

# Triglyceride values after six months of lopinavir/ritonavir therapy as an indicator for pancreatitis risk

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# **PREFACE**

The dissertation for this research project was completed in article format. The format abides by the guidelines and standards provided by the North-West University (NWU).

The dissertation is formatted into five chapters:

- Chapter 1: Research protocol
- Chapter 2: Literature review
- Chapter 3: Article manuscript
- Chapter 4: Results
- Chapter 5: Conclusion, recommendations and limitations

Chapter 3 of the dissertation contains the results of the research project in the form of an article manuscript. The manuscript is submitted for peer review and possible publishing in the South African Journal of Medicine.

The reference list and annexures follow Chapter 5. The reference list for the dissertation was written according to the Harvard NWU referencing style. The reference list for the article manuscript was written in the Vancouver referencing format as required by the South African Journal of Medicine.

The article was co-authored by the study supervisor, co-supervisor and statistician. Consent has been given that the article may be included in the dissertation.

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## ABSTRACT

**Title:** Triglyceride levels after six months of lopinavir/ritonavir therapy as an indicator for pancreatitis risk

**Key words:** Hypertriglyceridaemia, triglycerides, serum amylase, pancreatitis, lopinavir, ritonavir

Pancreatitis is the inflammation of the pancreas with varying aetiology. Hypertriglyceridaemia (HTG) is known to cause pancreatitis at triglyceride (TG) values of 11.3 mmol/L and beyond. The protease inhibitors (PIs) – a class of antiretroviral drugs that form part of some triple drug regimens indicated for the treatment of infection with the human immunodeficiency virus (HIV) – are known to induce HTG. Lopinavir/ritonavir (LPV/r) is the PI combination in a single dosage form that was relevant to this study.

The primary aim of this study was to investigate the risk of pancreatitis after the first six months of LPV/r therapy. The study included adult patients, treated at the Centre for Disease Control (CDC) at a public regional hospital in KwaZulu-Natal, older than the age of 18 years that were on LPV/r-based therapy (n=194). Data collected were TG values, serum (s)-amylase values and CD<sub>4</sub> counts after the first six months of LPV/r therapy. Risk was determined by the probability of pancreatitis in the study sample. Association between the cases of pancreatitis and cases of HTG was tested by a chi square statistical test. The chi square test was also used to determine whether there is an association between gender and pancreatitis and CD<sub>4</sub> count and pancreatitis.

No cases of pancreatitis were detected in the study sample. The mean triglyceride values (standard deviation [SD]) were 1.94 mmol/L (1.30). The mean s-amylase levels (SD) were 111.00 U/L (46.38). Both TG and s-amylase mean values were notably increased and there was a clear tendency to increase for both triglyceride levels and s-amylase levels. The mean CD<sub>4</sub> count (SD) was 364.76 cells/ $\mu$ L (226.85). There was a statistically significant difference between the mean TG values (SD) of males (n=50) and that of females (n=144) at 2.36 mmol/L (1.74) and 1.79 mmol/L (1.08) respectively.

There is therefore negligible risk of pancreatitis after the first six months of LPV/r therapy. However, the elevated mean triglyceride values may still warrant intervention and continuous monitoring, especially for the male population.

## UITTREKSEL

**Titel:** Triglisieried waardes na ses maande van lopinavir/ritonavir terapie as 'n indikator vir pankreatitis risiko.

**Trefwoorde:** Hipertriglisieriedemie, triglisieriede, serum amilase, pankreatitis, lopinavir, ritonavir

Pankreatitis is 'n inflammatoriese toestand van die pankreas met verskillende oorsake. Verhoogde triglisieried (TG) vlakke van 11.30 mmol/L en hoër kan pankreatitis veroorsaak en die protease inhibeerders (PIs) – 'n klas antiretrovirale middels vir die behandeling van infeksie met die menslike immuniteitsgebrek virus (MIV) – is bekend vir hul TG verhogende effek. Die PI kombinasie van lopinavir/ritonavir (LPV/r) was relevant tot hierdie studie.

Die primêre doel van hierdie studie was om vas te stel wat die risiko van pankreatitis is na die eerste ses maande van LPV/r gebaseerde terapie. Slegs volwasse pasiënte op LPV/r terapie met 'n ouderdom van 18 jaar of meer wat behandeling ontvang by die Centre for Disease Control (CDC) by 'n publieke streekshospitaal in KwaZulu-Natal was by die studie ingesluit (n=194). Die data wat versamel was het ingesluit TG waardes, serum (s)-amilase waardes en CD<sub>4</sub> tellings soos geneem na die eerste ses maande van LPV/r gebaseerde terapie. Die risiko word bereken deur die waarskynlikheid van pankreatitis te bepaal in die steekproef. Die chi kwadraat toets is gebruik om 'n moontlike verbintenis te bepaal tussen gevalle van pankreatitis en gevalle van verhoogde TG waardes. Die chi kwadraat toets is ook gebruik om 'n moontlike verbintenis te ondersoek tussen geslag en pankreatitis asook CD<sub>4</sub> tellings en pankreatitis.

Daar was geen gevalle van pankreatitis opgemerk in die steekproef nie. Die gemiddelde TG waarde (standard afwyking [SA]) was 1.94 mmol/L (1.30). Die gemiddelde s-amilase waarde (SA) was 111.0 U/L (46.38). Beide die TG en s-amilase gemiddelde waardes was verhoog. Die waardes in die steekproef het 'n duidelike tendens van verhoging gewys. Die gemiddelde CD<sub>4</sub> telling (SA) was 364.76 cells/ $\mu$ L (226.84). 'n Statistiese betekenisvolle verskil was gevind tussen die gemiddelde TG waardes (SA) van mans (n=50) en vrouens (n=144) met TG waardes van 2.36 mmol/L (1.74) en 1.79 mmol/L (1.08) onderskeidelik.

Die risiko vir pankreatitis na die eerste ses maande van LPV/r terapie is dus weglaatbaar klein. Triglisieried waardes was wel genoegsaam verhoog om 'n ingreep sowel as herhaalde monitering te regverdig, veral vir die manlike populasie.

## LIST OF ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immunodeficiency syndrome
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ART	Antiretroviral therapy
ARTEMIS	Antiretroviral Therapy with TMC114 ExaMined In naïve Subjects
ARV	Antiretroviral
AST	Aspartate transferase
ATV	Atazanavir
AZT	Zidovudine
CDC	Centre for Disease Control
CD <sub>4</sub>	Cluster of differentiation 4
CD <sub>8</sub>	Cluster of differentiation 8
DDI	Didanosine
EFV	Efavirenz
FDC	Fixed drug combination
FTC	Emtricitabine
HAART	Highly active antiretroviral therapy
HbA <sub>1c</sub>	Haemoglobin A <sub>1c</sub>
HBV	Hepatitis B virus
HCV	Hepatitis C virus

HDL	High density lipoprotein
HIV	Human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HTG	Hypertriglyceridaemia
HREC-NWU	Human Research Ethics Council North-West University
IRIS	Immune reconstitution inflammatory syndrome
LDL	Low-density lipoprotein
LFT	Liver function test
LPV/r	Lopinavir/ritonavir
MIV	Menslike immuniteitsgebrekvirus
Mkat/L	Millikatal per litre
Mmol/L	Millimol per litre/liter
NDoH	National Department of Health (South Africa)
NHLS	National Health Laboratory Service
NIAID	National Institute of Allergy and Infectious Diseases
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitor
NVP	Nevirapine
NWU	North-West University
PHRC	Provincial Health Research Council
PI	Protease inhibitor/inhibeerder
S	Serum
SA	Standaard afwyking

SD	Standard deviation
SREBP	Sterol regulating binding protein
SRE	Sterol regulating element
TDF	Tenofovir
TG	Triglyceride(s)/triglyceride(s)
U/L	Units per litre
VLDL	Very low-density lipoprotein



## LIST OF DEFINITIONS

AIDS infection	AIDS is an acronym for acquired immunodeficiency syndrome. AIDS is a very advanced stage of HIV (Aidsinfo, 2015).
Anaemia	Deficiency of haemoglobin or red blood cells (Merriam-Webster, 2016).
Antiretrovirals	A term used to refer to the combination of drugs used to treat the HIV infection (Aidsinfo, 2015).
CD <sub>4</sub> cells serve	Specific type of white blood cells (T lymphocytes) that as the natural host for HIV in the human body. The replication of HIV inside the CD <sub>4</sub> cells leads to the destruction of the cell and consequently an acquired immunodeficiency syndrome (Palmisano & Vella, 2011:47).
Human immunodeficiency virus lead to (AIDS) (McCutchan, abbreviated to virus itself or the infection	A retrovirus that, when infecting the human host, destroys specific white blood cells (CD <sub>4</sub> cells), which may acquired immunodeficiency syndrome (AIDS) (McCutchan, 2015). Human immunodeficiency virus is abbreviated to HIV, which is used to refer to the virus itself or the infection caused by the virus (Aidsinfo, 2015).
Hypertriglyceridaemia	A state in which triglyceride levels are higher than the normal of 1.7mmol/L (Rosenson, 2016).
Pancreatitis	Inflammation of the pancreas (Freedman, 2016).
Protease inhibitor protein that unable Perry,	A class of antiretroviral drug that inhibits HIV protease enzymes preventing the splitting of the gag-pol leads to the production of immature viruses that are to infect and multiply in other CD <sub>4</sub> cells (Croxtall & Perry, 2010:1887).
Serum amylase	Serum amylase is a digestive enzyme secreted by the pancreas and salivary glands (Mathew, 2015).

Side-effect drug's	An unwanted effect of the drug that occurs within the therapeutic range (Tarloff, 2015).
Steatorrhoea	An excess of fat secreted in the stool (Merriam-Webster, 2016).
Toxicity	The characteristic of being poisonous or toxic (Merriam-Webster, 2016).
Triglycerides	Lipid compounds that consist of three fatty acid chains and one glycerol molecule (Morris <i>et al.</i> , 2014).
Tuberculosis infected	Bacterial infection caused by <i>Mycobacterium tuberculosis</i> , characterised by the growth of nodules in the tissue, especially the lungs (Merriam-Webster, 2016).
Viral load	The amount of HIV particle copies per millilitre of blood (AIDS.gov, 2016).
Virological failure therapy	A viral load of above 50 copies/ml after 24 weeks of (Antinori <i>et al.</i> , 2012:122).
Virological resistance to	When a virus is no longer susceptible to an antiviral drug which it was previously susceptible (Aidsinfo, 2016).
Xanthoma	Irregular yellow patches of lipid deposits under the skin (Merriam-Webster, 2016).

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# **CHAPTER 1: RESEARCH PROTOCOL**

## **1.1 Introduction and background**

More than 30 years have passed since it was proven that the human immunodeficiency virus (HIV) is a causing agent of an acquired immunodeficiency syndrome (AIDS) (Barré-Sinoussi *et al.*, 1983:868). The human immunodeficiency virus is a retrovirus whose natural host in humans, for the purposes of self-replication, is the CD<sub>4</sub> T lymphocyte. The replication of the virus inside the CD<sub>4</sub> cell destroys the cell. Since lymphocytes play an important role in human immunity, the destruction of these cells will lead to an acquired immunodeficiency (Palmisano & Vella, 2011:43). In the years following the association of HIV with AIDS, reports of infection increased from around the world, and it was only in 1987 that the Food and Drug Administration of the United States of America approved the first antiretroviral (ARV) drug, zidovudine, for the treatment of HIV (Arts & Hazouda, 2012:2; Palmisano & Vella, 2011:47).

## **1.2 Antiretroviral therapy – past and present**

Monotherapy with zidovudine was the start of the ARV era. Soon, it became clear that a combination of at least three ARVs is required to decrease the likelihood of the virus developing resistance against the drugs (WHO, 2015), with each of the three drugs interfering in a different stage of viral replication (Palmisano & Vella, 2011:46). The combination of three different ARV drugs is referred to as combination antiretroviral therapy, highly active antiretroviral therapy (HAART), or simply just ART.

Antiretroviral therapy (ART) regimens in the public sector in South Africa are well defined by the National Department of Health (NDoH), and the selection of an ART regimen for a patient is done according to strict guidelines (NDoH, 2014:67). Antiretroviral therapy is divided into first-, second- and third-line regimens. The first-line regimens are the starting point for newly initiated patients. It usually consists of two nucleotide/nucleoside reverse transcriptase inhibitors (tenofovir, abacavir or zidovudine with lamivudine) and one non-nucleoside reverse transcriptase inhibitor (efavirenz or nevirapine) (NDoH, 2014:67). Regimen one may fail due to the development of viral resistance. This warrants initiation on second-line ART, which includes two nucleoside reverse transcriptase inhibitors and a protease inhibitor (PI) combination (lopinavir and ritonavir or atazanavir [ATV] and ritonavir) as the third agent (NDoH, 2014:68).

Since the first implementation of combination antiretroviral therapy, it has proven to be very successful and beneficial. However, clinicians are often limited by unfavourable side effects caused by ART (Tebas *et al.*, 2007:193). Lopinavir/ritonavir (LPV/r) is a potent PI combination formulated together in the same dosage form. The LPV/r combination has proven its efficacy (Cvetkovic & Goa, 2003:777); however, as with many other ARVs, this formulation is not without its disadvantages with regard to side effects.

### **1.3 Lopinavir/ritonavir – a brief profile**

Fifteen years after its introduction to HIV treatment regimens worldwide (Camacho & Rivero, 2014:31), LPV/r remains an effective drug combination and a mainstay in the treatment of the human immunodeficiency virus 1 and 2 (HIV-1 and HIV-2) respectively. Its safety during pregnancy has also made it useful for the prevention of transmission of HIV from mother to child and has a relatively low risk of hepatotoxicity in patients with liver disease (Aidsinfo, 2015; Camacho & Rivero, 2014:31).

Lopinavir and ritonavir inhibit HIV protease enzymes from preventing the splitting of the gag-pol protein that leads to the production of immature viruses that are unable to infect and multiply in other CD<sub>4</sub> cells (Croxtall & Perry, 2010:1887). Lopinavir is a ritonavir analogue, but with potency, ten times greater *in vitro* than that of ritonavir (Camacho & Rivero, 2014:31). The combination of LPV/r also decreases the probability of the particular mutations of HIV that lead to resistance (Camacho & Rivero, 2014:32; De Lagarde *et al.*, 2014:15; Kearney *et al.*, 2006:278; Ribera *et al.*, 2011:385) and inhibits 93% of the protease activity of the wild-type virus (Croxtall & Perry, 2010:1887).

Lopinavir/ritonavir undergoes rapid first-pass oxidative metabolism via CYP3A4. Ritonavir is a potent inhibitor of CYP3A4, thereby inhibiting the metabolism of lopinavir and boosting the lopinavir plasma concentration and bioavailability (Croxtall & Perry, 2010:1890; Cvetkovic & Goa, 2003:794; Kearney *et al.*, 2006:278). Ritonavir is not included in the formulation for its antiretroviral activity, but solely for its ability to increase the plasma concentration of lopinavir, also referred to as pharmacokinetic enhancer or booster. The plasma ritonavir concentration is usually about ten times less than that of lopinavir, which means the antiretroviral activity of LPV/r can be attributed to lopinavir alone (Croxtall & Perry, 2010:1887; Medscape, 2015). Most of the metabolised LPV/r is eliminated via the faecal route (Medscape, 2015), with only a small percentage via the renal route. This makes for the safe administration of LPV/r in patients with renal insufficiency (Camacho & Rivero, 2014:33).

Lopinavir/ritonavir is metabolised in the liver, which means that a patient's liver needs to be competent to deal with the metabolism and elimination of the drug. A liver function test (LFT)

may be performed to assess whether the patient's liver can manage the metabolic load of metabolising LPV/r. Elevated levels of alanine transaminase (ALT) and in particular, five times above the upper normal limit of aspartate transaminase (AST), have been reported in patients on LPV/r therapy (Croxtall & Perry, 2010:1906; Orkin *et al.*, 2013:55). Agents that affect liver metabolism, such as ketoconazole (inhibition), tuberculosis treatment and certain anti-epileptics (liver enzyme inducers) will therefore also affect the plasma levels of LPV/r.

### **1.3.1 Side-effects of LPV/r**

Tarloff (2015) describes an adverse effect as an unwanted, undesired and possibly dangerous effect that a drug causes at an elevated plasma concentration. A side effect is described as an unwanted effect of the drug that occurs within the drug's therapeutic range. For the purposes of this study, the term 'side-effect' will be used when referring to the unwanted effects for LPV/r, since the unwanted effects occur at the normal dosage of the drugs in question.

The most frequent and obvious side effects among patients taking LPV/r are diarrhoea, headaches, asthenia and skin rashes (Croxtall & Perry, 2010:1905; Cvetkovic & Goa, 2003:770; Hawkins, 2010:202). In addition to these directly observable side effects, there are also more subtle and less obvious side effects caused by LPV/r on a metabolic level. Lopinavir/ritonavir has shown to cause serum lipid abnormalities in patients taking this combination of ARV (Croxtall & Perry, 2010:1907; Hawkins, 2010:205; Orkin *et al.*, 2013:57).

The National Institute of Allergy and Infectious Diseases (NIAID) of the United States of America, developed a grading of severity of adverse effects (or side effects, as they are described for the purposes of this study) that groups adverse events into clinical subdivisions and then grades each adverse event into four grades of severity (NIAID, 2014:19). Grade 1 represents a mild adverse event, grade 2 moderate, grade 3 severe and grade 4 potentially life-threatening (NIAID, 2014:19).

The Antiretroviral Therapy with TMC114 ExaMined in naïve Subjects (ARTEMIS) study reported grade 4 and 3 levels of triglyceride (TG) and total cholesterol after 96 weeks of LPV/r therapy respectively (Arathoon *et al.*, 2013:14).

### **1.3.2 Hypertriglyceridaemia and pancreatitis**

Hypertriglyceridaemia and HIV infection are both proven causes of pancreatitis (Drakovic, 2013:422). The pathophysiology of hypertriglyceridaemia (HTG)-induced pancreatitis is uncertain, but patients with TG levels of higher than 11.3 mmol/L have a high risk of contracting pancreatitis (Baron, 2013).

Pancreatitis is the inflammation of the pancreas, an organ that is located behind the stomach in the abdomen. Pancreatitis may be acute or chronic (Friedman, 2013: 711-715). Patients with acute pancreatitis usually recover completely; however, chronic pancreatitis may leave tissue damage, which can impair the organ's endocrine and exocrine functions (Freedman, 2012). Clinically, a patient with pancreatitis will present with severe epigastric pain that may radiate towards the back, accompanied by nausea, vomiting and weakness (DiPiro & Schwinghammer, 2015:244; Freedman, 2012; Friedman, 2013: 711). The patient may have previously suffered from pancreatitis previously, which could be associated with alcohol consumption. Laboratory results will reveal an increased leukocyte count and increased serum (s)-amylase and s-lipase (DiPiro & Schwinghammer, 2015:245). Elevated bilirubin and alkaline phosphatase (ALP) may be present, but are not specific enough to diagnose pancreatitis. Alanine transaminase may be elevated above 150 U/L, which could suggest biliary pancreatitis. Serum-amylase and s-lipase are usually increased threefold above the normal values of 104 U/L and 160 U/L, respectively (Freedman, 2012; Friedman, 2013:711). The causes of pancreatitis are various. Many are due to the consumption of alcohol and diseases of the biliary tract. Other causes include hypercalcaemia, HTG and trauma to the abdomen. Pancreatitis can also be caused by certain drugs such as azathioprine, pentamidine, didanosine, valproic acid, tetracycline, metronidazole, isoniazid, dapsone, tamoxifen and oestrogen (DiPiro & Schwinghammer, 2015: 244; Friedman, 2013:711). Other risk factors that play a role in the risk of pancreatitis are gender and CD<sub>4</sub> counts (Drakovic, 2014:422). Low CD<sub>4</sub> counts increase the risk of pancreatitis and females have a much higher propensity for pancreatitis than males (Drakovic, 2014:422).

### **1.3.3 Is LPV/r use, a risk indicator for pancreatitis?**

Lopinavir/ritonavir has shown to increase TG and total cholesterol in various studies (Calza *et al.*, 2003:54; De Lagarde, *et al.*, 2014:15; Drakovic, 2013:422; Orkin, 2012:55; Reyskens *et al.*, 2013:1;). Co-trimoxazole has also been shown to increase TG when in combination with LPV/r in rats (Elias *et al.*, 2014:642). It would therefore, be reasonable to anticipate an increase in pancreatitis among patients on LPV/r therapy. However, it has been shown that the prevalence of pancreatitis is relatively low among patients who are on LPV/r therapy (Chandwani & Shuter, 2008:1030; Cvetkovic & Goa, 2003:775), with some studies not recording a single case (Calza *et al.*, 2003:54). Manfredi *et al.* (2004:537) conducted a case control study to assess the risk of

pancreatitis among patients on ART. The Manfredi study linked the various risk factors, including PIs, to pancreatitis, via abnormalities in pancreatic enzyme levels (elevations in lipase, pancreatic isoamylase, and amylase). Very few of the subjects were diagnosed with acute symptomatic pancreatitis, but several of the asymptomatic patients showed pancreatic enzyme levels that suggested pancreatitis. Therefore, many cases of pancreatitis may be subclinical and may go undetected.

For the purposes of this study, an s-amylase value of three times above the normal value (Manfredi *et al.*, 2004:538) of 104 U/L will be considered as pancreatitis (the upper normal limit of the National Health Laboratory Service [NHLS]). Hypertriglyceridaemia is defined as a TG level of higher than 1.7 mmol/L (Berglund *et al.*, 2014:3) and will be used as the upper normal limit of TG for the purposes of this study.

#### **1.4 Problem statement**

The prevalence of HIV infection in KwaZulu-Natal is the highest in South Africa at 16.9% (HSRC, 2014:37) and is highest among the population aged between 25 and 39 years (HSRC, 2014:40). Data collected in August 2015 shows that the public regional hospital where the study was conducted currently provides ART to 4 272 HIV-infected patients of all age groups every month. Infection with HIV is a definite risk factor for pancreatitis, which is considerably more prevalent among patients infected with HIV than those who are not (Dowel *et al.*, 1996:46; Dutta *et al.*, 1997:2045). In a population where HIV infection is quite common, the prevalence of pancreatitis could consequently be relatively high. Elevated s-amylase levels can be used as an indicator of pancreatitis. The assessment of the risk of pancreatitis among adults on LPV/r therapy can prove useful in the proactive reduction and management of PI-induced pancreatitis.

#### **1.5 Research aims and objectives**

##### **1.5.1 Research aims**

The primary aim was to assess the risk of TG-induced pancreatitis after the first six months of LPV/r therapy among HIV-infected patients.

## **1.5.2 Specific research objectives**

For the purposes of accomplishing the aims of this study, the following research objectives were met:

- Determination of the prevalence of TG-induced pancreatitis after the first six months of LPV/r therapy among males and females by using s-amylase levels elevated above three times the upper normal limit as diagnostic criteria for pancreatitis.
- Assessment of the contributing risk of a low CD<sub>4</sub> count to pancreatitis, after six months among patients on LPV/r therapy.

## **1.6 Research methodology**

### **1.6.1 Study design**

The study was in the form of a cross-sectional, retrospective and observational study.

Cross-sectional studies are performed by collecting all relevant data at a single point in time in order to investigate a subject as it was at that particular moment (Brink *et al.*, 2014:100). Since this study assessed certain variables for each patient as they were after the first six months of LPV/r therapy, a cross-sectional study design was the appropriate study design. Retrospective studies investigate an outcome or effect by looking back in history – in this case, the medical history of the patients – in order to analyse what was associated with this outcome or effect prior to the collection of data (Brink *et al.*, 2014:102). As an observational study, data were collected by directly observing and collecting descriptive data retrospectively, according to specific criteria (Brink *et al.*, 2010:150). With this method of data collection, no variable and therefore outcome was manipulated, controlled and randomised by the researcher as with an experimental study design (Brink *et al.*, 2010:103-104).

The relevant data recorded in each patient's medical history after the first six months of LPV/r therapy was collected in order to assess certain variables at that particular stage of therapy. Since the variables relevant to this study were retrieved from patients' medical history, they cannot be altered or manipulated. Data were therefore collected in an observational and retrospective manner.

## **1.6.2 Study setting**

The study took place in the Centre of Disease Control (CDC) at a public regional hospital in KwaZulu-Natal, a public sector facility. The hospital has approximately 500 beds and various specialist services in ophthalmology, internal medicine, family medicine, surgery and orthopaedics. A primary healthcare clinic situated at the entrance of the premises renders primary healthcare services and refers patients to the hospital for more specialised care at the hospital's outpatient department. Approximately 800 patients pass through outpatients and the pharmacy on a daily basis. A large part of the daily outpatients pass through the CDC, which is dedicated to the management and treatment of HIV/AIDS. The hospital has an NHLS on the premises.

## **1.6.3 Target and study population**

The target population included all patients above the age of 18 years who were taking LPV/r as a part of their antiretroviral treatment regimen. The target population only included black Africans, since the ethnic representation of the study population at the study setting to which the researcher had access, was almost exclusively black African. From a pharmacogenomics perspective, a homogenous population may be significant, as any results that this study may yield may not be applicable to another ethnic group. In studies conducted by Raza *et al.* (2013:12) and Riedel *et al.* (2008:3), investigating the incidence and risk factors of pancreatitis among people with HIV/AIDS, the prevalence of pancreatitis was highest among African-Americans. It was therefore necessary to include only black Africans in order to rule out genetic confounders.

This study population included all black African patients above the age of 18 years who had a TG and s-amylase assay result six months after the commencement of LPV/r therapy at the public regional hospital in KwaZulu-Natal. The TG and s-amylase assay results were collected from as far back as the database described in 1.7.1 allowed, until present.

### **1.6.3.1 Inclusion criteria**

- Black African adult patients ( $\geq 18$  years) attended to at the CDC of the public regional hospital on LPV/r therapy, on the standard 400mg/100 mg twice-daily dosage.
- Black African adult patients ( $\geq 18$  years) who had a TG and s-amylase assay done after the first six months of LPV/r therapy.
- Black African adult patients with a CD<sub>4</sub> count after the first six months of LPV/r therapy.

### 1.6.3.2 Exclusion criteria

- Patients who are not attended to at the CDC of the public regional hospital. These patients will not have medical records at the facility, which are required for data collection.
- All patients with existing acute or chronic pancreatic disease diagnosed before the initiation of LPV/r therapy. These cases would have had exposure to risk factors for pancreatitis, unrelated to the intended risk factor to be investigated in this study, i.e. TG levels after the first six months of LPV/r therapy. Any cases of existing pancreatitis diagnosed before LPV/r therapy was initiated will therefore be unrelated to LPV/r-induced HTG. If such cases were to be included in the sample, it would mean that no conclusion could be made regarding the relationship between LPV/r induced HTG and pancreatitis.
- Patients already taking fibrates for the lowering of TG levels. Any therapy that lowers TG will lower the risk of pancreatitis and possibly negate the effect that LPV/r may have on TG levels.
- Patients taking ketoconazole. Lopinavir/ritonavir is extensively metabolised in the liver (Croxtall & Perry, 2010:1890) and ketoconazole is a liver enzyme inhibitor (Rossiter, 2014:316). Therefore, if taken concomitantly with LPV/r, ketoconazole may increase the plasma levels of LPV/r and therefore alter its effect at the normal dosage.
- Patients on antiepileptic medication. Carbamazepine and phenobarbitone are potent liver enzyme inducers (Rang & Dale, 2007:581-582). As LPV/r is metabolised in the liver (Croxtall & Perry, 2010:1890), carbamazepine and phenobarbital will accelerate the metabolism of LPV/r and consequently reduce the plasma levels of LPV/r. The effects of LPV/r at the normal dosage will therefore be altered.
- Patients taking azathioprine, pentamidine, didanosine, stavudine, valproic acid, tetracycline, metronidazole, isoniazid, dapson, tamoxifen and oestrogen. These are all drugs that may cause pancreatitis (Friedman, 2013:711). Taken concomitantly with LPV/r, these drugs may obfuscate the risk that LPV/r may contribute to pancreatitis after six months of therapy.
- Patients with alanine transaminase (ALT) elevated above 150 U/L before commencing LPV/r therapy to eliminate the possibility of biliary pancreatitis, which is of different aetiology than pancreatitis, caused by high levels of TG.
- Those patients identified with pancreatitis that consumed alcohol at the time that the TG and s-amylase assays were performed. Alcohol is a risk factor for pancreatitis (Friedman, 2013:711). The possibility of alcohol-induced pancreatitis should therefore be ruled out since it would be of different aetiology than TG-induced pancreatitis. These patients can be identified by revising the medical records of those patients identified



during data collection, who have an s-amylase assay three times higher than the upper normal limit, to verify whether the attending doctor diagnosed alcohol-induced pancreatitis or not.

- Patients with hepatic disease and renal failure. Nephrotic syndrome causes increased production of apolipoprotein B, which may lead to elevated TG levels (Berglund *et al.*, 2012:2975). Acute hepatitis may also increase TG values (Berglund *et al.*, 2012:2976).
- Patients with familial HTG. The cause of HTG in these patients is genetic and therefore unrelated to the risk factors investigated by this study. Therefore, those patients whose medical records reflect familial HTG diagnosis will be excluded.

#### **1.6.4 Sampling**

##### **1.6.4.1 Sampling technique**

Simple random sampling was used.

##### **1.6.4.2 Determination of sample size**

Data from August 2015 showed at the time the study was conducted, there were 807 adult patients on LPV/r therapy at the public regional hospital. The population of 807 was obtained from dispensing statistics in the pharmacy. Performing a power analysis based on chi-square as the main method of analysis of association, indicated a required sample size of 197 with an effect size of 0.2. A sample size of 200 patients were used after the data was collected retrospectively.

#### **1.7 Measurement and data collection**

##### **1.7.1 Data sources**

Existing databases accessible on the premises were used to collect the following data:

- The TIER.Net database: A database used by NDoH to keep a national record of all patients that are on ART.
- National Health Laboratory Service online database: This is an online archive where the NHLS stores laboratory results for all patients of the Department of Health.
- Patient medical records: These will only be used in cases where data from the TIER.Net or NHLS databases are incomplete.

### **1.7.2 Specific type of data collected**

The data that were collected from the NHLS database are the results from the analysis of blood chemistry assays performed by the NHLS. The particular analytes relevant for this study were:

- Triglycerides measured in mmol/L (millimoles per litre)
- S-amylase measured in U/L (units per litre)

The instrument of measurement used to analyse all blood chemistry in the NHLS laboratory was the Beckman Coulter Unicel DxC. The blood chemistry analysis performed with this instrument included the TG and s-amylase assays. The results from the blood chemistry assays were loaded onto the NHLS online database by NHLS staff.

Other data relevant to the study that were collected from the TIER.Net database:

- Gender
- Age
- Previous ARV regimen. Since LPV/r form part of the second-line regimen, all previous regimens will be first-line that consist of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) such as tenofovir (TDF), abacavir (ABC), or zidovudine (AZT) with lamivudine (3TC) and one non-nucleoside reverse transcriptase inhibitor such as efavirenz (EFV) or nevirapine (NVP)
- CD<sub>4</sub> count after the first six months of LPV/r therapy
- Current ARV regimen

The data from the NHLS online database and the TIER.Net database were collected using the data collection tool (Annexure A). The data collection tool features all variables in tabular form. The data were entered for each patient into the relevant columns.

### **1.7.3 Development of data collection tool**

The retrospective data collection tool was developed with participant anonymity and confidentiality foremost in mind. No personal information was recorded on the data collection tool, in order to ensure anonymity and confidentiality.

The data required to conduct this study determined the design and relevant fields that required population on the document. Consideration was then given to the specific format of the data collection tool and an effort was made to fit the document onto a single page in order to make it easy to work with for those who recorded the required data, as well as the statistical analysis of the data. The data required were entered into columns under the relevant headings. Once the

data were transferred from the hard copy to an electronic format of the data collection tool, the statistician was able to easily gather the data from each column for analysis.

#### Validity and reliability of measurement instrument and data collection tool

- The data collection tool (Annexure A) was designed to capture all the required information for the study. It was discussed with the doctors at the CDC to ensure clarity with regard to the data that needed to be collected. Since two doctors, who were permanently employed at the CDC, populated the document, there was a risk of random error that could compromise the reliability and validity. However, since not much data needed to be collected at that time and the data were not complex, human error was most unlikely.
- The measurement instrument used by the laboratory had certain standards set to ensure validity and reliability (the researcher does not have access to the measuring instrument and does not operate it). The Unicel DxC was maintained and calibrated by trained laboratory staff on a daily basis, to ensure validity. Different calibration and maintenance procedures were performed on daily, weekly, twice-weekly, monthly, two-monthly, three-monthly, four-monthly and six-monthly intervals. The newly maintained and calibrated instrument was then tested with high, medium and low controls. This was repeatedly performed to ensure that the instrument could readily detect and analyse analytes from a very low to a very high concentration in order to avoid any system errors.
- Subject variation: The TG assays were done on a random blood sample after the first six months on LPV/r therapy. A random sample implies that the patient's fasting or fed status is not taken into consideration. Ng (1993:12) points out that the small differences between fasting and total cholesterol analyses are negligible due to the very small diurnal coefficient of variation of 2.5%.
- Setting of data collection: A public regional hospital in KwaZulu-Natal.
- Who collected the data? The two doctors who were permanently appointed at the CDC collected all data and recorded it on the data collection tool.
- Who worked with the data? The doctors in the CDC saw the data and to which patients it belongs. They only collected the data and did not use the data for research purposes. The researcher was the only other person to work with the data but only after it was anonymised for the purposes of this study. The study supervisor, co-supervisor and statistician also had access to the data.
- When were the data collected? Data collection started as soon as approval to conduct the study was obtained from the Provincial Health Research Council (PHRC) of KwaZulu-Natal and the Human Research Ethics Committee at the Faculty of Health Sciences of the North-West University (HREC-NWU).

#### **1.7.4 Data collection method**

It is standard practice at the CDC of the public regional hospital to request a serum lipid assay and a liver function assay when a patient has completed the first six months of LPV/r therapy. A serum lipid assay included levels of total cholesterol, high-density lipoproteins, low-density lipoproteins and TG. Only the TG levels were required for this study. The LFT includes the levels of various liver enzymes, bilirubin and s-amylase. Only s-amylase levels were required for this study. The laboratory analyses all blood chemistry panels (which include the urea and electrolytes, serum lipid assay and LFT), regardless of what the doctor requested. However, only the results that the doctor requested are printed out and included in the patient's medical records; in this case, the results of the serum lipid assay and liver function assay.

The data generated by the blood chemistry analysis by the NHLS are returned to the patient's file as per hospital regulations and loaded onto the NHLS's online archives. The doctors at the CDC then collect the data and the same doctors populate the data collection tool (Annexure A).

#### **1.7.5 Persons to collect the data**

The doctors (permanently appointed) at the CDC collected the data. The results of this study will not only benefit the medical community in general, but may have a direct impact on operations at the CDC. The results may also assist the doctors in improving the clinical service they render to the public. Since the study was conducted at the facility where they are employed and they are both HIV/AIDS care specialists, the study and its results are of particular interest to them. For these reasons, they were willing to participate and satisfied with the roles they played in the study. The data collection process is described in detail in 1.8.

#### **1.7.6 Setting of data collection**

All the data were collected at the CDC of the public regional hospital in KwaZulu-Natal.

#### **1.7.7 Time of data collection**

The two CDC doctors collected the data when convenient during normal working hours. The doctors were on duty from Monday to Friday. Normally, all patients who report to the CDC on a daily basis are cleared by lunch. Data were collected from after lunch to the end of the working day at 16:00.

Data collection commenced once approval to conduct the study was granted by HREC-NWU. Sufficient time (two months) was allowed for the doctors at the CDC to collect the data in order to ensure that it does not negatively affect their duties at the hospital. Data collection commenced at the beginning of April 2016 and was completed at the end of April 2016.

## **1.8 Data collection process**

1. The head of department at the CDC (Dr F Amod), who was also one of the two doctors who collected the data, drew a list from TIER.Net of all the patients who were taking LPV/r. The list represented the study population of approximately 807 patients. Information on this list consisted of, *inter alia*, patient name and surname, file number, ART regimen and date of commencement of ART.
2. The TIER.Net program numbered the patients on the list. The head of department at the CDC then generated random numbers that fell within the range of the population list. Each number generated, randomly selected a patient. This process was repeated until the sample size was achieved.
3. The file number of each patient selected was recorded. The list of file numbers of the sample was then divided into two halves – one-half for each of the two doctors permanently appointed at the CDC. Each doctor then accessed the NHLS online archives on a computer at the hospital and retrieved the TG and s-amylase values for the assays performed at six months after commencement of LPV/r therapy for each patient. Patient age, gender and CD<sub>4</sub> count were also recorded. Previous regimens were retrieved from the TIER.Net database where possible. If not, the patient's file that contains his/her medical records was consulted. The computer that was used by the two doctors was located in the office of the head of department of family medicine. Access to the computer was therefore controlled and the location was in a private space with no access for other staff members without permission. The data were collected during the time periods described in 1.7.7.
4. The retrieved data were recorded on a hard copy of the data collection tool (Annexure A). The patient's name and file number were not recorded to ensure anonymity and confidentiality.
5. Once each doctor recorded all the information for the sample on the data collection tool, the data were handed to the researcher for analysis.

### **1.8.1 Recruitment of participants**

Since this was a cross-sectional, observational retrospective study, there was no recruitment done. Patients' information was randomly selected as explained in 1.8 above.

## **1.9 Management of data**

When the two doctors completed the data collection, the data were handed over to the researcher. The researcher transferred the data from each sheet of the data collection tool onto an electronic variation in Microsoft Excel<sup>®</sup> format. During the study, the hard copies of the

populated data collection tool were kept locked up in a file cabinet at the researcher's residence. The electronic copies in Excel® format of the data collection tool were stored on the researcher's personal computer. The Excel® documents were password protected and the computer had adequate antivirus software to protect the data against electronic threats. Access to the computer was protected with a 10-digit password.

During the period of analysis of the data, only Mr W Greffrath (researcher), Dr JM du Plessis (supervisor), Dr M Viljoen (co-supervisor) and Ms M Cockeran (statistician) had access to the data.

After the study was completed, hard and electronic copies of the populated data collection tool were stored at the offices of MUSA (Medicine Usage in South Africa) for a period of at least seven years, after which MUSA staff will destroy both hard and soft copies of the data. The researcher deleted the electronic copies on his personal computer.

### **1.9.1 Data monitoring and quality assurance**

As this was a retrospective study, any missing data from the patients' medical records could not be recreated. The accuracy and consistency of the data required, depended on the maintenance and calibration of the analytical equipment of the NHLS and the person maintaining the TIER.Net database.

In order to ensure the quality of the data, two quality control measures were taken:

1. Both Dr Amod and Dr Sultana performed their own quality checks while collecting data from the patients' medical records and recording it on the data collection tool. Data entered onto the data collection tool were double-checked in order to ensure accuracy and to eliminate recording errors.
2. A person other than the researcher and Drs Amod and Sultana performed a 20% re-entering of data when the data recorded on the data collection tool were transferred to the electronic version of the data collection tool on an Excel® spread sheet. The 20% was randomly selected by this person to exclude undue influence from the researcher. This was the study supervisor, Dr JM du Plessis.

These measures ensured the value and integrity of the data management in this study and excluded errors. It also assisted the validity with possible publication of results.

## 1.10 Statistical analysis

The IBM SPSS Statistics for Windows, version 22.0, released in 2013, was used for the statistical analysis of the data. Ms M Cockeran, the statistician employed at MUSA, Faculty of Health Sciences at the North-West University, performed the statistical analysis.

For statistical analyses, the following tests were performed to determine the association between the identified risk factors and pancreatitis:

- Odds: Odds can be defined as the ratio of the number of positive events, to the number of negative events, with the total number of events being the sum of the positive and negative events. Within the context of this study, the number of patients with pancreatitis (as diagnosed by means of s-amylase levels), compared to the number of patients without pancreatitis, reflects the odds of developing pancreatitis after the first six months of LPV/r therapy. The results obtained did not require the odds ratios to be calculated.
- Probability: Probability can be defined as the ratio of detected events to the total amount of events. Within the context of this study, the ratio of patients with pancreatitis (as diagnosed by means of s-amylase levels), to the total number of patients in the sample reflect the probability of pancreatitis after the first six months of LPV/r therapy.
- Chi-square: A statistical test used to determine association between two independent groups of categorical data. Three sets of independent categorical data were tested for association in this study; pancreatitis and the presence of HTG (for the purposes of this study, a TG level of more than 1.7 mmol/L will indicate the presence of HTG), pancreatitis and gender, pancreatitis and CD<sub>4</sub> count, respectively.

The variables that were investigated in this study included:

### Independent variables

- Patients
- Gender
- Age
- Triglyceride levels (for chi square test)
- Presence of pancreatitis (for the chi square test)

## Dependent variables

- S-amylase values were a dependent variable in determining the odds ratio and probability of pancreatitis in the sample population after the first 6 months of LPV/R therapy
- CD<sub>4</sub> count

The objectives of this study and the corresponding statistical test and relevant variables are summarised in table 1.1.

**Table 1.1: Objectives, statistical tests and variables**

Objective	Statistical test	Variables
Prevalence: S-amylase levels elevated 3 times above upper limit among patients on LPV/r	Frequency	Independent variable: patients Dependent variable: s-amylase values
Association between pancreatitis and HTG among patients on LPV/r	Chi square	Categorical data: prevalence of pancreatitis and presence of HTG.
Association between gender and pancreatitis among patients taking LPV/r	Chi square	Categorical data: Gender and presence of pancreatitis
Pancreatitis association with CD <sub>4</sub> count	Correlation analysis	Continuous data: CD <sub>4</sub> and s-amylase values

### 1.11 Ethical considerations

Due to the stigma of HIV/AIDS in South Africa, it was important to ensure the confidentiality and anonymity of each patient.

- Preliminary approval from hospital management was obtained from the facility where the study will be conducted (Annexure B).
- Further approval was obtained from the PHRC of KwaZulu-Natal (Annexure C) once this research proposal was approved by MUSA.
- Final approval to conduct the study was obtained from HREC-NWU (Ethics number NWU-00356-15-A1) (Annexure D).
- Confidentiality: The patient's privacy was of paramount importance as enshrined by the National Health Act no 61 of 2003. Confidentiality was critical since a breach would have also compromised anonymity and hold serious legal ramifications.
- Anonymity: No information that may potentially reveal that a patient's data were used for this study was recorded or revealed at any stage of the study.
- Data were collected retrospectively and no recruitment will be done.



### **1.11.1 Confidentiality**

Law protects the doctor-patient relationship and any information that arises from this relationship is legally protected and remains confidential. The list of the study population retrieved from TIER.Net that contained the patients' names and hospital numbers was kept in a file in the doctors' consultation rooms. Once sampling was performed and the retrospective data recorded on the data collection tool, the list of the study population was destroyed. The data required for this study were anonymised to ensure that no information used or generated by this study was disclosed in a manner that will compromise the patients' privacy. While doctors were in the process of populating the data collection tool, the documentation was kept in a file in the doctors' consultation rooms and contained no personal information of any of the patients.

Once the data collection process was completed, the completed data collection tool for each doctor was handed to the researcher.

The researcher kept the completed data collection tool at his residence in a locked file cabinet. The data were entered into a Microsoft Excel sheet on a computer for purposes of analysis. The computer used was the personal computer of the researcher located in his study at his residence. Access to the computer was protected with a 10-digit password.

After the study was completed, hard copies of the populated data collection tool were stored at the offices of MUSA for a period of seven years, after which the research assistant will destroy it.

### **1.11.2 Anonymity**

The doctors recorded neither the patient's name nor other personal information on the data collection tool. The file containing the partially completed data collection tool was in the consultation room, which remained locked when the doctor was not on duty.

Whenever there was a need for one of the two doctors, permanently appointed at the CDC, to consult the file containing the medical records of a patient, no reference of participation in the study was made in or on the patient's file. The file was not marked on the outside in a way that revealed participation. The patient's file was returned and stored in archives to which only authorised staff have access. The staff that handled the patient files in archives were not able to tell whether the patient's data were being used for the study or not. Furthermore, they were not aware of the study in the first instance.

### **1.11.3 Justice**

There were no special appointments or commitments to be honoured, since this was a cross-sectional, retrospective and observational study. The burden was entirely with the researcher for the execution of the study and the doctors who are permanently appointed at the CDC to collect the data.

### **1.11.4 Justification of research study**

Knowing the probability and risk of pancreatitis among adult patients on LPV/r therapy can help clinicians to act proactively in the management of acute pancreatitis as a side effect.

### **1.11.5 Benefit-risk ratio analysis**

The benefits that were gained by conducting this study may provide insight into the risk of pancreatitis among patients taking LPV/r. This may improve the management and care of these patients. This study was classified by HREC-NWU as a medium risk study, as personal health records were assessed. As in any study, the patients' anonymity and confidentiality needed to be protected. The data collection process and storage of data were designed in such a way that nullified the risk of breach of anonymity and confidentiality. None of the parties concerned with the study experienced any discomfort or pain.

The benefits therefore outweighed the risk for this study.

#### **1.11.5.1 Direct benefits**

- Patient – No direct benefits.
- Medical community – No direct benefits.

#### **1.11.5.2 Indirect benefits**

- Patient – Those patients that could be identified with pancreatitis during the data collection process could be followed up by the doctors at the CDC for assessment.
- Possible intervention in the cases where pancreatitis may have been overlooked during the specific consultation, after the first six months of LPV/r therapy.
- The relevant public regional hospital, the Department of Health and the medical community. The medical community may gain insight into the prevalence and risk of pancreatitis after the first six months on LPV/r therapy in adult patients

### 1.11.6 Anticipated risks and precautions

#### 1.11.6.1 Anticipated risks to the participants and precautions taken

The potential risk to the participants and precautionary measures taken are summarised in table 1.2.

**Table 1.2: Risk to participants and precautions taken**

Risk	Precautions
Anonymity and confidentiality	Only the doctors at the CDC had access to the data and data collection tool where they recorded the required data for the purposes of this study. The patients' names and other personal information were not recorded on the data collection tool to ensure anonymity and confidentiality

#### 1.11.6.2 Anticipated risks to the researcher and precautions taken

The potential risks to the researcher and precautionary measures taken are summarised in table 1.3.

**Table 1.3: Risks to researcher and precautions taken**

Risk	Precaution
Loss of patient files and records	If access to one of either the NHLS online archive or physical patient records was compromised, the other could have been used to retrieve the data. The two doctors employed at the CDC, who collected the data, were unable to prevent the loss of patient records before they are required to be accessed for data collection purposes since neither have any influence in the management of NHLS database nor the archives where physical records are kept. In cases when neither the NHLS records nor physical records could be obtained for a particular patient, the patient was replaced in the sample by selection of another patient by the procedure explained in 1.8.
The two doctors at the CDC who will collect the data had to do so during working hours	The task was time-consuming and data collection was done in the afternoons when the CDC was quiet in order to not affect service delivery.

### **1.11.7 Reimbursement of study participants**

No party carried any form of expense for the carrying out of this study. It was a retrospective study in which the patient visited the CDC as per normal appointment schedules and was treated according to the KZN Department of Health guidelines and that of the NDoH.

### **1.11.8 Dissemination of research results**

The results of this study were compiled in a mini-dissertation and the publication of an article in a peer-reviewed scientific journal. The results were also presented to the medical staff at the public regional hospital during a formal presentation event held once per week at the hospital. The results were further discussed in detail with the medical staff at the CDC of the hospital. It will also be disseminated at a relevant medical or pharmaceutical conference.

### **1.11.9 Role of the members in the research team**

The researcher (Mr W Greffrath) is permanently employed as a pharmacist at public regional hospital in the position of assistant pharmacy manager. His duties included the procurement and management of pharmaceutical stock and the coordination and management of pharmacy staff and activities. The researcher was responsible for all matters related to study design and execution (except for the actual data collection). The researcher carried any cost incurred at any stage of the study.

The doctors at the CDC, Drs F Amod (MBBS, Head of Department, CDC) and MS Sultana (MBBS), were responsible for obtaining a list of possible study participants who complied with the inclusion criteria. They then collected the relevant data of the patients that the sample consisted of and recorded the information on the data collection tool.

The doctors have expressed that they have no interest to be co-authors in this study. They are satisfied with their role in assisting the researcher in collecting the data for the study as they considered the access to the results of the study to be in their interest. See written agreements in annexures E and F.

They are furthermore qualified and experienced medical officers at the facility. Both have some research experience from small studies done at the CDC where they are appointed on a full-time basis.

Dr JM du Plessis – Study supervisor employed by the North-West University. Dr du Plessis is a medical practitioner, has published numerous research articles and serves as a reviewer for various peer-reviewed journals, and therefore has vast experience and knowledge in this field.

Dr M Viljoen – Co-supervisor employed by the North-West University. Dr Viljoen is a practising pharmacist with a PhD in pharmacology. She has published and authored various research articles related to ARV therapy in international journals and therefore has vast experience and knowledge in this field.

Ms M Cockeran – She is a subject specialist in statistics in the niche area: Medicine Usage in South Africa (MUSA) at the NWU Potchefstroom Campus. She has recently completed her MSc in Statistics.

#### **1.11.10 Conflict of interest**

The student researcher was employed at the public regional hospital where the study was conducted.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Areas of investigation and sources consulted

The literature review covered the following subjects:

- Clinical management of HIV/AIDS in adults (including ART).
- ART guidelines in South Africa.
- The role of PIs in ART.
- Co-morbidities caused by HIV infection.
- The relationship between HIV and pancreatitis.
- Pathophysiology and aetiology of pancreatitis, particularly the role of TG and s-amylase.
- The effect LPV/r has on metabolism, specifically serum lipids and liver enzymes.
- The prevalence of metabolic changes caused by LPV/r in other populations with regard to ethnicity and age.
- Confounders that may independently influence TG and total cholesterol, and whether their influence carries any clinical significance.
- What range of change in serum lipids and liver enzymes warrants intervention?

Websites such as the following were consulted:

- Medscape
- EBSCOhost®
- Scopus®
- Pubmed®
- Aids Reviews
- Aids Info
- WHO
- Google Scholar™

Keywords that were used in combination or as individual entities: 'HIV', 'lopinavir/ritonavir', 'pancreatitis', 'protease inhibitors', 'side effects', 'adverse effects', 'metabolic changes', 'dyslipidaemia', 'hypertriglyceridaemia', 'triglycerides', 's-amylase', 'metabolic co-morbidities'

## 2.2 Introduction

The relationship between HIV and the human immune system was first described in the early 1980's (Barré-Sinoussi *et al.*, 1983:868). As reports of HIV infection increased from around the world, so did the need for appropriate treatment and it was only in 1987 that the Food and Drug Administration of the United States of America approved the use of the first ARV, zidovudine (Arts & Hazouda, 2012:2; Palmisano & Vella, 2011:47). In hindsight, it is now clear how little was known about the HIV infection and its management. Today, almost 3 decades later, it is well-known that treatment with a single ARV is not effective nor sustainable in the long term – a combination of at least three ARVs is required for adequate viral suppression (WHO, 2015).

As the HIV infection turned into a global pandemic, massive research and development became necessary for the successful treatment of the HIV infection. In the subsequent years after the introduction of zidovudine, several more ARVs of different classes were developed. Today almost 20 different ARVs spanning 6 different pharmacological classes are available for the treatment of HIV (Volberding & Deeks, 2010:49). In addition to the pharmacological treatment of HIV, comprehensive non-pharmacological treatment and management of the HIV infection became equally important for the management of the pandemic and prevention of further transmission. The World Health Organization (WHO) published its first guidelines for the treatment of HIV in 2001 (WHO, 2015). Several subsequent updates followed with the latest edition published in 2013 where the WHO refers to the broad continuum of care for HIV patients. This continuum of care refers to all aspects of HIV management – both pharmacological and non-pharmacological - for all age groups and different populations (WHO, 2015).

This comprehensive approach has drastically influenced the epidemiology of the pandemic. Antiretroviral therapy has improved the prognosis of the HIV infection from a fatal disease to a chronic illness (Volberding & Deeks, 2010:49). With the improved survival and prolonged life expectancy of HIV-infected patients, we are now seeing the HIV-infected population aging and it is estimated that more than half of the HIV population will be older than 50 years of age in 2015 (Warriner *et al.*, 2014:457). As a result, the HIV-infected population can be observed for much longer periods of time and with this the long-term co-morbidities of HIV and side effects of its treatment are becoming more apparent (Warriner *et al.*, 2014:457). HIV itself causes several non-AIDS-related co-morbidities such as cardiovascular disease, renal disease, bone disease, diabetes mellitus as well as pancreatitis (Mocroft *et al.*, 2009). The long-term side effects of many of the specific antiretroviral drugs also became apparent over the years. These include insulin resistance, bone loss, renal dysfunction and dyslipidaemia (Warriner *et al.*, 2015:458).

The long-term toxicity, as it relates to lipid metabolism of the PI combination of LPV/r, is of particular interest in this study. Dyslipidaemia, specifically HTG, is a well-known side effect of LPV/r (Croxtall & Perry, 2010:1907; Hawkins, 2010:205; Orkin *et al.*, 2013:57). Since HTG and HIV infection are both proven causes of pancreatitis (Drakovic, 2013:422), in a population where the prevalence of HIV is high as well as the relative usage of LPV/r as part of ART regimens, the prevalence of pancreatitis may also be noteworthy. The risk of TG-induced pancreatitis after a certain period of LPV/r treatment may therefore be worth investigating.

## **2.3 General guidelines for the treatment and management of HIV infection**

### **2.3.1 First contact with the patient**

The process of caring for adults that suffer from HIV/AIDS consists of several different stages and is continuous as with any chronic condition. Since no cure to HIV is available yet, the only endpoint to the care process is the death of the patient. The caring process starts the moment first contact is made at any medical facility where HIV care is available. Counselling and testing is the first step of the process and is the patient's crucial link to treatment and support (WHO, 2013:68).

Testing may be performed under different circumstances; voluntary counselling and testing of individuals, serodiscordant couples, survivors of sexual assault, drug addicts that use needles, partners of drug addicts, and pregnant mothers for the prevention of mother to child transmission (NDoH, 2014:20). Either the patient or the caregiver may initiate counselling and testing and may only be performed with the patient's consent (NDoH, 2014:20 & WHO, 2013:69). Rapid antibody tests are used to analyse the blood drawn from a finger prick (NDoH, RSA, 2014:21). A single test is not adequate in the case of a positive result. There should always be a second test to confirm the results of the first in case of a false positive result of the first, in order to avoid false diagnosis (NDoH, 2014:20; WHO, 2013:68). Should the second test result be negative, an enzyme-linked immunosorbent assay (ELISA) test should be performed in a laboratory for definite confirmation (NDoH, 2014:21). The confirmation of the patient's HIV status is very important to avoid unnecessary harm to the patient. The assurance of the quality of these tests is therefore very important to minimise any false positives and false negatives (WHO, 2013:68).

It is important to note that the testing is not only for the purposes of identifying those that are infected with HIV, but equally those that are not infected, because these individuals must be educated and counselled to maintain their negative status (NDoH, 2014:33).

According to the World Health Organization (2013:68), about half of the HIV-positive population are unaware that they are infected. With no knowledge of their infection, transmission of the



virus will obviously continue unabated. For this reason, large scale counselling and testing is of paramount importance in fighting the global pandemic of HIV. Access to treatment and adherence to treatment is important for the suppression of viral replication (Volberding & Deeks, 2010:49). Viral suppression and in turn, reduction in risky behaviour, are important for the prevention of transmission of HIV (Volberding & Deeks, 2010:50). Unfortunately, complete viral suppression is no guarantee to the restoration to perfect health, as virally suppressed individuals live shorter than those who are not infected. The exact reason why this is so remains elusive (Volberding & Deeks, 2010:49).

### **2.3.2 Disease progression**

The initial infection with HIV produces rather non-specific symptoms such as fever, malaise, diarrhoea, pharyngitis and rash (Volberding & Deeks, 2010:50). After the initial infection, an asymptomatic phase follows, that may last for years, and the patient will only start experiencing symptoms once the CD<sub>4</sub> count has dropped below 350 cells/μl. The progression of the HIV infection is characterised by the decline in the patient's CD<sub>4</sub> count and the generation of T-lymphocytes can be restored with ART (Sauce *et al.*, 2011:5142). Some patients with a low viral load continue to have a low CD<sub>4</sub> count. It therefore remains unclear exactly how the virus causes abnormally low lymphocyte counts in the blood (Sauce *et al.*, 2011:5142).

Depleted CD<sub>4</sub> numbers increase the risk of opportunistic, AIDS-defining infections (Volberding & Deeks: 2010:50). Most infected people will inevitably die from HIV infection, but there are a few people – known as controllers – that naturally control their HIV infection (Volberding & Deeks: 2010:50). These individuals are therefore of great interest for the developing of a vaccine for HIV.

### **2.3.3 Baseline testing**

Once a patient tests positive for HIV, a thorough personal and family history needs to be researched, a physical examination taken, and a systems review done (Aberg *et al.*, 2014:2; Aidsinfo, 2014:19). In addition, various other diagnostic tests need to be performed to establish the patient's immediate status regarding certain diagnostic markers that might be subject to change as the disease progresses and may be used to monitor response to treatment. In South Africa, the NDoH recommends the following for baseline testing:

- Confirmation of HIV status if no previous results are available.
- Clinical staging according to WHO guidelines. This is necessary to determine the urgency to initiate the patient on ART.
- CD<sub>4</sub> count – This is important not only to monitor future response to ART, but also to determine the urgency to initiate ART. A patient is eligible for ART with a CD<sub>4</sub> count of

less than 500 cells/ $\mu$ L. Lower than that and the patient may be prioritised ( $>350$  cells/ $\mu$ L), or fast tracked ( $>200$  cells/ $\mu$ L). With a  $CD_4 < 100$  cells/ $\mu$ L, the patient should be tested for cryptococcal antigen with cryptococcal latex agglutination.

- Pregnancy test – This is to identify women eligible for treatment for the prevention of mother to child transmission.
- Blood pressure and urine glucose – Screening for chronic comorbidities.
- Tuberculosis screening – Diagnose those with tuberculosis and assess eligibility for isoniazid prophylaxis.
- Hepatitis B virus – Those that test positive for the HBsAg should be initiated on HAART, regardless of  $CD_4$  count.
- Full blood count – HIV infection decreases haemoglobin levels (Meidani *et al.*, 2012:2). Anaemia can be diagnosed and treated. A patient's haemoglobin levels are also important when zidovudine is being considered for treatment because of the myelosuppressive effects of the drug (Aidsinfo, 2014:L-2).
- Creatinine – Renal sufficiency must be assessed if tenofovir is considered for treatment.
- Serum lipid panel – Increased cholesterol and serum lipid levels may increase if the patient is initiated on LPV/r (Chandwani & Shuter, 2008:1030; Croxtall & Perry, 2010:1906).
- Syphilis and sexually transmitted diseases.

Interestingly, the NDoH only requires a viral load at month six and none at baseline testing, as recommended by Aidsinfo and Aberg *et al.* (2014:2). Aidsinfo and Aberg *et al.* (2014:2), recommend a more elaborate panel of baseline tests. In addition to tests recommended by the NDoH, the following tests are also recommended:

- Viral load - A quantitative viral load test measures the amount of viral RNA copies in the blood. This can be done with a polymerase chain reaction (Volbering & Deeks; 2010:52). The baseline viral load is useful to monitor the response to treatment or to detect treatment failure (Aberg *et al.*, 2013:15-16). The primary objective of ART is to suppress the viral load to an undetectable level (Volbering & Deeks; 2010:52). Measuring the viral load is therefore a direct indication of treatment success or failure.
- Serology for hepatitis A, B and C – vulnerable patients should be vaccinated against hepatitis B and A (Aberg *et al.*, 2013:19).
- Fasting blood glucose – Recommended due to the increased prevalence of diabetes in the HIV-infected population (Aberg *et al.*, 2013:16-17).
- Genotype resistance testing – A virus resistant to a certain drug may be transferred from one individual to another (Aberg *et al.*, 2014:2). For the purposes of testing for resistance, genotype testing is preferred over phenotype testing (Antinori *et al.*,

2013:115). Since genotype testing depends on the availability of viral RNA in the blood, the test might not always be successful with patients with less than 500-1000 copies/ml (Aidsinfo, 2014:19). The results of the resistance test will have bearing on the regimen that patient should be initiated on (Antinori *et al.*, 2013:).

- HLAB\*5701 – There is an association between the HLAB\*5701 haplotype and abacavir hypersensitivity reactions. Hypersensitivity reactions are more common among white patients than black patients (Aidsinfo, 2014:C-22). Patients that test positive for HLAB\*5701 have a high risk of experiencing a hypersensitivity reaction in which case abacavir should be avoided (Aberg *et al.*, 2014:17).
- Co-receptor tropism assay - This test should be performed when the use of a CCR5 antagonist is being considered (Aberg *et al.*, 2014:16). Most HIV positive patients in the primary stages of the infection have a virus that binds to the CCR5 co-receptor to enter the host CD<sub>4</sub> cell. This variation is called the R5 virus. As the disease progresses, the patient also develops a variation of the virus that binds to the CXCR4 co-receptor to enter the host CD<sub>4</sub> cell – the X4 virus. CCR5 inhibitors like maraviroc will therefore only be effective for the R5 tropism of the virus (Volbering & Deeks, 2010:52-53).
- Toxoplasmosis - *Toxoplasmosis gondii* can be detected with an IgG assay (Aberg *et al.*, 2014:5).
- Cytomegalovirus.
- Cervical cancer – Cervical cancer is described as one of the AIDS-defining malignancies (Aidsinfo, 2014:E-7). Aberg *et al.* (2013:6) recommend a pap smear for all HIV-infected women.
- Human papilloma virus – Patients that are infected with the human papilloma virus have an increased risk of malignancies (Aberg *et al.*, 2014:21).

#### **2.3.4 When to initiate ART**

There used to be debate about when to initiate an HIV-infected person on ART. Some argue for an early and intensive start as opposed to a delay in initiation in order to minimise cost and avoid unwanted side effects from the medication (Volberding & Deeks: 2010:53).

Presently, most countries worldwide follow the WHO recommendations regarding the right time to initiate a patient on ART. For adults, the WHO recommends initiation of ART when a patient's CD<sub>4</sub> count drops below 500 cells/μL (WHO, 2013:92).<sup>1</sup> Those with a CD<sub>4</sub> count below 350 cells/μL should receive preference and for some patients ART should be initiated regardless of their CD<sub>4</sub> count. These include patients infected with tuberculosis, pregnant or breastfeeding

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<sup>1</sup> The recommended CD<sub>4</sub> count when to initiate ART was correct at the time of writing. The criteria for initiating ART has subsequently changed to include any patient that is tested HIV positive, regardless of CD<sub>4</sub> count.

(lactating) women, patient classified in stage 3 or 4 of HIV infection (NDoH, 2014:40; WHO, 2013:92), and the HIV-positive partner in a serodiscordant relationship. According to the WHO (2013:95), the mean CD<sub>4</sub> count upon initiation of ART is below 350 cells/μL on both high and low income countries. Most patients therefore seek help in the advanced stages of the HIV infection, which indicates how critical early testing is.

It is more beneficial for patients to start ART sooner rather than later to minimise HIV-associated co-morbidities (WHO, 2013:92). High viraemia increases the risk of cardiovascular, liver, renal, malignancies and neurocognitive co-morbidities (Aidsinfo, 2014:E-1).

Aidsinfo (2014:E-2) states that there is proof of benefit for starting ART at any CD<sub>4</sub> count; below 350 cells/μL, between 350 cells/μL and 500 cells/μL, and above 500 cells/μL. Starting ART in patients with a CD<sub>4</sub> count above 500 cells/μL is also rational since viraemia will be suppressed and transmission can be prevented.

The NDoH adopted the WHO recommendations of when to start ART, almost to the letter, judging by the patient's CD<sub>4</sub> count. The NDoH recommended that patient readiness should also be taken into account (NDoH, 2014:65) and that patients infected with tuberculosis should only be started on ART eight weeks after tuberculosis treatment has commenced (NDoH, 2014:66). This is mostly to avoid the debilitating effects of immune reconstitution inflammatory syndrome (IRIS) (NDoH, 2014:65). The phenomenon of IRIS manifests as a result of the recovering immune system that starts to recognise an undetected infection. It usually happens among patients with a CD<sub>4</sub> count of less than 200 cells/μL and during the first three months of ART treatment. Tuberculosis is the most common of these undetected infections along with herpes zoster, cytomegalovirus and herpes simplex (NDoH, 2014:65).

However, the current guidelines regarding the most suitable time to initiate ART are subject to change in the near future due to the preliminary findings of a trial conducted by the National Institute of Allergy and Infectious Diseases of the United States of America (the NIAID START trial), which found that the immediate start of treatment regardless of CD<sub>4</sub> count is most beneficial for HIV-positive patients (NIAID, 2015). A sample of 4685 ARV naive adults with CD<sub>4</sub> counts above 500 cells/μL were randomised into a group that received immediate ART and a second group for which ART was delayed until the CD<sub>4</sub> count was below 350 cells/μL. Serious AIDS-related events and non-AIDS related events were measured. A 53% reduction in adverse comorbidities was observed for those participants who started ART treatment with a CD<sub>4</sub> count higher than 500 cells/μL (NIAID, 2015).

### 2.3.5 What ARV combination to initiate for adults

As with any therapeutic regimen, the least toxic, most simplified, convenient and tolerable combination of drugs should be chosen for the treatment of HIV. The first line backbone of ART should be a combination of two NRTIs such as TDF (nucleotide reverse transcriptase inhibitor) with either 3TC or FTC (both nucleoside reverse transcriptase inhibitors) in combination with one NNRTI such as EFV or NVP (Aidsinfo, 2014:F-1; Volberg & Deeks: 2010:55; WHO, 2014:113). The TDF/FTC/EFV combination is the most ideal since it has a low pill burden (single dose tablet). The TDF/FTC combination has a high genetic barrier (Antinori *et al.*, 2012:119) against resistance development and EFV has a more favourable side effect profile than that of NVP, especially among pregnant woman with a CD<sub>4</sub> count of 250 cells/μL and more (WHO, 2013:113-115).

The HIV treatment guidelines have consistently evolved over the last decade as more became known about each antiretroviral agent and new drugs and fixed dose combinations (FDC) became available. The main changes affected in consecutive South African treatment guidelines for adults are summarised in the table below. Human immunodeficiency virus treatment guidelines in South Africa are consistent with the recommendations of the WHO and Aidsinfo. The combination TDF/FCT/EFV is the definite point of departure as first line of therapy in a single dose tablet. From here, the regimen will be individualised if needed, depending on the patient's laboratory results and the side effect profiles of each of the drugs.

The development of the South African standard treatment guidelines for antiretroviral treatment is summarised in table 4.

**Table 2.1: Standard treatment guidelines for antiretroviral treatment in South Africa since 2010**

South African antiretroviral treatment guidelines 2010 (NDoH, 2010)	
Comment	1 <sup>st</sup> line regimen
All new patients eligible for ART, pregnant women included	TDF + 3TC/FTC + EFV/NVP
Present d4T regimen without side effects	d4T + 3TC + EFV
Contraindication to TDF	AZT + 3TC + EFV/NVP
Comment	2 <sup>nd</sup> line
Failure on d4T or AZT based 1 <sup>st</sup> line regimen	TDF + 3TC/FTC + LPV/r
Failure on TDF based 1 <sup>st</sup> line regimen	AZT + 3TC + LPV/r
The South African antiretroviral treatment guidelines 2013 (NDoH, 2013)	
Comment	1 <sup>st</sup> line regimen

**Table 2.2: Standard treatment guidelines for antiretroviral treatment in South Africa since 2010 (continued)**

<b>South African antiretroviral treatment guidelines 2010 (NDoH, 2010)</b>	
<b>All new patients eligible for ART, pregnant women included</b>	<b>TDF + 3TC/FTC + EFV FDC preferred</b>
<b>EFV contraindicated</b>	<b>TDF + 3TC/FTC + NVP</b>
<b>TDF contraindicated</b>	<b>AZT + 3TC/FTC + EFV/NVP</b>
<b>TDF and AZT contraindicated</b>	<b>d4T + 3TC/FTC + EFV/NVP</b>
<b>Current d4T based regimen</b>	<b>TDF + 3TC/FTC + EFV/NVP FDC preferred</b>
<b>Comment</b>	<b>2<sup>nd</sup> line regimen</b>
<b>Failed TDF based 1<sup>st</sup> line regimen</b>	<b>AZT + 3TC + LPV/r</b>
<b>Failed d4T based 1<sup>st</sup> line regimen</b>	<b>TDF + 3TC/FTC + LPV/r</b>
<b>LPV/r induced dyslipidaemia and diarrhoea</b>	<b>Replace LPV/r with ATV/r</b>
<b>National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults 2014 (NdoH, 2014)</b>	
<b>Comment</b>	<b>1<sup>st</sup> line regimen</b>
<b>Adolescents &gt; 15 years weighing &gt; 40 kg Adults All tuberculosis co-infection All hepatitis B co-infection</b>	<b>TDF + 3TC/FTC + EFV as FDC</b>
<b>Adults and adolescent on d4T</b>	<b>Replace d4T with TDF (No patient should be on d4T)</b>
<b>Adolescents &lt; 15 years and &lt; 40 kg</b>	<b>ABC + 3TC + EFV</b>
<b>EFV contraindicated</b>	<b>TDF + 3TC/FTC + NVP or LPV/r</b>
<b>TDF contraindication</b>	<b>ABC + 3TC + EFV/NVP</b>
<b>Comment</b>	<b>2<sup>nd</sup> line regimen</b>
<b>Failed TDF based 1<sup>st</sup> line regimen</b>	<b>AZT + 3TC + LPV/r</b>
<b>Failed d4T and AZT based regimens</b>	<b>TDF + 3TC/FTC + LPV/r</b>
<b>LPV/r induced dyslipidaemia and diarrhoea</b>	<b>Replace LPV/r with ATV/r</b>
<b>Anaemia and renal failure</b>	<b>ABC + 3TC/FTC + LPV/r</b>

### **2.3.6 Monitoring response to treatment**

The ultimate objective of ART is to prolong the life of the infected patient and prevent or reduce associated morbidities. This is achieved by restoration of immunity by suppressing viral replication (Aidsinfo, 2014:D-1). The patient's CD<sub>4</sub> count and viral load are the main markers used to monitor the patient's response to treatment.

According to the NDoH guidelines of 2014, a CD<sub>4</sub> count must be done upon diagnosis of the HIV infection and repeated after 1 year of ARV treatment. The NDoH does not recommend a viral load assay to be done upon diagnosis of HIV infection, but only after month 6, month 12 and then every 12 months after initiation of ART.

In addition to asking the patient about possible drug-induced side effects at each visit, monitoring certain physiological parameters to detect the long-term side effects of certain ARVs is also important. A full blood count should be done for those patients on AZT on months 3 and 6, and serum creatinine for the patients on TDF at months 3, 6, 12 and then every 12 months. Interestingly, in the context of the results of this study, a fasting cholesterol and TG assay is only required at month 0 and month 3 if the patient is on an LPV/r-based regimen (NDoH, 2014).

A genotype test may be done in the case of virological failure to test for resistance (Aberg *et al.*, 2013:2).

### **2.3.7 When to change regimen**

A patient's ART regimen should be changed when the patient can either not tolerate the toxicity of any one or more of the ARVs in the combination, when certain health threatening side effects develop, when immunological failure develops, or when virological resistance develops (Volberding & Deeks, 2010:56, 200).

Toxicity and side effects can be easily detected by direct observation of the patient and the analysis of laboratory results.

In immunological failure the CD<sub>4</sub> counts will remain below 200 cells/μL in the advanced stages of the infection (Volberding & Deeks; 2010:200) despite viral suppression.

The first sign of resistance will be virological failure with a high viral load. Virological failure can be defined as a viral load of above 50 copies/ml after 24 weeks of therapy (Antinori *et al.*, 2012:122). Some factors like adherence, interruption of drug supply and prescribing habits may offer early warnings to potential resistance development (WHO, 2013:220-226).

## **2.4 Co-morbidities**

The effect that HIV has on the host's immune system, the CD<sub>4</sub> count in particular, has been well-documented (Barré-Sinoussi *et al.*, 1983:868). The resulting immunodeficiency syndrome makes the host susceptible to numerous opportunistic infections, which may be bacterial, viral, fungal or any combination of the aforementioned (Aidsinfo, 2016). Apart from the effect that HIV has on the immunity of its host, it also has a profound effect on some organs and systems in the

body that may result in cardiac, renal, hepatic, neurocognitive, metabolic, pancreatic and psychological disorders (Warriner *et al.*, 2014:457).

It is therefore possible to classify the co-morbidities of HIV into the non-infectious and the infectious kinds.

#### **2.4.1 Non-infectious co-morbidities**

With the dawn of the antiretroviral age, the prognosis of the HIV infection improved drastically from a fatal disease to that of a long-term chronic disease (Volberding & Deeks, 2010:50). The HIV-infected population is therefore living longer and as a consequence the long term, non-infective co-morbidities of the infection – as well as of ART – are becoming apparent (Warriner *et al.*, 2014:457). These can affect several organs and systems in the body:

- Cardiovascular disease - The inflammatory and immunological response related to HIV infection increases the risk of cardiovascular disease and subclinical atherosclerosis (Shrestha *et al.*, 2014); higher viral load is also associated with higher levels of pro-inflammatory interleukin-6 and D-dimer (Duprez *et al.*, 2012; Kuller *et al.*, 2008:1496), and ART naïve patients show higher levels of serum pro-coagulants and lower levels of anticoagulants (Baker *et al.*, 2013). Furthermore, increased TG and very low-density lipoprotein (VLDL) and lowered HDL have been observed in ART naive HIV-infected patients (El-Sadr *et al.*, 2005), which may have further metabolic or cardiovascular implications.
- Diabetes mellitus - The prevalence of diabetes mellitus among the HIV-infected population is as high as 14% (Warriner *et al.*, 2014:464). Regardless of ART, HIV-infected patients have greater odds for developing diabetes mellitus than the uninfected population (Tien *et al.*, 2008). Antiretroviral therapy – particularly multiple NRTI therapy – is associated with insulin resistance (Brown *et al.*, 2005) and a well-documented side effect of the PIs is hyperinsulinaemia and hyperglycaemia (Chandwani & Shuter, 2008:1030).
- Pancreatitis - The incidence of pancreatitis is less among those with asymptomatic HIV infections but increases as the disease progresses to the later stages (Dutta *et al.*, 1997:2045) with a definite correlation between full-blown AIDS and pancreatitis (Manfredi *et al.*, 2004:539). The aetiology of pancreatitis associated with HIV can be divided into three groups: opportunistic infections, drug induced and HIV itself, which will be discussed in detail in the following pages.
- Renal disease - Antiretroviral therapy has decreased the prevalence of HIV-associated nephropathy and renal failure (Warriner *et al.*, 2014:460), but regardless of adequate viral suppression, about 30% of patients infected with HIV still suffer from renal



dysfunction (Gupta *et al.*, 2005). However, ART related nephrotoxicity is common (Röling *et al.*, 2006); TDF causes proximal tubular damage and mitochondrial toxicity, and indinavir and atazanavir cause crystal deposits in the kidney (Warriner *et al.*, 2014:461). Nephrotoxicity is increased when TDF is co-administered with a ritonavir boosted PI by means of decreased flow of TDF from renal tubular epithelium, thereby assisting tubulopathy (Gallant & Moore, 2009).

- Bone disease - Decreased bone mineral density appear earlier in the HIV-infected population than the rest of the population, making fractures and osteoporosis more likely (Warriner *et al.*, 2014:462). Normal risk factors such as low body mass index, age and menopause also contribute. Hepatitis C infection and intravenous drug use are also associated with loss of bone mineral density and these factors are more common among the HIV-infected population (Warriner *et al.*, 2014:462). There is evidence that PIs cause osteopenia and osteoporosis (Tebas *et al.*, 2000).
- Obesity - In a study conducted in rural South Africa, it was found that obesity is 6 times more common among the HIV-infected population (Malaza *et al.*, 2012).

#### **2.4.2 Infectious co-morbidities**

- Tuberculosis - The most prevalent opportunistic infection suffered by people living with HIV/AIDS is tuberculosis (Makhado *et al.*, 2014:2). Tuberculosis is a bacterial infection caused by *Mycobacterium tuberculosis*. Other mycobacterium species also contribute to the disease burden. These include *M. avium complex*, *M. kansasii* and *M. xenopi* (Lawn *et al.*, 2005:361). According to the WHO (2015), tuberculosis is second only to HIV as the single infectious agent that causes the most deaths globally. *Mycobacterium tuberculosis* infects mostly the lungs but also other organs (WHO, 2015). About one third of the global population has latent tuberculosis and people living with HIV are about 30 times more likely to develop active tuberculosis than people that are not infected by HIV, with Africa having the highest incidence at 281 cases per 100 000 (WHO, 2015).
- Cryptococcal meningitis - Infection by *Cryptococcus*, in particular *C. neoformans*, is another massively prevalent opportunistic infection among people infected with HIV (Jarvis & Harrison, 2007:2119). *Cryptococcus neoformans* is a saprophyte that is common in pigeon droppings and is contracted via inhalation (Jarvis & Harrison, 2007:2119). The infection is not contagious and may lay dormant for years before activation (NDoH, 2014:89). Smokers and people that work outdoors are vulnerable and particularly those with a very low CD<sub>4</sub> count (NDoH, 2014:89; Jarvis & Harrison, 2007:2119). The most common cause of meningitis in Africa is caused by *C. neoformans* and accounts for 10-12% of all HIV associated deaths on sub-Saharan Africa (Jarvis *et al.*, 2014:736-736). As with tuberculosis, immediate initiation of ART is not

recommended, especially among those with a CD<sub>4</sub> count of less than 100 cells/ $\mu$ L as IRIS is a real possibility and may be life-threatening.

- Hepatitis B and C - About 5-20% of people living with HIV is affected by the hepatitis B virus (HBV). The prevalence of hepatitis C virus (HCV) is a little less at 5-15% and is more common among people that use injectable drugs (WHO, 2013:166). The WHO (2013:92) recommends ART for patients with HBV and chronic liver disease, regardless of CD<sub>4</sub> count. HIV affects the natural progression of HBV by inhibiting spontaneous clearance, causing more disease chronicity and liver fibrosis and increasing the risk of cirrhosis and hepatocellular carcinoma (WHO, 2013:98). Among patients that require a change of regimen from a TDF containing regimen it is important to screen for HBV before discontinuing the TDF in order to prevent a fatal hepatitis flare. Such a patient should continue with TDF as a fourth drug in their new regimen (NDoH, 2014:46).
- Candidiasis - Infection in the oropharynx and oesophagus is a very common co-morbidity of HIV. Before the ART era, 90% of patients infected with HIV suffered from candidiasis at some stage during their illness (Lortholary *et al.*, 2012:69). However, candidiasis is still problematic among those with treatment failure (Lortholary *et al.*, 2012:69).
- Herpes Zoster - Infection of herpes zoster occurs after the reactivation of varicella zoster (Weitzman *et al.*, 2013:464). People infected with HIV are particularly more susceptible for reactivation, but with the restoration of immunity with ART, the burden of this co-morbidity may be reduced (Blank *et al.*, 2012).
- Human papilloma virus - Long-term infection with human papilloma virus increases the risk for cervical cancer (Mbulalwa *et al.*, 2012:15). Cervical cancer is preventable and treatable if a human papilloma virus infection is diagnosed early (WHO, 2013:168).
- Malaria - Large parts of Africa is endemic to malaria and patients living with HIV are always in danger of developing serious complications from malaria infection. Patients infected with malaria should be treated quickly and monitored closely because of toxicities that the anti-malaria and ART could possibly share (WHO, 2013:167).

### **2.4.3 HIV and pancreatitis**

Pancreatitis can be associated with HIV, both directly and indirectly, and the aetiology of pancreatitis among people infected with HIV is diverse and may vary widely over geographical regions, as found by Sekimoto *et al.* (2006:13). Along with cardiovascular disease, liver disease, renal disease and HIV-associated malignancies, pancreatitis is one of the non-AIDS defining illnesses that are becoming more common (Mocroft *et al.*, 2009).

The incidence of pancreatitis is less among those with asymptomatic HIV infections but increases as the disease progresses to the later stages (Dutta *et al.*, 1997:2045), with a definite correlation between full-blown AIDS and pancreatitis (Manfredi *et al.*, 2004:539).

Bush & Kosmiski (2003:e1) estimated pancreatic abnormalities among patients infected with HIV as high as 35-800 times higher, compared to an uninfected population. However, obvious cases of pancreatitis among HIV-infected patients are not frequent and very few severe cases are reported. Elevated s-amylase levels are common among people infected with HIV, but as s-amylase values increase, the number of cases of increased values decrease (Manfredi & Calza, 2008:100). However, as evidenced by extensive post mortem studies, the prevalence of pancreatitis among people living with HIV/AIDS is much higher than that reported among those patients who are still alive. A thorough post mortem study done in the pre-ART era, indicated a 33.9% prevalence of pancreatitis among people living with HIV/AIDS and most cases were due to opportunistic infections (Manfredi & Calza, 2008:100). In another post mortem study cited by Manfredi & Calza (2008:100), the prevalence of pancreatitis was as high as 90% and showed further morphological abnormalities in the pancreas such as HIV related malignancies (Manfredi *et al.*, 2004:541) that could be associated with HIV itself. Therefore, many cases of pancreatitis go unnoticed and are subclinical, only detected post mortem (Manfredi *et al.*, 2004:541).

Evaluating the aetiology of pancreatitis among the HIV-infected population, two distinct time periods need to be considered; the pre-combination ART era and the post-combination ART era. The latter was the start of successful suppression of viral replication and the consequent restoration of immunity with a small incidence of opportunistic infections, but it is more difficult to monitor because combination ART introduces its own long-term toxicity and pathophysiology for pancreatitis, especially the nucleoside analogues (Manfredi & Calza, 2008:101; Manfredi *et al.*, 2004:537). Pathology that stems from lipid and glucose abnormalities, metabolic abnormalities, lactic acidosis and hepatic steatosis are now increasingly common (Manfredi & Calza, 2008:103).

In the pre-ART era, the suppression of viral replication was still inadequate and without the contributing side effects of combination ART to the incidence of pancreatitis. During this era, it is easier to examine the aetiology of pancreatitis among people living with HIV/AIDS and the effects of HIV itself – directly and indirectly, in the form of opportunistic infections – on the incidence of pancreatitis.

Alcohol and gall stones are common causes of pancreatitis among people infected with HIV (Raza *et al.*, 2013:13; Riedel *et al.*, 2008:2; Sekimoto *et al.*, 2006:10) just as among the general population, but they certainly do not account for all cases of pancreatitis among people infected by HIV. The specific pathophysiology of HIV infection, introduced several other aetiologies.

Given the effect that HIV has on the host CD<sub>4</sub> count, infective aetiology can be anticipated. The most common opportunistic infections of the pancreas, as identified by several studies over the last two decades (Dowel *et al.*, 1996:44; Manfredi & Calza, 2008:100; Raza *et al.*, 2013:13; Riedel *et al.*, 2008:5), include:

- Cytomegalovirus
- Varicella zoster
- *Toxoplasma gondii*
- *Cryptococcus neoformans*
- Cryptosporidiosis
- *Mycobacterium tuberculosis*
- *Mycobacterium avium*
- Herpes simplex virus
- *Pneumocitis jirovecii*

Drug-induced pancreatitis may be due to several ARVs, as well as other drugs used for the management of HIV infection (Dowel *et al.*, 1996:46; Dutta *et al.*, 1997:2047; Manfredi & Calza, 2008:103; Manfredi *et al.*, 2004:540; Moore *et al.*, 2001:617; Raza *et al.*, 2013:13-14; Sekimoto *et al.*, 2006:14-15):

- Didanosine
- Stavudine
- Zidovudine
- Pentamidine
- Co-trimoxazole
- Dapsone
- Antituberculosis drugs
- Azathioprine
- Mercaptopurine
- Aminosalicylates
- Valproic acid

Opportunistic infections and drug-induced pancreatitis are aetiologies of pancreatitis brought on by the fact that a patient is HIV-infected. However, HIV itself has also proven to be pancreotoxic. It has been found that the cell infiltrate of chronic pancreatitis contains macrophages carrying CCR5 receptors that will facilitate the inflammatory process in the pancreas (Manfredi & Calza, 2008:103, Manfredi *et al.*, 2004:538).

The severe metabolic changes caused by the HIV infection may also play a role in the histology of pancreatitis that makes a patient more prone to pancreatitis (Riedel *et al.*, 2008:5). Infection with HIV also causes lymphoma and Kaposi sarcoma of the pancreas (Dutta *et al.*, 1997:2044).

The decrease in CD<sub>4</sub> count and consequent immunosuppression invites opportunistic infections, but CD<sub>4</sub> cells are also important in the prevention of acinar cell necrosis, which leads to pancreatitis (Riedel *et al.*, 2008:5). The CD<sub>4</sub> count can therefore also be considered a risk factor for pancreatitis (Manfredi & Calza, 2008:100; Raza *et al.*, 2013:14; Ueda *et al.*, 2006:783). The CD<sub>4</sub> count is so closely linked to pancreatitis that it can be used as predictor of pancreatitis with 72% specificity and 77.8% sensitivity (Ueda *et al.*, 2006:783). Similarly, the CD<sub>8</sub> count can also be used with 69.2% specificity and 77.8% sensitivity (Ueda *et al.*, 2006:783). The ability of the CD<sub>4</sub> and CD<sub>8</sub> counts to predict pancreatitis is as good as a conventional scoring system used for the diagnosis of pancreatitis (Ueda *et al.*, 2006:783).

## **2.5 Aetiology, pathophysiology, diagnosis and treatment of pancreatitis**

The pancreas is an elongated organ located in the abdomen behind the stomach and performs an endocrine and exocrine function (Hopkins Medicine, 2015).

As an exocrine gland, the pancreas secretes various digestive enzymes into the duodenum via the common bile duct. The enzymes are secreted by the exocrine acinar cells (Freedman, 2012) and include trypsin, chymotrypsin and carboxypeptidase for the digestion of proteins; ribonuclease and deoxyribonuclease for the digestion of nucleic acid; and lipase and amylase for the digestion of fat and polysaccharides respectively (Vander *et al.*, 1999:577). The pancreas also secretes bicarbonate into the duodenum to neutralise the acid from the stomach (Hopkins Medicine, 2015). These enzymes play a role in the pathophysiology of pancreatitis and some are used as markers for the diagnosis of pancreatitis.

The endocrine function of the pancreas is the secretion of insulin and glucagon by the beta cells directly into the bloodstream for the regulation of blood glucose (Hopkins Medicine, 2015).

Pancreatitis is inflammation of the pancreas and surrounding tissue (Freedman, 2012) and may be acute or chronic. Acute pancreatitis usually resolves with no permanent tissue damage, but chronic pancreatitis causes permanent tissue damage that ultimately progresses to pancreatic failure, which can affect both the exocrine and endocrine functions of the pancreas (Freedman, 2012). Pancreatitis is potentially fatal with a 5% mortality rate (Philip *et al.*, 2014:158). Early diagnosis is therefore important for timely intervention to ensure a favourable prognosis.

### **2.5.1 Aetiology and risk factors of pancreatitis**

There are numerous causing factors of pancreatitis. In the case of acute pancreatitis, the majority of cases can be ascribed to biliary tract disease (gall stones) or alcoholism (Freedman, 2012; Lankisch *et al.*, 2015:85). Some cases of chronic pancreatitis are caused by alcoholism, but many cases are completely idiopathic and less common causes such as hyperparathyroidism and obstruction of the main pancreatic duct also contributing to morbidity (Freedman, 2012). Many people also have a genetic predisposition for pancreatitis (Phillip *et al.*, 2014:159).

Several drugs have been linked to pancreatitis – the WHO has listed more than 500 – but only about 30 cause pancreatitis when a patient is re-challenged (Wu & Banks, 2013:1277). Some of the more common drugs considered to definitely contribute to the risk of pancreatitis are paracetamol, azathioprine, carbamazepine, cimetidine, didanosine, enalapril, erythromycin, oestrogens, furosemide, hydrochlorothiazide, itraconazole, lamivudine, metronidazole, opiates, pentamidine, simvastatin, steroids, sulfasalazine, co-trimoxazole and olanzapine (Lankisch *et al.*, 2015:86).

Other contributing factors that may increase the risk of pancreatitis are smoking, obesity, ages of about 60 years and older (Phillip *et al.*, 161; Wu & Banks, 2013:1274). Also dysfunction of the sphincter of Oddi, females and a previous history of pancreatitis (Lankisch *et al.*, 2015:86). Co-morbidities such as diabetes mellitus type 2 (Lankisch *et al.*, 2015:85), cancer, heart failure, kidney and liver disease also contribute to risk and an unfavourable prognosis (Wu & Banks, 2013:1274).

Hypertriglyceridaemia – a risk factor particularly relevant to this study – is also a well-documented risk factor of pancreatitis (Brock *et al.*, 2013:7236; Lankisch *et al.*, 2015:87; Phillip *et al.*, 2015:159). Lopinvir/ritonavir is known to cause HTG (Chandwani & Shuter, 2008:1030; Croxtall & Perry, 2010:1906), which in theory, should increase the risk of pancreatitis. Insulin resistance is also a well-known side effect of LPV/r (Chandwani & Shuter, 2008:1030; Lee *et al.*, 2004:6). Considering that diabetes mellitus type 2 is also a risk factor for pancreatitis (Lankisch *et al.*, 2015:85) and patients taking LPV/r are almost invariably also taking lamivudine, it can be expected that the risk of pancreatitis is higher among the diabetic population.

### **2.5.2 Pathophysiology of pancreatitis**

Regardless of aetiology, the pathophysiology of acute and chronic pancreatitis is similar, except that with acute pancreatitis, there is complete recovery with no tissue damage whereas chronic pancreatitis progresses to fibrosis and permanent tissue damage. The pancreatic enzymes become activated inside the pancreas and the resulting tissue damage, due to auto-digestion,

activates the complement system and inflammatory response. The resulting symptoms include inflammation, oedema and sometimes tissue necrosis (Freedman, 2012).

There are various reasons why pancreatic enzymes become activated inside the organ itself. In the case of alcoholic pancreatitis, precipitation of the proteins of pancreatic enzymes, in the small pancreatic ductules, may cause premature activation of pancreatic enzymes when alcohol is consumed (Freedman, 2012). In chronic pancreatitis, the protein plugs are caused by an excessive secretion of glycoprotein-2 or a deficient excretion of lithostatin, which inhibits calcium precipitation in the pancreas (Freedman, 2012). Alcohol itself also has a direct toxic effect on the pancreas by possibly causing contraction of the sphincter of Oddi, but also formation of calculi, which causes obstruction preventing the pancreatic digestive enzymes from leaving the pancreas. Alcohol also has an effect on the pancreatic stellate cells that increase synthesis of cytokines, which lead to inflammation (Lankisch *et al.*, 2015:86).

Obstruction of the sphincter of Oddi or anywhere along the bile duct, by gallstones or microlithiasis, will cause a physical obstruction and increase in ductal pressure (Brock *et al.*, 2013:7235), which prevents the digestive enzymes from leaving the pancreas (Lankisch *et al.*, 2015:86). This obstruction consequently impedes the exocytosis of the zymogen granules that contain the digestive enzymes from the pancreatic acinar cells (Lankisch *et al.*, 2015:86), which leads to auto-digestion.

Pancreatitis can also be inherited. Certain mutations in the cationic trypsinogen gene (SPINK1) cause pancreatitis in up to 89% of carriers and have a very clear familial trend (Brock *et al.*, 2013:7235; Freedman, 2012). SPINK1 is a serine PI gene, which prevents the activation of trypsinogen to active trypsin – and consequently the cascade activation of other pancreatic enzymes – inside the pancreas. A mutation in this gene compromises the inhibitory effect of SPINK1, which leads to auto-digestion (Brock *et al.*, 2013:7234).

Pancreatitis may also be caused by an autoimmune response, but the pathogenesis of autoimmune pancreatitis is thus far, not well understood (Brock *et al.*, 2013:7236).

The damage to the acinar cells due to auto-digestion activates the cellular response that leads to the release of tissue necrosis factor alpha and cytokines, and the infiltration of leukocytes and macrophages. The prognosis of the resulting inflammation has varying degrees of severity. In mild cases, the inflammation is limited to the pancreas but in the more severe cases the inflammation is systemic with necrosis and haemorrhage, and the necrotic tissue may become infected (Freedman, 2012). The systemic inflammation increases capillary permeability and decreases the tone in the vasculature, which leads to oedema, respiratory distress and renal failure (Freedman, 2012).

The damage to the pancreatic tissue may leave organic debris in the organ. Some of this debris clears up spontaneously and some form pseudocysts in the pancreas. The pseudocysts are fibrous capsules without a lining of epithelial cells that may rupture, haemorrhage or become infected (Freedman, 2012).

In chronic pancreatitis, the persistent inflammation and tissue damage over years lead to fibrosis and eventual loss of exocrine and endocrine function (Freedman, 2012).

In extreme cases, death may occur due to shock and renal failure (Freedman, 2012; Brock *et al.*, 2013:7231).

### **2.5.3 Diagnosis of pancreatitis**

Acute pancreatitis is characterised by a sudden onset of epigastric pain that may radiate towards the back. There may also be nausea, vomiting and excessive sweating (Wu & Banks, 2013:1272). Some cases may also present with Grey Turner sign and the Cullen sign – ecchymosis of the flanks and umbilical areas respectively with usually no elevation in temperature, a quick pulse and shallow, fast breathing (Freedman, 2012).

In chronic pancreatitis the epigastric pain may last for days. In advanced stages of the disease, when the acinar cells have been destroyed and no more enzymes are secreted, there will be no pain. The absence of lipase in the duodenum will result in fatty stools and steatorrhoea (Freedman, 2012).

It is often difficult to clinically diagnose acute pancreatitis because of several other diseases that present with the same symptoms. It is therefore important to rule out the differential diagnoses like acute cholecystitis, penetrating duodenal ulcer, myocardial infarction and bowel obstruction (Wu & Banks, 2013:1272).

Laboratory results will help a great deal in confirming the diagnosis. A urine trypsinogen-2 test has both sensitivity and specificity greater than 90%. Serum amylase and lipase will be significantly elevated, bilirubin may be elevated and serum calcium falls due to the formation of soaps with the fatty acids produced by lipase (Freedman, 2012). Serum amylase values of 3 times the upper normal limit were used as diagnostic criteria for this study. The upper normal limit for s-amylase of the NHLS is 104 U/L.

Imaging can also be used to confirm diagnosis and assist with the determination of the aetiology. An X-ray will reveal the calcification of the ducts in the case of chronic pancreatitis and ultrasonography will be able to confirm gall stone pancreatitis. An abdominal CT scan will



reveal tissue necrosis, fluid collection and the formation of pseudocysts (Freedman, 2012; Lankisch *et al.*, 2015:89; Phillip *et al.*, 2012:160).

Once pancreatitis has been diagnosed it is important to determine the degree of severity in order to appropriately treat the condition. The Revised Atlanta classification is widely used to classify pancreatitis into three degrees of severity (Acevedo-Piedra *et al.*, 2014:311):

- Mild with no organ failure
- Local complications only with transient organ failure
- Systemic complications with organ failure

#### **2.5.4 Clinical management of pancreatitis**

Treatment of pancreatitis is usually only supportive with careful management of complications. Fluid resuscitation is very important to reduce mortality and ensure adequate microcirculation (Phillip *et al.*, 2014:163). Analgesia also plays an important role as well as nutritional support (Lankish *et al.*, 2015:90; Phillip *et al.*, 2014:163; Wu & Banks, 2013:1274-1276). In chronic pancreatitis, the pseudocysts can be drained and enzyme supplements can be taken to treat steatorrhea and proton pump inhibitors, or H<sub>2</sub>-antagonists to reduce acid secretion in stomach. The reduction of acid secretion will cause less secretin secretion, which in turn will cause less pancreatic enzyme secretion (Freedman, 2012). Elimination of risk factors such as smoking, alcohol consumption or an offending drug is also important (Phillip *et al.*, 2014:163).

#### **2.5.5 The relationship between pancreatitis and HTG**

Triglycerides are one of the main sources of energy to the body. Once TG are ingested they are partially hydrolysed, then resynthesised into TG in the gut mucosa and then secreted into the lymphatic system in the form of chylomicrons (Berglund *et al.*, 2014:424; Brock *et al.*, 2013:7236). Lipoprotein lipase, an enzyme present on the luminal surface of the endothelium, hydrolyses the TG of the chylomicrons into free fatty acids that is then absorbed into the tissue (Berglund *et al.*, 2014:424).

Many patients with elevated serum lipids present with HTG, but it is the elevated levels of chylomicrons that ultimately cause pancreatitis (Valdivielso *et al.*, 2014:689). It is a well-documented fact that HTG causes pancreatitis, but the exact mechanism by which this happens is still being debated (Valdivielso *et al.*, 2014:690). The most widely accepted theory postulates that as the chylomicron levels rise – not just from ingested TG, but also from synthesis in the liver – it becomes more difficult for the body to clear the high levels of chylomicrons from the bloodstream after a sharp post prandial peak (Friedewald *et al.*, 2013:1134). These high levels of chylomicrons cause the blood to be more viscous, which leads to ischemia and acidosis in

the pancreas thereby causing tissue damage (Friedewald *et al.*, 2013:1134; Valdivielso *et al.*, 2014:690). Ischemia along with the pancreatic digestive enzymes, lead to auto-digestion of the pancreas (Friedewald *et al.*, 2013:1134). The high levels of TG hydrolysed by lipoprotein lipase, also produce high levels of free fatty acids that exceed the binding capacity of albumin. As a result, the free fatty acids aggregate into micelles with detergent properties, which cause damage to the capillaries in the pancreas and the pancreatic acinar cells (Valdivielso *et al.*, 2014:690). One of the symptoms of chylomicronaemia is the formation of xanthoma on the skin. A biopsy of the xanthoma will reveal inflammation with leukocytes, monocytes and macrophages. This suggests that TG are also pro-inflammatory which may contribute to the inflammation of pancreatitis (Friedewald *et al.*, 2013:1134).

Although the most common aetiologies of pancreatitis are gallstones or alcohol, TG induced pancreatitis is not all that uncommon (Valdivielso *et al.*, 2014:689). Up to 10% of acute pancreatitis cases are due to HTG and the odds of pancreatitis increase as TG levels increase (Toth *et al.*, 2014:795). Some primary causes of HTG are hereditary or familial and also a deficiency of lipoprotein lipase (Sandhu *et al.*, 2014:1). Secondary causes of hypertriglyceridemia may be other disease conditions or certain medication. Uncontrolled diabetes mellitus and pregnancy, elevate TG and medications such as retinoids, beta-blockers, oestrogens, hydrochlorothiazide, tamoxifen, and certain antiretroviral drugs also cause elevated TG (Berglund *et al.*, 2014:427-429; Sandhu *et al.*, 2011:1).

Clinically, TG-induced pancreatitis does not present any different from pancreatitis of other aetiology and the treatment mostly remains the same (Valdivielso *et al.*, 2013:691). Naturally, it would be necessary to limit the predisposing factors of pancreatitis in order to limit the progression of the disease and also prevent future incidents. In the case of TG-induced pancreatitis, much can be done in the form of pharmacologic and non-pharmacologic interventions to lower TG levels. To achieve an immediate lowering of TG, plasmapheresis may be performed. Although there is no data from a randomised controlled trial that the removal of TG by plasmapheresis improves morbidity and mortality, the fact remains that this technique does remove the causative agent from the blood (Valdivielso *et al.*, 2013:691). Heparin and insulin infusions are also used to reduce TG. Heparin releases lipoprotein lipase from the endothelial cells, which produces a short term lowering of TG. However, continuous use of heparin may deplete lipoprotein lipase and as consequently lead to raised TG levels once again, precipitating acute pancreatitis (Valdivielso *et al.*, 2013:691). Insulin is used for the role it plays in the synthesis of lipoprotein lipase. Again there is no clear evidence from randomised controlled trials on the use of heparin and insulin, but they are used in practise none the less (Valdivielso *et al.*, 2014:591).

Long-term management of HTG include lifestyle and dietary changes. The dietary intake of saturated and unsaturated fats must be reduced (Valdivielso *et al.*, 2014:691). Exercise and weight loss are also important interventions in the management of HTG (Berglund *et al.*, 2014:431). Pharmacologically, TG can be reduced by HMG-CoA reductase inhibitors, fibrates, niacin and omega-3 fatty acids (Berglund *et al.*, 2014:432; Valdivielso *et al.*, 2014:691). Ultimately, the goal is to maintain TG as low as possible to avoid the sharp postprandial increase in TG that may precipitate acute pancreatitis (Valdivielso *et al.*, 2014:691).

## **2.6 The side effects of LPV/r and how they compare to other PIs**

The most common observable side effects of LPV/r are related to the gastrointestinal tract such as diarrhoea, nausea and vomiting (Chandwani & Shuter, 2008:1029; Croxtall & Perry, 2010:1904; Lee *et al.*, 2004:5). Mild to moderate diarrhoea occurs in approximately 10-15% of patients taking LPV/r (Camacho & Rivero, 2014:32). Nausea and diarrhoea are often dosage related, occurring more among patients who take a once-daily dose of LPV/r and less so among patients with a twice-daily dosage (Croxtall & Perry, 2010:1904). More significant though, are the metabolic side effects of LPV/r, which, in the long term, may have serious health implications for the patient. The changes that LPV/r affect on certain metabolic parameters have been well documented. These include significant changes in TG levels and total cholesterol (Chandwani & Shuter, 2008:1030; Croxtall & Perry, 2010:1906), hyperglycaemia and insulin resistance (Chandwani & Shuter, 2008:1030; Lee *et al.*, 2004:6), elevation in serum ALT and AST (Croxtall & Perry, 2010:1906).

All PIs cause hyperlipidaemia, lipodystrophy, hyperglycaemia and insulin resistance in general (Arathoon *et al.*, 2013:12; Calza *et al.*, 2003:57). Hypertriglyceridaemia is the most common form of dyslipidaemia and occurs in up to 90% of patients. Hypercholesterolaemia occurs in up to 50% of patients, with hyperglycaemia and hyperinsulinaemia being less common (Calza *et al.*, 2003:57). Of interest, is how the different PIs compare among each other in terms of the changes affected on the different components of serum lipids. Indinavir, sequinavir and amprenavir have no effect on lipoproteins and are more associated with insulin resistance (Lee *et al.*, 2004:6). Other PIs, such as ritonavir and LPV/r have a more profound effect on serum lipids. Ritonavir in particular, causes marked elevation in TG, VLDL and apolipoprotein A and B (Lee *et al.*, 2003:57). The elevation of serum lipids is time and dose related, and seems fastest with treatment involving ritonavir (Calza *et al.*, 2003:57). The effect of ritonavir on serum lipids is also confirmed where it is the common denominator in combination PI therapy. Lopinavir/ritonavir therapy compared to darunavir/ritonavir (DRV/r) therapy, with the same dose of ritonavir, causes a larger increase of TG (Arathoon *et al.*, 2013:15) and also a more significant effect on serum lipids than atazanavir/ritonavir (Chandwani & Shuter, 2008:1031;

Croxtall & Perry, 2010:1905). Both DRV/r and LPV/r cause slight hyperglycaemia and hyperinsulinaemia (Arathoon *et al.*, 2013:15).

The specific effects the PIs have on serum lipids therefore appear to vary from drug to drug, and therefore are more drug-specific than class-specific. This may have a significant bearing on the selection of PI-based therapy for a particular patient where metabolic co-morbidities have to be taken into account.

The metabolic side effects of PIs have been well documented, but to what extent are these changes in lipid metabolism attributable to the PIs and how much does the change of body composition or disease status influence these changes? Lee *et al.* (2004) studied the effect of LPV/r on lipid metabolism in HIV-negative subjects in order to eliminate the possible effects of the HIV-infection. Eight out of the 10 subjects showed an 84% increase in TG and VLDL. They also found a 14% decrease in intravenous fat clearance and some glucose intolerance. Regardless of the effects of the HIV disease state itself, LPV/r still causes significant changes in TG and VLDL levels. Lee *et al.* (2004:6) further elaborated on the possible cause of the elevated TG levels; the HTG could not have been caused by the 14% decrease in TG clearance since such a small decrease in clearance could not account for the large 88% increase in TG. Lipoprotein lipase and hepatic lipase levels remained the same, therefore a decrease in lipolysis was ruled out. Consistent with Berthold *et al.* (1999:570-571), Lee *et al.* suggested that the increase in TG levels were due to an increase in VLDL production. Ritonavir causes a decrease in apolipoprotein A, which leads to an increase in VLDL and consequently TG (Lee *et al.*, 2004:6).

The increase in VLDL production by PIs is due to the effect that PIs have on HMG-CoA reductase lipid biosynthesis. Protease inhibitors cause the activation, an accumulation of sterol regulating binding protein (SREBP) (Reyskens *et al.*, 2013:1; Riddle *et al.*, 2001:375-14). The SREBP in turn binds to the promoter sequences contained in the sterol regulating element (SRE) in the lipogenic and cholesterologenic genes, such as 3-hydroxy3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), which results in the increase of low density as well as high density lipoproteins (Reyskens *et al.*, 2013:1). Furthermore, the ubiquitin-proteasome system responsible for elimination of defective proteins, is inhibited by ritonavir which leads to an inhibition of the degradation of apolipoprotein B. Increased apolipoprotein B increases serum lipids and is therefore another mechanism by which PIs elevated TG (Reyskens, 2013:2).

The increase in TG can have a widespread impact on the health of the patient. Secondary to HTG, insulin resistance may develop and consequently hyperglycaemia (Calza *et al.*, 2003:57; Carr *et al.*, 1998:F55). The increased TG levels will also have atherogenic implications to

consider (Berthold *et al.*, 1999:574). For the purposes of this study, the most significant implication of PIs induced (i.e. LPV/r induced) HTG is pancreatitis.

## **2.7 Lopinavir/ritonavir, HTG, HIV and pancreatitis**

People living with HIV/AIDS can be up to 800 times more likely to suffer from pancreatitis than the uninfected population (Bush & Kosmiski, 2003:e1). Furthermore, HTG has been well documented as a causative factor for pancreatitis (about 4% of cases) (Bush & Kosmiski, 2003:e1). Considering the significant increase in TG levels in patients treated with LPV/r, it can be reasonably expected that the prevalence of HTG-induced pancreatitis could be significant in this population (Bush & Kosmiski, 2003:e1).

However, several studies and reviews conducted over the last 10 years offer mixed and inconclusive results. Calza *et al.* (2003:58) conducted a prospective study on 212 patients on different PI regimens and observed not a single case of pancreatitis. Bush and Kosmiski (2003:e1) conducted a large retrospective study over six years with the beginning of the study before the introduction of LPV/r to ART, and the completion of the study some years after the introduction of LPV/r. This study had the advantage of comparing the prevalence of pancreatitis during the time before and after the introduction of LPV/r. It was found that there was not much change in the prevalence in pancreatitis after LPV/r was introduced in 1996 and that those cases with HTG as the cause, were 1% and 2% before and after 1996 respectively (Bush & Kosmisky, 2003:e2). Croxtall & Perry (2010:1903) state that LPV/r-induced HTG may have risk for pancreatitis and although some serious cases of pancreatitis, due to LPV/r have been reported, no causal relationship between LPV/r and pancreatitis has been reported yet. Riedel *et al.* (2008:9) investigated the aetiology of pancreatitis in HIV-infected patients admitted to their facility retrospectively over a period of 10 years from 1996 to 2006 – the first ten years after the introduction of LPV/r. Only two of the 86 patients admitted for pancreatitis were taking LPV/r. It is worth noting that the Riedel *et al.* study considered patients that clinically presented with pancreatitis, with supporting biochemical information. Therefore, all the patients who had subclinical pancreatitis were not detected, which means the prevalence may be higher than expected. It is important to note that the criteria for the diagnosis of pancreatitis varied between these studies.

## **2.8 Prevalence of metabolic changes caused by LPV/r in other populations with regards to ethnicity**

The field of pharmacogenomics is the study of how a patient's genetic makeup determines and influences the pharmacologic effect of a specific drug in that patient (USNLM, 2015). Knowledge regarding a particular patient's response to a specific drug may therefore help

clinicians to adjust pharmacotherapy exactly according to that patient's response as determined by the relevant gene. It will also enable clinicians to predict a pharmacological response (USNLM, 2015).

The study population for this study is homogenous black African. It may be useful to exclude the genetic influence that other population groups might have on the result. On the other hand, having a homogenous population also means that the responses of different population groups to LPV/r therapy cannot be analysed and compared. Results will therefore only be applicable to black Africans and comparative data for a pharmacogenomic comparison has to be gained with another study.

In studies conducted by Raza *et al.* (2013:12), Riedel *et al.* (2008:3) and Oliveira *et al.* (2014:117), investigating the incidence and risk factors of pancreatitis among people with HIV/AIDS the prevalence of pancreatitis was highest among African-Americans. No studies could be found that specifically investigated the pharmacogenomics response of black Africans to LPV/r therapy in terms of raised TG levels. However, since there are considerable indications that black Africans infected with HIV are more at risk for pancreatitis than other population groups, it can be expected that they will consequently have a higher risk of HTG-induced pancreatitis as well.

## **2.9 Confounders that may independently influence TG and total cholesterol**

The origin of all TG in the plasma is either exogenous or endogenous. Exogenous TG come from dietary intake of fats and is transported in the plasma in the form of chylomicrons. The endogenous source of TG is production in the liver in the form of VLDL (Yuan *et al.*, 2007:1113). Immediately after a meal, the levels of circulating TG in the form of chylomicrons will be raised. Conversely, in between meals while fasting, most TG will be due to liver production of VLDL (Yuan *et al.*, 2007:1113). Triglyceride catabolism is affected by apolipoprotein CII mediated lipoprotein lipase in the muscle and fatty tissue (Berglund *et al.*, 2012:2974; Yuan *et al.*, 2007:1113). Thus, anything that affects the exogenous or endogenous pathways of TG to central circulation will affect TG levels. Increased dietary fat uptake will increase intestinal TG production. Increased liver production is achieved, but up regulation of TG synthetic pathways and decreased peripheral catabolism will also lead to an increase in TG (Yuan *et al.*, 2007:1113).

The state of HTG can be classified as primary or secondary. The causes of primary HTG are hereditary and include (Berglund *et al.*, 2012:2972; Yuan *et al.*, 2007:1114):

- Familial HTG (elevated VLDL)
- Familial combined HTG (unclear aetiology)

- Familial dysbetalipoproteinaemia (elevated IDL)
- Familial chylomycronaemia (deficiency of LPL and apolipoprotein CII)
- Familial hypoalphalipoproteinaemia (unclear aetiology)

The prevalence of the hereditary HTG conditions varies among different authors but normally remains low. Familial HTG occurs in 1% of the population according to Berglund *et al.* (2014:2976), but 5-10% according to Yuan *et al.* (2007:1115). Familial combined HTG occur in 1% of the population according to Berglund *et al.* (2012:2976) and between 2-5% according to Yuan *et al.* (2007:1115). Familial dysbetalipoproteinaemia is rare and occurs only in 1 in 20 000, and familial chylomicronaemia in 10% of the population (Yuan *et al.*, 2007:1115).

Secondary causes of HTG include untreated diabetes mellitus type 2, obesity, excess alcohol intake, liver disease, renal disease and pregnancy and certain drugs (Berglund *et al.*, 2012:2972). The effect of acute hepatitis in TG levels will cease once the hepatitis has been cleared and will therefore be short lived. Pregnancy in the third trimester may have elevated TG levels up to three times higher than normal. However, this physiological elevation in TG is of little clinical consequence (Yuan *et al.*, 2007:1116).

## **2.10 What magnitude of change in serum lipids warrants intervention?**

The threshold plasma concentration of serum TG after which treatment should be commenced, is generally accepted to be 1.7 mmol/L (Sahebkar & Watts, 2015:364; Rosenson, 2016). Treatment of TG values between 1.7 and 11.3 mmol/L, is mainly aimed at reducing cardiovascular risk whereas treatment of TG values beyond 11.3 mmol/L will include the prevention of pancreatitis (Berglund *et al.*, 2014:3).

Initial treatment of HTG should be non-pharmacological, focussing on changes in lifestyle (Rizzo *et al.*, 2013:1869). These include reduced dietary intake of fat and carbohydrates, weight loss, cessation of smoking, restricted intake of alcohol and increased exercise (Berglund *et al.*, 2014:11-12; Sahebkar & Watts, 2015:364; Sandhu *et al.*, 2011:6). Strict glycaemic control should be exercised where diabetes mellitus is present (Rosenson, 2016).

At serum TG values of more than 5.65 mmol/L, TG saturation exceeds the removal thereof from the blood. In such cases, postprandial spikes will be rapid and high and pharmacological treatment is indicated (Berglund *et al.*, 2014:3; Rosenson, 2016). Pharmacologic intervention is strongly advised when TG values exceed 5.6 mmol/L, especially if a patient has a prior incident of pancreatitis or carries cardiovascular risk factors (Calza *et al.*, 2003:57).

Drugs used for the treatment of HTG include statins, fibrates, niacin and omega-3 fatty acids (Berglund *et al.*, 2014:13). The statins have a modest TG lowering effect and are indicated for

treatment of mild to moderate HTG. They are therefore indicated for the reduction of cardiovascular risk and will not be useful used alone for the treatment of severe HTG (Berglund *et al.*, 2014:12) and therefore prevention of pancreatitis. The fibrates have much more potent TG lowering effect than the statins and are indicated for the treatment of moderate to severe HTG (Berglund *et al.*, 2014:12).

## **2.11 Conclusion**

As the diverse co-morbidities of HIV infection itself, and its treatment become apparent, the body of knowledge required to manage such diversity in co-morbidities needs to expand. The prevalence of infective co-morbidities secondary to AIDS is declining (Warriner *et al.*, 2014:458) as a result of the successful impact of ART. Non-AIDS related co-morbidities are becoming more prevalent now due to the aging of the HIV-infected population (Warriner *et al.*, 2014:458). Many organ systems may be affected, one of them the pancreas. Both HIV and the side effects of LPV/r therapy may increase the risk of pancreatitis. This study will attempt to quantify the risk of pancreatitis after the first six months of LPV/r therapy.



## CHAPTER 3: ARTICLE MANUSCRIPT

In this chapter, the results and discussion thereof are presented in an article format. The article was prepared according to the author's guidelines as set out for the South African Medical Journal ([www.samj.org.za](http://www.samj.org.za)) and submitted November 2016 (manuscript number: SAMJ12212).

### 3.1 Article: Hypertriglyceridaemia and the risk of pancreatitis and atherosclerosis 6 months post LPV/r initiation

#### 3.1.1 Article

## Hypertriglyceridaemia and the risk of pancreatitis and atherosclerosis 6 months post LPV/r initiation

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**Background.** Hypertriglyceridaemia (HTG) is an important risk factor for pancreatitis and cardiovascular disease (CVD), depending on severity. Hypertriglyceridaemia is common in human immunodeficiency virus (HIV) infection and also a common complication of lopinavir/ritonavir (LPV/r).

**Objectives.** To evaluate the risk of pancreatitis and atherosclerosis associated with HTG in patients 6 months post initiation of LPV/r-based therapy in a regional public hospital.

**Methods.** Triglyceride (TG), serum amylase (s-amylase) and CD<sub>4</sub> values were retrospectively investigated 6 months post LPV/r-based initiation. Age, gender, previous antiretroviral regimen and period since HIV diagnosis were also recorded.

**Results.** The final sample consisted of 194 patients, 50 males and 144 females; mean ( $\pm$  standard deviation [SD]) age 39.52 ( $\pm$ 9.98) years and the mean ( $\pm$ SD) period since HIV diagnosis was 91.32 ( $\pm$ 25.18) months. Normal TG levels ( $< 1.7$  mmol/L) were detected in only 55% of patients and the rest presented with some degree of HTG. The mean ( $\pm$ SD) TG for the entire sample was elevated at 1.94 ( $\pm$ 1.30) mmol/L with the mean ( $\pm$ SD) of the males at 2.36 ( $\pm$ 1.74) - statistically higher compared to the females at 1.79 ( $\pm$ 1.08) mmol/L, ( $p=0.034$ ). No cases of pancreatitis were recorded and the time since HIV diagnosis did not indicate any statistically significant differences in the means of the TG, serum-amylase or CD<sub>4</sub> values.

**Conclusion.** Triglyceride levels were not elevated substantially enough to induce pancreatitis at 6 months post initiation of LPV/r, but the levels were elevated above the accepted upper normal limit of 1.70 mmol/L, implying risk for atherosclerosis. Continuous monitoring of TG and s-amylase is therefore recommended after every six months.

Keywords: Hypertriglyceridaemia, pancreatitis, lopinavir/ritonavir, atherosclerosis, cardiovascular risk.

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Massive advancements in the understanding and treatment of the human immunodeficiency virus (HIV) have changed the prognosis of HIV infection from a fatal disease to a chronic illness.<sup>[1]</sup> The prolonged life expectancy of the HIV-infected population elucidated not only the long-term pathophysiologic effects of the disease itself, but also the long-term side effects of antiretroviral therapy.<sup>[2]</sup> Infection with HIV itself causes co-morbidities unrelated to acquired immunodeficiency syndrome (AIDS) such as cardiovascular, renal and bone disease, as well as diabetes mellitus and pancreatitis. The long-term side effects of antiretroviral therapy include loss of renal function, loss of bone density, insulin resistance and dyslipidaemia with saquinavir, indinavir, nelfinavir and lopinavir/ritonavir (LPV/r) that are well known for increasing triglycerides (TG).<sup>[2,3]</sup> Pancreatitis and atherosclerosis are well documented consequences of hypertriglyceridaemia (HTG).<sup>[4-6]</sup> Since the HIV infection has no cure and consequently its therapy is lifelong, the effects of each on, inter alia, the pancreas and cardiovascular system, becomes important in order to correctly manage and treat the related risks that may arise in the long-term.<sup>[2,7]</sup> Management of HTG-associated risk factors and co-morbidities should not only include the reactive treatment thereof, but also the aggressive prevention thereof.<sup>[2]</sup>

Triglycerides are a major source of energy to the body. Once exogenous TG arrive in the gut, they are hydrolysed to free fatty acids, then resynthesised to TG in the gastric mucosa after absorption, and transported in the lymphatic system in the form of chylomicrons.<sup>[4,8]</sup> Hypertriglyceridaemia can be brought about by primary or secondary factors. The primary factors are genetic and include various types of hereditary HTG.<sup>[6]</sup> Secondary factors are diverse and may include several HTG inducing drugs such as ethanol, thiazide diuretics, beta blockers, oestrogens and several antiretroviral drugs,<sup>[9,10]</sup> particularly certain protease inhibitors.<sup>[3,11,12]</sup> Diabetes mellitus (type 2), hypothyroidism and HIV also cause an increase in TG, as well as several lifestyle factors such as physical inactivity and a diet high in fat and carbohydrates.<sup>[9-11]</sup> The LPV/r combination is useful in decreasing the probability of the development of viral resistance.<sup>[13-16]</sup> A well-documented metabolic side effect of LPV/r is increased TG.<sup>[3,18]</sup> Related metabolic side effects of LPV/r include, hypercholesterolaemia, hyperglycaemia and insulin resistance.<sup>[3]</sup> Hypertriglyceridaemia is a known cause of pancreatitis.<sup>[19,20]</sup> Although TG-induced pancreatitis is not the main aetiology of pancreatitis, it is not uncommon,<sup>[21]</sup> with as much as 10% of cases of acute pancreatitis due to HTG.<sup>[22]</sup>

Triglyceride plasma concentrations above 1.7, 2.3 and 5.6 mmol/L are classified as mild, moderate and severe respectively,<sup>[5,11]</sup> with very severe HTG above 11.3 mmol/L.<sup>[4]</sup> Triglyceride values of > 20 mmol/L are usually due to a combination of primary and secondary factors.<sup>[9]</sup> The main consequences of HTG are increased risk of pancreatitis and atherosclerosis.<sup>[4,6]</sup> The risk for pancreatitis seems to increase with an increase in TG,<sup>[20]</sup> but only becomes a significant concern at values greater than 11.3 mmol/L.<sup>[4,5,23]</sup>

The exact mechanism by which HTG causes pancreatitis remains unclear.<sup>[21]</sup> The mechanism most widely accepted suggests that as TG values rise in the form of chylomicrons due to primary and secondary HTG inducing factors, the body becomes less able to clear the chylomicrons from circulation after post prandial peaks,<sup>[19]</sup> and the blood becomes more viscous which can lead to ischaemia and acidosis in the pancreas, resulting in tissue damage<sup>[19,21]</sup> as the pancreatic enzymes cause autodigestion of the pancreas.<sup>[19]</sup>

In acute pancreatitis, serum amylase and serum lipase (s-amylase and s-lipase respectively) are elevated three times higher than the upper normal limit.<sup>[25,26]</sup> The upper normal limit for this study was 2.08  $\mu$ kat/L (104 U/L as defined by National Health Laboratory Service [NHLS]).

At mild to moderate HTG, the risk for cardiovascular disease (CVD) becomes more prevalent.<sup>[11]</sup> Hypertriglyceridaemia is a characteristic sign of atherogenic dyslipidaemia,<sup>[27]</sup> but the exact manner of contribution of TG to cardiovascular risk is still unclear.<sup>[5,6]</sup> It is not clear whether

there is a causal relationship between HTG and atherosclerosis,<sup>[20]</sup> but it is now accepted that TG is at least indirectly associated with cardiovascular risk.<sup>[6]</sup>

Long-term proactive monitoring and management of HTG is therefore important to prevent HTG-induced pancreatitis and CVD. This is especially true in a population with very high prevalence of HIV infection and the presence of other associated risk factors of pancreatitis and cardiovascular disease and it can be expected to be much higher compared to the general population. The risk of pancreatitis is also further increased by opportunistic infections and certain drugs that the patient may have to take for the management of HIV infection such as co-trimoxazole, lamivudine,<sup>[28]</sup> and zidovudine.<sup>[7]</sup>

The focus of this study was on the effects of the combination of LPV/r on TG levels and to assess the risk of TG induced pancreatitis and atherosclerosis 6 months post initiation.

## Methods

This was a cross-sectional, retrospective, observational study. The following data were collected from patients' medical history: age, gender, s-amylase, TG, CD<sub>4</sub> count, period since date of HIV diagnosis to present and prior antiretroviral regimens. Simple random sampling was used to select 200 patients from the TIER.Net database. The TIER.Net system (previously known as the HIV electronic register or eRegister) is a database of all patients receiving antiretroviral therapy countrywide and is maintained by the National Department of Health (NDoH). Data used from the pharmacy dispensing statistics indicated that as of August 2015, 807 adult patients received LPV/r-based treatment from the hospital. A power analysis based on chi-square analysis of association, indicated a minimum sample size of 197 equal to an effect size of 0.2. The data were retrospectively collected during April 2016.

The study was conducted at a public sector hospital of approximately 500 beds in KwaZulu-Natal. The Centre for Disease Control (CDC), a department within this hospital, is dedicated to the management and treatment of patients suffering from HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome) on an outpatient basis.

All black adult patients older than 18 years who were on LPV/r-based treatment that accessed the services of the CDC at any time in the past, provided that they were entered into the Tier.Net data base with TG, s-amylase and CD<sub>4</sub> results six months post LPV/r initiation, were eligible to be included.

This study population was of homogenous black race in order to rule out genetic confounders as both Raza and co-workers,<sup>[29]</sup> and Riedel and co-workers<sup>[30]</sup> indicated that pancreatitis was highest among HIV-infected African-Americans. Any factor or pre-existing disease that may independently increase TG or precipitate pancreatitis was excluded where possible. Medication that may induce or inhibit the metabolism of LPV/r (such as ketoconazole, antiepileptic agents), fibrates or other cholesterol lowering agents, or the use of alcohol during the same time blood was drawn for TG or s-amylase, were excluded.

Descriptive statistics mean (standard deviation [ $\pm$ SD]) and median (interquartile range [IQR]) were calculated for all the variables for the entire sample, and separately for males and females. An independent t-test was performed to investigate a possible significant difference in TG, s-amylase and CD<sub>4</sub> counts between males and females. In addition, the duration since HIV diagnosis was categorised (0-79, 80-104 and > 104 months) to investigate any differences in the means of the TG, s-amylase and CD<sub>4</sub> counts. Statistical significance was set at  $p < 0.05$  and practical significance was tested with Cohen's d-value (0.2 = small effect; 0.5 = medium effect, 0.8 = large effect).

## Results

The TG, s-amylase and CD<sub>4</sub> counts for 194 patients were statistically analysed, as six patients had to be removed from the original sample since they were already on LPV/r based regimens.

Table 1 reflects the demographic details of 144 (74%) female and 50 (26%) male patients. The mean ( $\pm$ SD) age for the entire sample was 39.52 ( $\pm$ 9.98) years. The mean ( $\pm$ SD) period for all the patients since first diagnosis of HIV infection until the time of data collection was 91.32 ( $\pm$ 25.18) months. The majority of patients were on regimen 1, containing stavudine (d4T) ( $n=157$ , 81.93%), with the second most common previous regimen being the tenofovir (TDF) based regimen 1 that replaced the d4T regimen 1 ( $n=36$ , 18.56%) from 2010 onwards.

	Females	Males	Total
<i>N</i>	144	50	194
Mean age ( $\pm$ SD)	38.87 ( $\pm$ 9.23)	41.40 ( $\pm$ 11.77)	39.52 ( $\pm$ 9.98)
Mean months (SD) since HIV diagnosis	90.08 ( $\pm$ 25.45)	94.92 ( $\pm$ 24.29)	91.32 ( $\pm$ 25.18)
Previous regimen frequency			
d4T/3TC/EFV	59	32	91
d4T/3TC/NVP	61	5	66
TDF/3TC/EFV	7	13	20
TDF/3TC/NVP	16	0	16
AZT/3TC/NVP	1	0	1
Total	144	50	194

3TC, lamivudine; AZT, zidovudine; d4T, stavudine; ddI, didanosine; EFV, efavirenz; NVP, nevirapine, TDF,tenofovir

Table 2 summarises the mean ( $\pm$ SD) values of TG, s-amylase and CD<sub>4</sub> count of the female, male and total group but also indicates the statistical and practical significance between the two genders. The mean ( $\pm$ SD) TG was 1.94 ( $\pm$ 1.30) mmol/L and median (interquartile range [IQR]) for the whole population was 1.48 (1.35) mmol/L. The mean ( $\pm$ SD) TG values for females and males were 1.79 ( $\pm$ 1.08) mmol/L and 2.36 ( $\pm$ 1.74) mmol/l respectively, which was statistically significant ( $p=0.034$ ).

	Female ( $n=144$ )		Male ( $n=50$ )		t-test	Total ( $n=194$ )	
	Mean ( $\pm$ SD)	Median (IQR)	Mean ( $\pm$ SD)	Median (IQR)	$p^*$ (Cohen's d-value)†	Mean ( $\pm$ SD)	Median (IQR)
TG mmol/l	1.79 ( $\pm$ 1.08)	1.44 (1.04)	2.36 ( $\pm$ 1.74)	1.89 (2.79)	0.034* (0.330)	1.94 ( $\pm$ 1.30)	1.48 (1.35)
S-amylase $\mu$ kat/L	2.19 ( $\pm$ 0.90)	1.99 (0.96)	2.30 ( $\pm$ 1.01)	2.08 (1.19)	0.463 (0.11)	2.22 ( $\pm$ 0.93)	2.04 (1.05)
CD <sub>4</sub> x 10 <sup>3</sup> /L	392 ( $\pm$ 222)	361 (281)	287 ( $\pm$ 226)	232 (268)	0.004* (0.47)	365 ( $\pm$ 227)	328 (283)

\*  $p < 0.05$  statistically significant  
 †Cohen's d-value (practical significance) 0.2=small effect; 0.5=medium effect; 0.8=large effect

Only 1% of the patients in this study had severe HTG values of above 5.6 mmol/L. A further 26% had moderate HTG with values between 2.3 mmol/L and 5.6 mmol/L. Mild HTG with values between 1.7 and 2.3 mmol/L occurred in 19% of the patients. The remaining 55% were all

below 1.7 mmol/L. A significant total of 45% of the patients suffered from some degree of HTG with 42% of females and 54% of the male population suffering from HTG.

The criterion for pancreatitis for this study was an elevation of s-amylase three times above the upper normal limit of 2.08  $\mu\text{kat/L}$ . No cases of pancreatitis were recorded after 6 months of LPV/r treatment, mean ( $\pm\text{SD}$ ) s-amylase value for the entire sample was 2.22 ( $\pm 0.93$ )  $\mu\text{kat/L}$  and a median (IQR) of 2.04 ( $\pm 1.05$ )  $\mu\text{kat/L}$ . There was a small and insignificant difference ( $p=0.463$ ) in the mean ( $\pm\text{SD}$ ) s-amylase values between females (2.19 [ $\pm 0.90$ ]  $\mu\text{kat/L}$ ) and males (2.31 [ $\pm 1.01$ ]  $\mu\text{kat/L}$ ).

The mean ( $\pm\text{SD}$ )  $\text{CD}_4$  count for the entire sample after the first 6 months of LPV/r-based therapy was 365 ( $\pm 227$ )  $\times 10^3/\text{L}$ . There was a statistical ( $p=0.004$ ) as well as a practical significant difference (0.47) in the mean ( $\pm\text{SD}$ )  $\text{CD}_4$  counts between the female and male (392 [ $\pm 222$ ]  $\times 10^3/\text{L}$  and 287 [ $\pm 226$ ]  $\times 10^3/\text{L}$  respectively). The elapsed time since the date of diagnosis of the HIV infection until the date of data collection was divided into 3 categories (0 - 79 months, 80 - 104 months and 104 > months). Variation between the mean values and mean rank values for TG, s-amylase and  $\text{CD}_4$  counts for each time period was investigated. Even though there was a mathematical increase in mean and mean rank values of TG and s-amylase as a function of time, there was no statistically significant difference between any of the values (all  $p > 0.05$ ) from the corresponding time periods.

Table 3. Difference between means and mean peak respectively per time period since HIV diagnosis

One-way ANOVA	Mean values per time period ( $\pm\text{SD}$ )			
	0-79 months ( $n=65$ )	80-104 months ( $n=65$ )	104 > months ( $n=64$ )	p
TG mmol/L	1.83 ( $\pm 1.14$ )	1.92 ( $\pm 1.25$ )	2.07 ( $\pm 1.500$ )	0.591
S-amylase $\mu\text{kat/L}$	2.11 ( $\pm 0.82$ )	2.26 ( $\pm 1.07$ )	2.29 ( $\pm 0.88$ )	0.495
$\text{CD}_4 \times 10^3/\text{L}$	349.15 ( $\pm 236.07$ )	381.29 ( $\pm 243.74$ )	363.82 ( $\pm 200.37$ )	0.723
Kruskal-Wallis	Mean rank per period			
	0-79 months ( $n=65$ )	80-104 months ( $n=65$ )	104 > months ( $n=64$ )	p
TG mmol/L	95.18	95.41	101.98	0.738
S-amylase $\mu\text{kat/L}$	92.07	95.77	104.77	0.418
$\text{CD}_4 \times 10^3/\text{L}$	91.71	100.43	100.41	0.594

## Discussion

### Hypertriglyceridaemia and the risk of pancreatitis

The exact value at which the risk for acute pancreatitis starts is still not clear – it may be as low as 1.7 mmol/L<sup>[4]</sup> – but the risk seems to increase with TG values and becomes more evident above 11.3 mmol/L<sup>[4,5,23]</sup> with clinically detectable symptoms at 22.6 mmol/L.<sup>[20]</sup>

The results from this study suggest that the risk for pancreatitis after the first 6 months of LPV/r is negligible since not a single case of acute pancreatitis was recorded. None of the TG values recorded were > 11.3 mmol/L in order to possibly induce pancreatitis. In fact, the mean TG value was a mere 1.94 mmol/L. Even though no cases of pancreatitis were identified according to the criteria of this study, the s-amylase values showed a tendency to increase at the six months mark with some individual values close to reaching the value requisite for pancreatitis for this study. A total of 3.1% ( $n=6$ ) of the patients had s-amylase values above double the upper normal value. However, this does not necessarily mean that no cases were present. According to Oliveira and co-workers,<sup>[7]</sup> and Manfredi and co-workers,<sup>[31]</sup> post mortem evidence of pancreatitis is very common in the HIV-infected population. Many cases of pancreatitis may therefore be subclinical and may go unnoticed.<sup>[31]</sup> Given the low mean TG value of this study sample, if there were cases of undetected subclinical pancreatitis, they were probably not due to HTG. It is estimated that pancreatitis pathology is as much as 800 times more common in the HIV-infected population than otherwise,<sup>[23]</sup> which this study could not corroborate. Asymptomatic elevations in s-amylase and s-lipase are quite common, but cases of acute pancreatitis are not frequently reported among HIV-infected patients.<sup>[32]</sup> Oliveira and co-workers<sup>[7]</sup> confirmed that drug-induced pancreatitis may therefore be frequently overlooked when mild but clinically meaningful increases in s-amylase and s-lipase values are present. Considering that several other factors may precipitate pancreatitis in an HIV-infected population, physicians should therefore be highly suspicious of mildly elevated s-amylase values as was suggested by Oliveira and co-workers.<sup>[7]</sup> Triglyceride values of > 20 mmol/L are usually due to a combination of primary and secondary factors.<sup>[9]</sup> Carefully considered exclusion criteria made sure to exclude as many primary and secondary factors that may cause HTG or pancreatitis in this study population in order to establish the effect of LPV/r alone on TG. The TG values of this study are therefore relatively free of confounders. In an uncontrolled environment, where other factors that may precipitate acute pancreatitis may be present in any combination – in addition to LPV/r therapy – it can be reasonably expected that the risk for pancreatitis in the study population may be significantly higher than the results suggest.

The mean TG value for males was a notable 32% higher than that of the females. Factors such as age, gender and ethnicity affect TG values.<sup>[4]</sup> Triglyceride values are normally higher in males than in females,<sup>[4]</sup> and there was a statistically significant difference between the means of males and females. This raises questions regarding the susceptibility of males compared to females to the HTG-inducing effects of LPV/r. It also raises questions regarding the possible differences in dietary habits and prevalence of obesity in the male population – factors that were not known at the time of observation for this study. Ford and co-workers<sup>[33]</sup> also found the prevalence of HTG among African-American women lower than that among men (14.4% and 21.2% respectively) – consistent with the findings of this study (54% for males and 42% for females). The sample investigated by Ford and co-workers<sup>[33]</sup> was taken from the general population to investigate the prevalence of metabolic syndrome among adults in the United States regardless of aetiology. The sample from this study was exclusively HIV-infected and on LPV/r based therapy. The difference in prevalence of HTG between the two populations stratified by gender may therefore be due to HIV infection and LPV/r therapy. However, it must be kept in mind that the dietary habits and prevalence of obesity may vary between the populations of this study and that of Ford and co-workers.<sup>[33]</sup>

The difference in mean s-amylase levels between males and females was small and insignificant, suggesting that neither gender was more susceptible to pancreatitis than the other in this study.

However, Oliveira and co-workers<sup>[28]</sup> as well as Riedel and co-workers<sup>[30]</sup> both found female gender to be a risk factor for pancreatitis among HIV-infected patients, especially those of lower body weight. In both these studies, they cited several studies with very large sample sizes of several thousand participants and the study durations were also longer than 6 months. A possible shortcoming of this study was the relatively short period of observation (6 months) and population size. A much larger population may therefore be required to better determine the risk of pancreatitis in a given population.

Where CD<sub>4</sub> counts are concerned, it has been well established that lower CD<sub>4</sub> counts correlate with higher risk of pancreatitis.<sup>[7,30]</sup> This study found the males to have a significantly lower mean CD<sub>4</sub> counts compared to females, which puts them at a higher risk of pancreatitis. This is contrary to the findings of both Oliveira and co-workers,<sup>[7]</sup> and Riedel and co-workers<sup>[30]</sup> regarding the risk contributed by gender. Given the lopsided prevalence of females in this study population, as well as the higher mean CD<sub>4</sub> count of females – it raises questions regarding the compliance of males with ART.

Furthermore, in the HIV clinical cohort study conducted by Riedel and co-workers<sup>[30]</sup> to investigate the risk factors for pancreatitis, of the cases of acute pancreatitis recorded during the period of their study, 88.2% were black African and 51.8% were male. This would suggest that ethnicity is a factor. The median population age was 36 years, which was similar to the median age of 38 years for this study, which also suggests that the male population investigated for this study carried more risk of acute pancreatitis than not only the general population, but perhaps also other HIV positive populations.

In this study, a significant total of 45% of the patients suffered from some degree of HTG, which may largely be attributed to LPV/r therapy, since factors that may independently increase TG values were excluded from the sample as far as possible. This was slightly lower than the results of Calza,<sup>[3]</sup> who found HTG in 60-90% of patients on protease inhibitors. Baseline TG values will therefore be important in order to quantify the exact impact of LPV/r therapy on TG values, which was not available for this study. Considering the prevalence of HTG and other factors unaccounted for among the 194 patients – early investigation and intervention are indicated. The apparent time-dependent increase in TG in patients' LPV/r,<sup>[3]</sup> the apparent faster increase in TG with an LPV/r containing regimen<sup>[3]</sup> combined with primary causes and other secondary causes of HTG, TG values may well reach high enough values to precipitate acute pancreatitis in the long term, beyond the first six months of LPV/r therapy, and significantly contribute to the risk of cardiovascular disease.<sup>[6]</sup>

Other risk factors include the total period infected with HIV (mean of 91.3 months in this study population) and a CD<sub>4</sub> count of less than 200.<sup>[7]</sup> In this study, the comparative mean values of TG, s-amylase and CD<sub>4</sub> did not significantly vary over the three consecutive categorical periods of HIV infection duration. However, even though statistically insignificant, the mean values of TG and s-amylase did increase over the three categorical periods, which may suggest that proactive monitoring of these parameters would be wise, starting from the day of HIV infection diagnosis and continuing throughout the duration of treatment.

### **Hypertriglyceridaemia and risk of atherosclerosis**

The risk for TG induced pancreatitis only becomes relevant at 11.3 mmol/L,<sup>[4,5,23]</sup> but cardiovascular risk apparently starts much lower at 1.7 to 2.3 mmol/L.<sup>[6,19]</sup> The mean TG value for this entire sample was 1.94 mmol/L, and 1.79 and 2.36 mmol/L for females and males respectively. Overall, only mild HTG was present, with a notable difference between females (mild HTG) and males (moderate HTG). Triglyceride values were not critically high, but mild to moderately elevated nonetheless, which may contribute to cardiovascular risk later on.<sup>[6]</sup> Since most contributing factors that may also elevate TG values have been removed from the study sample, it is reasonable to accept that an uncontrolled population with other co-morbidities and HTG-inducing drugs may be more at risk. In addition to possible primary factors such as familial

HTG.<sup>[6,10]</sup> other secondary HTG inducing factors include obesity, diabetes mellitus, hypothyroidism and pregnancy. In addition, cardiovascular risk occurs earlier and at a higher rate in black Africans.<sup>[12]</sup> With HTG, insulin resistance increases – and therefore type 2 diabetes mellitus<sup>[4,9]</sup> – along with the fat content in myocytes.<sup>[19]</sup> In turn, insulin resistance may aggravate HTG by increasing de novo hepatic lipogenesis and secretion of TG rich VLDL and chylomicron biogenesis.<sup>[5]</sup> Therefore, protease inhibitor associated insulin resistance,<sup>[12]</sup> and the resulting contribution to metabolic syndrome<sup>[19]</sup> and its associated cardiovascular risk should be closely monitored. Since ARV therapy is lifelong, the cardiovascular and other metabolic consequences of HTG will also be long term.

## Summary

Almost half (45%) of the patients in this study had elevated TG values after the first 6 months of LPV/r treatment. Triglyceride values were not elevated enough to cause a realistic risk of pancreatitis. However, co-morbidities and drugs that increase TG in uncontrolled circumstances should not be disregarded. Hypertriglyceridaemia contributes to cardiovascular risk, starting at lower values required to cause pancreatitis, which may place a considerable part of the study population at risk of cardiovascular disease. The current guidelines of the NDoH require screening for hypertension and diabetes mellitus at the time of the diagnosis of the HIV infection.<sup>[34]</sup> This ensures that any co-morbidities are identified and managed even before antiretroviral therapy is initiated. The guidelines from the NDoH also recommend a fasting TG and cholesterol assay to be done when LPV/r therapy is indicated, then only repeated again after 3 months of LPV/r therapy. This approach will identify the patients most at risk during the formative stages of LPV/r-based therapy. However, as Calza and co-workers demonstrated,<sup>[3]</sup> the HTG inducing effect of LPV/r is time-dependent and TG values can more than double from 3 months post initiation LPV/r therapy to 12 months post initiation. Patients with acceptable TG values at 3 months may therefore not remain optimal, and more frequent TG testing every 6 months when the patient and chronic prescription are reviewed, could therefore be beneficial to proactively monitor TG values over the long term. In addition, 6-monthly s-amylase and s-lipase assays are indicated since many cases of pancreatitis are subclinical and may go unnoticed.<sup>[31]</sup> Considering the relationship between HTG and insulin resistance,<sup>[4,9]</sup> constant blood glucose monitoring may also be wise. Intervention and vigilance is therefore indicated from the first day of therapy on LPV/r and at continued intervals to reduce both the risk of pancreatitis and cardiovascular disease.

## Study limitations

The lack of baseline values for TG, s-amylase and CD<sub>4</sub> counts meant that the effect of LPV/r on the change of these variables could not be quantified. Body mass index values and dietary habits (including alcohol consumption) were also not consistently and reliably available, which could influence TG values. The more specific s-lipase instead of s-amylase would have enabled a more accurate diagnostic criterion for cases of pancreatitis. Hypothyroidism was not part of the exclusion criteria. The observation period of 6 months was too short to predict the long-term effects of LPV/r induced HTG and possibly pancreatitis; therefore, future studies should be expanded to include, at minimum, 12 months' duration on LPV/r-based regimens.

## Approvals

This study was approved by the HREC-NWU on 14/03/2016 (NWU-00356-15-A1) and also approved by the KwaZulu-Natal Department of Health's Directorate of Health Research and Knowledge Management (KZ\_2015RP22\_110), and the Medical Manager at the facility where the study was conducted.

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### **3.1.2 Author guidelines**

The author guidelines as provided by the South African Journal of Medicine were used (<http://www.samj.org.za/index.php/samj/about/submissions#authorGuidelines>).

### **3.1.3 Statements**

The writing and preparation of the dissertation and article manuscript was supervised and co-supervised by Dr JM du Plessis and DR M Viljoen respectively. All statistical data were generated by Ms M cockeran. The writing and preparation of the dissertation and manuscript was done by WP Greffrath.

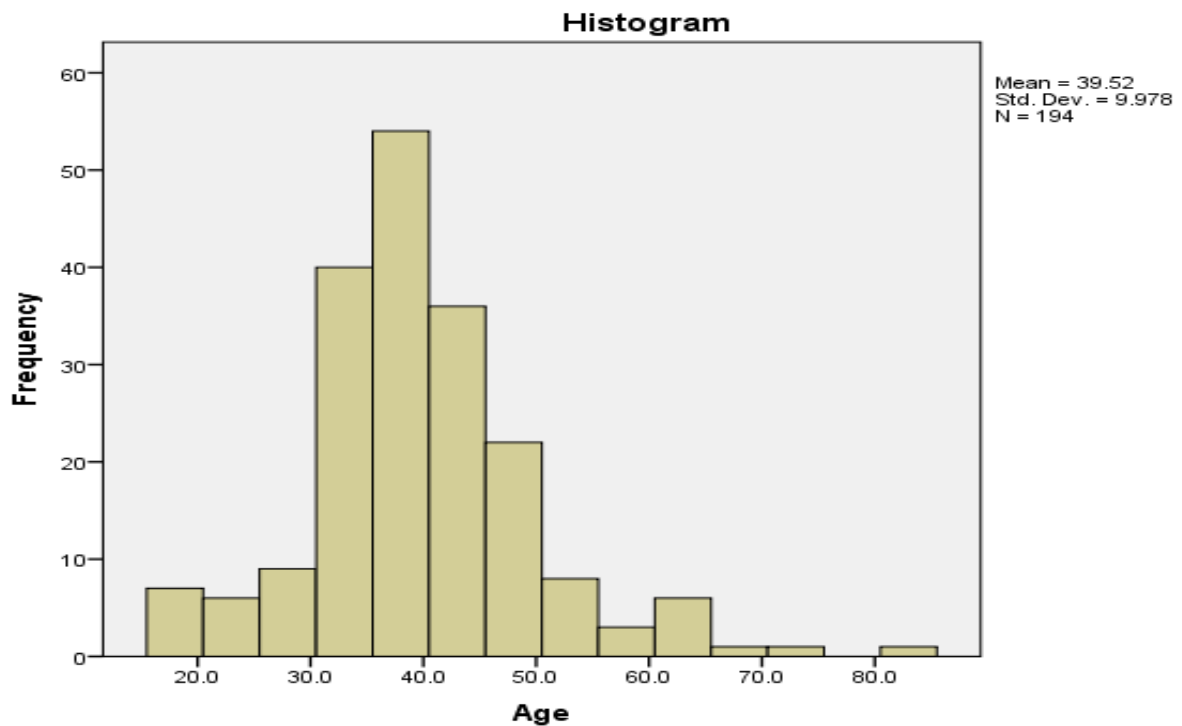
# CHAPTER 4: RESULTS

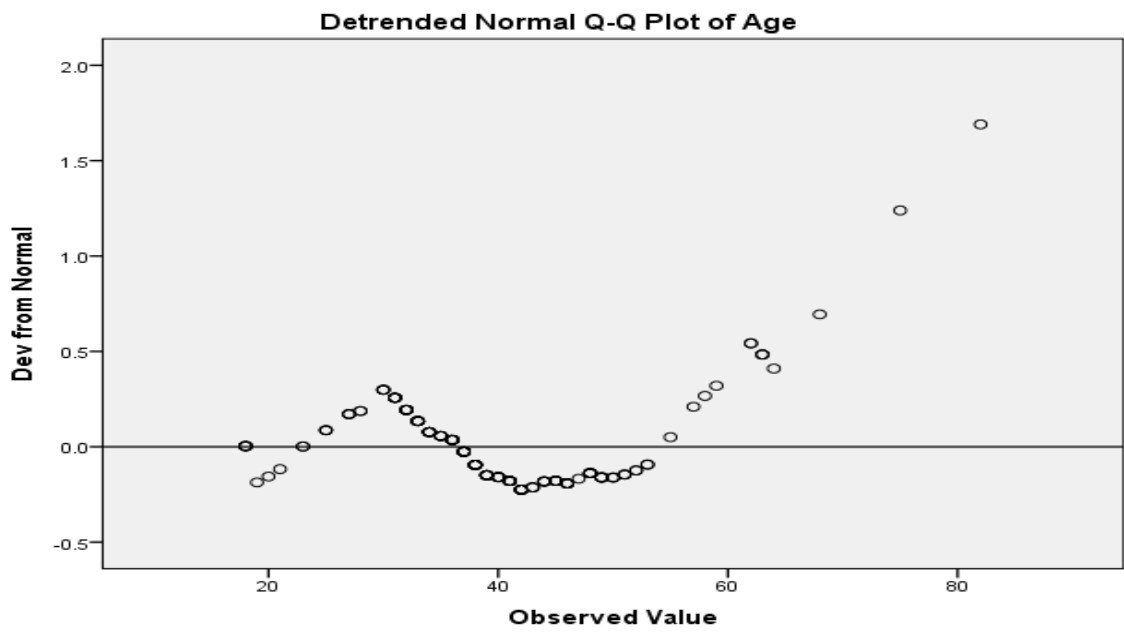
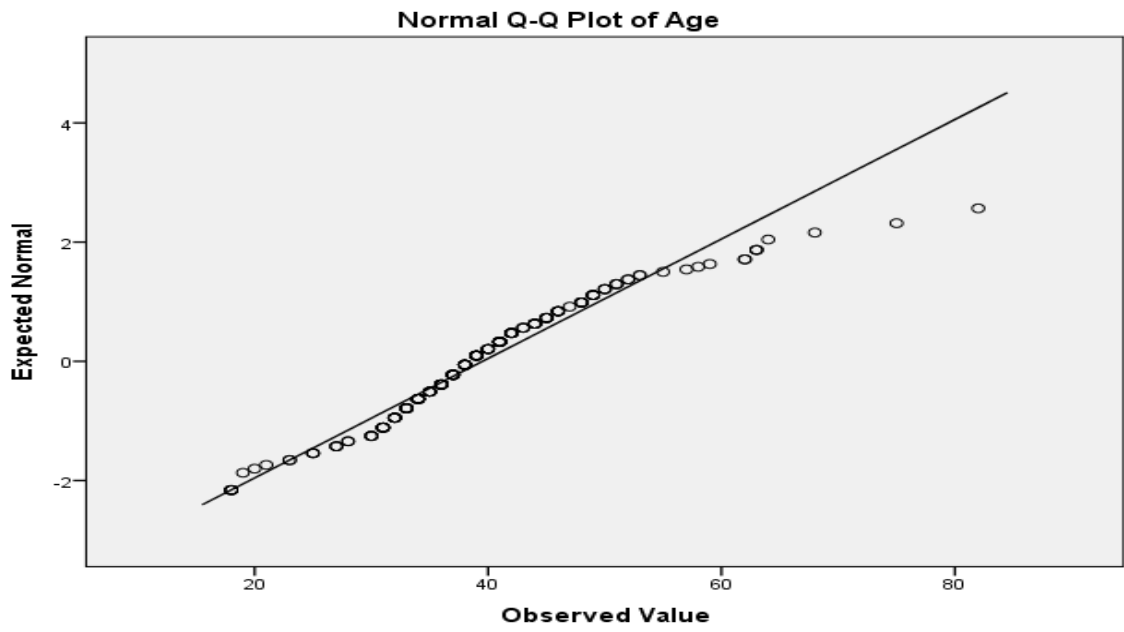
## 4.1 Descriptive statistics

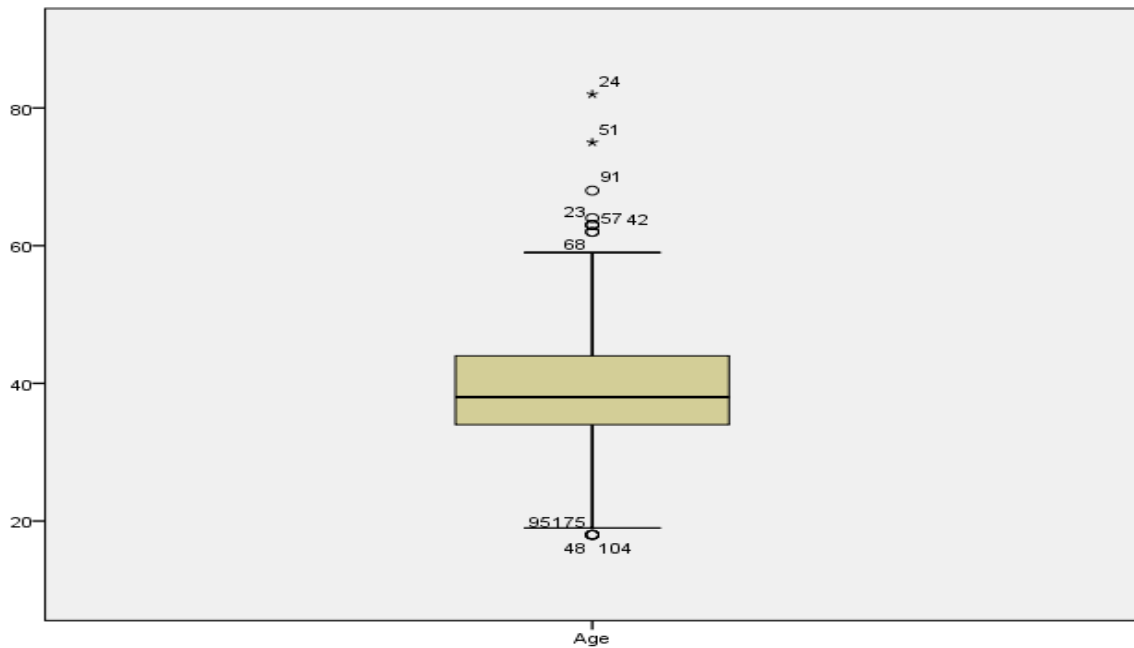
Certain parts of the descriptive results, as well as the results from specific statistical tests that were not required for the preparation of the article manuscript, are discussed in this chapter.

## 4.2 Combined descriptive statistics

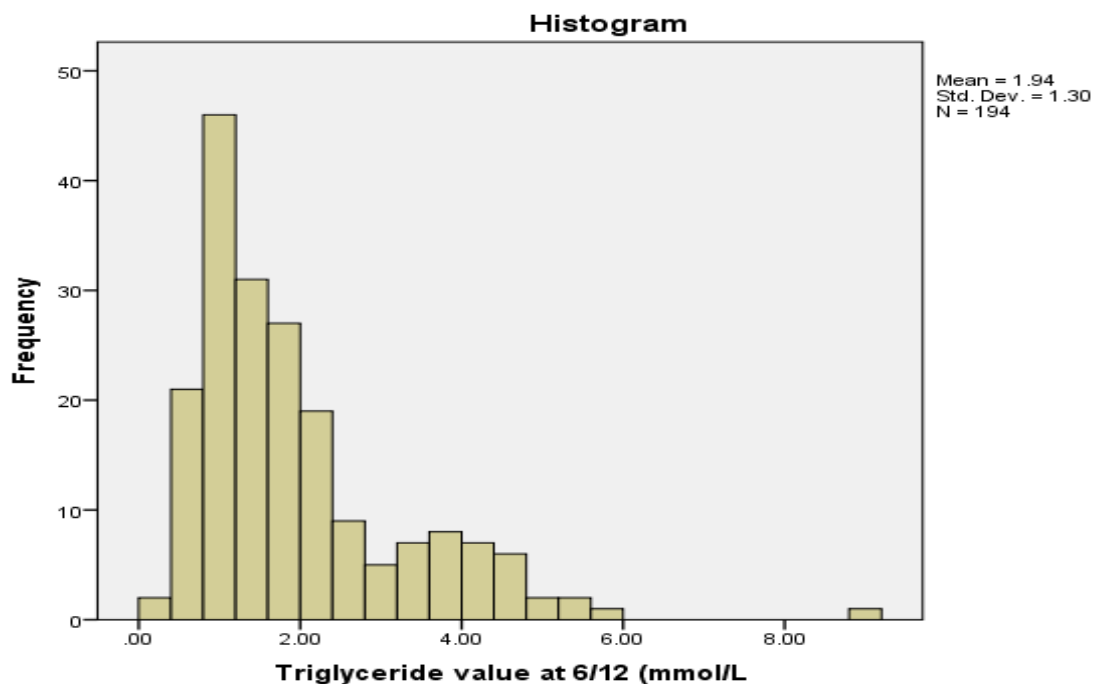
- Age: The lower and upper bound 95% confidence intervals for the mean were 38.11 and 40.93 years respectively. The oldest patient was 84 years of age and the youngest 18 with a range of 64 years. The variance was 99.56 and the interquartile range 10.3. Most patients were therefore in their 30's and early 40's. There was skewness of 0.82 and kurtosis of 2.36. Slight skewness was illustrated by the histogram, normal Q-Q plot and detrended Q-Q plot. The box plot confirms slight skewness with more outliers above the 75% quartile than below the 25% quartile.

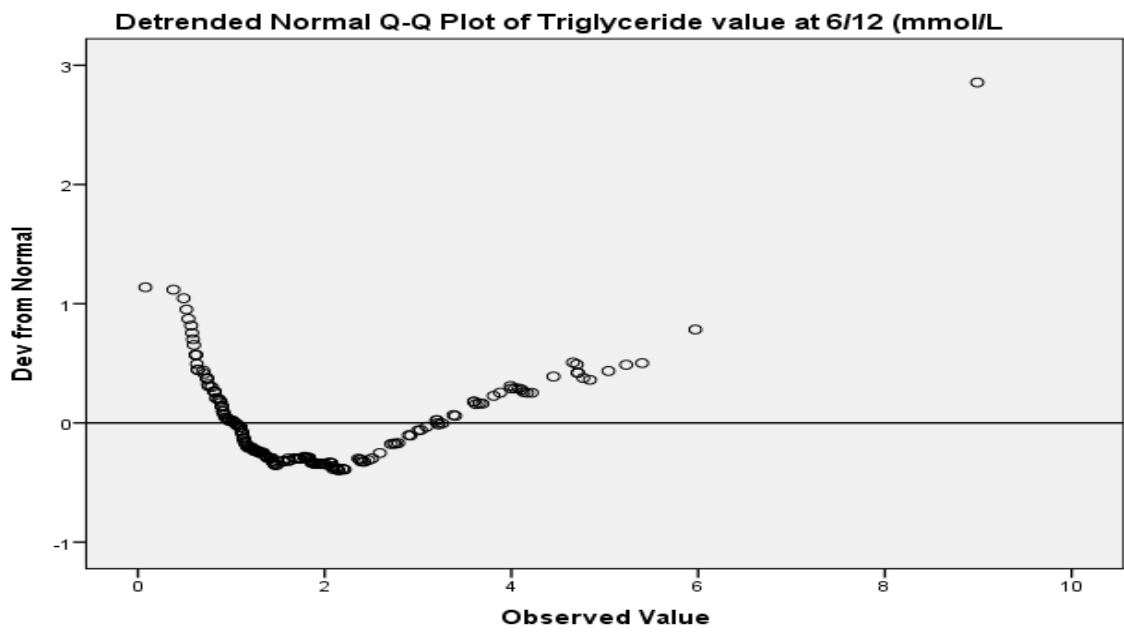
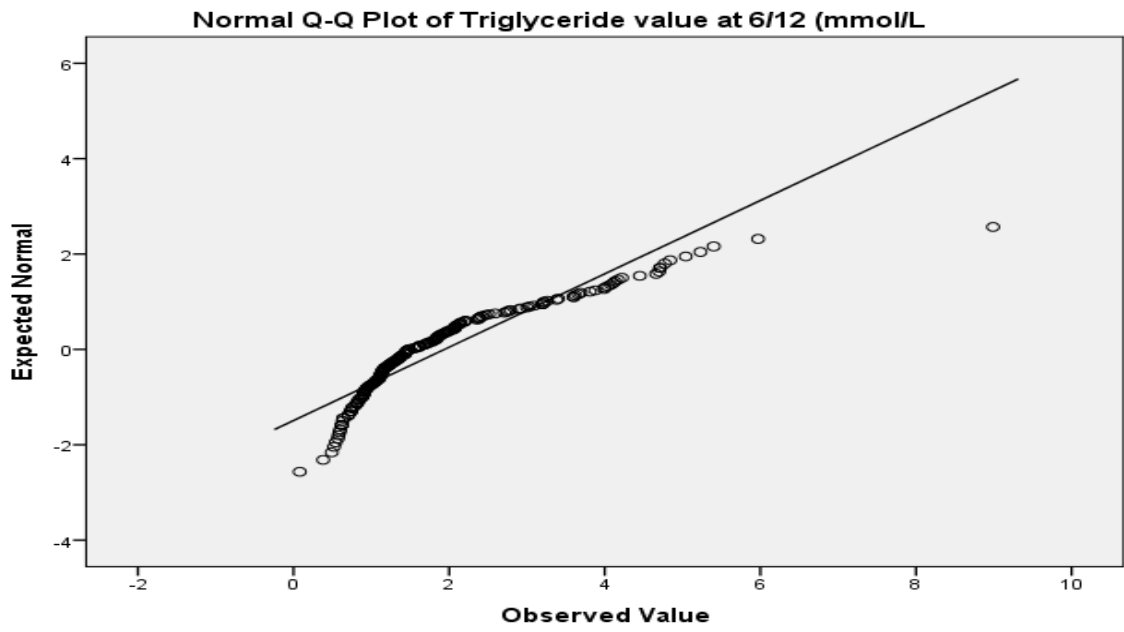


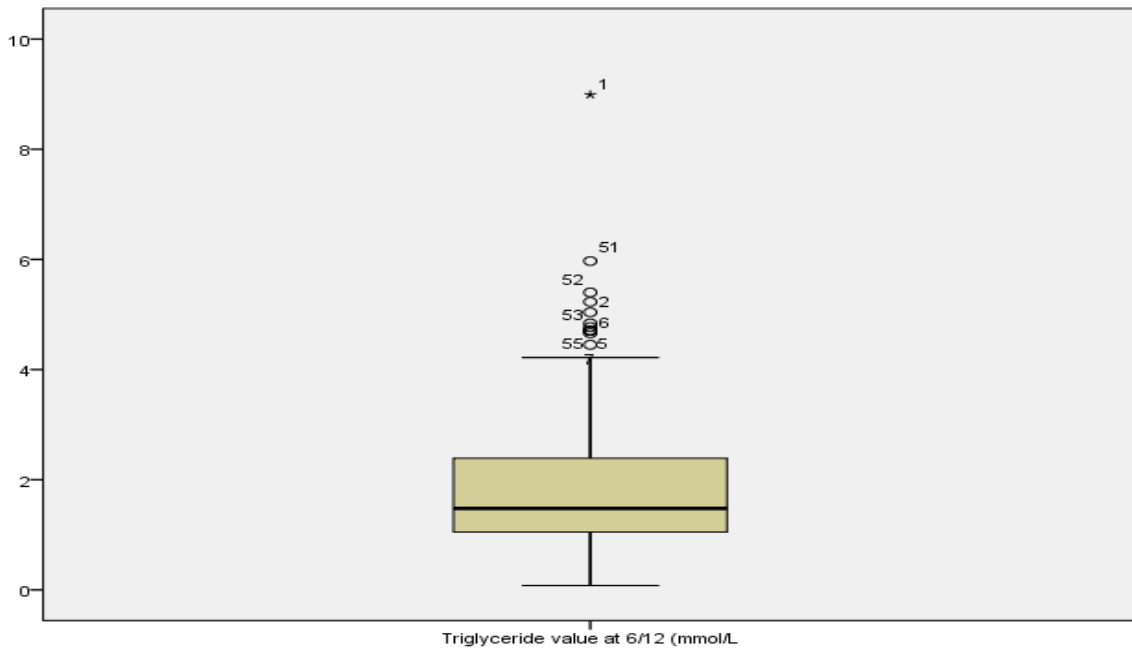




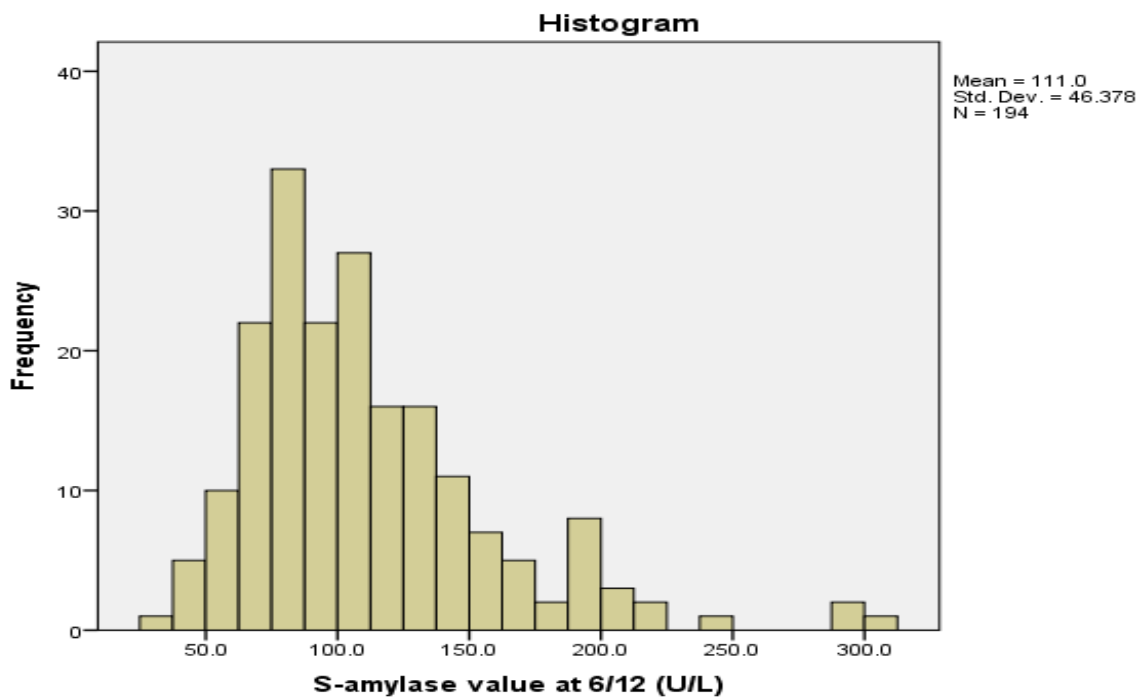
- Triglycerides: The lower and upper bound 95% confidence intervals for the mean of 1.94 mmol/L were 1.75 and 2.12 mmol/L respectively. The lowest value was 0.80 mmol/L and the highest value was 8.99 mmol/L with a range of 8.91 mmol/L. The variance was 1.69 and the interquartile range 1.35. Left skewed distribution of data is further indicated by skewness of 1.69 and kurtosis of 4.22. The histogram, normal and detrended Q-Q plots as well as the box plot show clear left skewed distribution of data and therefore a tendency to increase. All the outliers in the sample are also located above the 75% quartile.



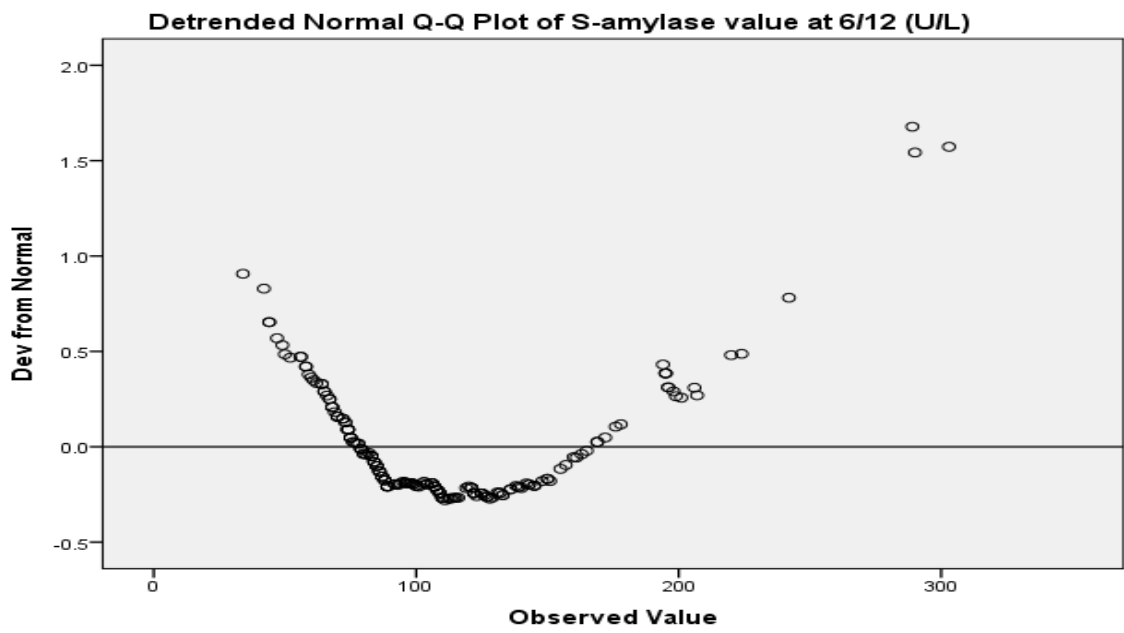
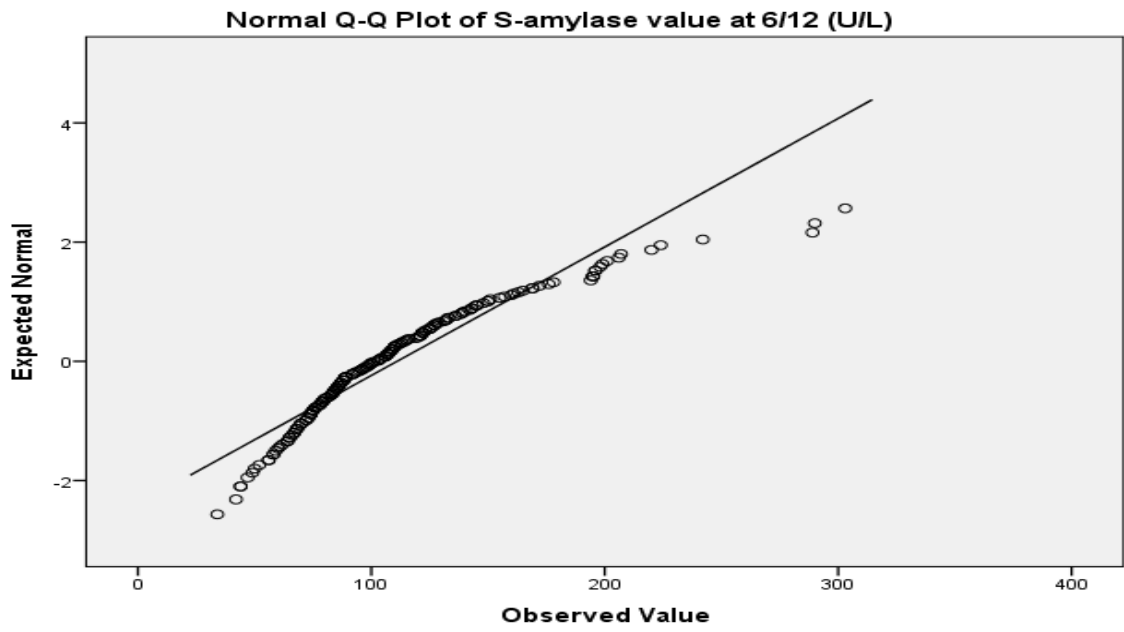


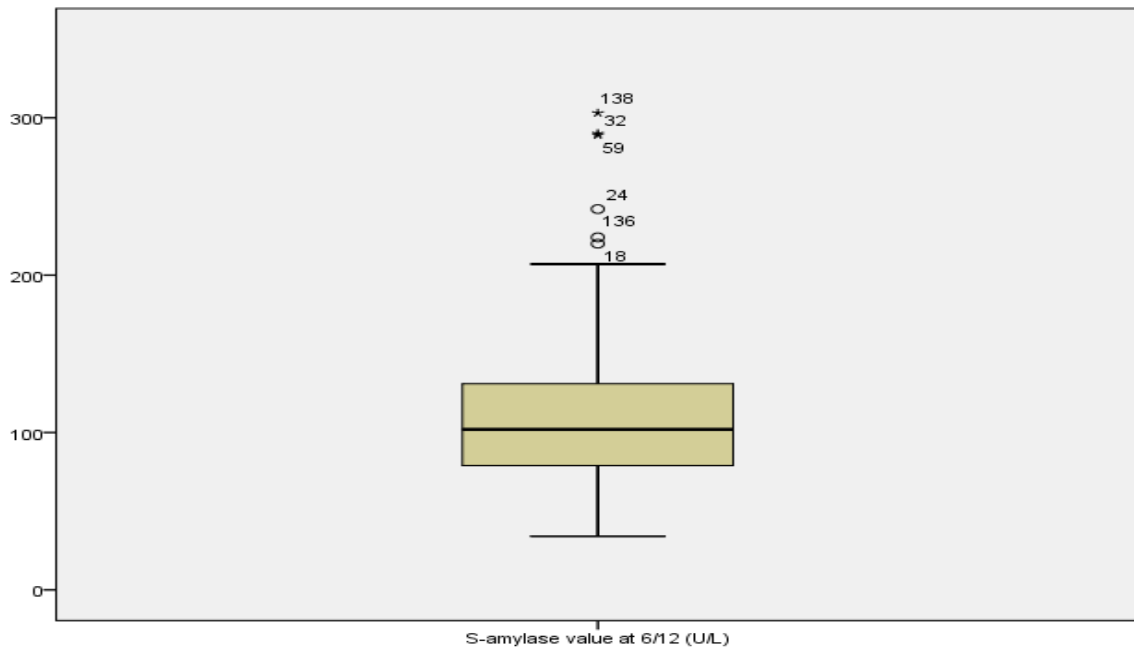


- Serum amylase: The lower and upper 95% confidence intervals for the mean were 104.43 and 177.56 U/L respectively. The lowest value was 34.00 U/L and the highest 303.00 U/L. The highest value was very close to the diagnostic requisite for pancreatitis of 312 U/L. The variance was 2150.91 and interquartile range 52.30. Left skewed distribution of data indicated by skewness of 1.43 and kurtosis of 2.83. The box plot shows all the outliers are above the 75% quartile.

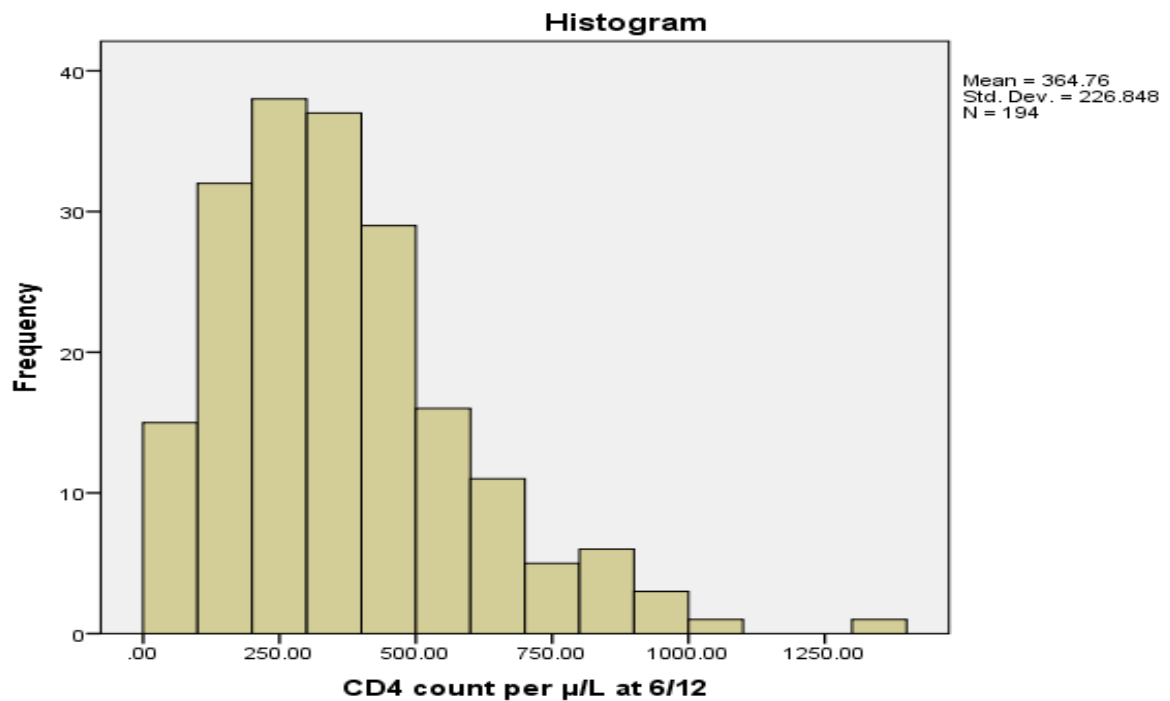


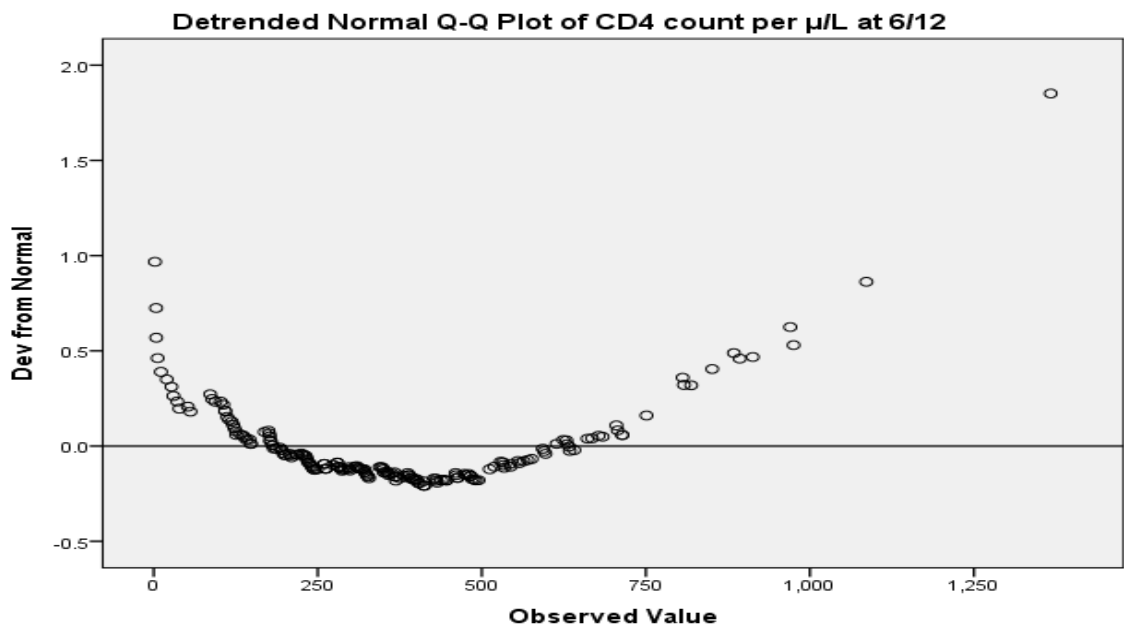
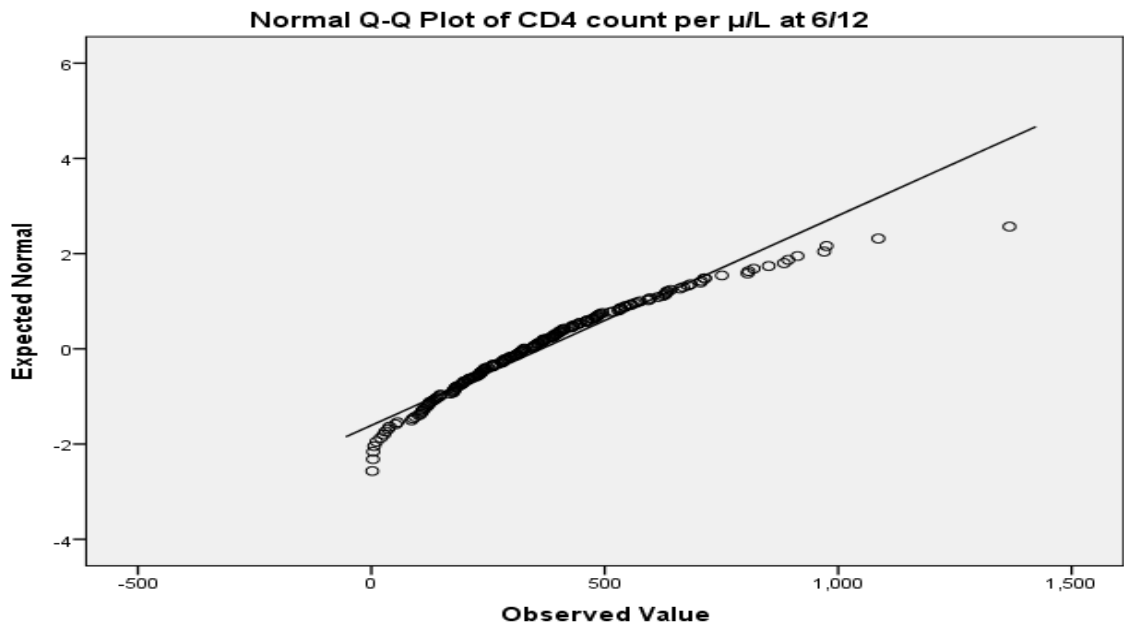


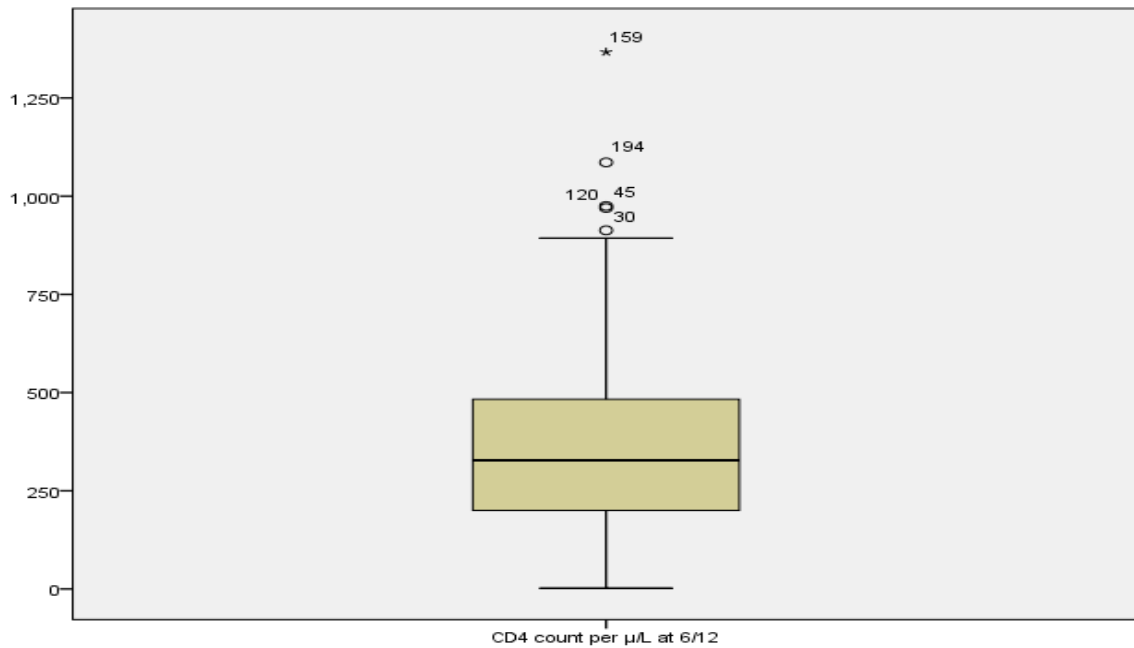




- CD<sub>4</sub> count: The lower and upper 95% confidence intervals for the mean were 332.64 and 396.88 cells/ $\mu$ L respectively. The lowest CD<sub>4</sub> count was 34 cells/ $\mu$ L and the highest 303 cells/ $\mu$ L. There was large variance of 51460.00 and an interquartile range of 283.00. Left skewed distribution of data indicated by skewness of 1.04 and kurtosis of 1.82. The histogram, Q-Q plots and box plots also demonstrate left skewed distribution of data.

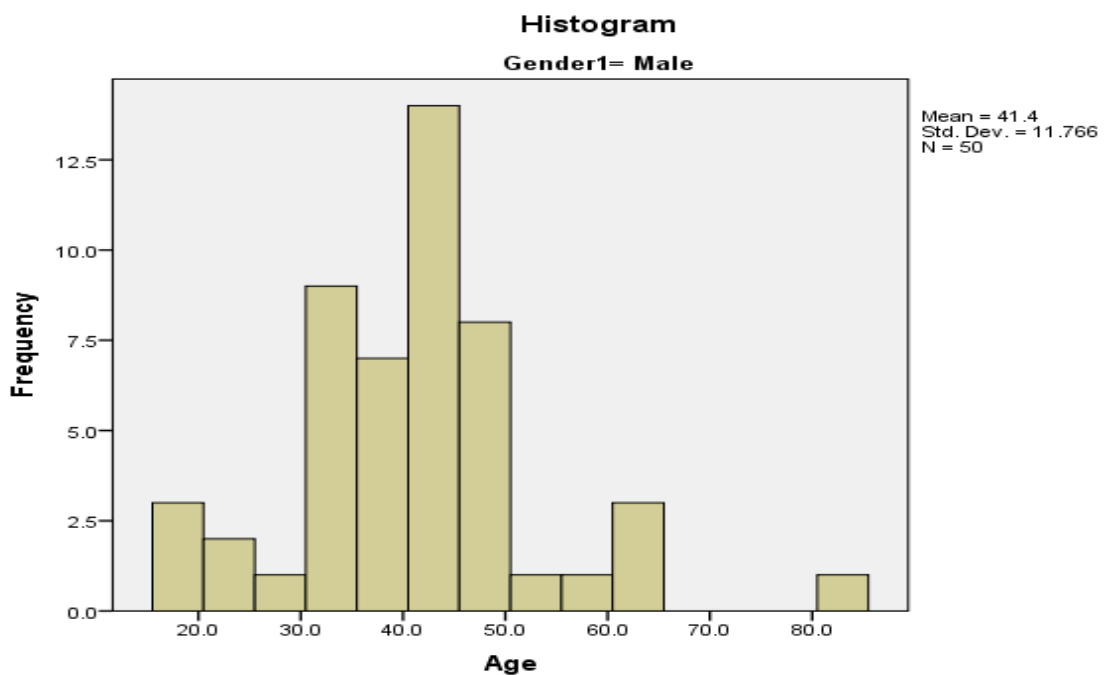




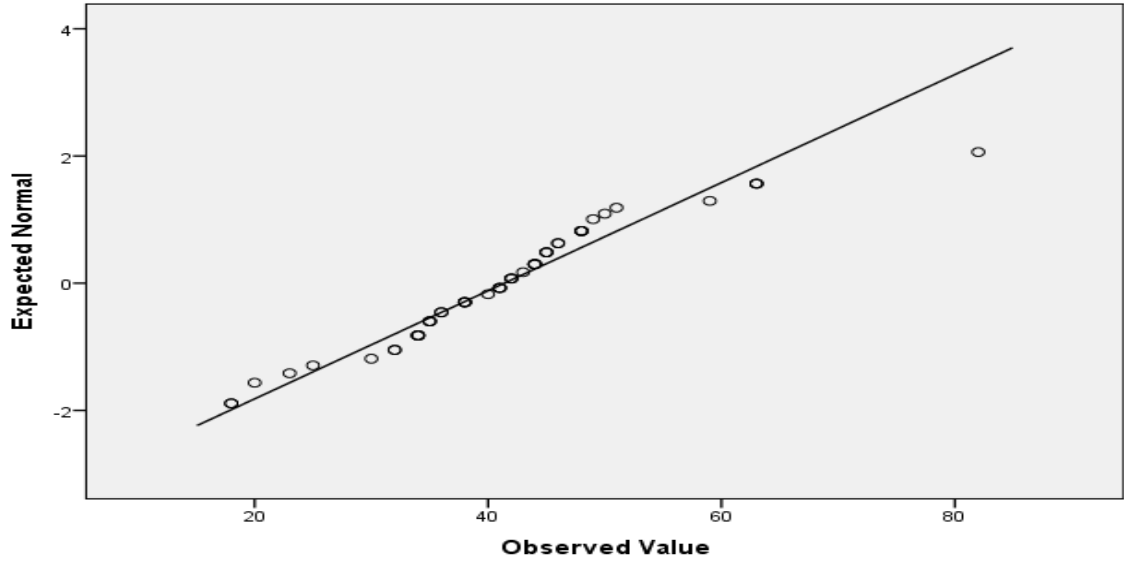


### 4.3 Results for males

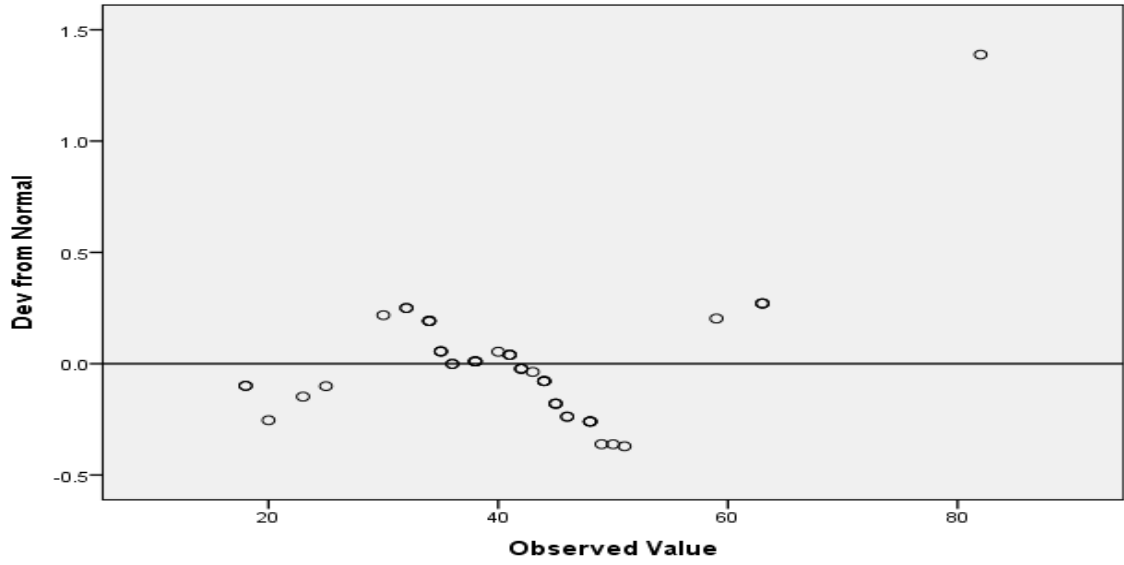
- Age: The 95% confidence interval lower bound was 38.06 and the upper bound was 44.74 for the mean. The minimum age was 18 years and the maximum age was 82 years. The mean age was 41.40 and the median 41.06 indicating negligible skewness. The variance was 138.44 and interquartile range 11.80. Skewness was at 0.71 and kurtosis 2.27. The Q-Q plots (both normal and detrended) showed very little skewness. The box plot showed quite even distribution of values, as did the histogram.

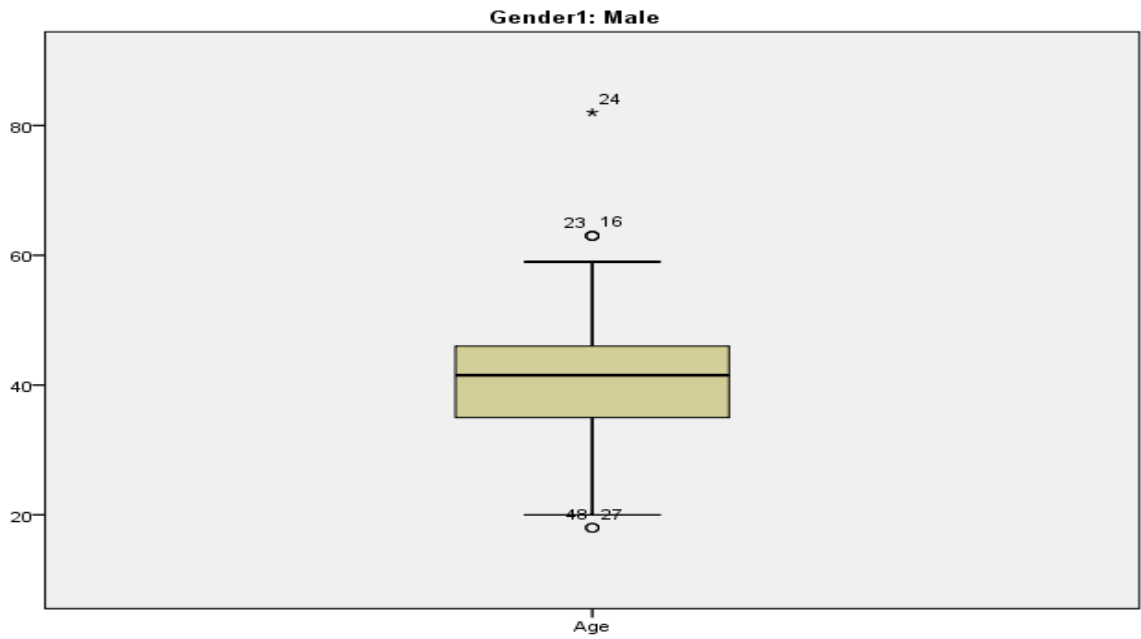


Normal Q-Q Plot of Age  
Gender1= Male

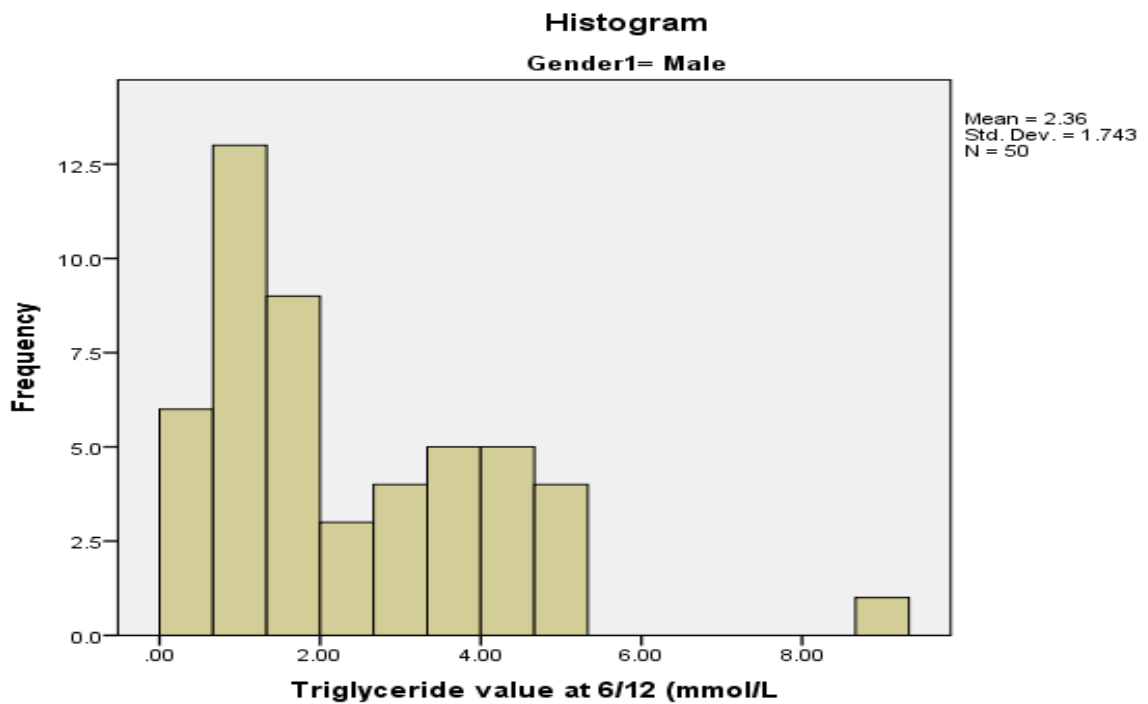


Detrended Normal Q-Q Plot of Age  
Gender1= Male

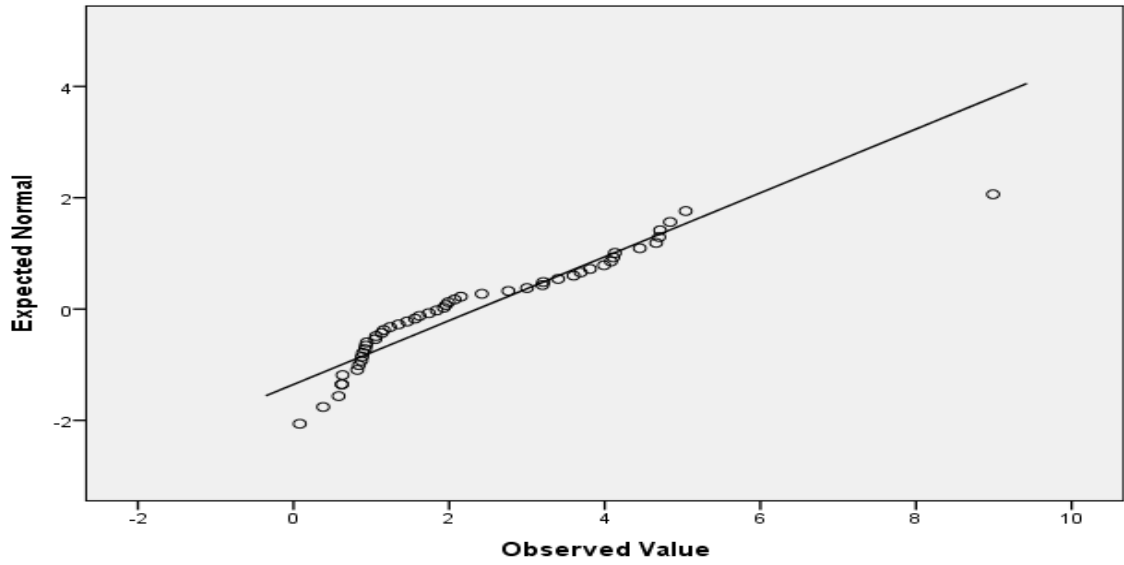




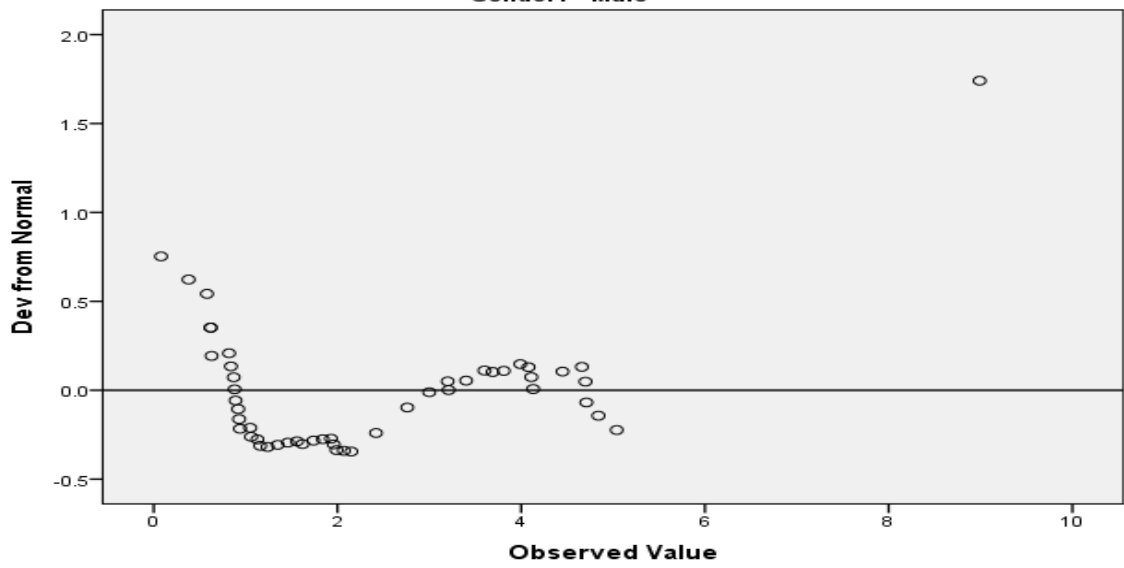
- Triglycerides: The upper and lower bound 95% confidence intervals for the mean were 2.86 and 1.87 respectively. The variance was small at 3.04. Skewness and kurtosis were 1.29 and 2.57 respectively. Left skewed data were confirmed by the histogram, normal and detrended Q-Q plots and box plot. The box plot shows all the outliers above the 75% quartile.

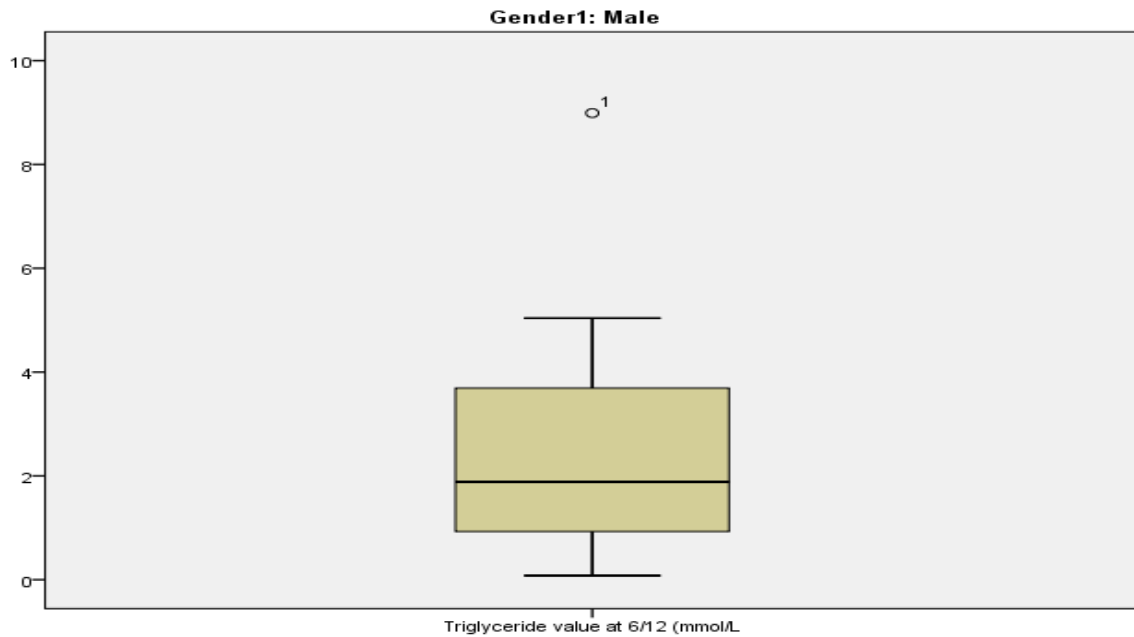


Normal Q-Q Plot of Triglyceride value at 6/12 (mmol/L)  
Gender1= Male

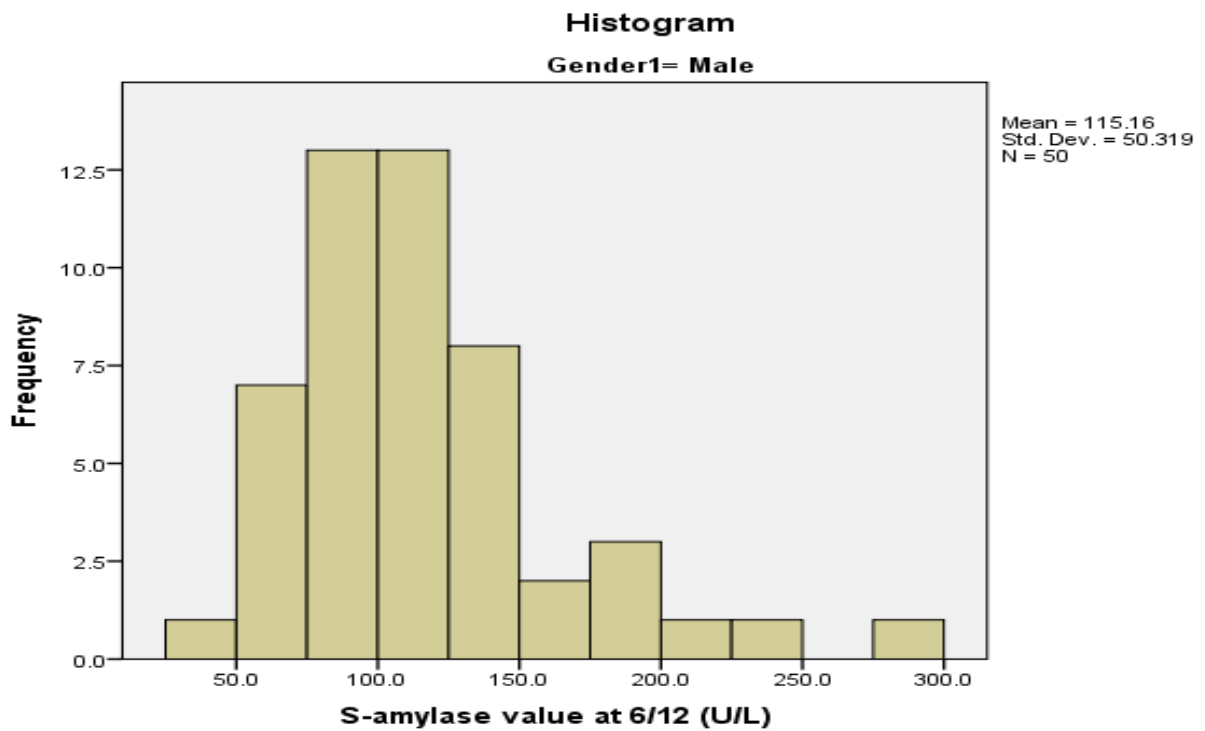


Detrended Normal Q-Q Plot of Triglyceride value at 6/12 (mmol/L)  
Gender1= Male





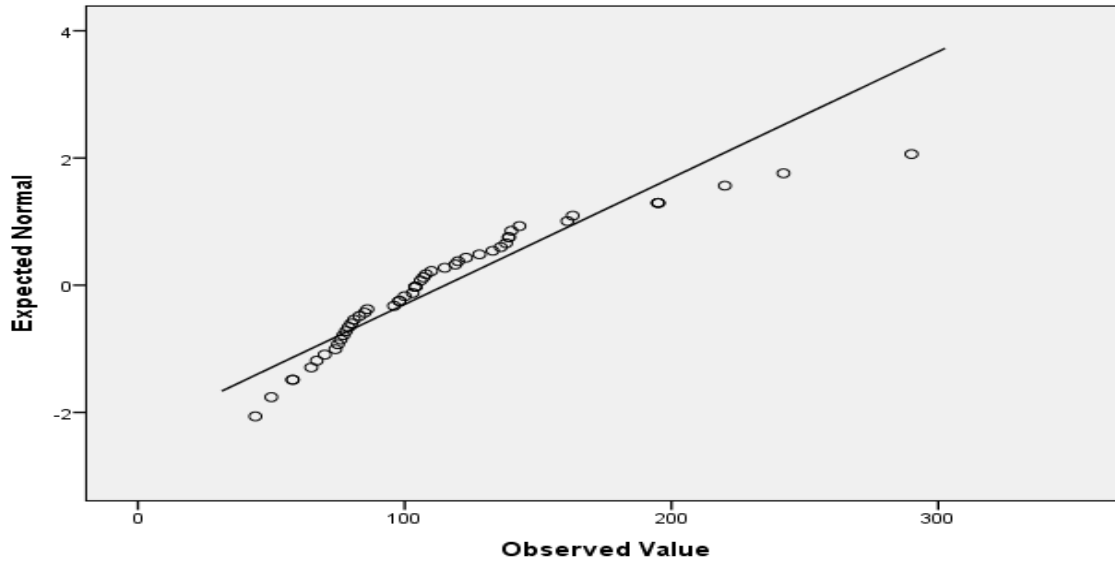
- Serum amylase: The upper and lower 95% confidence intervals for the mean were 129.18 and 100.86 respectively. Variance was considerable at 2531.97. The mean was to the left of the median indicating left skewed distribution of the data. Skewness and kurtosis were 1.44 and 2.38 respectively. The histogram, normal and detrended Q-Q plots and the box plot further demonstrated skewness. The box plot showed most values above the median, demonstrating a slight tendency to increase.





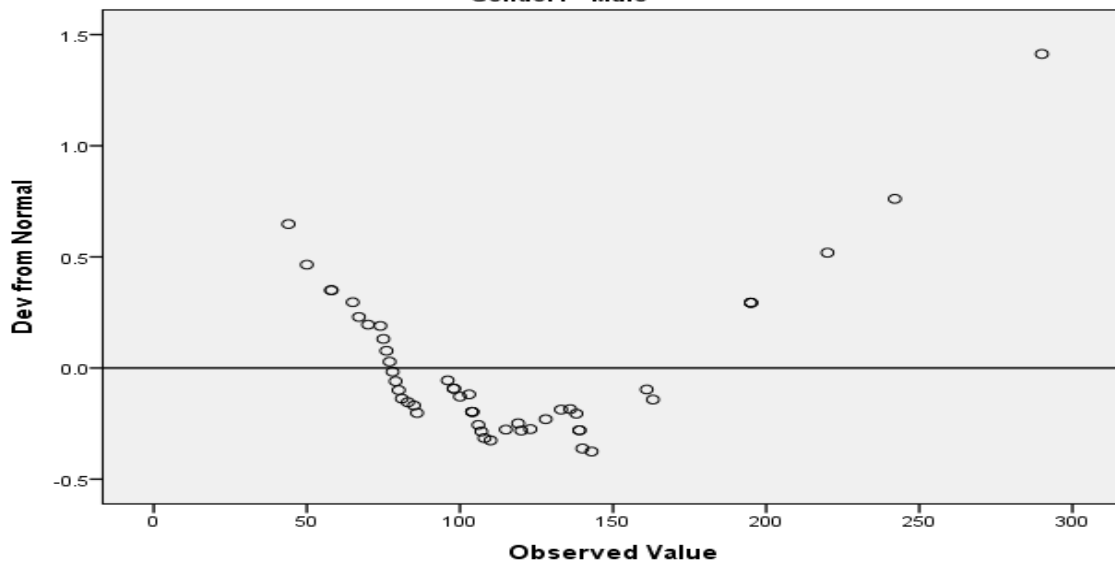
Normal Q-Q Plot of S-amylase value at 6/12 (U/L)

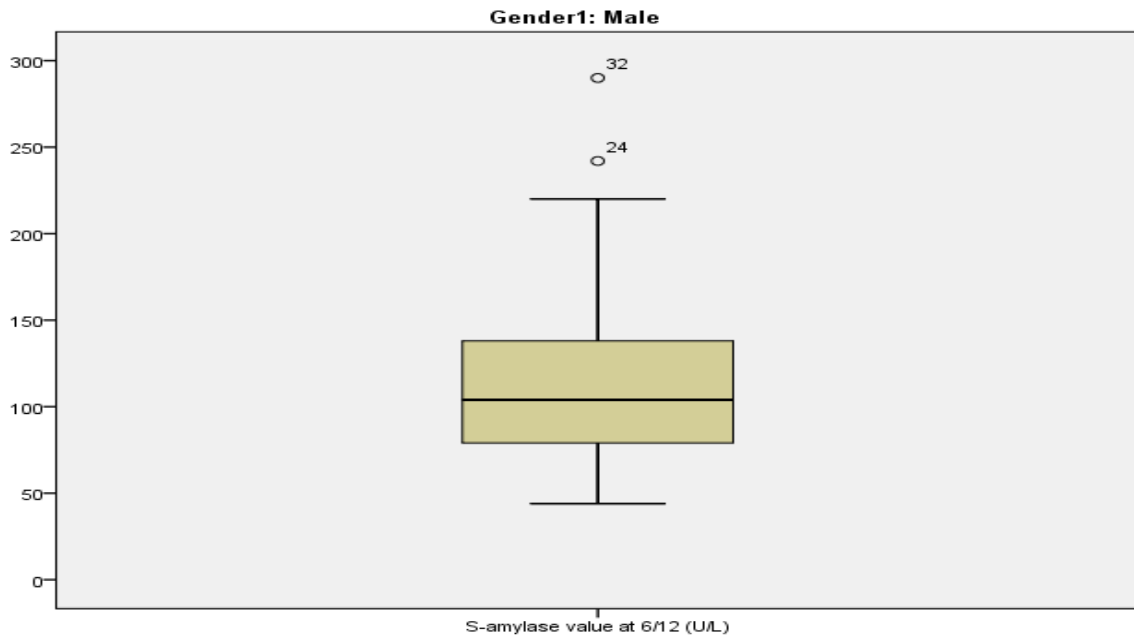
Gender1= Male



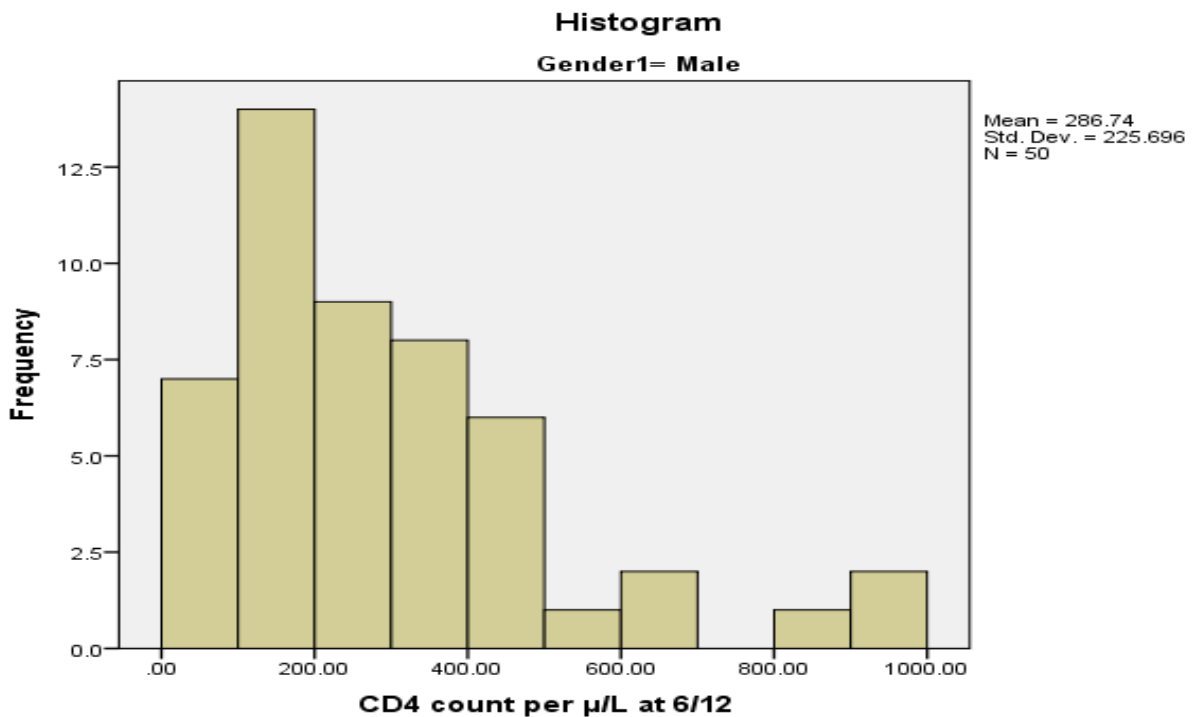
Detrended Normal Q-Q Plot of S-amylase value at 6/12 (U/L)

Gender1= Male



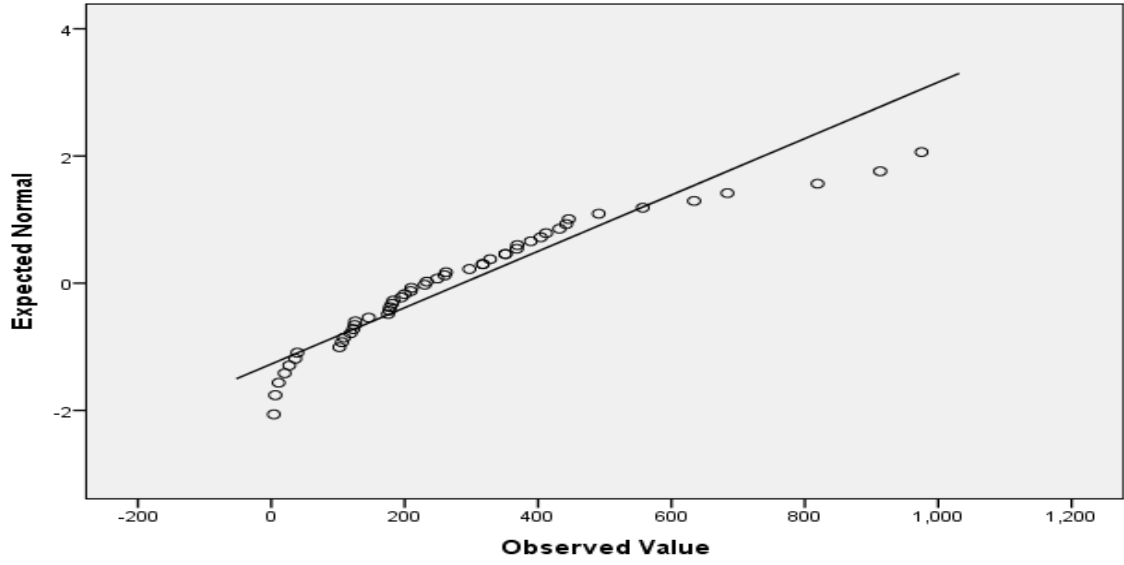


- CD<sub>4</sub> count: The upper and lower 95% confidence intervals were 350.88 and 222.59 respectively. Variance was large at 50938.71. The mean was to the left of the median indicating left skewed distribution of the data. Skewness and kurtosis were 1.28 and 1.70 respectively. The histogram, normal and detrended Q-Q plots and the box plot further demonstrated skewness. The box plot showed most values above the median, demonstrating a slight tendency to increase with three outliers beyond the 75% quartile.



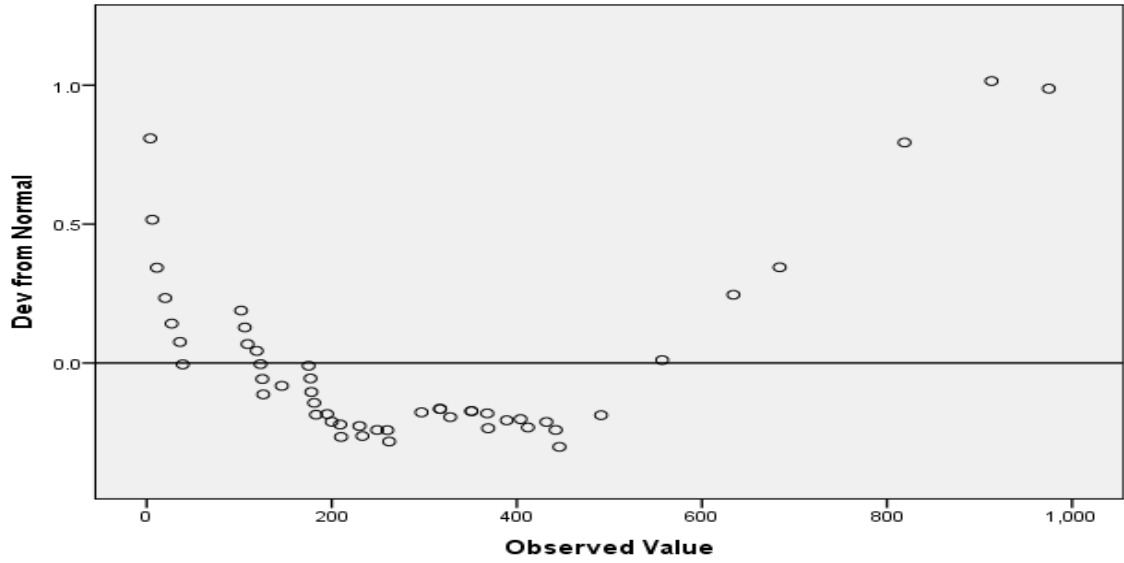
Normal Q-Q Plot of CD4 count per  $\mu\text{L}$  at 6/12

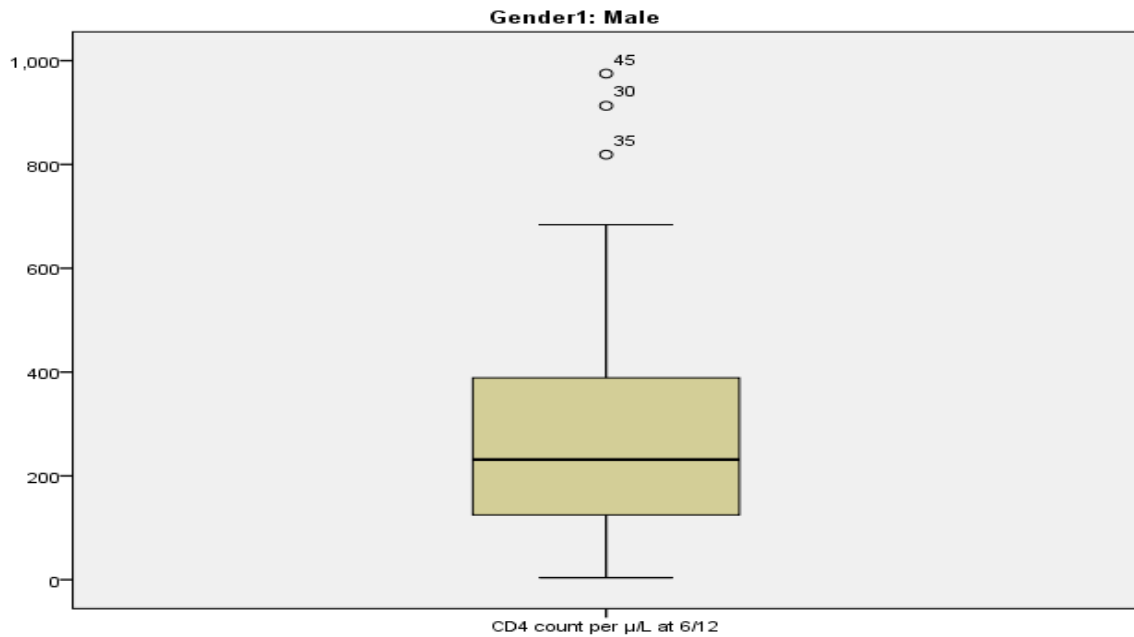
Gender1= Male



Detrended Normal Q-Q Plot of CD4 count per  $\mu\text{L}$  at 6/12

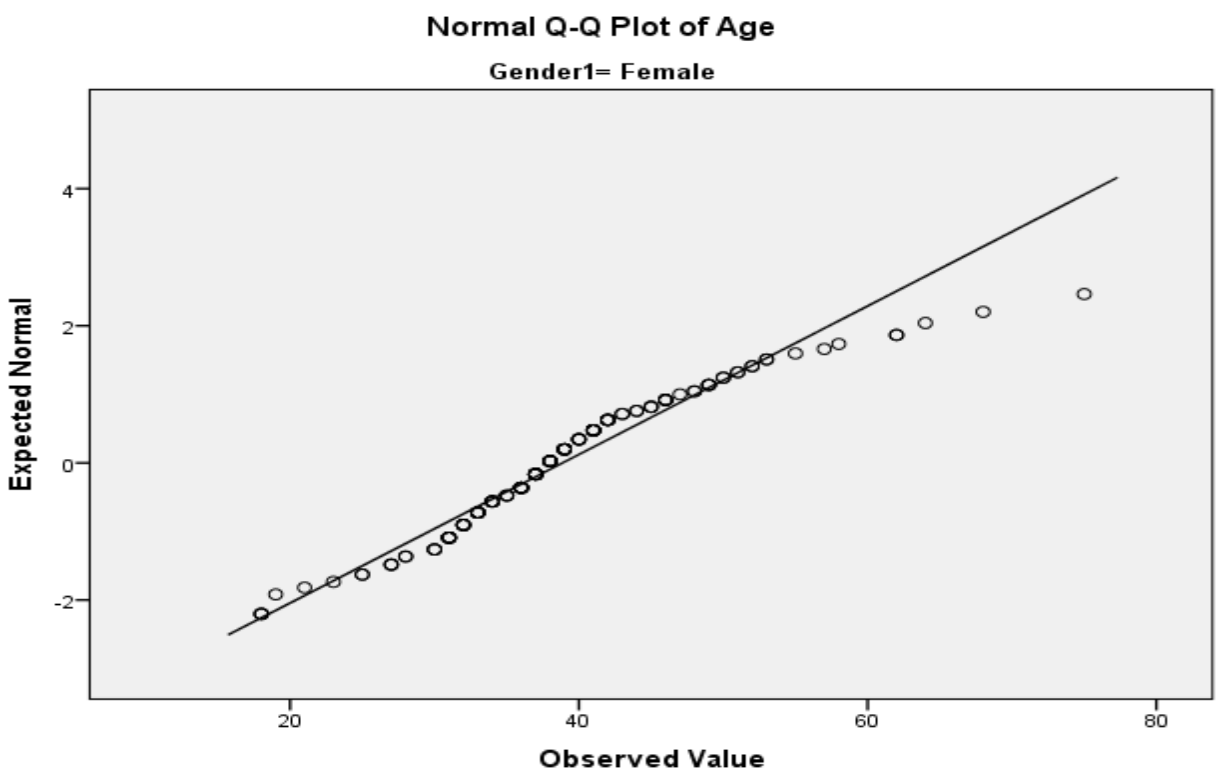
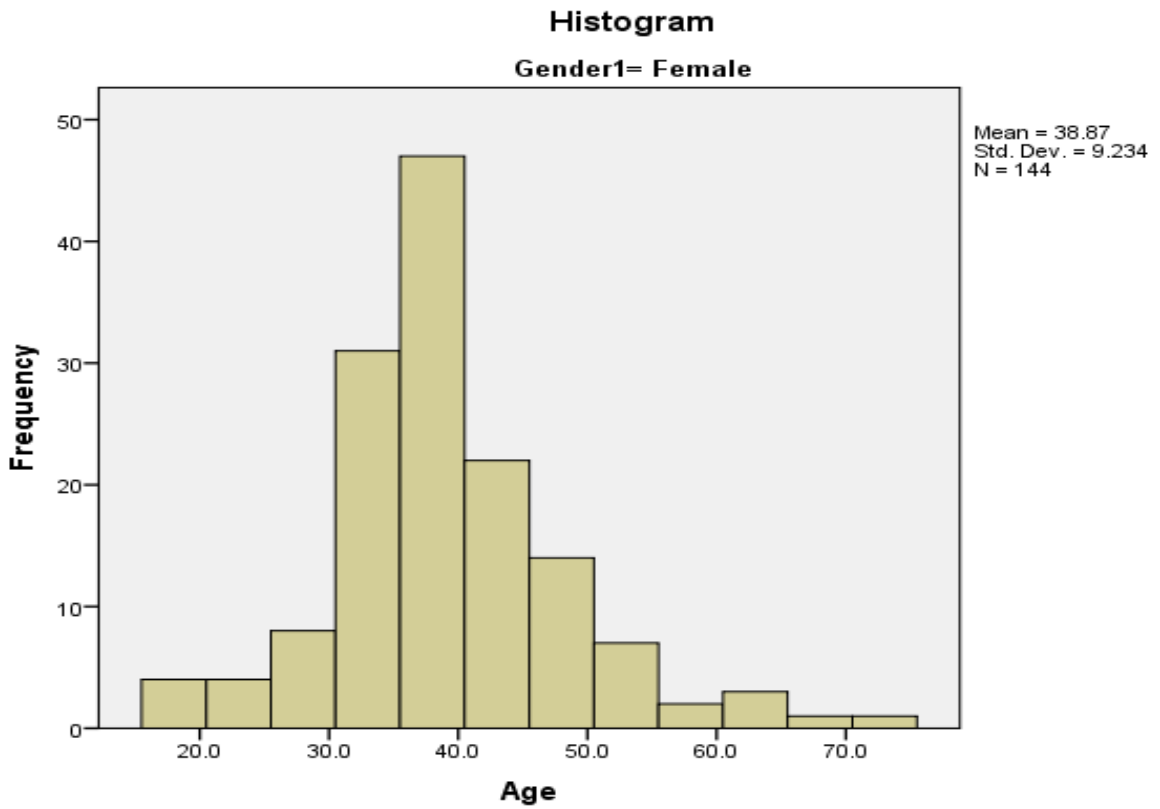
Gender1= Male





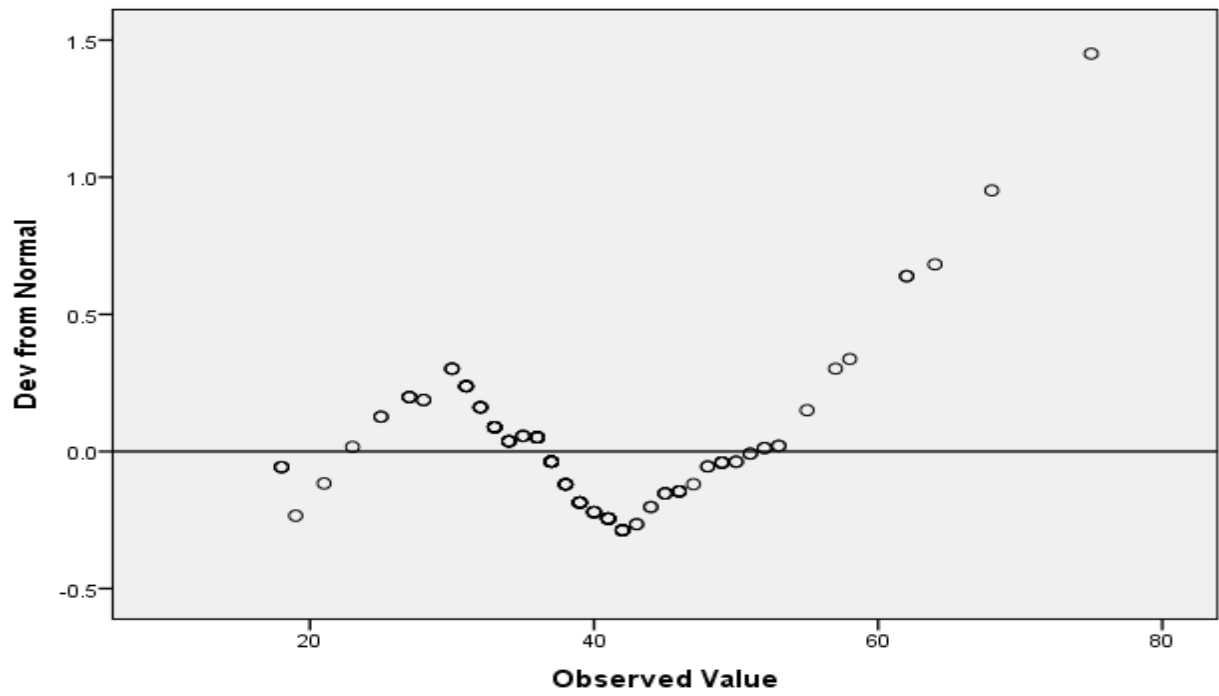
#### 4.4 Results for females

- Age: The lower and upper bound 95% confidence interval for the mean were 37.35 and 40.39 respectively. There was very little difference between the mean (38.89) and median (38.00) indicating negligible skewness. The youngest patient was 18 years of age and the oldest 75 years with a range of 57 years. The interquartile range was narrow at 9 years. Skewness and kurtosis were slight at 0.79 and 2.08 respectively. The histogram demonstrates fairly normal distribution. The normal and detrended Q-Q plots indicate very little skewness. The box plot indicates fairly normal distribution, but notably showed many more outliers above the 75% quartile than below the 25% quartile.

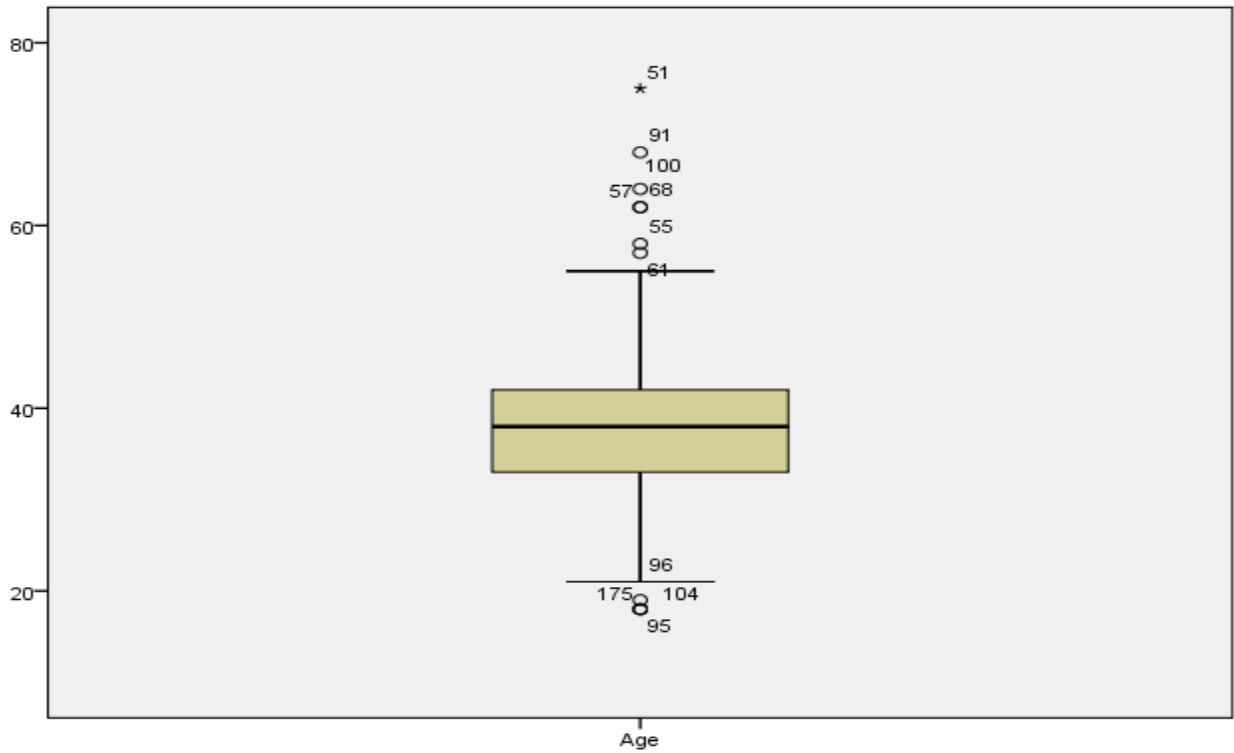


### Detrended Normal Q-Q Plot of Age

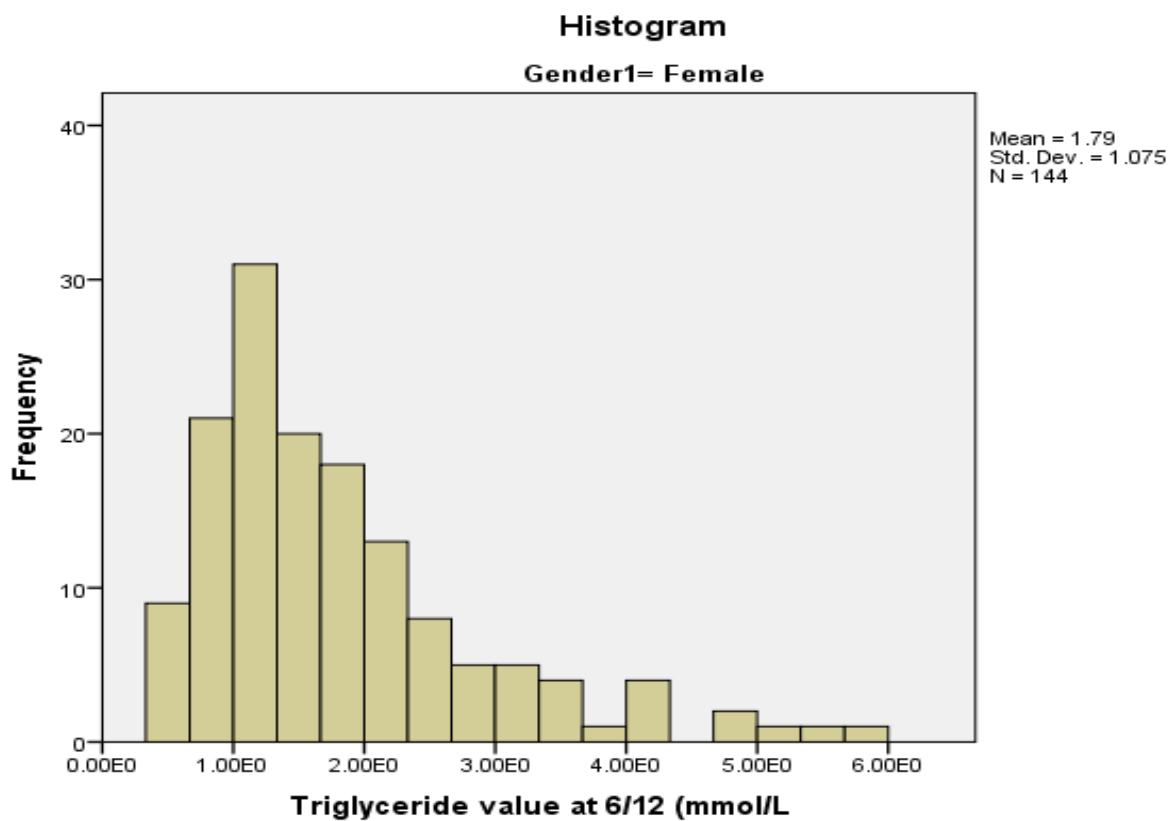
Gender1= Female



Gender1: Female

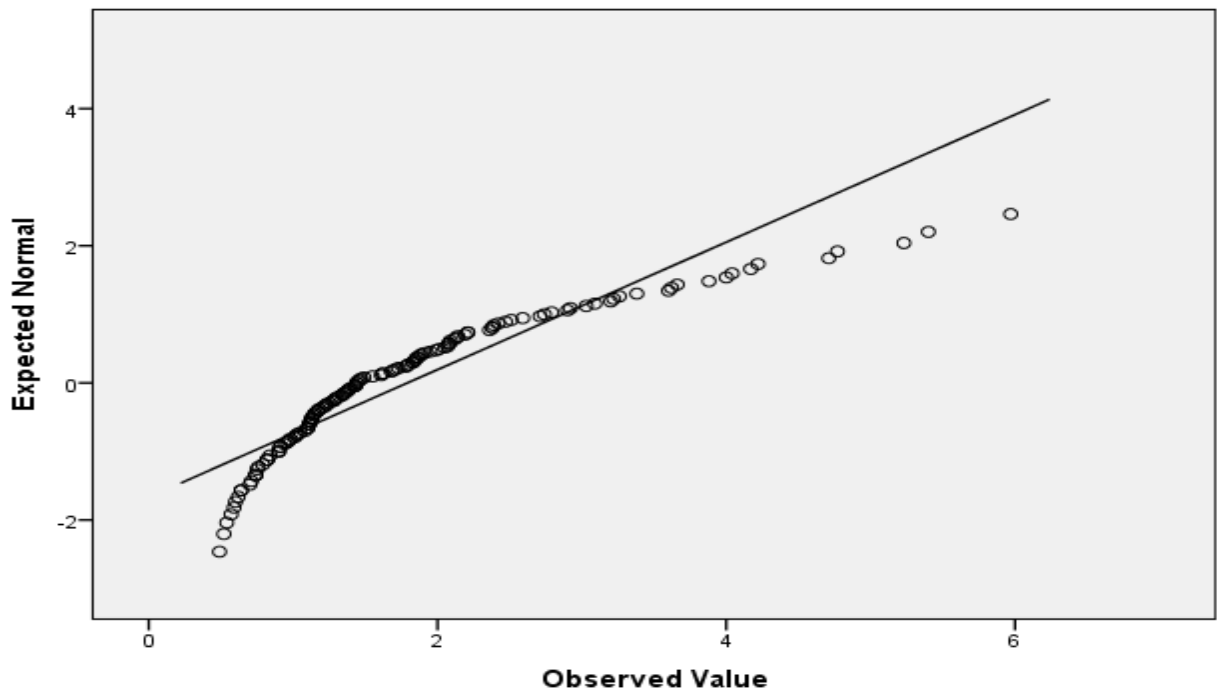


- Triglycerides: There is a definite difference between the mean (1.79 mmol/L) and median (1.44 mmol/L) indicating significant left skewed data. The lower and upper 95% confidence intervals for the mean were 1.61 and 1.97 respectively. Variance was significant at 1.16. The lowest value was 0.49 mmol/L and the highest value was 5.97 mmol/L with a range of 5.48 mmol/L. Skewness and kurtosis were 1.54 and 2.44 respectively. The histogram, normal and detrended Q-Q-plots, as well as the box plot showed obvious left skewed distribution of data and a tendency to increase. The box plot shows several outliers above the 75% quartile.



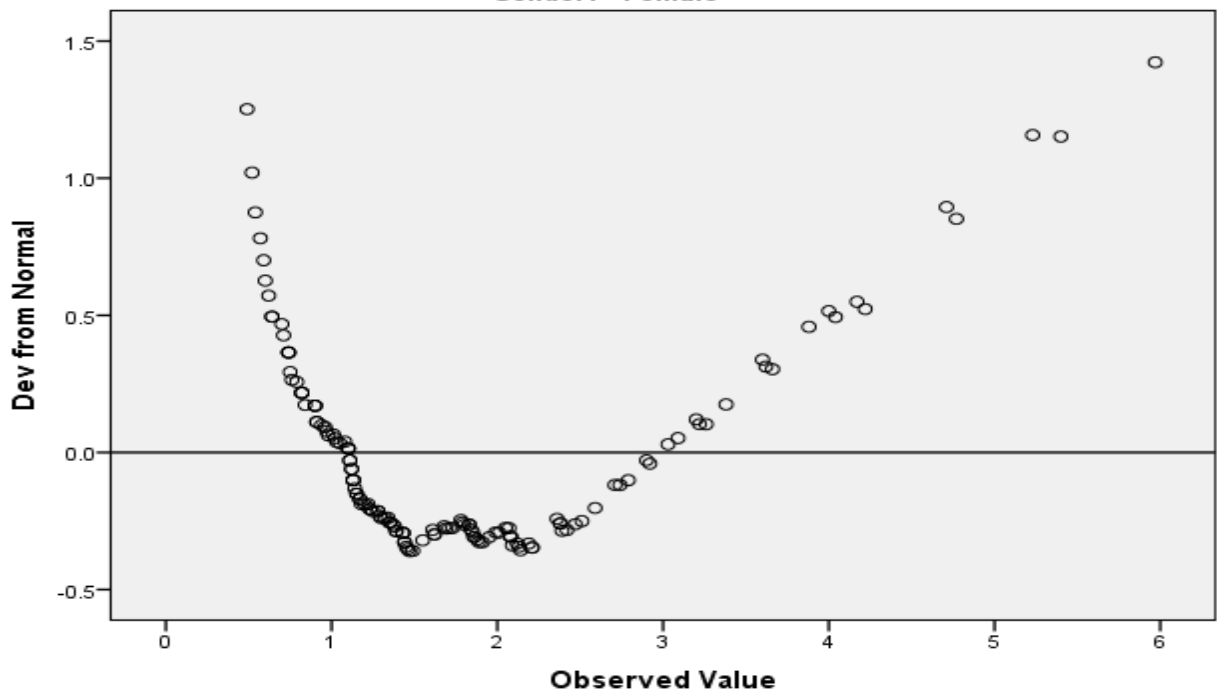
Normal Q-Q Plot of Triglyceride value at 6/12 (mmol/L)

Gender1= Female

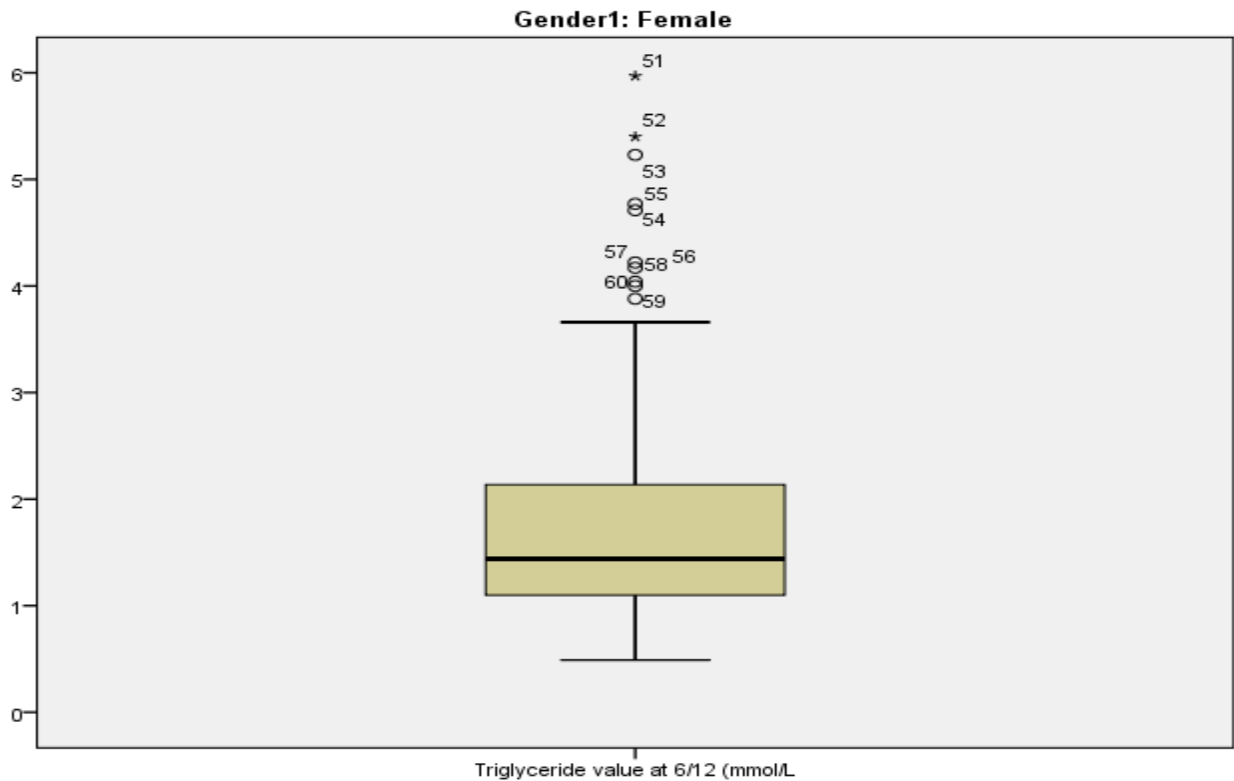


Detrended Normal Q-Q Plot of Triglyceride value at 6/12 (mmol/L)

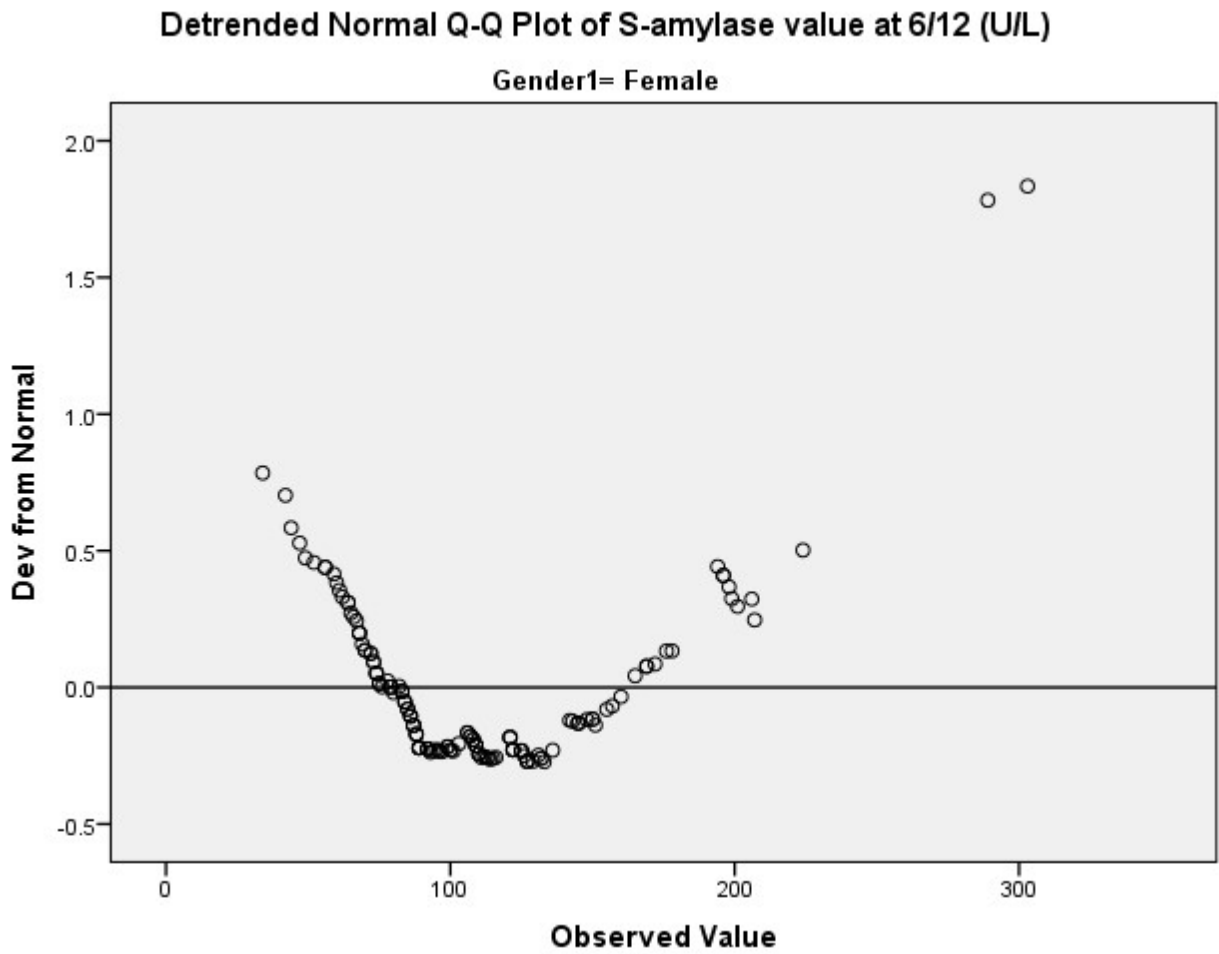
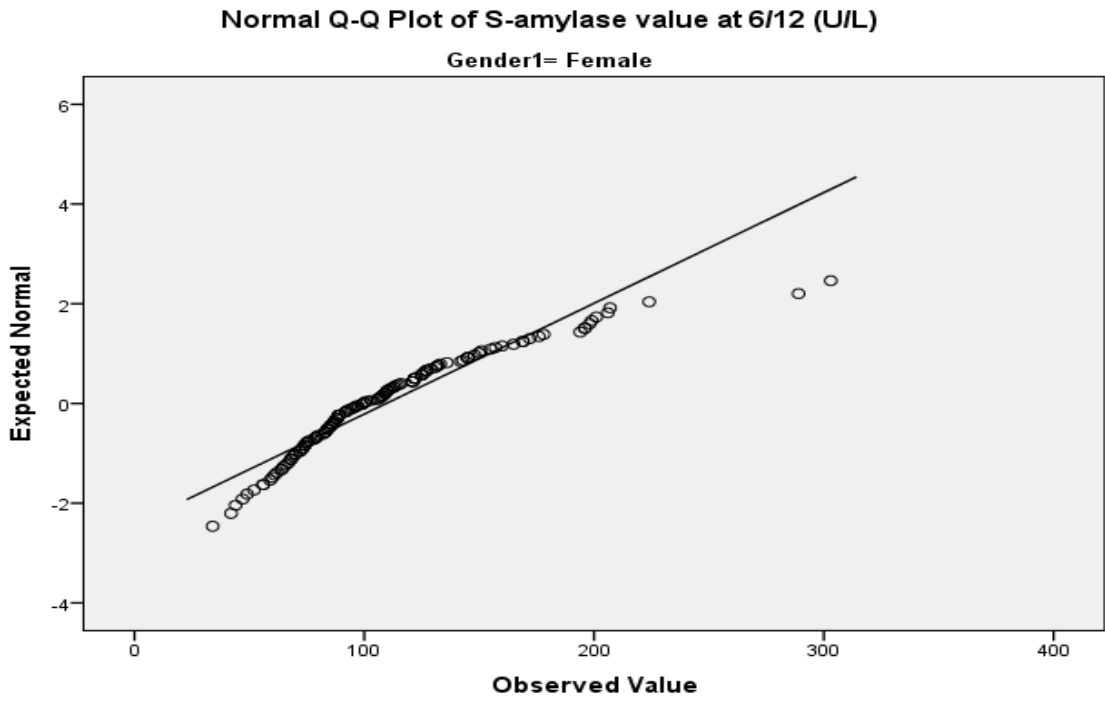
Gender1= Female



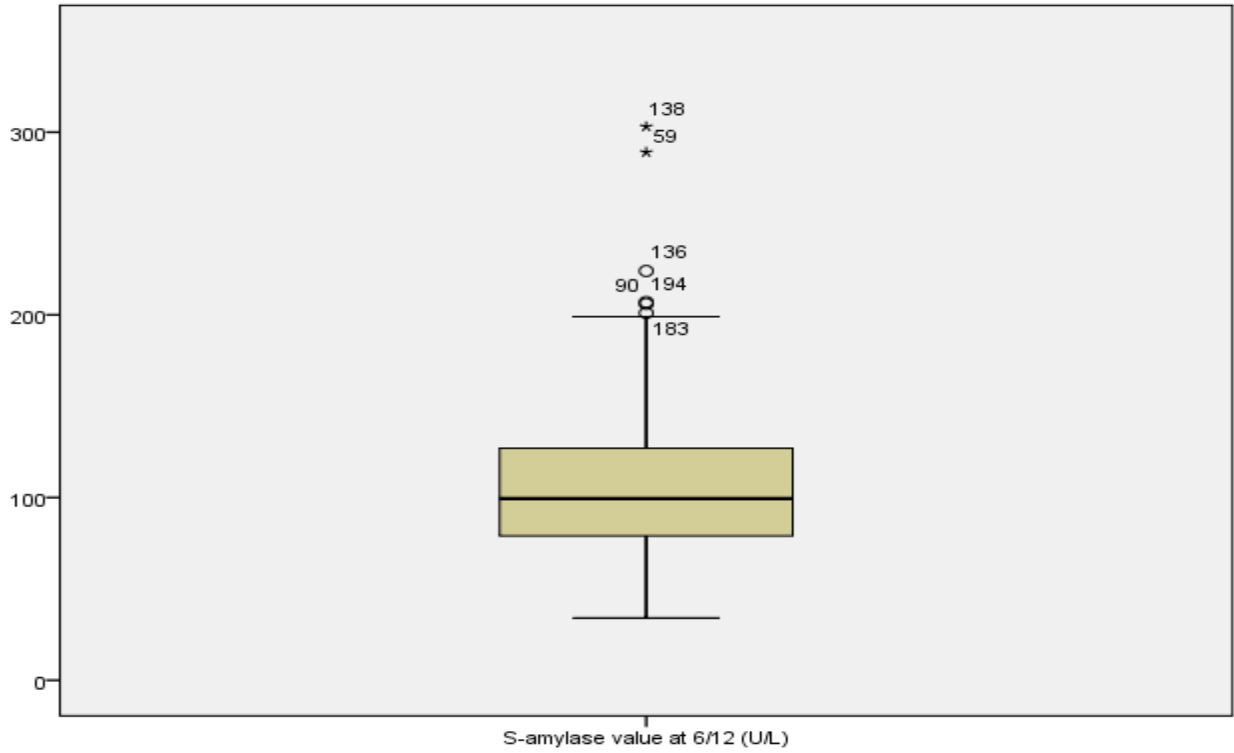




- Serum Amylase: The difference between the mean (109.56) and median (99.50) showed slight left skewed distribution of data. The lower and upper 95% confidence intervals for the mean were 102.14 and 116.97 U/L respectively. There was significant variance at 2027.23 with the smallest value at 34.00 U/L and the highest value at 303.00 U/L with a range of 269.00. The interquartile range was 48 U/L with skewness and kurtosis at 1.44 and 3.12 respectively. The histogram, normal and detrended Q-Q plots and the box plot showed notably left skewed distribution of data. All the outliers were above the 75% quartile with one below the 25% quartile.

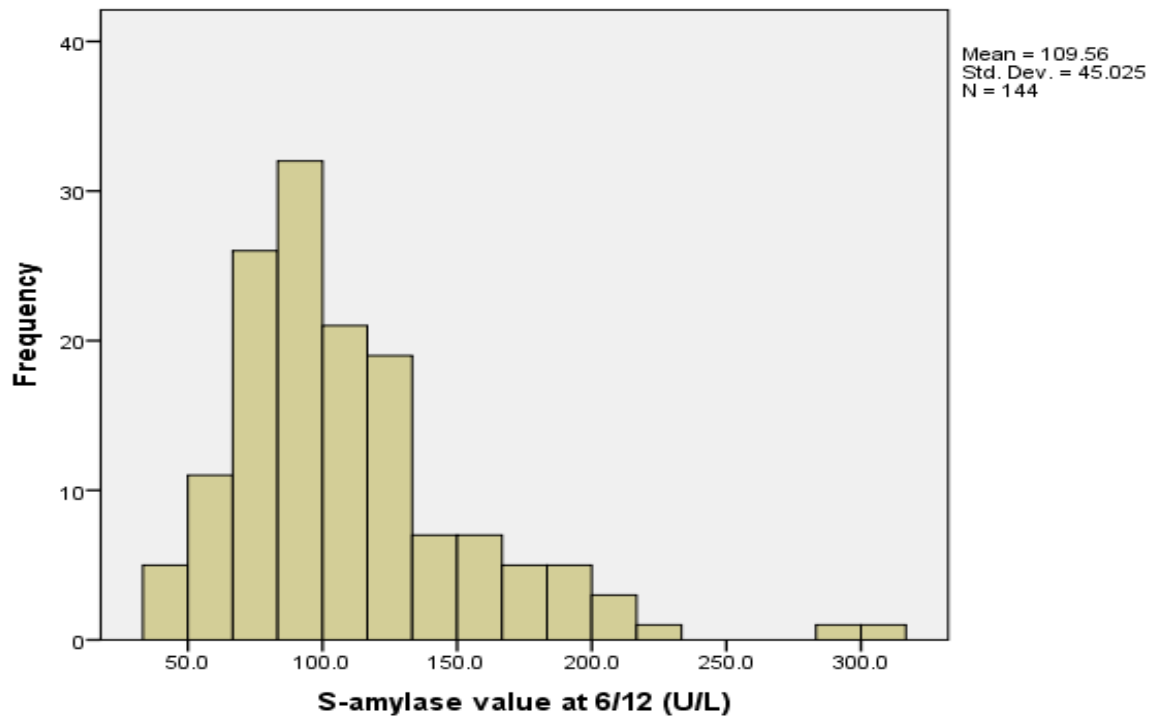


**Gender1: Female**

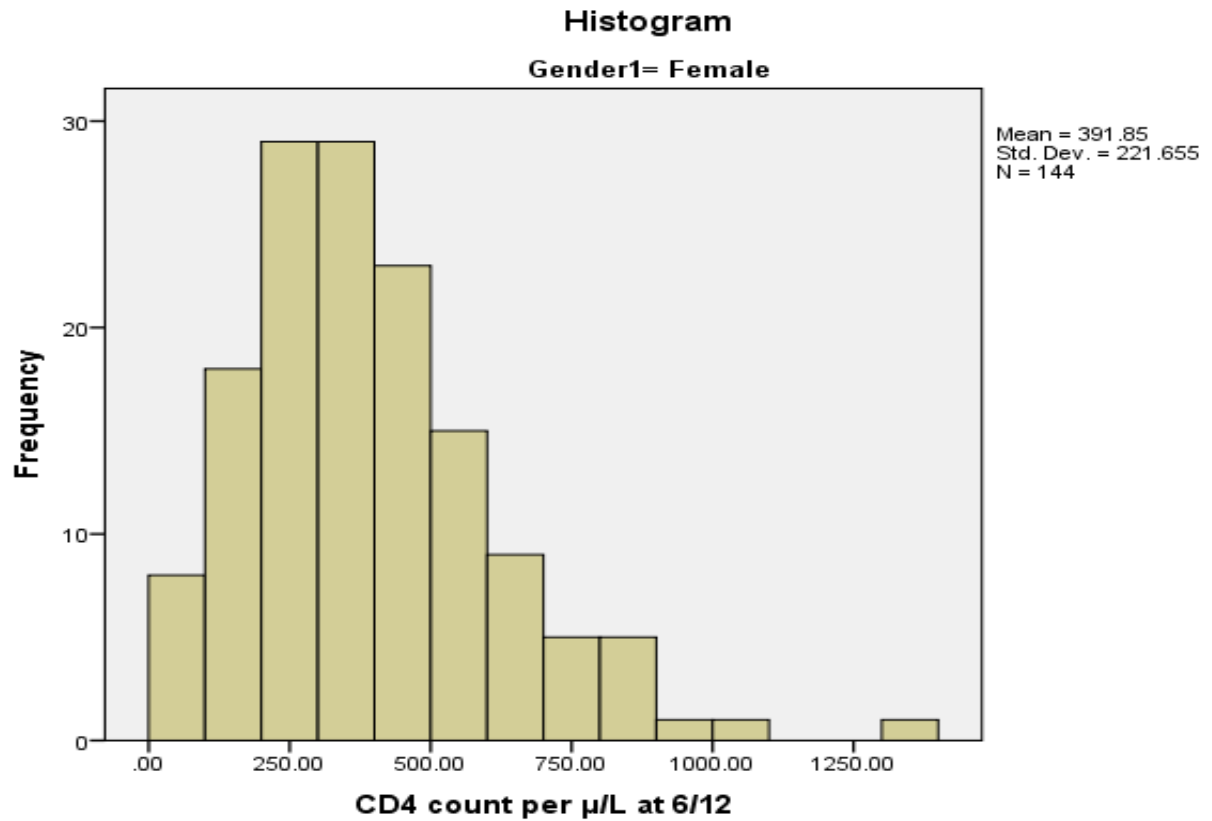


**Histogram**

**Gender1= Female**

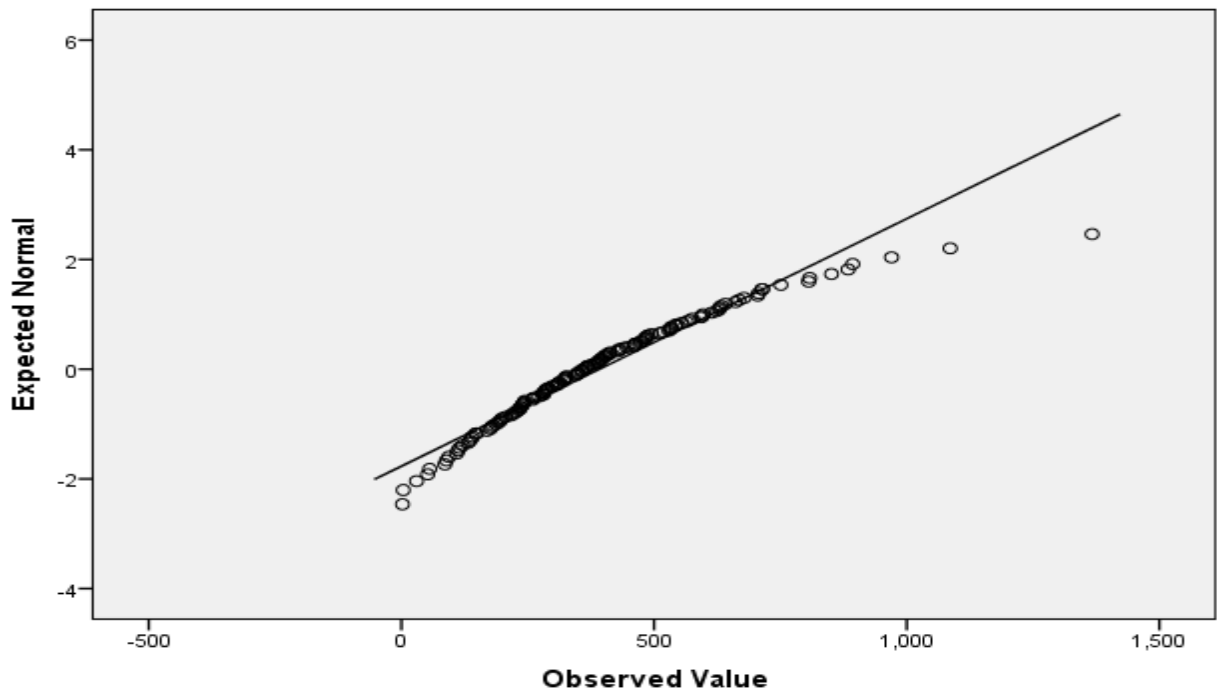


- CD<sub>4</sub> count: The difference between the mean (391.85) and the median (360.50) indicated slight left skewed distribution of data. The lower and upper 95% confidence intervals for the mean were 355.34 and 428.36 respectively. There was significant variance at 49131.16. The smallest value was 1.93 cells/ $\mu$ L and the highest value was 1367.07 cells/ $\mu$ L. Skewness and kurtosis were 1.09 and 2.29 respectively. The histogram, normal and detrended Q-Q plots and box plot showed slight left skewed distribution of data. The box plot showed the only outliers above the 75% quartile.



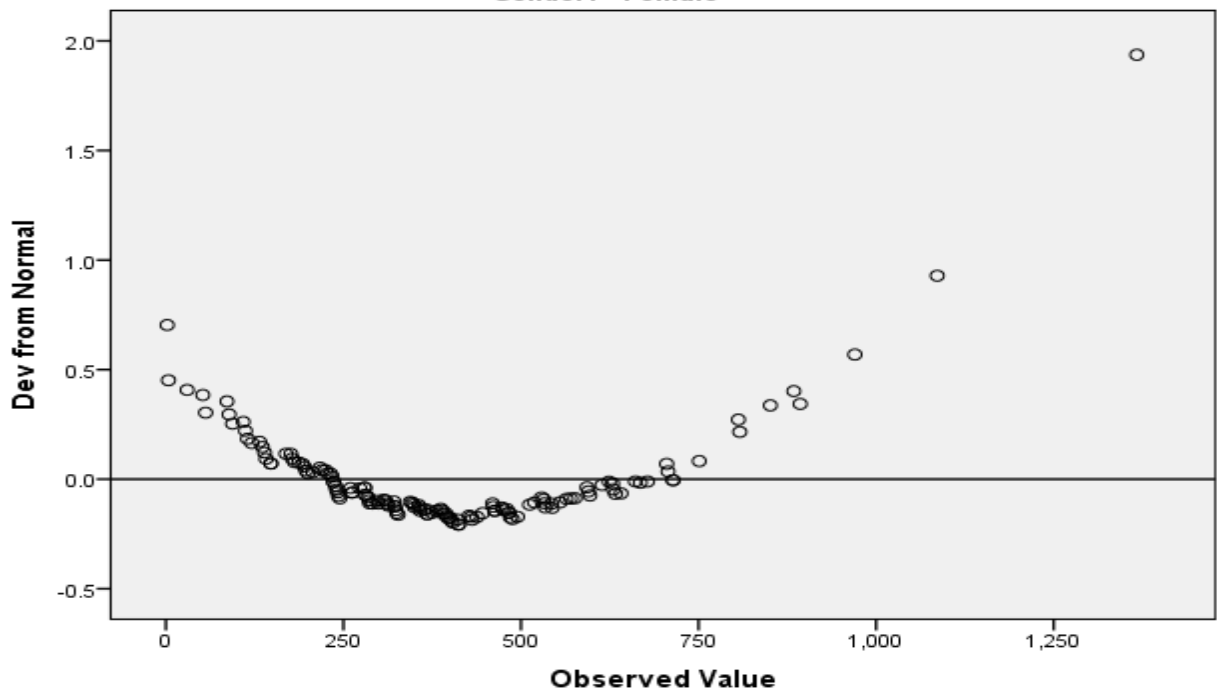
Normal Q-Q Plot of CD4 count per  $\mu\text{L}$  at 6/12

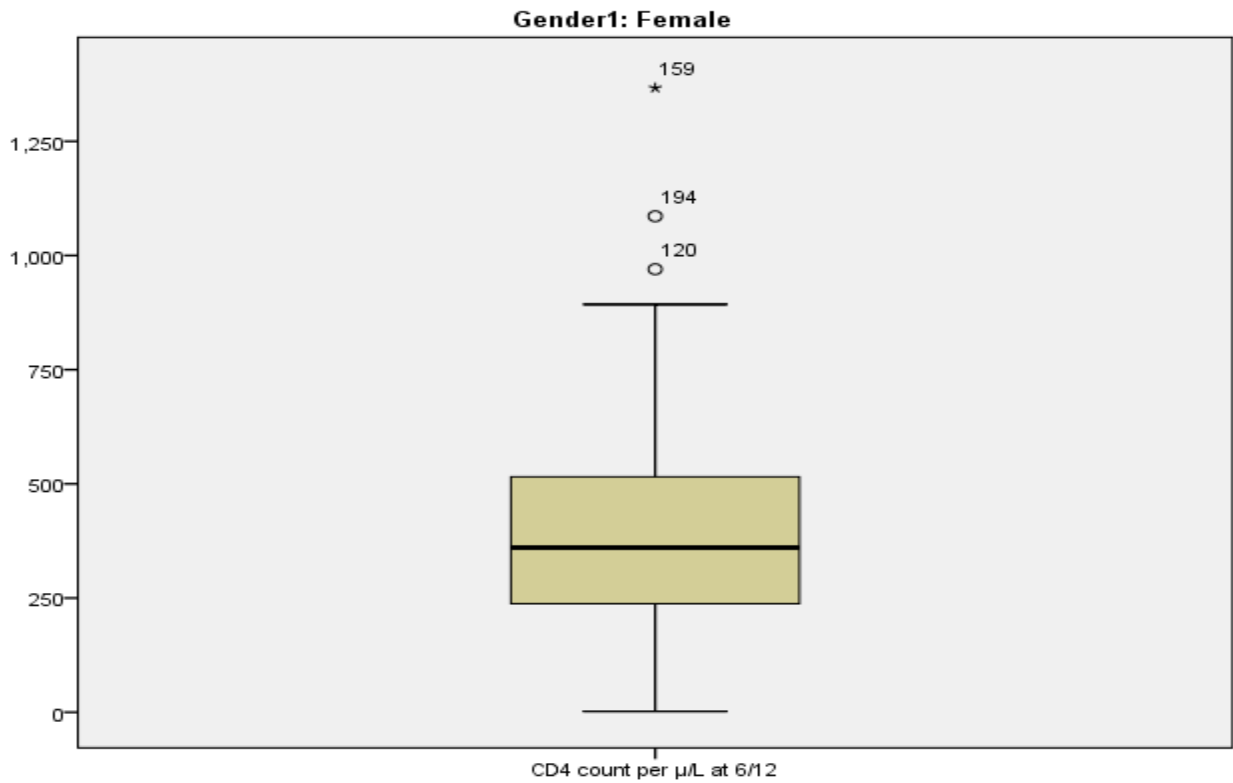
Gender1= Female



Detrended Normal Q-Q Plot of CD4 count per  $\mu\text{L}$  at 6/12

Gender1= Female

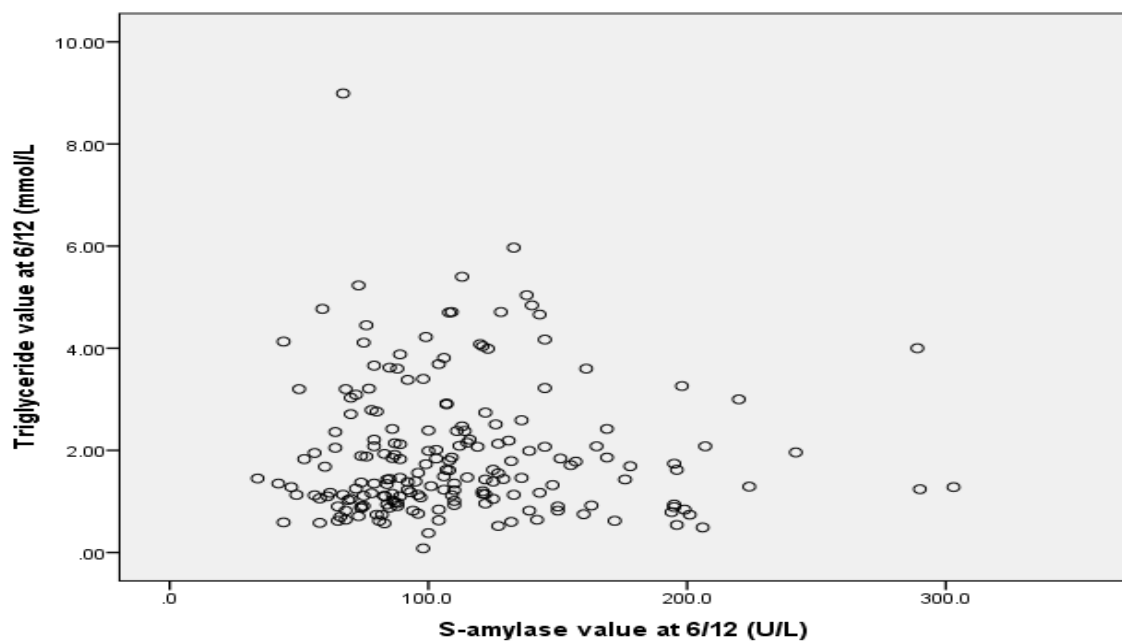




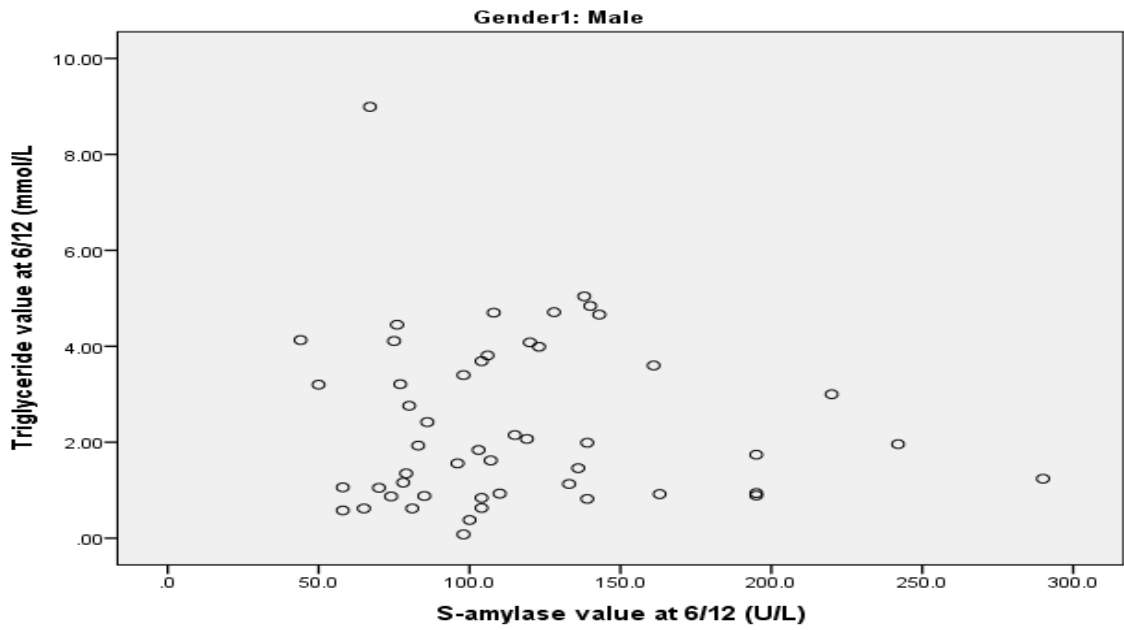
#### 4.5 Correlations analysis

Correlation analyses were done to investigate whether there was a linear correlation between the following:

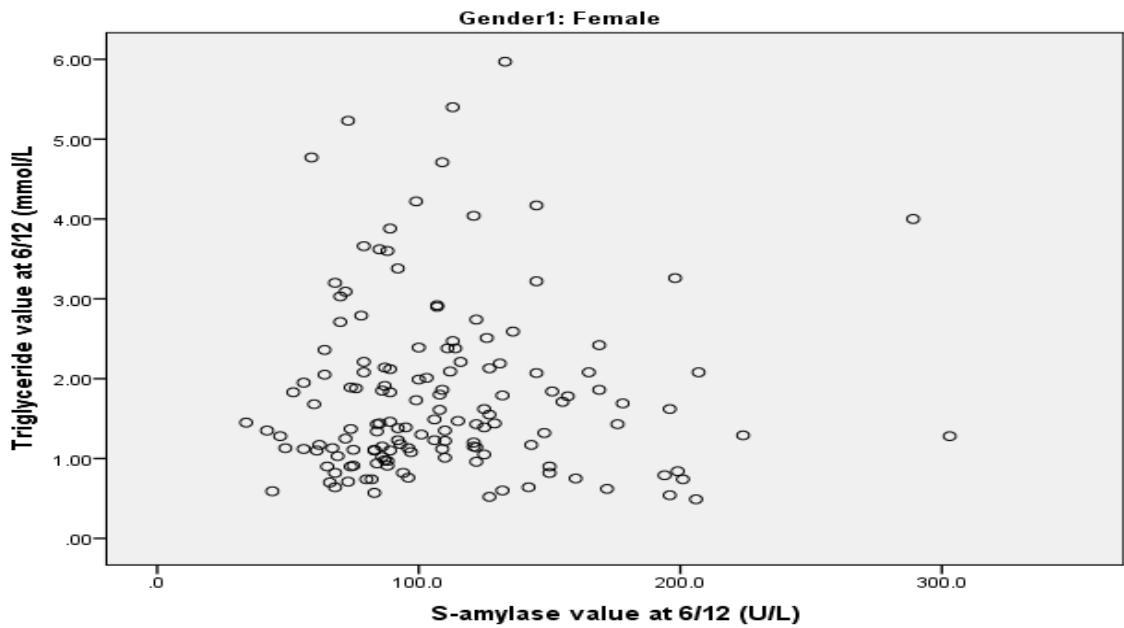
- Total TG values and s-amylase values: Pearson's correlation of -0.013.



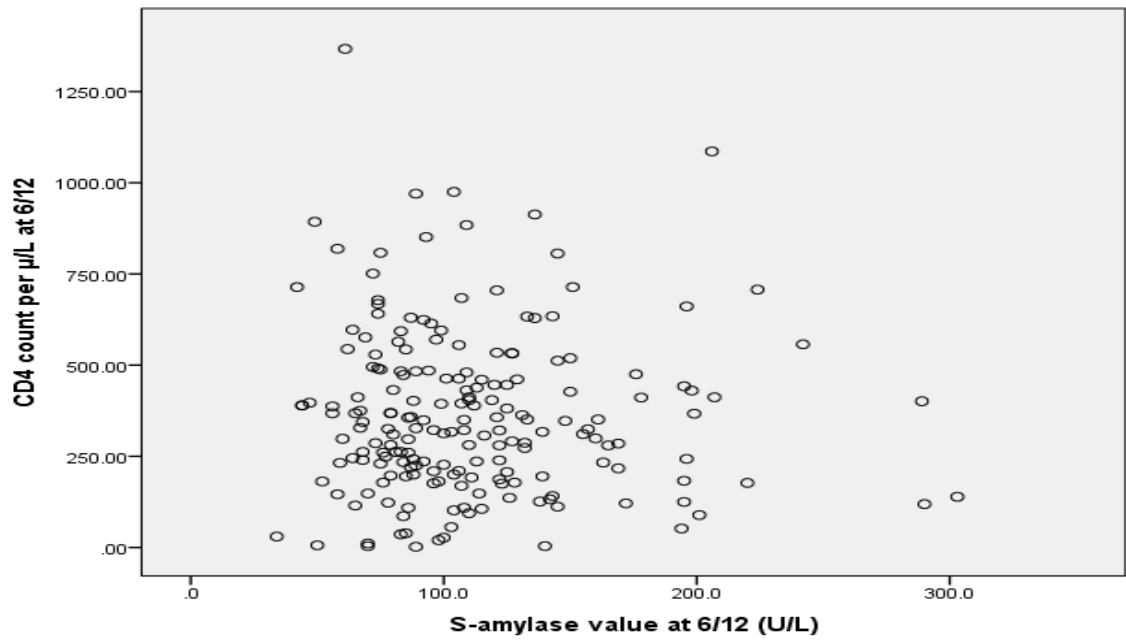
- Male TG values and s-amylase values: Pearson's correlation value -0.084.



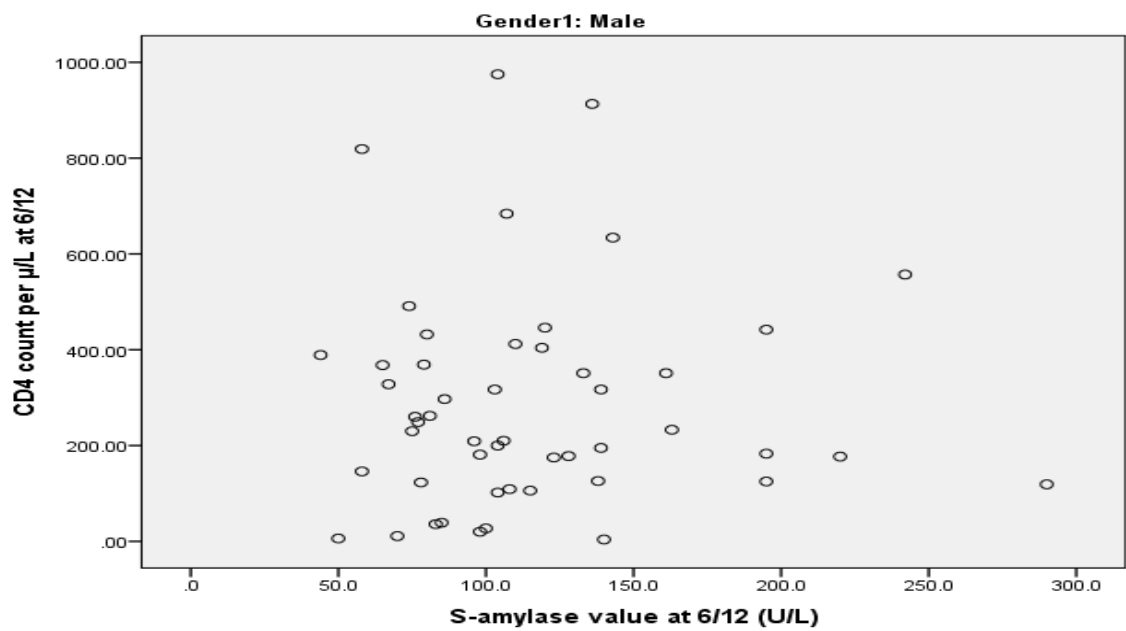
- Female TG levels and s-amylase values: Pearson's correlation value -0.014.



- Total CD<sub>4</sub> counts and s-amylase values: Pearson's correlation of -0.042.

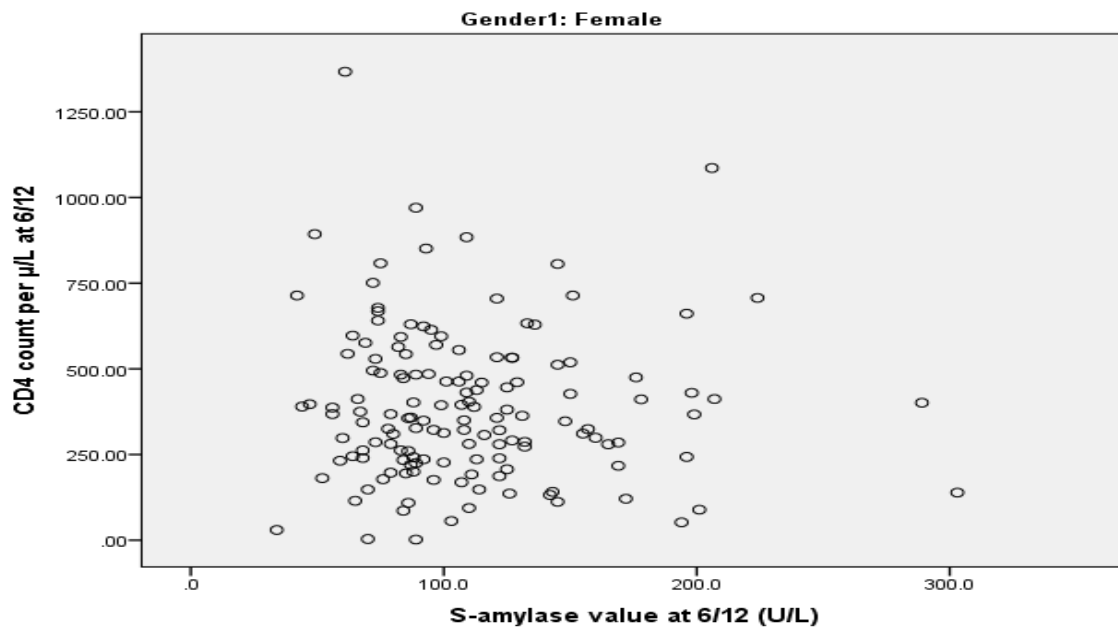


- Male CD<sub>4</sub> counts and s-amylase values: Pearson's correlation value of 0.011.



- Female CD<sub>4</sub> counts and s-amylase values: Pearson's correlation value of -0.048.





There was no statistically meaningful linear correlation between any of the variables in the six correlation analyses.

#### 4.6 Graded TG levels

The upper normal limit of TG values for this study is 1.7 mmol/L. The prevalence of TG values above 1.7 mmol/L was investigated for the entire population as well as for males and females separately. It was found that out of the entire sample (n=194, males n=50, females n=144) 54.6% (n=106) had TG values below 1.7 mmol/L and 45.4% (n=88) had values above 1.7 mmol/L and therefore HTG. Fifty four per cent (n=27) of the males had values above 1.7 mmol/L and the remaining 46% (n=23) had values below 1.7 mmol/L, whereas 42.2% of the females (n=61) had TG values above 1.7 mmol/L and 57.6% (n=83) had values below 1.7 mmol/L. Almost half of the sample therefore had HTG. Hypertriglyceridemia was more prevalent among the male population than the female population.

#### 4.7 Graded s-amylase levels

The upper normal limit of s-amylase values for this study was 104 U/L. The prevalence of amylase values above the upper normal limit and above twice the upper normal limit were investigated for the whole population as well as for both females and males separately. Out of the entire sample (n=194, males n=50, females n=144), 49% (n=95) had s-amylase values below 104 U/L and 51% (n=99) had amylase values above 104 U/L. Fifty four per cent (n=27) of the males had s-amylase values below 104 U/L and 46 % (n=23) had s-amylase values above 104 U/L. The females had 47.2% (n=68) of the s-amylase values below 104 U/L and 52.8%

n=76 above 104 U/L. The overall distribution of the s-amylase values was therefore quite well balanced below and above the upper normal limit of 104 U/L.

The s-amylase values were also analysed relative to twice the upper normal limit of 208 U/L. Out the entire sample (n=194), 3.1% (n=6) had s-amylase values above 208 U/L and 96.9% (n=141) had amylase values above 208 U/L. Only 6% (n=3) of the males had s-amylase values above 208 U/L and 94% (n=23) had s-amylase values below 208 U/L. A total of 2.1% (n=3) of the females had s-amylase values above 208 U/L and 97.9% (n=141) below 208 U/L. As the s-amylase values increase, the cases with elevated s-amylase decrease. There were no values above three times the upper normal limit of 312 U/L.

# CHAPTER 5: CONCLUSION, RECOMMENDATIONS AND LIMITATIONS

## 5.1 Introduction

The results of this cross-sectional retrospective study of 194 patients were analysed and the conclusions reached because of the empirical investigation and literature review discussed in this chapter. The primary and secondary aims as set out in chapter 1 are also reported on. The recommendations that sprouted from conclusions that were reached and the limitations to this study are also discussed in this chapter.

## 5.2 Conclusion

### 5.2.1 Pancreatitis and HTG

The risk of HTG-induced pancreatitis increases with elevated TG values, but only becomes realistic with TG values above 11.2 mmol/L (Berglund *et al.*, 2014:3; Bush & Kosmiski, 2002:e3; Sahebkar & Watts, 2015:363) and clinically detectable symptoms above 22.6 mmol/L (Lederle & Bloomfield, 2012:662). Triglyceride values therefore have to increase far beyond the upper normal limit of 1.7 mmol/L before HTG-induced pancreatitis will occur.

This study did not detect a single case of pancreatitis after the first six months of LPV/r-based therapy. The results therefore suggest that HTG-induced pancreatitis is unlikely to occur after the first 6 months of LPV/r therapy. The mean TG value was 1.94 mmol/L – significantly short of the accepted threshold of 11.3 mmol/L for pancreatitis. However, s-amylase values demonstrated a clear tendency to increase at month 6 of LPV/r-based therapy, even though there were no cases of pancreatitis detected. The diagnostic criteria for pancreatitis for this study was s-amylase values elevated 3 times above the upper normal limit of 104 U/L, i.e. 312 U/L, and only 3.1% of the patients (n=6) had s-amylase values 2 times above the upper normal limit.

Oliveira *et al.* (2014:113) and Manfredi *et al.* (2004:541) state that post mortem evidence of pancreatitis is quite common among the population infected with HIV. From this, it can be inferred that although there were no cases of pancreatitis detected, according to the diagnostic criteria of this study, there may have been cases of subclinical pancreatitis present that went unnoticed, as suggested by Manfredi *et al.* (2004:541) in their study. However, even though there may have been cases of subclinical pancreatitis in the sample of this study, it could not have been induced by HTG, since the mean TG value was a mere 1.94 mmol/L and the highest TG values of the sample at 8.99 mmol/L.

Bush & Kosmiski (2003:e1) state that pancreatitis among the HIV-infected population may be as much as 800 times more common than the general population, but this could not be confirmed by this study. Cases of acute pancreatitis are not frequently reported, notwithstanding, frequent yet asymptomatic elevations of s-amylase and s-lipase (Manfredi & Calza, 2008:100). Oliveira *et al.* (2014:117) suggest that drug-induced pancreatitis may therefore, be frequently overlooked when mild but clinically meaningful increases in s-amylase and s-lipase values are present and considering that the HIV-infected population is exposed to several other factors that may cause pancreatitis, physicians should be very suspicious of mildly elevated s-amylase and s-lipase values (Oliveira *et al.*, 2014:117).

Very high TG values elevated above 20 mmol/L, are usually due to both primary and secondary factors (Sandhu *et al.*, 2011:1). In order to determine the effect of LPV/r alone on TG, the exclusion criteria of the study were made as exhaustive as possible in order to eliminate as many primary and secondary HTG-inducing factors as possible. In a setting where most primary and secondary HTG-inducing factors were not removed, and may be present in any combination in addition to LPV/r therapy, the risk of HTG-induced pancreatitis may be higher than the results of this study suggest.

Age, gender and ethnicity also affect TG values (Berglund *et al.*, 2014:4). The mean TG values for males in this study were a notable 32% higher than the mean of the females with a statistically significant difference between the respective means. Normally, male TG values are higher than that of females (Berglund *et al.*, 2014:4). This suggests that males may be more susceptible to the HTG-inducing effects of LPV/r therapy. In addition, it may also lead to questions regarding the prevalence of obesity among the male population - a variable that was not available for the purposes of this study. The prevalence of HTG was also higher in the male population than in the female population of this study. The findings of Ford *et al.* (2009) regarding the prevalence of HTG among males, as compared to women, was consistent with the findings of this study in that HTG was more prevalent in males than females; in an African-American study population, Ford *et al.*, (2009) found that the prevalence of HTG was 14.4% for the females as opposed to the 21.2% for the males (compared to 42% and 54% for males and females respectively for this study). However, Ford *et al.* (2009) investigated the prevalence of metabolic syndrome among the general population regardless of aetiology, whereas this study focussed only on HIV-infected patients on LPV/r-based therapy. Differences in the prevalence of HTG between the study population of Ford *et al.* (2009) and that of this study, may therefore be ascribed to the HIV infection and LPV/r therapy. However, possible differences in the prevalence of obesity and dietary habits between the two populations must be taken into account.

Oliveira *et al.* (2014:117) and Riedel *et al.* (2008:5) both found female gender to be a risk factor for pancreatitis among HIV-infected patients, especially the females with low body weight. The results of this study could not corroborate these findings. In this study, the difference between the s-amylase values of males and females was small and insignificant. The studies of Oliveira *et al.* (2014:117) and Riedel *et al.* (2008:5) used very large study samples, over long periods of time, as opposed to the sample size of this study. A larger sample over a longer period of time, may better determine the risk of pancreatitis in the studied population.

It has been well documented that lower CD<sub>4</sub> counts are associated with an increased risk of pancreatitis (Oliveira *et al.*, 2014:115; Riedel *et al.*, 2008:11). The mean CD<sub>4</sub> count for males in this study was significantly lower than that of the females, which indicated that the males have a higher risk of pancreatitis than the females. This is in conflict with the results of Oliveira *et al.*, 2014:115 and Riedel *et al.*, 2008:11, regarding the risk contributed by gender. The higher number of females in the sample of this study and the higher mean CD<sub>4</sub> of females raise questions regarding the compliance with ART by males.

Ethnicity may also be a risk factor for pancreatitis. As demonstrated in an HIV clinical cohort study conducted by Riedel *et al.* (2008:3), the cases of pancreatitis detected during the study were 88.2% black Africans and 51.8% were male. The median age of the population was 36 years – similar to the median age of 38 years for this study. The results of Riedel *et al.* (2008:3) are therefore consistent with the findings of this study in that males in an HIV-infected population carry a higher risk of pancreatitis than females.

A large proportion (45%) of the patients in this study suffered from some degree of HTG. This may mainly be attributed to the effects of LPV/r since most other HTG inducing factors were largely excluded. These results were slightly lower than those of Calza *et al.* (2003:57), who detected HTG in 60-90% of patients on PI-based therapy. Although an increase in TG is apparent in the patients in this study, to be able to quantify the effect of LPV/r in TG, baseline values were required but not available for the purposes of this study. Given the prevalence of HTG, as well as other factors unaccounted for among the patients of this study, early screening and intervention is indicated.

The increase of TG in patients on LPV/r-based therapy seems to be time-dependent (Calza *et al.*,2003:57). This, combined with various primary and secondary causes of HTG, may lead to an increase in TG values high enough to cause acute pancreatitis in the long term beyond the first six months of LPV/r therapy and also contribute to the risk of atherosclerosis (Rosenson, 2016). Other risk factors include the total period infected with HIV (mean of 91.3 months in this study population) and a CD<sub>4</sub> count of less than 200 cells/ $\mu$ L (Oliveira *et al.*, 2014:117). The mean respective values of TG, s-amylase and CD<sub>4</sub> counts over three consecutive periods

during the period of HIV infection did not significantly vary. Even though statistically insignificant, the mean values of TG and s-amylase did increase over the three categorical periods, which may suggest that pro-active monitoring of these parameters would be wise, starting from the day of HIV infection diagnosis and not only from the day of LPV/r initiation.

### **5.2.2 Atherosclerosis and HTG**

The risk of atherosclerosis starts at much lower TG values of 1.7 mmol/L to 2.3 mmol/L (Friedewald, 2003:1134; Rosenson, 2016), compared to TG values of higher than 11.3 mmol/L required for HTG-induced pancreatitis (Berglund *et al.*, 2014:3; Bush & Kosmiski, 2002:e3; Sahebkar & Watts, 2015:363). The mean overall TG values were 1.94 mmol/L - above the upper normal limit 1.7 mmol/L. There was a clear difference between the mean TG values for males and females (1.79 and 2.30 mmol/L respectively). These values classify the male population with moderate HTG and the females with mild HTG. Although the TG elevations are not extreme, the risk for atherosclerosis is present (Rosenson, 2016).

Since most HTG-inducing factors have been removed from the study population it may be anticipated that in an uncontrolled setting were other co-morbidities, primary and secondary factors in different combinations, may elevate HTG values even more. Possible primary factors include familial HTG (Rosenson, 2016, Scherer *et al.*, 2014:4), and possible secondary HTG-inducing factors including obesity, diabetes mellitus, hypothyroidism and pregnancy (Dubé *et al.*, 2003:618). Furthermore, cardiovascular risk occurs earlier and at a higher rate in black Africans (Dubé *et al.*, 2003:614).

High TG values lead to insulin resistance and may therefore precipitate type 2 diabetes mellitus (Berglund *et al.*, 2014:3; Sandhu *et al.*, 2011:5). Increased insulin resistance and type 2 diabetes mellitus, in turn, may increase TG values by means of hepatic lipogenesis and the release of TG and VLDL into the blood (Sahebkar & Watts, 2015:364). The consequent PI-associated insulin resistance (Dubé *et al.*, 2003:616) and the resulting effect on the risk factors of metabolic syndrome (Friedewald, 2013:1134) should be closely monitored. Since ART is lifelong, the metabolic and cardiovascular risk factors should be monitored throughout.

Nearly half of the patients reviewed in this study had TG values above 1.7 mmol/L after the first six months of LPV/r therapy. Although TG values did not reach high enough levels to induce pancreatitis, the contribution of co-morbidities and certain pancreotoxic drugs should be taken into account. The risk of atherosclerosis starts at much lower TG than required for pancreatitis, which indicates that a considerable portion of the study population may be at risk of cardiovascular disease. Current ARV treatment guidelines from NDoH (2014:67) require screening for hypertension and diabetes mellitus upon diagnosis of HIV in order to ensure that

the relevant co-morbidities are managed before initiating ART. The guidelines also recommend a fasting cholesterol and triglyceride assay when LPV/r is initiated, and then only again on month 3 after initiation. This approach will identify patients at risk of HTG and associated pathology during the initial stages of ART. However, as demonstrated by Calza *et al.* (2003:57), the HTG-inducing effect of LPV/r is time-dependent and values can more than double from month 3 after initiation of LPV/r to month 12 post-initiation. Those patients with acceptable TG values in the initial stages of LPV/r therapy may therefore not remain so. Continuous monitoring of TG values and s-amylase values is therefore indicated, not only from the first day of LPV/r treatment, but throughout the duration of LPV/r therapy in order to reduce the risk of HTG-induced pancreatitis and atherosclerosis.

### **5.3 Recommendations**

Prior to initiating patients on LPV/r, baseline tests and screening tests should be done to quantify risk of both pancreatitis and cardiovascular disease.

The current NDoH guidelines recommend a TG and cholesterol assay before initiation of LPV/r therapy, then again after the first 3 months of LPV/r-based therapy, but no HbA<sub>1c</sub> or thyroid function test. This may be inadequate monitoring in light of the results of this study. In addition, co-morbidities that may contribute to acute pancreatitis or cardiovascular disease should not only be diagnosed and under control before initiation of LPV/r, but also periodically monitored once diagnosed. These include diabetes mellitus, dyslipidaemia, hypothyroidism, obesity and any existing cardiovascular co-morbidity. The guidelines from NDoH recommend screening for hypertension and diabetes mellitus, but no thyroid function test. Drugs that may increase TG values, or drugs that may cause pancreatitis by some other mechanism should be used with caution.

The following assays are recommended every six months throughout the duration of LPV/r-based therapy:

- Full lipogram
- Serum amylase and s-lipase
- Thyroid function
- HbA<sub>1c</sub>

#### **5.4 Limitations**

Baseline TG values, s-amylase values and CD<sub>4</sub> counts were not routinely done for the study population. In the case of TG, the magnitude of the HTG inducing effect of LPV/r could not be determined. Baseline TG levels would have made it possible to determine the mean percentage increase in TG which may have superior prognostic value than merely knowing that LPV/r increases TG values. Even though there are several factors that may cause pancreatitis in the HIV-infected patient, having the baseline TG and s-amylase values available at the time of initiating the patient on LPV/r, may enable the clinician to establish the aetiology of acute pancreatitis if detected at 6 months post-initiation.

Cigarette smoke and ethanol are well-known factors that may cause pancreatitis. Although these factors were part of the exclusion criteria, acute consumption at any time during the period prior to the moment of the relevant assays being performed, cannot be completely ruled out. The attending doctor may also not have questioned the patient regarding the use of tobacco or ethanol, which means that such data would not be recorded in the patient's medical records. Since this was a retrospective observational study, it was not possible to know exactly what the patient consumed during the hours before their visit to the CDC after the first six months of LPV/r therapy. Elevations in s-amylase values at the time blood samples were taken could well have been caused by cigarette smoke or ethanol consumption.

The exclusion criteria catered for comprehensive exclusion of any drugs that may cause pancreatitis. However, it was still possible for a patient to be included into the sample while taking a pancreatitis-inducing drug. A good example is the patients taking sodium valproate. The CDC and outpatients department (OPD) of the hospital keep separate archives for their patients. When a patient starts treatment for epilepsy, before becoming a patient of the CDC (or vice versa), it is possible for that patient to have two separate files for a period before having their medical history from the two departments combined. If the data of such a patient was collected for this study during this period, the possible pancreatitis inducing effect of sodium valproate could not be ruled out because the persons tasked with data collection may only have revised the CDC file while the OPD file was in the OPD archives. Lamivudine is another drug



that is known to possibly cause pancreatitis. However, lamivudine could not be excluded with the exclusion criteria since it is the common denominator in the antiretroviral regimen of every single patient.

Obesity and diet are factors that elevate TG values. A patient's body mass index, weight and what was eaten are not routinely recorded at the time of LPV/r initiation and neither after the first six months of LPV/r-based therapy. The effect of these two factors could therefore not be excluded from the data of this study.

Serum lipase values do not form part of the standard panel of liver function assays as s-amylase does. Serum-lipase is much more organ-specific than s-amylase for the purposes of diagnosing pancreatitis and would therefore be more accurate in predicting pancreas pathology than s-amylase.

According to the exclusion criteria for this study, patients diagnosed with familial HTG should be excluded from the study. However, this could only be done if their medical history stated as much. Those patients with undiagnosed familial HTG at the time of data collection could therefore not be accounted for and were excluded from the study sample.

Several other studies that investigated some aspect of the HTG induction properties of LPV/r and the prevalence of pancreatitis among the HIV-infected population, used study samples of several thousand patients, investigated over long periods of time.

Hypothyroidism may exacerbate HTG and was not part of the exclusion criteria for this study.

The patients did not always report at exactly six months after commencement of LPV/r therapy. Due to logistical and financial factors, they may have reported to the CDC a few days before or after the date that marks six months.

## **5.5 Final conclusion**

The consequences of the HTG inducing effect of LPV/r are minimal after the first 6 months of therapy. According to the diagnostic criteria for pancreatitis for this study, there is no significant risk for HTG-induced pancreatitis. However, since subclinical pancreatitis is often present in the HIV-infected population, the possibility of pancreatitis should not be entirely dismissed. The clinician should remain mindful of the possibility of pancreatitis in the uncontrolled environment at month 6 of LPV/r-based therapy – not only due to HTG, but also other aetiologies. The mean TG values at the 6 month mark do hold significant risk for atherosclerosis. Since TG values will increase as a function of time on LPV/r therapy, constant monitoring and vigilance is recommended; not only of TG and s-amylase, or s-lipase values, but also other markers of

related comorbidities and contributing factors such as HbA<sub>1</sub>C and thyroid function. Furthermore, the recommended monitoring should be implemented from day one of LPV/r therapy and sustained throughout the course of therapy, repeated every 6 months with the renewal of the patient's chronic prescription.

## **5.6 Study reflection**

Even after three decades of studying HIV/AIDS there is still much to be learned. This study focussed on only a small part of the HIV continuum of care and elucidated a very specific aspect with regard to ART and associated complications and comorbidities. The results will hopefully bring about a better understanding of a patient's status at the relevant period during treatment and enable the clinician to pre-empt adverse events related to HIV and ART, rather than react to adverse events. In turn, this may lead to a better life expectancy for the patient infected with HIV. As this study relied on the work done by others regarding this specific subject, it is hoped that the results may also open more avenues of investigation to other researchers.

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# ANNEXURE B: APPROVAL FROM HOSPITAL



health  
Department: \_\_\_\_\_ c  
Health  
PROVINCE OF KWAZULU-NATAL

**LADYSMITH PROVINCIAL HOSPITAL**  
Address: Private Bag X 9928, LADYSMITH, 3370  
Postal Address: 36 Malcolm Road, LADYSMITH, 3370  
Tel: 036 637 2111  
Fax.: 036 631 4221  
**OFFICE OF THE SENIOR MANAGER - MEDICAL SERVICES**  
Email: bongani.mabaso@kznhealth.gov.za  
www.kznhealth.gov.za

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**Enquiries: Dr B.A. Mabaso**  
**Telephone: 0833884747**  
Date: 05/06/2015

Wilhelm Greffrath  
Assistant Pharmacy Manager  
Ladysmith Provincial Hospital  
Tel 036 637 2111 ext 219  
Fax 036 637 5934

RE: PERMISSION TO CONDUCT RESEARCH AT THE HOSPITAL

I have pleasure in informing you that permission has been granted to you by the hospital to conduct research on "Triglyceride levels after six months of lopinar/ritonavir therapy as an indicator for pancreatitis risk"

Please note the following:

1. Please ensure that you adhere to all the policies, procedures, protocols and guidelines of the Department of Health with regard to this research.
2. This research will only commence once this office has received confirmation from the Provincial Health Research Committee in the KZN DOH.
3. Please ensure this office is informed before you commence your research.
4. The hospital will not provide any resources for this research.
5. You will be expected to provide feedback on your findings to the hospital.

Thank you.

.....  
**DR. B.A. MABASO**  
Senior Manager: Medical Services

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uMnyango Wezempilo. Departement van Gesondheid

*Fighting Disease, Fighting Poverty, Giving Hope*



# ANNEXURE C: APPROVAL FROM PHRC KWAZULU-NATAL



**health**  
Department:  
Health  
PROVINCE OF KWAZULU-NATAL

330 Langalibalele street,  
Private Bag X9051 PMB, 3200  
Tel: 033 395 2805/3189/3123 Fax: 033 394 3782  
Email: [hrkm@kznhealth.gov.za](mailto:hrkm@kznhealth.gov.za)  
[www.kznhealth.gov.za](http://www.kznhealth.gov.za)

DIRECTORATE:

Health Research & Knowledge  
Management (HKRM)

Reference: HRKM360/15  
KZ\_2015RP22\_110

22 March 2016

Dear Mr W Greffrath  
(University of North West)

**Subject: Approval of a Research Proposal**

1. The research proposal titled 'Triglyceride levels after six months of lopinavir/ritonavir therapy as an indicator for pancreatitis risk' was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

The proposal is hereby **approved** for research to be undertaken at Ladysmith Hospital.

2. You are requested to take note of the following:
  - a. Make the necessary arrangement with the identified facility before commencing with your research project.
  - b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.
3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to [hrkm@kznhealth.gov.za](mailto:hrkm@kznhealth.gov.za)

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely



**Dr E Lutge**

Chairperson, Health Research Committee

Date: 23/03/16.

# ANNEXURE D: APPROVAL FROM HREC



NORTH-WEST UNIVERSITY  
YUNIBESITI YA BOKONE-BOPHIRIMA  
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom  
South Africa 2520

Tel: (018) 299-4900  
Faks: (018) 299-4910  
Web: <http://www.nwu.ac.za>

## Institutional Research Ethics Regulatory Committee

Tel +27 18 299 4849  
Email [Ethics@nwu.ac.za](mailto:Ethics@nwu.ac.za)

### ETHICS APPROVAL CERTIFICATE OF FULL SINGLE APPLICATION

Based on approval by **Health Research Ethics Committee (HREC)**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

<b>Project title:</b> Triglyceride levels after six months of lopinavir/ritonavir therapy as an indicator for pancreatitis risk.																																														
<b>Project Leader:</b>	Dr JM du Plessis																																													
<b>Student:</b>	WP Greffrath																																													
<b>Ethics number:</b>	<table border="1"><tr><td>N</td><td>W</td><td>U</td><td>-</td><td>0</td><td>0</td><td>3</td><td>5</td><td>6</td><td>-</td><td>1</td><td>5</td><td>-</td><td>A</td><td>1</td></tr><tr><td colspan="3">Institution</td><td colspan="5">Project Number</td><td colspan="2">Year</td><td colspan="5">Status</td></tr><tr><td colspan="15"><small>Status: S = Submission, R = Re-Submission, P = Provisional Authorisation, A = Authorisation</small></td></tr></table>	N	W	U	-	0	0	3	5	6	-	1	5	-	A	1	Institution			Project Number					Year		Status					<small>Status: S = Submission, R = Re-Submission, P = Provisional Authorisation, A = Authorisation</small>														
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<b>Approval date:</b>	2016-03-14																																													
<b>Expiry date:</b>	2017-03-13																																													
<b>Risk</b>	<b>Medium</b>																																													

#### Further conditions of the approval (if any):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.
- Any further information and any report templates is obtainable from Carolien van Zyl at [Carolien.VanZyl@nwu.ac.za](mailto:Carolien.VanZyl@nwu.ac.za).

#### General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC and HREC:
  - annually (or as otherwise requested) on the progress of the project, and upon completion of the project
  - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
  - Annually a number of projects may be randomly selected for an external audit.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the HREC and NWU-IRERC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
  - request access to any information or data at any time during the course or after completion of the project;
  - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
    - any unethical principles or practices of the project are revealed or suspected,
    - it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented,
    - the required annual report and reporting of adverse events was not done timely and accurately,
    - new institutional rules, national legislation or international conventions deem it necessary.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis  
DN: cn=Linda du Plessis, o=NWU,  
ou=Vaal Triangle Campus,  
email=linda.duplessis@nwu.ac.za,  
c=ZA  
Date: 2016.03.15 11:45:22 +02'00'

Prof Linda du Plessis

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

# ANNEXURE E: DECLARATION OF SUPPORT – DR AMOD



health

Department:  
Health  
PROVINCE OF KWAZULU-NATAL

LADYSMITH REGIONAL HOSPITAL  
Centre for Disease Control (CDC)

36 MALCOLM ROAD

LADYSMITH 3370

27/05/2015

## CLINICAL SUPPORT FOR LOPINAVIR/RITONAVIR STUDY

To whom it may concern,

I wish to state that I am willing to assist the researcher, Mr. Wilhelm Greffrath, with the data collection for the purposes of conducting this study as required for a master's degree in advanced clinical pharmacy.

I understand what Mr. Greffrath expects from me and have no objection assisting him for his research and I do not wish to co-author the study. The study is designed around normal clinical procedures in our department (CDC) and will have no impact on operations or the delivery of service to our patients.

Sincerely,

MBBS  
HPCSA NO 0274238

Dr. F. Amod, MBBS

Head of Department: Centre for Disease Control

Ladysmith Regional Hospital

Tel: 036 637 2111

uMnyango Wezempilo . Departement van Gesondheid

*Fighting Disease, Fighting Poverty, Giving Hope*

# ANNEXURE F: DECLARATION OF SUPPORT – DR SULTANA



health

Department:  
Health  
PROVINCE OF KWAZULU-NATAL

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LADYSMITH REGIONAL HOSPITAL  
Centre for Disease Control (CDC)

36 MALCOLM ROAD

LADYSMITH 3370

27/05/2015

## CLINICAL SUPPORT FOR LOPINAVIR/RITONAVIR STUDY

To whom it may concern,

I wish to state that I am willing to assist the researcher, Mr. Wilhelm Greffrath, with the data collection for the purposes of conducting this study as required for a master's degree in advanced clinical pharmacy.

I understand what Mr. Greffrath expects from me and have no objection assisting him for his research and I do not wish to co-author the study. The study is designed around normal clinical procedures in our department (CDC) and will have no impact on operations or the delivery of service to our patients.

Sincerely,

*Sultana* MP 0601675

Dr. M.S. Sultana, MBBS

Centre for Disease Control

Ladysmith Regional Hospital

Tel: 036 637 2111

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uMnyango Wezempilo . Departement van Gesondheid

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