THE PREVALENCE OF HBV, HTLV, HIV AND CONCURRENT INFECTIONS IN BLOOD RECIPIENTS OF THE SOUTH AFRICAN NATIONAL BLOOD SERVICE (SANBS)

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ABSTRACT

Background: Currently, the South African National Blood Services are not testing for HTLV and HTLV screening is not mandated by the WHO or by regulatory standards in South Africa. Looking at the uniquely high prevalence of HIV and HIV / HBV co-infections in the South African population and taking into account the literature that suggests that most of these infected patients will be receiving blood, exposing these patients to an additional burden like HTLV can result in an increased disease progression of HIV to AIDS and a poor prognosis in these infected patients.

Study design and methods: A blinded cross-sectional study was performed. 7015 specimens were collected from all blood transfusion laboratories across South Africa excluding the Western Cape Blood Transfusion Service laboratories. The specimens collected were tested using the ABBOTT Alinity S® Immunochemiluminescent autoanalyser. All test results were confirmed with the Roche Cobas® E801 and E411 auto analyser.

Results: Over all prevalence for HIV was 39.39% (N=2763), HBV 7.57% (n=531) and HTLV 0.70% (N=49). Concurrent infection for HIV/HBV 4.92% (N=345), HIV/HTLV 0.36% (N=25), HBV/HTLV 0.09% (N=6) and HIV/HBV/HTLV 0.07% (N=5).

Conclusion: This study confirmed an overall high prevalence of HIV and HBV infections among patients receiving blood products from the SANBS. Compared to the general population, the HIV prevalence in blood recipients was two-fold higher. Patients receiving a blood transfusion from the SANBS have high rates of HIV, HBV and HTLV which should be taken into consideration when determining donor screening strategies.

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CHAPTER 1
PROBLEM SETTING

1.1. INTRODUCTION

The prevalence of HBV, HTLV, HIV and concurrent infections of these viruses in patients receiving blood and blood products is currently unknown. Although the literature indicates an increase in the prevalence of concurrent infection with HIV / HTLV / HBV in hospital patients, transfusing HIV/HBV infected patients with HTLV can possibly worsen their prognosis and accelerate the clinical progression to AIDS that is untested in South African communities.

The human T-cell lymphotropic virus (HTLV) is the first human retrovirus to be linked to disease in man (Gonçalves et al., 2010). The most serious disorders caused by this virus are HTLV-associated myelopathy (HAM) or tropical spastic paraparesis (TSP), and adult T-cell leukaemia (ATL) (Gonçalves et al., 2010).

Human immunodeficiency virus (HIV) is a retroviral infection that weakens the immune system and is a subsequent causative agent of acquired immuno-deficiency syndrome (AIDS) (Dube et al., 2018). The outcome of infection and duration for disease progression with clinical symptoms may vary greatly between individuals, but often it progresses slowly (Naif, 2013). It takes several years from primary infection to the development of symptoms of advanced HIV diseases and immunosuppression. Symptomatic stage of disease indicates the late phase of HIV disease (AIDS) where individuals may be susceptible to other opportunistic infections (Naif, 2013).

Among adults with normal immune status, most (94%–98%) recover completely from newly acquired HBV infections, eliminating the virus from the blood and producing neutralizing antibodies that confer immunity from future infection (Abara et al., 2017). In infants, young children, and immunosuppressed persons, most newly acquired HBV infections result in chronic infection (Abara et al., 2017). Infants are at greatest risk, with a 90% chance of developing chronic infection if infected at birth (Abara et al., 2017). Although the consequences of acute hepatitis B can be severe, most of the serious sequelae occur in persons in whom chronic infection develops (Abara et al.,

2017). Chronic liver disease develops in two-thirds of these persons, and approximately 15%–25% die prematurely from cirrhosis or liver cancer (Abara et al., 2017).

The problem of HIV / HTLV concurrent infection has emerged as a worldwide health problem in the last 20 years (He et al., 2016). It is estimated that rates of HTLV or HTLV-2 co-infections in HIV-infected individuals are at least 100 to 500 times greater than in the general population (Isache et al., 2016). HTLV may be detrimental to the HIV-infected individual with increased risk for the development of neurologic complications including TSP/HAM, leukaemia, and lymphoma (Beilke, 2012). HTLV Tax protein up-regulates HIV expression, as well as expression of various cytokines and cytokine receptors involved in T-cell activation, thereby providing a favourable condition for HIV infection and exacerbates the cytopathic effects of HIV that accelerate the clinical progression to acquired immune deficiency syndrome in individuals infected with both HIV and HTLV (Casoli et al., 2007).

The natural history of HBV disease progression is modified by HIV co-infection (Thio, 2003). A prospective study of 5293 gay men, 326 of whom had chronic hepatitis B, demonstrated that mortality attributable to liver disease was significantly increased in those who were co-infected with HIV and HBV compared with those with HBV alone (Thio, 2003). The investigators found that individuals with lower CD4 counts appeared to be at greatest risk (Thio, 2003).

The available data suggest limited or no effect of HBV / HTLV on progression to various HTLV / HBV outcomes (Moreira et al., 2013).

The main modes of transmission of these viruses are vertically from mother to child, horizontally by sexual intercourse, and by transfusion of blood and blood cellular products (Tweteise et al., 2016). Transfusion is the most infectious mode of transmission as it delivers a large viral load, resulting in seroconversion rates of 44–63% and the interval from infection to disease is also shortened (Tweteise et al., 2016).

All three of these viruses are envelope viruses. HIV and HTLV has no cure and these viruses will remain with the infected individual for their life span. Treatment of HIV and

HTLV with antiretroviral agents help to control the virus replication to undetectable levels but does not eliminate the virus, whereas HBV can be prevented through early childhood vaccination and most adults clear the infection spontaneously (Procop & Pritt, 2014). Early antiviral treatment may be required in fewer than 1% of HBV infected people, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised (Procop & Pritt, 2014). The treatment lasts from six months to a year, depending on medication and genotype (Zammitt & O'Brien, 2017). Treatment duration when medication is taken by mouth, however, is more variable and usually longer than one year (Zammitt & O'Brien, 2017).

1.2. INCIDENCE OF HEPATITIS B (HBV) BURDEN

1.2.1. Global

Schweitzer and colleagues (2015) reported on the most robust estimates to date of the prevalence of chronic Hepatitis B virus (HBV) by country and region. Their findings highlight both the substantial global burden of chronic HBV and the concentration of the epidemic in specific countries. This systematic review collated data from hepatitis B surface antigen (HBsAg) seroprevalence studies including 109 415 627 individuals, across 161 countries, published between 1965 and 2013. Overall Schweitzer *et al.* (2015) estimated that 3.61% (95% CI 3.61–3.61) of the global population is living with chronic HBV infection. HBV infection is the most common chronic viral infection in the world. An estimated 2 billion people have been infected and more than 350 million are chronic carriers of the virus (Trépo *et al.*, 2014). HBV infection ranked in the top health priorities in the world and was the tenth leading cause of death (786 000 deaths per year) (Trépo *et al.*, 2014). Despite this, comprehensive reviews of data regarding the burden of chronic HBV at regional and national levels have been scarce.

1.2.2. Africa

Zampino *et al.* (2015) suggested that Africa is overall considered to have a high HBV endemicity. HBV infection is hyperendemic and more than 8% of hepatitis B surface antigen (HBsAg) are chronic carriers in the general population of sub-Saharan African countries such as Nigeria, Namibia, Gabon, Cameroon, Burkina Faso (Zampino *et al.*, 2015). Other countries like Kenya, Zambia, The Ivory Coast, Liberia, Sierra Leone and

Senegal are considered areas of intermediate endemicity 2%-8%, while Egypt, Tunisia, Algeria and Morocco, located in the north of the continent, show a low endemicity level less than 2% (Zampino *et al.*, 2015). The lifetime risk of HBV is over 60% in the infected population, who are at risk of progressive liver disease and hepatocellular carcinoma (Howell *et al.*, 2014).

1.2.3. South Africa

Spearman and Sonderup (2014) suggested that an estimated 65 million people in Africa are chronically infected and 2.5 million of them are in South Africa. According to Schweitzer *et al.* (2015) the HBsAg seroprevalence was estimated at 6.7%, pointing to high intermediate endemicity with an estimated 3.5 million individuals chronically HBV infected in South Africa. According to Vermeulen *et al.* (2017), the prevalence of the hepatitis B virus in the first-time donor population was found to be 0.66%, which are significantly lower compared to other studies within the general population.

1.3. INCIDENCE OF HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV) BURDEN

1.3.1. Global

Although the exact number of HTLV seropositive individuals in the world is not known it is estimated that about 15 to 20 million persons live with HTLV infection worldwide (Gonçalves *et al.*, 2010). The seroprevalence rates differ according to geographic area, the socio-demographic composition of the population studied and individual risk behaviours. However, information on prevalence rates from representative samples of the general population is rare (Gonçalves *et al.*, 2010).

Overall, relatively high HTLV seroprevalence rates in the general population or specific groups of individuals as pregnant women and/or blood donor candidates are found in south-western Japan (up to 10%), several countries in the Caribbean basin including Jamaica, Trinidad (up to 6%), in several sub-Saharan Africa countries for example Benin, Cameroon, Guinea-Bissau (up to 5%) and localized areas of Iran and Melanesia (less than 5%) (Gessain & Cassar, 2012a).

To a certain extent lower seroprevalence rates are found in several countries in South America, although to our knowledge no studies from representative samples of the general population have been conducted so far in South America (Gessain and Cassar 2012). Data from Argentina, Brazil, Colombia and Peru are for the most part restricted to blood donors (up to 2% of seropositivity to HTLV-I/II), pregnant women and samples of specific native populations as well as intravenous drug users (IDU) from Brazil (Gessain & Cassar, 2012a).

For nonendemic geographic areas such as Europe and North America, HTLV-I infection is mainly found in immigrants from endemic areas, their offspring and sexual contacts, among sex workers and IDU. For blood donors in North America and Europe, seroprevalence is very low for example 0.01–0.03% in USA, Canada 0.002% in Norway and 0.0056% in Greece (Gessain & Cassar, 2012a). Prevalence rates for HTLV-I and II were six-fold higher in pregnant women (4.4 per 10,000) than in blood donors (0.07 per 10,000) in the United Kingdom (Gessain & Cassar, 2012a; Gonçalves *et al.*, 2010).

1.3.2. Africa

HTLV is widespread in sub-Saharan Africa, particularly in West and Central Africa. In West Africa, the reported rates of seropositivity for anti-HTLV antibodies vary from 1% to more than 5% among blood donors and/or pregnant women (Zehender *et al.*, 2008).

1.3.3. South Africa

A 2013 cross-sectional study of HTLV prevalence among 46 752 South African blood donors confirmed that HTLV prevalence was 0.16% in Black donors, 0.02% in both White and Coloured donors and 0% in South Asian donors, for an overall prevalence of 0.062% extrapolated to the current blood donor population (Vermeulen *et al.*, 2019b). Amongst 46 752 donors that were screened in a 1996 study, 3 tested positive for HTLV. The number of HTLV-positive donors increased to 60 in the most recent survey of 2013 conducted on 46 752 individuals (Vermeulen *et al.*, 2019b). The increasing number of donors found to be HTLV positive is cause for concern, especially considering the efficiency with which the virus is transmitted via exposure

to blood and blood products (Vermeulen *et al.*, 2019b). The study done by Bhigjee *et al.* (1993) showed a seroprevalence of HTLV in the study patient population of KwaZulu Natal to be 2.6%.

1.4. INCIDENCE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) BURDEN

1.4.1. Global

According to the World Health Organisation in 2015 approximately 37.6 million people worldwide were living with HIV, including 1.8 million children under the age of 15. Additionally, 1.9 million adults and 190,000 children were newly infected with the disease (HIV/AIDS, 2015). In 2016, an estimated 36.7 million people were living with HIV (including 1.8 million children) – with a global HIV prevalence of 0.8% among adults. Around 30% of these same people do not know that they have the virus (HIV/AIDS, 2015).

1.4.2. Africa

The HIV/AIDS pandemic marks a severe development crisis in Africa, which remains by far the worst affected region in the world. Forty-two million people now live with HIV/AIDS of which 29.4 million (70.0%) are from sub-Saharan Africa (Kharsany & Karim, 2016). Approximately 5 million new infections occurred in 2002 and 3.5 million (70.0%) of these were also from sub-Saharan Africa (Kharsany & Karim, 2016). In 2002, the epidemic claimed about 2.4 million lives in Africa, more than 70% of the 3.1 million deaths worldwide. The average life expectancy in sub-Saharan Africa is now 47 years whereas it would have been 62 years without AIDS (Kharsany & Karim, 2016).

1.4.3. South Africa

In 2016, South Africa had 270 000 new HIV infections and 110 000 AIDS-related deaths (UNAIDS, 2017). There were 7 100 000 people living with HIV in 2016, among whom 56% were accessing antiretroviral therapy (UNAIDS, 2017). An estimated 12 000 children were newly infected with HIV due to mother-to-child transmission

(UNAIDS, 2017). Among people living with HIV, approximately 45% had suppressed viral loads (UNAIDS, 2017). South Africa has the largest HIV epidemic in the world, with 19% of the global number of people living with HIV, 15% of new infections and 11% of AIDS-related deaths (UNAIDS, 2017). According to a report released by Statistics South Africa, the estimated HIV prevalence rate is approximately 13,1% among the South African population (Stats SA, 2018). According to Vermeulen *et al.* (2019a) there were 0.2% HIV-positive donations over a 10-year period. The prevalence of HIV in first-time blood donors within South Africa was 1.13% (Vermeulen *et al.*, 2019b).

1.5. CONCURRENT INFECTIONS OF HBV, HTLV AND HIV

1.5.1. HTLV and HIV

The problem of HIV/HTLV co-infection has emerged as a worldwide health problem in the last 20 years with increasing numbers of HIV/HTLV infected individuals in South America and Africa (He *et al.*, 2016). It is estimated that the rates of HTLV or HTLV-2 co-infections in HIV-infected individuals are at least 100 to 500 times greater than in the general population (Isache *et al.*, 2016). In some geographic regions 5–10% of HIV-infected individuals may be co-infected with HTLV or HTLV-2. HTLV may be detrimental to the HIV-infected individual, with increased risk for the development of neurologic complications including TSP/HAM, leukaemia, and lymphoma (Beilke, 2012). This often occurs in the scenario of a patient with relatively normal or even elevated CD4+ T cell counts (Beilke, 2012).

In HIV infected individuals, HTLV may precipitate the transition from M- to the T-tropic phenotype that is associated with HIV disease progression (Casoli *et al.*, 2007). In addition, HTLV Tax protein upregulates HIV expression, as well as expression of various cytokines and cytokine receptors involved in T-cell activation, thereby providing a favourable condition for HIV infection and gives an indication that HTLV infection exacerbates the cytopathic effects of HIV and accelerates clinical progression to AIDS in these co-infected individuals (Casoli *et al.*, 2007).

1.5.2. HBV and HIV

The natural history of HBV disease progression is modified by HIV co-infection (Thio, 2003). A prospective study of 5293 men who have sex with men (MSM), 326 of whom had chronic hepatitis B, demonstrated that mortality attributable to liver disease was significantly increased in those who were co-infected with HIV and HBV compared with those with HBV alone (Thio, 2003). The investigators found that individuals with lower CD4 counts appeared to be at greatest risk (Thio, 2003).

In HIV-infected persons, clearance rates of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) are decreased compared with those without HIV infection and may be related to the degree of immunosuppression (Thio, 2003). HIV infection is also associated with reactivation of HBV, accelerated loss of anti-HBs, higher levels of HBV DNA, and lower Alanine Aminotransferase (ALT) levels. The lower ALT levels suggest less hepatocyte destruction because of a depressed immune response and may contribute to the decreased effectiveness of anti-HBV therapy in co-infected individuals (Thio, 2003).

1.5.3. HTLV and HBV

The available data suggest limited or no effect of HBV / HTLV on progression to various HTLV / HBV outcomes; however, the study populations were small.

1.6. CONTEXT OF THE STUDY

The study will be carried out at the South African National Blood Services (SANBS) and will include all the regions in which the SANBS operates namely the Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West and Northern Cape regions. This study will be funded by the SANBS (Annexure A). This study was ethically approved by the human ethics research committee of the SANBS and the Vaal University of Technology (VUT) (2017/13 Annexure B).

1.7. PROBLEM STATEMENT

The prevalence of HBV, HTLV, HIV and concurrent infections of these viruses in patients receiving blood and blood products is currently unknown.

Investigating the incidence of HIV, HBV, HTLV and concurrent infection burden within the patient population of the SANBS by using an observational study that analyses data from the patient population within the SANBS at a specific point in time. This analysis is essential in controlling the residual risk of transfusing HTLV to HIV patients, the risk of not testing for HTLV in the donor population of the SANBS and understanding the impact of blood transfusion within the patient population of the SANBS.

Although the literature indicates an increase in the prevalence of concurrent infection with HIV / HTLV / HBV in hospital patients, transfusing HIV/HBV infected patients with HTLV can possibly worsen their prognosis and accelerate the clinical progression to AIDS that is untested in South African communities.

1.8. SIGNIFICANCE

Currently, the South African National Blood Services are not testing for HTLV and HTLV screening is not recommended by the WHO or by regulatory standards in South Africa (Ingram *et al.*, 2015). This study will therefore provide much-needed insight into the prevalence of HIV, HTLV and HBV in patients receiving blood and blood products.

The investigation will have a twofold significance the first is to measure the burden of these diseases within the patient population of the SANBS and the second is to look at the impact of possible transfusion of HTLV to HIV patients.

Looking at the uniquely high prevalence of HIV and HIV / HBV co-infections in the South African population (Mayaphi *et al.*, 2012) and taking into account the literature that suggests that most of these infected patients will be receiving blood (Ntusi & Sonderup, 2011), exposing these patients to an additional burden like HTLV can result in an increased disease progression of HIV to AIDS and a poor prognosis in these infected patients (Beilke, 2012).

1.9. AIMS AND OBJECTIVE

1.9.1. Aim

The aim of this study is to evaluate the prevalence of HIV, HTLV, HBV and concurrent infections of these viruses in patients receiving blood from the South African National Blood Service.

1.9.2. Objectives

The objectives of this study are:

1) To determine the prevalence of HIV infection in patients that require blood donation (recipients)
2) To determine the prevalence of HBV infection in patients that require blood donation (recipients)
3) To determine the prevalence of HTLV infection in patients that require blood donation (recipients)
4) To evaluate the number of recipients who have concurrent infections of HIV, HBV and HTLV

1.10. STRUCTURE OF THE DISSERTATION

This study structure will consist of an Introduction which will outline the content and broad overview of the study. The Literature Review will provide an overview of the major theories and philosophies related to this study. The Methodology Chapter of this study outlines the methods used in the study and will provide a justification for the chosen methodology and a detailed explanation of its practical application. The Analysis of Findings will be the main body of the study and will investigate the findings and results of the study. The Conclusion and Recommendations chapter will give an overview of the results and recommendations on what the resulting risk could be for SANBS based on the proven results.

1.11. CONCLUSION

This study is fully funded by the SANBS to provide the SANBS with information on the prevalence of HIV, HTLV and HBV in the patient population serviced by the SANBS. This will provide the management team of the SANBS with the information on the resulting risk of not testing for HTLV in the donor population of the SANBS.

CHAPTER 2
LITERATURE REVIEW

2.1. INTRODUCTION

Human immunodeficiency virus (HIV), hepatitis B virus (HBV) and human T-cell lymphotropic virus (HTLV) represent major public health problems throughout the world.

2.2. HTLV

2.2.1. Pathophysiology of HTLV

Human T-lymphotropic virus (HTLV) is a human retrovirus that preferentially infects CD4+ lymphocytes (Bangham & Matsuoka, 2017). The virus causes a haematological malignancy known as adult T-cell leukemia and an inflammatory disease in the central nervous system (CNS) called HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gudo *et al.*, 2015). Approximately 0.3% of HTLV-infected individuals develop HAM/TSP. HAM/TSP patients show spastic paraparesis and sphincter dysfunction, as well as sensory disturbance of the lower extremities, which corresponds to pathological lesions in the spinal cord.

Although the majority of HAM/TSP patients progress slowly, this disease progresses rapidly in some patients (Gudo *et al.*, 2015). An increased HTLV proviral load is more common in HAM/TSP patients than in asymptomatic HTLV carriers and is considered to be a strong risk factor for HAM/TSP development (Ikezu & Gendelman, 2016). A prominent cellular immune response in HAM/TSP patients is a significantly elevated number of HTLV Tax-specific CD8+ cytotoxic T lymphocytes (CTL) in peripheral blood mononuclear cells compared to asymptomatic HTLV carriers. Additionally, CD4+ and CD8+ lymphocytes accumulate in the perivascular areas of the spinal cord in HAM/TSP patients.

Viral Deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA) and proteins are detected only in infiltrating CD4+ T cells, but not in neural cells. A high proportion of HTLV-specific CTL infiltrates the CNS (Ikezu & Gendelman, 2016). Furthermore, some neural cells surrounding the CTL predominantly the oligodendrocytes undergo apoptosis. These findings suggest that the pathogenesis that the HTLV-specific inflammation induced by the interaction of HTLV-infected CD4+ T cells and HTLV-specific CD8+ CTL causes bystander damage in the CNS (Ikezu & Gendelman, 2016).

2.2.2. Characteristics of the HTLV

2.2.2.1. Virological characteristics

HTLV is an enveloped virus that contains two identical copies of a plus single-stranded RNA genome and an outer envelope containing protruding viral glycoproteins (Handin *et al.*, 2003). This virus is known as a retrovirus because the RNA genome directs the formation of a DNA molecule, which ultimately acts as the template for the synthesis of viral mRNA (Handin *et al.*, 2003). Since most retroviruses do not kill their host cells infected cells can replicate producing daughter cells with integrated proviral DNA (Sirica, 2012). These daughter cells continue to transcribe the proviral DNA and bud progeny virions. Some retroviruses contain cancer-causing genes called oncogenes (Sirica, 2012).

Cells infected by such retroviruses are oncogenically transformed into tumor cells and are usually specific for certain cell types. HTLV is known to cause leukemia and lymphoma and primarily infects certain cells of the immune system known as CD4+ T-cells (Sirica, 2012). It is considered a difficult virus to work with; however, because although HLTV has the capacity to infect a number of cell types including T cells, B cells, and endothelial cells, the only cells susceptible to HTLV transformation are primary T-lymphocytes (Handin *et al.*, 2003). These specified cells have cell-surface receptors that interact with viral proteins which account for the host-cell specificity of the virus (Handin *et al.*, 2003).

2.2.2.2. Pathological

Due to the long incubation period between viral exposure and disease onset, the exact mechanism of HTLV pathogenesis remains unclear (Ikezu & Gendelman, 2016). Research has shown that the host cell's control of HTLV replication is the primary determinant of virus expression and subsequent disease (Martins *et al.*, 2012). It is also known that the transmission of HTLV occurs through perinatal transmission by blood or breast milk, sexual transmission, or exposure to contaminated blood products and that the infected cells must be passed from the infected individual or material because HTLV is transmitted via cell to cell contact (Martins *et al.*, 2012).

The process begins when an infected cell contacts an uninfected cell, forming a microtubule-organising center (MTOC) that is polarised at the cell-cell junction (Nejmeddine *et al.*, 2009). A virological synapse is then formed at the interface and is triggered by the Tax protein located in the upstream open reading frame IV (4) (Nejmeddine *et al.*, 2009). Tax protein plays a central role in enhancing the transcription of viral and cellular gene products that promote viral replication and transformation of human T lymphocytes (Nejmeddine *et al.*, 2009). The HTLV GAG-complex and viral genomic RNAs then accumulate at the synapse and are released into the uninfected cell (Nejmeddine *et al.*, 2009). The engagement of intercellular adhesion molecule 1 (ICAM1) increases the polarisation of the MTOC at the point of contact, indicating that the interaction of ICAM1 and lymphocyte function-associated antigen 1 (LFA1) is important for HTLV infection (Nejmeddine *et al.*, 2009).

Tax plays an important role in the pathogenesis of HTLV by stimulating viral gene expression and by deregulating the expression of cellular genes (Kannian & Green, 2010). The Tax has also been shown to activate transcription of a number of cellular genes involved in cell proliferation, and the expression of these growth-related genes has been implicated in contributing to the establishment of HTLV associated pathogenesis (Kannian & Green, 2010). While Tax does not bind directly to DNA, it appears to stimulate RNA synthesis through protein-protein interactions with host cell transcription factors (Kannian & Green, 2010).

2.2.3. Immune response to HTLV

2.2.3.1. Cellular Immune Response to HTLV

A number of immunologic parameters have been described in HAM/TSP patients including high HTLV proviral load, increased spontaneous lymphoproliferation, high antibody titers to HTLV both in sera and cerebrospinal fluid (CSF), and increased cytokine production Rafatpanah *et al.* (2013). Since these immune abnormalities are more often observed in patients with HAM/TSP than in HTLV infected asymptomatic carriers, Rafatpanah *et al.* (2013) suggest that immune dysregulation may be associated with the pathogenesis of HTLV associated neurologic disease.

Increased levels of the cytokines Interferons (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 have been reported in the sera and CSF, and mRNA for IL-1β, IL-2, TNF-α, and IFN-γ is up-regulated in HAM/TSP peripheral blood lymphocytes (PBL) (Quaresma *et al.*, 2015). An ELISPOT Assay is also a form of immunostaining since it is classified as a technique that uses antibodies to detect a protein analyte, with the word analyte referring to any biological or chemical substance being identified or measured (Kalyuzhny, 2005). An ELISPOT assay has shown significant elevation of IL-2, IFN-γ, and IL-4 in peripheral blood mononuclear cells (PBMC) isolated from HAM/TSP patients compared with both asymptomatic carriers and seronegative normal donors (Quaresma *et al.*, 2015). Abnormalities in cellular immune responses of HAM/TSP patients have also been identified. Natural killer cells tend to be diminished in both number and activity in HAM/TSP. In particular, the phenomenon of spontaneous lymphoproliferation defined as the ability of PBMC to proliferate ex vivo in the absence of antigenic stimulation or IL-2 has been well described in HAM/TSP PBL, in HTLV asymptomatic carriers, and in HTLV infected persons (Quaresma *et al.*, 2015).

However, the magnitude of this spontaneous lymphoproliferation is typically higher in HAM/TSP PBL. The spontaneous lymphoproliferation of HTLV infected PBL is thought to consist of the proliferation of HTLV infected CD4 cells and the expansion of CD8 cells based on the demonstration of an increase in virus-expressing cells concomitant with an increase in the percentage of CD8+CD28+ lymphocytes (Ikezu & Gendelman,

2008). Spontaneous lymphoproliferation from the PBMC of HAM/TSP patients involves both IL-2 and IL-15 (Ikezu & Gendelman, 2008).

2.2.3.2. Humoral Response to HTLV

Anti-Tax antibody might be associated with subsequent inflammation following initial tissue damage and disruption of the blood-brain barrier which is probably caused by the antiviral immune responses to HTLV and induces the release of autoantigens. In HTLV infection, HAM/TSP patients generally have a higher anti-HTLV antibody (Ab) titer than asymptomatic carriers (ACs) with a similar HTLV proviral load (Saito & Bangham, 2012). These anti-HTLV Abs often include IgM in both ACs and patients with HAM/TSP. These findings suggest that there was a persistent expression of HTLV proteins in vivo and the existence of an augmented humoral immune response to HTLV in HAM/TSP patients (Saito & Bangham, 2012).

Although Ab responses to the immunodominant epitopes of the HTLV envelope proteins were similar in all of the three clinical groups (HAM/TSP, ATL, and ACs) reactivity to four Tax immunodominant epitopes was higher in HAM/TSP patients (71%–93%) than in ATL patients (4%–31%) or ACs (27%–37%) (Ikezu & Gendelman, 2008). Among these anti-HTLV antibodies, anti-Env Ab is particularly important since some anti-envelope (Env) Abs have neutralising activity against HTLV. Antisera raised against recombinant HTLV Env polypeptides, vaccinia virus containing HTLV Env gene, immunisation with neutralising epitope peptides, and passive transfer of human IgG that has neutralising activity were all shown to neutralise HTLV infectivity (Ikezu & Gendelman, 2008).

2.2.4. Prognosis and treatment

No treatment intervention exists for acute or chronic human T-cell lymphotropic virus (HTLV) infection (Gonçalves *et al.*, 2010). Thorough neurological and ophthalmologic examinations, in addition to a complete physical examination, should be performed in patients infected with HTLV. Good oral care and routine dental follow-up are recommended. All patients with HTLV-1 or HTLV-2 infection should be counselled extensively on the lifelong implications of their infection (Gonçalves *et al.*, 2010).

The treatment of adult T-cell leukemia (ATL) is the same irrespective of the presence or absence of HTLV infection (Gonçalves *et al.*, 2010). Treatment should begin immediately in patients diagnosed with the aggressive types of ATL (Niederhuber *et al.*, 2013). Patients diagnosed with an indolent type of ATL (chronic or smoldering) should be monitored closely for disease progression (Niederhuber *et al.*, 2013). Chronic ATL usually progresses to the acute form within two years. Mogamulizumab, a defucosylated humanised anti-CCR4 IgG1 monoclonal antibody was approved in Japan as a new therapy for ATL (Niederhuber *et al.*, 2013). ATL cells usually express chemokine receptor CCR4. Mogamulizumab is pending phase 2 results in the United States. Chemotherapy is the primary treatment approach for adult T-cell leukemia. CHOP (cyclophosphamide, doxorubicin [hydroxydaunomycin], vincristine [Oncovin], and prednisone) or a similar regimen is used.

However, no known regimen increases the median survival time of 2 years (Niederhuber *et al.*, 2013). Complete remission can occur, but relapse is common. Other regimens include interferon alfa, topoisomerase inhibitors, zidovudine plus interferon alfa, arsenic trioxide plus interferon alfa, blockade of NF-κB with several experimental agents, and monoclonal antibodies against the IL-2R and other receptors on ATL cells (Niederhuber *et al.*, 2013). Allogenic hematopoietic stem cell transplantation has yielded varying results in HTLV infection.

HTLV–associated myelopathy/tropical spastic paraparesis (HAM/TSP) treatment options are even more limited and focus on symptomatic therapy as follows: studied therapies have included corticosteroids, plasmapheresis, cyclophosphamide and interferon all of which may produce temporary improvement in signs and symptoms associated with HAM/TSP (Niederhuber *et al.*, 2013). Mogamulizumab, a defucosylated humanised anti-CCR4 IgG1 monoclonal antibody is also under study for the treatment of HAM/TSP. This antibody effectively reduced HTLV proviral load, spontaneous proliferation, and production of proinflammatory cytokines. Further studies are needed if this treatment will translate into clinical treatment of HAM/TSP (Niederhuber *et al.*, 2013).

2.3. HIV

2.3.1. Pathophysiology of HIV

There are several viral and host factors determining the variability in HIV infection outcomes and in rates of disease progression in HIV infected individuals. Cellular tropism defines viral phenotype and receptor (Naif, 2013). Co-receptors that determine viral entry into various cell types are the major factors influencing HIV pathogenesis. The exact mechanism of how these factors contribute to the dramatic loss of CD4+ T cells and the persistence of R5 and X4 strains during the AIDS status is still not well identified (Naif, 2013). Infection with HIV starts without symptoms or ill-feeling and is accompanied by slight changes in the immune system. This stage can be as short as 21 days and spans up to three months after infection until seroconversion where HIV-specific antibodies can be detected in individuals following recent exposure (Naif, 2013).

The outcome of infection and duration for disease progression with clinical symptoms may vary greatly between individuals, but often it progresses fairly slowly (Naif, 2013). It takes several years from primary infection to the development of symptoms of advanced HIV diseases and immunosuppression. During primary infection, although individuals may look healthy, the virus is actively replicating in the lymph nodes and bloodstream of infected individuals (Naif, 2013). As a result, the immune system may get slowly damaged by the burst of viral load in their bodies. Symptomatic stage of disease indicates the late phase of HIV disease (AIDS) where individuals may be susceptible to other opportunistic infections, such as infections with *Mycobacterium avium*, *Mycobacterium tuberculosis*, *Pneumocystis carinii*, CMV, toxoplasmosis and candidiasis (Naif, 2013). It is agreed that infected individuals develop an AIDS status when their plasma HIV load is high and the CD4+ T count is less than 200 ul (Naif, 2013).

2.3.2. Characteristics of HIV

2.3.2.1. Virological Characteristics of HIV

The mature virus consists of a bar-shaped electron-dense core containing the viral genome, two short strands of ribonucleic acid (RNA) each 9200 nucleotide bases long, encased with the enzymes reverse transcriptase, protease, ribonuclease, and integrated within an outer lipid envelope derived from a host cell (Klatt, 2016). This envelope has 72 surface projections or spikes containing the antigen gp120 that aids in the binding of the virus to target cells with CD4 receptors (Klatt, 2016).

A second gp41 glycoprotein binds gp120 to the lipid envelope. Electron microscopy shows that the plasma membrane of an infected CD4+ lymphocyte exhibits budding virus particles approximately 100 nanometers in diameter (Klatt, 2016). The virion has an asymmetric core consisting of a conical capsid (a geometric “fullerine cone”) with a broad electron-dense base and a hollow tapered end. Virions bud from plasma membranes or from cytoplasmic vacuoles of infected host cells (Klatt, 2016). Spikes are inserted into the membrane of the developing virion which buds to a complete sphere (Klatt, 2016). Aberrant virion formation is common, including double buds, giant virions, empty nucleoids, and misplaced electron-dense material (Klatt, 2016).

The genome of HIV is similar to retroviruses, in general, and contains three major genes namely: gag, pol, and Env (Parija, 2014). These genes code for the major structural and functional components of HIV including envelope proteins and reverse transcriptase (Parija, 2014). The structural components encoded by Env include the envelope glycoproteins: outer envelope glycoprotein gp120 and transmembrane glycoprotein gp41 derived from glycoprotein precursor gp160 (Parija, 2014). Components encoded by the gag gene include core nucleocapsid proteins p55 (a precursor protein), p40, p24 (capsid, or “core" antigen), p17 (matrix), and p7 (nucleocapsid); the important proteins encoded by pol are the enzyme proteins p66 and p51 (reverse transcriptase), p11 (protease), and p32 (integrase) (Parija, 2014).

Although most of the major HIV viral proteins, which include p24 (core antigen) and gp41 (envelope antigen), are highly immunogenic the antibody responses vary

according to the virus load and the immune competence of the host (Parija, 2014). The antigenicity of these various components provides a means for detection of the antibody, the basis for most HIV testing. Accessory genes carried by HIV include tat, rev, nef, vif, vpr, and vpu (for HIV) or vpx (for HIV-2) (Parija, 2014). The rev gene encodes for a regulatory protein that switches the processing of viral RNA transcripts to a pattern that predominates with established infection, leading to production of viral structural and enzymatic proteins (Parija, 2014).

The long terminal repeat (LTR) serves as a promoter of transcription and the tat (trans-activator of transcription) gene plays multiple roles in HIV pathogenesis (Parija, 2014). It produces a regulatory protein that speeds up transcription of the HIV provirus to full-length viral mRNAs (Parija, 2014). It functions in the transactivation of viral genes. In addition, tat modulates host cell gene expression (Klatt, 2016). The effects of such modulation may include enhanced immune suppression, apoptosis, and oxidative stress (Parija, 2014). The nef (negative factor) gene produces a regulatory protein that modifies the infected cell to make it more suitable for producing HIV virions, by accelerating endocytosis of CD4 from the surface of infected cells. The vif, vpr, and vpu genes encode proteins that appear to play a role in generating infectivity and pathologic effects (Parija, 2014). Vif, vpu, and vpr protein products link to members of a superfamily of modular ubiquitin ligases to induce the polyubiquitylation and proteasomal degradation of their cellular targets.

More specifically, vpr (viral protein r) has the ability to delay or arrest infected cells in the G2 / M phase of the cell cycle and facilitates infection of macrophages, and it promotes nuclear transport of the viral preintegration complex (Parija, 2014). Vif antagonizes the antiviral effect of apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G, or the protein product of the gene APOBEC3G (A3G). Vpu enhances efficient release of virions from infected cells (Klatt, 2016).

2.3.2.2. Pathological Characteristics of HIV

After entering the body, the viral particle is attracted to a cell with the appropriate CD4 receptor molecules where it attaches by fusion to a susceptible cell membrane or by endocytosis and then enters the cell (Wilen *et al.*, 2012). The probability of infection is

a function of both the number of infective HIV virions in the body fluid which contacts the host as well as the number of cells available at the site of contact that have appropriate CD4 receptors (Wilen *et al.*, 2012).

HIV infection can occur through oropharyngeal, cervical, vaginal, and gastrointestinal mucosal surfaces, even in the absence of mucosal disruption (Schust *et al.*, 2012). Routes of HIV entry into mucosal lamina propria include dendritic cells, epithelial cells, and microfold cells (Schust *et al.*, 2012). Dendritic cells can bind to gp120 through a C type lectin, suggesting that dendritic cells that squeeze between tight epithelium may capture HIV and deliver it to underlying T cells, resulting in dissemination to lymphoid organs (Belyakov & Berzofsky, 2004). HIV can cross a tight epithelial barrier by transcytosis during contact between HIV-infected cells and the apical surface of an epithelial cell (Belyakov & Berzofsky, 2004).

The presence of mucus on epithelial surfaces further retards viral entry, particularly in the endocervix where there is just a single columnar epithelial cell layer (Belyakov & Berzofsky, 2004). HIV can transmigrate across fetal oral mucosal squamous epithelium that has few layers, 5 or less. HIV-infected macrophages, but not lymphocytes, are able to transmigrate across fetal oral epithelia (Belyakov & Berzofsky, 2004). HIV-infected macrophages and, to a lesser extent, lymphocytes can transmigrate across fetal intestinal epithelia (Belyakov & Berzofsky, 2004).

Efficient viral transmission through adult mucosal epithelia is difficult because of a mechanical barrier of stratified epithelia with tight junctions that prevent penetration of virions into the deeper layers of the epithelium and from the expression of the anti-HIV innate proteins HBD2, HBD3, and SLPI that inactivate virions (Belyakov & Berzofsky, 2004). Transcytosis of virions through intact epithelium is favoured via surface expression of syndecans and chemokine receptors by epithelial cells (Belyakov & Berzofsky, 2004). However, the efficiency of transcytosis is poor, with only 0.02% of the original inoculum of HIV able to navigate across genital epithelium (Belyakov & Berzofsky, 2004). Thus, intact epithelium is a significant barrier to HIV infection, but the presence of antigen processing cells and inflammatory cells increases HIV transmission.

Exposure to HIV can upregulate pro-inflammatory cytokine production by genital epithelial cells, including tumor necrosis factor (TNF)-α that impairs the tight junction barrier, allowing HIV and luminal bacteria to translocate across the epithelium (Nazli *et al.*, 2010). Endothelium may also harbor HIV virions following parenteral transmission and during HIV viremia following infection (Nazli *et al.*, 2010). Endothelial cells express surface syndecans that mediate adsorption of HIV by binding of viral gp120 to heparan sulfate chains of syndecan (Nazli *et al.*, 2010). Although syndecan does not substitute for HIV entry receptors, it enhances infectivity and preserves virus infectivity for a week, whereas unbound virus loses its infectivity in less than a day (Nazli *et al.*, 2010).

The ligand for E-selection (CD62L) is incorporated into the virion during budding and can enhance virion attachment to endothelial cells and accelerate transfer of HIV to CD4 cells (Nazli *et al.*, 2010). Galactosylceramide expressed by human monocyte derived immature dendritic cells as well as dendritic cells isolated from blood and mucosal tissue and in situ on mucosal tissue can act as a mucosal epithelial receptor for gp41 on HIV (Magérus-Chatinet *et al.*, 2007). HIV entry into cells can occur independently of CD4 receptor interaction (Magérus-Chatinet *et al.*, 2007). Such entry is less efficient and less extensive and the entry has been described for renal tubules, gut enterocytes, vascular endothelium, cardiac myocytes, and astrocytes (Magérus-Chatinet *et al.*, 2007). Infection of these cells may play a role in the pathogenesis of HIV-related diseases occurring at tissue sites with those cells (Magérus-Chatinet *et al.*, 2007).

2.3.3. Immune response to HIV

The three stages of HIV infection are an acute infection, latent infection, and AIDS. Acute infection occurs immediately after the contraction of the virus and can be associated with influenza-like illness (Finton, 2014; Tarafdar, 2014). HIV is carried by the antigen-presenting cells to CD4 T cells and the infected CD4 T cells then traffic to the lymph nodes and disseminate within 4 to 11 days (Finton, 2014; Tarafdar, 2014). In the following few months, the viral load increases exponentially and is accompanied by the death of numerous CD4 T cells (Finton, 2014; Tarafdar, 2014). The elevated viral load is finally stabilised, predominately by the innate immune response (at one to

two weeks) and, secondarily, by the adaptive immune response (at four to eight weeks) (Finton, 2014; Tarafdar, 2014).

The viral load set point is established at a median time of two months (Finton, 2014; Tarafdar, 2014). During the second phase, the latent or chronic phase of infection, which lasts eight years on average, the viral load is kept relatively constant due to the efforts of the host immune system and the rapid turnover of infected cells (Finton, 2014; Tarafdar, 2014). CD4 T cell numbers decrease and virus sequence diversity increases (Finton, 2014; Tarafdar, 2014). The third phase is defined once the CD4 T cell count falls below 200/μL or with the occurrence of certain opportunistic infections and is characterised by dramatic increases in viral load and loss of CD4 T cells (Finton, 2014; Tarafdar, 2014).

2.3.3.1. Cellular Immune Response to HIV

The body's response to HIV in the first few weeks following infection plays a critical role in determining the viral set point and consequently disease progression (Ganesan *et al.*, 2010). At this time, the innate immune system is responsible both for initial control of the virus and also for inducing the adaptive immune response (Ganesan *et al.*, 2010). Cells of the innate immune system include natural killer cells, and natural killer T cells (cytolytic cells) capable of direct killing of pathogen-infected cells, dendritic cells, macrophages, monocytes (phagocytes) capable of antigen clearance, dendritic, macrophages, B cells (professional antigen-presenting cells) able to capture and present foreign antigens to cells of the adaptive immune system, eosinophils, basophils, neutrophils (granulocytes), γδ T cells, and mast cells (Clark & Kupper, 2005).

These cells patrol the body and respond to foreign antigens through receptor engagement without the need for prior exposure to the antigen (Clark & Kupper, 2005). Receptor activation results in the release of cytokines such as interferons (IFNs) that create an antiviral environment, recruit immune cells and shape the quality of the adaptive immune response (Clark & Kupper, 2005). Among the innate immune cell types, natural killer cells in the mucosa are first in the line of defense against HIV infected cells (Mata *et al.*, 2014).

Natural killer cells, as well as macrophages, neutrophils, and eosinophils, can also exert protective effects through antibody-dependent cell-mediated cytotoxicity and antibody-dependent cell-mediated virus inhibition (Clark & Kupper, 2005). Antibody-dependent cell-mediated cytotoxicity is a mechanism whereby innate effector cells bind to the fragment crystallisable (Fc) portion of an antibody that is bound to virally infected cells and cause lysis of the infected cell through the release of cytotoxic granules (Clark & Kupper, 2005). Antibody-dependent cell-mediated virus inhibition refers to viral inhibition as a result of the death of the infected cell (Clark & Kupper, 2005).

The importance of Antibody-dependent cell-mediated cytotoxicity / antibody-dependent cell-mediated virus inhibition in HIV infected individuals is demonstrated by the finding that disease progression is inversely correlated to high titers of glycoprotein (gp)120-specific (Envelope glycoprotein 120 is a glycoprotein exposed on the surface of the HIV envelope) antibody-dependent cell-mediated cytotoxicity antibodies (Mata *et al.*, 2014).

2.3.3.2. Humoral immune Response to HIV

Antibodies to HIV can be detected soon after acute infection, often as early a few days after exposure to the virus, but generally within 1 to 3 months (Baum, 2010). These antibodies can be found in the blood, on mucosal surfaces, and in various body fluids (Baum, 2010). Immunoglobulin G (IgG)1 is a key player in host defense at all stages of infection and helps to combat the virus with antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and neutralising and blocking responses (Baum, 2010). Antibody-dependent cellular cytotoxicity is directed against sites on the viral envelope, especially against sites on the V3 loop and the extracellular domain of gp41 (Baum, 2010).

All the other antibody (Immunoglobulin (IG) isotypes (IgM, IgA, IgG2, IGG4 and IGD) vary in their levels throughout the course of infection, and there is no known correlation between isotype appearance and stage of disease (Baum, 2010). Neutralising antibodies offer some protection by limiting viral replication during the early

asymptomatic stages, but overall their titers tend to remain too low to clear the HIV infection (Finton, 2014). The low titer is probably a result of the fact that the virus's envelope epitopes have a highly dynamic configuration, which changes often, depending on the state of activation and binding to cellular receptors (Finton, 2014). The low titer also favours the emergence of resistant mutants during active replication (Finton, 2014).

Patients often produce many neutralising bodies, but these antibodies are specific to the earlier virus isolates and cannot neutralise these "escape mutants" as effectively (Finton, 2014). Neutralising antibodies bind to specific sites on the viral envelope complex, SU-TM. The V3 loop on SU is one of the chief targets of these immune molecules (Finton, 2014). Anti-V3 antibodies block co-receptor interactions that occur after the virus attached to the major CD4 receptor (Finton, 2014).

Antibodies to this region tend to be very strain specific because of the high sequence variation in this segment (Finton, 2014). As a result, V3 is not a good target for vaccines or antiretroviral therapy, even though it has suitably high antigenicity. Another group of neutralising antibodies blocks the binding of SU to CD4 receptors and are more broadly reactive than anti-V3 antibodies (Finton, 2014). Broad neutralising activity against the carbohydrate-containing regions of the viral envelope protein has also been detected (Finton, 2014).

2.3.4. PROGNOSIS AND TREATMENT

2.3.4.1. Prognosis

HIV/AIDS has become a chronic rather than an acutely fatal disease in many areas of the world (Colvin, 2011). Prognosis varies between people, and both the CD4 count and viral load are useful for predicted outcomes (Korenromp *et al.*, 2009). Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype (Johnson *et al.*, 2017). After the diagnosis of AIDS, if treatment is not available, survival ranges between 6 and 19 months (May *et al.*, 2014). The primary causes of death from HIV/AIDS are opportunistic

infections and cancer, both of which are frequently the result of the progressive failure of the immune system (Bonnet *et al.*, 2002).

Early symptoms are like a common cold, flu, general fatigue, weakness, loss of appetite, fever, etc. As the disease worsens, symptoms increase in numbers, such as *Candida albicans* infection of the skin and mucous membrane, appearance of herpes simplex, herpes zoster, purple plague, blood blisters, and congestion spots; later, visceral organs are gradually violated, which leads to unexplained persistent fever up to 3–4 months; also cough, shortness of breath, difficulty breathing, persistent diarrhea, hemafecia, hepatosplenomegaly, malignancy and the like will occur (Lv *et al.*, 2016).

Clinical symptoms are complex and changeful, but each patient does not have all the above symptoms. In case of violation of the lung, difficulty in breathing, chest pain, and cough will occur; gastrointestinal violation can cause persistent diarrhea, abdominal pain, weight loss and weakness; violations of the nervous system and cardiovascular system can also be caused (Lv *et al.*, 2016). As AIDS will cause long-term disfunction of patients' body organs and most patients show systemic organ failure at death (Lv *et al.*, 2016).

2.3.4.2. Treatment

The World Health Organisation, the United States and South Africa recommends antiretrovirals in people of all ages including pregnant women as soon as the diagnosis is made regardless of CD4 count (WHO, 2018b). Once treatment is begun it is recommended that it is continued without breaks or "holidays". Many people are diagnosed only after treatment ideally should have begun (WHO, 2018b).

Treatment is accomplished through numerous combinations of antiretroviral agents from four classes: nucleoside analogue reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, and the newest class, nucleotide analogues (McDanirl *et al.*, 2000). Benefits of treatment include a decreased risk of progression to AIDS and a decreased risk of death (McDanirl *et al.*,

2000). In the developing world treatment also improves physical and mental health (McDanirl *et al.*, 2000).

Additional benefits include a decreased risk of transmission of the disease to sexual partners and a decrease in mother-to-child transmission (WHO, 2018b). At present, there is no cure for HIV infection, and although recent treatment strategies have achieved undetectable plasma viral loads, it is increasingly unlikely that the HIV virus can be eliminated entirely from an infected individual (Jordon *et al.*, 2000). There are candidate vaccines in trials, but the main strategy of treatment is to reduce the speed of disease progression and to treat any opportunistic infections that arise (Jordon *et al.*, 2000).

2.4. Hepatitis B virus (HBV)

2.4.1. Pathophysiology of HBV

Hepatitis B virus (HBV) may be directly cytopathic to hepatocytes (Samji, 2017). However, immune system-mediated cytotoxicity plays a predominant role in causing liver damage (Samji, 2017). The immune assault is driven by human leukocyte antigen (HLA) class I–restricted CD8 cytotoxic T lymphocytes that recognise hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg) on the cell membranes of infected hepatocytes (Samji, 2017).

2.4.1.1. Acute infection

The incubation period of HBV infection is 40-150 days; however, DNA can be detected in 15 days and the infection can be transmitted through blood transfusion (Islam, 2017). The clinical illness associated with acute HBV infection may range from mild disease to a disease as severe as a fulminant hepatic failure (FHF) (Islam, 2017). After acute hepatitis resolves, 95% of adult patients and 5-10% of infected infants ultimately develop antibodies against hepatitis B surface antigen (HBsAg) and fully recover (Islam, 2017).

About 5% of adult patients, 90% of infected infants, and 30-50% of children infected at age 1-5 years develop chronic infection (Samji, 2017). Some patients, particularly individuals who are infected as neonates or as young children, have elevated serum levels of HBV DNA and a positive blood test for the presence of hepatitis B e-antigen (HBeAg) but have normal alanine aminotransferase (ALT) levels and show minimal histologic evidence of liver damage (Samji, 2017). These individuals are in the so-called "immune-tolerant phase" of the disease (Samji, 2017).

Years later, some but not all these individuals may enter the "immune-active phase" of the disease, in which the HBV DNA may remain elevated as the liver experiences active inflammation and fibrosis. An elevated ALT level is also noted during this period (Samji, 2017). Typically, the immune-active phase ends with the loss of HBeAg and the development of antibodies to HBeAg (anti-HBe). Individuals who seroconvert from an HBeAg-positive state to an HBeAg-negative state may enter the "inactive carrier state" or "healthy carrier state" (Liaw, 2009). Such individuals are asymptomatic, have normal liver chemistry test results, and have normal or minimally abnormal liver biopsy results (Liaw, 2009). Blood test evidence of HBV replication should be nonexistent or minimal, with a serum HBV DNA level in the range of 0 to 2000 IU/mL (Liaw, 2009).

Inactive carriers remain infectious to others through parenteral or sexual transmission. Inactive carriers may ultimately develop anti-HBs, clear the virus and become immune (Liaw, 2009). However, some inactive carriers develop chronic hepatitis, as determined by liver chemistry results, liver biopsy findings, and HBV DNA levels. Inactive carriers remain at risk for hepatocellular carcinoma (HCC), although the risk is low (Liaw, 2009). At this point, no effective antiviral therapies are available for patients in an inactive carrier state. Other patients who seroconvert may enter the "reactivation phase" of disease (Liaw, 2009). These individuals remain HBeAg-negative but have serum HBV DNA levels higher than 2000 IU/mL and show evidence of active liver inflammation. These patients are said to have HBeAg-negative chronic hepatitis (Liaw, 2009).

2.4.1.2. Chronic infection

The spectrum of chronic HBV infection ranges from the asymptomatic carrier state to chronic hepatitis B, liver cirrhosis and hepatocellular carcinoma (Maheshwari *et al.*, 2005). Chronic hepatitis B patients have abnormal liver chemistry results, blood test evidence of active HBV replication, and inflammatory or fibrotic activity on liver biopsy specimens (Samji, 2017). Patients with chronic hepatitis may be considered either HBeAg-positive or HBeAg-negative (Samji, 2017). Patients with HBeAg-negative chronic hepatitis were presumably infected with wild-type virus at some point (Samji, 2017). Over time, they acquired a mutation in either the precore or the core promoter region of the viral genome (Samji, 2017). In such patients with a precore mutant state, HBV continues to replicate, but HBeAg is not produced (Samji, 2017). Patients with a core mutant state appear to have down-regulated HBeAg production. The vast majority of patients with HBeAg-negative chronic hepatitis B have a serum HBV DNA level greater than 2000 IU/mL (Samji, 2017).

2.4.1.3. HBV and hepatocellular carcinoma (HCC)

An approximately 8-20% of untreated adults with chronic hepatitis B go proceed to develop cirrhosis within 5 years; of these individuals, 20% annually develop hepatic decompensation and 2-5% annually develop HCC (Niederau, 2014). Globally, an estimated 30% of cases of cirrhosis and 45% of cases of HCC are attributed to HBV (Samji, 2017). The incidence of HCC parallels the incidence of HBV infection in various countries around the world. Worldwide, up to 1 million cases of HCC are diagnosed each year (Samji, 2017). Most appear to be related to HBV infection. In HBV-induced cirrhosis, as in cirrhosis due to other causes, hepatic inflammation and regeneration appear to stimulate mutational events and carcinogenesis. However, in HBV infection, in contrast to other liver diseases, the presence of cirrhosis is not a prerequisite for the development of HCC (Samji, 2017). The integration of HBV into the hepatocyte genome may lead to the activation of oncogenes or the inhibition of tumor suppressor genes (Samji, 2017). Successful suppression of HBV infection by antiviral therapy can decrease the risk of developing HCC (Samji, 2017).

2.4.2. VIROLOGICAL AND PATHOLOGICAL CHARACTERISTICS OF HBV

2.4.2.1. Virological Characteristics of HBV

Hepatitis B virus is a member of the hepadnavirus family. The virion consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. These virions are 30 - 42 nm in diameter (Patient *et al.*, 2007). The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity (Patient *et al.*, 2007). The outer envelope contains embedded proteins that are involved in viral binding of, and entry into, susceptible cells (Patient *et al.*, 2007). The virus is one of the smallest enveloped animal viruses. The 42 nm virions, which are capable of infecting liver cells known as hepatocytes, are referred to as "Dane particles" (Patient *et al.*, 2007).

In addition to the Dane particles, filamentous and spherical bodies lacking a core can be found in the serum of infected individuals (Patient *et al.*, 2007). These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigens (HBsAg) and is produced in excess during the life cycle of the virus (Howard, 1986). The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded (Howard, 1986).

One end of the full-length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full-length strand) and 1700–2800 nucleotides long (for the short length-strand) (Howard, 1986). The negative-sense (non-coding) is complementary to the viral mRNA (Howard, 1986). The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by the completion of the (+) sense strand and removal of a protein molecule from the (−) sense strand and a short sequence of RNA from the (+) sense strand (Liaw & Zoulim, 2015). Non-coding bases are removed from the ends of the (−) sense strand and the ends are re-joined (Liaw & Zoulim, 2015).

There are four known genes encoded by the genome, called C, X, P, and S (Liaw & Zoulim, 2015). The core protein is coded for by gene C (HBcAg), and its start codon is

preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced (Liaw & Zoulim, 2015). HBeAg is produced by proteolytic processing of the pre-core protein. In some rare strains of the virus known as Hepatitis B virus precore mutants, no HBeAg is present (Liaw & Zoulim, 2015). The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg).

The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Due to the multiple start codons, polypeptides of three different sizes called large, middle and small (S) are produced (Liaw & Zoulim, 2015). The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer (Liaw & Zoulim, 2015). It stimulates genes that promote cell growth and inactivates growth regulating molecules (Liaw & Zoulim, 2015).

2.4.2.2. Pathological Characteristics of HBV

Hepatitis B virus primarily interferes with the functions of the liver by replicating in hepatocytes (Boltjes *et al.*, 2014). There is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D (Boltjes *et al.*, 2014). The virions bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalised by endocytosis (Boltjes *et al.*, 2014). HBV-preS-specific receptors are expressed primarily on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells (Boltjes *et al.*, 2014).

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance (Liaw & Zoulim, 2015). Although the innate immune response does not play a significant role in these processes, the adaptive immune response, in particular virus-specific Cytotoxic T-Lymphocyte (CTLs), contributes to most of the liver injury associated with HBV infection (Liaw & Zoulim, 2015). CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes (Liaw & Zoulim, 2015). Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can

worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver (Liaw & Zoulim, 2015).

2.4.3. IMMUNE RESPONSE TO HBV

2.4.3.1. The innate immune response

A typical feature of HBV infection acquired in adult life is that HBV remains quiescent for some weeks before starting an active and exponential phase of replication, which can lead to high viraemia levels and the infection of virtually all hepatocytes (Ferrari, 2015). When the infection is self-limited, the peak of HBV replication is followed by a rapid decline in viraemia, which generally starts before maximal ALT elevation (Ferrari, 2015). This early virus control is probably sustained by non-cytolytic mechanisms and type I interferons represent the most likely candidates for this antiviral activity (Ferrari, 2015). Human hepatocytes infected with HBV were shown to stimulate Kupffer cells to produce IL-6 with anti-HBV activity (Ferrari, 2015).

The predominant view is that HBV has developed specific strategies to evade recognition by the innate immunity (Wang & Ryu, 2010). In particular, the use of a transcriptional template that is sequestered within the nucleus of infected cells in its replication cycle may allow HBV to escape detection by the innate DNA sensing cellular machinery (Wang & Ryu, 2010). The production of polyadenylate viral mRNA that resembles the normal cellular transcripts and protection of newly transcribed genomes within viral capsides in the cytoplasm may further contribute to hide the virus from host immune recognition (Wang & Ryu, 2010). In addition to escaping innate immunity, HBV seems also to be able to actively inhibit innate responses (Wang & Ryu, 2010). Secretory HBV proteins (HBsAg, HBeAg) have been reported to suppress Toll-like receptors (TLR) expression and to abrogate TLR-induced responses, further contributing to impair initial antiviral control (Wang & Ryu, 2010).

2.4.3.2. The adaptive immune response

Due to the poor induction of innate intracellular immunity, adaptive responses are efficiently and timely induced immediately after active virus replication begins (Bauer

et al., 2011). Although HBV-specific T cells become detectable several weeks after infection, these responses are only apparently delayed in relation to the time of infection because of the initial quiescence of HBV, which probably does not provide enough stimulation to prime and expand HBV-specific T cells (Bauer *et al.*, 2011). A key role in CD8 cell recruitment into the liver is played by platelet activation within the infected liver, which can facilitate platelets/ cytotoxic T lymphocyte interaction and the egress of the latter from the bloodstream with accumulation in the infected parenchyma (Bauer *et al.*, 2011).

Cytokines released by liver infiltrating HBV-specific T cells, especially CD8 cells, at the site of infection are believed to be the main cause of the early non-cytolytic clearance of HBV (Bauer *et al.*, 2011). In self-limited infections, HBV-DNA decreases by more than 90% within 2–3 weeks after peak viral replication and before the detection of liver damage, as indicated by the increase in ALT (Bauer *et al.*, 2011). Therefore, a large quantity of viral DNA is eliminated without liver cell destruction (Bauer *et al.*, 2011). Intrahepatic recruitment of HBV-specific CTL obviously also leads to the killing of infected hepatocytes with subsequent infiltration of antigen non-specific cells, which is facilitated by the secretion of chemokines and by platelet activation which amplifies hepatocellular damage and liver disease that are initially triggered by HBV-specific mechanisms (Ferrari, 2015).

HBV-specific T-cell responses in the acute phase of infection are typically multispecific, Th1 oriented, poly-functional and much stronger than those detectable in chronic infection (Bauer *et al.*, 2011; Ferrari, 2015). Natural killer (NK) cells that can produce antiviral cytokines such as IFN-γ and TNF-α should obviously contribute to the early non-cytolytic control of infection (Ferrari, 2015). Even in the absence of an early induction of pro-inflammatory cytokines directly triggered by HBV infection, NK cells remain well-poised to respond to acute HBV infection in the liver given the very low basal levels of MHC class I expression by liver cells and given the high NK cell frequency in the normal liver, where NK cells constitute 30–40% of intrahepatic lymphocytes (Ferrari, 2015).

In contrast to the timely activation of T cells, NK cell activation seems to be delayed in overt acute infections, from the time of first HBsAg and HBV-DNA detection revealing

suppressed NK cell activation and effector function at the time of viral load increase with the peak of NK cell frequency and function occurring once viraemia resolves (Bauer *et al.*, 2011; Ferrari, 2015). This inhibition of NK cell activation and effector potential was temporally associated with an induction of IL-10, again raising the possibility that HBV can actively evade immune responses. To avoid excessive immune pathology, mechanisms of T-cell inhibition sustained by the effect of soluble factors, such as arginase released by necrotic liver cells are activated at the time of the ALT peak, when HBV-specific T cells appear to be highly activated but almost totally inhibited in their function (Bauer *et al.*, 2011; Ferrari, 2015).

This functional inhibition is only transient, because resolution of infection is associated with a decline of activated T cells, an increasing expression of CD127 molecules, a decline of PD-1 on HBV-specific CD8 cells and a restoration of the T-cell function with maturation of long-lasting protective memory T-cell responses that are maintained by the occult persistence of HBV, which is never totally eliminated from the infected host, even after successful resolution of acute hepatitis (Bauer *et al.*, 2011; Ferrari, 2015). In HBV infection, long-lasting T-cell responses are not sustained by typical antigen-independent memory T cells persisting in the absence of antigen but are rather maintained by continuous exposure of T cells to minute quantities of viral antigens (Russo, 2015). This mechanism can activate good quality HBV-specific antibody and T-cell responses, which are protective but not sterilising (Russo, 2015).

2.4.3.3. The humoral immune response

Antibody production is critical for the neutralization of free HBV particles and for the interference with virus entry into the host cells (Ferrari, 2015). Therefore, protection by antibodies is most important before invasion of the host cells; thereafter, antibodies can contribute to limit cell to cell spread of viral particles but elimination of intracellular virus becomes the principal task of HLA class I restricted CTL and other effector cell types non-specifically recruited to the site of infection (Ferrari, 2015).

Indeed, recognition of virally infected cells by antibodies and their killing through complement-mediated cytotoxicity or antibody-dependent cell cytotoxicity are less efficient than activation of HLA class I restricted CTL that require no more than 100

peptide/HLA complexes for activation. This high efficiency is in contrast with the millions of antibody molecules that are needed to destroy virally infected cells through a complement-mediated mechanism (Ferrari, 2015). The antibody production by B cells is generally a T cell-dependent phenomenon that requires the helper effect of CD4+ T cells through both cognate interaction and release of cytokines needed for differentiation of B lymphocytes into antibody-producing cells (Ferrari, 2015).

This property is explained by the structure of HBcAg (Ferrari *et al.*, 2003). The clustering of individual HBcAg subunits gives rise to spikes distributed over the surface of the HBcAg shell that project out of it (Ferrari *et al.*, 2003). The orientation of these spikes, on the top of which the dominant B cell epitopes are located, may be optimal for cross-linking the B cell membrane receptor and can explain the exceptional B cell activation capacity of the HBV core molecule (Ferrari *et al.*, 2003). These features can also explain the clinical evidence that anti-HBc antibodies are produced by virtually all HBV infected patients, regardless of their clinical status, and that their level is particularly high during chronic HBV infection as a likely result of a continuous B cell stimulation by HBcAg (Ferrari *et al.*, 2003).

2.4.4. PROGNOSIS AND TREATMENT

2.4.4.1. Prognosis

Although many patients with chronic HBV infection have mild disease and minimal morbidity or mortality (Procop & Pritt, 2014), approximately, one third develop significant consequences including cirrhosis or hepatocellular carcinoma. The risk of progression to cirrhosis and HCC depends in part on both viral (genotype, DNA level, and hepatitis C co-infection) and host factors (male gender, increased age, and alcohol use).

An estimated 30% of patients develop cirrhosis at an overall incidence of 2% to 3% per year. Although patients with cirrhosis are at greatest risk for developing HCC, non-cirrhotic patients are also at risk (Procop & Pritt, 2014).

2.4.4.2. Treatment

Acute hepatitis B infection does not usually require treatment and most adults clear the infection spontaneously (Procop & Pritt, 2014). Early antiviral treatment may be required in fewer than 1% of people, whose infection takes a very aggressive course (fulminant hepatitis) or who are immunocompromised (Procop & Pritt, 2014). On the other hand, treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer (Procop & Pritt, 2014). Chronically infected individuals with persistently elevated serum alanine aminotransferase, a marker of liver damage, and HBV DNA levels are candidates for therapy (Zammitt & O'Brien, 2017). The treatment lasts from six months to a year, depending on medication and genotype (Zammitt & O'Brien, 2017).

Treatment duration when medication is taken by mouth, however, is more variable and usually longer than one year (Zammitt & O'Brien, 2017). Although none of the available drugs can clear the infection, they can stop the virus from replicating, thus minimising liver damage (Zammitt & O'Brien, 2017). Current treatment options for chronic HBV include nucleoside analogue inhibitors of the HBV reverse transcriptase (lamivudine and adefovir dipivoxil) (Zammitt & O'Brien, 2017). Also available are telbivudine, entecavir and tenofovir (in certain countries), and interferon. Side effects, cost, duration of treatment, and possible selection of resistant mutants are all considered in determining a treatment strategy for an individual patient (Procop & Pritt, 2014).

Liver transplantation is an option for patients with end-stage liver disease and some patients with HCC. Response to treatment differs between the genotypes (Zammitt & O'Brien, 2017). Interferon treatment may produce an e antigen seroconversion rate of 37% in genotype A but only a 6% seroconversion in type D (Zammitt & O'Brien, 2017). Genotype B has similar seroconversion rates to type A while type C seroconverts only in 15% of cases (Zammitt & O'Brien, 2017). Sustained e antigen loss after treatment is 45% in types A and B but only 25–30% in types C and D (Zammitt & O'Brien, 2017).

2.5. CONCURRENT INFECTIONS OF HTLV, HBV AND HIV

2.5.1. PATHOPHYSIOLOGY

2.5.1.1. HTLV and HIV

The problem of HIV/HTLV co-infection has emerged as a worldwide health problem in the last 20 years as the numbers of HIV/HTLV infected individuals in South America and Africa has increased (He *et al.*, 2016). It is estimated that rates of HTLV or HTLV-2 co-infections in HIV-infected individuals are at least 100 to 500 times greater than in the general population (Isache *et al.*, 2016). In some geographic regions 5–10% of HIV-infected individuals may be co-infected with HTLV-1 or HTLV-2 (Gudo *et al.*, 2009). HTLV may be detrimental to the HIV-infected individual, with increased risk for the development of neurologic complications including TSP/HAM, leukaemia, and lymphoma (Beilke, 2012).

This often occurs in the scenario of a patient with relatively normal or even elevated CD4+ T cell counts (Beilke, 2012). In HIV infected individuals, HTLV may precipitate the transition from M- to the T-tropic phenotype that is associated with HIV disease progression (Casoli *et al.*, 2007). In addition, HTLV Tax protein upregulates HIV expression, as well as expression of various cytokines and cytokine receptors involved in T-cell activation, thereby providing a favourable condition for HIV infection and gives an indication that HTLV infection exacerbates the cytopathic effects of HIV and accelerates clinical progression to AIDS in these co-infected individuals (Casoli *et al.*, 2007).

2.5.1.2. HBV and HIV

The natural history of HBV disease progression is modified by HIV co-infection (Thio, 2003). A prospective study of 5293 gay men, 326 of whom had chronic hepatitis B, demonstrated that mortality attributable to liver disease was significantly increased in those who were co-infected with HIV and HBV compared with those with HBV alone (Thio, 2003). The investigators found that individuals with lower CD4 counts appeared to be at greatest risk (Thio, 2003).

In HIV-infected persons, clearance rates of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) are decreased compared with those without HIV infection and may be related to the degree of immunosuppression (Thio, 2003). HIV infection is also associated with reactivation of HBV, accelerated loss of anti-HBs, higher levels of HBV DNA, and lower ALT levels. The lower ALT levels suggest less hepatocyte destruction because of a depressed immune response and may contribute to the decreased effectiveness of anti-HBV therapy in co-infected individuals (Thio, 2003).

2.5.1.3. HTLV and HBV

The available data suggest limited or no effect of HBV / HTLV on progression to various HTLV / HBV outcomes; however, the study populations were small (Moreira *et al.*, 2013).

2.6. TRANSFUSION TRANSMISSION

2.6.1. Transfusion transmission of HBV

Over the last four decades, the risk of transfusion-transmitted hepatitis B virus has steadily reduced, yet HBV transmission remains the most frequent transfusion-transmitted viral infection (Candotti & Allain, 2009). The residual risk of HBV transfusion transmission is mainly related to blood donations negative for HBsAg that have been collected either during the pre-seroconversion window period which is defined as the time between infection and detection of a viral antigen or antibody marker or during the late stages of infection (Candotti & Allain, 2009). Clinical observations suggest a limited transmission rate of occult HBV compared to window period (Candotti & Allain, 2009). Low transmission rate might be related to low viral load observed in the occult carriage of HBV infection (OBI) or to the presence of mutants associated with occult carriage (Candotti & Allain, 2009).

In a recent study done by Vermeulen *et al.* (2012), reported that the risk of HBV transmission by low viral load OBI carriers is 3% or less. OBIs carrying detectable anti-HBs (~50%) are essentially not infectious by transfusion (Candotti & Allain, 2009).

However, recent data suggest that the neutralising capacity of low anti-HBs may be inefficient when overcome by exposure to high viral load (Candotti & Allain, 2009). Therefore a number of blood services will only transfuse a donation with occult HBV if the Viral load is undetectable in a highly sensitive (95% LOD <50 copies/ml) assay, HBsAg is non-reactive and the anti-HBs titre is greater than 200 IU/mL. (Hoshi *et al.*, 2019).

Immunodeficient elderly and patients receiving immunosuppressive treatments may be susceptible to infection with lower infectious dose even in the presence of anti-HBs (Candotti & Allain, 2009). The immune status of blood recipients should be taken into consideration when investigating “post-transfusion” HBV infection (Candotti & Allain, 2009). Pre-transfusion testing and post-transfusion long-term follow-up of recipients, and molecular analysis of the virus infecting both donor and recipient are critical to definitively incriminate transfusion in the transmission of HBV (Candotti & Allain, 2009; Vermeulen *et al.*, 2012).

Transfusion transmission can be documented by (1) notifying and testing donors that have been implicated in a case of possible post-transfusion hepatitis B (traceback or look back); (2) notifying and testing recipients after the administration of potentially infectious HBV-containing blood products (traceback or look back); and (3) prospective phylogenetic analysis testing of donor–recipient pairs (Candotti & Allain, 2009; Vermeulen *et al.*, 2012). HBV incubation time can be considerably prolonged (up to 13 months) in unusual circumstances involving an impaired immune system and active or passive neutralising antibodies to HBV in the recipient. There is preliminary evidence that immunocompromised patients are not only more susceptible to lower infectious dose including in the presence of anti-HBs but also at higher risk of developing chronic infection (Candotti & Allain, 2009). The ± 50% mortality rate within 6–12 months post-transfusion reported in transfusion recipients may also limit the identification of HBV transmission (O'Flaherty *et al.*, 2018).

Lookback of recipients who were transfused with donations from a newly identified HBV-infected repeat donor is often used to identify recipients at risk of HBV transfusion transmission (O'Flaherty *et al.*, 2018; Vermeulen *et al.*, 2012). Evidence of transfusion transmission of HBV may not be obtained because pre-transfusion samples are rarely

available, HBV infections might have resolved before they are identified by lookback, and often recipients cannot be traced or samples obtained (Candotti & Allain, 2009).

As HBV marker levels may fluctuate over time, follow-up of suspected donors and testing of archived samples are necessary to exclude recent infection and false-positive (Candotti & Allain, 2009). Testing for archived donations and recipient samples requires access to a frozen repository of donor/recipient samples (Candotti & Allain, 2009). Such system exists in several national blood services. Post-transfusion hepatitis B is not necessarily transfusion-transmitted and iatrogenic sources of infection should be systematically investigated before concluding that HBV-infected blood donors are involved in viral transmission (O'Flaherty *et al.*, 2018; Vermeulen *et al.*, 2012).

2.6.2. Transfusion transmission of HTLV

Human T-cell leukemia viruses (HTLV-1 and HTLV-2) are associated with a variety of human diseases. Transfusion transmission of HTLV through cellular blood components is undeniable (Murphy, 2016). Transfusion-transmitted HTLV is associated with the accelerated onset of HTLV associated myelopathy (HAM), a debilitating spinal cord condition and with case reports of adult T-cell leukemia/lymphoma (ATL) (Murphy, 2016).

Antibody screening for HTLVs was introduced in many countries and remains in place today, although this is not a requirement from the World Health Organisation (WHO) (Murphy, 2016). In addition to its primary purpose of preventing transfusion transmission, such screening also provides a public health resource in allowing estimation of the population prevalence of HTLV infection (Murphy, 2016). Paradoxically, most countries performing HTLV screening of blood donors have very low prevalence and incidence, while certain countries in Africa with probable high HTLV prevalence in their donors do not currently perform antibody screening due to costs and the low incidence among HTLV carriers in progressing to ATL or HAM/TSP (Martin *et al.*, 2016; Murphy, 2016; Vermeulen *et al.*, 2019c).

An HTLV prevalence study was done by the SANBS in 2013 on a total of 46,716 blood donors sampled and the overall number of confirmed infections and estimated prevalence in SANBS blood donors was 624 units (0.062%) (Vermeulen *et al.*, 2019b). HTLV-1/2 screening is not recommended by the WHO or by regulatory standards in South Africa; however, a number of countries perform routine screening on all blood donations (Vermeulen *et al.*, 2019b). In the USA screening of donors started in 1988 and recently about 0.02% of first-time donors were found to be infected (Vermeulen *et al.*, 2019b). About 75% of these were HTLV. In 1991 testing of blood donors became mandatory in France followed by the Netherlands, Denmark, Sweden and Finland (Vermeulen *et al.*, 2019b).

Other countries in Europe have tested parts of their blood donor population such as performing selected screening on new donors only (Vermeulen *et al.*, 2019b). Eleven countries in Europe implemented HTLV-1/2 screening of some kind and Norway and Finland have stopped testing either due to low prevalence, the introduction of leucoreduction or pathogen inactivation (Vermeulen *et al.*, 2019b). None of the countries in Africa perform routine screening of HTLV (Vermeulen *et al.*, 2019b).

2.6.3. Transfusion transmission of HIV

According to Vermeulen *et al.* (2019a), it was estimated that a significant rate (34 per million) of infectious window period donations could be entering the blood supply in 1998, a projection supported by confirmed reports of transfusion-transmitted (TT) HIV infections each year. A structured risk management program, which included stringent education, product triage, and donor race-ethnicity as risk markers, was implemented in 1999, and these measures were successful at reducing the estimated residual risk of TT HIV infection from 34 per million to 26 per million red blood cell (RBC) transfusions (Vermeulen *et al.*, 2019a).

The introduction of ID-NAT in 2005 in the SANBS has significantly enhanced the safety of the blood supply, but the careful recruitment and selection of low-risk donors remain crucial to the prevention of transfusion-transmitted infections (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009). NAT testing has further reduced the HIV window period to about 6 - 11 days (Ledwaba *et al.*, 2008). The window period was responsible for HIV

transfusion-related HIV infections between 2001 and 2005. Since the introduction of NAT testing in October 2005, there has been one HIV transmission by blood transfusion reported to the haemovigilance program (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009). The Transfusion Transmissible Infection (TTI) Look Back Programme was established in 1986 within the SANBS and have been incorporated into the Haemovigilance Programme since 2005 (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009).

Blood Transfusion Services in South Africa screen all blood donations for HIV, hepatitis C and hepatitis B by both serological tests and by individual donor nucleic acid amplification testing (ID-NAT) (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009). The Look Back Programme aims to trace all patients who are identified as recipients of blood from donors who test positive for a transfusion-transmissible infection on a subsequent donation, where the previous negative unit may possibly have been donated in a window period (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009).

In a donor-triggered look back investigation the recipient/s of the previous negative units are identified and their treating doctor notified (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009). As far as possible, the patient is recalled, counselled and tested for the relevant viral marker and the result reported to the Blood Service (Vermeulen *et al.*, 2019a; Vermeulen *et al.*, 2009). Between 2010 and 2015, 2887 lookback investigations (of 5.8 million HIV-negative transfusions) were initiated based on lapsed or repeat donors who seroconverted to HIV antibody positivity (Vermeulen *et al.*, 2019a).

Of these, 1166 cases (40%) remained unresolved (i.e., no recipient outcome data were provided by hospitals), 396 (14%) patients died after transfusion, 262 (9%) patients tested HIV positive prior to the transfusion, 236 (8%) patients tested HIV negative following investigation, 23 (0.85%) declined further testing, and 15 (0.5%) patients tested HIV positive following transfusion without documentation of their infection status prior to the implicated transfusion (Vermeulen *et al.*, 2019a). Of these 15 HIV-positive patients identified as possible TT cases, eight showed no genetic linkage between the donor and recipient viruses, two were unlikely transfusion related due to a very short time interval to HIV positive results following transfusion (<6 days), three were unresolved but were unlikely to be TT cases (one was already on ART 3

months after the transfusion and could not be amplified, and two never returned for testing), and one case was a confirmed HIV transmission following transfusion of a WP RBC unit (Vermeulen *et al.*, 2019a).

2.7. HIV, HBV AND HTLV METHODS OF DETECTION

The laboratory test methods are the same for HTLV, HBV and HIV (Amini *et al.*, 2017). Most blood transfusion services have a first-line serological test which is one of the immunoassay principles and nucleic-acid amplification tests (NAT) as the second line test (Amini *et al.*, 2017). The human body produces antibodies to HTLV, HBV and HIV which can be tested for using enzyme immunoassays (EIA), electrochemiluminescence immunoassays (ECLIA), microparticle enzyme immunoassays (MEIA) and chemiluminescent microparticle immunoassays (CMIA) (Amini *et al.*, 2017). HBsAg can also be detected in this manner.

2.7.1. IMMUNOASSAY

2.7.1.1. Principle

- Radioimmunoassay

Radioimmunoassay (RIA) is the first generation of serological techniques and is a suitable tool to detect antibodies towards HIV, HTLV and HBV (Heiat *et al.*, 2014). In RIA a cold antigen (a non-radioactive one) competes with a hot antigen (an antigen conjugated with radioactive materials) for binding to antibodies (Heiat *et al.*, 2014). Therefore, target concentration (non-conjugated target) and radiation have an inverse relation. Nowadays RIA is an extensively used technique to diagnose all types of HIV, HBV and HTLV in a commercial kit format (Heiat *et al.*, 2014).

- Immunochemiluminescent Assay

According to Heiat *et al.* (2014) the Chemiluminogenic reagent was used for the first time in 1976 as a non-isotopic label in an immunoassay system, the chemiluminogenic

label were categorised not a consumed and non-consumed labels which was both employed for detection of HBV, HTLV and HIV. The oxidation of chemiluminogenic reagents is associated with a measurable light emission which resulted in the immunochemiluminescent assay showing an equal and even higher level of sensitivity in comparison with RIA (Heiat *et al.*, 2014). Comparative studies on enzyme immunoassay (EIA) and the Immunochemiluminescent assay have confirmed that the Immunochemiluminescent assay has equivalent sensitivity but relatively higher specificity, predictive value and fewer false-positive results in HBV, HTLV and HIV diagnosis procedures (Heiat *et al.*, 2014). The high stability after conjugation, low consumption of reagents, high sensitivity and high safety are advantages of the Immunochemiluminescent assay (Heiat *et al.*, 2014).

- Lateral Flow Immunoassay

Lateral flow immunoassay (LFIA or LFA), due to its excellent advantages such as high sensitivity and specificity, ease of interpretation, simplicity of use and design and not requiring special instruments, has obtained extensive acceptance in clinical laboratories and is considered as a popular detection test (Heiat *et al.*, 2014). LFA works as a rapid test on the basis of fluid capillary movement through the sandwich (antigen or antibody sandwich) and commutative formats (Heiat *et al.*, 2014). LFA strip structure is composed of different overlapped layers include sample pad, conjugate pad, analytical membrane and absorbance pad (Heiat *et al.*, 2014). The liquid sample migrates through the strip from the sample pad to the absorbance pad (Heiat *et al.*, 2014). Based on LFA format the appearing and disappearing of test lines determine the presence or absence of a target in the sample (Heiat *et al.*, 2014).

2.7.1.2. Advantages

A wide variety of immunoassay technologies are commercially available, many of which rely on the use of enzyme-, radioisotopc-, or fluorescent-labelled species for detection purposes (Lynch & Duval, 2010). Some of these tests are highly automated, allowing high sample throughput on the order of several hundred tests per day, whereas others are more labour intensive and are less readily automated (Lynch &

Duval, 2010). Many of the highly automated assays are homogeneous in nature and can be performed in one step without the need for separation (Lynch & Duval, 2010).

Immunoassays that require a separation step to remove unbound from the bound drug are called heterogeneous assays (Lynch & Duval, 2010). These tests usually take longer to complete, are not readily automated, and are more technically demanding (Lynch & Duval, 2010). However, because of the separation step that takes place before detection, heterogeneous immunoassays may be less susceptible to endogenous and exogenous interferences than their homogeneous counterparts (Lynch & Duval, 2010). Heterogeneous assays are also less susceptible to matrix effects and are more amenable to blood and alternative fluids, often without sample pre-treatment (Lynch & Duval, 2010).

2.7.1.3. Disadvantages

Due to the nature of the antibody-antigen reaction, immunoassays offer limited specificity (Lynch & Duval, 2010). When the antibody recognises a structural conformation or epitope on the molecule, it may bind producing a positive result (Lynch & Duval, 2010). Immunoassays are very rarely truly specific to the target molecules because structurally similar molecules may also bind to the antibody to varying degrees (Lynch & Duval, 2010). However, the degree of specificity may not necessarily limit the usefulness of the test as some immunoassays are designed to be nonspecific in order to cross-react with several molecules within a given class (Lynch & Duval, 2010).

2.7.2. NUCLEIC-ACID AMPLIFICATION TEST (NAT)

The Nucleic Acid Amplification Testing (NAT) is a highly sensitive method of testing blood that is used to detect most viruses in the blood (Lorincz, 2016). A nucleic acid amplification test is a technique utilised to detect a particular nucleic acid of the virus which acts as a pathogen in a sample (Lorincz, 2016).

NAT detects genetic materials rather than antigens or antibodies and thus reduces the window period by detecting low levels of viral genetic materials that are present soon

after infection but before the body has had a chance to start producing antibodies (Lorincz, 2016). Since the amount of a certain genetic material is usually very small, NAT includes an amplification step of the genetic material (Lorincz, 2016). There are several ways of amplification including polymerase chain reaction (PCR), strand displacement assay (SDA), or transcription-mediated assay (TMA) (Lorincz, 2016).

Virtually all nucleic acid amplification methods and detection technologies utilise the specificity of Watson-Crick base pairing; single-stranded probe or primer molecules capture DNA or RNA target molecules of complementary strands and therefore, the design of probe strands is highly significant to raise the sensitivity and specificity of the detection (Lorincz, 2016).

2.7.2.1. Principle

Transcription-based amplification methods are modelled after the replication of retroviruses (Rifai *et al.*, 2018). These methods are known by various names, including transcription-mediated amplification NA sequence—based amplification and self-sustained sequence replication (3SR) assays (Rifai *et al.*, 2018). They amplify their target without temperature cycling (isothermally) and use the collective activities of reverse transcriptase, RNase H, and RNA polymerase (Rifai *et al.*, 2018).

The most widely used is TMA (Rifai *et al.*, 2018). Two primers, a reverse transcriptase, and an RNA polymerase are used (Rifai *et al.*, 2018). The primer complementary to the RNA target has a 5'-tail that includes a promoter sequence for RNA polymerase (Rifai *et al.*, 2018). This primer anneals to the target RNA and is extended by the reverse transcriptase, creating an RNA—DNA duplex. The RNA strand is degraded by the RNAse H activity of the reverse transcriptase, allowing the second primer to anneal (Rifai *et al.*, 2018). The reverse transcriptase then extends the second primer to create double-stranded DNA (dsDNA) that includes the promoter (Rifai *et al.*, 2018). RNA polymerase recognises the promoter and initiates transcription, producing 100 to 1000 copies of RNA for each DNA template (Rifai *et al.*, 2018).

Each strand of RNA then binds and extends the second primer, forming an RNA—DNA hybrid; the RNA in the hybrid is degraded, the promoter primer binds and extends

to produce dsDNA that can be transcribed, and the cycle repeats (Rifai *et al.*, 2018). As in PCR, all reagents are included, and amplification is exponential with completion in less than 1 hour (Rifai *et al.*, 2018). Unlike PCR, these methods do not require temperature cycling (except for an initial heat denaturation if a DNA template is used) (Rifai *et al.*, 2018). They are particularly advantageous when the target is RNA (e.g. human immunodeficiency virus (HIVI and hepatitis C virus IHCVI in blood bank NA testing) (Rifai *et al.*, 2018).

2.7.2.2. Advantages

Nucleic acid amplification testing shortens this window period of HIV, HTLV and HBV (Chigurupati & Murthy, 2015). NAT technologies have the potential to detect viremia earlier than current screening methods, which are based on seroconversion (Chigurupati & Murthy, 2015). This offering blood centres and diagnostic laboratories a much higher sensitivity for detecting viral infections and is accomplished by direct detection of viral DNA or RNA (Chigurupati & Murthy, 2015).

2.7.2.3. Disadvantages

False-positive results may be caused by the fact that DNA (and RNA) amplification is highly sensitive and thus prone to contamination (Vaneechoutte & Van Eldere, 1997). The most likely sources of contamination are other samples and products from previous amplifications (Vaneechoutte & Van Eldere, 1997). Processing of negative samples along with each extraction batch can control for contamination by sample carryover, and a convenient solution to sample carryover during extraction procedures might be the application of automated nucleic acid extraction techniques (Vaneechoutte & Van Eldere, 1997). The high cost associated with NAT is also a major disadvantage for most blood transfusion services and diagnostic laboratories (Vaneechoutte & Van Eldere, 1997).

2.8. CONCLUSION

The chapter looked into the literature of HIV, HBV and HTLV. These viruses have the same mode of infection and the body produces antibodies to each of these viruses.

The diagnostic test is done on the same principles, namely, serological testing using immunoassays and biological testing using nuclei acid amplification testing.

CHAPTER 3

RESEARCH DECISION & METHODOLOGY

3.1. INTRODUCTION

The World Health Organisation recommended a retrospective double-blinded experimental study design for the screening of donated blood for transfusion transmissible infections in Africa (WHO, 2010). This recommendation also covers the surveillance of transfusion transmissible infections within the general population which is necessary to improve the quality of the blood transfusion service for the benefit of the community (WHO, 2010). This study followed the recommendations of the WHO to collect 7015 samples from all blood transfusion laboratories across South Africa excluding the Western Cape Blood Service transfusion laboratories. The specimens collected were tested using the ABBOTT Alinity S® Immunochemiluminescent auto analyser.

3.2. ETHICAL APPROVAL

The study was carried out at the South African National Blood Services (SANBS) and included all the regions in which the SANBS operates. This study was funded by the SANBS (Annexure A). This study was ethically approved by the human ethics research committee of the SANBS and the Vaal University of Technology (VUT) (2017/13 Annexure B).

The specimen of this study was stored frozen for two months before testing commenced, making this study a retrospective experimental study and the recipient's custodians of the study. This study was also double-blinded and all identification of recipients delinked from the samples once the demographic data were collected from the samples for this study based on the recommendations of the WHO (2010) and the human research ethics committee of the SANBS and VUT.

3.3. STUDY PERIOD

The sample collection took place over a year period and each zone was sampled separately. The samples were stored at the donation testing laboratory for two-months after all samples was collected before testing started. Testing was done over a two-month period and all reactive samples was confirmed using the Roche Cobas E801 for HIV and HBV. The Roche Cobas E401 was used for HTLV. The study was completed over a three-year period from 2017 to 2019.

3.4. SAMPLE CHARACTERISTICS

Patient specimen collected for compatibility testing were separated and frozen. The number of samples was matched to the number and proportion of patient samples received per blood bank nationally to enable the estimation of a national prevalence.

3.4.1. Sample size calculations and Power

The sample size of this study was estimated using an equation described by Arya *et al.* (2012).

$$n = \frac{N (Z^2) P (1-P)}{(d^2) (N-1) + (Z^2) P (1-P)}$$

n is the sample size, N is the population size (Total number of samples received at the SANBS for one month), Z is the statistic for the level of confidence at 95% and value is 1.96, since 95 % of a normal distribution would lie within ±1.96 standard deviations on either side of the mean, P is the expected proportion or prevalence of disease and d is the allowable margin of error that is set at 5%. A common recommendation is to set d = P/2.

According to Hlela *et al.* (2009), the prevalence of HTLV in Africa suggests that P will be 6.6% for HTLV. According to the prevalence of HIV in Africa, P will be 17.8%

(Lewis, 2011) and based on the HBV prevalence in Africa, P will be 8% (Franco *et al.*, 2012).

$$n \text{ for HTLV} = \frac{58862\ (1.96^2)\ 0.066\ (1-0.066)}{(0.033^2)\ (58862-1) + (1.96^2)\ 0.066\ (1-0.066)} = 217 \text{ specimens}$$

$$n \text{ for HIV} = \frac{58862\ (1.96^2)\ 0.178\ (1-0.178)}{(0.089^2)\ (58862-1) + (1.96^2)\ 0.178\ (1-0.178)} = 71 \text{ specimens}$$

$$n \text{ for HBV} = \frac{58862\ (1.96^2)\ 0.088\ (1-0.088)}{(0.044^2)\ (58862-1) + (1.96^2)\ 0.088\ (1-0.088)} = 159 \text{ specimens}$$

A minimum sample size of 217 is therefore required for this study**.** The SANBS took a management decision to sample at least 1000 samples per zone and matched to the number and proportion of patient samples received per blood bank nationally to enable the estimation of a national prevalence.

3.4.2. Sampling strategies

The sampling in this study took the form of stratified random sampling, the researcher attempts to stratify the population in such a way that the population within a stratum is homogeneous with respect to the characteristic on the basis of which it is being stratified (Daniel, 2011:133-136). In proportionate stratified sampling, the number of elements selected in the sample from each stratum is in relation to its proportion in the total population (Daniel, 2011:133-136).

The SANBS has 85 blood transfusion laboratories in 8 provinces, namely, Eastern Cape, Free State, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, North West and

Northern Cape. These provinces have been divided into 7 zones of operations in the SANBS, namely, Eastern Cape, Egoli, KwaZulu Natal, Mpumalanga, Northern, Vaal, Free-state and Northern Cape. The specimen was proportionally stratified between the blood transfusion laboratories of each zone based on their percentage of patient specimens received in each of the respective zones as per Table 1.

***Table 1*: SANBS zones and specimen distribution.**

Zone:	Private Hospitals (33%)	Government Hospitals (67%)	Total samples to be collected
Eastern Cape (8.35%)	193	391	591
Egoli (23.17%)	535	1087	1625
Free state / Northern Cape (5.98%)	137	284	421
KwaZulu Natal (18.01%)	418	844	1262
Mpumalanga (6.19%)	144	278	422
Northern (23.88%)	550	1128	1678
Vaal (14.45%)	339	677	1016
Total	2322	4693	7015

As shown in table 1 the total number of samples was divided between the blood testing laboratories based on the percentage of samples received within each laboratory. The Eastern cape received 8.35% of the total samples within the SANBS and based on this percentage the Eastern Cape will collect 591 samples which will be divide per hospital class 33% (n = 193) from private hospital patients and 67% (n = 391) from provincial hospital patients. The Egoli zone receives 23.17% of the total number of samples within the SANBS and will collect 1652 samples which will be divided between private hospital patients (33%, n = 535) and provincial hospital patients (67%, n = 1087). The Free State or Northern zone receives 5.98% of samples within the SANBS and based on this percentage will need to collect 421 samples, 137 (33%) from private hospital patients and 284 (67%) from provincial hospital patients. KwaZulu Natal receives 18.01% of samples within the SANBS and will have to collect 1262

samples, 418 (33%) samples from private hospital patients and 844 (67%) from provincial hospital patients. Mpumalanga receives 6.19% of the samples within the SANBS and will have to collect a total of 422 samples, 144 (33%) from private hospital patients and 278 (67%) from provincial hospital patients. The Northern zone receives 23.88% of the samples within the SANBS and will have to collect 1678 samples, 550 (33%) samples from private hospital patients and 1128 (67%) samples from provincial hospital patients and the Vaal zone receives 14.45% of the samples within the SANBS and will have to collect 1016 samples, 339 (33%) samples from private hospital patients and 677 (67%) samples from provincial hospital patients.

Specimens received from hospitals were processed according to standard operating procedures for pre-transfusion testing. This required samples to be stored for 5 days at 2 to 6˚C in the blood bank fridge for possible referrals. The samples were then placed into a specimen transport hamper and sent to the SANBS donation testing laboratory. A study management system (SMS) was designed by the SANBS IT department and was used to capture patients' demographic information (race, gender, age, blood group, diagnosis and previous transfusion history).

The plasma was separated from the red cells and stored frozen below -18˚C until analysed. After ensuring that all the demographic information was captured on each sample, the samples were double-blinded (the SANBS laboratory number was removed from the Excel spreadsheet and the SMS) and tested in the laboratory. A total of 7015 samples was tested outside of normal operating hours of the donation testing laboratory. Virology test results were uploaded into the SMS and copied onto an Excel spreadsheet.

3.4.3. Inclusion and exclusion criteria

Inclusion criteria:

- Patients receiving blood and blood products from all the SANBS Blood Banks
- 7000 Specimens will be collected randomly from each of the 7 zones based on the percentages of specimens received from the hospitals to give a true reflection of total received specimens within the SANBS.

Exclusion criteria:

All samples that have insufficient volumes for testing and samples that cannot be tested because of sample quality; for example, haemolysed samples.

3.5. STUDY DESIGN

The design of the study is a double-blinded, retrospective experimental study on a convenience selected sample, based on the recommendations by the World Health Organisation on screening donated blood for transfusion transmissible infection in Africa (WHO, 2010) and was ethically approved by human ethics research committee of the SANBS and the Vaal University of Technology (VUT) (2017/13 Annexure B).

3.6. BLOOD TEST PARAMETERS

3.6.1. Sample preparation and limitations

3.6.1.1. Sample preparation

Samples were stored frozen (-18˚C and below) for two months as per sampling strategy. Samples were thawed before testing which resulted in fibrin clots and particulate matter due to sample quality. Samples were centrifuged at 3000 rpm for 5 min as per packet insert recommendations for the ABBOTT Alinity S instrument and fibrin clots and particulate matter was removed with a sterile applicator stick as per packet insert recommendation for the Alinity S instrument (Alinity S Operation Manual, 2016).

3.6.2. Measuring instruments

3.6.2.1. ABBOTT's Alinity S®

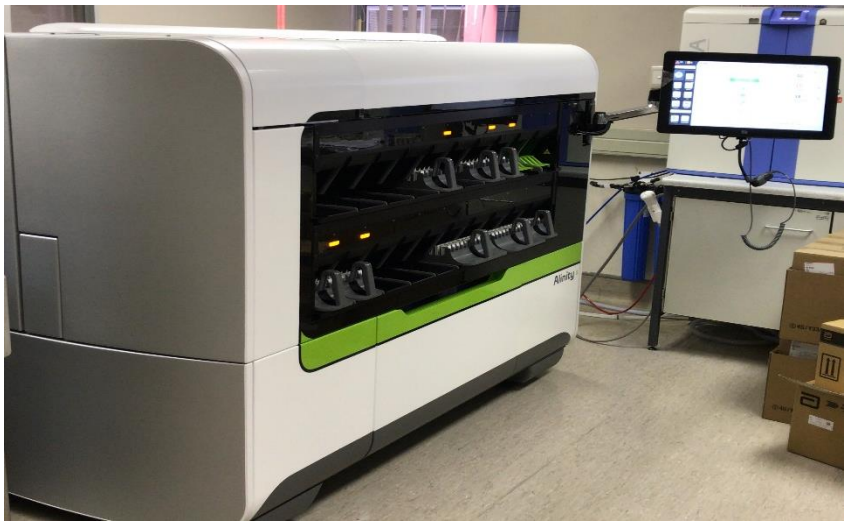

***Figure 1*: ABBOTT Alinity S® Immunochemiluminescent auto-analyser**

The ABBOTT Alinity S® system is the only instrument available in Africa and was housed in the evaluation and validation laboratory of the SANBS. ABBOTT sponsored all the reagents and consumables for this study with no conditions except for the publication which will be generated out of this study as a contribution to scientific academic growth within South Africa. The advantages and disadvantages of Immunochemiluminescent assays were discussed in chapter 2.7.1.2. and 2.7.1.3. This method improves the chances for the detection of the 3 viruses in patients currently on antiretroviral treatment as the treatment prevents the virus RNA to replicate but does not prevent the body from making antibodies to these viruses which can be detected with this method compared to Nucleic acid amplification testing (NAT) (WHO, 2016).

3.6.2.1.1. Test method, principal and machine processes

❖ Method and Principal

The test method and principal were discussed in chapter 2.7.1.1. under Immunochemiluminescent Assay.

❖ Machine Processes

1. The sample and the paramagnetic microparticles coated with capture molecules are dispensed into the reaction vessel (RV). The vortex mixes the reaction mixture (Alinity S Operation Manual, 2016).

   - This assay is a two-step immunoassay for the qualitative detection of HIV p24 antigen, antibodies to HIV-1 (group M and group O), and antibodies to HIV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample, HIV-1/HIV-2 antigen and HIV p24 antibody (mouse IgG, monoclonal) coated paramagnetic microparticles, and assay diluent is combined.

   - This assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using the chemiluminescent microparticle immunoassay (CMIA) technology. Sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labelled conjugate is combined to create a reaction mixture and incubated at 31.6°C to 35.6°C. The HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium labelled conjugate.

   - This assay is a two-step immunoassay for the qualitative detection of antibodies to HTLV I and HTLV II in human serum and plasma using the chemiluminescent microparticle immunoassay (CMIA) technology. Sample, HTLV I/HTLV II coated paramagnetic microparticles, and assay diluent is combined and incubated. The antibodies to HTLV I/HTLV II present in the sample bind to the HTLV I/HTLV II synthetic peptides and HTLV II recombinant antigen-coated microparticles.

2. During incubation of the reaction mixture, the analyte in the sample binds to the capture molecules on the paramagnetic microparticles and forms an immune complex (Alinity S Operation Manual, 2016).

3. A magnet attracts the paramagnetic microparticles (which are bound to the specific analyte) to a wall of the RV. The wash zone assembly washes the reaction mixture

to remove unbound materials. Additional assay processing can now occur (Alinity S Operation Manual, 2016).

4. The pipettor dispenses a chemiluminescent, acridinium-labelled conjugate into the RV. The conjugate binds to the immune complex to complete the reaction mixture. The vortex mixes the reaction mixture. The reaction mixture incubates (Alinity S Operation Manual, 2016).

5. The wash zone assembly washes the reaction mixture to remove unbound materials (Alinity S Operation Manual, 2016).

6. The Pre-Trigger Solution nozzle dispenses the Pre-Trigger Solution (hydrogen peroxide) into the reaction mixture. The vortexer mixes the reaction mixture (Alinity S Operation Manual, 2016).

   - The Pre-Trigger Solution:
     - Creates an acidic environment to prevent the early release of energy (light emission).
     - Helps to prevent any clumping in microparticles.
     - Separates the acridinium dye from the conjugate that is bound to the microparticle complex. This action prepares the acridinium dye for the next step.
     - The CMIA optical system performs a background read.

7. The Trigger Solution nozzle dispenses the Trigger Solution (sodium hydroxide) into the reaction mixture. The Trigger Solution creates an alkaline environment that, with the exposure to peroxide in the Pre-Trigger Solution, causes the acridinium dye to undergo an oxidative reaction. The oxidative reaction causes a chemiluminescent reaction to occur. N-methylacridone forms and releases energy (light emission) as N-methylacridone returns to its ground state (Alinity S Operation Manual, 2016).

   The CMIA optical system measures the chemiluminescent emission (activated read) over a predefined time period to determine a result (Alinity S Operation Manual, 2016).

3.6.2.1.2. Results validation

The ABBOTT Alinity S® Immunochemiluminescent auto analyser automatically tests all the samples found to be inconclusive or reactive two times to confirm the results obtained in the first stage of testing. All samples which are inconclusive or possible reactive and unable to confirm after the third test was marked on the exception and results page for further investigation by the machine operator (Alinity S Operation Manual, 2016).

3.6.2.1.3. Sensitivity and specificity

According to a study done by Vokel *et al.* (2017), the Alinity s HBsAg, Anti-HCV and Syphilis assays, all samples tested initially non-reactive, and the specificity of these assays was determined to be 100%. The Alinity s HIV Ag/Ab Combo assay had one initially reactive sample that turned negative upon retesting in duplicate, for a specificity of 100% (Vokel *et al.*, 2017). Nine samples were found repeatedly reactive upon testing with Alinity s Anti-HBc, all of which were confirmed positive upon subsequent testing (Vokel *et al.*, 2017). For Alinity s HTLV I/II, two samples were initially and repeatedly reactive but negative by confirmatory testing, therefore reducing the specificity of this test to 99, 92% (Vokel *et al.*, 2017). Coleman *et al.* (2018) showed the same results in their conference presentation, showing that the specificity for HIV was 99.97% and for HBsAg was 99.90%. The sensitivity for these markers was 100% (Coleman *et al.*, 2018).

3.6.2.1.4. Reagents and consumables

Figure 2: Reagent Cartridges (Alinity S Operation Manual, 2016)

Reagent cartridges

Reagent cartridges have two or more reagent components for the Alinity s System. A maximum of 500 tests per cartridge can be run. Cartridges can be loaded and unloaded during sample processing (Alinity S Operation Manual, 2016).

HIV

This assay is a two-step immunoassay for the qualitative detection of HIV-1 p24 antigen, antibodies to HIV-1 (group M and group O), and antibodies to HIV-2 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology (Alinity S Operation Manual, 2016).

HBV

This assay is a one-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology (Alinity S Operation Manual, 2016).

HTLV

This assay is a two-step immunoassay for the qualitative detection of antibodies to HTLV I and HTLV II in human serum and plasma using the chemiluminescent microparticle immunoassay (CMIA) technology (Alinity S Operation Manual, 2016).

Figure 3: **Triger, Pre Triger and Wash buffer (Alinity S Operation Manual, 2016)**

Bulk solutions

Bulk solutions are liquid solutions that are provided in large quantities for use during assay processing. The Alinity S System uses three bulk solutions. Each bulk solution is loaded in the bulk solution drawer (Alinity S Operation Manual, 2016).

Pre-Trigger Solution (1 L bottle)

Included a hydrogen peroxide solution that separates the acridinium dye from the conjugate that is bound to the microparticle complex. This action prepares the acridinium dye for the addition of Trigger Solution (Alinity S Operation Manual, 2016). The Pre-Trigger Solution is sensitive to light, is stored at a temperature of 2°C to 8°C, and is stable in the system for 28 days. A new bottle can be loaded in the bulk solutions drawer without any interruption in system operation (Alinity S Operation Manual, 2016).

Trigger Solution (1 L bottle)

This is a sodium hydroxide solution that produces the chemiluminescent reaction that provides the final read (Alinity S Operation Manual, 2016). This solution is stored at a temperature of 2°C to 30°C and is stable on the system for a maximum of 28 days (Alinity S Operation Manual, 2016). A new bottle can be loaded in the bulk solutions drawer without any interruption in system operation (Alinity S Operation Manual, 2016).

Concentrated Wash Buffer (10 L container)

This solution contains a phosphate-buffered saline (Alinity S Operation Manual, 2016). This solution is diluted tenfold by the system and then is pumped to the sample and

reagent pipettor assemblies and to wash zones during assay processing (Alinity S Operation Manual, 2016). This solution is stored at a temperature of 15°C to 30°C and is stable on the system until the expiration date is reached. A new cubitainer can be loaded in the bulk solutions drawer without an interruption in system operation (Alinity S Operation Manual, 2016).

Figure 4: **Reaction Vessel (Alinity S Operation Manual, 2016)**

Reaction vessels

Reaction vessels (RVs) are disposable containers in which the CMIA reaction occurs. The operator can add RVs at any time (Alinity S Operation Manual, 2016).

3.6.2.1.5. Maintenance and procedures

Calibration procedures

Calibration is required when a new assay is installed, every 14 days after an active calibration curve is generated, and when a new lot number of a reagent kit is present (Alinity S Operation Manual, 2016). One replicate of the negative and positive assay controls must be run after the calibration and once every 24 hours when the instrument is being used (Alinity S Operation Manual, 2016). Control values must be within the acceptable ranges specified in the product documentation. If a control value is out of range the associated test results are invalid. Recalibration may be required when control values are out of range (Alinity S Operation Manual, 2016).

The Alinity S System uses the cutoff assay data reduction method, which measures the relative light unit (RLU) values and calculates a cutoff value (Alinity S Operation Manual, 2016). This method uses a one- or two-point calibration to calculate the point

(cutoff) value where reactive and nonreactive samples are differentiated (Alinity S Operation Manual, 2016). To calculate the sample-to-cutoff ratio, the sample RLU value is divided by the cutoff value (Alinity S Operation Manual, 2016).

The system software uses the following calibration methods:

One-point qualitative (index formula) method

This method uses one calibrator in the index formula. The following example is a one-point cutoff formula where *a* and *b* are assay-specific constants:

Cutoff = *a* x Calibrator RLU + *b*

Two-point qualitative (index formula) method

This method uses two calibrators in the index formula. The following example is a two-point cutoff formula where *a* and *b* are assay-specific constants:

Cutoff = *a* x Calibrator 1 RLU + Calibrator 2 RLU – *b*

Reference method

This method uses calibration values generated from another assay (reference assay) to calculate results (Alinity S Operation Manual, 2016).

Daily Maintenance

The daily maintenance procedure is performed to clean the wash zone 1 probe and zone 2 probes (Alinity S Operation Manual, 2016). The sodium hypochlorite solution is diluted by the instrument and pipetted into the RVs. Wash zone 1 probe is cleaned by immersion in the RVs that contains the 0.5% sodium hypochlorite solution (Alinity S Operation Manual, 2016). Wash zone 1 and 2 probes are then washed with wash buffer (Alinity S Operation Manual, 2016).

Pipettor Probe Replacement

This is performed as an As-Needed maintenance procedure to replace a sample, R1, R2, or all pipettor probes (Alinity S Operation Manual, 2016). For the sample and R1 pipettor probe options, the upper loading area opens to access the pipettors. The sample pipettor moves to the sample pipettor access point, and the R1 pipettor moves to the front of the incubation track (Alinity S Operation Manual, 2016). For the R2

pipettor probe option, the operator can open the rear access doors to access the pipettor. The R2 pipettor moves to the process path conjugate dispense area (Alinity S Operation Manual, 2016).

Bulk Solutions Purge

This is performed as-needed maintenance procedure to purge the bulk solutions when it is necessary to change lots or empty the reservoirs (Alinity S Operation Manual, 2016). The operator can select to purge the Trigger Solution, the Pre-Trigger Solution, or the diluted wash buffer (Alinity S Operation Manual, 2016). The Trigger Solution and Pre-Trigger Solution are purged until the reservoirs are empty. The operator has the option to purge the diluted wash buffer until empty or to purge to a liquid level percent value (Alinity S Operation Manual, 2016).

Reagent Carousel Temperature Tool

This is performed as-needed in the maintenance procedure to remove and replace the reagent carousel temperature tool (Alinity S Operation Manual, 2016). The procedure also provides the ability to print a report of the temperature data measured by the reagent carousel temperature tool according to the laboratory operating procedures (Alinity S Operation Manual, 2016).

Incubation Temperature Test

This is performed as-needed in the maintenance procedure to measure temperatures in the incubation track, process path 1, and process path 2 with an independent temperature tool (Alinity S Operation Manual, 2016). This procedure reads and evaluates the Alinity s System incubation temperatures at the various temperature zones (Alinity S Operation Manual, 2016). The independent temperature tool consists of sensors, each incorporated into three RVs that travel through the incubation sequence (Alinity S Operation Manual, 2016). Each zone temperature is read, evaluated, and printed in a report. The time at each zone is composed of temperature stabilization and measurement (Alinity S Operation Manual, 2016).

3.6.2.1.6. Quality control

Figure 5: Assay Controls (Alinity S Operation Manual, 2016)

Assay controls

The system requires an Alinity S System assay control to validate the assay calibration. Assay controls that have multiple bottles are linked together. The number of bottles that are linked depends on the assay (Alinity S Operation Manual, 2016). Assay controls are run once a day and every time a reagents lot changes.

Figure 6: **Release Controls (Alinity S Operation Manual, 2016)**

Release controls

Release controls are controls that are run to release test results (Alinity S Operation Manual, 2016). No results will be released before the release controls are run.

Figure 7: **Calibrators (Alinity S Operation Manual, 2016)**

Calibrators

Calibrators are samples that are used to determine the assay cutoff (Alinity S Operation Manual, 2016). Calibrators that have multiple bottles are linked together. The number of bottles that are linked together depends on the assay (Alinity S Operation Manual, 2016). Calibrators are run every 14 days unless the reagent lot changes.

3.7. DEMOGRAPHIC INFORMATION

This study focused on the patient population of the South African national blood service, the socio-demographics, namely age, race, gender, blood group, hospital class (private or provincial) and zone representation are reported in this study as it included patients from all the provinces and social classes within the regions in which the South African National Blood Service operates.

3.7.1. Demographic data collection and analysis

All the specimens received in the blood transfusion laboratory for this study were processed according to the SANBS standard operating produces which requires the specimen demographics and information to be entered on to the Blood Establishments computer system (BECS). Meditech is the BECS used in the SANBS. All patient demographics have been recorded on to the BECS before the specimen was processed. The demographics information of all specimens was extracted from the BECS to an excel spreadsheet for use in chapter 4 of this study.

3.8. DATA ANALYSIS

Microsoft Excel was used to capture the data, after which the data was transferred to SPSS® version 25 software and SAS university edition. Descriptive statistical analyses were conducted using a crude chi-square test and a logistic regression test as all data were categorical data and these were the most appropriate tests to be used.

The bivariate chi-square (χ^2) statistic test can be used to analyse data measured on a nominal scale, such as gender, where there are two or more discrete categories (Ravid, 2014). It can also be used to analyse other types of numerical data (such as data measured on an interval scale) that are first divided into logically defined and generally agreed-upon categories (Ravid, 2014). In applying the chi-square test, two types of frequencies are used: observed and expected. The observed frequencies are based on actual (empirical) observations and or prevalence (Ravid, 2014).

The expected frequencies are theoretical or based on prior knowledge (academic theoretical knowledge) (Ravid, 2014). The observed and expected frequencies can be expressed as actual headcounts or as percentages. The chi-square test is used to decide whether there is a significant difference between the observed and expected frequencies, and both types of frequencies are used in the computation of the chi-square value (Ravid, 2014). The Crude Odds ratio (OR) is obtained by considering the effect of only one predictor variable.

A Logistic regression was performed taking all variables into consideration (confounder variables for the said relationship) to give the adjusted Odds Ratio, which considers the effect due to all the additional variables included in the analysis.

The Chi-square test was used in SPSS 25 as a bivariate test to predict the significant interaction between HIV, HBV, HTLV, concurrent infections and race, age, gender, blood group, zone and hospital class.

SAS university edition was used to run a logistic regression with a likelihood logit model. Logistic regression results were displayed as odds ratios or as probabilities.

HIV, HBV and HIV-HBV concurrent infection reactive samples were modelled where males were used as a reference and compared to females and unknown gender samples. The white race was used as a reference and compared to black, coloured, Asian or Indian and unknown race. Egoli zone was used as a reference and compared to Eastern Cape, Vaal, Free State or North Cape, Northern, Mpumalanga and KwaZulu Natal. The 31-40 age group was used as reference and compared to 0-10, 11-20, 21-30, 41-50, 51-60, 61-70, 71-80, 80+ and unknown age groups. The AB blood group was used as reference and compared to A, B, O and unknown blood groups. The private hospital patients were used as reference and compared to provincial hospital patients.

HTLV had no reactive samples in the white, coloured race group, and AB blood group. The 31-40 age group had a very low number of reactive samples. HTLV reactive samples were modelled and the black race group was used as a reference and compared to Asian or Indian and the unknown age group. The 41-50 years age group was used as a reference and compared to the other age groups. A blood group patient were used as a reference and compared to the other blood groups.

HBV-HTLV concurrent infection, HIV-HTLV concurrent infection and HIV-HBV-HTLV concurrent infection had very low numbers of reactive samples as seen in Tables 6, 7 and 8. HIV-HTLV concurrent infection reactive samples were modelled and the black race group was used as a reference and compared to Asian or Indian and the unknown age group. The 41-50 years age group was used as a reference and compared to the other age groups. AB blood group patients were used as a reference and compared to the other blood groups.

HBV-HTLV concurrent infection reactive samples were modelled and the black race group was used as a reference and compared to Asian or Indian and the unknown age group. 41-50 years age group was used as a reference and compared to the other age groups. AB blood group patients were used as a reference and compared to the other blood groups.

HBV-HTLV concurrent infection reactive samples were modelled and the black race group was used as a reference and compared to Asian or Indian and the unknown

age group. KwaZulu Natal was used as a reference and compared to the other zones. The 41-50 years age group was used as a reference and compared to the other age groups. The B blood group patients were used as a reference and compared to the other blood groups.

HIV-HBV-HTLV concurrent infection reactive samples were modelled and the black race group was used as a reference and compared to Asian or Indian and the unknown age group. KwaZulu Natal was used as a reference and compared to the other zones. The 41-50 years age group was used as a reference and compared to the other age groups. B blood group patients were used as a reference and compared to the other blood groups.

3.9. CONCLUSION

In this chapter, the ethical approval, sample strategies and sample preparation were discussed. All specimen was analysed and stored according to supplier recommendations and the SANBS standard operating procedures to ensure and adhere to good laboratory practices. The machine methods, principals, reagents, maintenance, processes and quality control were discussed. The study demographics information, consideration and collection procedures were also discussed in this chapter.

CHAPTER 4

RESULTS

4.1. INTRODUCTION

In this chapter, the results of the study are presented and discussed with reference to the aim of the study, which was to determine the prevalence of HIV, HBV and HTLV in the patient population of the SANBS. This chapter comprises the analysis, presentation and interpretation of the findings resulting from this study.

4.2. DEMOGRAPHICS

4.2.2.1. Age distribution of the sample

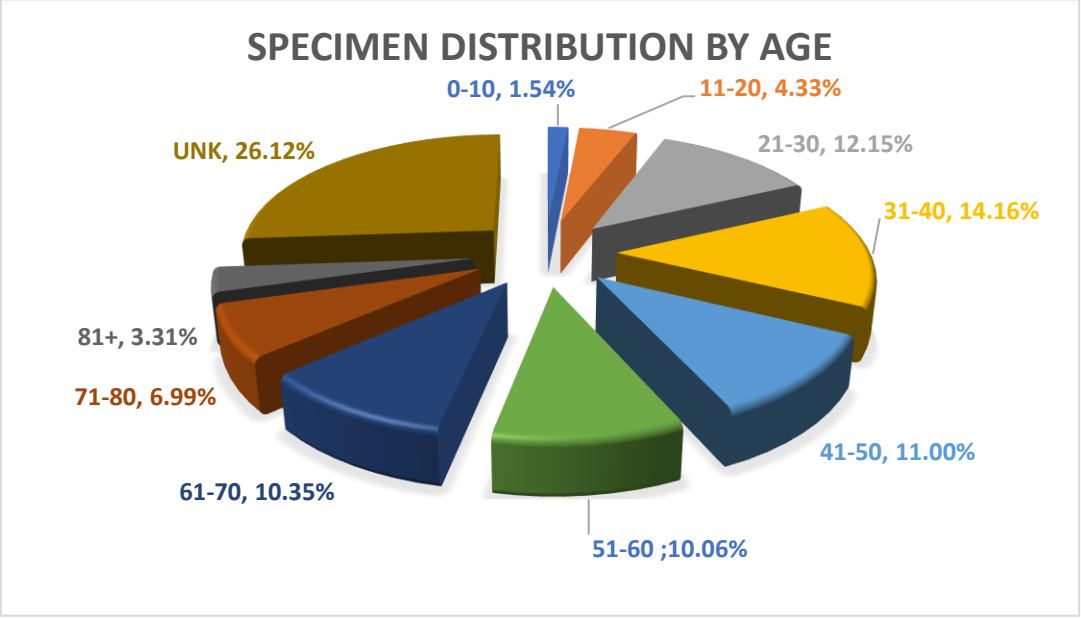

Figure 8: Specimen distribution by age

Presented in Figure 8, 1.5% (N = 108) was collected from age group 0 to 10 years, 4.3% (N = 304) was collected from 11 to 20 years old, 12.1% (N = 852) was collected from 21 to 30 years old, 14.2% (N = 993) was collected from 31 to 40 years old, 11% (N = 772) was collected from 41 to 50 years old, 10.1% (N = 706) was collected from 51 to 60 years old, 10.3% (N = 726) was collected from 61 to 70 years old, 7% (N =

490) was collected from 71 to 80 years old, 3.3% (N = 232) was collected from 81 upwards and 26.1% (N = 1832) was collected from patients with unknown age.

4.2.2.2. Race distribution of the sample

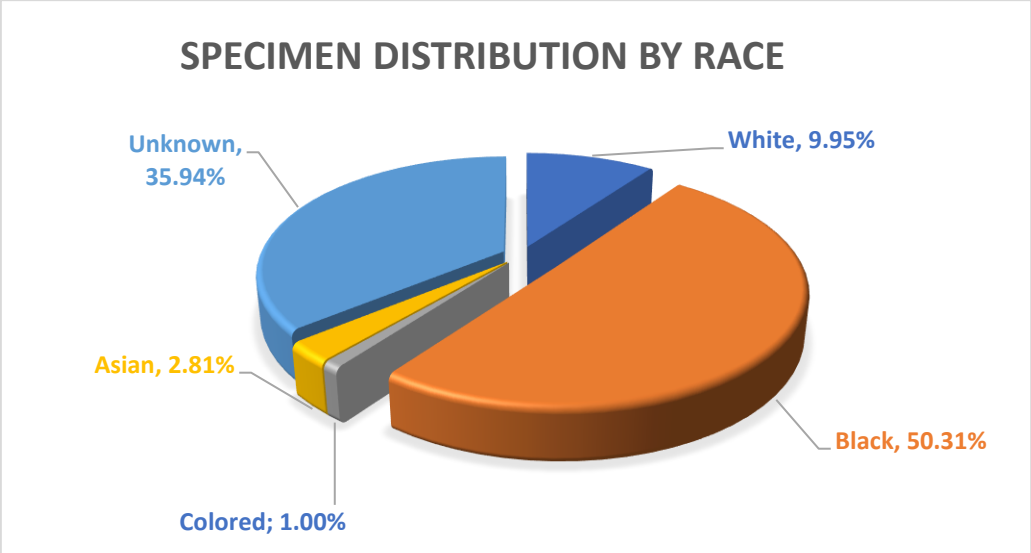

Figure 9: Specimen distribution by race

10% (N = 698) was collected from Caucasian's (White), 50.3 (N = 3529) was collected from Black, 1% (N = 70) was collected from Coloured, 2.8% (N = 197) was collected from Asians or Indians and 35.9% (N = 2521) of the specimens race was unknown (Figure 9).

4.2.2.3. Gender distribution of the sample

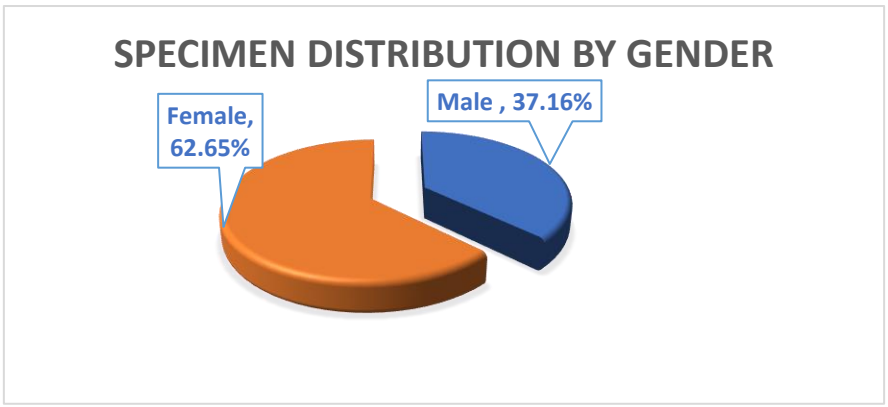

Figure 10: Specimen distribution by gender

37.2% (N = 2607) of the specimens was from Male patients, 62.7% (N = 4395) was collected from female patients and 0.2% of the sample's gender was unknown (Figure 10).

4.3. QUALITY ASSURANCE

4.3.1. HIV, HTLV and HBV reagent quality control

As outlined in chapter 3, assay controls were performed daily, and release controls were done before any results were released. Westgard rules were used to ensure the accuracy and reliability of results in this study and results are shown in Table 2 and Figure 11 (Halim, 2019). Table 2 and Figure 11 provided evidence that all assay and release controls were within a 1 standard deviation range and acceptable according to Westgard quality control rules.

Table 2: Standard deviation (SD) of quality control, assays control minimum and maximum ranges

Control Name	Assay Name	SD	Control Min Range	Control Max Range
Assay controls	HBsAg Neg	0,07	0.00	0.70
Assay controls	HBsAg Pos1	0,19	1,42	7,25
Assay controls	HIV Combo Neg	0,02	0	0,61
Assay controls	HIV Combo Pos1	0,23	1	7,05
Assay controls	HIV Combo Pos2	0,11	1,29	6,6
Assay controls	HIV Combo Pos3	0,11	1,29	6,6
Assay controls	HIV Combo Pos4	0,11	1,16	5,9
Assay controls	HTLV I/II Neg	0,05	0	0,71
Assay controls	HTLV I/II Pos1	0,08	1,18	6
Assay controls	HTLV I/II Pos2	0,11	1,35	6,89
Release controls	HBsAg-Rel Pos1	0,19	1,42	7,25
Release controls	HIV Combo-Rel Pos1	0,09	1,29	6,6
Release controls	HTLV I/II-Rel Pos1	0,17	1,18	6

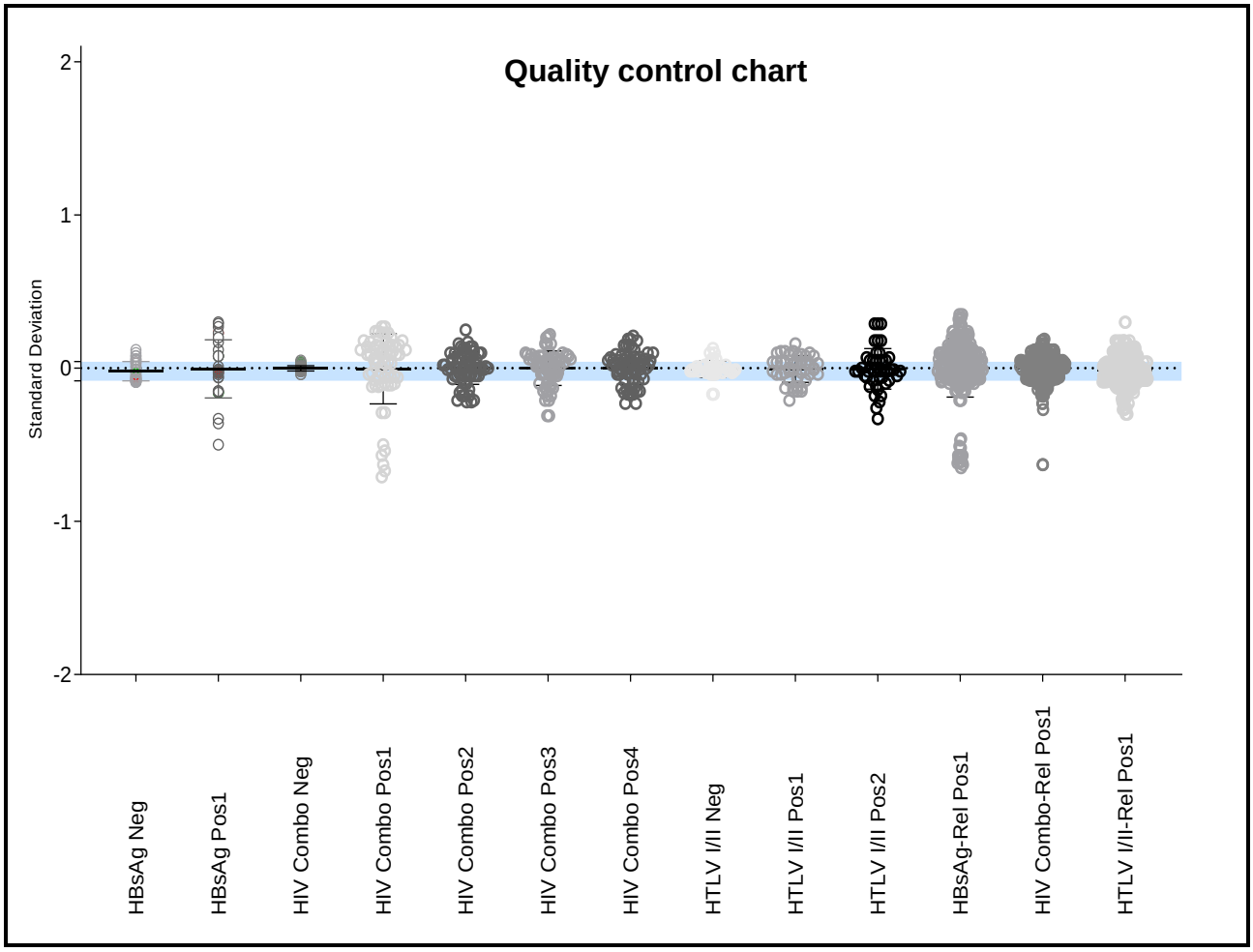

Figure 11: Quality control chart with the standard deviation of each control

4.4. RESULTS VALIDATION

As discussed under 3.6.2.1.2. results validation: 1882 (26,82%) of the samples were inconclusive or possible reactive and unable to confirm after the third test and was marked on the exception and results page for further investigations by the machine operator. The 1882 samples were re-run and the barcode and sample identification were rectified before the rerun. 82 (1.16%) of the samples was not tested of which 66 samples did not have enough sample volume and 16 samples barcodes was unreadable. Of these 1882 samples that was re-run on the Abbott Alinity S automated instrument, 1481 was HIV positive, 246 was HBV positive, 22 was HTLV positive, 82 samples were not tested, and 51 samples came up negative for all markers.

4.5. PREVALENCE OF HIV INFECTION

In the bivariate analysis presented in Table 3 females have a significantly higher HIV prevalence than males (42.75% VS 33.76%), (OR 1.466, 95% CI, 1.325-1.621, P<0.001). In the logistic regression model the significance remains with an adjusted OR (OR 1,196, 95% CI, 1,067-1,340).

The black ethnic group had the highest prevalence of HIV (49,11%, crude OR 11,062, 95% CI, 8,353-14.651, P<0,001, adjusted OR 5,430, 95% CI, 4,040-7,426) followed by the unknown ethnic group (36,61%, crude OR 6,622, 95% CI, 4,980-8,804, P<0,001, adjusted OR 3,863, 95% CI, 2,817-5,379), coloured (30%, crude OR 4,913, 95% CI, 2,752-8,772, P<0,001, adjusted OR 3,263, 95% CI, 1,715-6,101), Asian / Indian (15,23%, crude OR 2,059, 95% CI, 1,281-3,312, P=0,003, adjusted OR 1,292, 95% CI, 0,771-2,134) when compared to White reference group (8.02%)

All zones except Northern zone were associated with a higher HIV prevalence than Egoli Zone the reference zone. Even after adjusting the significance remained. The highest prevalence was in the Mpumalanga zone (53,50%, crude OR 2,014, 95% CI, 1,627-2,493, P<0,001, adjusted OR 1,829, 95% CI, 1,446-2,317) followed by Free State / Northern Cape (44,99%, crude OR 0,839, 95% CI, 0,670-1,050, P=0,139, adjusted OR 0,848, 95% CI, 0,653-1,098), KwaZulu Natal (44,90%, crude OR 1,426, 95% CI, 1,230-1,653, P<0,001, adjusted OR 1,556, 95% CI, 1,309-1,850), Eastern Cape (41,67%, crude OR 1,250, 95% CI, 1,024-1,527, P=0,032, adjusted OR 1,091, 95% CI, 0,877-1,355), Vaal (40,32%, crude OR 1,183, 95% CI, 1,005-1,392, P=0,048, adjusted OR 1,276, 95% CI, 1,063-1,533), Egoli (36,36%, and the lowest prevalence was in the Northern zone (35,48%, crude OR 0,962, 95% CI, 0,837-1,105, P=0,605, adjusted OR 1,030, 95% CI, 0,869-1,222).

Comparing age groups, the highest HIV prevalence was in the 31 to 40 years age group (60,73%), when using this age group as the reference the younger age groups and much older age groups had significantly lower prevalence. 21 to 30 years age group (45,66%, crude OR 0,543, 95% CI, 0,451-0,654, P<0,001, adjusted OR 0,503, 95% CI, 0,415-0,610), 11 to 20 years age group (25,66%, crude OR 0,223, 95% CI, 0,167-0,297, P<0,001, adjusted OR 0,202, 95% CI, 0,150-0,270), 0 to 10 years age

group (16,67%, crude OR 0,129, 95% CI, 0,077-0,218, P<0,001, adjusted OR 0,126, 95% CI, 0,072-0,210), 51 to 60 year age group (30,59%, crude OR 0,379, 95% CI, 0,311-0,463, P<0,001, adjusted OR 0,346, 95% CI, 0,279-0,429), 61 to 70 years age group (14,46%, crude OR 0,109, 95% CI, 0,086-0,139, P<0,001, adjusted OR 0,143, 95% CI, 0,111-0,183), and the lowest prevalence was in the 71 to 80 years and 81+ years age groups (7,76%, crude OR 0,054, 95% CI, 0,038-0,078 and 0,033-0,089, P<0,001, adjusted OR 0,090 and 0,091, 95% CI, 0,062-0,129 and 0,053-0,148). With only the 41 to 50 years age group (54,53%, crude OR 0,776, 95% CI, 0,641-0,939, P=0,010, adjusted OR 0,847, 95% CI, 0,695-1,033), having a similar prevalence.

There was no difference in prevalence amongst the blood groups when compared to blood group AB, with patients with an unknown blood group having the highest prevalence (46.43%, crude OR 1,279, 95% CI, 0,965-1,695, P=0,100, adjusted OR 1,312, 95% CI, 1,,040-1,655), followed by the B blood group patients (41,72%, crude OR 1,187, 95% CI, 0,913-1,540, P=0,224, adjusted OR 1,112, 95% CI, 0,956-1,293), O blood group patients (38,40%, crude OR 1,033, 95% CI, 0,807-1,323, P=0,843, adjusted OR 1,009, 95% CI, 0,956-1,293), AB blood group patients (37,63%,) and the lowest prevalence was in the A blood group patients (37,28%, crude OR 0,985, 95% CI, 0,765-1,269, P=0,960, adjusted OR 1,065, 95% CI, 0,932-1,215).

Patients receiving transfusions at provincial hospital were more likely to be HIV positive with a HIV prevalence (46,00%, crude OR 2,210, 95% CI, 1,990-2,454, P<0,001, adjusted OR 1,250, 95% CI, 1,096-1,425) compared to private hospital patients (27,82%,).

Table 3: Bivariate and logistic regression of demographic variables for HIV Infection

Exposure Variable:	Outcome Variable: HIV Infection								
Demographic Data	**Reactive N**	**Reactive %**	**Total N**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	2763	39,39	7015	-	-	-	-	-	-
Male	880	33,76	2607	ref	ref	ref	ref	ref	ref
Female	1879	42,75	4395	1,466	1,325-1,621	55,48	P<0,001	1,196	1,067-1,340
Unknown	4	30,77	13	0,872	0,268-2,840	0,052	P=0,946	0,544	0,142-1,763
Race	2763	39,39	7015	-	-	-	-	-	-
White	56	8,02	698	ref	ref	ref	ref	ref	ref
Black	1733	49,11	3529	11,062	8,353-14,651	402,95	P<0,001	5,430	4,040-7,426
Coloured	21	30,00	70	4,913	2,752-8,772	34,063	P<0,001	3,263	1,715-6,101
Asian / Indian	30	15,23	197	2,059	1,281-3,312	9,184	P=0,003	1,292	0,771-2,134
Unknown	923	36,61	2521	6,622	4,980-8,804	211,12	P<0,001	3,863	2,817-5,379
Zone / Region	2763	39,39	7015	-	-	-	-	-	-
Egoli	641	36,36	1763	ref	ref	ref	ref	ref	ref
Eastern Cape	215	41,67	516	1,250	1,024-1,527	4,796	P=0,032	1,091	0,877-1,355
Vaal	375	40,32	930	1,183	1,005-1,392	4,072	P=0,048	1,276	1,063-1,533
Free State / North Cape	139	44,99	429	0,839	0,670-1,050	2,358	P=0,139	0,848	0,653-1,098
Northern	601	35,48	1695	0,962	0,837-1,105	0,305	P=0,605	1,030	0,869-1,222
Mpumalanga	229	53,50	428	2,014	1,627-2,493	42,291	P<0,001	1,829	1,446-2,317
KwaZulu Natal	563	44,90	1254	1,426	1,230-1,653	22,274	P<0,001	1,556	1,309-1,850
Age	2763	39,39	7015	-	-	-	-	-	-
0-10	18	16,67	108	0,129	0,077-0,218	76,89	P<0,001	0,126	0,072-0,210
11-20	78	25,66	304	0,223	0,167-0,297	114,77	P<0,001	0,202	0,150-0,270
21-30	389	45,66	852	0,543	0.451-0.654	41,882	P<0,001	0,503	0,415-0,610
31-40	603	60,73	993	ref	ref	ref	ref	ref	ref
41-50	421	54,53	772	0,776	0.641-0.939	6,836	P=0.010	0,847	0,695-1,033
51-60	216	30,59	706	0,379	0.311-0.463	93,176	P<0,001	0,346	0,279-0,429
61-70	105	14,46	726	0,109	0.086-0.139	370,537	P<0,001	0,143	0,111-0,183
71-80	38	7,76	490	0,054	0.038-0.078	375,125	P<0,001	0,090	0,062-0,129
81+	18	7,76	232	0,054	0.033-0.089	211,08	P<0,001	0,091	0,053-0,148
Unknown	877	47,87	1832	0,594	0.508-0.695	42,656	P<0,001	0,522	0,436-0,624
Blood Group	2763	39,39	7015	-	-	-	-	-	-
A	705	37,28	1891	0,985	0.765-1.269	0,013	P=0.960	1,065	0,932-1,215
B	499	41,72	1196	1,187	0.913-1.54	1,642	P=0.224	1,112	0,956-1,293
O	1142	38,40	2974	1,033	0.807-1.323	0,068	P=0.843	1,009	0,766-1,326
AB	111	37,63	295	ref	ref	ref	ref	ref	ref
Unknown	306	46,43	659	1,279	0.965-1.695	2,941	P=0.100	1,312	1,040-1,655
Hospital Class	2763	39,39	7015	-	-	-	-	-	-
Private	710	27,82	2552	ref	ref	ref	ref	ref	ref
Provincial	2053	46,00	4463	2,210	1.990-2.454	224,751	P<0,001	1,250	1,096-1,425

4.6. PREVALENCE OF HBV INFECTION

In the bivariate analysis shown in table 4 females (6.85%) have a significantly lower HBV prevalence than males (8,78%, Crude OR 0,763, 95% CI, 0,638-0,913, P=0,003, Adjusted OR 0,687, 95% CI, 0,571-0,828).

Black ethnicity is associated with HBV prevalence when modelled in the bivariate analysis (8,25%, crude OR 1.870, 95% CI, 1.286-2.720, P=0,001) however once adjusted in the logistic regression the association goes away (adjusted OR 1,345, 95% CI, 0,900-2,066). The other race groups showed no significance when compared to white patients. unknown ethnic group (7,77%, crude OR 1,755, 95% CI, 1,196-2,575, P=0,004, adjusted OR 1,542, 95% CI, 0,998-2,440), coloured (7,14%, crude OR 1,601, 95% CI, 0,603-4,250, P=0,509, adjusted OR 1,462, 95% CI, 0,471-3,751), white (4,58%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) and the lowest prevalence was in the Asian / Indian ethnic group (3,55%, crude OR 0,767, 95% CI, 0,330-1,765, adjusted OR 0,541, 95% CI, 0,213-1,205).

The Mpumalanga zone (9,81%, crude OR 1,503, 95% CI, 1,040-2,174, P=0,037, adjusted OR 1,476, 95% CI, 1,004-2,134) and KwaZulu Natal (8,93%, crude OR 1,355, 95% CI, 1,035-1,773, P=0,031, adjusted OR 1,466, 95% CI, 1,103-1,949), were associated with increased HBV infection compared to Egoli zone (6,92%). The other zone showed no difference. Eastern Cape (8,53%, crude OR 1,288, 95% CI, 0,898-1,847, P=0,200, adjusted OR 1,277, 95% CI, 0,878-1,828), Vaal (7,31%, crude OR 1,090, 95% CI, 0,800-1,485, P=0,641, adjusted OR 1,111, 95% CI, 0,806-1,519), Northern (7,20%, crude OR 1,062, 95% CI, 0,817-1,381, P=0,701, adjusted OR 0,954, 95% CI, 0,710-1,282), and the lowest prevalence was in the Free State / North Cape zone (4,90%, crude OR 0,711, 95% CI, 0,442-1,145, P=0,139, adjusted OR 0,747, 95% CI, 0,442-1,207).

Comparing age groups, the highest HBV prevalence was in the 31 to 40 years age group (9,37%), followed by the unknown age group (9,12%, crude OR 0,971, 95% CI, 0,744-1,267, P=0,048, adjusted OR 0,925, 95% CI, 0,688-1,013), only the very old >50 and very young < 10 years were associated with significantly lower prevalence 51

to 60 years age group (6,52%, crude OR 0,674, 95% CI, 0,467-0,974, P=0,043, adjusted OR 0,666, 95% CI, 0,454-0,964), 61 to 70 years age group (6,06%, crude OR 0,624, 95% CI, 0,430-0,906, P=0,016, adjusted OR 0,641, 95% CI, 0,434-0,933), 81 and more years age group (4,74%, crude OR 0,482, 95% CI, 0,253-0,915, P=0,032, adjusted OR 0,544, 95% CI, 0,688-1,247), 71 to 80 years age group (3,88%, crude OR 0,390, 95% CI, 0,235-0647, P<0.001, adjusted OR 0,422, 95% CI, 0,243-0,698) and the lowest prevalence was in the 0 to 10 years age groups (3,70%, crude OR 0,372, 95% CI, 0,134-1,033, P=0,073, adjusted OR 0,341, 95% CI, 0,102-0,842). The other age groups showed no difference in association; 11 to 20 years age group (8,88%, crude OR 0,943, 95% CI, 0,602-1,478, P=0,887, adjusted OR 0,965, 95% CI, 0,604-1,496), 41 to 50 years age group (7,64%, crude OR 0,801, 95% CI, 0,570-1,126, P=0,232, adjusted OR 0,788, 95% CI, 0,557-1,107), 21 to 30 year age group (7,16%, crude OR 0,746, 95% CI, 0,533-1,045, P=0,104, adjusted OR 0,771, 95% CI, 0,547-1,081),

There was no difference in HBV prevalence amongst the blood groups when compared to the AB blood group patients (8,81%,)B and unknown blood group patients (8,03%, 8,56%, crude OR 0,903 and 0,831, 95% CI, 0,574-1,421 and 0,506-1,366, P=0,746 and P=0,547, adjusted OR 1,072 and 0,903, 95% CI, 0,857-1,422 and 0,600-1,348), A blood group patients (7,40%, crude OR 0,827, 95% CI, 0,534-1,282, P=0,464, adjusted OR 1,072, 95% CI, 0,857-1,339) and the lowest prevalence of HBV was in the O blood group patients (7,13%, crude OR 0,794, 95% CI, 0,519-1,216, P=0,344, adjusted OR 1,266, 95% CI, 0,807-1,913).

Provincial hospital patients were more likely to have an HBV infection (8,25%, crude OR 1,317, 95% CI, 1,088-1,595, P=0,005, adjusted OR 1,106, 95% CI, 0,884-1,387) and private hospital patients had the lowest prevalence (6,39%,).

Table 4: Bivariate and logistic regression of demographic variables for HBV Infection

Exposure Variable:	Outcome Variable: HBV Infection								
Demographic Data	**Reactive N**	**Reactive %**	**Total N**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	531	7,57	7015	-	-	-	-	-	-
Male	229	8,78	2607	ref	ref	ref	ref	ref	ref
Female	301	6,85	4395	0,763	0.638-0.913	8,76	P=0.003	0,687	0,571-0,828
Unknown	1	7,69	13	0,865	0.112-6.685	0,019	P=0.724	0,693	0,038-3,617
Race	531	7,57	7015	-	-	-	-	-	-
White	32	4,58	698	ref	ref	ref	ref	ref	ref
Black	291	8,25	3529	1,870	1.286-2.720	11,07	P=0.001	1,345	0,900-2,066
Coloured	5	7,14	70	1,601	0.603-4.250	0,908	P=0.509	1,462	0,471-3,751
Asian / Indian	7	3,55	197	0,767	0.33-1.765	0,392	P=0.668	0,541	0,213-1,205
Unknown	196	7,77	2521	1,755	1.196-2.575	8,453	P=0.004	1,542	0,998-2,440
Zone / Region	531	7,57	7015	-	-	-	-	-	-
Egoli	122	6,92	1763	ref	ref	ref	ref	ref	ref
Eastern Cape	44	8,53	516	1,288	0.898-1.847	1,899	P=0.200	1,277	0,878-1,828
Vaal	68	7,31	930	1,090	0.800-1.485	0,298	P=0.641	1.111	0,806-1,519
Free State / North Cape	21	4,90	429	0,711	0.442-1.145	1,985	P=0.193	0,747	0,442-1,207
Northern	122	7,20	1695	1,062	0.817-1.381	0,202	P=0.701	0,954	0,710-1,282
Mpumalanga	42	9,81	428	1,503	1.040-2.174	4,747	P=0.037	1,476	1,004-2,134
KwaZulu Natal	112	8,93	1254	1,355	1.035-1.773	4,932	P=0.031	1,466	1,103-1,949
Age	531	7,57	7015	-	-	-	-	-	-
0-10	4	3,70	108	0,372	0.134-1.033	3,887	P=0.073	0,341	0,102-0,842
11-20	27	8,88	304	0,943	0.602-1.478	0,065	P=0.887	0,965	0,604-1,496
21-30	61	7,16	852	0,746	0.533-1.045	2,917	P=0.104	0,771	0,547-1,081
31-40	93	9,37	993	ref	ref	ref	ref	ref	ref
41-50	59	7,64	772	0,801	0.570-1.126	1,638	P=0.232	0,788	0,557-1,107
51-60	46	6,52	706	0,674	0.467-0.974	4,462	P=0.043	0,666	0,454-0,964
61-70	44	6,06	726	0,624	0.430-0.906	6,245	P=0.016	0,641	0,434-0,933
71-80	19	3,88	490	0,390	0.235-0.647	14,153	P<0,001	0,422	0,243-0,698
81+	11	4,74	232	0,482	0.253-0.915	5,176	P=0.032	0,544	0,688-1,247
Unknown	167	9,12	1832	0,971	0.744-1.267	0,048	P=0.879	0,925	0,688-1,013
Blood Group	531	7,57	7015	-	-	-	-	-	-
A	140	7,40	1891	0,827	0.534-1.282	0,723	P=0.464	1,072	0,857-1,339
B	96	8,03	1196	0,903	0.574-1.421	0,195	P=0.746	1,107	0,857-1,422
O	212	7,13	2974	0,794	0.519-1.216	1,129	P=0.344	1,266	0,807-1,913
AB	26	8,81	295	ref	ref	ref	ref	ref	ref
Unknown	57	8,65	659	0,831	0.506-1.366	0,534	P=0.547	0,903	0,600-1,348
Hospital Class	531	7,57	7015	-	-	-	-	-	-
Private	163	6,39	2552	ref	ref	ref	ref	ref	ref
Provincial	368	8,25	4463	1,317	1.088-1.595	8,015	P=0.005	1,106	0,884-1,387

4.7. PREVALENCE OF HTLV INFECTION

According to Gessain and Cassar (2012b), the HTLV prevalence in South Africa is only 0.2% and this study found a higher HTLV prevalence of 0.70%. Even if the number of specimens in this study is increased, the HTLV prevalence will still not be significant statistically as the number of reactive specimen will be too low to have a statistically significant impact.

Comparing gender, Table 5 shows that female patients (0,84%, crude OR 1,836, 95% CI, 0,956-3,527, P=0,088, adjusted OR 1,883, 95% CI, 0,995-3,820) have a twofold higher prevalence of HTLV compared to males (0.46%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

HTLV Prevalence was the highest in the Asian or Indian population (1.52%, crude OR 1,804, 95% CI, 0,546-5,962, P=0,555, adjusted OR 1,283, 95% CI, 0,276-4,372), followed by Black population (0.85%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) and Unknown race specimens (0.63%, crude OR 0,745, 95% CI, 0,405-1,370, P=0,423, adjusted OR 0,635, 95% CI, 0,239-1,684).

The HTLV prevalence was the highest in Free State or North Cape (1.40%, crude OR 3,112, 95% CI, 1,074-9,016, P=0,062, adjusted OR 3,755, 95% CI, 1,150-11,822) followed by KwaZulu Natal (1.20%, crude OR 2,656, 95% CI, 1,123-6,284, P=0,035, adjusted OR 2,416, 95% CI, 0,970-6,425), Mpumalanga (0.93%, crude OR 2,070, 95% CI, 0,620-6,905, P=0,398, adjusted OR 1,707, 95% CI, 0,446-5,579), Egoli (0.45%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference), Eastern Cape (0.39%, crude OR 0,854, 95% CI, 0,181-4,032, P=0,848, adjusted OR 0,718, 95% CI, 0,107-2,921), Northern (0,71%, crude OR 1,564, 95% CI, 0,638-3,836, P=0,446, adjusted OR 1,859, 95% CI, 0,683-5,282) and Vaal zone had the lowest HTLV prevalence (0.22%, crude OR 0,473, 95% CI, 0,100-2,231, P=0,525, adjusted OR 0,505, 95% CI, 0,075-2,060).

The HTLV prevalence was the highest in the 41 to 50 years old (1.42%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI,

reference), followed by 11 to 20 years old (1.32%, crude OR 0,922, 95% CI, 0,291-2,920, P=0,879, adjusted OR 0,763, 95% CI, 0,208-2,289), 0 to 10 years old (0.93%, crude OR 0,647, 95% CI, 0,083-5,058, P=0,980, adjusted OR 0,733, 95% CI, 0,039-3,957), 61 to 70 years old (0.83%, crude OR 0,577, 95% CI, 0,212-1,567, P=0,396, adjusted OR 0,715, 95% CI, 0,240-1,931), Unknown age specimens (0.76%, crude OR 0,533, 95% CI, 0,241-1,179, P=0,174, adjusted OR 0,522, 95% CI, 0,201-1,346), 51 to 60 years old (0.57%, crude OR 0,394, 95% CI, 0,125-1,244, P=0,166, adjusted OR 0,410, 95% CI, 0,112-1,220), 81 plus years old (0.43%, crude OR 0,299, 95% CI, 0,038-2,332, P=0,380, adjusted OR 0,542, 95% CI, 0,029-2,928), 71 to 80 years old (0.41%, crude OR 0,284, 95% CI, 0,063-1,285, P=0,145, adjusted OR 0,424, 95% CI, 0,064-1,630), 21 to 30 years old (0.35%, crude OR 0,244, 95% CI, 0,068-0,880, P=0,038, adjusted OR 0,208, 95% CI, 0,047-0,681) and the lowest prevalence of HTLV was in the 31 to 40 years old patients (0.30%, crude OR 0,210, 95% CI, 0,058-0,754, P=0,017, adjusted OR 0,192, 95% CI, 0,043-0,621).

The unknown blood group patients (0.91%, crude OR 1,104, 95% CI, 0,392-3,110, P=0,934, adjusted OR 1,899, 95% CI, 0,500-6,692) had the highest HTLV prevalence, followed by O blood group patients (0.74%, crude OR 1,077, 95% CI, 0,541-2,142, P=0,971, adjusted OR <0,001, 95% CI, 0,00-0,978), A blood group patients (0.69%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) and B blood group patients (0.67%, crude OR 0,973, 95% CI, 0,402-2,354, P=0,870, adjusted OR 0,866, 95% CI, 0,339-2,082).

Provincial hospital patients had the highest HTLV prevalence (0.74%, crude OR 1,181, 95% CI, 0,649-2,149, P=0,692, adjusted OR 0,894, 95% CI 0,454-1,814) compared to Private hospital patients (0.63%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

Table 5: Bivariate and logistic regression of demographic variables for HTLV Infection

Exposure Variable:	Outcome Variable: HTLV Infection								
Demographic Data	**Reactive N**	**Reactive %**	**Total N**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	49	0,70	7015	-	-	-	-	-	-
Male	12	0,46	2607	ref	ref	ref	ref	ref	ref
Female	37	0,84	4395	1,836	0.956-3.527	3,428	P=0.088	1,883	0,995-3,820
Unknown	0	0,00	13	0,000	0,000	0	0,00	0,000	0
Race	49	0,70	7015	-	-	-	-	-	-
White	0	0,00	698	0,000	0,000	0	0,00	0,000	0
Black	30	0,85	3529	ref	ref	ref	ref	ref	ref
Coloured	0	0,00	70	0,000	0,000	0	0,00	0,000	0
Asian / Indian	3	1,52	197	1,804	0.546-5.962	0,962	P=0.555	1,283	0,276-4,372
Unknown	16	0,63	2521	0,745	0.405-1.370	0,904	P=0.423	0,635	0,239-1,684
Zone / Region	49	0,70	7015	-	-	-	-	-	-
Egoli	8	0,45	1763	ref	ref	ref	ref	ref	ref
Eastern Cape	2	0,39	516	0,854	0.181-4.032	0,04	P=0.848	0,718	0,107-2,921
Vaal	2	0,22	930	0,473	0.100-2.231	0,938	P=0.525	0,505	0,075-2,060
Free State / North Cape	6	1,40	429	3,112	1.074-9.016	4,854	P=0.062	3,755	1,150-11,822
Northern	12	0,71	1695	1,564	0.638-3.836	0,971	P=0.446	1,859	0,683-5,282
Mpumalanga	4	0,93	428	2,070	0.620-6.905	1,462	P=0.398	1,707	0,446-5,579
KwaZulu Natal	15	1,20	1254	2,656	1.123-6.284	5,339	P=0.035	2,416	0,970-6,425
Age	49	0,70	7015	-	-	-	-	-	-
0-10	1	0,93	108	0,647	0,083	0,175	P=0.980	0,733	0,039-3,957
11-20	4	1,32	304	0,922	0.291-2.920	0,019	P=0.879	0,763	0,208-2,289
21-30	3	0,35	852	0,244	0.068-0.880	5,454	P=0.038	0,208	0,047-0,681
31-40	3	0,30	993	0,210	0.058-0.754	6,958	P=0.017	0,192	0,043-0,621
41-50	11	1,42	772	ref	ref	ref	ref	ref	ref
51-60	4	0,57	706	0,394	0.125-1.244	2,704	P=0.166	0,410	0,112-1,220
61-70	6	0,83	726	0,577	0.212-1.567	1,194	P=0.396	0,715	0,240-1,931
71-80	2	0,41	490	0,284	0.063-1.285	3,039	P=0.145	0,424	0,064-1,630
81+	1	0,43	232	0,299	0.038-2.332	1,492	P=0.380	0,542	0,029-2,928
Unknown	14	0,76	1832	0,533	0.241-1.179	2,493	P=0.174	0,522	0,201-1,346
Blood Group	49	0,68	7015	-	-	-	-	-	-
A	13	0,69	1891	ref	ref	ref	ref	ref	ref
B	8	0,67	1196	0,973	0.402-2.354	0,004	P=0.870	0,866	0,339-2,082
O	22	0,74	2974	1,077	0.541-2.142	0,044	P=0.971	<0,001	0-0,978
AB	0	0,00	295	0,000	0,000	0	0,00	1,026	0,519-2,108
Unknown	6	0,91	659	1,104	0.392-3.110	0,035	P=0.934	1,899	0,500-6,692
Hospital Class	49	0,70	7015	-	-	-	-	-	-
Private	16	0,63	2552	ref	ref	ref	ref	ref	ref
Provincial	33	0,74	4463	1,181	0.649-2.149	0,296	P=0.692	0,894	0,454-1,814

4.8. CONCURRENT INFECTIONS

4.8.1. HIV – HBV Concurrent infections results

As established in Table 6, HIV - HBV concurrent infection is significantly higher in males (5.68%,) than in females (4.46%, crude OR 0,776, 95% CI, 0,623-0,966, P=0,026, adjusted OR 0,658, 95% CI 0,524-0,827). Only 1 unknown patient tested positive for concurrent infection of HIV - HBV (7.69%, crude OR 1,385, 95% CI, 0,179-10,720, P=0,773, adjusted OR 0,920, 95% CI 0,050-4,892).

The black population had the highest HIV - HBV concurrent infection prevalence (6.04%, crude OR 6,341, 95% CI, 2,973-13,522, P=<0,001, adjusted OR 3,254, 95% CI 1,593-7,844), followed by unknown race specimens (4.72%, crude OR 4,891, 95% CI, 2,271-10,530, P<0,001, adjusted OR 3,914, 95% CI 1,838-9,695), coloured population (2.86%, crude OR 2,903, 95% CI, 0,591-14,253, P=0,428, adjusted OR 2,043, 95% CI 0,292-9,082), Asian or Indian (2.03%, crude OR 2,046, 95% CI, 0,593-7,061, P=0,429, adjusted OR 1,128, 95% CI 0,287-3,872) and Caucasians had the lowest prevalence of HIV - HBV concurrent infection (1%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

Mpumalanga had the highest HIV - HBV concurrent infection prevalence (7.01%, crude OR 1,628, 95% CI, 1,054-2,516, P=0,036, adjusted OR 1,577, 95% CI 0,995-2,443) followed by KwaZulu Natal (6.62%, crude OR 1,531, 95% CI, 1,114-2,104, P=0,010, adjusted OR 1,677, 95% CI 1,196-2,357), Vaal (5.38%, crude OR 1,227, 95% CI, 0,853-1,767, P=0,313, adjusted OR 1,310, 95% CI 0,896-1,901), Eastern Cape (4.46%, crude OR 1,008, 95% CI, 0,626-1,622, P=0,928, adjusted OR 0,999, 95% CI 0,604-1,595), Egoli (4.48%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference), Northern (3.90%, crude OR 0,875, 95% CI, 0,626-1,223, P=0,486, adjusted OR 0,757, 95% CI 0,517-1,106) and Free State / North Cape had the lowest prevalence of HIV - HBV concurrent infection (3.26%, crude OR 0,729, 95% CI, 0,408-1,300, P=0,346, adjusted OR 0,862, 95% CI 0,452-1,538).

The 31 to 40 years old had the highest prevalence of HIV - HBV concurrent infection (7.75%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by the unknown age group (6.39%, crude OR 0,812, 95% CI, 0,602-1,094, P=0,195, adjusted OR 0,832, 95% CI 0,594-1,168), 41 to 50 year olds (6.09%, crude OR 0,771, 95% CI, 0,530-1,123, P=0,205, adjusted OR 0,760, 95% CI 0,517-1,106), 11 to 20 years old (5.26%, crude OR 0,661, 95% CI, 0,380-1,151, P=0,178, adjusted OR 0,697, 95% CI 0,386-1,186), 21 to 30 years old (4.93%, crude OR 0,617, 95% CI, 0,419-0,909, P=0,017, adjusted OR 0,655, 95% CI 0,439-0,965), 51 to 60 years old (4.11%, crude OR 0,510, 95% CI, 0,329-0,790, P=0,003, adjusted OR 0,508, 95% CI 0,319-0,787), 0 to 10 years old (2.78%, crude OR 0,340, 95% CI, 0,105-1,096, P=0,089, adjusted OR 0,314, 95% CI 0,076-0,868), 61 to 70 years old (1.38%, crude OR 0,166, 95% CI, 0,085-0,323, P<0,001, adjusted OR 0,177, 95% CI 0,085-0,332), 81 plus years old (0.86%, crude OR 0,103, 95% CI, 0,025-0,424, P<0,001, adjusted OR 0,141, 95% CI 0,023-0,463) and the age group with the lowest prevalence for HIV - HBV concurrent infection was 71 to 80 years old (0.41%, crude OR 0,049, 95% CI, 0,012-0,199, P<0,001, adjusted OR 0,061, 95% CI 0,010-0,196).

The AB blood group patients had the highest prevalence (6.78% crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by B blood group patients (5.60%, crude OR 0,816, 95% CI, 0,487-1,368, P=0,525, adjusted OR 1,250, 95% CI 0,915-1,693), Unknown blood group patients (5.61%, crude OR 0,702, 95% CI, 0,394-1,249, P=0,291, adjusted OR 0,782, 95% CI 0,469-1,228), A blood group patients (4.87%, crude OR 0,703, 95% CI, 0,427-1,159, P=0,213, adjusted OR 1,210, 95% CI 0,914-1,596) and O blood group patients had the lowest prevalence of HIV - HBV concurrent infections (4.34%, crude OR 0,623, 95% CI, 0,383-1,015, P=0,076, adjusted OR 1,685, 95% CI 0,999-2,708).

The provincial hospital patients had the highest prevalence (5.56%, crude OR 1,489, 95% CI, 1,171-1,893, P=0,001, adjusted OR 0,977, 95% CI 0,743-1,291) compared to private hospital patients (3.80%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

Table 6: Bivariate and logistic regression of demographic variables for HIV – HBV Concurrent Infection

Exposure Variable:	Outcome Variable: HIV-HBV Infection								
Demographic Data	**Reactive N**	**Reactive %**	**Total N**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	345	4,92	7015	-	-	-	-	-	-
Male	148	5,68	2607	ref	ref	ref	ref	ref	ref
Female	196	4,46	4395	0,776	0.623-0.966	5,192	P=0.026	0,658	0,524-0,827
Unknown	1	7,69	13	1,385	0.179-10.720	0,098	P=0.773	0,920	0,050-4,892
Race	345	4,92	7015	-	-	-	-	-	-
White	7	1,00	698	ref	ref	ref	ref	ref	ref
Black	213	6,04	3529	6,341	2.973-13.522	29,917	P<0,001	3,254	1,593-7,844
Coloured	2	2,86	70	2,903	0.591-14.253	1,889	P=0.428	2,043	0,292-9,082
Asian / Indian	4	2,03	197	2,046	0.593-7.061	1,336	P=0.429	1,128	0,287-3,872
Unknown	119	4,72	2521	4,891	2.271-10.530	20,086	P<0,001	3,914	1,838-9,695
Zone / Region	345	4,91	7015	-	-	-	-	-	-
Egoli	79	4,48	1763	ref	ref	ref	ref	ref	ref
Eastern Cape	23	4,46	516	1,008	0.626-1.622	0,001	P=0.928	0,999	0,604-1,595
Vaal	50	5,38	930	1,227	0.853-1.767	1,219	P=0.313	1,310	0,896-1,901
Free State / North Cape	14	3,26	429	0,729	0.408-1.300	1,156	P=0.346	0,862	0,452-1,538
Northern	66	3,90	1695	0,875	0.626-1.223	0,609	P=0.486	0,757	0,517-1,106
Mpumalanga	30	7,01	428	1,628	1.054-2.516	4,911	P=0.036	1,577	0,995-2,443
KwaZulu Natal	83	6,62	1254	1,531	1.114-2.104	6,986	P=0.010	1,677	1,196-2,357
Age	345	4,92	7015	-	-	-	-	-	-
0-10	3	2,78	108	0,340	0.105-1.096	3,58	P=0.089	0,314	0,076-0,868
11-20	16	5,26	304	0,661	0.380-1.151	2,17	P=0.178	0,697	0,386-1,186
21-30	42	4,93	852	0,617	0.419-0.909	6,064	P=0.017	0,655	0,439-0,965
31-40	77	7,75	993	ref	ref	ref	ref	ref	ref
41-50	47	6,09	772	0,771	0.530-1.123	1,846	P=0.205	0,760	0,517-1,106
51-60	29	4,11	706	0,510	0.329-0.790	9,38	P=0.003	0,508	0,319-0,787
61-70	10	1,38	726	0,166	0.085-0.323	35,493	P<0,001	0,177	0,085-0,332
71-80	2	0,41	490	0,049	0.012-0.199	35,108	P<0,001	0,061	0,010-0,196
81+	2	0,86	232	0,103	0.025-0.424	14,807	P<0,001	0,141	0,023-0,463
Unknown	117	6,39	1832	0,812	0.602-1.094	1,884	P=0.195	0,832	0,594-1,168
Blood Group	345	4,91	7015	-	-	-	-	-	-
A	92	4,87	1891	0,703	0.427-1.159	1,924	P=0.213	1,210	0,914-1,596
B	67	5,60	1196	0,816	0.487-1.368	0,597	P=0.525	1,250	0,915-1,693
O	129	4,34	2974	0,623	0.383-1.015	3,679	P=0.076	1,685	0,999-2,708
AB	20	6,78	295	ref	ref	ref	ref	ref	ref
Unknown	37	5,61	659	0,702	0.394-1.249	1,463	P=0.291	0,782	0,469-1,2287
Hospital Class	345	4,92	7015	-	-	-	-	-	-
Private	97	3,80	2552	ref	ref	ref	ref	ref	ref
Provincial	248	5,56	4463	1,489	1.171-1.893	10,705	P=0.001	0,977	0,743-1,291

4.8.2. HBV – HTLV Concurrent infections results

As presented in Table 7, there were only six HBV - HTLV reactive specimen and are not enough to test for significant interaction between the study demographics and HBV – HTLV concurrent infection prevalence.

Of the six specimens four were female (0.09%, crude OR 1,489, 95% CI, 1,171-1,893, P=0,001, adjusted OR 0,977, 95% CI 0,743-1,291) and two was male (0.08%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

Two specimens were from the black population (0.06%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference), Asian or Indian population (1.02%, crude OR 18,087, 95% CI, 2,534-129,089, P=0,003, adjusted OR 8,416, 95% CI 0,656-146,70) and Unknown race specimens (0.08%, crude OR 1,400, 95% CI, 0,197-9,947, P=0,865, adjusted OR 1,941, 95% CI 0,075-58,20).

Three specimens was from KwaZulu Natal (0.24%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference), two specimens from Northern (0.12%, crude OR 0,493, 95% CI, 0,082-2,953, P=0,735, adjusted OR 0,620, 95% CI 0,017-26,466) and one specimen from Mpumalanga (0.23%, crude OR 0,977, 95% CI 0,977, P=0,579, adjusted OR 1,883, 95% CI 0,076-31,428).

Three specimens was from 41 to 50 years old (0.39%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference), One specimen from 11 to 20 years old (0.033%, crude OR 0,846, 95% CI, 0,088-8,165, P=0,680, adjusted OR 0,817, 95% CI 0,038-7,181), 61 to 70 years old (0.14%, crude OR 0,354, 95% CI, 0,037-3,407, P=0,660, adjusted OR 0,295, 95% CI 0,014-2,528) and Unknown age (0.05%, crude OR 0,140, 95% CI, 0,015-1,348, P=149, adjusted OR 0,262, 95% CI 0,011-3,69).

Comparing blood groups, three specimen was from B blood group patients (0.25%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95%

CI, reference) and three from O blood group patients (0.10%, crude OR 0,402, 95% CI, 0,081-1,992, P=0,81, adjusted OR <0,001, 95% CI 0).

Three specimen was from private hospital patients (0.12%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) and three was from provincial hospital patients (0.07%, crude OR 0,572, 95% CI, 0,115-2,834, P=0,787, adjusted OR 0,777, 95% CI 0,092-6,029).

Table 7: Bivariate and logistic regression of demographic variables for HBV - HTLV Concurrent Infection

Exposure Variable:	Outcome Variable: HBV-HTLV Infection								
Demographic Data	**Reactive N**	**Reactive %**	**Total N**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	6	0,09	7015	-	-	-	-	-	-
Male	2	0,08	2607	ref	ref	ref	ref	ref	ref
Female	4	0,09	4395	1,187	0,217	0,039	P=0.822	1,268	0,231-9,517
Unknown	0	0,00	13	0,000	0,000	0	0,00	0,000	0
Race	6	0,09	7015	-	-	-	-	-	-
White	0	0,00	698	0,000	0,000	0	0,00	0,000	0
Black	2	0,06	3529	ref	ref	ref	ref	ref	ref
Coloured	0	0,00	70	0,000	0,000	0	0,00	0,000	0
Asian / Indian	2	1,02	197	18,087	2.534-129.089	15,987	P=0.003	8,416	0,656-146,70
Unknown	2	0,08	2521	1,400	0.197-9.947	0,114	P=0.865	1,941	0,075-58,20
Zone / Region	6	0,09	7015	-	-	-	-	-	-
Egoli	0	0,00	1763	0,000	0,000	0	0,00	0,000	0
Eastern Cape	0	0,00	516	0,000	0,000	0	0,00	0,000	0
Vaal	0	0,00	930	0,000	0,000	0	0,00	0,000	0
Free State / North Cape	0	0,00	429	0,000	0,000	0	0,00	0,000	0
Northern	2	0,12	1695	0,493	0.082-2.953	0,626	P=0.735	0,620	0,017-26,466
Mpumalanga	1	0,23	428	0,977	0.101-9.414	0	P=0.579	1,883	0,076-31,428
KwaZulu Natal	3	0,24	1254	ref	ref	ref	ref	ref	ref
Age	6	0,09	7015	-	-	-	-	-	-
0-10	0	0,00	108	0,000	0,000	0	0,00	0,000	0
11-20	1	0,33	304	0,846	0.088-8.165	0,021	P=0.680	0,817	0,038-7,181
21-30	0	0,00	852	0,000	0,000	0	0,00	0,000	0
31-40	0	0,00	993	0,000	0,000	0	0,00	0,000	0
41-50	3	0,39	772	ref	ref	ref	ref	ref	ref
51-60	0	0,00	706	0,000	0,000	0	0,00	0,000	0
61-70	1	0,14	726	0,354	0.037-3.407	0,884	P=0.660	0,295	0,014-2,528
71-80	0	0,00	490	0,000	0,000	0	0,00	0,000	0
81+	0	0,00	232	0,000	0,000	0	0,00	0,000	0
Unknown	1	0,05	1832	0,140	0.015-1.348	3,951	P=0.149	0,262	0,011-3,069
Blood Group	6	0,09	7015	-	-	-	-	-	-
A	0	0,00	1891	0,000	0,000	0	0,00	0,000	0
B	3	0,25	1196	ref	ref	ref	ref	ref	ref
O	3	0,10	2974	0,402	0.081-1.992	1,335	P=0.481	<0,001	0
AB	0	0,00	295	0,000	0,000	0	0,00	0,000	0
Unknown	0	0,00	659	0,000	0,000	0	0,00	0,000	0
Hospital Class	6	0,09	7015	-	-	-	-	-	-
Private	3	0,12	2552	ref	ref	ref	ref	ref	ref
Provincial	3	0,07	4463	0,572	0.115-2.834	0,481	P=0.787	0,777	0,092-6,029

4.8.3. HIV – HTLV Concurrent infections results

As obtained from Table 8, twenty-five specimen tested positive for HIV - HTLV concurrent infection.

Females have a higher prevalence of HIV - HTLV concurrent infection prevalence (0.48%, crude OR 3,124, 95% CI, 1,071-9,112, P=0,046, adjusted OR 2,897, 95% CI 1,082-10,048) than males (0.15%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

The Asian / Indian population have the highest prevalence of HIV - HTLV concurrent infection (1.02%, crude OR 2,252, 95% CI, 0,514-9,863, P=0,562, adjusted OR 2,127, 95% CI 0,288-10,045) followed by the black population (0.45%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) and unknown race (0.28%, crude OR 0,611, 95% CI, 0,251-1,488, P=0,377, adjusted OR 0,687, 95% CI 0,160-2,744).

KwaZulu Natal had the highest prevalence of HIV - HTLV concurrent infection (0.72%, crude OR 2,117, 95% CI, 0,752-5,963, P=0,234, adjusted OR 1,838, 95% CI 0,592-6,073) followed by Mpumalanga (0.70%, crude OR 2,067, 95% CI, 0,515-8,299, P=0,531, adjusted OR 1,623, 95% CI 0,333-6,425), Free State / North Cape (0.47%, crude OR 1,372, 95% CI, 0,276-6,820, P=0,963, adjusted OR 1,670, 95% CI 0,230-8,169), Egoli (0.34%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference), Northern (0.24%, crude OR 0,693, 95% CI, 0,195-2,459, P=0,799, adjusted OR 0,792, 95% CI 0,172-3,338) and Vaal had the lowest prevalence of HIV - HTLV concurrent infection (0.11%, crude OR 0,315, 95% CI, 0,038-2,622, P=0,465, adjusted OR 0,327, 95% CI 0,017-1,976),

The 41 to 50 years old have the highest prevalence of HIV - HTLV (0.91%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by 11 to 20 years old (0.66%, crude OR 0,724, 95% CI, 0,150-3,504, P=0,974, adjusted OR 0,591, 95% CI 0,087-2,518), unknown age (0.44%, crude OR 0,479, 95% CI, 0,173-1,326, P=0,244, adjusted OR 0,449, 95% CI 0,130-1,511), 81 plus years old (0.43%, crude OR 0,473, 95% CI, 0,058-3,865, P=0,769,

adjusted OR 0,760, 95% CI 0,039-4,636), 21 to 30 years old (0.35%, crude OR 0,386, 95% CI, 0,100-1,499, P=0,267, adjusted OR 0,315, 95% CI 0,067-1,170), 61 to 70 years old (0.28%, crude OR 0,302, 95% CI, 0,063-1,458, P=0,212, adjusted OR 0,363, 95% CI 0,052-1,582) and 31 to 40 years old (0.20%, crude OR 0,221, 95% CI, 0,046-1,065, P=0,084, adjusted OR 0,198, 95% CI 0,029-0,831).

B blood group patients had the highest prevalence of HIV - HTLV concurrent infection (0.42%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by O blood group patients (0.37%, crude OR 0,884, 95% CI, 0,307-2,551, P=0,960, adjusted OR <0,001, 95% CI 0-1,997), unknown blood group patients (0.46%, crude OR 0,725, 95% CI, 0,140-3,748, P=0,991, adjusted OR 1,608, 95% CI 0,242-8,875) and A blood group patients (0.32%, crude OR 0,758, 95% CI, 0,231-2,490, P=0,882, adjusted OR 0,932, 95% CI 0,319-2,471).

The provincial hospital patients have the highest HIV - HTLV concurrent infection prevalence (0.40%, crude OR 1,472, 95% CI, 0,614-3,530, P=0,506, adjusted OR 1,002, 95% CI 0,375-2,934) compared to private hospital patients (0.27%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

Table 8: Bivariate and logistic regression of demographic variables for HIV - HTLV Concurrent Infection

Exposure Variable:	Outcome Variable: HIV-HTLV Infection								
Demographic Data	**Reactive N**	**Reactive %**	**Total N**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	25	0,36	7015	-	-	-	-	-	-
Male	4	0,15	2607	ref	ref	ref	ref	ref	ref
Female	21	0,48	4395	3,124	1.071-9.112	4,84	P=0.046	2,897	1,082-10,048
Unknown	0	0,00	13	0,000	0,000	0	0,00	0,000	0
Race	25	0,36	7015	-	-	-	-	-	-
White	0	0,00	698	0,000	0,000	0	0,00	0,000	0
Black	16	0,45	3529	ref	ref	ref	ref	ref	ref
Coloured	0	0,00	70	0,000	0,000	0	0,00	0,000	0
Asian / Indian	2	1,02	197	2,252	0.514-9.863	1,225	P=0.562	2,127	0,288-10,045
Unknown	7	0,28	2521	0,611	0.251-1.488	1,199	P=0.377	0,687	0,160-2,744
Zone / Region	25	0,36	7015	-	-	-	-	-	-
Egoli	6	0,34	1763	ref	ref	ref	ref	ref	ref
Eastern Cape	0	0,00	516	0,000	0,000	0	0,00	0,000	0
Vaal	1	0,11	930	0,315	0.038-2.622	1,273	P=0.465	0,327	0,017-1,976
Free State / North Cape	2	0,47	429	1,372	0.276-6.820	0,15	P=0.963	1,670	0,230-8,169
Northern	4	0,24	1695	0,693	0.195-2.459	0,326	P=0.799	0,792	0,172-3,338
Mpumalanga	3	0,70	428	2,067	0.515-8.299	1,095	P=0.531	1,623	0,333-6,425
KwaZulu Natal	9	0,72	1254	2,117	0.752-5.963	2,109	P=0.234	1,838	0,592-6,073
Age	25	0,36	7015	-	-	-	-	-	-
0-10	0	0,00	108	0,000	0,000	0	0,00	0,000	0
11-20	2	0,66	304	0,724	0.150-3.504	0,163	P=0.974	0,591	0,087-2,518
21-30	3	0,35	852	0,386	0.100-1.499	2,036	P=0.267	0,315	0,067-1,170
31-40	2	0,20	993	0,221	0.046-1.065	4,259	P=0.084	0,198	0,029-0,831
41-50	7	0,91	772	ref	ref	ref	ref	ref	ref
51-60	0	0,00	706	0,000	0,000	0	0,00	0,000	0
61-70	2	0,28	726	0,302	0.063-1.458	2,497	P=0.212	0,363	0,052-1,582
71-80	0	0,00	490	0,000	0,000	0	0,00	0,000	0
81+	1	0,43	232	0,473	0.058-3.865	0,511	P=0.769	0,760	0,039-4,636
Unknown	8	0,44	1832	0,479	0.173-1.326	2,095	P=0.244	0,449	0,130-1,511
Blood Group	25	0,36	7015	-	-	-	-	-	-
A	6	0,32	1891	0,758	0,231-2490	0,21	P=0.882	0,932	0,319-2,471
B	5	0,42	1196	ref	ref	ref	ref	ref	ref
O	11	0,37	2974	0,884	0.307-2.551	0,052	P=0.960	<0,001	0-1,997
AB	0	0,00	295	0,000	0,000	0	0,00	0,000	0
Unknown	3	0,45	659	0,725	0.140-3.748	0,148	P=0.991	1,608	0,242-8,875
Hospital Class	25	0,36	7015	-	-	-	-	-	-
Private	7	0,27	2552	ref	ref	ref	ref	ref	ref
Provincial	18	0,40	4463	1,472	0.614-3.530	0,761	P=0.506	1,002	0,375-2,934

4.8.4. HIV - HBV – HTLV Concurrent infections results

The bivariate and logistic regression analysis illustrated in Table 9 revealed that there were only five reactive specimens for HIV, HBV, HTLV concurrent infection and five samples are not enough to test for significance.

One specimen was reactive for males (0.04%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) and four specimens was reactive for females (0.09%, crude OR 2,374, 95% CI, 0,265-21,252, P=0,737, adjusted OR 2,384, 95% CI 0,301-51,67).

The prevalence for HIV, HBV and HTLV concurrent infections was the highest in the Asian or Indian population (1.02%, crude OR 18,087, 95% CI, 2,534-129,089, P=0,003, adjusted OR 18,290, 95% CI 1,076-561,45), unknown race specimens (0.40%, crude OR 0,700, 95% CI, 0,063-7,722, P=0,769, adjusted OR 1,936, 95% CI 0,023-130,74) followed by Black population (0.06%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference).

KwaZulu Natal the highest HIV – HBV - HTLV concurrent infections prevalence (0.24%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by Mpumalanga (0.23%, crude OR 0,977, 95% CI, 0,101-9,414, P=0,579, adjusted OR 2,117, 95% CI 0,081-55,291) and the Northern zone (0.06%, crude OR 0,246, 95% CI, 0,026-2,369, P=0,418, adjusted OR 0,330, 95% CI 0,002-28,833).

The 41 to 50 years old age group had the highest HIV – HBV - HTLV concurrent infection prevalence (0.39%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by the 11 to 20 years old group (0.33%, crude OR 0,846, 95% CI, 0,088-8,165, P=0,680, adjusted OR 0,733, 95% CI 0,034-6,795) and the unknown age group specimens (0.05%, crude OR 0,140, 95% CI, 0,015-1,348, P=0,149, adjusted OR 0,188, 95% CI 0,008-2,248).

The B blood group patients highest prevalence of HIV - HBV- HTLV concurrent infections (0.25%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by the O blood group patients (0.07%, crude OR 0,268, 95% CI, 0,045-1,604, P=0,149, adjusted OR <0,001, 95% CI 0-3,674).

The prevalence of HIV – HBV - HTLV concurrent infections was the highest in the private hospital patients (0.08%, crude OR reference, 95% CI, reference, P= reference, adjusted OR reference, 95% CI, reference) followed by the provincial hospital patients (0.07%, crude OR 0,858, 95% CI, 0,143-5,136, P=0,766, adjusted OR 1,987, 95% CI 0,162-35,616).

Table 9: Bivariate and logistic regression of demographic variables for HIV - HBV - HTLV Concurrent Infection

Exposure Variable:	Outcome Variable: HIV-HBV-HTLV Infection								
Demographic Data	**Reactive N (%)**	**Non-Reactive N (%)**	**Total N (%)**	**Crude OR (95% CI)**		**X2**	**P-Value**	**Adjusted OR (95% CI)**	
Gender	5	0,07	7015	-	-	-	-	-	-
Male	1	0,04	2607	ref	ref	ref	ref	ref	ref
Female	4	0,09	4395	2,374	0.265-21.252	0,636	P=0.737	2,384	0,301-51,67
Unknown	0	0,00	13	0,000	0,000	0	0,00	0,000	0
Race	5	0,07	7015	-	-	-	-	-	-
White	0	0,00	698	0,000	0,000	0	0,00	0,000	0
Black	2	0,06	3529	ref	ref	ref	ref	ref	ref
Coloured	0	0,00	70	0,000	0,000	0	0,00	0,000	0
Asian / Indian	2	1,02	197	18,087	2.534-129.089	15,987	P=0.003	18,290	1,076-561,45
Unknown	1	0,04	2521	0,700	0.063-7.722	0,086	P=0.769	1,936	0,023-130,74
Zone / Region	5	0,07	7015	-	-	-	-	-	-
Egoli	0	0,00	1763	0,000	0,000	0	0,00	0,000	0
Eastern Cape	0	0,00	516	0,000	0,000	0	0,00	0,000	0
Vaal	0	0,00	930	0,000	0,000	0	0,00	0,000	0
Free State / North Cape	0	0,00	429	0,000	0,000	0	0,00	0,000	0
Northern	1	0,06	1695	0,246	0.026-2.369	1,729	P=0.418	0,330	0,002-28,833
Mpumalanga	1	0,23	428	0,977	0.101-9.414	0	P=0.579	2,117	0,081-55,291
KwaZulu Natal	3	0,24	1254	ref	ref	ref	ref	ref	ref
Age	5	0,07	7015	-	-	-	-	-	-
0-10	0	0,00	108	0,000	0,000	0	0,00	0,000	0
11-20	1	0,33	304	0,846	0.088-8.165	0,021	P=0.680	0,733	0,034-6,795
21-30	0	0,00	852	0,000	0,000	0	0,00	0,000	0
31-40	0	0,00	993	0,000	0,000	0	0,00	0,000	0
41-50	3	0,39	772	ref	ref	ref	ref	ref	ref
51-60	0	0,00	706	0,000	0,000	0	0,00	0,000	0
61-70	0	0,00	726	0,000	0,000	0	0,00	0,000	0
71-80	0	0,00	490	0,000	0,000	0	0,00	0,000	0
81+	0	0,00	232	0,000	0,000	0	0,00	0,000	0
Unknown	1	0,05	1832	0,140	0.015-1.348	3,951	P=0.149	0,188	0,008-2,248
Blood Group	5	0,07	7015	-	-	-	-	-	-
A	0	0,00	1891	0,000	0,000	0	0,00	0,000	0
B	3	0,25	1196	ref	ref	ref	ref	ref	ref
O	2	0,07	2974	0,268	0.045-1.604	2,401	P=0.291	<0,001	0,0-3,674
AB	0	0,00	295	0,000	0,000	0	0,00	0,000	0
Unknown	0	0,00	659	0,000	0,000	0	0,00	0,000	0
Hospital Class	5	0,07	7015	-	-	-	-	-	-
Private	2	0,08	2552	ref	ref	ref	ref	ref	ref
Provincial	3	0,07	4463	0,858	0.143-5.136	0,028	P=0.766	1,987	0,162-35,616

4.9. DISCUSSION

When comparing the study population to the general population we have to indicate that the study population is a non-healthy population and could be receiving transfusions for the symptoms associated with these viruses, hence the higher prevalence rate and the comparisons to general population estimates are merely to bring the figures into context.

4.9.1. Global comparison

Since the beginning of the epidemic, more than 70 million people have been infected with the HIV virus and about 35 million people have died of HIV. Globally, 36.9 million [31.1–43.9 million] people were living with HIV at the end of 2017. An estimated 0.8% [0.6-0.9%] of adults aged 15–49 years worldwide are living with HIV, although the burden of the epidemic continues to vary considerably between countries and regions.

The WHO reported that the African region remains most severely affected, with nearly 1 in every 25 adults (4.1%) living with HIV and accounting for nearly two-thirds of the people living with HIV worldwide (WHO, 2018a). When comparing this estimation with this study, we can see that the HIV prevalence in the patient population (39.38%) of the SANBS is 49 times higher than the global estimate and 9.6 times higher than the African estimates of the WHO. As seen in chapter 1 of this thesis, Schweitzer *et al.* (2015) estimated that 3.61% of the global population is living with chronic HBV infection. When comparing these estimated numbers to this study results it is shown that the HBV prevalence in the patient population (7.56%) of the SANBS is twofold higher compared to the estimates by Schweitzer *et al.* (2015) of the global population (3.61%).

According to Schweitzer *et al.* (2015) the worldwide distribution of HTLV may be slightly or well above 10 million infected individuals. Furthermore, the true prevalence of HTLV worldwide has not been covered previously as epidemiological studies mostly cover only blood donors, pregnant women, or hospital-based studies of different selected patients or high-risk groups such as IDUs, HIV, and other hematologic

patients, neurologic patients or prostitutes, rather than the general population (villages, towns, cities, states, provinces regions or geopolitical zones of a country).

The prevalence of HTLV is different for different parts of the world (Anyanwu *et al.*, 2018). It is generally categorised into three strata - regions of low (less than 1%), average or moderate (1% to 5%), and high (greater than 5%) prevalence rates (Anyanwu *et al.*, 2018). The results if this study showed that the observed 0.69% prevalence is generally categorised in the low prevalence region of the global HTLV prevalence.

4.9.2. Africa comparison

According to Wang *et al.* (2016), six countries (Botswana, Lesotho, Namibia, Swaziland, South Africa, and Zimbabwe) had an HIV prevalence of more than 10% of the entire population. Nine countries in sub-Saharan Africa (Central African Republic, Cameroon, Equatorial Guinea, Kenya, Mozambique, Malawi, Tanzania, Uganda, and Zambia) had a prevalence of more than 2 - 5% of the entire population (Wang *et al.*, 2016). Outside sub-Saharan Africa, a further 11 countries (the Bahamas, Belize, Bermuda, Dominican Republic, Guyana, Haiti, Cambodia, Portugal, Suriname, Trinidad and Tobago, and Saint Vincent and the Grenadines) had prevalence rates between 0 - 5% and 2 - 5% (Wang *et al.*, 2016).

Comparing the results from this study to the African prevalence of HIV it is observed that the prevalence of HIV in the patient population of the SANBS is significantly higher as shown in Chapter 4 (39.38%). Zampino *et al.* (2015) suggested that Africa is overall considered to have a high HBV endemicity. HBV infection is hyperendemic and there are more than 8% hepatitis B surface antigen (HBsAg) chronic carriers in the general population of sub-Saharan African countries such as Nigeria, Namibia, Gabon, Cameroon, Burkina Faso (Zampino *et al.*, 2015).

The HBV prevalence results from this study (7.56%) are in line with the study of Zampino *et al.* (2015) (8%) for the Sub-Saharan African population. Anyanwu *et al.* (2018) reported on HTLV prevalence in Africa and estimated the prevalence to be between 6.6% and 8.5% in Gabon, 1.05% in Guinea, 3.2% in Congo, 5.5% in Nigeria,

2.7% and 19.5% in Kenya. The prevalence lies between 0.5% and 4.2% in Ghana, 0.9% in Cameroon, 1.5% in Mozambique, 0.6% in Central African Republic, 1% in South Africa, 0.63% in Malawi and >15% in Seychelles. The prevalence of 0,69% in the patient population of the SANBS are in line with Zampino *et al.* (2015) findings.

4.9.3. South Africa comparison

According to Stats SA (2018), the estimated overall HIV prevalence rate is approximately 13,1% among the South African population. The total number of people living with HIV is estimated at approximately 7.52 million in 2018. In chapter 4 of this study, the HIV prevalence in the SANBS patient population is threefold higher (39.38%) compared to the estimates of Stats SA (2018) for the general population of South Africa (13.1%). According to Vermeulen *et al.* (2019a), there were 0.2% HIV-positive donations over a 10-year period. According to Parikh and Veenstra (2008) an HIV prevalence of 25.7% was found among patients visiting primary health care facilities across four South African provinces which is slightly below what was found in this study and we need to take into account that the patients in this study might have been receiving blood due to the causative symptoms of these viruses. When comparing the results of the two primary health care facilities clinics which was mentioned in Parikh and Veenstra (2008), the HIV prevalence was found to be 34% and 36% which are in line with the findings within this study.

The prevalence of HBV in first-time blood donors within South Africa was 1.13% (Vermeulen *et al.*, 2019b). According to Schweitzer *et al.* (2015) the HBsAg seroprevalence was estimated at 6.7%, pointing to high intermediate endemicity with an estimated 3.5 million individuals chronically HBV infected in South Africa. Based on the results of this study there is a slightly higher prevalence of HBV in the patient population of the SANBS (7.56%) compared to the general population of South Africa (6.7%). With the introduction of the mandatory HBV vaccine in 1995 (Prabdial-Sing *et al.*, 2019), it was expected that there would have been a noticeable decrease in HBV prevalence in the younger age groups of 30 years and below (7,27%) compared to the age groups 31 years and above (6,94%) which was not the case within this study and again reference must be made to this studies population as it is patients in need of a blood transfusion and we can speculate that this can be as a result of the symptoms

associated to these viruses and cannot be compared to the South African general population.

According to Vermeulen *et al.* (2017) the prevalence of the HTLV virus in the first time blood donor population represented 0.66% of their study population, which are significantly lower compared to other studies within the general population. Anyanwu *et al.* (2018) estimated the HTLV prevalence in South Africa to be 1%, which is slightly higher than what was found in this study (0.69%). A study was done on new blood donors in South Africa and the prevalence in these donors was estimated at 0.062% in 2013 (Vermeulen *et al.*, 2019b), which is much lower compared to the results in chapter 4 of this study.

This can be due to blood donors being a healthy cohort and that the donor questionnaire is designed to remove at risk populations which is why the prevalence in donors is much lower than the study and general population. When comparing the study population to the general population we can also speculate that the study population is a non-healthy population and could also be receiving transfusions for anaemia hence the higher prevalence rate.

4.10. CONCLUSION

This chapter highlighted the prevalence of HIV, HBV, HTLV and concurrent infections which was compared to gender, race, age, location or zone, blood group and hospital class with reactive and non-reactive samples and used logistic regression to find the probabilities using one group within each category as a reference. The overall results have shown the patient population of SANBS has a higher prevalence of HIV, HBV, HTLV and concurrent infections compared to the general population.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1. INTRODUCTION

This chapter presents the summary of the findings, conclusions and recommendations based on the data analysed in the previous chapter. Through addressing the aims and objectives, the findings of this study brought insight into the prevalence of HIV, HBV, HTLV and concurrent infections in the patient population of the SANBS. The findings in this study have proved, patients receiving a blood transfusion from SANBS have high rates of HIV, HBV and HTLV which should be taken into consideration when determining donor screening strategies and some limitations have been identified.

5.2. RESEARCHER'S CONTRIBUTION

Ethical clearance was obtained from VUT and the SANBS as mentioned in Chapter 1. The researcher is an HPCSA registered medical technologist specialised in blood transfusion technology (MT0114219) and took responsibility for the planning, analysis and reporting of this study, starting with problem identification, study design and proposal writing under the leadership and guidance of the supervisors Dr C.J. Grobler and Mrs. M. Vermeulen. The researcher planned, presented and coordinated the specimen collection within all the zones.

All samples were tested according to standard operating procedures and supplier recommendations by the researcher under the guidance of ABBOTT laboratory, Dr C.J. Grobler and Mrs M. Vermeulen. Data analysis and statistical interpretation of results were performed by the researcher using IBM SPSS® Statistics version 25 software. The results were analysed and interpreted by the researcher with guidance from the supervisor Dr C.J. Grobler. The assembling of the dissertation towards obtaining MTech Biotechnology qualification and the articles to be published was also done by the researcher. Additionally, an abstract for presentation at the 35th South African National Blood Transfusion Congress in 2019 was also submitted.

5.3. LIMITATIONS

After the particulate matter and fibrin clots were removed, 843 samples did not have the required sample volume for testing. All the samples which were greater than 1 ml, but less than 2 ml of serum or plasma were diluted to 2 ml with saline and all samples which had less than 1 ml of serum or plasma were removed and not tested. A total of 66 samples was not tested due to sample volume.

Note that there is a possibility that of the 123 HIV negative, 731 HBV negative and 839 HTLV negative samples which were diluted with saline could have had a low antibody titre to HBV, HTLV and/or HIV which could have present as false-negative results. This can result in an under presentation of the prevalence of HIV, HTLV and HBV in this study.

This study was conducted on the transfusion recipient patient population of the South African National Blood service and only and did not include other patients.

5.4. MAIN FINDINGS

The main findings of this study are outlined as follows:

5.4.1. Problem and setting

- Currently, the South African National Blood Services are not testing for HTLV and HTLV screening as it is not recommended by the WHO or by regulatory standards in South Africa (Ingram et al., 2015).
- The prevalence of HBV, HTLV, HIV and concurrent infections of these viruses in patients receiving blood and blood products is currently unknown.
- Transfusing HIV/HBV infected patients with HTLV can possibly worsen their prognosis and accelerate the clinical progression to AIDS that is untested in South African communities.

5.4.2. Literature review

- The main modes of transmission of HTLV are vertically from mother to child, horizontally by sexual intercourse, and by transfusion of blood and blood cellular products (Tweteise *et al.*, 2016).
- Transfusion is the most infectious mode of transmission as it delivers a large viral load, resulting in seroconversion rates of 44–63%, and the interval from infection to disease is also shortened (Tweteise *et al.*, 2016).
- Vermeulen *et al.* (2019b) found in their study, an overall prevalence of 0.062% among blood donors of the SANBS and concluded that the increasing number of donors found to be HTLV positive is cause for concern, especially considering the efficiency with which the virus is transmitted via exposure to blood and blood products.
- HTLV may be detrimental to the HIV-infected individual, with increased risk for the development of neurologic complications including TSP/HAM, leukaemia, and lymphoma (Beilke, 2012).
- HTLV Tax protein up-regulates HIV expression, as well as expression of various cytokines and cytokine receptors involved in T-cell activation, thereby providing a favourable condition for HIV infection, and exacerbates the cytopathic effects of HIV that accelerate the clinical progression to acquired immune deficiency syndrome in individuals infected with both HIV and HTLV (Casoli *et al.*, 2007).
- Laboratory test methods are the same in HTLV, HBV and HIV (Amini et al., 2017). Most blood transfusion services have a first-line serological test which is one of immunoassays principles and nucleic-acid amplification tests (NAT) as the second line test (Amini et al., 2017).
- The human body produces antibodies to HTLV, HBV and HIV which can be tested for using enzyme immunoassays (EIA), electrochemiluminescence immunoassays (ECLIA), microparticle enzyme immunoassays (MEIA) and chemiluminescent microparticle immunoassays (CMIA) (Amini et al., 2017).

5.4.3. Methodology

- This study was ethically approved and applied principles of autonomy, beneficence, non-maleficence and equal distribution of risk throughout the study.
- Sample size was greater than the minimum sample size required to determine the statistical significance of the prevalence of HIV, HBV, HTLV and concurrent infections in the patient population of the SANBS, as calculated using the power calculation.
- The study was performed on patients receiving blood from all the regions or zones of the SANBS.
- Design of the study was a double-blinded, retrospective experimental study on a convenience selected sample, based on the recommendations by the World Health Organisation on screening donated blood for transfusion transmissible infection in Africa (WHO, 2010).
- Specimens which was collected were tested using the ABBOTT Alinity S® Immunochemiluminescent auto analyser to determine the HIV, HTLV, HBV and concurrent infections within the patient population of the SANBS.
- Raw data was captured on Microsoft Excel and imported to IBM SPSS® Statistics version 25 software for descriptive and inferential statistical analysis.

5.4.4. Results and interpretation

- Female patients had the highest prevalence of HIV, HTLV, HBV – HTLV concurrent infection, HIV – HTLV concurrent infection and HIV – HBV – HTLV concurrent infection.
- Male patients had the highest prevalence of HBV and HIV – HBV concurrent infections.
- The black population had the highest prevalence of HIV, HBV, HTLV and HIV – HBV concurrent infections and the second highest prevalence of HIV – HTLV concurrent infections.
- Asian or Indian populations had the highest prevalence of HIV – HTLV concurrent infection, HBV- HTLV concurrent infection and HIV – HBV – HTLV concurrent infection.

- Unknown race specimens had the second-highest prevalence of all infections except for HIV – HTLV concurrent infection.
- Mpumalanga had the highest prevalence of HIV, HBV and HIV – HBV concurrent infection and the second-highest HIV – HTLV concurrent infection, HBV – HTLV concurrent infection and HIV – HBV – HTLV concurrent infection.
- KZN had the highest prevalence of HIV – HTLV concurrent infection, HBV – HTLV concurrent infection and HIV – HBV – HTLV concurrent infection.
- Free State and Northern Cape had the highest prevalence of HTLV.
- 31 to 40-year-old age group had the highest prevalence of HIV, HBV and HIV – HBV concurrent infections.
- 41 to 50-year-old age group had the highest prevalence of HTLV, HIV – HTLV concurrent infection, HBV – HTLV concurrent infection and HIV – HBV - HTLV concurrent infection.
- Unknown blood group patients had the highest prevalence of HIV and HTLV.
- AB blood group patients had the highest prevalence of HBV and HIV – HBV concurrent infection.
- B blood group patients had the highest prevalence of HIV – HTLV concurrent infection, HBV – HTLV concurrent infection and HIV – HBV – HTLV concurrent infection.
- Provincial hospital patients had the highest prevalence of HIV, HBV, HTLV, HIV – HBV concurrent infection and HIV – HTLV concurrent infection.
- Private hospital patients had the highest prevalence of HBV – HTLV concurrent infection and HIV – HBV – HTLV concurrent infection.

5.5. CONCLUSION AND RECOMMENDATIONS

In conclusion, the results show a higher prevalence of HIV, HBV, HTLV and concurrent infection in the patient population compared to the donor population (refer to Chapter 4: 4.6). The results of this study are in agreement with other studies except for HIV which had a significantly higher prevalence in this study (refer to Chapter 4: 4.6). This study confirmed an overall high prevalence of HIV and HBV infections among patients receiving blood products from SANBS. Unlike HBV and HTLV, the prevalence of HIV among the general population is well known.

Compared to the general population, the HIV prevalence in blood recipients was three-fold higher. This may be due to HIV positive patients becoming anaemic and requiring blood transfusions, increasing their risk of acquiring HTLV through transfusion. Patients receiving a blood transfusion from SANBS have high rates of HIV, HBV and HTLV which should be taken into consideration when determining donor screening strategies.

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Annexure A: SANBS Human Research Ethics Approval Letter

SOUTH AFRICAN NATIONAL BLOOD SERVICE NPC

Human Research Ethics Committee

SANBS
South African National Blood Service
Association Incorporated Under Section 21
Registration No. 2000/026390/08

OHRP Number : IORG0006278
FWA Registration Number : IRB00007553
SA NHREC Registration Number : REC-270606-013

Secretariat: Tel: 011 761 9135 | Fax: 011 761 9137 | felicity.lew@sanbs.org.za

To : Mr Reynier Willemse
E-mail : reynier.willemse@sanbs.org.za

Dear Mr Willemse,

DATE OF COMMITTEE MEETING	**:**	21 February 2017
PROJECT TITLE	**:**	The Prevalence of HBV, HTLV and HIV In Patients receiving Blood and Possible Implications Of Transfusion Transmitted HTLV
DECISION OF THE COMMITTEE	**:**	Approved
CLEARANCE CERTIFICATE NO	**:**	2017/13

1. Execution of the study must be compliant with applicable guidelines and policies.
2. Any amendment, extension or any other modifications to the protocol must be submitted to this Ethics Committee for approval prior to implementation.
3. The Committee must be informed of any serious adverse event, planned and unplanned termination of the study.
4. A progress report should be submitted yearly for studies longer than a year and a final report at completion of the study for both short term and long term studies.
5. Kindly refer to the SANBS HREC clearance certificate number on all future correspondence on this study to the HREC secretariat.
6. This approval is valid for 5 years from the date stated above.

COMMITTEE GUIDANCE DOCUMENTS:

- International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guideline (ICH, 1996), Ethics in Health Research: Principles, Structures and Procedures (SA Department of Health, 2015); Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa (SA Department of Health, 2006); Ethical Principles for Medical Research Involving Human: Declaration of Helsinki (World Medical Association, 2013); Reviewing Clinical trials: A Guide For Ethics Committees (Karlberg and Speers, 2010).

CHAIRPERSON: Prof J.N. Mahlangu

24 March 2017
DATE

Annexure B: Nexus Database Letter

VUT
Vaal University of Technology

To whom it may concern

This letter serves to inform that literature search has been performed on behalfWillemse, R (214142523) on the Topic:

"THE PREVALENCE OF HBV, HTLV, HIV AND CONCURRENT INFECTIONS IN BLOOD RECIPIENTS OF SOUTH AFRICA NATIONAL BLOOD SERVICE"

Currently there are no records on the NRF Nexus Database. The search was conducted by Thomas, NG on the 29-May-2017.

Signed .....

VUT
Vaal University of Technology
Your world to a better future

Zodwa Thomas | Information Specialist: |
Library Information Services
Client Services: Applied and Computer Sciences
Email: zodwa@vut.ac.za |x9650

Annexure C: VUT Proof Of Registration

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Tel: 054 332 3304
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WILLEMSE RJ
PO BOX 7621
SECUNDA
2302

214142523
29 March 2019

Proof of Registration: Qualification and Subject Information

615003: M TECH: BIOTECHNOLOGY

Subject Code	Description	Qual Code	OT Exam Type	Date Registered	Exam Year	Exam Mnth	Date cancelled	Exempt/ Enrolm	Primary Enrolm
	YEAR Subjects								
ABBRPSA	RESEARCH PROJECT AND DISSERTATION	615003	VP NORMAL EXAM	29-MAR-2019	2019	11		BOTH	Y

End Of Report

Annexure D: VUT Approval and Acceptance Letter

Vaal University of Technology
Your world to a better future

VUT

To: Faculty Research and Innovation Committee
From: Dr. T. A. Walmsley
Date: 19th June 2017
Subject: Proof that student has presented their proposal at a Departmental/Faculty seminar

This letter serves to confirm that Mr R Willemse (student number: 214142523) has presented his MTech proposal to the Department of Biotechnology. The research project meets the minimum requirements for the qualification and may now be submitted to the Higher Degrees Committee for final approval and acceptance.

Dr. T. A. Walmsley
(Senior Lecturer, Department of Biotechnology)

Ms. C. S. Van Wyk
(Acting HOD, Department of Biotechnology)