An Investigation of Saccadic Eye Movement Abnormalities in Children with HIV/AIDS on Highly Active Antiretroviral Therapy

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Dedication

To my parents, sister, brother-in-law, dear friends and colleagues for their continued words of encouragement and support

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to all the children living with HIV/AIDS that had participated in this research and those that may have passed on during the writing of this thesis, my heartfelt gratitude to them, for which this study would not have been possible. May they be blessed.

Abstract

Introduction: The Human Immuno-deficiency Virus (HIV) and the consequent Acquired Immuno-Deficiency Syndrome (AIDS) have cost the lives of millions of people globally over the past 30 years since the first cases of illness appeared. Due to the overlap in areas in the brain that are damaged by the HIV with those that control saccadic eye movements, screening of eye movement functions in children with HIV/AIDS could thus be a valuable early indicator of a declining neurological and immunological state. Therefore, movement testing through non-invasive means may give the optometrist valuable insight into the developing central nervous system (CNS) in HIV-infected children.

Aim: To determine if abnormal saccadic eye movements in children with HIV/AIDS on HAART could be a predictor of the status of their immune system.

Methodology: The study population comprised of 128 conveniently selected subjects aged 5 to 14years diagnosed with HIV/AIDS on HAART. This prospective study, used a descriptive design. The two significant biological parameters such as CD4 count and viral load (VL) data of patients were accessed and subjects performed the DEM test, which is a visual-verbal reading speed test, used to detect oculomotor function as well as automaticity skills. The subjects were then classified according to the different 'behaviour types' as is specified in the DEM test based on their test performances. Statistical Analysis Software (SAS) version 9.2 was used to analyse the data.

Results: Nine year olds were the most prevalent comprising of 23% of the sample. Subjects were categorised into three categories of their VL and CD4 count parameters from minimal to severe immunosuppression. Seventy eight percent (78%) of subjects had minimal immunosuppression with CD4 counts $\geq$500cells/mm^3 with a median value of 778.5 cells/mm^3. Sixty five percent (65%) of the subjects had undetectable VL (<40 copies/mm^3) with the median value of <40 copies/mm^3 in the sample. With the DEM test, 93% had vertical and 92% had horizontal times that were outside of the standardised DEM norm. The classification of subjects into behaviour types revealed that 53% were type 3 – automaticity problems, 22% type 4 – oculomotor problems and automaticity problems, 8% type 1 – normal performance and 3% were type 2 – oculomotor dysfunction. Fourteen percent were in the unspecified

behaviour type category. The relationship between the VL with behaviour types (p=0.2) and the CD4 count against the behaviour types (p=0.17) were neither statistically nor clinically significant, hence no relationship could be established.

Discussion: Since the cognitive functioning in children with HIV/AIDS was moderately affected, the DEM test could be a valuable tool, if not to only detect eye movement problems but to assess the automaticity skills, which shows the impact on their neurodevelopment. It therefore does prove to be worthwhile for optometrists and other health professionals to use the DEM test as part of a battery of neurodevelopmental tests to assess different neurocognitive functions, specifically in children with HIV/AIDS.

Recommendation: DEM norms for a South African paediatric population should be established as the characteristics of this population differ from the population of English-speaking American children on which this test was standardised.

Conclusion: Immunologic and virologic statuses in children with HIV/AIDS on HAART cannot be predicted from abnormal saccadic eye movements. Performances across all age groups were significantly below the standard DEM norms. Saccadic eye movement abnormalities were the least prevalent and automaticity deficiencies were the most prevalent across the sample with no associations to the CD4 count and viral load.

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List of Acronyms

ADC	**AIDS Dementia Complex**
ANI	Asymptomatic Neurocognitive Impairment
ARV	Anti-retroviral
BBB	Blood Brain Barrier
BG	Basal Ganglia
BIPAI	Baylor College of Medicine International Pediatric AIDS Initiative
CDC	Centre for Disease Control and Prevention
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DEM	Developmental Eye Movement
DHHS	Department of Health and Human Services
DLPFC	Dorsolateral Prefrontal Cortex
DNA	Deoxyribonucleic Acid
DOH	Department of Health
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FEF	Frontal Eye Fields
HAART	Highly Active Anti-Retroviral Therapy
HAD	HIV-Associated Dementia
HAND	HIV-Associated Neurocognitive Disorder
HSRC	Human Sciences Research Council
II	Integrase Inhibitor
K-D	King-Devick Test
KZNDOH	KwaZulu Natal Department Of Health
MND	Mild Neurocognitive Disorder
MTCT	Mother To Child Transmission
NHLS	National Health Laboratory Services
NDOH	National Department of Health
NNRTI	Non-Nucleotide Reverse Transcriptase Inhibitor
NRTI	Nucleotide Reverse Transcriptase Inhibitor
NSUCO	Northeastern State University College of Optometry
NYSOA K-D	New York State Optometric Association King-Devick Test
PCR	Polymerase Chain Reaction

PEF	Parietal Eye Fields
PI	Protease Inhibitor
RAN	Rapid Automatic Naming
RNA	Ribonucleic Acid
SANAC	South African National AIDS Council
SCCO	Southern California College of Optometry
SEF	Supplementary eye fields
UNICEF	Unites Nations Children's Fund
UNAIDS	Joint United Nations Programme on HIV/AIDS
VL	Viral Load
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

Medical science is continuously searching for quicker, pragmatic, less invasive, and preferably cost-effective modes of investigation to study human behaviour and disease processes. This is done in pursuit of finding quicker answers with a focussed goal of promoting longevity. Modern medicine has widely accepted the concept that the sooner a disease or disorder is diagnosed, the sooner treatment interventions could be implemented. This would result in better outcomes to sustain and prolong healthier living with minimal compromise. This paradigm is a key concept in the approach to dealing with the burden of the Human Immunodeficiency Virus (HIV) and Acquired Immuno-Deficiency Syndrome (AIDS) worldwide.

Clinicians are often challenged when investigating the central nervous system (CNS) to assess for defects without having to expose the patient to continuous and regular radiological investigations. Some of these investigative techniques are invasive, costly and unfeasible in resource-limited settings and may sometimes not be conclusive of abnormalities in the very early stages of disease. Eye movement testing, as a means to investigate CNS function, may not satisfy all of the desirable factors that medical science wants to achieve to study human behaviour and pathophysiology. However, for the past three decades, eye movement testing has been used more frequently as an experimental tool to gain insight into HIV and AIDS-related diseases, learning-related disorders, psychiatric disorders, neuromuscular disorders and

neurological disease (Leigh and Kennard, 2004; Kumra et al., 2001; Rayner, 1998; Amador et al., 1995; Currie et al., 1988; Hoffman and Rouse, 1980).

The motive for placing value in studying eye movements in different diseases are for the very simple reason that eye movement control centres are located in the brain, providing us with a window to the CNS. Eye movement testing is intrusive yet non-invasive. It has been said that activity related to eye movements can be found in every corner of the brain (Leigh and Kanna, 2006; Leigh and Zee 2006). The study of eye movements has been a useful source of information to clinicians and scientists where functional impairments can be a clue in localization of disease processes and the monitoring of existing disorders or diseases (Leigh and Zee 2006). Leigh and Kanna (2006) further stated that eye movements are being used as an experimental tool in neuropsychological studies of memory and cognition.

With the introduction of Highly Active Anti-Retroviral Therapy (HAART) in 1996 (The CASCADE Collaboration, 2000), HIV has become as manageable as a chronic disease to those who have access to medication (Palella et al, 1998). The overarching goals of antiretroviral therapy are to reduce HIV-related morbidity, improve quality of life and prolong survival of infected individuals. The main drivers of the pandemic in South Africa are sexual transmission in adults and by mother-to-child-transmission (MTCT) via breast milk in children (Baylor College of Medicine International Pediatric AIDS Initiative (BIPAI) 2010, pp.1-6).

An inability to detect the virus in the blood while on HAART does not indicate clearance or absence of the virus from the body but that it is in a very low concentration, below the assay/sample threshold for that specific instrument used for analysis. It may be found in other parts of the body, especially the CNS where it is in higher concentrations because of the variable blood-brain penetrative abilities of anti-retroviral drug therapy (Kolson, Lavi and Gonzalez-Scarano, 1998).

During the primary stage of infection of the human body by the HIV, the virus rapidly progresses to the central nervous system where it remains in a viral reservoir as found in cerebrospinal fluid (CSF) studies (Pilcher et al, 2001; De Luca et al, 2002). A consequence of this neuro-invasion is the development of abnormal neurological and neurocognitive manifestations (Brouwers, Walters and Civitello 1998, pp.293-308) broadly termed HIV encephalopathy (Pilcher et al, 2001) with manifested eye movement abnormalities as one of the later signs. HAART has resulted in the lower incidence of HIV-related encephalopathy (Tardieu, 2000), however children still exhibit neurocognitive decline in the presence of HAART. This reinforces the need for the development of screening and diagnostic tools to assess the neurocognitive and CNS functioning in HIV-infected children while the challenge to develop effective CNS-penetrating antiretrovirals continues.

Special attention must be paid to the early detection of neurologic problems in children since their CNS is not completely developed and therefore vulnerable to damage. Early detection and appropriate treatment of HIV-associated neurologic

problems in children often leads to favourable outcomes (BIPAI 2010, pp. 194-205). Infected children develop neurological dysfunction more frequently from HIV encephalopathy whereas infected adults develop neurologic dysfunction more frequently through opportunistic infections as the CNS as they harbour latent pathogens which produce disease when HIV-related immunologic depression occurs (Civitello 2005, pp.431-44).

What remains vague and insufficiently researched is the pathogenic process of HIV progression through the developing CNS of children. It is also still unknown at which point a neurologic impairment occurs, that is observable and measurable through non-invasive techniques, while on HAART. In addition, a consistent predictive marker of early and progressive neurologic impairment is yet to be established in this HAART era.

Saccadic-type of eye movements are rapid eye movements that occur to align the visual axes of both eye with objects of interest where the eyes make a conjugate movement to jump from one object to the next (Johnson and Everling, 2008). Typically, about three saccadic eye movements are made every second in everyday life without even being aware of it (Rayner, 1998) making it the most common type of eye movement action that we voluntarily and involuntarily use in everyday life. Saccades are used to scan our visual world as a way of gathering information through visual search (Leigh and Zee 2006, pp. 3-19). The first use of the word *saccade* to describe this observed rapid eye movement that occurred during reading was by visual

scientist, Javal in 1879 (as cited by Caldara and Miellet, 2011) and has continued to be used and researched today over a century onwards, as an authentic tool to explore an array of diseases and paradigms in the neurosciences (Leigh and Kennard, 2004).

Screening for ocular motility abnormalities by assessing saccadic eye movements forms part of a basic optometric examination (Scheiman et al, 2002). Assessments are done by gross observation through subjectively-induced motility tasks with rating scales to grade the eye movements (Maples, Atchley and Ficklin, 1992). In a clinical setting, an alternative to the routinely employed gross observation methods of saccadic testing is the use of psychometric tests. The Developmental Eye Movement test (DEM) is a simple psychometric test that is recommended as an appropriate oculomotor assessment tool in optometric clinical practice for school-aged children (Garzia et al, 1990; Tassinari and Deland, 2005).

Manifestation of saccadic irregularities in HIV positive children in an optometric examination may indicate HIV-related neurologic complications and subsequently lead to early referral for further investigation and intervention. The DEM test could be used as a non-invasive screening tool to monitor CNS disease progression as it is established that the architecture and control centre of eye movements is located in the brain. This approach is analogous to the use of fundus photography as a screening and monitoring tool in patients with diabetic retinopathy (Williams et al, 2004). Alternatively, the CD4 count and viral load may be able to predict if a child would have saccadic dysfunctions indicative of a declining neurological state.

Neurological preservation is one of the key outcomes of antiretroviral therapy therefore ocular motor testing and its performance rates could be used along with other cognitive, perceptual and functional tests, as a non-invasive CNS-probing aid in children to monitor treatment efficacy in achieving and sustaining neurological preservation. These DEM test scores in children could be an adjunct investigation to neuropsychological testing and other concomitant investigations as part of a battery of tests to examine a single parameter of neurologic functioning. The results may be consolidated to provide prognostic evidence to implement the appropriate interventions to achieve a better clinical outcome.

By investigating saccadic eye movements in HIV positive children, the researcher sought to find out if saccadic eye movement disorders can be a marker for a declining neurological and neuropsychological state in HIV infected children on HAART. Secondly, the researcher intended to explore if manifestations of these ocular motility abnormalities correlate well with the progressive nature of the disease by evaluating its association to biomarkers such as CD4 count and viral load in the presence of HAART.

Paediatric research in neurocognitive and neurodevelopmental anomalies in HIV/AIDS remains scarce as there are currently no published studies on saccadic eye movement abnormalities in HIV positive children in South Africa and Sub-Saharan Africa. Considering the current situation, this study has legitimate scientific value in

contributing to the body of knowledge on the role of child-based saccadic eye movement testing in HIV/AIDS for health professionals and health scientists. Insight into this is crucial in the overall interest of longevity and neurological preservation with healthy cognitive development and maturation of an infected child.

HIVAIDS remains a major concern to all South Africans as this epidemic not only significantly affects the South African health infrastructure by straining human and medical resources, but also has resulted in a plunge in the life expectancy of the South African population (Mba, 2007). It further carries a heavy social and economic burden to South Africa. As health professionals and researchers, we need to find ways, within our own scopes of practice, to support and contribute towards universal health care to confront the challenges of HIV/AIDS that face South Africa.

Saccadic eye movement screening has contributed significantly in other areas of human biology in the understanding of disease processes. For this reason, it is thus prudent to investigate the value and reliability of this tool in contributing knowledge in HIV/AIDS, as it is currently our most relevant and magnanimous disease burden to date.

CHAPTER TWO

LITERATURE REVIEW

This chapter is divided into two sections. The first section provides a foundational framework that defines the disease, an overview of its epidemiology with global and local perspectives on health care strategies and implementation. The second section provides a focused review on the various published scholarship related to this study, namely, saccadic eye movement testing, tools of CNS investigation in CNS disorders and their application in HIV/AIDS and universal health care.

2.1 THEORETICAL FRAMEWORK

2.1.1 HIV/AIDS: Global and National Epidemiological Outlook

In 1981, in the United States of America (USA) and elsewhere, a new disease, an unnamed syndrome appeared in a few homosexual men and was primarily characterised by a deficiency in the immune system. The virus called HIV was first cultured in 1983 and subsequently identified in 1984 as the causative viral agent that weakens the immune system (Barré-Sinoussi et. al, 1983; Popovic et al, 1984) and the cause of the disease called AIDS (Barré-Sinoussi, 1996; Singh, Bairy and Shivananda, 2003). Early observations showed that the common modes of transmission of HIV were spread through intimate sexual contact e.g. genital fluids, blood and MTCT (Jaffe, Bregman and Selik, 1983).

According to the World Health Organisation (WHO) for the purposes of HIV case definitions for reporting and surveillance, a child is defined as less than 15 years of age and an adult as 15 years and over (WHO, 2007). Current global figures of the HIV epidemic estimates are that there are 34.2 million [31.8 – 35.9] people living with HIV in the world today of which 3.4 million [3.0 – 3.8] are children (UNAIDS, 2012b). It is estimated that 90% of all HIV positive children live in Sub-Saharan Africa where the global burden of HIV remains the greatest (WHO, UNAIDS and UNICEF, 2010; UNAIDS, 2010). In 2010, 1.8 million people died globally through AID-related illness of which 250 000 were children (WHO, UNAIDS and UNICEF, 2010). There are 8 million people that have access to antiretroviral treatment in middle to low economic countries with 562 000 of those being children (UNAIDS, 2012a). The WHO declared that the lack of sufficient access to HIV treatment is a global health emergency and called for urgent strides across all nations to ensure increased global access to treatment (WHO, 2004). There is an international drive to reach 15 million people by 2015 with antiretroviral therapy as set out by the 2011 Political Declaration on HIV and AIDS with unanimous support from the United Nations' member states (UNAIDS, 2012a).

Since the first recorded case of HIV/AIDS in South Africa in 1982 (Karim and Karim, 2002), the same year the term 'AIDS' was coined, it has reached pandemic levels on its 30th anniversary with 5.38 million South Africans infected (Statistics SA, 2012a). A 2010 report by the WHO placed the number of people living with HIV and AIDS in South Africa at 5.6 million [5.4 – 5.9] (WHO, UNAIDS and UNICEF, 2010, UNAIDS, 2012a). South Africa had a HIV prevalence rate of 10.6% in 2011

(Statistics SA, 2012a) with the highest recorded absolute number of HIV positive adults and children in the world, which exceeds the number of infected people in all of Asia. There was an estimated number of 377 000 children between the ages of 0 and 14 that are in need of HAART with an estimated number of 105 000 children in the same age category that were receiving it in 2011 (Statistics SA, 2012a). The UNAIDS recorded 29 100 new HIV infections in children under 15 years of age in 2011 in South Africa (UNAIDS, 2012a).

In the Free State province, there were 345 000 people living with HIV in 2009 (UNAIDS, 2012b). As of June 2011, there were 152 ARV treatment facilities in the province with the number of children registered on HAART being 9643. Due to attrition for a number of reasons, the actual number was reduced to 8020 (Republic of South Africa (RSA). Free State Department of Health, 2011). Although these figures do paint a bleak picture on the global outlook, the tide is turning with a steady decline in AIDS-related deaths due to HAART as well as a decline in the incidence of new HIV infections in South Africa and abroad (WHO, UNAIDS and UNICEF, 2010; UNAIDS 2012a, 2012b). Global figures of children living with HIV seem to be levelling off as well (WHO, UNAIDS and UNICEF, 2010). Even though South Africa has made significant gains in addressing the HIV/AIDS epidemic over the past 10 years, there unfortunately still remains a significant gap in treatment distribution between the number of adults versus the number of children, globally and locally (UNAIDS 2012a, 2012b).

This three-decade long epidemic has claimed millions of lives and will continue to claim many more but a hope for a cure is insight as reported in an article by 2008 Nobel Laureate of Medicine, French researcher Francoise Barré-Sinoussi, who was part of a team that discovered HIV (SAPA-AFP, 2012). She further shared some optimism by citing a case of a patient that was cured of HIV in Berlin after having a bone marrow transplant in 2009 (SAPA-AFP, 2012). There has been documented cases of less than 5 people in the world that have been cured from HIV.

2.1.2 Testing, Diagnosis and the Classification System of HIV/AIDS in Children, globally and in South Africa.

Diagnosis of HIV infection in children born to HIV-infected mothers is not as simple when compared to that in adults. The complication exists by the presence of maternal anti-HIV IgG antibodies in the blood of the new borns due to trans-placental transmission to the fetus (Center for Disease Control and Prevention (CDC), 1994). These maternal antibodies usually remain in a child up to 18 months of age so HIV antibody testing within this window period would be unreliable in confirming the diagnosis of HIV infection (Simpson and Andiman, 1994). Children born to HIV infected mothers are classified as 'HIV exposed' prior to the confirmation of HIV status. It is only after this period of 18 months that the status can be changed to a 'seroreverter' or HIV negative if enzyme immunoassay (EIA) testing reveals absence of HIV antibodies (CDC, 1994). Polymerase Chain Reaction (PCR) and virus culture are probably the most sensitive laboratory assays for detecting HIV infection in

children born to infected mothers prior to 18 months of age as these tests directly investigate the presence of viral genetic material in the blood of new borns (CDC, 1994; BIPAI 2010, pp. 7-14).

Initially in 1990, the WHO developed a four-stage clinical HIV/AIDS staging system for adults. The WHO thereafter developed a pre-HAART classification system for infants and children for clinical staging and immunological profiling of HIV infection and related disease. This revised four-stage system that was originally intended for surveillance and case reporting (WHO, 2007; BIPAI 2010, pp.15-44; RSA, Kwa-Zulu Natal Department of Health (KZN DOH), 2010; USA, Department of Health and Human Services (DHHS), 2011) was then developed for other purposes. This updated system was intended to assist in clinical management of HIV, determination of prognosis, strengthening of the clinical diagnosis, guidance in decisions regarding treatment initiation and to facilitate the scaling up of access to antiretroviral therapy (WHO, 2007). The WHO classification system closely relates to the system used CDC, which has classified HIV infection in children into three domains which were, Infectious Status, Immunologic Status and Clinical Status that which are still used today (CDC, 1994).

A CD4 cell count is a direct indicator of how healthy the immune system is and is expressed as cells per microlitre (μL) or cubic millimeters (mm^3) (USA, DHHS, 2011; AIDSINFO, 2011). In children, the CD4 absolute count is expressed as a percentage of CD4 cells that make up the total lymphocyte count and not always as an

absolute value as is expressed with adults. Among children younger than 5 years of age, the absolute CD4 count tends to fluctuate more than the CD4 percentage as compared to children older than 5 years of age. It is for this reason that the measurement of the CD4 percentage was thought to be more reliable in younger children 5 years old or less (Raszka et al, 1994). This is due to the variability in the absolute value measurement which could be influenced by a series of physiological factors and concurrent illnesses (Raszka et al, 1994; WHO, 2007). However, the availability of laboratory equipment to produce the CD4 percentage in resource-limited settings is not always available whereas equipment to determine the absolute CD4 value is more readily available (WHO, 2007). The absolute CD4 cell count and CD4 percentage in uninfected infants are considerably higher than those observed in uninfected adults (WHO, 2007). The CD4 count then stabilises to adult levels by 6 years of age. Age must therefore be taken into account as a variable in considering absolute CD4 counts and CD4 percentages (Bunders, Cortina-Borja and Newell, 2005; Ochieng et al, 2006; Shah, 2006).

The HIV viral load (VL) is the best indicator of how active the virus is in the body. Viral load testing is a useful predictor of clinical disease progression (Marschner et al, 1998; Thiebaut et al, 2000). The higher the viral load in the blood plasma, the lower would the CD4 count be as the virus destroys the CD4 lymphocyte cells (Yeni et al, 2002). Viral load testing directly tests the quantity of viral genetic material in the blood plasma using PCR technology and is represented by its level of

replication as copies/ml (Rogers et al, 1989; Krivine et al, 1992; Food and Drug Administration (FDA), 2007).

In the South African public health sector, the Free State Department of Health utilizes the services of the National Health Laboratory Services (NHLS) with a regional branch located at Pelonomi Regional Hospital in Bloemfontein for the CD4 and viral load analysis as well as other HIV-related pathology analyses. The reference interval used by the NHLS for a normal CD4 count is 500 – 2010 cells/ µL. In both HIV-infected children and adults, HIV viral load, CD4 absolute count and percentage are independent predictors of disease progression and mortality risk but with the combined use of these two biomarkers, prognosis of infected individuals can be more accurately determined (Mellors et al, 1996; Mofenson, et al, 1997; Palumbo et al, 1998).

2.1.3 South African ART Programme and Policy Implementation Guidelines

The South African ART programme, known as the Comprehensive Care Management, Treatment and Support (CCMTS) programme was launched in 2003 (RSA. National Department of Health (NDOH)/ South African National AIDS Council (SANAC), 2010a). Although the programme achieved much success, ongoing high mortality from HIV/AIDS indicated that substantial changes were required in order to improve access to HIV care and the overall success of the programme (RSA. NDOH/SANAC, 2010a). One of the biggest improvements was the establishment of a

larger number of ART facilities which stood at 2 552 facilities in South Africa (RSA. NDOH/SANAC, 2012). A new treatment guideline was recommended and is in the implementation phase in South Africa. The proposal for an improved treatment protocol came from supported knowledge that HAART reduces the HIV-related morbidity and mortality if treatment was initiated earlier than was currently practiced subsequently improving the quality of life over an extended period (Palella et al, 1998). These recommendations would however mean a substantial increase in the number of patients who will require both treatment and early HIV testing (Lodi et al, 2011).

The progress update was officially announced on the 1st of December 2009 at the World Aids Day, by the Honourable President Jacob Zuma for new interventions for improving antiretroviral access in order to decrease the disease burden, to address maternal and child mortality and to improve life expectancy (RSA. NDOH/SANAC, 2010a; 2010b). The specific objectives of the new HAART programme in South Africa are to prioritize ARVs for patients with CD4 counts < 200 cells/mm^3 or with severe HIV-related opportunistic disease irrespective of CD4 count, patients co-infected with HIV and tuberculosis (TB) with CD4 < 350 cells/mm^3 and pregnant women with CD4 < 350 cells/mm^3 for lifelong ART and CD4 > 350 cells/mm^3 for prophylaxis (RSA. NDOH/SANAC, 2010a). One of the most sought after improvements recommended was to initiate therapy for all infected infants less than one year of age regardless of CD4 count or virologic standing (RSA. NDOH/SANAC, 2010a; 2010b).

2.1.4 Highly Active Anti-Retroviral Therapy (HAART) in HIV-Infected Children

The specific objectives of antiretroviral therapy are to target maximal suppression of viral load in the body and to preserve immunologic functions (USA. DHHS, 2013). Since the development and commercial use of Zidovudine (AZT) as mono-therapy in 1987, significant advances had been made in antiretroviral therapy with the introduction of HAART in 1996 (The CASCADE Collaboration, 2000; AIDSINFO, 2011). Currently, there are six drug classes of anti-retrovirals in existence with 24 different agents licensed for use, each with their own dosage forms and dose concentrations (Tang and Shafer, 2012). Each class of drugs are unique in their mechanism of action that interrupts at different stages, the life cycle of the virus (Rathbun, 2011; Tang and Shafer, 2012). It is a standardized international recommendation that the drug regimen for HAART initiation be comprised of a 3 drug regimen with 2 agents from the nucleoside reverse transcriptase inhibitor (NRTI) class and 1 agent from either the non-nulceoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI) or the integrase inhibitor (II) classes (WHO, 2004; WHO 2008; RSA, NDOH/SANAC, 2010a). As previously practised in South Africa, the first line drug regimen initiated with HIV positive children were Lamivudine, Stavudine and Efavirenz, however due to the side effects of Stavudine, it has been replaced by Abacavir as an alternative drug from the same class (RSA, NDOH/SANAC, 2010a; RSA KZNDOH, 2010). For those already on the Stavudine, if lipodystrophy is suspected in a child, it is immediately discontinued and substituted with Abacavir (RSA, NDOH/SANAC, 2010a).

Through viral suppression, this gives the body a chance to rebuild its immunity with progressive inclination of the CD4 cell count. An adequate CD4 response for most patients on therapy is defined as an increase in CD4 count in the range of 50–150 cells/mm3 per year, generally with an accelerated response in the first 3 months. Subsequent increases in patients with good virologic control show an average increase of approximately 50–100 cells/mm3 per year (Kaufmann et al, 2003). After the first line anti-retroviral therapy implementation, the viral load should become undetectable (<40 copies/ml) on a 4-6 month follow-up (USA. DHHS, 2011).

Virologic failure is the most common reason for treatment failure ie. an incomplete viral suppression, unsustained suppression or viral rebounding according to the DHHS ART Guidelines (USA. DHHS, 2013). The criteria to determine treatment failure are clinical disease progression, lack of sustained immunological recovery and the viral load values (WHO, 2007). The clinical category in determining treatment failure is the presence of recurrent opportunistic infections, loss of developmental milestones, growth failure or an additional disease from the same disease stage or a disease from the next disease stage. Immunologic failure is determined by a confirmed return of CD4 percentage to the baseline or a drop in 50% of the CD4 percentage from its peak. Virologic failure or failure of 1st line regimen is classified as viral load >1000 copies/ml when tested twice 3 months apart (RSA, KZNDOH, 2010). A second line regimen is implemented if treatment failure occurs, therefore viral load testing on an internationally accepted 6 month interval is crucial to allow timely switching to the next regimen (Médecins Sans Frontières/Doctors Without Borders, 2012). The

decision on when to switch from first-line to second-line therapy is critical as too early a decision would mean that the months or years of potential further survival-benefit from the first line regimen is lost. However, if it is made too late, the effectiveness of second-line therapy may be compromised and the patient is put at risk of a blunted recovery with increased mortality risk (WHO, 2007). Adherence to treatment in a paediatric population is a more complex situation due to an array of factors than that experienced with adult populations (Haberer and Mellins, 2009). The impact of non-compliance and treatment interruption on viral load and CD4 count are serious consequences, which contributes to treatment failure and poor recovery on subsequent treatments thereafter (Tesiorowski, 2001).

2.1.5 HIV Pathogenesis and Life Cycle

There are currently two types of HIV strains identified that are pathogenic to the human species called HIV-type 1 and HIV-types 2. The HIV-1, which is the more prevalent and virulent type is classified into 3 groups, M, N and O. Of these three groups, M is the largest group made up of 9 clades or subtypes namely, A, B, C, D, F, H, J and K. Subtypes E, G and I are classified as genetically mixed or recombinant forms (Levy, 2009). The most prevalent subtype in the world and in Southern Africa is the subtype C. Different subtypes are found to be the prevalent in different geographical regions across the world (BIPAI 2010, pp.7-14). The HIV-1 transmission rate and the pathogenic course of disease progression to AIDS are much quicker than is with the HIV-2 strain and infected individuals survive longer with HIV-2 (Whittle,

1994). The HIV-2 discovered approximately 2 years after the discovery of HIV-1 (Clavel et al, 1986), is more frequent in regions of Central and Western Africa (Kanki et al, 1994; Fanales-Belasio et al, 2010; BIPAI 2010, pp. 7-14). It is almost indistinguishable from the Simian Immunodeficiency Virus (SIV), which was found in its natural host, the sooty mangebey monkeys of West Africa (Dunham et al, 2006). The HIV originated from the SIV through animal-to-human (zoonotic transmission) from infected sooty mangabey monkeys to the human species (Hahn et al, 2000, BIPAI 2010, pp. 1-6). HIV belongs to the Lentivirus genus of the Retrovirus family (Fanales-Belasio et al, 2010). Typical of Lentiviruses, HIV exhibits a chronic insidious pathophysiological process of disease development with longer incubation periods and persistent viral replication (Fanales-Belasio et al, 2010).

T-lymphocytes are types of white blood cells that are a critical component of the body's immune system and are the primary targets of the HIV (BIPAI 2010, pp.7-14; RSA. NDOH/SANAC, 2010a). The HIV is fragile and has a short life span outside of the host but once entry occurs, the HIV life cycle persists through a six-stage process. The stages are binding and entry, reverse transcription, integration, replication, assembly, budding and maturation (BIPAI 2010, pp.7-14). The external structure of a HIV virion is that of a spherical bi-lipid layered envelope consisting of numerous, paired glycoprotein spikes bound together on its surface (BIPAI 2010, pp.7-14; Fanales-Belasio, 2010; Vogt and Moreno, 2012). These two glycoproteins gp120 and gp41 on the virus surface serve as detectors for susceptible host-invading cells with which the virion could attach itself to. Specifically, these glycoproteins bind to

CD4+ receptors on the surface of CD4+ lymphocytes similar to a lock and key fit and begin the process of host cell invasion (BIPAI, 2010, pp.7-14; Vogt and Moreno, 2012). Within the cytoplasm of the virus, are several accessory proteins that help the virion in numerous ways to carry out its functions to survive and proliferate (Stout et al, 2009).

Within the core of the virus is a cone-shaped protein structured 'capsid' housing the nucleocapsid layer that contains the RNA genetic material and the 3 most important triggering viral enzymes called reverse transcriptase, integrase and protease (Ganser-Pornillos, Yeager and Sundquist, 2008; Stout et al, 2009; BIPAI 2010, pp. 7-14). There are anti-HIV drugs that had been developed and currently are in use that block the action of these individual viral enzymes and are used in combination as a HAART regimen (Rathbun, 2011). HIV being a retrovirus, is typically a ribonucleic acid (RNA) virus that survives by evolving to a stage where it uses the host cell's DNA that serves as the machinery to produce vital structural and functional components of the virus (BIPAI 2010, pp. 7-14). However, unique from the genetic make-up of other retroviruses, HIV has 9 genes on two identical single stranded RNA that code for the all the necessary enzymes and proteins that make up a single virion (BIPAI 2010, pp. 7-14). HIV remains dormant within its host cell and only upon activation of the infected lymphocyte, would the HIV then commence the dynamic six-stage process that would eventually kill off the CD4+ host cell (Vogt and Moreno, 2012).

2.1.6 Neuroinvasion by HIV and CNS Damage

Invasion of the nervous system occurs in the early stages of HIV infection by infecting cells of the immune system (Powderly, 2000; Lindl et al, 2010). Even in the presence of HAART, the pathological features of HIV encephalitis persists as found with post-mortem analyses (Masliah et al, 2000). This only reinforces the mounting evidence that HAART is capable of controlling the disease but not eliminating it, especially in a sanctuary site like the CNS (Albright, Soldan and Gonzalez-Scarano, 2003). Progression to the CNS occurs very rapidly by crossing of the blood-brain barrier (BBB). The BBB is in two anatomical places, the first one is between the blood within the cerebral capillaries and the brain tissue (parenchyma) and the second is between the blood and the CSF in the choroid plexus (Zhang and Tuomanen, 1999). The BBB, an important portal of entry for the virus into the CNS, is however a physical impediment to antiviral drug delivery, hence limiting HIV eradication from the CNS (Albright, Soldan and Gonzalez-Scarano, 2003). The most common findings are that the infection of brain tissue occurs through cellular invasion by monocytes/macrophages, endothelial cells, astrocytes, microglia and CD4 lymphocytes, with the perivascular macrophages as the undisputed virus-carrying invader of brain tissue (Davis et al, 1999; Johnson et al, 1996; An et al, 1999; Trillo-Prazos et al, 2003; Lindl et al, 2010). Macrophages are cells that are a vital component of the human immune system, which scavenge debri and primarily phagocytose foreign material and pathogens in the body (BIPAI 2010, pp. 7-14). However, if infected by HIV, they have the potential to secrete neurotoxins that directly results in neural tissue destruction (Albright, Soldan and Gonzalez-Scarano, 2003).

Oligodendrocytes which are glial cells that surround neurons to provide structural support and speed up electrical signals, have also been implicated as cells involved in CNS infection (An, Giometto and Scaravilli, 1996; Bissel and Wiley, 2004; Genetics Science Learning Center (GSLC), 2012). Microglia which are the brain's immune cells have similar functions to that of macrophages and they play a role in sustaining brain development and brain physiology and neural circuit functions (Gehrmann, 1996; Tremblay et al, 2011; GSLC, 2012). Astrocytes, like microglia are glial cells that play a supportive role in maintaining structure of brain tissue with their long octopus-like processes (GSLC, 2012). Newer studies have shown that beyond their primary role, they are also responsible for vital neural circuitry functions as they respond intensely to CNS injury (Sofroniew and Vinters, 2010). Endothelial cells are cells that line blood vessel walls and are an important structural component of the BBB due to the tight cellular junctions that glues these cells together (Liu et al, 2002; Ballabh, Braun and Nedergaard, 2004). The currently accepted hypothesis of neuroinvasion by HIV is through the 'Trojan Horse' style of entry, were monocytes infected with HIV permeate through the BBB to enter the brain tissue (Liu et al, 2000; Bissel and Wiley, 2004). These infected monocytes later differentiate into macrophages (Albright, Soldan and Gonzalez-Scarano, 2003). At this point of the neuroinvasion, the virus establishes its reservoir within the brain parenchyma and the perivascular macrophages where it remains latent, impervious to certain antiretrovirals. Upon activation of the macrophage, begins the dynamic process of viral replication (Morris et al, 1999). Astrocytes, which are susceptible to HIV infection, appear not to be responsible for viral production. Although they harbour the virus, they potentially could be excluded

as cells involved in productive CNS infection (Gorry et al, 2003; Gonzalez-Scarano and Martin-Garcia, 2005). The CD4 lymphocytes are initially involved in the transmission of HIV into the CNS however, they are not the primary cells responsible for a productive infection in the CNS due to their short life span. Neurons are resistant to viral penetration and are not involved in viral production but they are destroyed due to host inflammatory reactions and the release of neurotoxins from viral proteins, an unfortunate consequence of the presence of the virus in the brain (McArthur et al, 2003; Gonzalez-Scarano and Martin-Garcia, 2005; Kaul et al, 2005).

The evidence-based consensus is that the brain macrophages and microglia are the most commonly infected cells in the CNS with high HIV-producing capabilities (Bagasra et al, 1996; Johnson et al, 1996). These cells are responsible for mediating neurological disease through viral protein production and neurotoxins (Kaul and Lipton, 1999). In a publication by Van Rie et al (2007), the differences between the CNS pathophysiology in HIV-infected adults and children have been tabulated. A valuable observation was that the target cells of HIV are similar but with astrocytes playing a more central role in the CNS infection in children than with adults (Van Rie et al, 2007). Further research in HIV neuroinvasion at cellular and molecular level are needed as the pathophysiological processes involved, for some parts, remain enigmatic (Harrington et al, 2005). This insight would be valuable in understanding the clinical manifestations of CNS damage that is observed in infected children.

2.2 Focussed Scholarship Review

2.2.1 Neuro-Cognitive impairment in HIV Infection

Human immunodeficiency virus invasion into the CNS is the aetiology of an array of neurological disorders collectively known as HIV-Associated Neuro-cognitive Disorders (HAND) (Lindl et al, 2010). A key consequence of neuronal cell death and neurodegeneration is the development of HAND (Lindl et al, 2010). HAND comprises of three syndromes namely, HIV-associated Asymptomatic Neurocognitive Impairment (ANI), HIV-associated Mild Neurocognitive Disorder (MND) and HIV-Associated Dementia (HAD) being the most severe form of HAND (Foley et al, 2008). The diagnostic criteria that characterise HAND are motor, behavioural and cognitive deficits that occur in individuals that fall into any of the three stages of HAND (Kaul et al, 2005). HIV-Associated Dementia is an AIDS-defining illness and has devastating consequences due to it being progressive and debilitating with cerebral and basal ganglia atrophy and diffuse white matter degeneration (Dal Pan et al, 1992).

Subcortical dementia is a sequale of HAD given the fact that the virus preferentially targets the basal ganglia and the deep white matter of the brain parenchyma (Boisse, Gill and Power, 2008). Neurocognitive factors that comprise of HAD are psychomotor slowing, changes in mood and anxiety levels, deficits in memory, verbal fluency, attention and information processing (Portegies et al, 1993). Systemic and CNS viral burden is a poor predictor of HAD and neurocognitive impairment in general, however viral load in the CSF may be predictive of HAD

(McArthur, Brew and Nath, 2005). Neuropsychologic testing and evaluation has been used as a reliable tool in confirming the diagnosis of HAD and in the response to antiretroviral therapy (Boisse, Gill and Power, 2008).

With the introduction and combination therapy of antiretrovirals, systemic infection is adequately controlled with a significant decrease in the incidence of HAD. However, due to HIV-infected patients' longer life expectancy due to HAART, patients still suffered from HAND with an increase in the prevalence of HAD and more in the milder forms of HAND (Sacktor et al 2004; Antinori et al, 2007). This is attributed to survival and persistence of HIV within the CNS compartment.

HIV classically affects immature brains in a different way than in adult brains. The condition that arises from the infection of developing brains is called HIV encephalopathy that is subdivided into static and progressive (Smith, Adnams and Eley, 2008). The CDC has defined progressive encephalopathy which is the most severe form of encephalopathy as having microcephaly, or failure to attain, or loss of developmental milestones, or loss of intellectual ability and/or acquired abnormalities in gross motor skills (CDC, 1994). Patients classified as having static encephalopathy display sustained and non-progressive abnormalities in gross motor functionality, learning abilities, or developmental delays in reaching the relevant milestones (Shanbhag et al, 2005), The Department of Psychiatry in California looked at the impact of HIV-associated neuropsychological deficits on everyday functioning and revealed a significant reduction in adequate performance of daily activities and

cognitive abilities (Havens and Mellins, 2008). The overall quality of life is compromised even with milder forms of HAND where activities of daily living are adversely affected (McArthur, 2004).

The white matter damage and volume reduction in brain matter is a common radiological feature seen in HIV infection. This is due to myelin sheath loss from neurons. This common finding implies that there is a tendency for subcortical white matter atrophy in CNS infection by HIV (Foley et al, 2008). The regions most commonly affected are the basal ganglia, deep white matter, hippocampus and cerebral cortex (Gorry et al, 2003). With neuroimaging techniques, the hallmarks of basal ganglia calcification and deep central white matter lesions have been identified in HIV infection (Decarli et al, 1993). From this consistent finding, reduction in specifically the caudate volume of the basal ganglia has been identified. Kieburtz et al (1996) found that signs of reduction in fine motor dexterity, psychomotor speed, verbal fluency and auditory attention have been linked to caudate degeneration. Children infected with HIV are at high risk for developmental delays and deterioration in their cognitive functioning and abilities (Zheng and Gendelman, 1997; Havens and Mellins, 2008). Developmental delays are frequent in the major domains of cognition, speech/language and motor functioning. (European Collaborative Study, 1990; Wolters et al, 1995; Coplan et al, 1998; Boisse, Gill and Power, 2008). Children on HAART, generally function within normal limits and succeed in school but may exhibit subtle delays and deficits in selected areas (Frank and Foley, 1997) such as processing speed (Blanchette et al, 2002) and working memory (Bisiacchi, Suppiej and Laverda 2000).

Risk factors for the development of neurologic disease in children include high CSF viral load and maternal high viral burden at the time of birth. Major consistent differences in the neuropathology of children from adults in this HAART era are the frequent infection of astrocytes together with basal ganglia calcification and cortical atrophy (Tardieu et al, 2000). HAART's neuroprotective benefits have shown through various studies with sustained improvement and recovery in adults in terms of neurocognitive functioning (Robertson et al, 2004). Unfortunately, little is known regarding the neurocognitive strengths and weakness in infected children and adolescents on HAART (Martin et al, 2006). Longitudinal studies assessing the long-term impact of HAART on the neurocognitive outcomes in infected children are still left wanting (Shanbhag et al, 2005). Furthermore, a meta-analysis of five Paediatric AIDS Clinical Trials Group studies have shown that viral load and not CD4 counts were predictive of cognitive decline in children beyond the infancy stage (Lindsey et al, 2000) whereas Shanbhag et al (2005) demonstrated that viral load and CD4 count are marginally predictive of neurocognitive testing outcomes.

2.2.2 Neural Control of Saccadic Eye Movements

Saccadic eye movements are elicited by motorneurons that discharge a burst of action potientials initiating the excitory phase followed by an inhibitory tonic discharge to maintain and hold fixation on a target (Robinson and Fuchs, 2001). For saccadic generation, cortical and sub-cortical regions are involved in the process of regulating

saccadic eye movements (Muri and Nyffeler, 2008). The cortical and sub-cortical areas in the brain involved in saccadic control are the visual cortex in the occipital lobe, frontal eye fields (FEF), supplementary eye fields (SEF) and the dorsolateral prefrontal cortex (DLPFC) in the frontal lobe, parietal eye fields (PEF) in the parietal lobe, basal ganglia (BG), thalamus, superior colliculi and the brainstem in the sub-cortical region as well as the cerebellum (Leigh and Zee 2006, pp. 3-19). Each of these structures comprises of the neurocircuitry that work together to produce accurate saccadic function. There are two major domains of saccadic eye movements: reflexive or involuntary and the voluntary type or volitional saccades (Muri and Nyffeler, 2008).

The PEF is mainly involved in reflexive saccades to sudden stimuli presented in the environment and disengaging fixation for the reflex action to occur (Muri and Nyffeler, 2008). The FEF are involved in initiation of an action for intentional saccadic movement of the eyes to a fixation target. The FEF and superior colliculi work closely together in voluntary saccadic eye movements and memory-guided saccades. Memory-guided saccades refers to saccadic movements that are initiated to a remembered fixation point which is kept in working memory even when that target is no longer present (Mitchell and Zipser, 2003). The SEF function in a similar manner to FEF to control the coordinated gaze movements of both eyes and head (Martinez-Trujillo, Wang and Crawford, 2003). Neural recordings in the SEF are shown to be involved in activities that are related to both vision and saccades, where similar findings have been identified with the FEF and superior colliculi. Newer research regarding SEF has shown that their functions in saccadic control are more specialised

and complex involving executive cognitive functions as well (Stuphorn and Schall, 2006).

The DLPFC is connected to a multitude of functional areas in the brain. These interconnections exists between the thalamus, parts of the basal ganglia (specifically, the dorsal caudate nucleus), the hippocampus, temporal, parietal, and occipital areas (Zelazo and Muller 2002, pp.445-469). The DLPFC serves as a crucial area responsible for executive functions such as motor planning, organization, and regulation. Due to its varied interconnections, it integrates sensory and motor information with regulation of intellectual function (Zelazo and Muller 2002, pp.445-469). The DLPFC is heavily involved in memory-guided saccade and inhibits reflexive saccades.

The BG is a functional area located at the base of the cerebrum comprising of numerous nerve cell nuclei that is considered to be necessary for voluntary control of body movements among other functions. The BG is composed of globuspallidus, substantia nigra, subthalamic nucleus and the striatum of which the latter is further sub-divided into the caudate nucleus and the putamen. From clinical observations it has been reported that patients exhibiting excessive and retarded bodily movements were diagnosed with BG disorders. Lesions in the BG lead to movement disorders ranging from the inability to initiate a movement to the inability to suppress an involuntary movement (Hikosaka, Takikawa and Kawagoe, 2000). Patients with diagnosed BG degeneration and cerebellar lesions as is in those with Parkinson's disease have difficulty generating predictive saccades (O'Driscoll et al, 2000). The

output of the BG is directed to the brainstem, with significant communication with the superior colliculi. Patients experience saccadic deficits with memory-guided saccades and visually-guided saccades in BG disorders. In terms of saccadic function the BG facilitate eye movements by preventing the inhibition signals, allowing movement and secondly, to enhance the inhibition of saccadic eye movements (Hikosaka, Takikawa and Kawagoe, 2000).

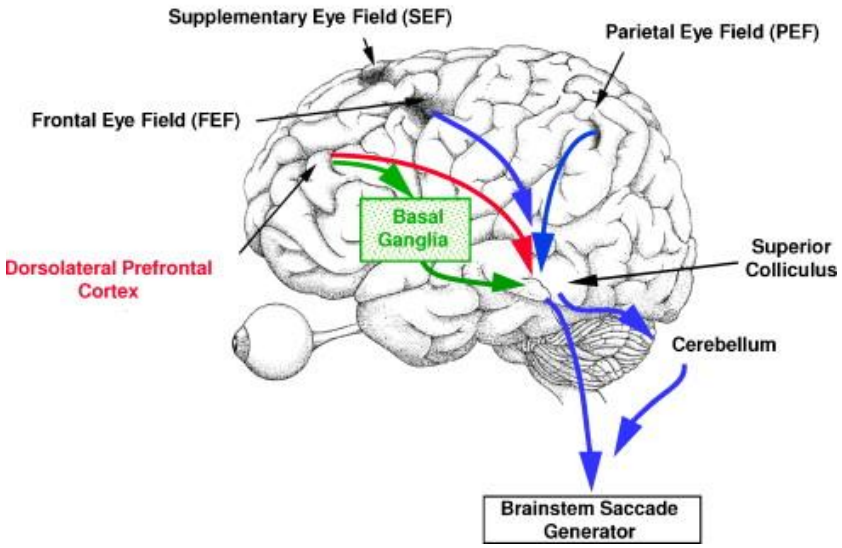


Figure 2.1: Illustration of the Saccadic Pathway extracted from an article by Gaymard (2012). *Cortical and sub-cortical control of saccades and clinical application.*

2.2.3 Eye Movement Testing as a Tool in Psychiatric and Neurodevelopmental Disorders

HIV-infection can be associated with CNS involvement and changes in the neurobehavioural status, particularly in advanced stages of the illness (Bornstein et al, 1992). Eye movements are one of the most thoroughly studied domains in the field of

cognitive neuroscience as they have proven to be a very useful neuroscientific tool that has allowed the psychiatric researcher to examine relationships between the brain and behaviour and the development of psychopathologies (Beatriz, Velanova and Geier, 2008). Researchers have assessed eye movements in an array of psychiatric disorders such as Autism Spectrum disorder, Attention-deficit Hyperactive disorder, Childhood-onset Schizophrenia, Tourette's Syndrome, Oppositional Deviant Disorder, Conduct Disorder and Obsessive Compulsive Disorder, to facilitate the understanding of the complex underlying neuropathophysiology of psychiatric disorders (Nanda, Van Der Stigchel and Sergeant, 2008). Specifically, voluntary control of saccades is particularly sensitive to psychopathology (Sweeney et al, 2004). Green et al (2007) further indicated that saccadic eye movement testing could be a useful tool in providing insight into the workings of the CNS in children diagnosed with Fetal Alcohol Spectrum disorders (FASD) (Green et al, 2007).

These findings may not necessarily be extrapolated to children and adolescent patients, since the clinical manifestation and aetiology of psychiatric disorders may differ between childhood-onset and adult-onset (Carlson, Bromet and Sievers, 2000). Eye movements themselves may differ substantially between children and adults (Karatekin, 2007).

2.2.4 Clinical Assessment of Oculomotor Function

Assessments of eye movement functioning in a clinical setting falls into three categories of tests: gross observation, psychometric tests and electrodiagnostic tests (Maples, Atchely and Ficklin, 1992). The latter two testing approaches are not routinely used in mainstream general practice but rather by practitioners whose focus is on paediatric, binocular vision and behavioural optometry.

Gross observation methods referring to chair skill testing is more widely used due to it being the quickest method without additional devices or materials which do not form part of the essential equipment routinely used in optometric practices in South Africa. The traditional gross observation methods used is where the examiner is seated at eye level in front of the patient holding two fixation targets approximately 40cm from the patient's midline. The examiner then provides instructions to alternate fixation between the targets as it is held in the horizontal, vertical and 2 oblique positions (Griffin and Grisham 1995, pp. 17 - 28; Carlson and Kurtz 2004, pp. 57-58). This technique is merely descriptive and not easily quantifiable.

Subjective tests developed by the Southern California College of Optometry (Hoffman and Rouse, 1980) and The Northeastern State University College of Optometry decided to improve on the traditional gross observation methods by developing assessment criteria and norms to standardise the testing (Maples, Atchely and Ficklin, 1992). These improved subjective, observational tests are referred to as the SCCO test (Hoffman and Rouse, 1980) and the NSUCO test (Maples and Ficklin,

1988). The SCCO test is similar to the NSUCO test which has four areas of performance for saccades and for pursuits that are scored out of a scale of five. This was an improvement on the accuracy of measurement of oculomotor function (Maples, Atchely and Ficklin, 1992).

The Visigraph devices and the Readalyzer are electro-oculographic tests (Orlansky et al, 2011) that use infrared emitters and detectors that are mounted onto goggles, which the patient has to wear during reading. It assesses the number of fixations, regressions and the reading speed (Okumura and Laukkanen, 2011). Since saccadic eye movements are made during reading, it is thus also assessing the quality of efficient saccadic eye movements. These instruments, although useful and reliable as objective eye movement recording instruments, are expensive hence they are not widely used and may be difficult to use with early-school going children (Rouse 2006, pp.335-368).

An alternative approach in oculomotor assessments is the use of psychometric tests that create a reading environment. This class of tests are based on the principle of verbalizing numbers that are read as quickly as possible without the use of a finger to guide the reading (Orlansky et al, 2011). Such tests based on this principle are the Pierce Saccadic Test, the King-Devick Test (K-D), New York State Optometric Association King-Devick Test (NYSOA K-D) test and the Developmental Eye Movement Test (DEM), which was later developed (Orlansky et al, 2011). The K-D test was determined to be a reliable test for athletes with head trauma and a rapid,

sideline screening tool for athletes with a concussion (Galetta et al, 2011). The validity of some of the earlier visual-verbal tests were questioned due to the fact that they were unable to differentiate between problems of automaticity and oculomotor problems (Richman, Walker and Grazia, 1983). Automaticity refers to rapid, accurate and effortless word identification at single word level (Hook and Jones, 2002) and in these psychometric tests, automatic naming or calling skills of numbers. It was for this challenge that lead to the development of the DEM which differentiates oculomotor dysfunction from automaticity problems (Garzia et al, 1990). The DEM test falls into the domain of Rapid Automatic Naming (RAN) tests where its purpose was to quantify the efficiency of saccadic eye movements based on the speed and accuracy that a series of single digit numbers could be recognized and verbalized (Rouse et al, 2004). The DEM examines the time taken for numbers to be read out aloud vertically and then horizontally. The errors made during the test are computed and used in the final calculation of the recorded times. A ratio score is determined by taking the horizontal time and dividing it by the vertical time. These four parameters are compared against the established norms of the test according to the chronological age of the child (Garzia et al, 1990)

The instructions on the DEM test version 1 is quite clear that the child should not use their finger while reading but to only use read eyes. The developers of the DEM reported that the test had good test-retest reliability for the horizontal and vertical times with fair-to-good reliability for the ratio scores (Orlanksy et al, 2011). An investigation on the DEM test indicated that performance on the test relates to certain

symptoms that are associated with oculomotor dysfunction (Tassinari and DeLand, 2005). Another study concluded that the DEM test performance did not correlate well with saccadic eye movement skills but related more closely to reading performance and visual processing speed in a simulated reading environment (Ayton et al, 2009). A study by the University of Melbourne that investigated the validity of the DEM as a clinical saccadic test, demonstrated results that argued against the DEM as an assessment tool for saccadic eye movements (Ayton et al, 2009). The European Academy of Optometry and Optics conducted a study that examined the relationship between the objective and subjective evaluation of eye movements using the DEM and the Readalyzer. The conclusion of this study was that the results pointed to the DEM remaining a valid test of ocular motility but that the correlation between the DEM and the Readalyzer decreased because of the different stimuli used in the different tests (Manzoli et al, 2010).

A study that evaluated the repeatability of the DEM by conducting the test three times at separate visits concluded that clinicians should be 'cautious' in using the DEM test in isolation in reaching a diagnosis (Orlansky et al, 2011). Medland, Walter and Woodhouse (2010) in their study determined that the horizontal score may be a reliable measure of a child's visual processing speed and be used as a predictor of children at risk of developing reading problems. This would imply that the DEM ratio, which is determined by the using the vertical and horizontal score, is a greater indicator of poor reading skills (Medland, Walter and Woodhouse, 2010).

The following studies examined the use of the DEM test in their different populations. A study on an Italian population of children that examined the validity of the DEM according to the four variables of the test as a function of the chronological age concluded that the DEM is a valid tool in differentiating ocular motility problems from naming problems in the developmental age (Facchin, Maffioletti and Carnevali, 2011).

A study on Spanish-speaking children tested whether the DEM scores changed with respect to language differences. Even though the norms for the Spanish-speaking children in the six year old category were lower than in the standardised norm for that age, it concluded that the DEM appears to be a reliable tool regardless of the language of the population being examined (Fernandez-Velazquez and Fernandez-Fidalgo, 1995). A study on Portuguese children using the standard American table of norms showed that a large number of these children had oculomotor problems and/or automaticity problems that highlighted the need for specific Portuguese-speaking guidelines for Portuguese children. It was further concluded that DEM scores might be affected by differences in language, educational systems and/or cultural differences (Bapista et al, 2011).

Pang (2004), from his dissertation on the DEM test with Cantonese-speaking children, proposed new norms for children of this language population. He further proposed an improved method of recording using a voice recording of the responses, which allow the examiner to re-listen and re-assess the recording times to enhance the accuracy of the results (Pang, Lam and Woo, 2010b). Cantonese-speaking children

performed better in the vertical and horizontal test as compared to the standard norm but the study further highlighted that differences in language, educational and cultural backgrounds were factors that contributed to variable performances in the test (Pang, Lam and Woo, 2010a).

2.2.5 Eye Movement Analyses in HIV-Infected Subjects

Due to the overlap between brain areas that control eye movements and that which are injured during HIV CNS infection, studies examining subjects with HIV/AIDS were conducted. Beyond the structural damage that occurs with the central white matter and deep grey matter structures, functional abnormalities such as ADC are a common sequale (Sweeney et al, 1991). Evaluating eye movements as part of neuropsychological assessments to study disturbances in attention and motor control that are early manifestations of HIV infection of the brain appeared to be a promising approach (Sweeney et al, 1991). Tervo et al (1986) indicated that ocular motility abnormalities may be an early sign of HIV CNS infection which was also emphasised by Feldon (1990) at the International Neuro-Ophthalmology Symposium. Ocular motility defects observed in HIV infected patients appeared to originate from brainstem compromise as initial clinical presentations in three patients (Hamed, Schatz and Galetta, 1988). Other motility disturbances reported were primarily saccadic dysfunctions, pursuit dysfunctions and alterations in optokinetic nystagmus (Roig and Iranzo, 1996). Sweeney et al (1991) concluded from their study on pursuits eye movements in HIV-1 infected individuals that oculomotor disturbances are present in

the HIV infected individuals prior to the establishment of the diagnosis of ADC. This implied that pursuit dysfunctions could be an early indicator of CNS infection in HIV infected individuals. Currie et al (1988) who focussed their study on saccadic eye movement functioning concluded that there was a correlation between saccadic deficits and the severity of ADC (Currie et al, 1988). Most of these publications reporting eye movement dysfunctions were in the pre-HAART era and none of which were conducted on children with HIV/AIDS.

CHAPTER THREE

METHODOLOGY

The methodology employed in this study was constructed to answer the primary research enquiry.

3.1 METHODOLOGICAL FRAMEWORK

3.1.1 RESEARCH QUESTION

Could the detection of abnormal saccadic eye movements in children from 6 to 13 years with HIV/AIDS on HAART, be a predictor of the status of their immune system?

3.1.2 PRIMARY OBJECTIVES

The primary objectives of this study were to determine the:

1. Prevalence of saccadic eye movement abnormalities in HIV positive subjects on HAART
2. Relationship between saccadic eye movement abnormalities and the immunologic (CD4 count) and virologic (viral load) indicators of HIV positive subjects.

3.1.3 SUPPORTIVE INVESTIGATIONS

The supportive investigations of this study were conducted to further explore the characteristics of this population to fully address the research enquiry. The supportive investigations were to:

1. Examine the association between the CD4 count and viral load.

2. Compare the prevalence of saccadic eye movement abnormalities between the different age groups of the study sample.
3. Evaluate the subjects' performance in the DEM test against the age-specified standardized normative values.

3.1.4 HYPOTHESIS

Null Hypothesis (H_0)	Abnormal saccadic eye movements detected with the DEM test cannot predict the immunologic and virologic status of children with HIV/AIDS on HAART.
Alternate Hypothesis (H_a)	Abnormal saccadic eye movements detected with the DEM test can predict the immunologic and virologic status of children with HIV/AIDS on HAART.

3.1.5 RESEARCH DESIGN

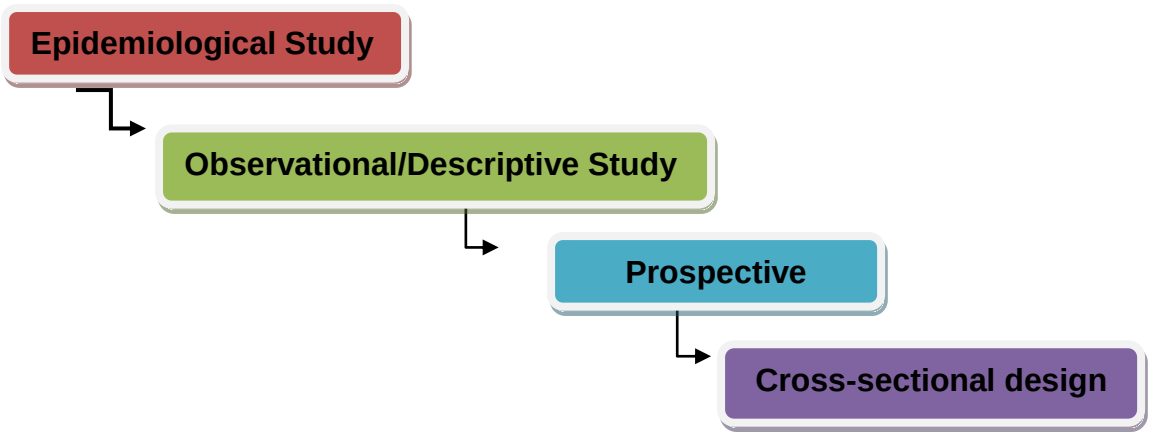


Figure 3.1: Study design framework of the research enquiry.

This study was epidemiological in nature that described the population of HIV/AIDS subjects on HAART. By investigating saccadic abnormalities, the researcher aimed to observe and describe the findings of the specified population in terms of eye movement performance without attempting to affect a change or use interventional methods to alter the characteristics of the population. The study was prospective where the subjects were individually tested with the DEM test from which conclusions about the population was drawn from the results. A cross-sectional design was adopted as the subjects were utilized only once and were not revisited. The DEM test was performed once and the results were compared against the latest immunologic and virologic findings of the subjects that were available at the time of the data collection.

Method of Data Collection

Simultaneous observation of the subjects by the researcher when the DEM test was conducted.

3.1.6 INCLUSION CRITERIA

- Children from the ages of 6 years to 13 years and 11 months were included in this study provided they met the criteria listed below:
    - Infected with HIV
    - HIV type 1 strain infection only
    - HIV infection through mother to child transmission (MTCT)

- Visual identification and verbalisation of numbersNear visual acuities of 6/9 and in each eye

3.1.7 EXCLUSION CRITERIA

- Subjects with HIV type 2 strain.
- Subjects with HIV type 1 & HIV type 2 infections.
- Illiterate subjects
- Subjects non-verbal in English
- Subjects on different ARV drug regimens
- Learning disabilities
- Subjects with:
  - Near visual acuity worse than 6/9
  - Strabismus
  - Extra-ocular muscle nerve palsies
  - Eye infections
  - Recently acquired HIV infections
  - Neurological diseases or disorders
- Subjects previously diagnosed and with existing psychiatric and neurodevelopmental disorders.
- Subjects previously diagnosed and existing central nervous system infections and cancerous diseases.

3.1.8 STUDY POPULATION

Children with HIV/AIDS on HAART from 6 years to 13 years and 11 months of age living in the Motheo and Xariep district of the Free State province were accessed from 8 data collection sites.

3.1.9 SAMPLE SIZE

One hundred and twenty eight (128) HIV positive children made up the final study sample. There were 185 subjects accessible to the researcher during the sample selection phases at the data collection sites of which 57 subjects did not meet inclusion criteria. According to the estimated number of subjects attending the data collection sites, a projected number of fewer than 200 subjects were targeted. This sample number was determined conveniently, as this was the ideal approach due to time availability and direction from the biostatistician.

There were unforeseen additional factors that warranted exclusion from the final sample pool outside of what was specified in the exclusion criteria. These additional unforeseen factors were incomplete or missing CD4 count and viral load data from the health records and absence of blood results due to testing not being done at a specific time. Attempts were made to acquire some of the information from the NHLS but with moderate success.

3.1.10 SAMPLING METHOD

Convenient sampling

One hundred and twenty eight HIV positive children who presented at the data collection sites for their routine treatment and health assessments were included in the study. This sample that was drawn was based on health record information and vision screening results all of which satisfied the inclusion criteria of this study. As this was a non-random sampling method, no statistical formulae was required to draw a sample from the population.

All 128 subjects were on HAART and none of the subjects used English as their home language. All subjects were of African origin and were Sotho and Afrikaans home language-speaking individuals. All subjects were however familiar with English and performed the DEM test by verbalizing the numbers in English as a standard.

3.1.11 SAMPLE SELECTION PROCESS

Phase 1: Review of health records to extract relevant data and investigate for the factors that warrants exclusion.

Phase 2: Subjects selected from phase 1 underwent a vision screening process to identify suitable candidates that satisfy the inclusion criteria and exclude those that did not fulfil the selection criteria

Phase 3: Subjects that were successful from phase 2 comprised of the final study sample and performed the DEM test.

Phase 1: Subject Recording Form (Appendix A) was used for all subjects.

1. Review of health records to assess for exclusion factors and to extract the relevant data such as:-
    - Personal details
    - Demographic information
    - Mode of transmission
    - Date of confirmed HIV infected status
    - Latest CD4 count and Viral load values
    - History or current systemic illness, neurological disease or disorders
    - Number of previous hospitalisations
    - Current ARV medication
    - Any non-ARV medication
    - Date HAART was initiated.

Phase 2: Appendix A was used for all subjects selected at the end of phase 1. Informed consent (Appendix B and C) was requested from the care-givers of the prospective subjects in order to participate in phase 2.

The following evaluations were conducted:-

- Distance and near visual acuities
- Retinoscopy
- Distance and near cover test
- Motility testing of pursuits, saccades and ductions
- Near point of convergence

- Ophthalmoscopy
- Knowledge of numeracy

Phase 3: DEM test and scoring sheet was used for subjects that passed the criteria in phase 2. The final results were recorded of the prepared DEM score sheet.

The following steps of Phase 3 were followed:-

- The DEM test was placed in front of the subject.
- The pre-test was explained and then the subject was allowed to perform the pre-test.
- Once the subject was familiar with the test, the main DEM test was carried out.
- The time taken by the subject to carry out Test A, Test B and Test C was recorded and the subject was released.

3.1.12 DATA COLLECTION SITES

The data collection sites were classified as ARV treatment centres (centres that dispense ARV medication to HIV-infected patients) in the province. They were (conveniently) purposefully selected according to the knowledge that they have a greater access to HIV positive children and better accessibility for the researcher. As the environment of the data collection sites were homogenous, generalized conclusions were made about the data. The nearest site was 9 km from the location of the researcher with the furthest being 150 km away. There were six chosen sites from the

Motheo District in central Free State and 2 sites from the Xariep District in the southern Free State.

List of data collection sites:

1. Manguang University Community Partnership Programme (MUCPP) Centre – Motheo District, Bloemfontein
2. Batho Primary Health Care (PHC) Clinic - Motheo District, Bloemfontein
3. Pelonomi Regional Hospital - Motheo District, Bloemfontein
4. Bloemspruit PHC Clinic - Motheo District, Bloemfontein
5. Botshabelo Hospital – Motheo District, Botshabelo
6. J.S Moroka Hospital –Motheo District, Thabanchu
7. Petrusburg PHC Clinic –Xariep District, Petrusburg
8. Ethembeni PHC Clinic – Xariep District, Koffiefontein

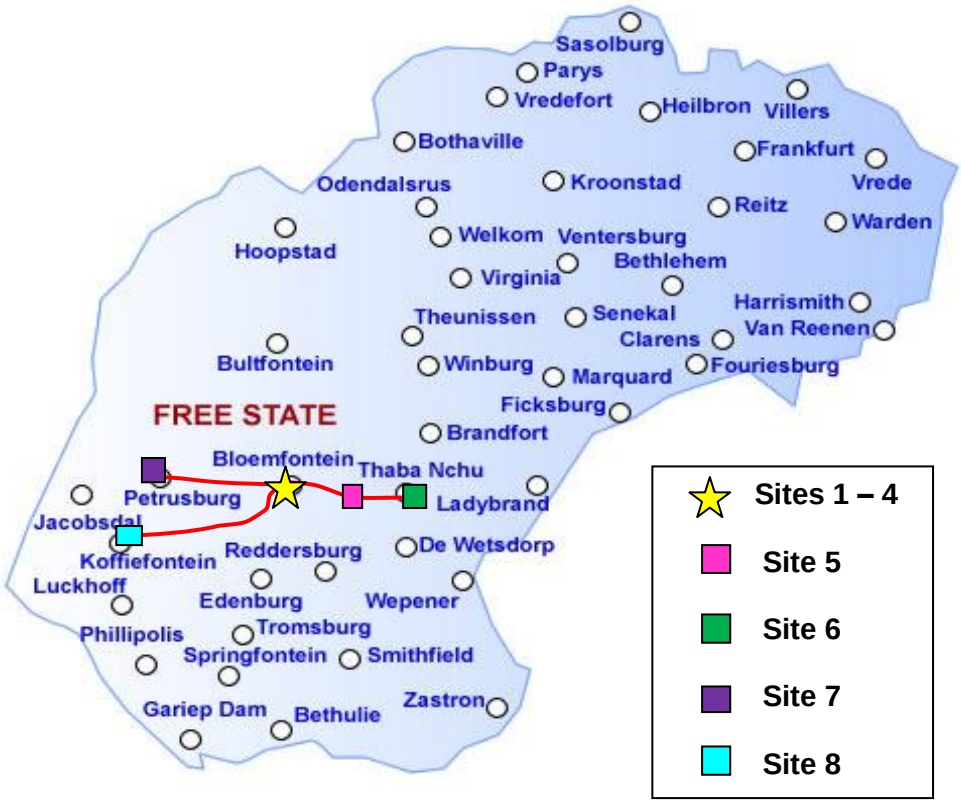


Figure 3.2: Map of the Free State Province indicating the towns where the data collection sites where situated in the province. Map sourced from Property in South Africa. *http://www.noagent.co.za/prov_map.php*

3.1.13 DATA COLLECTION INSTRUMENTS

- Developmental Eye Movement Test:-

To answer the research question most appropriately, the DEM test was selected to be the instrument of choice.

- Motivation

This type of test is claimed to be a valid instrument that is suitable for assessing eye movements in children by its developers and later by other researchers as a

superior test to its predecessors ie. Pierce Saccadic Test, the King-Devick Test (K-D) and the New York State Optometric Association King-Devick Test as it is able to effectively differentiate oculomotor dysfunction from automaticity problems.

The procedure of the DEM test is least invasive for a study sample of minors. It is less intimidating and less novel to a child's experience in his/her environment as these are school-going children that are currently literate and perform reading skills.

The duration of the DEM test and interaction with the subjects did not exceed 10 minutes making it least imposing to the subjects.

This test could be performed in any environment that is familiar to a child without any specific room or surroundings. In this study, the test was performed with the subjects seated at a table in the clinic or hospital where they receive their medication or other treatment when needed.

This test is not an automated device that is dependent on other factors for its functioning carrying minimal risk to the subjects.

- Components of the DEM Test
    - Booklet – version 1, 1987. Developed by Jack E. Richman O.D. and Ralph P. Garzia O.D. and distributed exclusively by

Bernell. It is 265mm x 215 mm in dimensions. Booklet comprises of 6 ring bound cards of which the first is the cover followed by the pre-test, vertical Test A, vertical Test B, horizontal Test C and the lastly the DEM Normative Table for the vertical time, horizontal time, errors and ratio for age groups from 6 years to 13 years and 11 months.

- DEM Score Sheet– This score sheet has Test A, B and C presented together on one sheet for the assessor to follow during testing. It has areas to capture the times of these tests as well as the errors and the ratio.

- Subject Recording Form (Appendix A) – This form was used to capture data collected from phase 1 and phase 2 by the researcher. This form contained the demographic information, medical history and current health status (CD4 count, viral load and ARV treatment) of the subjects as well as the results of their vision screening.

3.2 DEVELOPMENTAL EYE MOVEMENT (DEM) TEST

Description, Procedure, Analysis, Interpretation and Characteristics

3.2.1 DESCRIPTION

There is a pre-test consisting of 10 single digit numbers in a row in a random order. There are two vertical tests (Test A and Test B) composed of 40 single digit numbers

each arranged in 2 vertical columns of 20 digits. The two columns are 98mm apart. The horizontal test (Test C) comprises 80 single digit numbers arranged in a horizontal array of 16 rows of 5 single digits each. Test C is a sequential array of digits of Test A followed by digits of Test B but set out in a horizontally. The spacing between each digit in a row is not equal from row to row.

The vertical time represented the baseline performance and determined automaticity of number calling. The horizontal test assessed number calling skills but in a horizontal spatial array where adequate saccadic ability and oculomotor skill was required. The child is encouraged to read without using their finger to guide them but instead to merely follow with their eyes as they verbalise the numbers

3.2.2 PROCEDURE

The researcher was seated next to the subject at a table with the DEM test approximately 30cm in front of the subject. The procedure of the test was carried out as specified in the DEM test manual (Richman and Garzia, 1987).

(start) Pre-test → Test A → Test B → Test C (end)

Step1: The researcher asked the subject to carry out the DEM Pre-test (Figure 3.3) prior to performing the main DEM test. The subject was expected to read out aloud the single digit numbers as fast and accurately as possible on the pre-test. The pre-test assured the examiner that the subject was familiar with the numbers and instructions. No recording of time was need in this step.

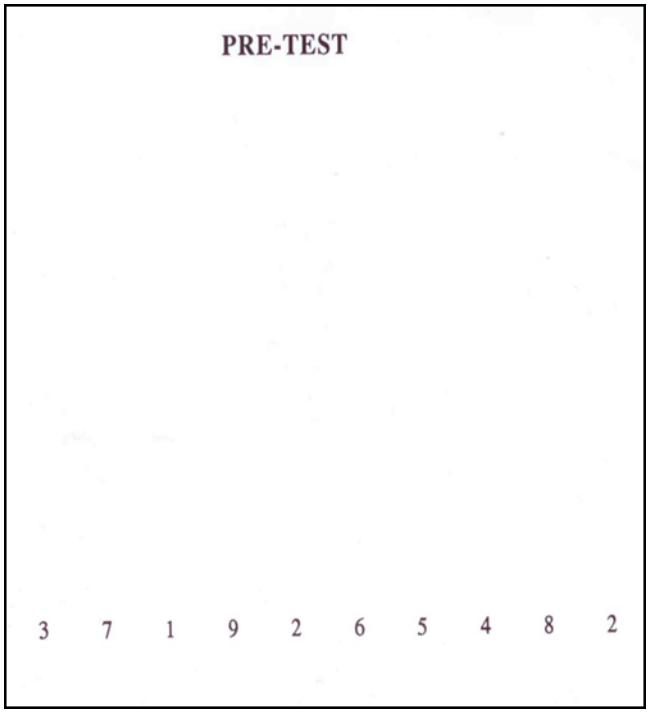
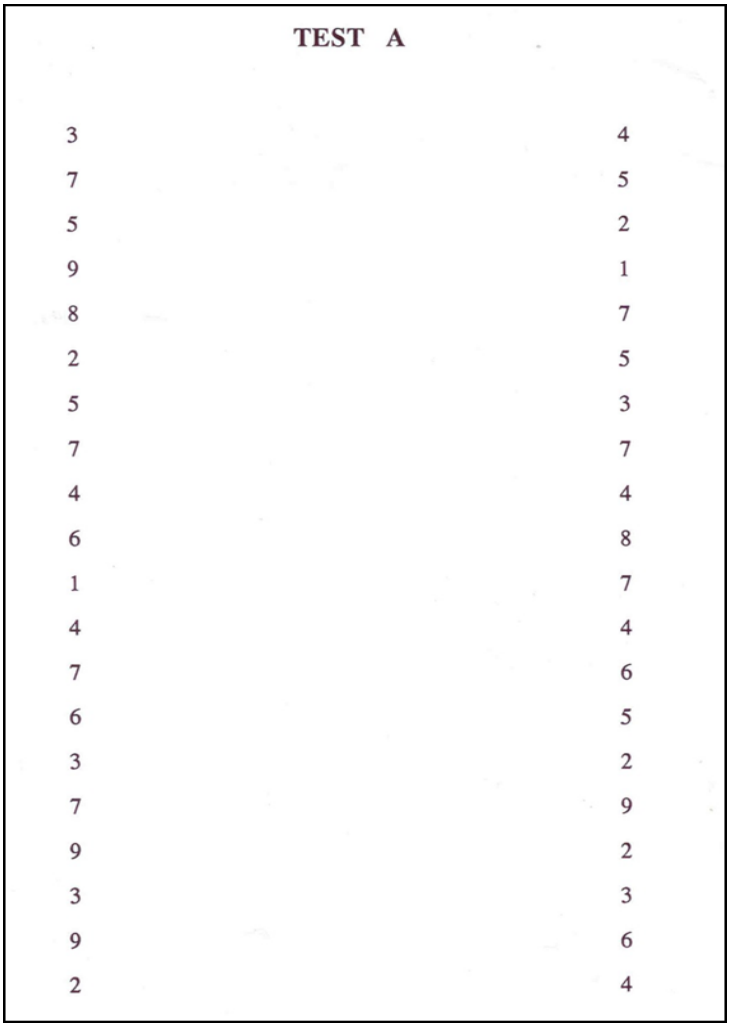


Figure 3.3: The DEM Pre-test. Extracted from the DEM test (Richman and Garzia, 1987).

Step 2: The examiner then had the child to complete Test A (Figure 3.4). The subjects were then timed with a stopwatch and the duration of time taken to complete Test A was recorded on the DEM Score Sheet.

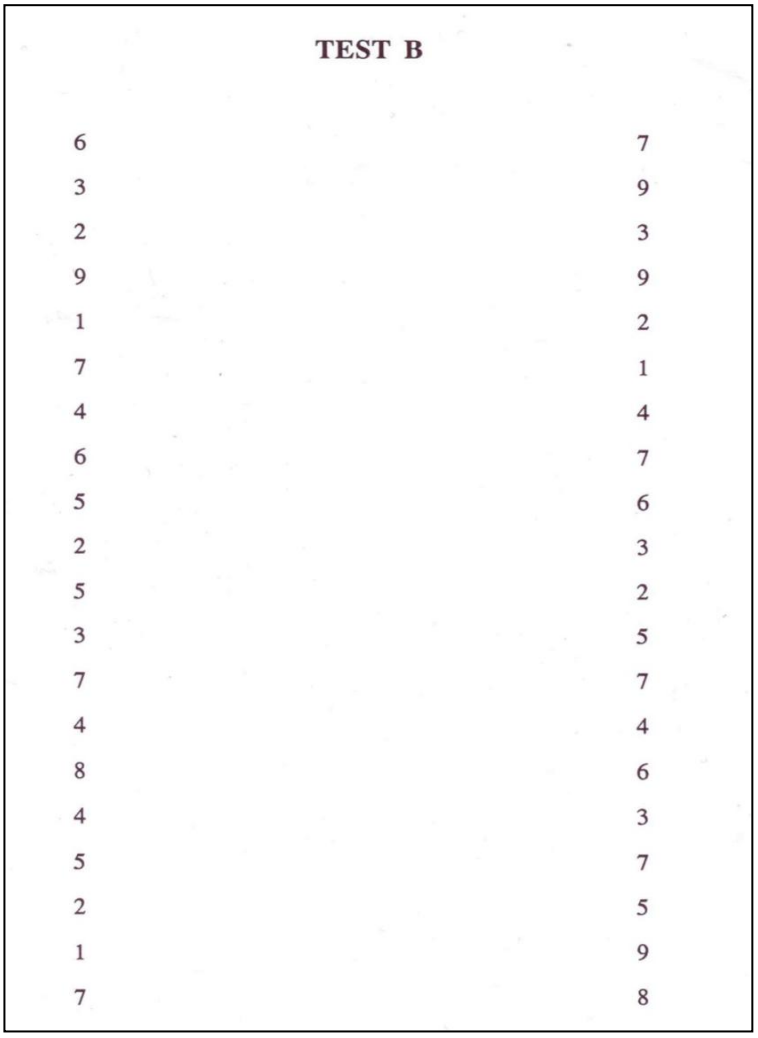


TEST A

3	4
7	5
5	2
9	1
8	7
2	5
5	3
7	7
4	4
6	8
1	7
4	4
7	6
6	5
3	2
7	9
9	2
3	3
9	6
2	4

Figure 3.4: The DEM Test A. Extracted from the DEM test (Richman and Garzia, 1987).

Step 3: The subject was then prompted to perform Test B (Figure 3.5) in the same manner that Test A was done. The time was recorded with a stopwatch and written down on the DEM Score Sheet. The digits of Test B are not the same as those of Test A.

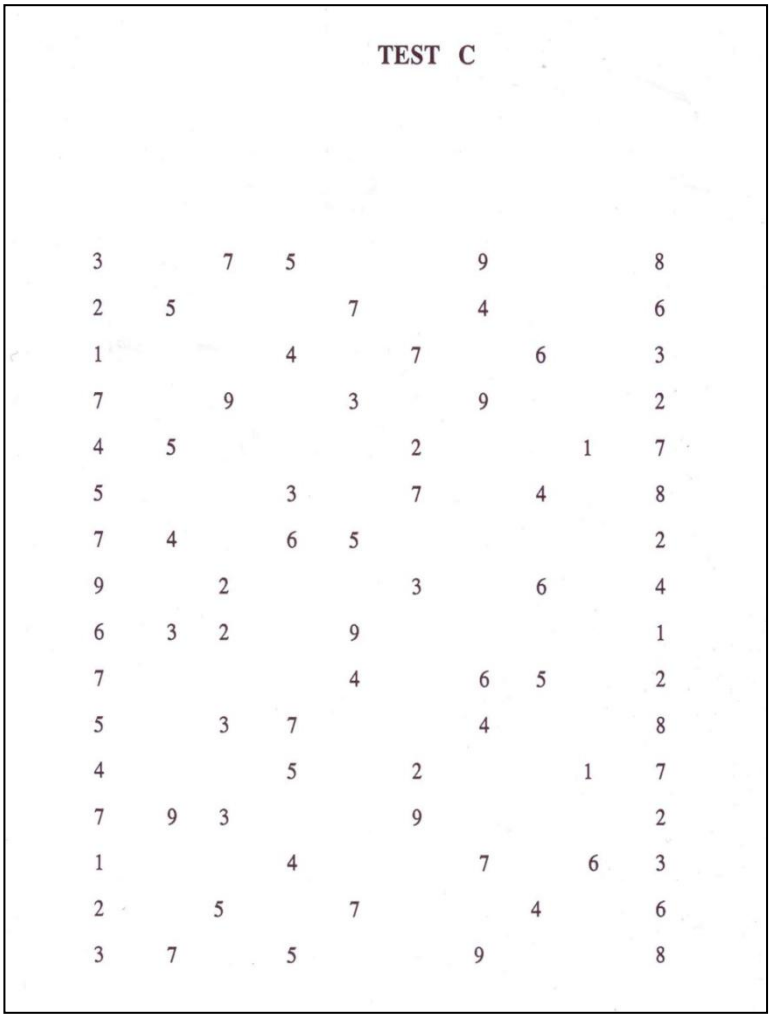


TEST B

6	7
3	9
2	3
9	9
1	2
7	1
4	4
6	7
5	6
2	3
5	2
3	5
7	7
4	4
8	6
4	3
5	7
2	5
1	9
7	8

Figure 3.5: The DEM Test B. Extracted from the DEM test (Richman and Garzia, 1987).

Step 4: In this final step, Test C (Figure 3.6) was carried out after an instruction was given on how to perform this horizontal test as it looked different from Test A and B. The subject was asked once again not to follow each digit using a finger. While the horizontal test was being carried out, the examiner carefully followed on the DEM Score Sheet and highlighted the errors that were made as the subject verbalised the test. There were different types of errors that were made which this test defines and accommodates for.



TEST C

3		7	5			9			8
2	5			7		4			6
1			4		7		6		3
7		9		3		9			2
4	5				2			1	7
5			3		7		4		8
7	4		6	5					2
9		2			3		6		4
6	3	2		9					1
7				4		6	5		2
5		3	7			4			8
4			5		2			1	7
7	9	3			9				2
1			4			7		6	3
2		5		7			4		6
3	7		5			9			8

Figure 3.6: The DEM Test C. Extracted from the DEM test.(Richman and Garzia, 1987)

3.2.3 ANALYSIS: TEST SCORE CALCULATION

Errors: There are four types of errors. The errors that the DEM took into account were omission (o), addition (a), substitution (s) and transposition (t). Omission refers to numbers that have been left out or skipped during the test. Additions refer to new numbers added to the list of numbers in a row. Substitution refers to calling out of numbers by the name of another number that is not present in the row being read.

Transposition refers to the reading out-of-sequence of the numbers in a row. Errors made in the vertical tests are ignored as they are a rare occurrence. Only errors made with the horizontal test was recorded and used in the determination of the adjusted horizontal time.

Vertical time: The two vertical scores/times (Test A and B) are added together to derive the total vertical time in seconds. There was no adjustment of the vertical time as was with the horizontal time.

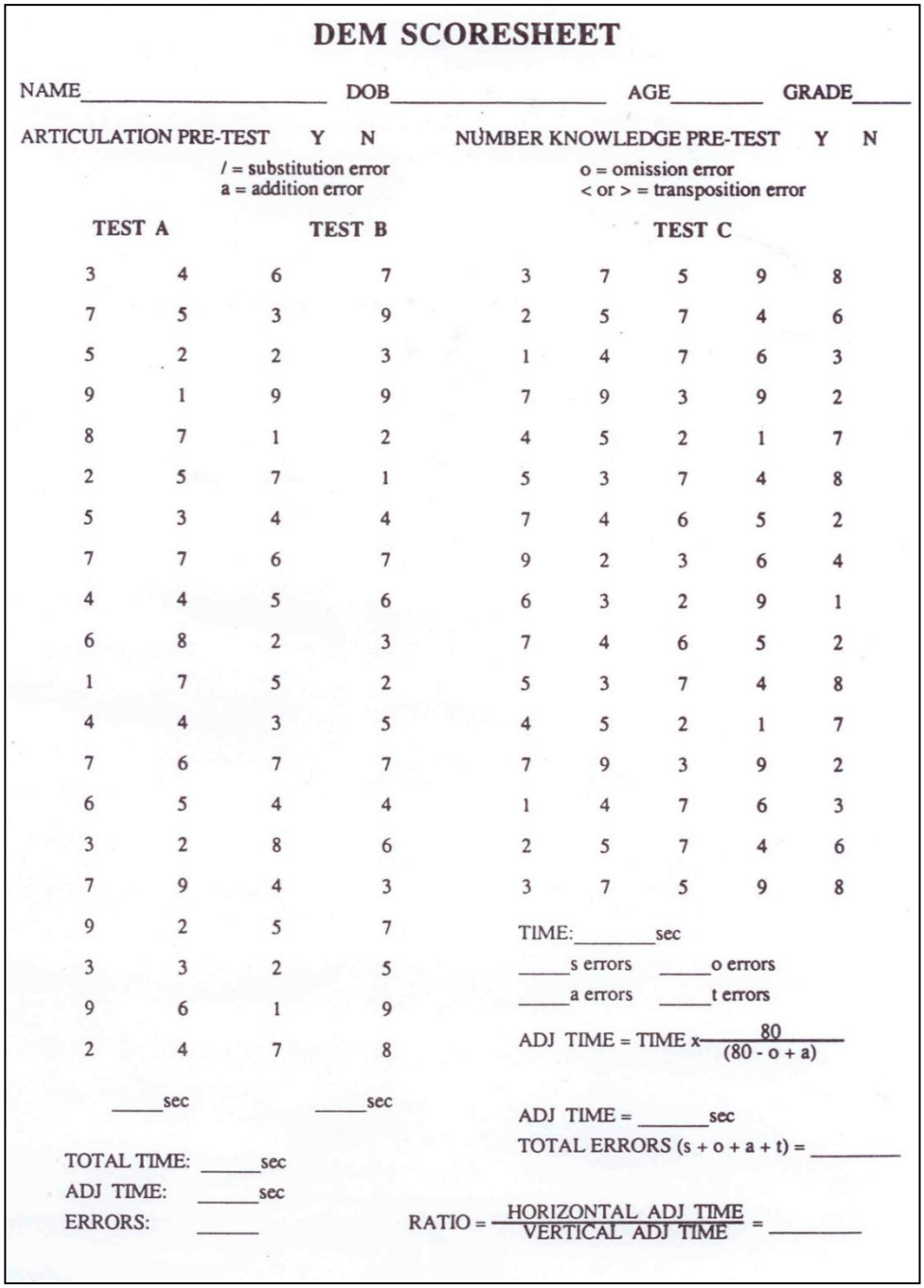
Adjusted Horizontal Time: The horizontal time (Test C) was converted to a more reliable '*adjusted horizontal time'* by compensating for the presence of errors made during this Test C. The adjustment was established by decreasing the original horizontal time when numbers are added and increasing the time when numbers were omitted. Omissions and additions were the only type of errors used in the calculation of the adjusted horizontal time. Since there were 80 digits of Test C, this number was used in the calculation to produce a more accurate reflection of the horizontal time of the subjects.

Calculation is as follows:

Adjusted Horizontal Time = horizontal time (sec) x [80/80 –o+a)]

o = omission, a = additions

Ratio: A ratio score was calculated by dividing the adjusted horizontal time by the total vertical time (H/V).



DEM SCORESHEET

NAME_____ DOB_____ AGE_____ GRADE_____

ARTICULATION PRE-TEST Y N

/ = substitution error
a = addition error

NUMBER KNOWLEDGE PRE-TEST Y N

o = omission error
< or > = transposition error

TEST A		TEST B		TEST C				
3	4	6	7	3	7	5	9	8
7	5	3	9	2	5	7	4	6
5	2	2	3	1	4	7	6	3
9	1	9	9	7	9	3	9	2
8	7	1	2	4	5	2	1	7
2	5	7	1	5	3	7	4	8
5	3	4	4	7	4	6	5	2
7	7	6	7	9	2	3	6	4
4	4	5	6	6	3	2	9	1
6	8	2	3	7	4	6	5	2
1	7	5	2	5	3	7	4	8
4	4	3	5	4	5	2	1	7
7	6	7	7	7	9	3	9	2
6	5	4	4	1	4	7	6	3
3	2	8	6	2	5	7	4	6
7	9	4	3	3	7	5	9	8
9	2	5	7					
3	3	2	5					
9	6	1	9					
2	4	7	8					
_____sec		_____sec						

TIME:_____sec

_____s errors _____o errors

_____a errors _____t errors

ADJ TIME = TIME x $\frac{80}{(80 - o + a)}$

ADJ TIME = _____sec

TOTAL ERRORS (s + o + a + t) = _____

TOTAL TIME: _____sec

ADJ TIME: _____sec

ERRORS: _____

RATIO = $\frac{\text{HORIZONTAL ADJ TIME}}{\text{VERTICAL ADJ TIME}}$ = _____

Figure 3.7: The DEM Score Sheet extracted from the DEM test (Richman and Garzia, 1987)

The DEM Score Sheet (Figure 3.7) which was used by the researcher to follow as the subject performed the tests. The recordings and calculations were done at the bottom of the score sheet, where indicated.

3.2.4 INTERPRETATION OF BEHAVIOUR TYPES

The clinician must then consult the normative tables that were established for each age category to determine if the subjects' total vertical time, adjusted horizontal time, ratio score and errors are within the DEM standard norm. For clinical purposes and interpretation, the results of the subjects have been categorised into one of four "behaviour types" (Table 3.1) based on the DEM normative scale.

Table 3.1: List of behaviour types with the rating of their vertical time, horizontal time and ratio.

Behaviour Type	Vertical Time	Horizontal Time	Ratio
Type 1	normal	normal	normal
Type 2	normal	high	high
Type 3	high	high	normal
Type 4	high	high	high

Table 3.2: Non-standardised behaviour type that is not specified in the DEM test.

Type 5	high	normal	Low to normal

3.2.5 CHARACTERISTICS OF BEHAVIOUR TYPES

Behaviour Type 1 showed normal performances in both the vertical test and horizontal test within the norms and standard deviations for their age. This resulted in a normal ratio. This behaviour type means that there were no oculomotor abnormalities or automaticity problems manifested with the DEM test.

Behaviour Type 2 comprised of subjects with normal vertical times and abnormally high horizontal times meaning their times were significantly increased above the norm. This would mean that the resultant ratio would be higher than the norm for their age category. This was interpreted according to the DEM test, as having problems with oculomotor function as opposed to automaticity skills.

Behaviour Type 3 is a category with an abnormal increase in both vertical and horizontal times but with a resultant normal ratio. This represented problems in automaticity or specifically in number calling skills.

Behaviour Type 4 is characteristic of subjects with increased vertical and horizontal times and with an abnormally high ratio. This indicated the subjects with deficiencies in both automaticity and oculomotor skills.

Behaviour Type 5 is a new category that is unlisted in the standardized DEM test. Even though the DEM specifies only four behaviour types there are other occurrences that are not classified in the DEM test by its developers. Subjects could have a high baseline performance ie. increased times with the vertical test but better and quicker times with the horizontal test. This was an unusual scenario as the horizontal test would characteristically take a longer time. This occurrence would then result in a low to normal ratio. In this study, this type of performance was classified as Behaviour

Type 5. This type of behaviour is similar to behaviour type 3 with respect to the ratio, which is normal. The implication of this type of behaviour is that automaticity deficiencies many still be the causative factor as opposed to eye movement problems as poor vertical times is a direct indicator of automaticity problems

Table 3.3 summarises the interpretation of the performances in the vertical and horizontal times and the resultant ratio that defines the behaviour type based on those 3 parameters as having normal automaticity skills and oculomotor skills, oculomotor dysfunction, deficient automaticity or a combination of deficient automaticity and dysfunctional oculomotor skills. Errors that were made during the testing were not a parameter that was used in the determination of a diagnosis or specification of a behaviour type.

Table 3.3: A **s**ummary of the list of behaviour types with a primary description of the characteristics that define them.

Behaviour Type	Characteristic
Type 1	Normal automaticity and oculomotor skills
Type 2	Oculomotor dysfunction
Type 3	Deficiencies in automaticity/number calling skills
Type 4	Deficiencies in automaticity and oculomotor skills
Type 5	*Unspecified/unclassified in the standard DEM test*

3.3 DATA ANALYSIS

The data analysis was prepared by a biostatistician based at the Department of Biostatistics at the University of the Free State.

The statistical system used was the Statistical Analysis Software (SAS) version 9.2. Specific statistical methods employed were dependant on the characteristics of the collated data as well as the best tools to express outcome of the hypotheses.

Below is a systematic overview of the analyses that were conducted:-

- A **demographic profile** of the sample was conducted using descriptive statistics to describe the frequencies (counts) and relative frequencies (percentages) of the categorical data such as gender and the frequency of subjects at the various data collection sites. Descriptive statistics were also used to express the numerical data of the age ranges of the sample through the expression of mean median and standard deviation.

- A **clinical profile** of the sample was described using descriptive statistics to express the numerical data presented in tables, which evaluated the:-
    - Time frame from birth until diagnosis of HIV infection was confirmed
    - Time frame from birth until HAART was initiated
    - Time frame from diagnosis until HAART was initiated
    - Duration on HAART
    - CD4 count distribution in the sample
    - Viral load distribution in the sample

- CD4 count distribution across the different age groups
- Viral load distribution across the different age groups

Non-parametric statistical methods were used to express the relationship between the variables as no distributional assumptions were made.

- A correlation analysis was conducted between the continuous variables of CD4 count, viral load, treatment duration and CD4 percentage, which were expressed using *Spearman's Correlation Coefficient*. Spearman Correlation Co-efficient was preferred over Pearson Correlation Co-efficient for non-normally distributed data and since a prediction of linearity between two variables was not one of the expectations, Spearman's was the better method of analysis.
- Hypothesis testing was done to validate or invalidate the claim, which was made with the null hypothesis. The precision of the estimates were assessed using 95% confidence intervals. If $p > 0.05$, this indicated weak evidence against the null hypothesis hence failure to reject the null. If $p < 0.05$, this indicated strong evidence against the null hypothesis hence it would be rejected. Statistical significance was implied indicating that some other factor besides chance was operating for the difference between the expected and the observed data to be so great.
- In this study the variables have been proportioned into specified categories were their associations were examined with Chi-square tables. Statistical significance testing between the categorical variables was evaluated

through the *Chi Square Test* as well as the *Fisher's Exact Test* where the status of the hypothesis was determined. For computation of the *p* value, where the Chi-square had a larger distribution of cells with counts less than 5, the Fisher's Exact test was used. As a rule of thumb, for deciding whether the Ch-squared approximation is good enough, the Chi- square test is not suitable when the expected values in any of cells of the table are less than 5. The Fisher's exact test is one from a class of 'exact tests' where the significance of the deviation between the observed and expected from the null hypothesis can be calculated exactly where the absolute p value is expressed. The exact p values were given for the benefit of the reader. It is useful for categorical data and examines the significance of the associations between different categorical variables.

- A **DEM analysis profile** was done on the results of the DEM test. The analysis was as follows:-
    - Vertical time, horizontal time, ratio and error scores of the full sample were examined to determine the percentage of subjects *that fell outside o*f the DEM standards.
    - Vertical time, horizontal time, ratio and errors scores where categorically expressed across the age groups and was simultaneously compared against the DEM age-specific norms.
    - The behaviour types where expressed from the results of the vertical time, horizontal time and ratio scores.

- The prevalence of the different behaviour types across the sample was determined.
- The viral load and CD4 count where independently put into three categories based on levels of severity in terms of quantity of the virus in the blood and in terms of strength of the immune system using the CD4 count values.
- The relationship between the two disease parameters where investigated with the Chi Square Test and the Fisher's Exact Test.
- The relationship between the behaviour types and the viral load and between the behaviour types and CD4 count categories were performed using a Chi Square Test. Fisher's Exact Test was further employed to determine probability.
- The prevalence of the different behaviour types in the different age categories were graphically represented and compared for assessment of trends from which conclusions.

3.4 ETHICAL REVIEW

An expedited ethics review was requested by the University of Kwa-Zulu Natal Biomedical Research Ethics Committee after submission of the research proposal. This type of review was requested as there were no invasive procedures to be performed during the data collection process. Clarification on the methodology was

requested prior to the final granting of full approval (Ethical Approval Number: HSS/0446/2010 M: Faculty of Health Sciences).

The participation of minors in this study was indispensable as this research and the instrument (DEM Test) was of relevance to children and could not be carried out with adults.

This study is classified as 'non-therapeutic' as by definition this intervention does not hold out the prospect of direct health-related benefit for the participant but the results produced may contribute significantly to knowledge in the general specified population regarding about the condition of these subjects (RSA. NDOH, 2004).

In terms of 'risk', this study places the participant at no more than minimal risk, which is also referred to as 'negligible risk'. Minimal risk is defined as that which is commensurate with daily life or routine medical or psychological examinations (RSA. NDOH, 2004). In this study no harm or discomfort was brought to the subjects as all procedures that were done on the participants are that which are routinely done in a basic eye examination along with the DEM test which was simply a reading test. All tests were non-invasive with no direct discomfort to the eyes. No pharmaceutical agents were used with the subjects during data collection.

3.5 ETHICAL CONSIDERATIONS

3.5.1 Informed Consent

Signed informed consent was obtained from all parents/guardians of the subjects (Appendix C). The adults responsible for the subjects were given information on an information sheet (Appendix B) which stated the aim of this study, the requests of the researcher and the expected role of the subjects in this study. Consent was stated as voluntary and no subjects were victimized if participation was refused as subjects and their caregivers were granted autonomy and were under no obligation to participate. The subjects had the right to withdraw from the study at any point and could request that their data not to be used. The presence of the adult responsible for the subject during the data collection process was also welcomed. This additional information was included in the information sheet. The consent form and information sheet was produced in three languages ie. English (B1,C1), Afrikaans (B2,C2) and Sotho (B3,C3) and was given to the respective care-givers in their preferred language.

3.5.2 Patient Confidentiality

The confidentiality of the subjects were upheld as no names were be used in the data collection form, but a code for each subject was allocated. There was a 7-digit code developed for each subject. The first 3 digits were abbreviations of the data collection site that the subject was examined at. The next 2 digits were the first letters of the subjects' surname and first name and the last 2 digits represented the number that the subjects were given while waiting in order to be seen. There was no

duplication of the subject codes and this was the manner in which the data of each subject was captured electronically.

The confidentiality of the subjects' health status and their medical history was also maintained as the knowledge of such was only held by the researcher who was the only data collection personnel along with the nursing staff that provided assistance during the process.

3.5.3 Moral Considerations and Conduct

Regardless of how the subject performed during the screening and the DEM testing phases, positive re-enforcement was used with every subject. There was no negative information or comments given to the subject during the data collection process.

Even though the researcher was under no obligation, every parent and guardian that accompanied the subjects on the day of data collection was given general feedback on the overall condition of the subjects' eyes. All subjects were advised to come for an eye exam once a year but if problems with their eyes occurred at any point, they should come as soon as possible to seek medical attention.

When there were subjects that co-incidentally were found to require further medical care during the data collection process, they were assisted at the same time

within the expertise of the researcher. Alternatively, they were referred to the National Hospital Optometry Clinic in Bloemfontein for further intervention or referred internally to the attending doctor at the data collection site.

3.5.4 Administrative Obligations

A letter of request to the provincial Head of Health in the Free State was submitted prior to commencement of the data collection process. This letter briefly explained the nature of the study and a request to use the indicated health facilities as the data collection sites. Approval was subsequently granted with recommendations (Appendix D).

3.6 METHOD OF REFERENCING

The citations and referencing in this study was done according to the Harvard system of referencing (De Montfort University, 2009).

CHAPTER FOUR

RESULTS

This chapter presents the results of the study by initially describing the study sample. This is followed by an in-depth analysis of the data with specific focus on the objectives and the supportive investigations that were conducted to address the scientific enquiry. The chapter concludes with a summary of the results presented.

4.1 DEMOGRAPHIC PROFILE

The data to follow under this section represents the frequency (absolute value) of subjects at the different data collection sites as well as the gender and age distribution of the sample.

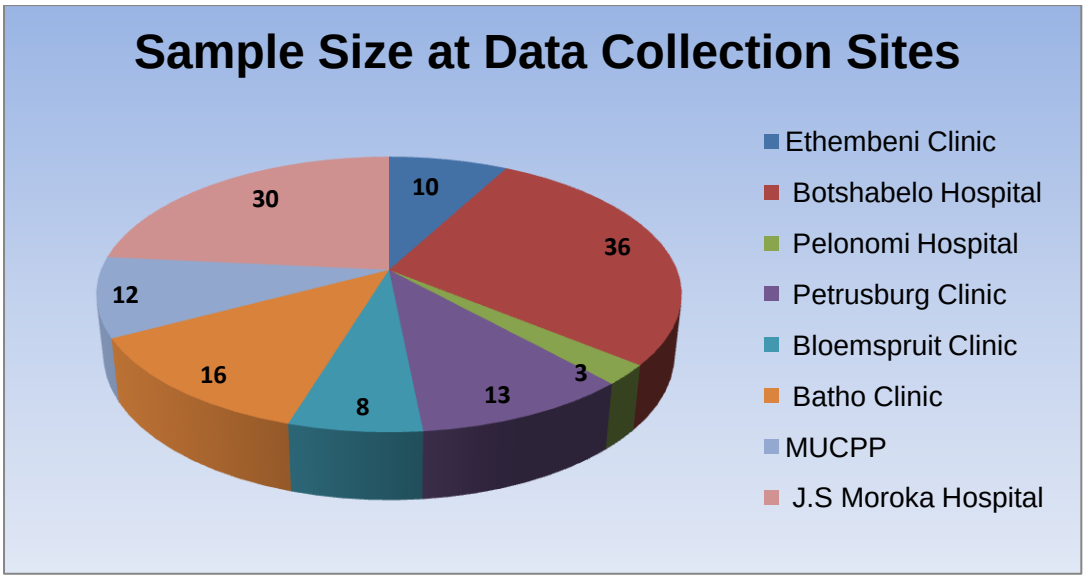


Figure. 4.1: The frequency of subjects that were conveniently selected at the different data collection sites.

Figure 4.1 demonstrated that there were 69 (54%) subjects seen in the three hospitals with the remaining 59 (46%) subjects accessed from clinics. Most of the subjects came from Botshabelo Hospital in Botshabelo and J.S Moroka Hospital in Thaba'nchu making up 52% of the total sample. There were 23(18%) subjects seen in the Xariep District and 82% of subjects were from the Motheo District. None of the subjects from these areas used English as their home language.

Table 4.1: Gender distribution of the total sample

GENDER DISTRIBUTION			
N	**Gender**	**Frequency (n)**	**Percent (%)**
128	**Male**	**61**	**48**
	Female	**67**	**52**

An almost even distribution of gender differences as shown in table 4.1 was not an intentional desire for the sample as is shown in Table 4.1, however the final sample pool shows a close to even gender spread across the sample of subjects. The ratio of male to female subjects selected, quite closely resembles the 1:1 ratio of the gender distribution of children aged 0-14 years in the Free State province (Statistics SA, 2012a).

Table 4.2: Represents the summary statistics of the age variable

AGE				
N	**Mean**	**Median**	**Minimum**	**Maximum**
128	**10yrs and 1 month**	**8yrs and 6 months**	**6yrs and 9 months**	**13 yrs and 11 months**

AGE				
N	**Mean**	**Median**	**Minimum**	**Maximum**
	(121 months)	**(102 months)**	**(81 months)**	**(167 months)**

In table 4.2, the mean (10yrs + 1 month) quite closely matches the centre value of the spread (median). The minimum age ranged from 6 years 9 months to 13 years 11 months as the maximum age, which was the restriction placed on the sample drawn from the population due to the DEM test only being applicable to candidates up to 13years and 11 months.

Table 4.3: The distribution of the ages in the sample ranging from 6 to 13 years and 11 months.

AGE DISTRIBUTION		
Age	**Frequency (n = 128)**	**Percent (%)**
6 (0 – 11 months)	**4**	**3**
7 (0 – 11 months)	**13**	**10**
8 (0 – 11 months)	**24**	**19**
9 (0 – 11 months)	**30**	**23**
10 (0 – 11 months)	**16**	**13**
11 (0 – 11 months)	**11**	**9**
12 (0 – 11 months)	**17**	**13**
13 (0 – 11 months)	**13**	**10**

Table 4.3 has shown that a large proportion of subjects were 9 years old (23%) with only 3% of subjects at age 6 years of age. The majority of the subjects were from 8 to 10 years of age (55%)

4.2 CLINICAL PROFILE

This section comprehensively describes the clinical profile of the sample using summary statistics by examining the virologic status, immunologic status as well as the diagnosis and treatment timelines ranges for each of the subjects. The data to follow are represented with tables expressing the time taken till the diagnosis of HIV infection was confirmed, the waiting period until treatment was initiated, the duration of subjects on HAART as well as the CD4 count and viral load distribution across the sample. More focused expression of the data regarding the viral load and CD4 count variables had been described for each age group. The second section presented the correlation analyses between the parameters of viral load, CD4 count and treatment duration.

4.2.1 Description of Variables

Table 4.4: The summary statistics of the time period from birth until a diagnosis of HIV infection was confirmed.

TIME FRAME FROM BIRTH UNTIL DIAGNOSIS CONFIRMED					
N	**Mean**	**Std. Dev.**	**Median**	**Minimum**	**Maximum**
81	**5yrs and 10 months (70.58 Months)**	**±3yrs (41.7 Months)**	**6yrs 1 month (73 Months)**	**1 month**	**13yrs**

The average time taken for the diagnosis to be confirmed as shown in table 4.4 was 5 years and 10 months with a centre value of 6 years and 1 month. This analysis was based on only 81 of the 128 subjects as there was insufficient data available in the remaining 47 subjects' health records of when the diagnosis was established.

Table 4.5: The summary statistics of the waiting period from diagnosis to the initiation of HAART

PERIOD FROM DIAGNOSIS TILL INITIATION OF TREATMENT					
n	**Mean**	**Std. Dev.**	**Median**	**Min**	**Max**
79	**2yrs 7 months (19.1 Months)**	**2 yrs (24.5 Months)**	**9.0 Months**	**0**	**10 yrs 7 months (127 Months)**

The numerical data displayed in table 4.5 is a description of the time frame for only 79 of the 128 subjects as the time of diagnosis was not always specified for all subjects in the subjects' health records. With the centre value (median) being a low 9 months as compared to the mean of 19.1 months, this indicated that 50% or more of the 79 subjects were put on HAART in under 12 months of being diagnosed.

Table 4.6: The summary statistics of the waiting period from birth to the initiation of HAART

PERIOD FROM BIRTH TILL INITIATION OF TREATMENT				
n	**Mean/Median**	**Std. Dev.**	**Min**	**Max**
127	**7yrs (83.9 Months)**	**±3yrs (35.46 Months)**	**10 month**	**13yrs and 6 months (162 Months)**

Both the mean and median time from a sample of 127 subjects indicated in table 4.6 that approximately 7 yrs (6yrs and 11 months) would be the waiting period that an HIV positive child from this sample had waited to be treated with HAART (Table 4.6).

Table 4.7: The summary statistics of the duration of HAART in the subjects

DURATION ON HAART					
n	**Mean**	**Std. Dev.**	**Median**	**Min**	**Max**
127	**3yrs 2 months (38.2 Months)**	**±3yrs (35.46 Months)**	**2yrs 10 months (34 months)**	**4 months**	**6yrs and 8 months (92 Months)**

The expression of the numerical data on the treatment duration variable ranged from 4 months up to 7 years and 8 months as shown in table 4.8. This represented a sample with uninterrupted treatment with the specified 3 drug regimen. The median and the mean are almost the same, which indicated a gradual climb from the minimum to the maximum duration time.

Table 4.8: The length of time at which each age group was on HAART

TREAMENT DURATION (MONTHS) ACROSS THE AGE CATEGORIES OF THE SAMPLE					
Age	**n**	**Mean**	**Std. Dev.**	**Min**	**Max**
6yrs	**3**	**46.67**	**±28.75**	**16**	**73**
7yrs	**13**	**44.15**	**±24.94**	**4**	**79**
8yrs	**24**	**42.04**	**±24.59**	**6**	**90**
9yrs	**30**	**38.30**	**±23.00**	**4**	**73**

TREAMENT DURATION (MONTHS) ACROSS THE AGE CATEGORIES OF THE SAMPLE					
Age	**n**	**Mean**	**Std. Dev.**	**Min**	**Max**
10yrs	**16**	**40.19**	**±28.21**	**5**	**92**
11yrs	**11**	**34.55**	**±20.30**	**8**	**73**
12 yrs	**17**	**29.53**	**±24.25**	**5**	**83**
13yrs	**13**	**35.00**	**±22.32**	**5**	**76**

Table 4.8 displays the numerical data of the treatment duration of subjects in the sample according to the different age groups. The pool of 6 year olds had the longest average time on HAART (46 months). Subjects in the 12 year old group with a frequency of 17 subjects averaged the lowest duration on HAART (29 months). By close inspection of all the means in the different age groups, children who were in the 6 and 7 year olds' age groups were the longest on HAART whereas the subjects in the 12 and 13 years age groups were the shortest on treatment.

Table 4.9: The distribution of the CD4 counts across the sample.

CD4 COUNT DISTRIBUTION			
Immunologic Categories	**CD4 Count (cells/mm^3) *Absolute Value***	**Frequency (n = 128)**	**Percent (%)**
Severe suppression	**<200**	**7**	**5.5**
Evidence of moderate suppression	**>200 ≤ 500**	**21**	**16.4**
Minimal/no evidence of suppression	**≥ 500**	**100**	**78.1**

The CD4 count range was broken up into 3 categories abridged from the CDC classification of infection control and shown in table 4.9 above. Seventy eight percent (78%) of subjects are within the 3rd category, indicating that over 3 quarters of the sample had an immune system that is being sustained within a normal range due. Any absolute CD4 count ranging from 500 cells/mm^3 to 2010 cells/mm^3 is taken to be a normal quantity of CD4 lymphocytes in serum. Only 5% of the sample was found to have counts of less than 200 cells/mm^3, implying that these subjects would be highly vulnerable to opportunistic infections and sporadic viral proliferation due to a severely compromised immune system.

Table 4.10: the summary statistics of the CD4 count and percentage across the sample.

CD4 COUNT SUMMARY						
N	**CD4 Count**	**Mean**	**Std. Dev.**	**Median**	**Min**	**Max**
128	**Absolute (cell/mm^3)**	**815.73**	**±419.50**	**778.5**	**17**	**2210**
89	**Percent (%)**	**25.92**	**±9.81**	**26.92**	**2.87**	**52.66**

The percentage row in table 4.10 does not represent the percentile equivalent of the absolute data as shown above, but is an independent parameter of a sample of 89 subjects. Ideally a CD4 percentage is used as the parameter to express CD4 count in children as opposed to the absolute CD4 count value but only 89 of the 128 subjects had results expressed as a percentage from the NHLS blood work-up as was found in the subjects' health records. In table 4.10, the absolute and percentage mean reflects that the sample was generally 'healthy' in terms of their immune status.

Table 4.11: The summary statistics of the absolute CD4 count variable for each age category.

ABSOLUTE CD4 COUNT ACROSS THE AGE CATEGORIES					
Age	**n**	**Mean**	**Std. Dev.**	**Min**	**Max**
6yrs	**4**	**858.50**	**±427.62**	**361**	**1263**
7yrs	**13**	**923.77**	**±581.23**	**17**	**2131**
8yrs	**24**	**866.83**	**±395.36**	**74**	**1588**
9yrs	**30**	**995.77**	**±388.12**	**147**	**2101**

ABSOLUTE CD4 COUNT ACROSS THE AGE CATEGORIES					
Age	**n**	**Mean**	**Std. Dev.**	**Min**	**Max**
10yrs	**16**	**687.50**	**±351.20**	**75**	**1266**
11yrs	**11**	**754.00**	**±568.19**	**325**	**2210**
12 yrs	**17**	**670.65**	**±299.01**	**183**	**1130**
13yrs	**13**	**584.54**	**±245.46**	**20**	**1000**

Table 4.11 shows that across all age groups the mean CD4 count was above 500 cell/mm^3. Since the normal absolute CD4 count is classified as being above 500 cells/mm^3, this would imply that all age groups had a satisfactorily functioning immune system, even though individually, not all subjects had a healthy functioning immune system. Subjects in the 9 year old age group are perceived to be the healthiest group with the 13 year old subjects found to be within the lower range of a healthy functioning immune system.

Table 4.12: The frequency of subjects in the different viral load categories

VIRAL LOAD DISTRIBUTION		
Viral Load (copies/ml) ***Absolute Value***	**Frequency (n = 125)**	**Percent (%)**
<40	**81**	**65**
>40 ≤ 1000	**32**	**25**
≥ 1000	**12**	**10**

The viral load data was categorized into 3 levels in table 4.12. These categories were determined by the resultant effect that the HAART had on the virus in the blood. In category 1, if the viral load is < 40 copies/ml, it is classified as *undetectable*. Table 4.12 indicates that 65% of the sample fall into category 1 meaning that this portion of the sample is adequately responding to the HAART, which is currently effective in suppressing the virus in the blood. If the viral load is > 1000 copies/ml, this is interpreted as *virologic failure* as the anti-retrovirals are ineffective at suppressing the viral load within the blood, which represents 10% of the sample. Any value, expressed between 40 copies/ml and 1000 copies/ml indicates that there is a moderate viral load in the blood serum. Twenty five percent (25%) of the sample was found to have a viral load between 40 copies/ml and 1000 copies/ml, which is not the desired goal.

Table 4.13: The summary statistics of the viral load variable across the complete sample of subjects.

VIRAL LOAD SUMMARY (copies/ml)					
N	**Mean**	**Std. Dev.**	**Median**	**Min**	**Max**
125	**7414.13**	**±42038.5**	**0**	**0**	**368391**

Table 4.13 summarized the description of the viral load variable of 125 subjects as 3 subjects didn't have viral load data in their health records at the time of assessment. As displayed in Table 4.13, the median and the mean differ significantly. Any value below 40 copies/ml is classified as undetectable and denoted with a zero

figure in this study. The median is the more accurate statistic as over 50% of the sample has an undetectable viral load (40 copies/ml).

Table 4.14: The summary statistics of the viral load variable across the different age categories.

VIRAL LOAD ACROSS THE AGE CATEGORIES						
Age	**n**	**Mean**	**Std. Dev.**	**Median**	**Min**	**Max**
6	**4**	**123.25**	**±246.50**	**0**	**0**	**493**
7	**12**	**70.83**	**±155.54**	**0**	**0**	**513**
8	**24**	**7269.50**	**±33595.85**	**0**	**0**	**164849**
9	**30**	**12563.37**	**±67212.56**	**0**	**0**	**368391**
10	**14**	**25670.57**	**±64292.00**	**0**	**0**	**197829**
11	**11**	**1099.73**	**±2456.92**	**0**	**0**	**7628**
12	**17**	**71.53**	**±130.86**	**0**	**0**	**515**
13	**13**	**104.77**	**±353.56**	**0**	**0**	**1279**

Table 4.14 shows that the 8, 9 and 10 year olds have more significant viral load averages, well above the undetectable threshold, with only the 7 and 12 year olds having viral loads averages close to being undetectable. The median across all age groups are zero indicating that 50% of all subjects in each age sample group had undetectable levels. This would then imply that in the 8 to 10 year age groups there were subjects with significantly high individual viral loads (<50% of the sample group) which elevated the viral load mean substantially. Evidence for this is further supported in the fact that the maximum viral load findings of subjects were in the 8 to 10 year age

group. In this case, the median is a more accurate description of the viral load average in the different age group.

4.2.2 Correlation Analysis

Table 4.15*:* Numerical display of the associations between the variables expressed via Spearman's Correlation Co-efficient.

RELATIONSHIP BETWEEN THE VARIABLES		
N	**VARIABLES**	**SPEARMAN'S CORRELATION COEFFICIENTS (r)**
125	**CD4 Count vs Viral Load**	**-0.26674**
127	**Treatment Duration vs CD4 Count**	**0.44084**
124	**Treatment Duration vs Viral Load**	**-0.14926**
89	**Treatment Duration vs CD4 %**	**0.60420**

CD4 count versus Viral Load

The co-efficient (r) that resulted when an association between the above 2 variables were compared was a negative correlation. Also, with the r = 0.26, this indicated a poor or **weak negative correlation** as the value fell outside of the 0.6 to 1.0 strong correlation range.

Treatment Duration versus CD4 Count

When the association between these 2 variables were investigated a **weak positive correlation** existed. Even though the analysis indicated that as treatment duration

continued the CD4 count would increase which is what is believed, the correlation of r = 0.44 did not indicate a linear relationship.

Treatment Duration versus Viral Load

In the analysis of the trend between the above variables, a **marginally weak negative correlation** existed. As treatment duration increased the viral load decreased which is an expectation of the HAART. However, a precise linear relationship did not exist.

Treatment Duration versus CD4 %

As was expected a positive association between treatment duration and CD4 % existed. A further interpretation of the co-efficient was that the association between the variables was strong. The conclusion was then that a **strong positive correlation** existed between those variables. It must also be noted that of the 128 subjects of the sample that had available absolute CD4 counts, it was only 89 subjects that had available CD4 % data.

4.3 DEM ANALYSIS PROFILE

This section is divided into two sections where a more complex analysis of the data of phase 3 of the data collection process is presented. In the first part, the researcher profiled the results of the DEM test. The researcher examined the vertical times, horizontal times, DEM ratio and errors of the subjects, and compared it to the normative scale of the DEM test. In the second part, the researcher thereafter

examined the prevalence of the behaviour types across the sample and focused the investigation for each age category. Correlations and associations between the primary variable of behaviour type and other variables (viral load, CD4 count) were investigated to determine the existence of any trends to help answer the research question.

4.3.1 Analysis of the DEM Test Results

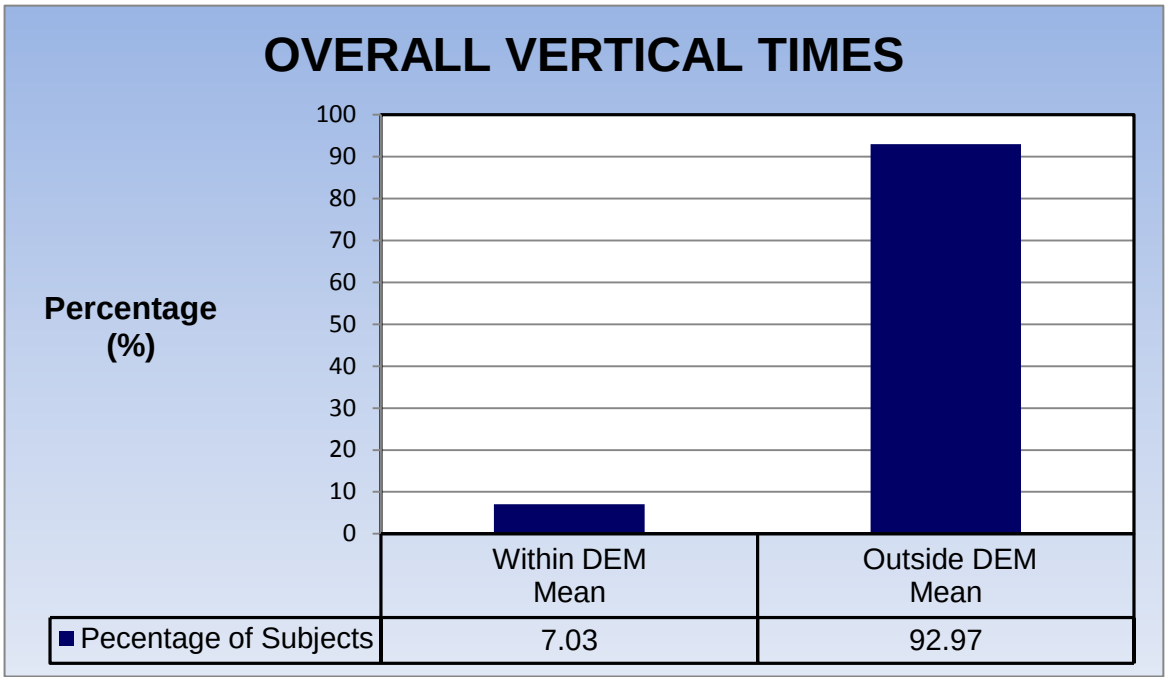
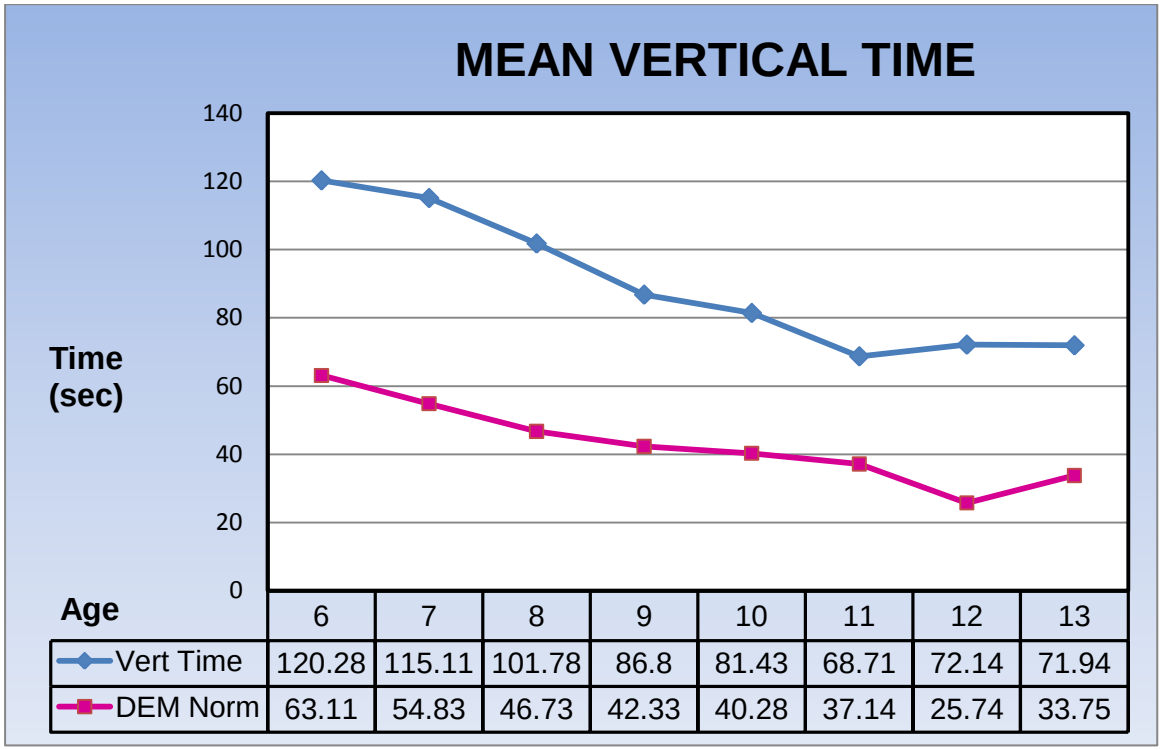


Figure 4.2: The vertical times across the sample that fell within and outside of the DEM normative value.

Figure 4.2 which illustrates the vertical times shows that almost 93% of the subjects had times that were outside of the DEM mean and standard deviation of the sample. The absolute value was 119 subjects outside of the standard norm and 9 subjects within the norm. 'Outside of the norm' refers to a combined time on subtest A and B being 'slower' than the specified mean time. The norms for vertical times are

age-specified norms and differ for different age groups. Therefore, figure 4.2 is reflective of the overall performance of individual subjects across the full sample calculated against the mean and standard deviation (±SD) for each age category. The ±SD was always taken into consideration to assess the range of the mean.



Age	6	7	8	9	10	11	12	13
Vert Time	120.28	115.11	101.78	86.8	81.43	68.71	72.14	71.94
DEM Norm	63.11	54.83	46.73	42.33	40.28	37.14	25.74	33.75

Figure 4.3: Mean vertical times for each age category against the DEM mean vertical time norm.

Figure 4.3 is appropriately presented with the use of trend lines, the mean performance of subjects of different age categories from 6 years to 13 years together with the presentation of a data table for immediate reference. The obvious interpretation from the graph is that the subjects'mean performance were well below the DEM mean norm as is verifiable by the data table.

By further inspection of the ±SD of the DEM standard mean and the study mean, the findings were still consistent in that the ranges were still beyond each other. The study vertical times were significantly slower than the norm and in some cases the mean vertical times were more than twice the DEM mean norm times. An inverse relationship between age and time is a normal trend and is apparent by trend lines of the DEM norm and study sample.

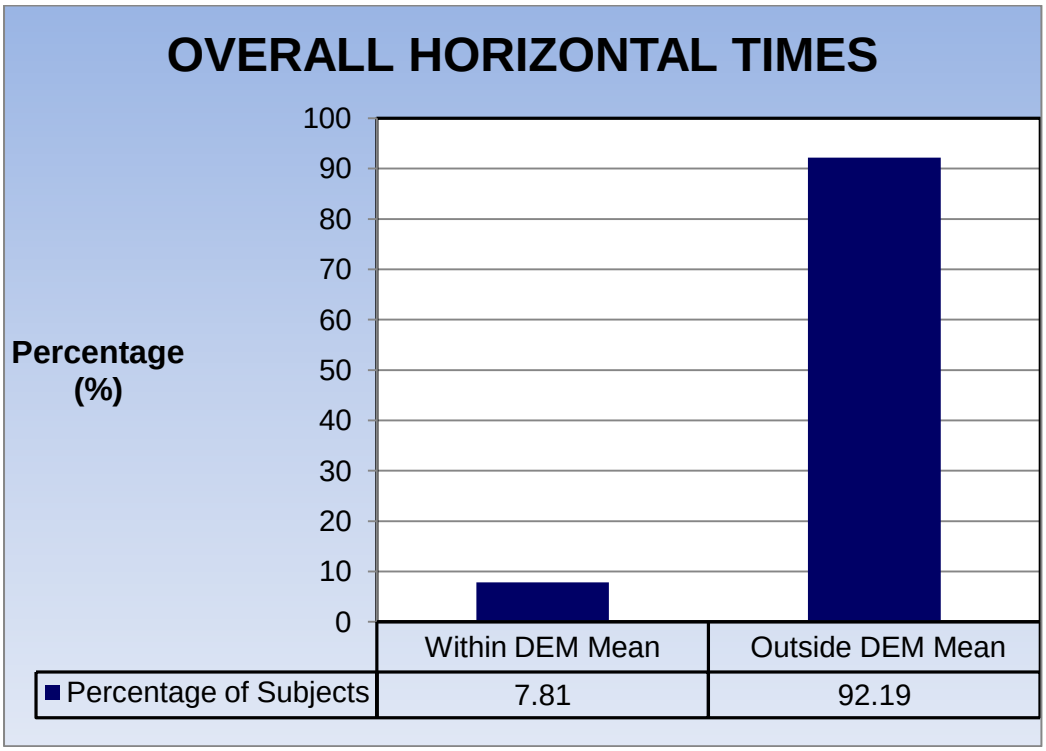


Figure 4.4: The percentage of individual horizontal times across the sample that fell within and outside of the DEM mean normative value for horizontal time.

Figure 4.4 which illustrates the horizontal times expressed that almost 92% of the 128 subject pool had times that were outside of the DEM Mean and ±SD, with almost 8% within the DEM mean norm. The absolute value was 118 subjects were outside of the

standard norm and 10 subjects within the norm. The results of the horizontal test (subtest C) almost mimic the performance on the vertical test. As with the vertical test, the norms (means and standard deviations) are age-specific and differ for different age groups.

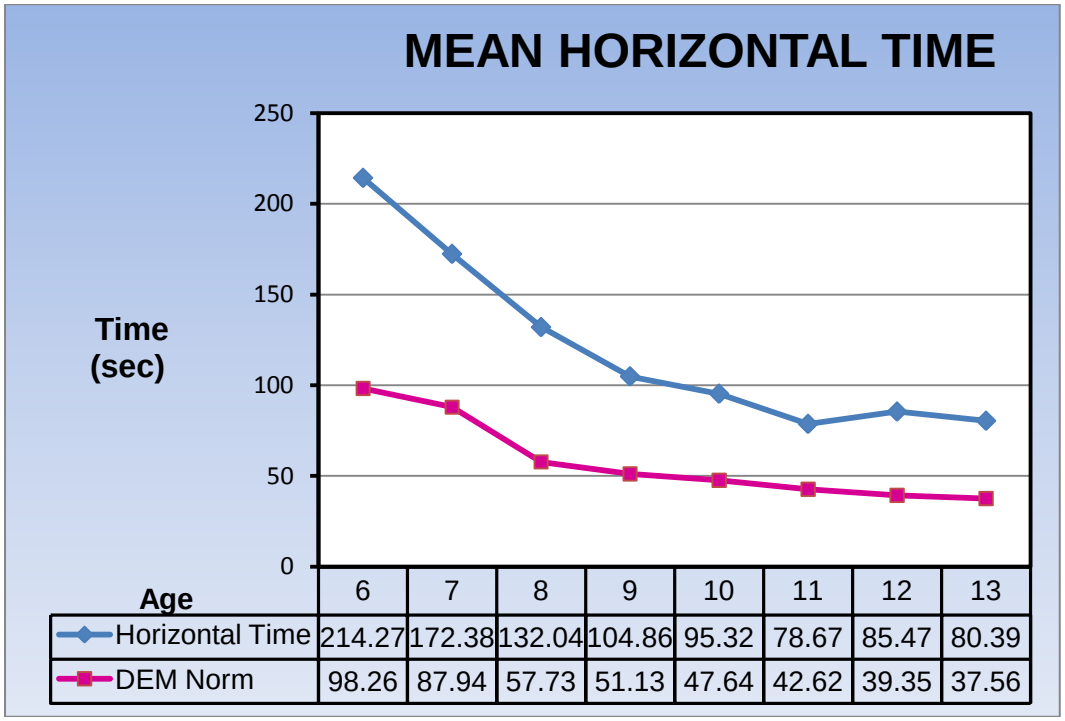


Figure 4.5: Mean horizontal times for each age category is presented with the DEM horzontal time norm.

Figure 4.5 demonstrates with the use of two trend lines joining the data points the subjects' mean performance at the different age categories against the DEM mean norm. The interpretation was that the resultant study means across the sample were below the norms as is evident with the presentation of a data table below the plot of figure 4.5. The strategic use of a data table helped to verify the graph presented and showed that the average horizontal times were much slower across all the ages norms.

A closer inspection of the standard deviations showed that the mean horizontal times for only the 9 year olds 104.86 (± 40.62) and 13 year olds 80.39 (± 35.98), marginally fell just within the standard deviation of the DEM norms for the 9 year olds 51.13 (± 13.30) and the 13 year olds 37.98 (± 7.23). The usage of the word 'marginally' in this context referred to the lower limit of the ±SD of the study sample overlapping the extreme upper limit of the DEM mean norm.

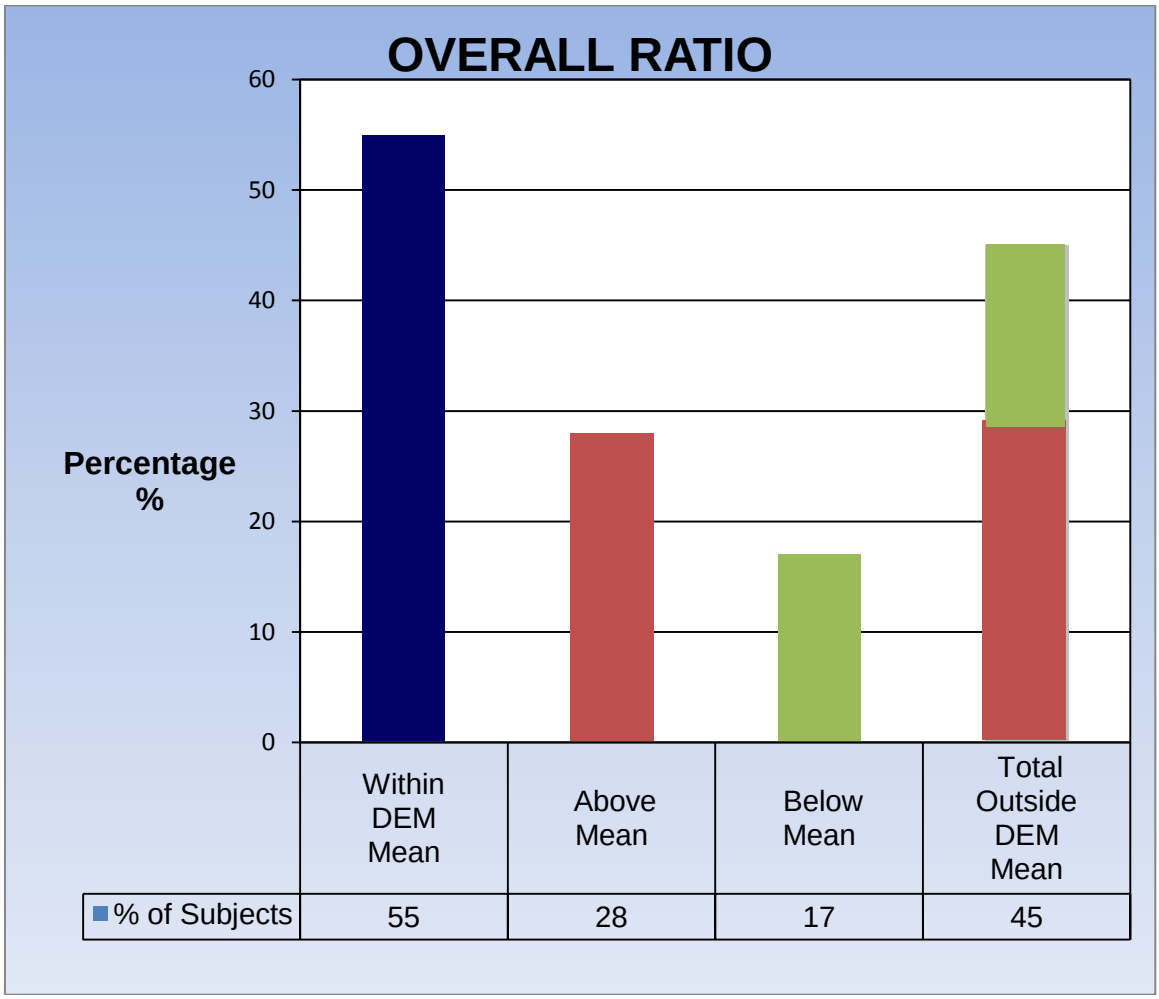
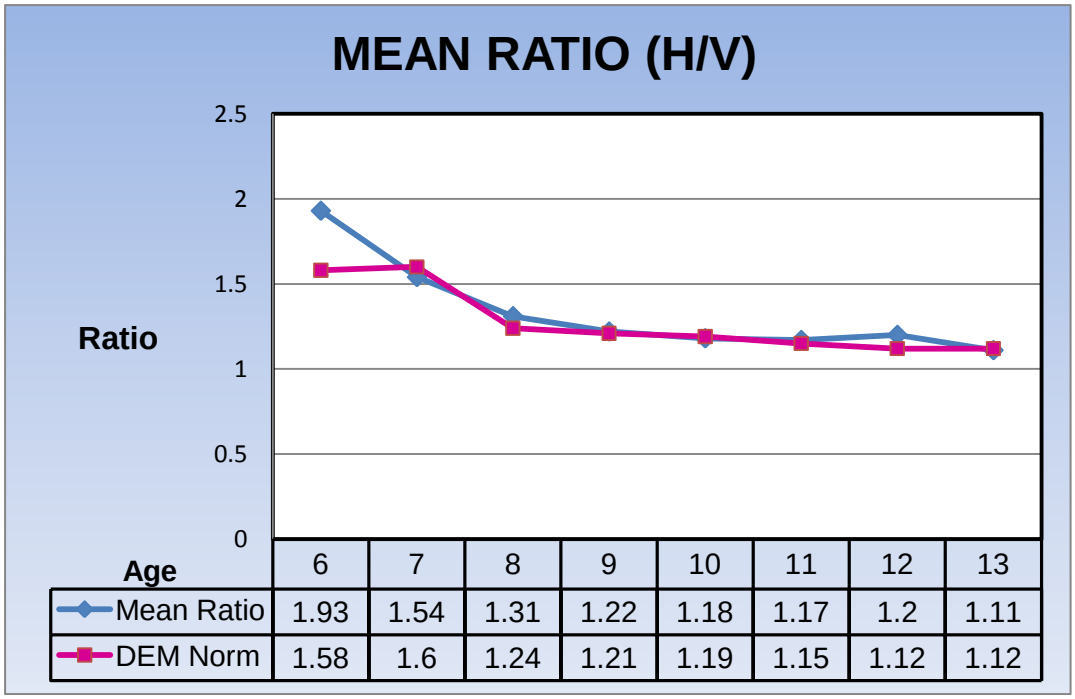


Figure 4.6: A display of the percentage of subjects whose individual DEM ratio scores fell within and outside of the specified DEM norm and ± SD.

In figure 4.6, a comparison of the percentage of subjects' absolute ratio scores against the DEM mean ratio norm, it is evident that 45% of 128 subjects had ratios outside of the mean and ±SD in the full sample of subjects (Figure 4.6). The absolute number of this being 57 subjects out of the 128. This would mean that 71 subjects had their ratios within the DEM ratio norm.

The 45% of subjects were further sub-divided for a more closer examination of the data to show that 'Total Outside DEM Mean' was a combined expression of those that fell both above and below the standard mean ratios standard deviation. An absolute number of 22 (17%) subjects of the 128 had uncharacteristic ratios below the DEM mean ratio and ±SD.



Age	6	7	8	9	10	11	12	13
Mean Ratio	1.93	1.54	1.31	1.22	1.18	1.17	1.2	1.11
DEM Norm	1.58	1.6	1.24	1.21	1.19	1.15	1.12	1.12

Figure 4.7: Mean ratio for each age category is presented with the DEM mean ratio norm.

The mean ratio graph in figure 4.7 shows a different picture as compared to the mean vertical time graph and the mean horizontal time graph. Even though the vertical times and horizontal times were much slower in the subjects as compared to the DEM norms, the mean ratios that are produced almost mimics the trend of the means for the DEM ratio.

In 100% of the age categories, the study mean ratio and ±SD is within the DEM ±SD for the mean norm.

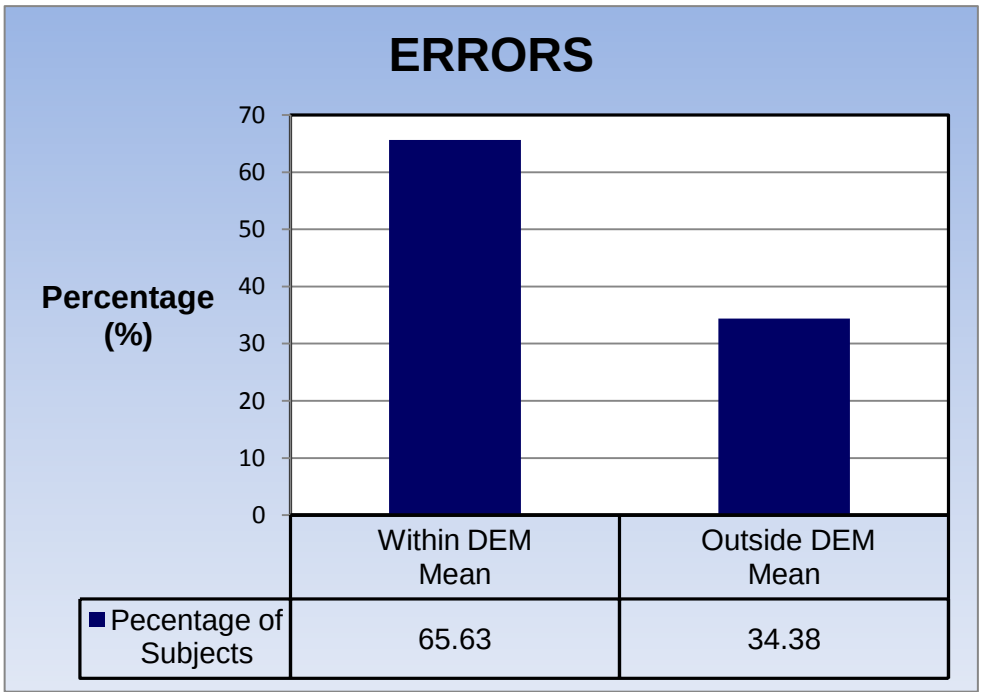
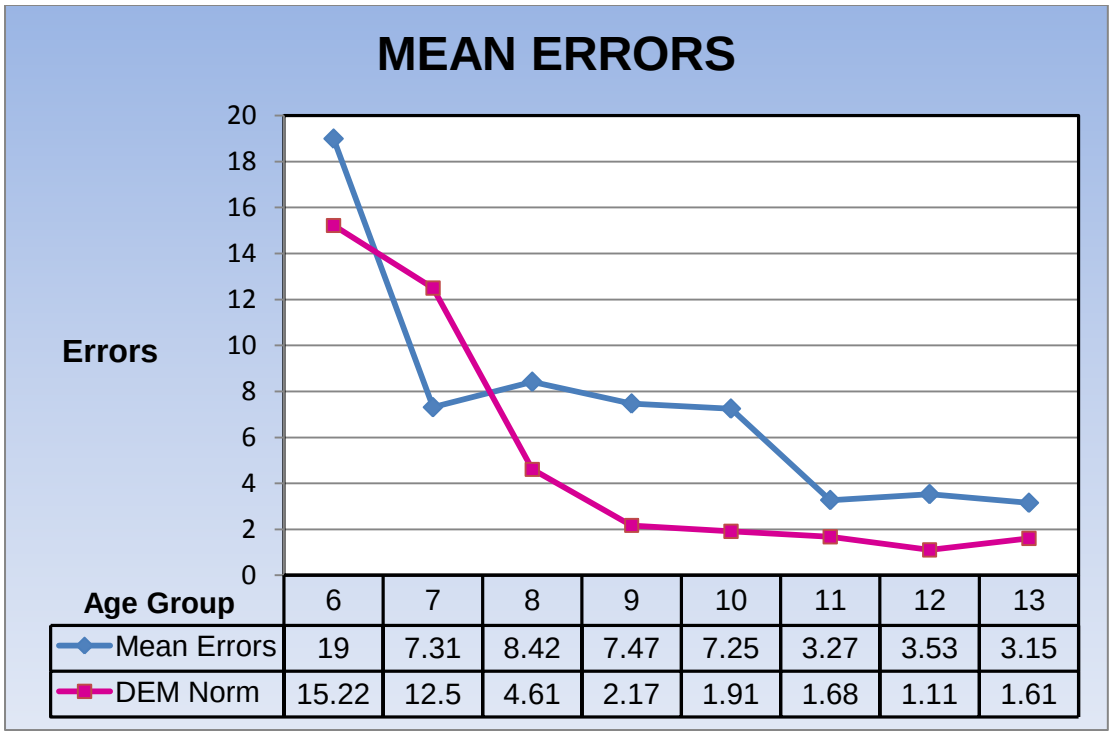


Figure 4.8: Representation of the percentage of subjects with error scores that fell within or outside of the DEM mean errors norm.

Figure 4.8 presents the error parameter of the DEM test and exhibited that 66% (84) of subjects made errors during testing that were within the ±SD of the DEM mean norm. As with all the other parameters the error range is age-dependent and there is a mean

error score and ±SD for each age group. The above graph is reflective of the full study sample.



Age Group	6	7	8	9	10	11	12	13
Mean Errors	19	7.31	8.42	7.47	7.25	3.27	3.53	3.15
DEM Norm	15.22	12.5	4.61	2.17	1.91	1.68	1.11	1.61

Figure 4.9: The mean errors and the DEM norm mean errors across the different age groups.

The Figure 4.9 above shows a higher mean error value in the younger age group and almost plateaus out from the 11 to 13 year olds range as is demonstrated by the trend line and supported by the data table. The inverse relationship of age and error is apparent with the DEM norm as well as with the study sample results. It is only in the age 7 category that for the 1st time the study sample average for the error mean falls significantly below the standard.

Table 4.16: DEM norms compared against the findings in this study for the different DEM parameters

RESULTS SUMMATION					
n	**Parameter**	**Mean**	**Std. Dev.**	**Min**	**Max**
128	V. Time	87.85	±29.19	34	177
534	DEM Norm	44.17	±8.39	-	-
128	H. Time	111.72	±47.48	44.2	257
534	DEM Norm	57.78	±14.93	-	-
128	Ratio	1.27	±0.34	0.56	2.89
534	DEM Norm	1.28	±0.22	-	-
128	Error	6.64	±6.37	0	24
534	DEM Norm	5.10	±5.47	-	-

Table 4.16 is a comprehensive display of the means and standard deviations of each of the 4 test parameters for the full sample in the study. The researcher inserted the DEM normative values for the purposes of comparison only.

The values for each parameter are not of any important significance as each age category has its own independent mean and ±SD. The purpose of this presentation of combining and averaging the data across the sample to produce one mean and ±SD was to demonstrate the overall differences in performance of the sample against the DEM standard norms for the reader to appreciate. The DEM test used a sample of 534 subjects in the development of the DEM normative scale for reference.

4.3.2 Analysis of Behaviour Types in Association with Other Variables

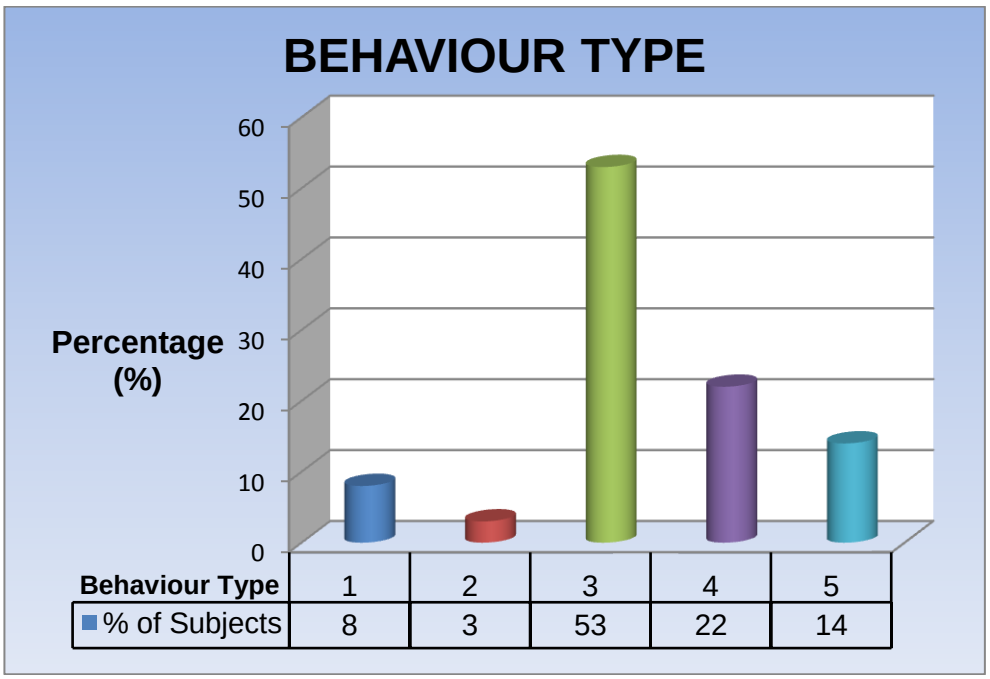


Figure 4.10: Percentage of subjects classified into the different behaviour types from 1 – 5.

As is displayed in figure 4.10, there are 5 behaviour types of which type one to four are the standard behaviour types (Table 3.1) as classified by the DEM test and type 5 represents the unclassified type (Table 3.1). The colours representing the different behaviour types will remain consistent through out this chapter for easier and quicker identification for the reader's convenience.

Behaviour type 3 was the most prevalent in the study sample making up 53% (68) of subjects and type 2 was the least prevalent in the sample making up only 3% (4) of subjects. A surprising 14% (18) of subjects fell into an unspecified category of behaviour types. The distinct characteristic of this type 5 is the low ratio. Of the 18 subjects in this category, 13 had increased horizontal and vertical times displaying similar characteristics to

type 3 and type 4 but with a low ratio outside of the norm for that subject's age. The remaining 5 subjects of the 18 had vertical times larger than the horizontal time.

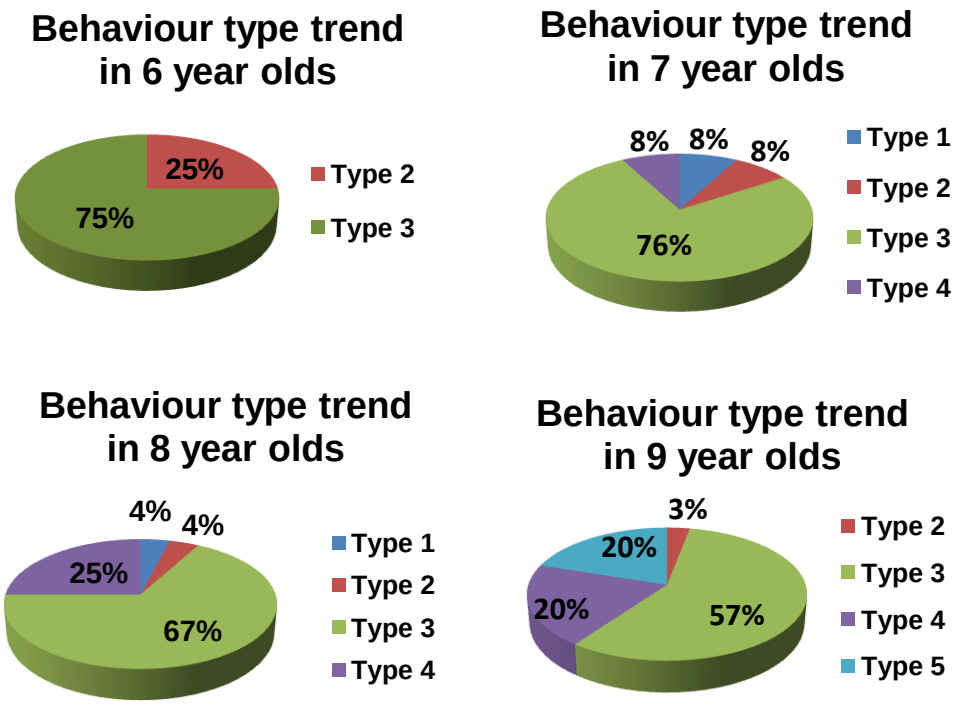


Figure 4.11a-d: A presentation of the behaviour type trends in the age groups 6 – 9 years old. a) Behaviour type trend in 6year olds. b) Behaviour type trend in 7 year olds. c) Behaviour type trend in 8 year olds. d) Behaviour type trends in 9 year olds.

The above four pie charts represent the prevalence of the different behaviour types in four age categories. A display of the next four age categories will be presented after this discussion. The reason the researcher opted to present four pie charts of the different age categories together and not seperately as one would traditionally expect to create an

immediate visual comparison and assessment of the data purely for the convenience of the reader.

In pie chart (a) displaying the behaviour trends in 6 year old subjects, the only behaviour types that are present is behaviour type 2 and behaviour type 3. The sample size (n) in this age category is only four which then means that one of the four exhibited pure oculomotor dysfunction.

In pie charts (b) – (d), the behaviour type 2 once again featured as the smallest percentage of the pool but yet again still represented only one subject. The reason the percentage of behaviour type 2 decreased with age is because the sample pool in the different age categories increased from n = 13 in 7 year olds, n= 24 in 8 year olds and n= 30 in 9 year olds. Consistently, it was noted that behaviour type 3 made up no less than 57% of the sample in the different age categories. Behaviour type 5 (light blue) featured at the age group 9 category representing 6 of the 30 subjects in this age group.

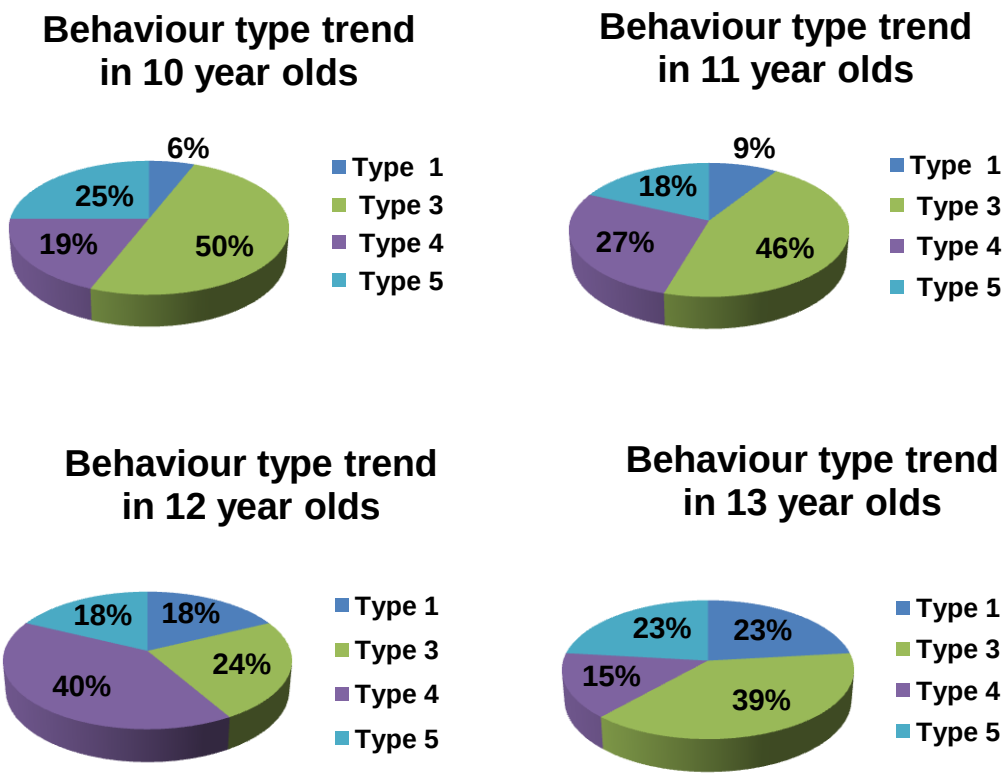


Figure 4.12a-d: A presentation of the behaviour type trends in the age groups 10 – 13 years old. a) Behaviour type trend in 10 year olds. b) Behaviour type trend in 11 year olds. c) Behaviour type trend in 12 year olds. d) Behaviour type trends in 13 year olds.

Figure 4.12 represents age groups from 10 years to 13 years. The immediate impression is that behaviour type 2 (red) is absent from these four age pools.

The size of the sample in the above age categories are as follows (*refer to Table 4.3)*:

10 year olds n = 16

11 year olds n = 11

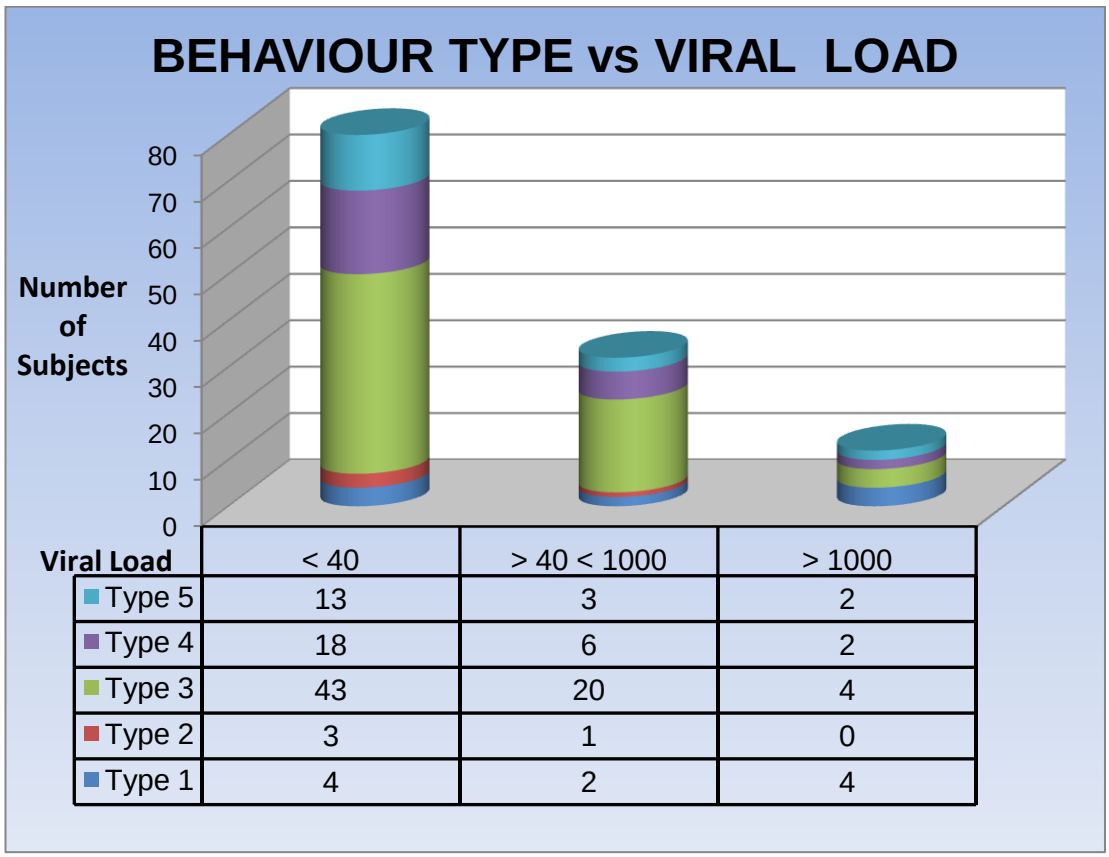
12 year olds n = 17

13 year olds n = 13.

Throughout these four age categories and the previous four age categories behaviour type 4 (purple) has never exceeded 41% or an absolute number of 7 subjects in any of the eight age categories and it has the highest prevalence in the 12 year old age category.

Behaviour type 1 (dark blue) appears to increase in prevelance consistently as the subject samples got older from the 9 year olds to the 13 year olds.

Behaviour type 5 (light blue) never seemed to exceed the quarter mark of the sample pool in each age category and never exceeded an absolute subject number of 6 in any of the age pools.



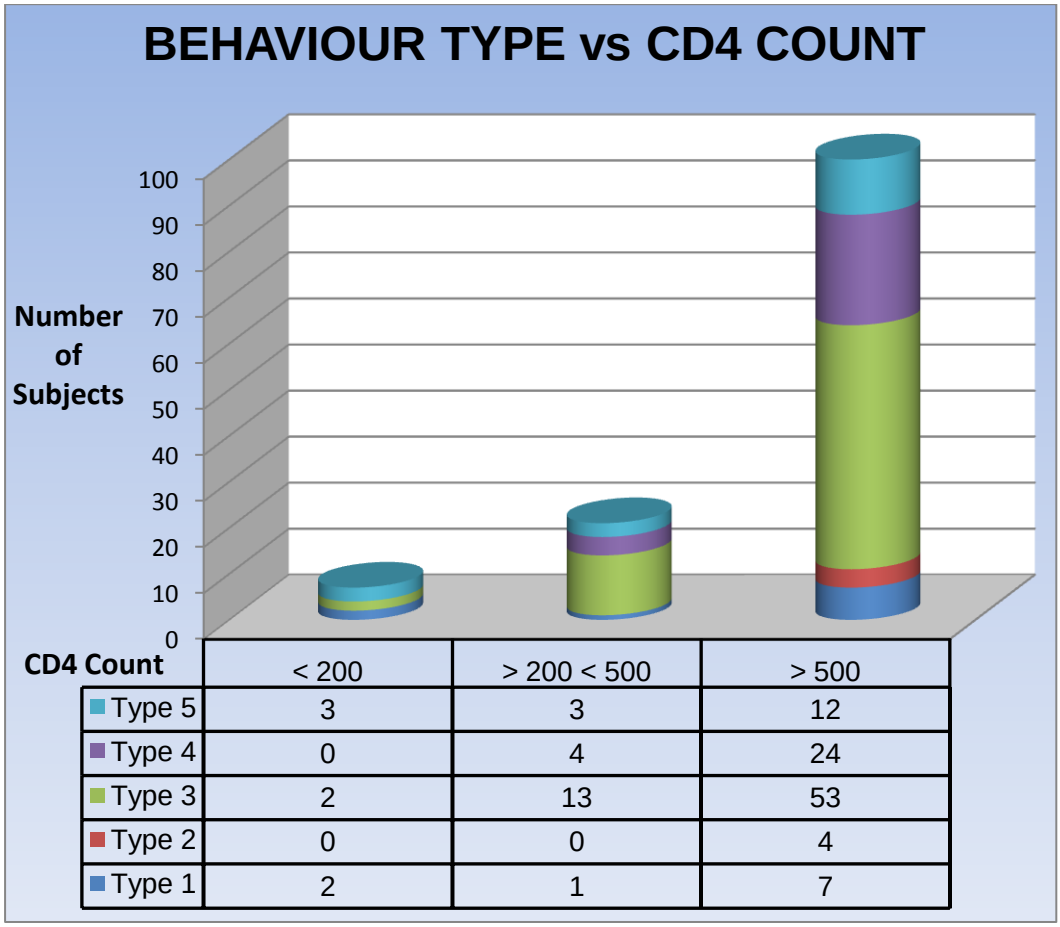
Viral Load	< 40	> 40 < 1000	> 1000
Type 5	13	3	2
Type 4	18	6	2
Type 3	43	20	4
Type 2	3	1	0
Type 1	4	2	4

Figure 4.13: A presentation of the prevalences of the different behaviour types in the three virologic status categories of the sample.

In Figure 4.13 each cylinder comprised of the different types of behaviour that is prevalent in the three virologic categories. The data table was presented beneath the graph to effectively express the data comprehensively for easy interpretation.

Subjects with an undetectable viral load are most prevalent of the three virologic categories as was previously presented, hence a much taller cylinder is seen in Figure 4.13 in the first of the viral load categories with a shortest cylinder in the virologic failure category. This category represented 12 subjects as can be identified with the use of the data table placed below the graph.

Behaviour type 3 (green) is the most prevalent type in all three virologic categories. Behaviour type 2 (red) is the least prevalent and is absent in the virologic failure category. The prevalence the individual behaviour types from 2 to 5 decreased as the viral load categories increased from undetectable levels to virologic failure (Figure 4.13). Behaviour type 1 (dark blue) is the only type that had the same number of subjects in category one and category three.



CD4 Count	< 200	> 200 < 500	> 500
Type 5	3	3	12
Type 4	0	4	24
Type 3	2	13	53
Type 2	0	0	4
Type 1	2	1	7

Figure 4.14: A presentation of the prevalences of the different behaviour types in the three immunologic status categories of the sample.

In Figure 4.14, each cylinder comprises of the different types of behaviour but that which is prevalent in three immunologic categories. The data table was presented beneath the graph to present a snapshot of the raw data for each behaviour type of the three categories for immediate referral, assessment and interpretation.

As was previously demonstrated in Table 4.9, subjects with minimal or no immune suppression were in the category with the highest number of subjects that reached 100 (78%) of 128 subjects. This is evident in Figure 4.14 with the highest

cylinder and confirmation of this is by adding the total behaviour types in the last column on the data table under the cylinder that graphically represents this data.

From the Figure 4.14 it is once again observed that behaviour type 3 (green) is most prevalent in category 2 (>200<500) and category 3 (>500). Behaviour type 5 is most prevalent in catergory 1 and 2 (<200) which describes the group with severe immune suppression. Behaviour type 2 is absent in categories 1 and 2 and only features in subjects in the 'healthy' 3rd category making up all 4 of the 128 subjects found with this behaviour type. Subjects with behaviour type 4 occur in category 2 and 3 having the 2nd highest prevalence in both the categories but is absent from category 1.

4.3.3 Probability

Behaviour Types and Viral Load

In the analysis between the viral load and the behaviour types a 3 x 5 table Chi Square analysis was performed. The viral load was categorised into 3 categories as was described in Table 4.12 and compared to the 5 behaviour types. Ideally a 2 x 2 table would be most appropriate for this type of analysis. Results of the test indicated poor reliability as 53% of the cells had expected counts that were less then 5.

Alternatively the Fisher's Exact test was conducted to determine probability. With n = 125, p >0.05. precise p value = 0.2. This larger p value indicated that there is weak evidence *against* the rejection of a null hypothesis, hence the null hypothesis is accepted. This indicated that there was no statistically significant difference between the categories of the behaviour types and the viral load variables. However, of the 4

subjects that had displayed type 2 behaviours, 3 of them were in the category of undetect viral load contrary to what was predicted.

Behaviour Types and the CD4 Count

A Chi Square test was performed using 3 x 5 tables where the CD4 count was compared to the different categories of behaviour types. The CD4 count was grouped into the categories as described in Table 4.9. The results showed that 67% of the cells had counts less than 5 so the use of the Chi square test in this case was not valid. The Fisher's Exact test revealed with n = 128, $p > 0.05$. Precise p value = 0.17. This p value indicated that there is weak evidence *against* the rejection of a null hypothesis. This indicated no statistical significance between the categories of the behaviour types and the CD4 count variables. However, of the 4 subjects exhibiting type 2 behaviours all fell into the category of minimal immune suppression as was with the viral load, contrary to what was predicted.

4.4 SUMMARY OF RESULTS

4.4.1 Demographic Profile:-

- **Figure 4.1:** 54% of subjects were accessed from hospital sites with 46% of subjects accessed from clinics.
- 18% of subjects were seen in the Xariep District and 82% of subjects from the Motheo District.
- **Table 4.1**: 48% male subjects and 52% female subjects
- **Table 4.2**: Median age of sample was 8.5years

- **Table 4.3**: 23% of subjects were 9 years 11 months as the most prevalent mean age and 3% were 6 years and 11 months as the least prevalent mean age.

4.4.2 Clinical Profile:-

- **Table 4.4**: Median time period from birth until the HIV status is confirmed was 6 years and 1 month.
- **Table 4.5**: Median time period from diagnosis of HIV to the commencement of HAART was 9 months.
- **Table 4.6**: Median time period from birth until HAART was initiated was 7 years.
- **Table 4.7**: Median duration of subjects on HAART was 2 year and 10 months.
- **Table 4.8**: 6 year old subjects were on HAART for the longest period of a mean of 44.67 months. The 12 year old subjects were on HAART for the shorted time with a mean of 29.53 months.
- **Table 4.9**: 78% of subjects had CD4 counts $\geq$ 500 cells/mm^3. 6% of subjects had severe immune suppression.
- **Table 4.10**: Median CD4 count of the sample was 778.5 cells/mm^3.
- **Table 4.11**: Highest mean CD4 count was in the 9 year old group and the lowest mean CD4 count was in the 13 year old group.
- **Table 4.12**: 65% of subjects had an undetectable viral load and 10% of subjects were in the virologic failure category.
- **Table 4.13**: Median viral load was 0 (<40 undetectable level) across the sample.

- **Table 4.14**: Median viral load across all age groups were 0 (<40 undetectable level).
- **Table 4.15**: CD4 count versus Viral Load – weak negative correlation.

  Treatment Duration versus CD4 Count – weak positive correlation.

  Treatment Duration versus Viral Load – marginally weak negative correlation.

  Treatment Duration versus CD4 % – strong positive correlation.

4.4.3 DEM Analysis Profile:-

- **Fig.4.2**: 93% of the subjects had vertical times that were outside of the DEM mean and standard deviation of the sample.
- **Fig.4.3**: 100% of the mean vertical times per age group were higher than the DEM age-specific mean and standard deviation.
- **Fig.4.4**: 92% of subjects had horizontal times that were outside of the DEM mean and standard deviation of the sample.
- **Fig.4.5**: 100% of the mean horizontal times per age group were higher than the DEM age-specific mean and standard deviation.
- **Fig.4.6**: 45% of subjects have ratio scores outside of the DEM mean with 55% within the specified mean norm.
- **Fig.4.7**: Subjects in 7 of the 8 age groups had ratios within the DEM age specified norms except for subjects in the 6 year old category which had results above the mean.

- **Fig.4.8**: 66% of subjects had errors that were within the DEM mean and standard deviation with 34% outside of the mean.
- **Fig.4.9**: Subjects in 7 of the 8 age groups had their age-specified means of error above the expected DEM norm expect for the subjects in the 7 year old category whom had errors below/less than the DEM age-specified norm.
- **Table 4.16**: DEM standardised norm values were determined with a sample of 534 subjects. Vertical and horizontal times were well above the DEM standardized norms. Errors and ratio was with the DEM standard norm values
- **Fig.4.10**: The highest prevalence of behaviour types of the sample was behaviour type 3 with 53% with type 2 behaviours (saccadic dysfunction) with the lowest prevalence of 3%.
- **Fig.4.11**: In the different age categories, behaviour type 3 was the most prevalent. Subjects with type 2 behaviours where present in age group 6 years, 7 years, 8 years and 9 years. The prevalence decreased with increasing age group.
- **Fig.4.12**: Subjects from 10 to 13 years showed no type 2 behaviours with an increase in type 1 behaviours (normal) with increasing age.
- **Fig.4.13**: Subjects with undetectable viral loads had the highest prevalence of all behaviour types except for the virologic failure category, which matched the undetectable viral load category for type 1 behaviours.

  Of the subjects displaying type 2 behaviours, 3 were in the undetectable viral load category with 1 subject being in the ‘moderate viral suppression’ category.

- **Fig.4.14**: Subjects in the ‘no/minimal suppression’ category of the CD4 count indicator, had the highest prevalence of all behaviour types.

  All subjects displaying type 2 behaviours had CD4 counts in the ‘minimal to no immune suppression’ category.

  The computation of the *p* value in assessing statistical significance indicated that in evaluating the relationship between the behaviour types and the viral load and CD4 count there was not a statistically significant difference between the different behaviours types against the categories of CD4 count and viral load.

CHAPTER FIVE

DISCUSSION, LIMITATIONS, CONCLUSION, RECOMMENDATIONS AND SUMMARY

5.1 DISCUSSION

5.1.1 Demographic Profile

The demographics of HIV positive children on treatment in this study with reference to the sample pool at each site is not representative of the geographic distribution of HIV positive children in the Free State. Due to the administrative systems at Botshabelo Hospital and J.S Moroka Hospital, patients had been more accessible at these sites than at the other sites hence a larger pool of subjects was selected from these two locations. This however did not bias the sample in any manner as the HIV/AIDS treatment and management prrogram for paediatrics in the province is consistent through all treatment centres and regulated by the Free State Department of Health.

The gender distribution of the sample closely mimics the true proportion of male and female children in the Free State Province. In the Free State, we have approximately 50.20% male and 49.70% females in the age category from 5 to 15 year olds (Statistics SA, 2012b).

With almost a quarter (23%) of the sample being 9 year old subjects, only 4 subjects were from the 6 to 6 years and 11 month old category. The reason for a small sample of 6 year old children was because they were largely illiterate and moderately familiar with verbalizing numbers and these factors required them to be excluded according to the specified exclusion criteria.

None of the 128 subjects used English as their home language. The home languages of the subjects were Sotho, Afrikaans and Tswana. Subjects from the Xariep district in the South-Western Free State used Afrikaans and Sotho as their home language, whereas subjects from the Motheo district used Sotho as their home language. Subjects from the Thabanchu area in the eastern region of the Motheo district, who attended the J. S Moroka Hospital, predominately used Tswana as their home language along with Sotho. However, English was used in school along with Afrikaans, Sotho and Tswana during the teaching process and informal communication outside of the classroom.

5.1.2 Clinical Profile

Even though it is noted in the health records that the mode of infection amongst all subjects in the sample is from mother-to-child-transmission, there is no guarantee that horizontal modes of infection are not possibilities. Such modes of transmission include sexual abuse, iatrogenic transmission from hospitals or clinics and receiving of breast milk from HIV positive mothers who are not the biological mothers (Brody et al, 2003; HSRC, 2008).

When a child is born from a mother that is HIV positive, that child is classified as "HIV-exposed" as the child will be carrying HIV antibodies from the mother due to trans-placental transmission. An antibody test (HIV ELISA and HIV Rapid Test) in an infant can only be done after 18 months of birth to confirm the diagnosis of HIV infection (RSA. KZNDOH, 2010). The reason for this is that a mother's HIV antibodies are still found in the infant up to 18 months of age. Alternatively, a PCR test is done to detect viral genetic material in a child at any age without any waiting period (Nutall, 2012).

As shown in **Table 4.4**, the median value of 6 years was the average waiting period for subjects in this study to be confirmed as HIV-infected even though this could have been done from 18 months of age with an antibody test. Among other factors, there are patient-centred reasons to why the average time of diagnosis was not sooner as one would have hoped for. Examples of these factors could include poor education, non-compliance with appointments, lack of urgency with collection of results and denial of circumstances, all of which play a role in the delay of confirmation of the diagnosis.

The waiting period for the initiation of treatment showed a median of 9 months with a maximum waiting period of 10 years and 7 months (**Table 4.5**). Possible operational and procedural reasons for the delay in treatment are:

1. Not all HIV-infected children were eligible to be on HAART according to the previous treatment guidelines for HAART initiation in children.
2. Inadequate access to treatment medication for children.
3. Poor training of health care personnel and knowledge on the drug regimens for children in the beginning stages of the roll out.
4. Care-givers not given the results of the diagnosis timeously
5. Roll out of ARVs commenced only in 2004 with slow focus on children and preparation (Michaels et al, 2006).

From an evaluation of 127 subjects, **Table 4.6** expressed the equal median and mean times for the time period from birth until the start of HAART, which was 7 years. The interpretation of this is that if children who are born to HIV positive mothers are eventually diagnosed with HIV infection and are put on HAART, this process will usually span a period of approximately 7 years. A study with a larger cohort should confirm if this is the current situation for a population of HIV/AIDS children in South Africa. The 2004 roll out of ARVs in South Africa may have significantly contributed to the long waiting period for subjects in this study. This delayed initiation has undesirable consequences, which may reduce the length of survival, affect morbidity and mortality rate and further influence the incidence of neurodevelopmental impairment. Hoffmann et al (2013) showed that the long-term clinical outcome would be better if initiation was started earlier at higher CD4 counts and lower baseline viral loads. Alternatively, McCombe et al (2013) stated that increased age and length of survival due to HAART are predictive factors of

symptomatic HAND. This would be a consequence of the long-standing presence of HIV in the sanctuary site of the CNS.

As described in **Table 4.7**, the maximum treatment duration of subjects in this study was 7 years and 8 months which can be explained by the roll out of ARVs commencing in 2004 and not prior to this date. Therefore, neither a child, nor an adult in the public health sector would have had access to HAART for more than 8 years in South Africa. In this study, the eldest subjects were 13 years and 11 months, born in 1999. When the roll out of ARVs commenced only in 2004 at a time when children were not prioritised for treatment, this lead to the understanding that the subjects of this study that were born from 1999 to 2003 did not receive treatment during these years regardless of whether they had satisfied the criteria. This situation had resulted in the waiting period for initiation of HAART being skewed to a lengthier time period for the subjects of this study.

The duration of HAART for subjects within the nine age categories ranging from 6 years to 13 years (**Table 4.8**) showed that the younger subjects had been on HAART for a longer time period that the older subjects. This evidence contributed to our understanding that the older children were not promptly started on treatment compared to the younger subjects. This also was a good indication that improvements have been made in the public health system where children are started on treatment from an earlier age compared to what had been previously practised.

Analysis of the CD4 count results revealed that the subjects in this sample are largely "healthy". This interpretation was derived from the fact that in **Table 4.9**, 78% of the subjects had CD4 counts above 500cells/mm^3, implying that their immune systems were adequately able to sustain their health. This was probably a result of the HAART that is sustaining their immunity to optimal levels. The average CD4 count of the whole sample is above 500 cells/mm^3 as is seen in **Table 4.10**, confirming that the subjects in this study are responding positively to the HAART in maintaining a healthy immune system.

With a closer inspection of the mean CD4 count in each age group (**Table 4.11)**, stronger immune systems were seen in subjects from 6 to 9 years old. Subjects with lower CD4 counts were seen in the 10 to 13 year old groups but still within the 'healthy' range. No age group averaged CD4 counts of below 500 cells/mm^3 even though subjects in the 13 year old age group had the lowest mean. The highest CD4 count and the longest duration of treatment was noticed in the younger age groups with the older subjects having shorter treatment durations and CD4 counts in the low-normal range.

A correlation analysis was conducted between treatment duration and CD4 count (**Table 4.15**) and it showed that a positive correlation did exist between those variables indicating that the CD4 count increased with longer sustained period of adherence to treatment, however it was not a strong association as the relationship between these 2 variables were not purely linear. Treatment interruption could

contribute in slowing the rate of increase in the CD4 count and may even blunt a full recovery to satisfactory elevated levels (Tesiorowski, 2001).

With the assessment of the subjects viral load in **Table 4.12**, 65% of the subjects had undetectable viral loads, which could be attributed to the HAART controlling viral replication in the blood. In all age categories, the median for the viral load was undetectable which meant that 50% or more of the subjects in each age group had undetectable viral loads as is demonstrated in **Table 4.14**. Expression of the viral load as log units would have been ideal and more valid as the absolute values of the viral load ranged from double digits to millions of copies which made data analysis challenging.

With only approximately 35% of subjects having detectable viral loads, of which 10% had virologic failure while on HAART, this implied that the overall subject pool had adequate viral suppression in the blood due to HAART. The reasons for virologic failure in subjects on HAART could range from poor treatment adherence to failure of the initial drug regimen resulting in viral rebounding due to activation of latent viral reservoirs. Poor adherences in children are due to many social and psychosocial reasons, which make compliance more difficult with children than with adults. A study by Haberer and Mellins (2009) that looked at paediatric adherence found that child characteristics differ from caregiver characteristics with non-adherence to treatment resulting in failure of HAART. A child may skip medication if they feel well or reach a point of compliance fatigue as the treatment is daily and

lifelong. Psychosocial factors of depression and denial of condition may occur with maturity into adolescence. Poor education of caregivers or collapse of the child-caregiver relationship may also be contributing factors. Furthermore, a Romanian study found that adherence under the care of non-related caregivers is very poor as there is a lack of emotional attachment to the HIV-infected child (Cupsa et al, 2000).

Theoretically, one would expect to see a strong negative correlation between CD4 count and viral load. However, in evaluating the correlation between these two critical biomarkers (**Table 4.15**), a negative but weak correlation was found in this study. This finding is also supported by earlier studies showing a modest to poor correlation between these two biomarkers (Jurriaans et al, 1994; Mellors et al, 1996; Mofenson et al, 1997) which is blamed on the insidious behaviour of the virus.

A correlation analysis between treatment duration and viral load shared a negative trend with a very weak correlation. This is evident from **Table 4.14**, where erroneously high viral loads were found in certain age categories preventing a non-linear negative trend. This could possibly be due to changes in the social environment of the subjects which affected the steady success of the HAART in suppressing the viral replication in the body and as well as interruption in treatment which could have blunted a sustained suppression of viral replication. Activation of the virus from its latent state in different sanctuary sites for whatever reason could have lead to the proliferation of the virus in the body in certain subjects. Even if treatment had been

interrupted or that there was poor treatment compliance in subjects, such information would reluctantly be divulged by the care-giver.

5.1.3 DEM Analysis Profile

The vertical (**Figure 4.3**) and horizontal (**Figure 4.5**) times were distinctly slower across all age groups in this sample when compared to the specified DEM norm. However, the mean ratios (**Figure 4.7**) and the mean errors (**Figure 4.9**) were within the specified norms. There are many intrinsic and extrinsic causative factors that may have contributed to the overall slower times. Blanchette et al (2002) indicated that in specific areas like information processing speed, there may be subtle deficiencies within this population, which is a plausible cause for the subnormal rates in both vertical and horizontal tests. Puthanakit et al (2010) studied neurocognitive function in Thai children aged 6 to 12 years using the Welscher Intelligence Scale for Children III (WISC III) and showed that neurocognitive functioning in HIV-infected children on HAART are lower than that of non-infected children.

A study by Martin et al (2006) concluded that HIV-infected children on HAART functioned within normal limits in certain neuropsychological tests, however, differences in performances did exist between subjects with varying levels of brain neuroimaging abnormalities. This study emphasised the importance of incorporating neuropsychologic assessments as part of medical care in children with HIV/AIDS.

Other intrinsic factors relate to the neuropathogenesis of the disease and how effective HAART is in controlling CNS viral load, which this study had limited insight into due to inadequate research in these areas of paediatric HIV neuropathogenesis within the African population. The reversal of neuropathologic deficits in infected children on HAART still remains unclear as studies by Chirboga et al (2005) and Lindsey et al (2007) showed modest improvement whereas a study by Nozyce et al (2006) showed persistent mild behavioural and cognitive impairment in the presence of HAART.

Variations in HIV subtypes and their neuropathogenesis may also be a factor that influenced the cognitive ability in HIV infected children as small genetic variants between HIV strain subtypes have different neurotoxic potential as discussed in a study by Strain et al (2005) and rates of dementia may also be different (Sacktor et al, 2009). As there are different HIV subtypes found in different regions of the world (Levy, 2009), clinical guidelines and management relating to neurodevelopmental impairments in children in Africa must not be dependent on results done in the United States and Europe. These regions have different HIV subtypes that are not characteristic to what is found in Southern Africa (Levy, 2009). Health professionals should not only be aware of the type of HIV strain that their patients have but of the clade or subtype they have contracted. Even though the treatment regimens remain the same regardless of strain and subtype, the knowledge of different subtypes which have different virulence in terms of neurcognitive impairment pathogenicity (Sacktor et al, 2009) may be helpful in identifying the risk factors and taking earlier precautions.

Extrinsic factors which may have contributed to a sub-standard performance demonstrated by the DEM test results, relate to the social environment and educational support that may have influenced the neurodevelopment and cognitive potential of the subjects. Most of the subjects in this study are from rural and semi rural areas that attend school in those areas and access the public health services. A South African study by Smith et al (2008) using a battery of standardized cognitive tests that described verbal and non-verbal intelligence scores, showed that HIV-infected children on HAART were much lower than those of non-infected children. The study further indicated that neurocognitive development in HIV/AIDS children are influenced by their socio-economic factors, poor household educational stimulation and nutritional profile.

The DEM test has standardised norms developed with American children who are English-speaking learners. The subjects in this study are all non-English speaking learners and all were from poor socio-economic backgrounds. Earlier, Fernandez-Velazquez and Fernandez-Fidalgo (1995) from their study on Spanish-speaking norms concluded that the DEM is a reliable tool independent of language differences. However, Pang, Lam and Woo (2010b) proposed DEM norms for Cantonese-speaking children and cautioned that population-specific norms must be established to minimise bias caused by factors such as language and education. Bapista et al (2011) described in their study using the DEM test in Portugese-speaking children that language, educational and cultural differences may influence the performance on the DEM test.

The implications of the conclusions drawn from the previous studies mentioned above does entertain the possibility that the findings in this current studies may possibly have been influenced by similar factors as was experienced in those studies in their different populations, or possibly not.

Behaviour type 3 was distinctly the most prevalent in the study (**Figure 4.10**) showing that RAN was a significant problem that was revealed in this population with eye movement dysfunction manifesting in an insignificant proportion of the subjects. Deficient automaticity skills may or may not be due to HIV, however, as described in this study, it does exist within the population of school-aged children with HIV/AIDS on HAART. The causes for the automaticity problems could be multifactorial ranging from pathological to socio-economic factors as was with the overall performance in the vertical and horizontal scores.

When behaviour type prevalences were compared to the age variable (**Figure 4.11**), an apparent age-dependant trend was visible. Subjects with eye movement abnormalities were most prevalent in the youngest age group but reduced as age increased and was absent from 10 years onwards (**Figure 4.12**). Normal DEM performances (type 1 behaviours) were absent in the 6 year old and 9 year old age groups but increased significantly from 10 years upwards with the highest prevalence in the 13 year old age group. Deficiencies in automaticity skills or RAN, which were the highest with the 6 and 7 year olds reduced significantly in the 12 and 13 year old groups showing that there is a trend towards normality as the subjects aged as is shown in **Figure 4.12** . This finding was independent of their virologic and immune statuses.

The subjects in the 6, 7 and 8 year old age groups who averaged the longest duration on HAART had high prevalences of type 2 and type 3 behaviours. The older subjects in the 11, 12 and 13 year old age groups averaged much lower treatment durations but had better performances on the DEM. The assumption that poor performances on the DEM test is linked to treatment duration or the effect of a longer duration on HAART were beyond the scope of this study but does warrant further investigation.

A significantly large portion of the sample had undetectable viral loads (65%) (**Figure 4.13**) and higher CD4 counts with minimal immunosuppression (78%) (**Figure 4.14**). Due to the high prevalence of subjects in these 'healthy' categories, all five behaviour types were most prevalent in these two categories rendering any viable comparison of different behaviour types to different viral load and CD4 count levels, inconclusive. This finding challenged the notion that subjects with higher viral loads would perform poorer on the DEM test and be symptomatic of eye movement problems. It was found that no subject with type 2 behaviours where found in the category with VL > 1000 copies/mm^3 and 4 of the 12 subjects in this viral load category had normal DEM performances (Type 1 behaviour). A similar result was demonstrated when comparing CD4 counts to different behaviour types as was with the comparison done with viral load. All behaviour types were significantly higher in the single category of minimal immune suppression with CD4 counts > 500 cell/mm^3.

Subjects classified as having saccadic eye movement problems (Type 2) were not severely immunocompromised, hence no link between their immune and virologic status to their diagnosis based on the DEM was established. This finding further did not support the expectation that unhealthy and severely immunocompromised subjects would have significantly lower performance rates on the DEM. It also did not support the preconceived notion that there would be a high prevalence of behaviour type 2 subjects in the lower immunity categories. There was no statistically significant difference between the different behaviour types and the CD4 count and viral load categories.

There were no reliable indications of immunologic and virologic biomarkers influencing the performance on the DEM test without a convincing relationship to eye movement problems in this population. A relationship between the performances on the DEM test and the disease biomarkers remains unlinked. The only finding that supports a possible relationship with the DEM performance was that of the age of the subjects with the behaviour type trends. There was a progressive increase in the normal scoring in the DEM test (Type 1) with the ageing of the subjects however, it was still below the established norms.

The DEM test showed different behaviour types in the sample population but it failed to show that those with poorly sustained immune systems and high viral burdens had oculomotor dysfunctions. The obvious finding that was evident in the study was the significant automaticity deficits of the population. Deficient automaticity skills

were the highest across all age groups but its prevalence decreased with ageing children. Furthermore, children with the longest duration on HAART had higher automaticity and eye movement problems but were in the younger age groups. There was no relationship established between poor automaticity skills and disease parameters of CD4 count and viral load in this study but relationships between these parameters to other neurocognitive functions by other neuropsychological tests have been demonstrated (Martin et al, 2006; Smith, Smirnoptopoulos and Rushing, 2008).

Efficient automaticity requires good cognitive ability as the DEM test is a visual-verbal test. The essence of the DEM test is that it is patient-reliant, hence it is a subjective instrument. Cognitive functions such as visual memory, visual discrimination, visual information processing, processing speed and verbalisation are important components for performing the DEM Test and these are cognitive-dependant skills. Automaticity problems may a predictor or risk factor of neurocognitive impairment as it is cognitive-dependent. Other neurocognitive testing would need to be conducted to confirm if the findings of the DEM test is a reliable indicator of neurocognitive impairment, which is known to occur in this population. The DEM test has value as a screening tool in a subtest of neuropsychological tests beyond its description as an eye movement test as the dependency of this test is heavily reliant on a series of cognitive skills. A study by Ayton et al (2009) concluded the DEM failed to correlate well with other objective measures of saccadic eye movements to be a reliable test of oculomotor function but that it is an indicator of children at risk of

reading and academic delays due to its reliance on cognitive functions such as verbalisation and information processing speed.

Strong immunity and low viral load as detected in the blood are not reliable indicators that the CNS is unaffected. Studies by Cysique, Maruff and Brew (2004) and Dore et al (2003) showed that since subjects have been started on HAART earlier, the incidence of HAD reduced but the increase in the prevalence of HAND occurred with increased survival due to HAART. A study by Ruel et al (2012) showed that HIV infected children with good CD4 counts, who were not eligible for HAART, manifested with neurocognitive and motor deficits which then questioned the WHO threshold guideline for eligibility for treatment. If eye movement dysfunction is not characteristic in this population of HIV-infected school-aged children on HAART but deficiency in RAN is evident it may be plausible that automaticity skill may be indicator of neurocognitive impairment or neurodevelopmental delays.

The DEM in essence still has validity and reliability as a screening tool in clinical practice as its function extends beyond the detection of eye movement dysfunctions and should be used in parallel with other tests of neurocognitive function that are used by optometrists. Screening of neurocognitive function in a school-aged patient is important for the monitoring of their neurodevelopment and for optimal multi-sensory learning in the HIV/AIDS population of school-aged children and even those unaffected by HIV/AIDS. The question arises as to whether the automaticity problems found with the subjects in this study are due to norms developed by a

different population or if it's related to HIV/AIDS with the possible existence of neurocognitive impairments in the current population of subjects. This study supports the suggestion by Martin et al (2006) in emphasising that neuropsychological testing needed to be done in all HIV-infected children regardless of their treatment status. Optometrists need to play a more significant role in screening and referring such children as part of a universal health care approach beyond just the attention to primary visual functions especially when confronted with children with HIV/AIDS on HAART.

5.2 LIMITATIONS

Below are the descriptions of the limitations of this study that was identified during and after the study.

- The incompleteness of subjects' health records at certain state health facilities posed a challenge, which resulted in a significant number of candidates being excluded from the study. Pre-existing neurological or neurocognitive disabilities, previous hospitalisation details and the time frame of such were unclear in the documentation. Not all subjects had absolute CD4 counts and CD4 percentage calculations as well as absolute viral load and log unit expressions, which would have made data analysis of these parameters easier and simpler.

- A pilot study should have been conducted which would have identified the challenges described above more efficiently.

- All subjects in this study were presumed to have contracted HIV through vertical mother-to-child transmission. Records did not indicate if MTCT was the mode of transmission or if contraction of HIV occurred through different modes beyond the infancy age. However, the mode of transmission of all subjects was accepted to be MTCT during the neonatal age of the subjects for this study.

The current caregivers of the subjects were not necessarily the biological mother as most biological mothers were deceased. Accurate and detailed case histories of the subjects were therefore limited.

- The health facilities had different procedural systems as to when subjects are to present themselves to the clinics which affected the sample size and rate at which subjects were acquired using the convenient sample selection process for this study. The restricted sample size could thus limit the ability to generalise findings in this study.

- There were a limited number of 6 year old subjects in this study due to poor numeracy skills hence the 6 year old age category was comparably small to the other age categories.

- None of the subjects had any neuropsychological or neurodevelopmental testing done to assess the cognitive state of the subjects prior to this study. This meant that the neurocognitive functioning of the HIV-infected subjects before and after HAART was unknown.

- Other variables that could have influenced the outcome of the study where social, socio-economic and educational backgrounds, none of which were measured in this study.

- As this study was a descriptive and not an analytical design, it limited a comparability approach to an HIV-uninfected control group.

5.3 CONCLUSION

This section states the conclusions drawn from the research enquiry of this study.

In answering the research question:-

I. Immunologic and virologic statuses in children with HIV/AIDS on HAART cannot be predicted from abnormal saccadic eye movements.

In addressing the primary objectives of the study:-

I. The prevalence of saccadic eye movement abnormalities in HIV positive paediatric subjects on HAART was very low.

II. There was no relationship between the saccadic eye movement abnormalities and the immunologic and virologic biomarkers in children with HIV/AIDS.

In the addressing the supportive investigations of this study:-

I. There was a weak negative correlation between the immunologic and virologic biomarkers.

II. The prevalence of eye movement abnormalities decreased from 6 years old to 9 years old and thereafter was absent in the older age categories.

III. Evaluation of the performance in the DEM test was subnormal for all age-specified norms.

Other conclusions drawn from this study:-

I. A clinically significant number of subjects from this specific population manifested with problems in automaticity skills.

II. Performance times in all 3 subtests of the DEM was significantly reduced in all age groups according to the specified norms.

The Null Hypothesis (H_0) was accepted.

5.4 RECOMMENDATIONS AND SUMMARY

The limitations of this study could be addressed by the following recommendations. Considerations for future studies are further proposed below.

- New norms for a DEM test based on non-English speaking children in a South African population should be established. South Africa is a nation of multiple home languages with diverse cultural backgrounds, it should ideally have standardised norms developed from and for its own population for adoption. This could strengthen the reliability and validity of the results that infer deficits in eye movement and automaticity problems for the South African population.

- Since social and educational factors influence performance on neurocognitive and neuropsychological tests, a study using the DEM test to compare children of different social, economic and educational backgrounds beyond language differences, should be undertaken.

- The DEM test can be used or tested as part of a battery of neuropsychological tests in school-aged children with vertically acquired HIV to determine if its results are consistent with other neuropsychological tests.

- A similar descriptive study with an analytical design should be done by comparing the cognitive ability in performing the DEM test amongst subjects who are treatment-naive HIV-infected subjects, HIV-infected subjects on

HAART and an HIV-uninfected control group. A control may be valuable to determine if HIV has a negative effect on a child's eye movements and neurocognitive development that is manifested through the DEM test.

- To enhance the accuracy of eye movement testing using the DEM test, instructions relating to controlling of head and upper body movements could be given additionally to the prescribed instructions stipulated in the DEM test manual. If newer tests or modifications for this test are researched, these instructions can be recommended to be included to strength the reliability of the results.

- Testing of eye movements in paediatric subjects should ideally be done objectively limiting the variability, improving reliability and repeatability of the results. For research purposes, visual tracking devices using infrared technology can be utilised as instruments of choice for analysing eye movements as the sensitivity of these instruments and objectivity is highly reliable.

As current research has shown that HIV-associated neurocognitive disorders are still persistent in the presence of HAART, children with developing systems are still at risk of CNS disease. In consideration of this, optometrists should take cognisance and use this knowledge in clinical practice through specific paediatric tests as it would be worthwhile to screen for and monitor neurodevelopmental anomalies beyond the assessment of just the primary visual functions. In the domain

of neurodevelopmental and neuropsychological testing there are an insurmountable number of tests that are used in paediatric assessments by various health professionals. The DEM test should be emphasised in this specific population along with a battery of other neuropsychological tests that optometrists currently use such as the Tests of Visual Analysis Skills (TVAS), Tests of Auditory Analysis Skills (TAAS), Tests of Visual Perceptual Skills (TVPS) and the Developmental Tests of Visual Motor Integration (Beery VMI).

The role of health care providers should be to actively identify at-risk patients to get the appropriate medical attention and rehabilitation that is needed at an earlier stage. This action is critical to prevent children from being handicapped by their condition as HIV-associated neurocognitive disorders could be debilitating. This holistic approach to health care could benefit children with HIV/AIDS in levelling the playing field to allow them the same opportunities, success and achievement as non-infected children.

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Appendix A

PATIENT RECORDING FORM

Sample Group | On Tx | None |

PHASE 1: DEMOGRAPHIC INFORMATION

Assessment Site: File No.:

Identification Code:

Age: Date of Birth: y y y y m m d d
Gender:

School grade/level:

MEDICAL HISTORY

1st Diagnosed:
HIV exposure: MTCT / other

History (general health):
_____

_____

Current Hospitalisation: Y N
Reason(s):

Clinical stage:_____

Viral load:_____ last tested:

CD4 %:_____ last tested:

Reason(s) for ARV initiation: _____
_____

Date of treatment commencement:

ARV med:	Duration:
3TC (Lamivudine) ___	_____
d4T (Stavudine) ___	_____
EFV (Efavirenz) ___	_____

Other ARV Medication:
Reason:
Other medication:
Reason(s):

PHASE 2: VISION SCREENING INFORMATION

1. Distance visual acuity – with distance Snellen E chart.

R:

L:

OU:

2. Near Visual Acuity – with reduced Snellen E chart:

R:

L:

OU:

3. Cover test

Distance:

Near:

4. NPC:

5. Motility testing :

Pursuits:
Ductions:

6. number recognition & fluency:

Numbers: Y N

9. Pupils:

7. Refractive error:

R:

L:

8. O'scopy:

R:

L:

PHASE 3: DEM TEST RESULTS

Developmental Eye Movement Test (DEM)

DEM score sheet (attached): ☐

Interpretation

Signature **Date**

Appendix B1

INFORMATION SHEET

Dear Parent/ Guardian/Care-giver

re: Research Study for Children that are HIV positive.

This is a request by myself, Mr N. Naicker for your consent for participation in a research study of the child in your care. I am an Optometrist (OP0033847) based at National Hospital in Bloemfontein and my study aims to investigate eye movements problems in children that are HIV positive.

The information on this sheet would help you understand what is expected of the child for you to decide whether you are willing to allow the child to participate.

Procedure: Firstly the child would have their vision checked, followed by the testing of their eye movements. Their eye movements would be tested by a reading test called the Developmental Eye Movement test (DEM). This test is made up of numbers that need to be read out aloud by the child and their eye movements are monitored.
The time spent with the child will take up to a maximum of 30 minutes in total for checking their eyes then getting him or her to read the DEM test.

Confidentiality of the children will be maintained throughout the study and after I am completed working with the child. No names of the children will be used in any publication or presentation.
Instead, each child will be given a code for identification and their names will not be used.

As the care giver and welfare responsible for this child, you are under no pressure to grant permission and the participation must be voluntary.

You have the right to not to allow the child to participate and you can withdraw from the study at anytime. If you feel so, you may request his or her information not to be included in this study.

I hope that you will agree and be willing to allow the child to be a part of this study.

For any further enquires, you are welcome to contact me using my details below.

N.Naicker
Senior Optometrist/Junior Lecturer
University of the Free State/ Free State Dept. of Health
Tel:051 4052684 Fax:051 4303346
naickern@fshealth.gov.za

INFORMASIE BYLAAG

Geagte Ouer / Voog

Re: informasie oor Navorsings projek

Ek, Mnr. N. Naicker, wil graag u toestemming vra dat die kind wat in u sorg geplaas is mag deelneem aan n navorsings- studie. Ek is n oogkundige (OP0033847) wat gebaseer is by die Nasionale Hospitaal in Bloemfontein en my studie beoog om die abnormaliteite in oog bewegings in n HIV positiewe populasie vas te stel.

Die informasie op hierdie bylaag sal u help om te verstaan wat verwag sal word van die kind en u moet dit eerstens lees voordat u toestemming gee daartoe.

Prosedure: Die kind se visie sal eerstens getoets word en daarna sal die oogbewegings ook getoets word. Om te bepaal of daar enige probleme is met die oogbewegings, word gebruik gemaak van n toets nl. Die “developmental eye Movement test (DEM)”. Elke deelnemer sal instruksies gegee word oor wat hy/sy moet doen voordat die toets begin. Die DEM toets bestaan uit n lys van nommers wat hardop gelees moet word deur die kind en die oogbewegings word dan deur myself gemonitor. Die kind se antwoorde sal opgeneem word op band vir my analise.

Die tyd benodig met elke kind sal op die meeste 30min wees in total. As die toets voltooi is, sal geen verdere deelname verlang word nie.

Vertroulikheid van die identifikasie van die deelnemers sal behou word gedurende die studie asook na die voltooing van die studie. Die identifikasie van die deelnemers sal ook nie gebruik word in enige publikasie of voordrag nie.

Gedurende die studie, sal n kode aan elke kind toegedien word, sodat geen name gebruik sal word nie. As die oog bewegings toets gedoen is by Leboni House en dit is die 5de kind wat getoets is wie se naam Jacob Moloi is, sal die kode wat geallokeer word wees:

U het die reg om deelname van die kind te weier en u kan die kind onttrek van die studie enige tyd gedurende die studie. As u so voel, mag u vra dat sy/haar resultate nie in die studie ingesluit word nie.

U is welkom om teenwoordig te wees gedurende die assessering van die kind as u sou wou.

Vir enige verdere navrae, kan u my kontak deur die besonderhede hieronder te gebruik.

Appendix B3

Pampitshana ya Tlhahiso Leseding

Ho Motswadi/Mohlokomedi

DIPATLISO TSA BANA BA NANG LE KOKWANAHLOKO YA HIV

Ena ke kopo ya ka Mr Naicker ho wena motswadi/mohlokomedi wa ngwana ho nka karolo le ho dumella ngwana ya tlhokomelong ya hao ho kena dipatlisisong tsa mahlo. Ke ngaka ya mahlo (Optometrist) (Mr Naicker-OP003347) ke sebetsa National H. Mangaung. Ke etsa dipatlisiso ka mahlo a bana ba nang le kokwana ya bosollatlhapi (HIV)

Tse latelang mona di tla o lemohisa hore o utlwisise hore ho lebelletwe eng ho wena haeba o dumella ngwana ho nka karolo dipatlisisong.

DITSAMAISO: Ngwana otla etsa diteko tsa mahlo mahala, ebe ho shejwa pono ya hae, motsamao wa mahlo a hae otla lekolwa ka hore a bale se bitswang (D.E.M)
Teko ena e etswa ka di tlhaku tse ballwang hodimo. Morero ke ho sheba kapa ho lekola hore mahlo a ngwana a tsamaya ha kae le ho sekamela ka lehlakoreng lefe ha a bala.
Hotla nka metsotso e mashome a mararo he pheta sena.
Lekunutu ka maemo angwana etlaba ya pele e baballwang le ka morao ho moo. Ha ho mabitso a bana atla sebediswa kapa ho phatlalatswa dipatlisisong tsena. Ngwana otla sebedisa nomoro ya boitsebiso ele ho baballa lebitso la hae lannete.
Jwale ka motswadi/mohlokomedi wa hae ha otlamellwe ho nka karolo empa oka enka ha feela o dumela.
Onale tokelo ya ho hanela ngwana wa hao ho nka karolo dipatlisisong tsena nako enngwe le enngwe, ha feela osa kgotsofala.

N.Naicker

Senior Optometrist/Junior Lecturer

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Appendix C1

CONSENT FORM

I, ……., am the parent/ legal guardian/ care giver of …….....and I hereby give permission for child above to participate in this research study. I have read the information provided to me on the information sheet. I fully understand my rights and what is expected of the child and I am therefore fully satisfied to grant permission.

_____ _____

Signature Date

Appendix C2

Toestemmings vorm

Ek, ……., is die ouer / wettige voog / versorger van ……en hiermee gee ek toestemming vir die bogenoemde kind om deel te neem aan hierdie navorsings-projek. Ek het die informasie aan my voorsien gelees. Ek verstaan ten volle my regte en wat verwag sal word van die kind en is sodoende ten volle tevrede om my toestemming te gee.

_____ _____

Handtekening datum

Appendix C3

Sehlomathisetswa sa A

FOROMO YA TUMELO

Nna,.....ke motswadi/mohlokomdi wa.....ke fana ka tumellano hokenya ngwana waka Dipatlisisong tsena. Ke badile le ho utlwasisa dikateng tsohle tse ka hodimo lengolong. Ebile ke utlwisisa ditokelo tsa ka jwaleka motswadi/mohlokomedi

Kekgotsofetse ka dihare tsa teng.

_____ _____

Signature Date