;
Online access: http://doi:10.1038/srep35368(2016)
(Accessed on 25th April 2019).

APPENDICES

Appendix 1: Participant Information Sheet

Appendix 2: Consent Form

Appendix 3: Data Collection Sheet

Appendix 4: Permission Letter to conduct research on orthopaedic patients at UTH

Appendix 5: Permission Letter to conduct research on orthopaedic patients at Levy Mwanawasa Teaching Hospital

Appendix 6: Permission Letter to conduct research on orthopaedic patients at Italian Hospital

Appendix 7: Permission Letter to conduct research on orthopaedic patients at Beit Cure Hospital

Appendix 8: Ethics Letter from UNZABREC

Appendix 1: Participant Information Sheet

Title of Research: Post Surgery Lactate levels in HIV negative and HIV positive patients on HAART Orthopaedic patients after Tourniquet Use at four Hospitals in Lusaka, Zambia

Principal Investigator: Dr. Logizomai E.K. Chipasha

Introduction: You are being invited to take part in a research study. This form explains the research you are being asked to join. Please review this form carefully and ask any questions about this research study if you would like more information or if there is anything that you do not understand before you join this study. You may also ask any questions in the course of the study for any clarifications should you choose to join and please be assured that you may withdraw from participation at any time you feel otherwise. We would like to stress that participation in this study is voluntary and not compulsory.
Thank you for reading this.

Purpose of this research study: This study will help us learn whether we need to be more careful and take more precautions on people with HIV who are on ARVs when they go operations on their arms and legs with use of a tourniquet to reduce them from bleeding excessively during their operation.

Who can join this study: All men and women with above 18 years with a broken bone or hip, knee or elbow joint problem who needs an operation on their legs, arms or any of their joints to correct the problem. All those who meet the above description are welcome to join. This is why you are requested to join because you are eligible.

Voluntary participation: Your participation in this study is completely voluntary. You have the right to withdraw from this study at any time. Please be assured that should you opt not to participate in the study, or at any time decide to withdraw from the study, you will still get the same amount of quality medical care available that you may have at this hospital.

The principal investigator (whose details appear below) will be available to answer any questions you may have at the onset or during the course of this study.

What happens when you join the study: agree to join the study, we will ask you some questions related to the bone problem you have come with and other questions about your health in general. We will draw some little blood from you in a syringe (5-10ml) so as to do some blood tests on you before you go for theatre. During the operation we will carry out a few more tests on you by drawing out some tiny amounts of blood to determine your lactate levels during the operation on your bones and joints. After this, no more tests will be done on you. The research team will share with you the results of their findings.

Payment for Taking part in the research: You will not be paid for joining in the study.

Risks in taking part: there are no perceived risks to you for participating in the study.

Benefits of participating in the study: you are operated early where possible. You also get immediate referral to other specialists in the hospital at UTH should any other medical problem be discovered in the course of the study.

Confidentiality: Only the investigators and the health workers who are sworn to secrecy will have access to your answers and your laboratory tests.

Results of the study: the study team will do their best to inform you of the findings of their research and any findings that can improve your medical care. The results of the study will be published in magazine for doctors (medical journal). All participants names will not published.

What will happen if I want to stop participating in the study? You are free to drop out any time without giving any reason.

Results up to the time of your withdraw may be used if you don't mind. Otherwise, they will be destroyed if that is what you wants.

Persons to contact: if you want to talk with someone about this research study, or you think you have not been treated fairly, or you have been hurt by joining the study, or you have any questions, please contact the principal investigator, and he will try to help you:

Dr. Logizomai E.K. Chipasha of the Department of Surgery at UTH
Cell number 0967 - 538 216 or
Email: logizo2002@yahoo.com

If you still feel unhappy or a have a complaint against the investigator or anyone connected to the research, then you should contact the University of Zambia Biomedical Research Ethics Committee (UNZABRECS) on telephone number 0211 - 256067.

Appendix 2: Consent Form

Consent Form

Version: 0.04.
Date: 19/02/15
Title of Research: **Post Surgery Lactate Levels in HIV Negative and HIV positive patients on HAART Orthopaedic patients after Tourniquet Use at four Hospitals in Lusaka, Zambia**
Researcher**:** Dr. Logizomai E.K. Chipasha
Tick in Box

1. I confirm that I have read and understood the information sheet for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected and that I can refuse to answer any questions I deem personal.	☐
3. I understand that I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish.	☐
4. I understand that I will not be identified or identifiable in any report subsequently produced by the researcher.	☐
5. I accept that taking part in an study intervention is voluntary and confirm that any risks associated with this have been explained to me	☐
6. I agree to take part in the above study.	☐

Participant Name:…….....……....Signature/ Right Thumb print…....…….

Date…….....…….....

Witness:…….....Signature…….....Date……

For Further Questions, Please Contact: Dr. Logizomai E.K. Chipasha at UTH.
Cell number: 0967538216 **Email: logizo2002@yahoo.com**

Appendix 3: Data Collection Sheet

Upon enrolling into the study

Demographics

1. Serial number
2. Age in years at last birthday
3. Sex:
    1. M
    2. F
4. Weight
5. Height
6. BMI

Current Surgical History

7. When did you sustain the fracture?
    1. 1 - 4 weeks ago
    2. 5 - 8 weeks ago
    3. 9 - 12 weeks ago
    4. 13 - 16 weeks ago
    5. > 16 weeks ago

Past Medical History

8. What is your HIV status?
    1. Negative
    2. Positive
    3. I don't know
9. If positive, for how long?
    1. Less than 6 months
    2. Between 6-12 months
    3. Between 12-24 months
    4. Between 24-48 months
    5. More than 48 months

10. If positive, are you on ARVs?
    1. Yes
    2. No

11. If on ARVs, for how long?
    1. Less than 6 months
    2. Between 6-12 months
    3. Between 12-24 months
    4. Between 24-48 months
    5. More than 48 months

12. If on ARVs, which combination?
    1. Stavudine/ Lamivudine/Nevirapine
    2. Stavudine / Lamivudine/Efavirenz
    3. Zidovudine/ Lamivudine/Nevirapine
    4. Zidovudine/ Lamivudine/Efavirenz
    5. Tenofovir/ Emtricitabine/Nevirapine
    6. Tenofovir/ Emtricitabine/Efavirenz
    7. Tenofivir/ Lamivudine/Efavirenz
    8. Abacavir/ Lamivudine/Nevirapine
    9. Abacavir / Lamivudine/Efavirenz
    10. Zidovudine/ Lamivudine/ Lopinavir/ Ritonavir
    11. Zidovudine/Tenofovir/Emtricitabine//Lopinavir/ Ritonavir
    12. Stavudine/ Lamivudine/ Lopinavir/ Ritonavir
    13. Abacavir/ Didanosine/ Lopinavir/ Ritonavir
    14. Tenofovir/ Emtricitabine/ Lopinavir/ Ritonavir
    15. Others

Are you suffering from any of the following conditions?

13. Diabetes mellitus
    1. Yes
    2. No
14. Renal failure
    1. Yes
    2. No

15. Liver disease
    1. Yes
    2. No

16. Alcoholism
    1. Yes
    2. No

17. Hyperlipidemia
    1. Yes
    2. No

Obstetric History (if female)

18. Are you pregnant?
    1. Yes
    2. No

19. When was your last menstrual period? .....

Socio-economic History

20. What is your occupation?
    1. Manual worker
    2. Office worker
    3. Student
    4. Others

21. Do you take alcohol?
    1. Yes
    2. No

23. If yes, how much per day?
    1. One
    2. Two
    3. Three
    4. Four
    5. More than four

24. Do you smoke?
    1. Yes
    2. No

Investigations

- **Pre-operative**

	Laboratory Parameter	Levels	Units
25.	Serum lactate		mmol/l
26.	Hemoglobin		g/dl
27.	FBS		mmol/l

- **Post -tourniquet deflation**

	Laboratory parameter				
		2 min	**5 min**	**15min**	**30min**
28.	Serum lactate levels				

Serum lactate levels post tourniquet deflation

29. At 2 min …….....
30. At 5min …….....
31. At15min …….....
32. At 30min. …….....

Tourniquet duration

33. Less than 1 hr. …….....
34. 1-2hrs …….....
35. 2-3hrs …….....
36. More than 3hrs …….....

Appendix 4: Permission Letter to conduct Research on Orthopaedic patients at UTH

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH

University Teaching Hospital

P/Bag Rw 1X
Lusaka - Zambia
Tel: +260 211 253947 (Switch Board)
+260 211 251451

Fax: +260 211 250305
e-mail: mduth@yahoo.com

OFFICE OF THE SENIOR MEDICAL SUPERINTENDENT

Our Ref:

Your Ref:

15th December, 2015

The Head Clinical Care
University Teaching Hospital
LUSAKA.

Dear Dr Logizomai EK. Chipasha

RE: PERMISSION TO CONDUCT A RESEARCH ON ORTHOPAEDIC PATIENTS AT OUR FACILITY.

I am glad to inform you that permission to do research entitled Post Surgery Lactate levels in HIV negative and HIV position on HAART Orthopaedic patients after Tourniquite use has been granted.

Please ensure that your participation is voluntary and respect patient's confidentiality when conducting a research.

Yours faithfully,

Dr Penius Tembo

Appendix 5: Permission Letter to conduct Research on Orthopaedic patients at Levy Mwanawasa Teaching Hospital

All Communications should be addressed to:
The Senior Medical Superintendent
Tel: +260 211 285451
Fax: +260 211 285462

In reply please quote

No.:.....

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH

LEVY MWANAWASA UNIVERSITY
TEACHING HOSPITAL
P.O. BOX 310084
LUSAKA

15th December, 2015

Dear Dr. Logizomai EK. Chipasha

REF: PERMISSION TO CONDUCT A RESEARCH ON ORTHOPAEDIC PATIENTS AT OUR FACILITY

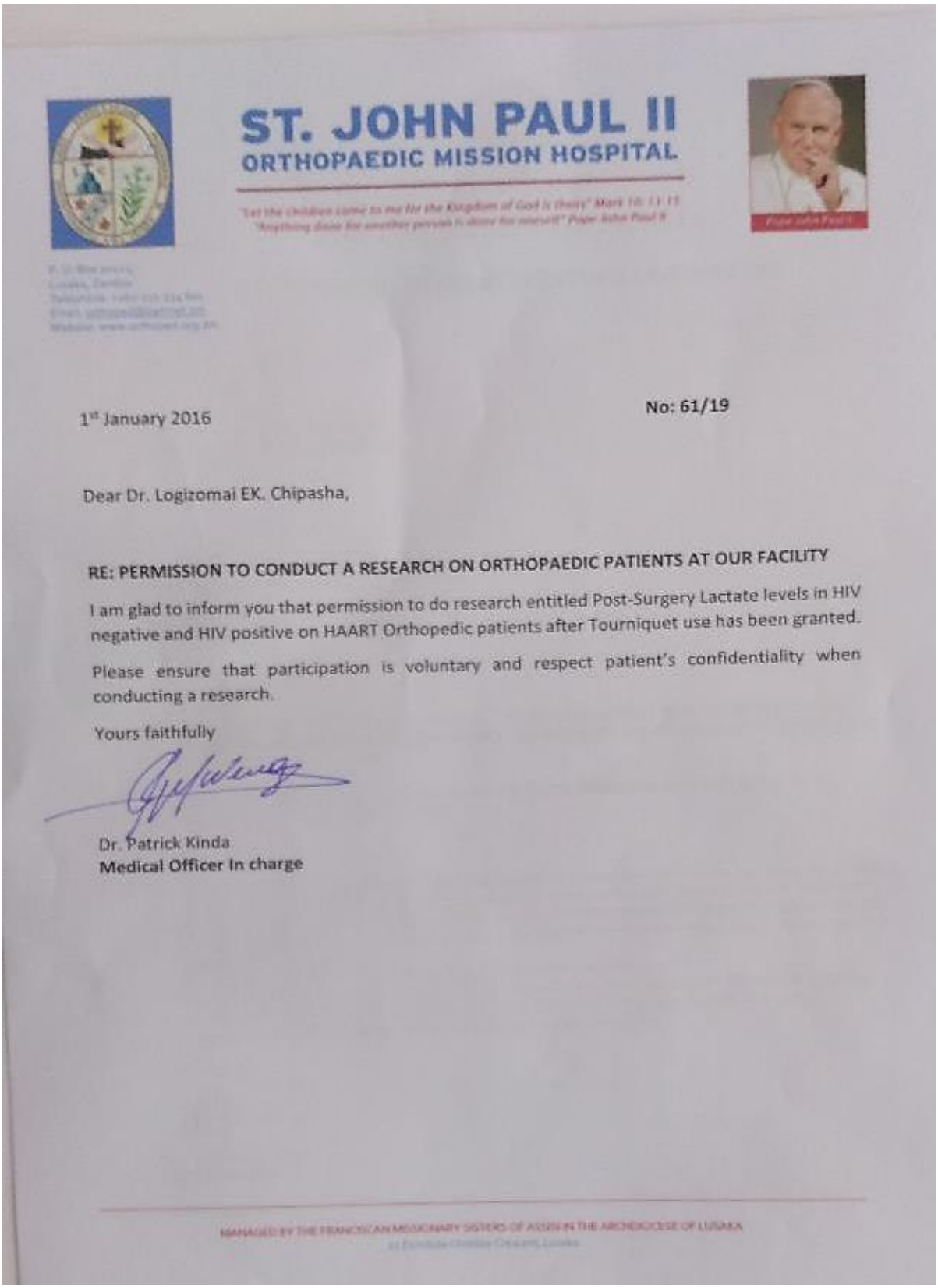
I' am glad to inform you that permission to do research entitled Post Surgery Lactate levels in HIV negative and HIV positive on HAART Orthopedic patients after Tourniquite use has been granted.

Please ensure that participation is voluntary and respect patient's confidentiality when conducting a research.

Yours faithfully,

Dr Chikoya

Appendix 6: Permission Letter to conduct Research on Orthopaedic patients at the Zambia / Italian Orthopaedic Hospital



ST. JOHN PAUL II
ORTHOPAEDIC MISSION HOSPITAL

"Let the children come to me for the Kingdom of God is theirs" Mark 10: 13-15
"Anything done for another person is done for oneself" Pope John Paul II

1st January 2016

No: 61/19

Dear Dr. Logizomai EK. Chipasha,

RE: PERMISSION TO CONDUCT A RESEARCH ON ORTHOPAEDIC PATIENTS AT OUR FACILITY

I am glad to inform you that permission to do research entitled Post-Surgery Lactate levels in HIV negative and HIV positive on HAART Orthopedic patients after Tourniquet use has been granted.

Please ensure that participation is voluntary and respect patient's confidentiality when conducting a research.

Yours faithfully

Dr. Patrick Kinda
Medical Officer In charge

MANAGED BY THE FRANCISCAN MISSIONARY SISTERS OF ASSISI IN THE ARCHDIOCESE OF LUSAKA

Appendix 7: Permission Letter to conduct Research on Orthopaedic patients at *Beit Cure* Hospital

Beit cure Hospital

20th December 2015

To Dr.Logizomai E.K.Chipasha

Universities Teaching Hospital

Lusaka, Zambia

Dear Dr.Chipasha

Re: Approval to Conduct Research at Beit CURE Hospital

I would to inform that your request to conduct research entitled " Post Surgery Lactate Levels in HIV negative and HIV positive on HAART orthopaedic patients after Tourniquet Use" at our hospital has been approved.

Please ensure to respect patient's confidentiality and obtain their consent before conducting the research.

Yours sincerely

Dr.Giorgio Lastroni

Medical Director

Beit Cure Hospital,

Zambia

cure
Dr. Giorgio Lastroni
Beit Cure Hospital
Lusaka-Zambia

Tel: 0977 740166 / 0950 798687
Email: zm.cureadmin@cureinternational.org
www.cure.org
P.O Box 36961, Lusaka, Zambia

Healing Changes Everything

Appendix 8: Ethics Letter from UNZABREC

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

26th November, 2015.

Our Ref: 006-07-15.

Dr. Logizomai E.K Chipasha,
University of Zambia,
School of Medicine,
Department of Surgery,
P.O Box 50110,
Lusaka.

Dear Dr. Chipasha,

RE: RESUBMITTED RESEARCH PROPOSAL: **"POST SURGERY LACTATE LEVELS IN HIV NEGATIVE AND HIV POSITIVE ON HAART ORTHOPEDIC PATIENTS AFTER TOURNIQUET USE" (REF. No. 006-07-15)**

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 6th November, 2015. The proposal is approved.

CONDITIONS:

- This approval is based strictly on your submitted proposal. Should there be need for you to modify or change the study design or methodology, you will need to seek clearance from the Research Ethics Committee.
- If you have need for further clarification please consult this office. Please note that it is mandatory that you submit a detailed progress report of your study to this Committee every six months and a final copy of your report at the end of the study.
- Any serious adverse events must be reported at once to this Committee.
- Please note that when your approval expires you may need to request for renewal. The request should be accompanied by a Progress Report (Progress Report Forms can be obtained from the Secretariat).
- **Ensure that a final copy of the results is submitted to this Committee.**

Yours sincerely,

M.C Maimbolwa PhD
CHAIRPERSON

Date of approval: 26th November, 2015. **Date of expiry:** 25th November, 2016.