The interaction between nutrition, immunity and co-infections with human immunodeficiency virus and intestinal parasites in South African adults: investigating the use of pre-albumin as a tool for nutritional assessment

by

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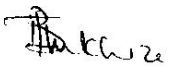
29 July 2019

DECLARATION

I certify that the work presented in this thesis to the best of my knowledge and belief, represents original work by myself, the author and has not been submitted in any form for any degree to any other university. Where work of others has been used, such work has been duly acknowledged in the text. I also certify that I have complied with the rules, requirements, procedures and policy of the university.

Signed  **Date: 29 July 2019**

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DEDICATION

To my family: my late husband Sibusiso ‘Bo’, and my children Kabelo, Nokuzola and Mandla

To my parents: my late father Wilson Themba Ndaba and my mother Elizabeth Ndaba

To my sister, my friend: Ziningi Kambule

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AAM	alternatively activated macrophages
ADCC	antibody-dependent cellular cytotoxicity
AIDS	acquired immunodeficiency syndrome
BCG	Bacillus Calmette-Guérin
BMI	body mass index
CCL	C-C motif chemokine ligand
CCR5	C-C chemokine receptor type
$CD4^+$	cluster of differentiation-4 positive
$CD8^+$	cluster of differentiation-8 positive
CDC	Centers for Disease Control and Prevention
cDNA	complementary deoxyribonucleic acid
CRP	C-reactive protein
CTL	cytotoxic T lymphocytes
CXCR4	C-X-C chemokine receptor type 4
DNA	deoxyribonucleic acid
^0C	degrees Celsius
EndoCAb	endotoxin core IgG antibody
ELISA	enzyme-linked immunosorbent assay
FGS	female genital schistosomiasis
Foxp3	forkhead box p3
GITR	glucocorticoid-induced tumour necrosis factor receptor
$\geq$	greater or equal to
HCT	HIV Counselling and Testing
HIV	human immunodeficiency virus
HIV/AIDS	human immunodeficiency virus infection and acquired immunodeficiency syndrome
HLA-DR	human leukocyte antigen- D related
ICOS	inducible T cell co-stimulator
IgE	immunoglobulin-E
IgG4	immunoglobulin-G subclass 4
iFABP	intestinal fatty acid-binding protein
IFN-γ	interferon-gamma
IL	interleukin

IL-1Ra	IL-1 receptor antagonist
KZN	KwaZulu-Natal
<	less than
LPS	lipopolysaccharide
MHC	major histocompatibility complex
MIP	macrophage-inflammatory protein
MMP-1	matrix metalloproteinase- 1
MTB	*Mycobacterium tuberculosis*
MTCT	mother-to-child-transmission
NARS	nutrient adequacy ratio
NK cell	natural killer cell
/	per
%	percent
PCR	polymerase chain reaction
PRR	pattern recognition receptor
RBC	red blood cell
RNA	ribonucleic acid
ROR-α	retinoic acid orphan receptor -alpha
RORγt	retinoic acid orphan receptor gamma t
SA	South Africa
sCD14	soluble CD14
sTNF-R	soluble TNF receptor
STATs	signal transducers and activators of transcription
TB	tuberculosis
T-bet	T-box expressed in T cells
TCR	T cell receptor
Th	T helper
TLR	Toll-like receptor
TNFα	tumour necrosis factor-α
TNF-β	tumour necrosis factor- β
Treg	T regulatory
ul	microliter
UKZN	University of KwaZulu-Natal
UNAIDS	The Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization

ABSTRACT

Highly prevalent HIV and helminth single infections continue to plague a significant proportion of the South African population. The geographic overlap of these infections lands to the expectation that high prevalence of co-infection with HIV and intestinal helminths exists, although this data for the South African adult population is lacking. Each of these single infections has an impact on the immune system, resulting in impaired responses due to the chronic activation. Also, both infections have an impact on the nutritional status, which may affect the potency of the immune responses, further compromising the immunity. A potent immune system requires adequate nutrition. Obesity, a form of malnutrition may mask micro- and macronutrient deficiency. Furthermore, obesity may result in low-grade inflammation, which may result is dysregulated responses. Therefore, malnutrition may start a cyclical process that may further predispose to infection, which in turn may result in malnutrition, where the cause-and-effect thread between malnutrition, infection and immune deficiency is indiscernible. Based on this, it was hypothesized that the HIV-intestinal helminth co-infection may have a deleterious effect on the nutrition and immunity of affected individuals, which may accelerate HIV progression. Thus, the aim of the study was to investigate the interaction between HIV and intestinal helminth single and co-infection with nutrition and immunity in an adult population (n = 263) in KwaZulu-Natal, a province with high prevalence of both HIV and intestinal helminths infections. The study expected to find an association between the co-infection with lower micro- and macro-nutrient levels, higher HIV viral load, increased immune activation, increased gene expression of Th2 and Treg cytokine responses and decreased Th1 cytokine responses compared to those singly infected and those uninfected with HIV and intestinal helminths.

However, the study found no significant association between HIV and intestinal helminth single or co-infection with micro- and macronutrient deficiency, although a general pattern of low intake of the nutrients was noted among the investigated cohort, who had a substantial proportion being overweight and obese. Difficulty in the assessment of nutritional status in the milieu of HIV and intestinal helminth co-infection, obesity and inflammation was noted. Furthermore, HIV-intestinal helminth co-infection was associated with an antiviral cytokine response profile of highly expressed IFN-γ and TNF-α cytokine genes and reduced viral load. The co-infected individuals with the $IgE^{hi}IgG4^{hi}$ intestinal helminth infection phenotype had a compromised immune profile of low CD4 counts. We recommend that antihelminthic interventions are included in the HIV management programmes, particularly in adults.

1.0 CHAPTER 1

Background and Literature Review

1.1 Background

Approximately 36.7 million of the world's population is infected with human immunodeficiency virus (HIV), of which an estimated 19.4 million are in sub-Saharan Africa (UNAIDS 2017a). Compounding to this plague, approximately 2 billion individuals (24%) are infected with intestinal helminth parasites globally, with high prevalence occurring in tropical and sub-tropical countries populated by poor and deprived communities, particularly in sub-Saharan Africa (World Health Organization 2016a). The geographic overlap between HIV, intestinal helminth infections and malnutrition, complicated by poverty has been well documented in sub-Saharan Africa (Himmelgreen *et al.* 2009; Hotez and Kamath 2009; Koethe and Heimburger 2010; Mwambete and Justin-Temu 2011; Noblick *et al.* 2011; Amare *et al.* 2015).

A substantial proportion of the South African population is exposed to both HIV and helminth infections, particularly in communities that live in poverty (Adams *et al.* 2006; Adeleke *et al.* 2015). Also considering that there is more than fifty percent of the South African population living under conditions of deprivation, it is reasonable to deduce that HIV and helminth co-infections are commonly overlapping with malnutrition in many parts of the country. KwaZulu-Natal (KZN), a province with the highest prevalence of HIV in South Africa has always been the epicentre of the global epidemic (Department of Health South Africa 2017). Furthermore, in certain regions of KZN, especially along the coast, high intestinal helminth infection rates have been found (Kwitshana *et al.* 2008). Warm climatic and/or moist environmental conditions which occur along the KZN coast, promote the persistence and transmission of intestinal helminths (Appleton *et al.* 1999; Brooker *et al.* 2006; Mascarini-Serra 2011). A geographical overlap of HIV and helminth infections is thus anticipated, since KZN also has a significant fraction of its population that live in poor conditions worsened by lack of adequate sanitation, potable water and poor personal hygiene (Statistics South Africa 2018). However, studies that have determined the interactions between immunity, HIV-helminth co-infections and nutrition in KZN are limited, especially among adults, where peak HIV infection occurs.

The current study, conducted in KZN, was premised on the hypothesis that the HIV-intestinal helminth co-infection has a deleterious effect on the nutritional status and the immune system, resulting in exacerbated HIV progression. It is suggested that chronic helminth infection may

induce immune system dysregulation as a result of persistent stimulation by helminth eggs, excretory/ secretory (ES) products that are released daily by the infecting parasites (Hewitson *et al.* 2009; Moreau and Chauvin 2010). ES products are actively exported through the secretory pathways of the helminths and those which may leak or diffuse from the intestines of adult worms (Hewitson *et al.* 2009). These helminth products increase the expression of C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) co-receptors in cluster of differentiation-4 positive ($CD4^+$) cells (Chachage *et al.* 2014). This constantly activated immune system is suggested to facilitate HIV entry more efficiently into the activated $CD4^+$ target cells that are increased in numbers (Jaspan *et al.* 2011; Woodham *et al.* 2016). The continued HIV acquisition may directly lead to more rapid loss of $CD4^+$ cells in HIV infected individuals (Vijayan *et al.* 2017).

Furthermore, HIV infection is associated with factors that can result in nutritional deficiency such as the inability of the infected individuals to consume and utilise food (Duggal *et al.* 2012). Likewise, intestinal helminths may compromise the nutritional status, which can be worse in HIV-intestinal helminth co-infected individuals (Gedle *et al.* 2017). The importance of adequate nutrition on the immune system has been established (Karacabey and Ozdemir 2012). Potent immune responses and adequate nutrition are essential to resist infectious agents. Many infections, such as HIV and intestinal parasites impair the nutritional status by various mechanisms. These include (i) the loss of appetite induced by cytokines such as tumour necrosis factor-alpha (TNF-α) produced during an infection (Paulsen *et al.* 2017); (ii) painful swallowing as occurs during HIV-induced oral thrush, thus decreasing the desire to eat; (iii) diarrhoea and disruption of the intestinal mucosa lining which impedes the absorption of nutrients and (iv) parasites such as hookworms that feed directly from the host's intestinal mucosa blood (Duggal *et al.* 2012). Unfortunately, in infected individuals from poor communities whose nutrient intake is already inadequate, the situation is worsened; malnutrition results in immune deficiency and further predisposition to infection leading to a vicious cycle of malnutrition, infection and immune deficiency such that the cause-and-effect thread is eventually lost (Rodriguez *et al.* 2011), as depicted in Figure 1, a model adapted with permission from Katona and Katona-Apte (2008).

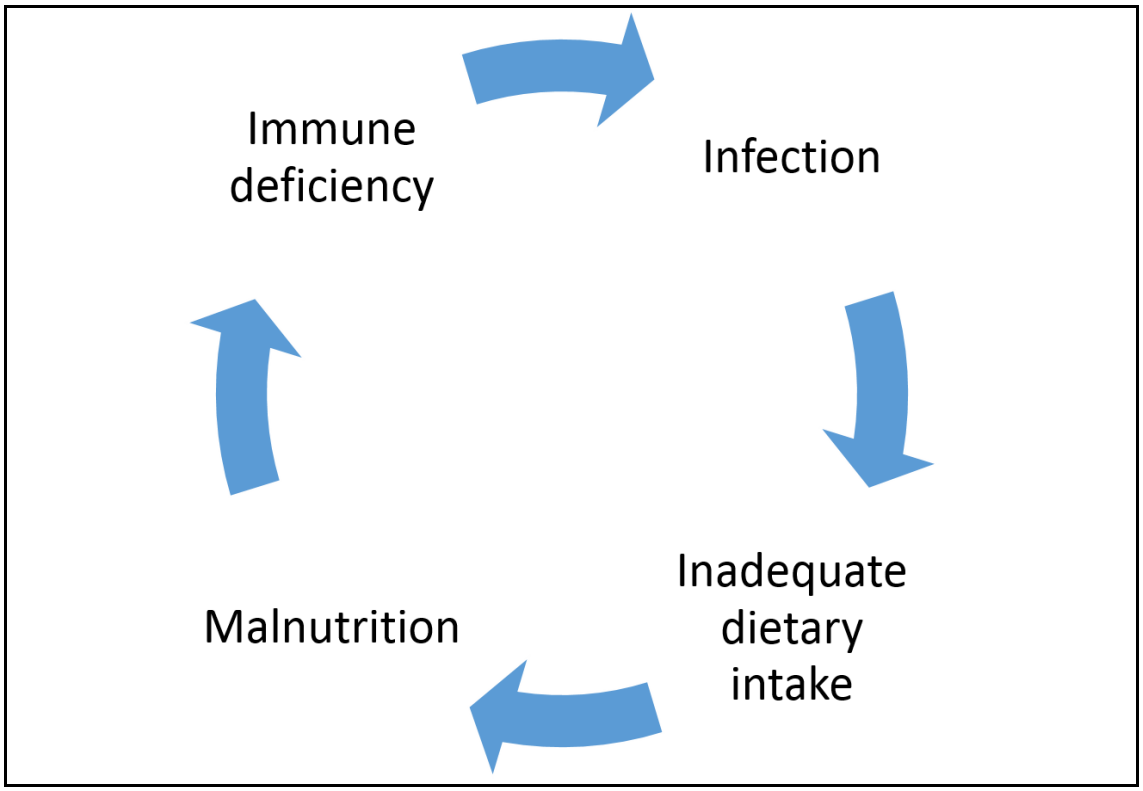

Figure 1: A conceptual model illustrating the cyclical interaction between immune deficiency, infections, inadequate nutrient intake and malnutrition

The milieu of HIV, intestinal helminth infections, malnutrition and poverty, may have an additive impact on the competency of the immune system which may lead to accelerated progression of the HIV and intestinal helminth infections. This may also facilitate increased transmission of HIV by promoting the susceptibility of the host to the virus or worsen the clinical course of the infection and/or augment viral replication in affected hosts (Karp and Auwaerter 2007; Assefa *et al.* 2009; Noblick *et al.* 2011). Neglecting to give the intestinal helminth infections the attention and priority they require contributes to the disease burden, especially in areas where there is high prevalence of HIV infection. Intestinal helminthiasis and schistosomiasis may be associated with healthcare challenges especially in poor resource communities, which further burden the public health system. Current literature on the impact of the HIV and intestinal helminths single and co-infections on the nutritional status and the immune system of those affected is discussed in this chapter.

1.2 Literature review

1.2.1 The prevalence of human immunodeficiency virus-1 infection in South Africa

The human immunodeficiency virus-1 (HIV-1) infection is continually spreading in many regions in spite of all the global campaigns and efforts that are deployed to curb the epidemic. Nearly 36.7 million individuals are infected globally, with 1.1 million HIV-related deaths in 2016 (World Health Organization 2016a). Sub-Saharan Africa remains the epicentre of the HIV pandemic (World Health Organization 2016a). Nearly two thirds of the world's HIV infected individuals live in this region, with 43% of the global new infections reported in sub-Saharan Africa in 2016 (UNAIDS 2017a).

South Africa (SA) is amongst countries in sub-Saharan Africa with the highest HIV-1 epidemic, with approximately 7.1 million individuals living with HIV (UNAIDS 2017b). Notably, amongst the South African provinces, KZN has always had the highest human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS) prevalence (Statistics South Africa 2015b). Moreover, KZN is reported to have had larger increases of HIV-1 prevalence over the years compared to the national prevalence of 10.2% (Statistics South Africa 2015a; Department of Health South Africa 2017). The prevalence amongst the KZN antenatal women in 2013 was 40.1%, rising to 44.4% in 2015 (Department of Health South Africa 2017). The virus affects mostly the 15 to 49 year old individuals, an age group which is reproductive and economically active (Shisana *et al.* 2014), with peak prevalence in females occurring in the 35 to 39 year old age group, whereas in males affecting mostly an older age group of between 45 to 49 years old (Simbayi *et al.* 2018).

1.2.1.1 Role of T-helper 1 (Th1) immune responses in intracellular infections

In a normal competent immune system that responds adequately to intracellular pathogens such as HIV and other viruses, and intracellular bacteria such as *Mycobacterium tuberculosis*, the antigen presenting cells such as monocytes, macrophages and dendritic cells of the innate system recognise, capture and process microbial antigens and present them as fragments to T helper (Th) cells that are relatively undifferentiated and naïve (Kidd 2003). The adaptive response to infection results in the recruitment of T lymphocytes to the site of infection, with all the T cell subsets functioning in synchrony. T cells then produce cytokines, which are central to the host defence against pathogens (Maizels *et al.* 2004). These cytokines induce differentiation and polarisation of the naïve T cells, from null Th (Th0) cells into either T helper 1 (Th1) or T helper 2 (Th2) cells, depending on the type of pathogen (Kidd 2003). Intracellular pathogens

induce differentiation into Th1 lineage while extracellular ones promote predominance of Th2 cells (Kaiko *et al.* 2007).

Intracellular pathogens stimulate the production of pro-inflammatory cytokines by Th1 cells. These cytokines elicit a cell mediated immune response, with the increased proliferation of cytotoxic T lymphocyte (CTL) responses against the pathogens (Becker 2004). Cytokines secreted by Th1 cells include interferon-gamma (IFN-γ); interleukin-2 (IL-2), IL-12 and tumour necrosis factor-alpha (TNF-α) or tumour necrosis factor-beta (TNF-β). These Th1 cytokines promote phagocytosis and intracellular killing by macrophages among other functions (Maizels and Holland 1998; Brown *et al.* 2004; Wang *et al.* 2008; Cavalcanti *et al.* 2012). IFN-γ production is key to the activation of macrophages (Diaz and Allen 2007). IFN-γ also recruits natural killer (NK) cells and CTLs to the site of virally infected cells, augmenting their antiviral activities (Pak-Wittel *et al.* 2013; Januskevica *et al.* 2016). IL-12 on the other hand, promotes the differentiation of Th1 cells and their ability to eradicate intracellular pathogens through the activation of the lineage specifying T-box expressed in T cells (T-bet) and STAT4 transcription factors (Gazzinelli *et al.* 1993; Anderson *et al.* 2003; Duhen *et al.* 2012). Furthermore, IL-12 augments the activation of CD8 T cells and promotes strong Th1 responses, enhancing the production of IFN-γ, mediated by C-C motif chemokine ligand 1 (CCL1) and CCL17 from dendritic cells and activated T cells (Henry *et al.* 2008).

1.2.1.2 HIV-1 infection induces Th1 immune responses

The direct effects of the presence of viral proteins during HIV infection is persistent stimulation of Th1 responses. During the primary infection Th1 responses induce the activity of $CD4^+$ Th1 cells, $CD8^+$ T cells, NK cells and antibody producing B cells, which contribute to a stable viral set-point (Walker and McMichael 2012; Salgame *et al.* 2013). HIV-1 infection elicits responses that are partially protective; they control the virus however, fail to eliminate it. T cell proliferation is stimulated, although the produced cells may have a limited protective benefit to the host. Particularly, in the chronic phase of the infection, the required Th1 cell proliferation and expansion is impaired and inadequate (Becker 2004; Shey *et al.* 2015). The levels of HIV-specific $CD8^+$ CTLs, NK cells and cytolytic perforin become reduced, resulting in a high viral load (Geldmacher *et al.* 2007). The impaired anti-viral function of $CD8^+$ T cells and NK cells may then lead to the inability of the immune system to control the perpetual HIV replication within the $CD4^+$ cells (Boasso *et al.* 2009). Furthermore, HIV may subvert the host defence mechanisms and use cell activation factors for further replication within the host cells. Activated host cells express transcription factors such as the nuclear factor of activated T cells (NFAT),

nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and the positive transcription-elongation factor complex (pTEFb) which bind to the HIV-1 long terminal repeat (LTR) promoter, thereby driving viral transcription and replication within $CD4^+$ cells (Kinoshita *et al.* 1998; Arendt and Littman 2001; Booiman *et al.* 2015).

Infectious pathogens and their products may continuously activate the immune system, and these include helminths, their eggs and excretory/secretory (ES) products; bacteria and their products, such as lipopolysaccharide (LPS) and HIV and other viruses such as chronic hepatitis B and C (Miedema *et al.* 2013). The persistent activation of $CD4^+$ and other immune cells drives viral replication (Jaspan *et al.* 2011). HIV replication is more efficient in activated than in naïve cells (Pan *et al.* 2013). The virus enters activated $CD4^+$ T cells, macrophages and dendritic cells using C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) co-receptors, respectively. Increased expression of these co-receptors at the surface of activated $CD4^+$ cells confers increased susceptibility to HIV acquisition (Levacher *et al.* 1992; Janeway *et al.* 2001; Nattrass 2009). The CCR5-activated cells in mucosal surfaces allow the virus direct access for entry into these cells and notably, individuals who have a 32 base pair deleted CCR5 gene are protected from acquiring HIV even when exposed to the virus (Janeway *et al.* 2001; Galvani and Novembre 2005; van Montfort *et al.* 2008). The chronic immune activation adds to the HIV disease challenge by providing more cells that are prone to more infection, which exhausts the immune system, resulting in hyporesponsiveness and anergy, characterised by the loss of proliferative capacity of potent effector cells (Reuter *et al.* 2012). Moreover, the continued proliferation that may occur, as an attempt of replenishing the depleted immune cells, may inadvertently increase the number of activated cells which also become targets for entry for HIV (Paiardini and Muller-Trutwin 2013).

Depleted $CD4^+$, $CD8^+$ and dendritic cells are also observed in the gastrointestinal (GIT) mucosa due to the immune activation and resultant inflammation (Klatt *et al.* 2013). These impaired responses fail to control incoming microbial pathogens in the GIT and this contributes further to the immune activation (Dandekar *et al.* 2010). The translocated pathogens then trigger elevated inflammatory responses, that result in immune activation (George *et al.* 2012). In chronic HIV infection, the translocated microbial products induce additive immune activation and dysfunction caused by the inflammation, resulting in exhausted intestinal macrophages and apoptotic enterocytes (Marchetti *et al.* 2013) that may result in the rapid progression of HIV infection (Klatt *et al.* 2013; Bi *et al.* 2016).

The activation and inflammatory responses induced by HIV and/or other infectious microbes persist throughout the course of the HIV disease (Janeway *et al.* 2001), resulting in immune dysregulated responses (Sonnenberg and Artis 2015). These dysregulatory mechanisms cause progressive and multifactorial impairment of all arms of the immune responses: innate and adaptive. These mechanisms include a decrease in the function of $CD4^+$ and $CD8^+$ cells and dendritic cells, induced by IL-10 and transforming growth factor–β1 (TGF-β) (Shey *et al.* 2015). Also, defective upregulation of the co-stimulatory accessory molecules: CD40 ligand, CD28 and CD80 may be activated, which results in hyporesponsive and anergic antigen presenting cells (APCs), $CD4^+$ and $CD8^+$ T cells (Borkow and Bentwich 2004). Furthermore, the upregulation of inhibitory molecules such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death-1 (PD-1) may also be activated (Boasso *et al.* 2009). These mechanisms are inhibitory and suppressive to anti-HIV responses and may lead to the accelerated progression of the HIV disease. The increasing viral load and persistent immune activation augments the progressive down-regulation of naïve and resting memory $CD4^+$ and $CD8^+$ cells through anergy and attrition (Grossman *et al.* 2002), which is associated with HIV disease progression. The activated $CD4^+$ and $CD8^+$ immune cells in response to the HIV challenge may express indicators of activation such as increased levels of human leukocyte antigen-D related (HLA-DR) and/or CD38 as well as Ki-67, a proliferation marker (Eggena *et al.* 2005; Paiardini and Muller-Trutwin 2013; Chachage *et al.* 2014).

1.2.1.3 The impact of HIV-1 infection on nutritional status

Furthermore, HIV infection is associated with factors that impact on the ability to consume and utilise food (Duggal *et al.* 2012). HIV may cause mild to moderate anaemia due to decreased or ineffective red cell production arising from nutritional deficiencies either from malnutrition or malabsorption as a result of gastrointestinal damage (Volberding *et al.* 2004; Gedle *et al.* 2015). This can lead to increased HIV disease progression. Micronutrient supplementation, in general, shows a significant slowing of HIV disease progression with better preservation of $CD4^+$ T cell count and lower viral loads (Fawzi *et al.* 2004). This supports the notion that adequate intake of micro- and macronutrients is essential for the host to be able to mount efficient immune responses.

Nutrients may augment the responsiveness of target cells or tissues to cytokines; for example fats may influence a change in fatty acid composition in cell membranes and proteins have an effect in protein metabolism during inflammation (Tesseraud *et al.* 2009). Furthermore, the synthesis of acute phase proteins and glutathione require an adequate intake of sulphur amino

acids (Grimble 2006). Therefore, infection may result in the depletion of nutrients, due to their increased utilisation during different metabolic processes following an invasion by a pathogen. For instance, protein energy malnutrition is associated with defects in the intrinsic components in the antigen presentation and the microbicidal activities in macrophages (Corware *et al.* 2014). Micronutrients, including zinc, selenium, iron, copper, β-carotene, folic acid, vitamins A, C, D and E are essential for the maintenance of metabolic and tissue function as well as a potent immune system (Erickson *et al.* 2000; Bhaskaram 2001; Shenkin 2006). The following illustrate the importance of the essential micronutrients for a competent immune system: vitamin A and zinc play a major role in the synthesis of nucleic acids in cells and tissues (Duggal *et al.* 2012). Vitamins A, D and E and trace elements are vital for phagocytic cells (macrophages and neutrophils) and NK cells activity against infectious agents (Erickson *et al.* 2000). Vitamin A supplementation increases the number of circulating $CD4^+$ lymphocytes and NK cells and increases antibody production, indicating potential benefit to HIV infected individuals (Villamor and Fawzi 2005).

Vitamin A deficiency is associated with increased susceptibility to gastrointestinal, respiratory and genitourinary tract infections, mostly impeding the innate immune response including the synthesis of lysozyme (Katona and Katona-Apte 2008). Vitamin A deficiency was associated with high mortality among HIV infected adult individuals (Mulu *et al.* 2011). The South African government has adopted a strategy of preventing vitamin A deficiency in children by instituting a programme that provides vitamin A supplementation (prophylactic and curative) to all children from the age of 6 months to five years (Department of Health South Africa 2012b).

Zinc deficiency is also associated with impaired cell-mediated responses, where IL-2 and IFN-γ cytokine production by the Th1 immune system are decreased and consequently, the cytolytic capacity of NK cells is also reduced (Foster and Samman 2012). Meanwhile, a potent Th1 response is essential for the control of HIV infection (Vingert *et al.* 2012). Both innate and adaptive immune responses induce the synthesis of molecules which require DNA replication, RNA expression, protein synthesis and secretion, all of which may consume nutrients and anabolic energy as well as catabolise the nutrients during inflammatory responses (Schaible and Kaufmann 2007). Hence, malnutrition may result in impaired innate and adaptive responses against pathogens (Chandrasekaran *et al.* 2017), particularly in HIV infection, even with effective antiretroviral therapy (ART) (Koethe *et al.* 2009; Duggal *et al.* 2012).

1.2.2 The epidemiology of intestinal helminth parasite infections in South Africa

Intestinal helminth infection, an additional health burden reported to be infecting approximately 2 billion individuals globally and similar to HIV, has the highest prevalence occurring in the sub-Saharan region (World Health Organization 2017b). Four countries in the region, namely Nigeria, Democratic Republic of Congo, South Africa (SA) and Tanzania carry 44% of the infections (Brooker *et al.* 2006; Hotez and Kamath 2009).

At least 342 species of helminths can infect humans (Crompton 1999; Adu-Gyasi *et al.* 2018). Intestinal helminths are multicellular worms of three taxonomic groups, which are cestode tapeworms, nematode roundworms and trematode flukes (McSorley and Maizels 2012). *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm species are the most common nematodes prevalent in poor communities in the tropics and subtropics including South Africa (CDC 2013). In addition, *Schistosoma haematobium* and *S. mansoni* are the most common trematode blood flukes that infect humans in South Africa (Schutte *et al.* 1981; Appleton and Miranda 2015; Hedley and Wani 2015).

South Africa (SA) has a significant percentage (approximately 53%) of its population that live under conditions of poverty, overcrowding and malnutrition, worsened by lack of clean water supplies, adequate sanitation and inevitable poor personal hygiene (Statistics South Africa 2016). Communities living in such conditions are more prone to intestinal helminth infections, owing to their oral-faecal route of transmission (World Health Organization 2017b). They are mostly transmitted through ingesting food, water or soil contaminated with the parasite eggs or larvae. These parasites are therefore known as soil-transmitted helminths, with a maturation stage in the soil (except for schistosomiasis which is transmitted through water bodies). Intestinal helminths can exist in human hosts in different developmental stages, as either eggs, larvae or adult worms, which can be detected in stool samples, except for *S. haematobium* detected in urine samples (Appleton and Miranda 2015). With the exception of the *Strongyloides* species, soil-transmitted helminths do not multiply within the host, however they produce eggs or larvae that infect the next host (Maizels and McSorley 2016).

The distribution of intestinal helminths in SA varies according to the climatic and/or environmental conditions, with highest infection rates found in coastal regions (Appleton *et al.* 1999). Figure 2, adapted from https://pixabay.com/en/south-africa-map-districts-country-42772/, shows the locations of the different provinces of South Africa and their proximity to the coast, noting those that are situated along the coast of the Indian Ocean, which are KwaZulu-

Natal and Eastern Cape. The Northern Cape is along the Atlantic Ocean and the Western Cape shares both the Indian and Atlantic Oceans.

Figure 2: The map of South Africa, showing the KwaZulu-Natal, Eastern Cape, Western Cape and Northern Cape provinces that are along the coast and in close proximity of the Indian and/or Atlantic Oceans (https://pixabay.com/vectors/south-africa-map-districts-country-42772/).

The KwaZulu-Natal (KZN) and Western Cape provinces are recorded to have high prevalence of soil-transmitted helminth infections (Ajoge *et al.* 2014) (Figure 3) (http://www.thiswormyworld.org/maps/distribution-of-soil-transmitted-helminth-survey-data-in-south-africa). However, the prevalence of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm species, the most common intestinal helminths, are mostly higher in KZN compared to other provinces (Appleton *et al.* 1999; Jinabhai *et al.* 2001; Saathoff *et al.* 2004a; Appleton and Kvalsvig 2006).

An investigation among adults in KZN found a prevalence of intestinal helminths to be 20.4 – 59%, the patients found to be mostly infected with *Ascaris lumbricoides* and *Trichuris trichiura* (Kwitshana *et al.* 2008). Others found in a household survey, a prevalence of 59% *Ascaris lumbricoides* and 48% *Trichuris trichiura* intestinal helminths (Tronnberg *et al.* 2010).

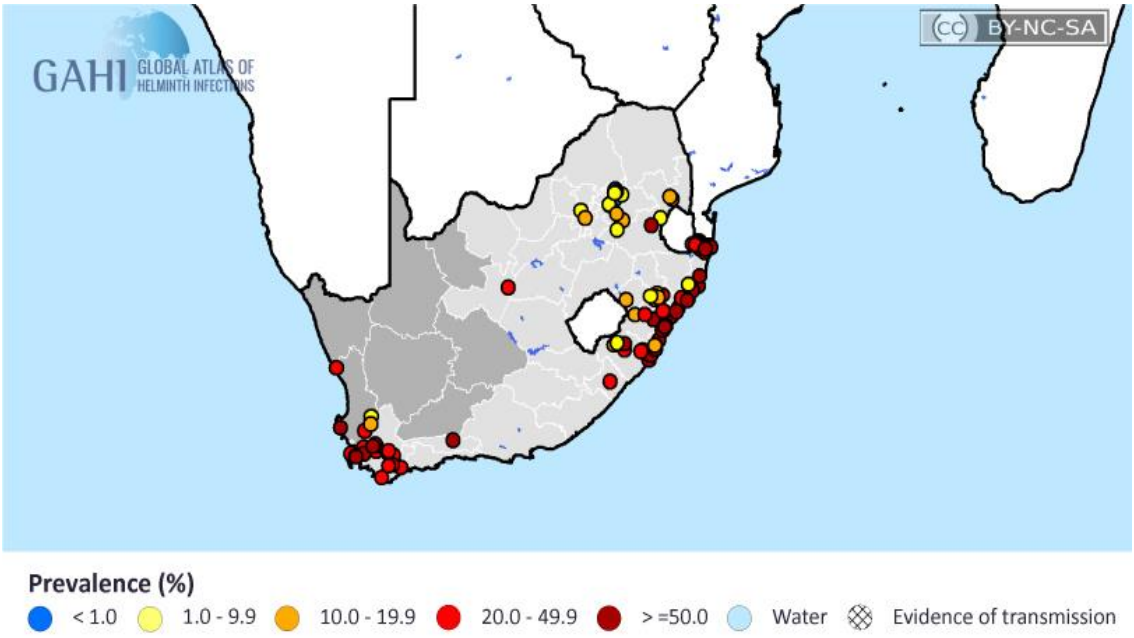

Figure 3: The percentage distribution of soil transmitted helminth infection in South Africa (http://www.thiswormyworld.org/maps/distribution-of-soil-transmitted-helminth-survey-data-in-south-africa).

The prevalence of intestinal helminths was reported as low in the inland parts of the country due to the lower temperatures and rainfall patterns (Mabaso *et al.* 2004). For example, Samie *et al.* (2009) found the most common intestinal helminths in adults and children who were diarrhoeal in Limpopo province as *Ascaris lumbricoides* (10.4%), *Trichuris trichiura* (10.4%), hookworm species (11.5%) and *S. mansoni* (11.9%). In Western Cape Province (coastal), Adams *et al.* (2005) found higher prevalence of *Ascaris lumbricoides* (24.8%), *Trichuris trichiura* (50.6%), and low prevalence of hookworm species (0.08%), *Hymenolepsis nana* (2.2%) and *Enterobius* (0.6%) among school-children. Similarly, in the coastal Eastern Cape, Nxasana *et al.* (2013) found higher prevalence of *Ascaris lumbricoides* (29%) and low prevalence of *Trichuris trichiura* (3.7%) and *Hymenolepsis nana* (4.9%) among school-children in Mthatha. These prevalence rates are generally lower than those recorded in KZN coastal regions. Table 1 indicates the high prevalence of intestinal helminth infections, higher than in other provinces in most cases, except for the higher *S. mansoni* reported in Limpopo (Samie *et al.* 2009).

Table 1: Previous prevalence studies conducted in KZN primary school-children indicating that *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm species are the most occurring intestinal helminths in north and south regions of KZN

KZN region	n	*Ascaris lumbricoides*	*Trichuris trichiura*	Hookworm species	*Schistosoma haematobium*	*Schistosoma mansoni*	Reference
Coastal	156	69%	89.5%	62.7%	NA	NA	(Appleton *et al.* 1999)
Rural South Coast	268	19.4%	54%	2.9%	21.9%	0.7%	(Jinabhai *et al.* 2001)
Rural North	1017	28.1%	57.2%	83.2%	NA	NA	(Saathoff *et al.* 2004a)
Northern	1109	NA	NA	NA	68.3%	NA	(Saathoff *et al.* 2004b)
Midlands	608	NA	NA	NA	7.2%	NA	(Johnson and Appleton 2005)
South	180034	65%	89%	25%	94%	NA	(Appleton and Kvalsvig 2006)
North East	153716	73%	88%	89%	98%	NA	(Appleton and Kvalsvig 2006)
Northern	320	NA	NA	NA	37.5%	NA	(Kabuyaya *et al.* 2017)
South Coast	428	55.9%	83.6%	59.4%	43.4%	NA	(Taylor *et al.* 2001)
South Coast	271	NA	NA	NA	43%	NA	(Thomassen Morgas *et al.* 2010)
South Coast	726	NA	NA	NA	36.9%	NA	(Molvik *et al.* 2017)

NA: data is not available

Schistosomiasis is also highly endemic in South Africa (Magaisa *et al.* 2015), with an estimated 5.5 million infected individuals (World Health Organization 2017a). It is prevalent in the north and east parts of SA, especially in Mpumalanga, Limpopo, Eastern Cape and KZN provinces (Moodley *et al.* 2003; Wolmarans and de Kock 2009; Meents and Boyles 2010; Magaisa *et al.* 2015).

An overall *S. haematobium* prevalence of 70.4% was reported in Limpopo, although the prevalence rates differed among the different age groups (Samie *et al.* 2010). The primary school-children were noted to have a *S. haematobium* prevalence of 42%, whilst it was 36% among the university students and the hospital out-patients (which included adults and children) ranged between 78% and 86% (Samie *et al.* 2010). In another investigation among school-children in Limpopo, nearly 80% were infected with *S. haematobium* (Wolmarans and de Kock 2009). The Eastern Cape province is also highly plagued with *S. haematobium* infection, with prevalence of 73.2% (Meents and Boyles 2010). Figure 4 indicates the Mpumalanga, Limpopo, Eastern Cape and KZN provinces which have high prevalence of schistosomiasis (http://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-south-africa).

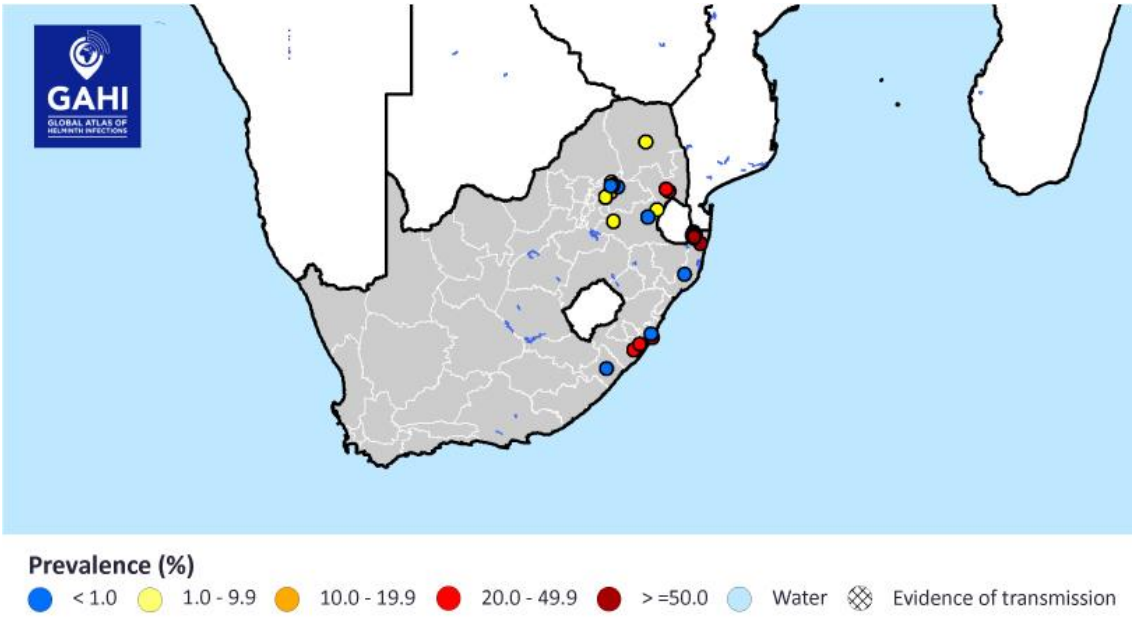

Figure 4: The percentage distribution of schistosome infection in South Africa (http://www.thiswormyworld.org/maps/distribution-of-schistosomiasis-survey-data-in-south-africa)

KwaZulu-Natal (KZN), the main focus of this work, is among the poorest provinces in SA (Statistics South Africa 2015b). It has a substantial percentage of its population living in conditions where there is generally a poor standard of living, with nearly 22.7% and 14.6% of the households lacking adequate sanitation and safe water supplies respectively (Statistics South Africa 2016). The poor living conditions predispose these communities to intestinal helminth infections, hence KZN is plagued by intestinal helminth infections and schistosomiasis. In addition, the persistence of the intestinal helminths in KZN is due to (i) the environmental conditions of warm temperature and moisture from rainfall seen throughout the year and (ii) its long coast which is along the Indian Ocean, both promoting the transmission of highly prevalent *Trichuris trichiura*, *Ascaris lumbricoides*, *Necator americanus*, *Taenia solium*, *Strongyloides stercoralis* and *S. haematobium* parasites (Appleton *et al.* 1999; Jinabhai *et al.* 2001; Hotez *et al.* 2003; Saathoff *et al.* 2004b; Braae *et al.* 2015; Molvik *et al.* 2017).

Most studies on the prevalence of intestinal helminths have been conducted in primary school-children. High numbers of intestinal helminths are harboured by primary school-children, with peak infection occurring in the five to ten year old age group, the intensity of infection declining in adulthood, whilst hookworm infections peak in adulthood (Hall *et al.* 2008). The ten to fifteen year old age group is mostly infected with schistosomiasis (Maseko *et al.* 2018).

With all the recorded data on the prevalence of intestinal helminths from investigations in school-children, there is however paucity of this data in the adult population (Kwitshana *et al.* 2008; Adeleke *et al.* 2015). A study on an adult population in Eastern Cape (Adeleke *et al.* 2015), found that 27.4% of the participants in Mthatha (n = 57) were infected with intestinal helminths. It was observed that they were infected with *Ascaris lumbricoides* (42.1%), *Trichuris trichiura* (5.26%), hookworm species (5.26%), *Hymenolepsis nana* (5.26%), *Diphylobothrium latum* (28.07%) and *Fasciolopsis buski* (8.8%) (Adeleke *et al.* 2015). Furthermore, a study conducted on an adult population in Cape Town found high prevalence of *Ascaris lumbricoides* (51%) (Adams *et al.* 2006). Another study on a KZN adult population observed high prevalence of intestinal helminths in the coast (35.9% in the south and 42.8% in the north coast) with moderate levels (20.4%) in eThekwini, where the current study site was situated (Kwitshana *et al.* 2008). This intestinal helminth prevalence data for the adult population, albeit limited, indicates that the intestinal helminths may also be highly prevalent in adults. Untreated infected adults may be a reservoir and a source of intestinal helminth transmission and infection in a community, where in most cases antihelminthic interventions are primarily implemented in schools (Bopda *et al.* 2016; Masaku *et al.* 2017; Silver *et al.* 2018).

1.2.2.1 Helminths induce strong Th2 immune responses

Large eukaryotic pathogens, such as the helminths induce strong Th2 humoral immune responses (Borkow and Bentwich 2004). The responses occur after the recognition of pathogen-associated molecular patterns (PAMP), which are extensively complex helminth worm-unique antigenic glycoconjugates (Maurya *et al.* 2012). They are recognised by Toll-like receptors (TLR) and/or pattern recognition receptors (PRR) expressed on dendritic cells (Everts *et al.* 2010). After the TLRs have detected microbial infection, they then induce innate and adaptive responses (Sakaguchi 2005).

Helminth infections initially elicit Th1 pro-inflammatory innate responses from macrophages soon after infection, where Th1 cytokines, including IFN-γ and TNF activate naïve T cells (Porthouse *et al.* 2006). In addition, Th17 cells, upon activation by helminths, secrete IL-17 cytokines which are pro-inflammatory and are indicated to be key regulators of inflammation (Wang *et al.* 2008). The retinoic acid orphan receptor gamma theta (RORγt) and ROR-alpha (ROR-α) transcription factors are required for Th17 cells (Duhen *et al.* 2012).

Thereafter over time, helminths induce Th2 responses. The Th2 cytokines secrete cytokines that typically include IL-4, IL-5, IL-10, IL-13 which result in B cell proliferation, immunoglobulin-

E (IgE) and immunoglobulin-G subclass 4 (IgG4) antibody production as well as activation of specific effector cell responses from eosinophils, basophils and mast cells (Wang *et al.* 2008; Fukumoto *et al.* 2009). The important role of both IL-4 and IL-13, of mediating protective responses or resistance to infection in the host leads to effective expulsion of the intestinal helminths (Filbey *et al.* 2014), facilitating the reduction of the helminth infection intensity (Turner *et al.* 2003). IL-4 and IL-13 signaling through the IL-4 receptor-α drive the expulsion of intestinal helminths, whereby mucosal mast cells and goblet cells secrete mucus to trap and eliminate the parasites (Maizels and Holland 1998). Additionally, IgE targets the helminths, which cannot be phagocytosed due to their large size. IgE coats them through FcεRI receptors for recognition and subsequent destruction by eosinophils, basophils and mast cells, using highly toxic granule proteins and free radicals as well inflammatory mediators (Janeway *et al.* 2001; Fitzsimmons *et al.* 2014). The IgE response can be further amplified by basophils, mast cells and eosinophils; when IgE is cross-linked by antigen on these cells. These effector cells express CD40 ligand which binds with CD40 on B cells, leading to the production of more IgE (Janeway *et al.* 2001). Eosinophils kill helminths through antibody-dependent cellular cytotoxity (ADCC) (Negrao-Correa 2001). Mastocytosis plays a role in the expulsion of intestinal helminths, by increasing permeability, smooth muscle contractility and intestinal epithelial cell fluid secretion (Maizels and Holland 1998; Turner *et al.* 2003). Although helminths stimulate stronger inflammatory Th2 cytokine responses, they however also induce complex immunomodulatory T regulatory (Treg) responses to attenuate these hostile Th2 host responses, for their survival within the human host (Girgis *et al.* 2013; McSorley *et al.* 2013; Johnston *et al.* 2016). The Th2 responses are important to the intestinal helminths for their survival in a human host, whereby the parasite and its products stimulate the production of anti-inflammatory IL-10 from Treg cells and eosinophils to downregulate the responses elicited against the parasite (Nutman 2015; Motran *et al.* 2018).

1.2.2.2 The modulated immune responses to intestinal helminth infections

Helminth infections result in the daily release of eggs and ES products that persistently stimulate immune responses (Walson and John-Stewart 2007) that end up causing immune-mediated inflammatory changes to the host (Borkow and Bentwich 2004). The intestinal helminths compromise the host epithelium to be able to feed, and they remain in preferred anatomic niches found in different locations which they migrate to so as to complete life cycles within the host (Porthouse *et al.* 2006; Boyett and Hsieh 2014). They induce modulatory mechanisms which enable them to evade and suppress the host responses to be able to live long within the host (Everts *et al.* 2010; Boyett and Hsieh 2014; Afifi *et al.* 2015), and to ensure

continuous transmission in a sequential passage between hosts (Klion and Nutman 2004). Helminths protect themselves by modulating responses mounted against them by host responses. These mechanisms protect the intestinal helminths against elimination and expulsion, such that they survive in the midst of the hostile host Th2 responses, with muted effector responses (Maizels and Balic 2004; Moreau and Chauvin 2010; Chatterjee and Nutman 2015; Smallwood *et al.* 2017).

Helminths activate an immune regulatory network, mounted by both the host and the parasite (Girgis *et al.* 2013). They control the way antigens are presented to dendritic cells and also downregulate the dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), a receptor required for entry of intracellular pathogens, such as HIV and *Mycobacterium tuberculosis* into dendritic cells (Kane *et al.* 2004; Manches *et al.* 2014; Chatterjee and Nutman 2015). They also produce cystatin molecules which inhibit cysteine proteases, required for antigen presentation to dendritic cells (Everts *et al.* 2010; Maizels and McSorley 2016). Helminths may also induce the immune system's own down-regulatory mechanisms (McSorley *et al.* 2013). This is achieved through a balance in responses between Th2 innate (eosinophils, basophils and mast cells), adaptive effector $CD4^+$ and $CD8^+$ T cells and T regulatory (Treg) cells (Belkaid and Rouse 2005; Everts *et al.* 2010).

Activated Treg cells suppress antigen presentation by down-modulating the expression of CD80 and CD86 ligands on APCs (Sakaguchi 2005). Defective antigen presentation suppresses effective activation of T cells, which requires costimulatory signalling through the ligation of the CD28 receptor to CD80 and CD86 ligands of APCs (Borkow and Bentwich 2004). To downregulateT cell activation, activated Treg cells secrete the inhibitory molecule CTLA-4, which binds to CD28 and thus inhibit T cell responses and proliferation of activated T effector cells, resulting in apopotosis and hyporesponsiveness (Borkow and Bentwich 2004). In this regard, Treg cells achieve the regulatory function by suppressing T cell proliferation and Th2 cytokine production in a cell-contact-dependent manner, in the quest of limiting host tissue immunopathology (Walker *et al.* 2005).

Fairly recently, the inducible T cell co-stimulator (ICOS) molecule has been indicated as homologous to the immune attenuator CTLA-4, and it is regulatory to the adaptive T cell responses (Wikenheiser and Stumhofer 2016). Furthermore, $CTLA\text{-}4^+$ Treg cells may induce dendritic cells to express the indoleamine 2.3-dioxygenase enzyme which catabolises tryptophan to kynurenine, which is toxic to dendritic cells (Sakaguchi *et al.* 2009). The lack of

the essential amino acid tryptophan inhibits T cell activation and promotes T cell apoptosis (Ricci *et al.* 2011). Furthermore, helminths may suppress intestinal mucosal Th1 and Th2 responses and regulate the intense mucosal inflammation, by inducing the activity of the Foxp3$^+$Treg cells (Nedim Ince *et al.* 2009; McSorley and Maizels 2012; McSorley *et al.* 2013). The balance between effector Th2 and Treg responses may be lost when the helminth-induced Treg cells suppress Th2 and Th1 effector responses, which may result in immunopathology (Belkaid and Rouse 2005; Everts *et al.* 2010; Girgis *et al.* 2013).

In addition to the modulating mechanisms to the host responses that helminths employ to survive within the host, they may also evade the host immune system using other complex mechanisms. They may employ strategies such as (i) antigenic variation to escape immune surveillance; (ii) acquiring surface molecules from the host such as blood group antigens to avoid immune recognition; (iii) sequestering antigens away from the processing pathway by the major histocompatibility complex (MHC) class II molecules; (iv) secreting proteases that cleave chemotactic factors secreted by eosinophils, produce superoxide dismutase and glutathione S-transferase that neutralise toxic oxide radicals; down-regulating T and B cell responses by inducing Treg cells or anti-inflammatory cytokines IL-10 and TGF-β (Constant and Bottomly 1997; van Riet *et al.* 2007; Moreau and Chauvin 2010).

Helminths may exhaust the host immune system through its chronic activation by helminth antigens, resulting in dysregulation of host T cell responses (Borkow and Bentwich 2004). This chronic activation may result in changes in T cell responses such as (i) decreased CD4$^+$ and increased CD8$^+$ cells; (ii) marked increase of CD4$^+$ and CD8$^+$ HLA-DR$^+$ (activated) cells; (iii) decreased CD4$^+$CD45RA$^+$ (naïve) cells (Borkow and Bentwich 2004); (iv) impaired expression of CD26 on T cells, a costimulatory molecule that influences T cell activity, and this results in compromised recruitment of T cells to infection sites (Rai *et al.* 2012) and (v) increased expression of lymphocyte apoptosis markers (Kalinkovich *et al.* 1998; Boasso *et al.* 2009). Notably, this persistent activation of immune cells by helminths may be substantially similar to those documented for HIV infection (Kassu *et al.* 2003).

In addition, in suppressing these effector cell responses, Th2 cytokines stimulated by helminths may also induce the development of immunoregulatory alternatively activated macrophages (AAMs) which downregulate Th1 cells, decrease the secretion of IL-12, control inflammation and inhibit IL-17 (Anthony *et al.* 2007; Cooke 2008; Wang *et al.* 2008). AAMs also downregulate excessive Th2 activity (Moreau and Chauvin 2010). In support of the notion that

helminths are down-regulatory to host responses, Turner *et al.* (2003) observed a link between increased intensity of *Ascaris lumbricoides* infection and polyclonal anergy of leukocytes in peripheral blood, activated by Treg cells in response to the helminths. Others observed increased IL-10 frequencies in both the $CD4^+$ and $CD8^+$ cells, which demonstrated Treg upregulation and a strong Th2 response in whole blood cultures from tuberculoid leprosy patients co-infected with intestinal helminths (Diniz *et al.* 2010). Thus, as has been highlighted, helminth antigens may induce the down-regulation of both Th1 and Th2 responses (Maizels and Yazdanbakhsh 2003).

1.2.2.3 The impact of intestinal helminth infections on nutritional status

Intestinal helminth infection may impair the nutritional status in infected individuals (Everts *et al.* 2010; Gedle *et al.* 2015). Malnutrition may result in impaired innate and adaptive responses against intestinal helminth infection (Franca *et al.* 2009; Elfstrand and Florén 2010; Mwambete and Justin-Temu 2011), resulting in lowered resistance to infection (Bhaskaram 2001; Faber and Wenhold 2007). In both children and adults, intestinal helminth infections have been associated with deficiency of most of the micronutrients such as vitamin A (Strunz *et al.* 2014; Arinola *et al.* 2015b). Vitamin A deficiency results in the down-regulation of Th2 cytokines, which may have an effect on the gut-associated lymphoid tissue, resulting in reduced expulsion of the intestinal helminths and prolonged survival within the host (Fekete and Kellems 2007; Ross 2012). An increase in vitamin A levels was observed in Kenyan school-children infected with *S. mansoni* upon concurrent deworming resulting in the reduction of the egg output (Mwaniki *et al.* 2002). This indicates the benefit of eliminating intestinal helminths that may influence nutritional status, which in turn may affect the competency of the immune system of the affected hosts. Furthermore, zinc deficiency is associated with impaired expulsion of helminths from the intestines (Kheirvari and Alizadeh 2012). *Ascaris lumbricoides* helminths obstruct the intestines, thus impeding the absorption of vitamin A and other nutrients (de Gier *et al.* 2014; World Health Organization 2017b).

The intestinal helminths may cause iron deficiency anaemia (Katona and Katona-Apte 2008) through various mechanisms such as (i) attachment of hookworm to the intestinal mucosa and sucking of the host blood, also resulting in loss of protein (Farham 2006; Osawuza *et al.* 2011); (ii) damage of intestinal blood vessels by the spined eggs of *S. mansoni* and (iii) urogenital tract irritation and inflammation by *S. haematobium*, leading to blood loss in stool and urine respectively (Friedman *et al.* 2005; Koukounari *et al.* 2008). Furthermore, intestinal helminth and schistosome infections can affect children by retarding their growth and development

(Adenowo *et al.* 2015) and impair their cognitive domains in learning, memory and verbal fluency (Colley *et al.* 2014). Some intestinal helminth infections can cause mild and transient pathology, while others result in significant clinical disease and mortality (Samie *et al.* 2009). It is thus put forward that potent responses against intestinal helminth infections are required for their elimination or else these helminths must be eradicated with efficient deworming therapy. This will be to prevent the development of sequelae of pathologies, as a result of trapped parasite eggs in the host's tissues that may lead to tissue damage and subsequent failure of various organs (King and Dangerfield-Cha 2008; Barsoum *et al.* 2013; Colley *et al.* 2014), and urogenital obstruction in women (Kjetland *et al.* 2012).

1.3 The interaction between nutrition and co-infection with HIV and intestinal helminths

The significance of adequate nutrition on the potency of the immune system has been well established (Karacabey and Ozdemir 2012). Single infections with HIV or intestinal helminths are associated with micro- and macronutrient deficiencies (Papathakis *et al.* 2007; Garba and Mbofung 2010; Arinola *et al.* 2015b). Furthermore, research suggests that co-infection with HIV and intestinal helminths has an additive effect, where individuals co-infected may have lower biochemical levels of micronutrients (Moreau and Chauvin 2010) as well as carbohydrate and protein macronutrients compared with uninfected controls (Katona and Katona-Apte 2008; Koethe and Heimburger 2010). Deficiencies of protein, energy and micronutrients impact on competent cell mediated and humoral responses, and the link to increased susceptibility to HIV and intestinal helminth co-infections in such cases has been demonstrated (Chandra 1997; Schaible and Kaufmann 2007). As a result, micronutrient and macronutrient deficiencies may predispose individuals to HIV and intestinal helminth infections as well as lead to exacerbated HIV progression. Therefore, both infections may predispose the affected individuals to malnutrition and immune deficiency. Malnutrition may therefore start a cyclical process that may further predispose to infection, which in turn may result in malnutrition (Schaible and Kaufmann 2007; Katona and Katona-Apte 2008); previously illustrated in Figure 1, where the cause-and-effect thread between malnutrition, infection and immune deficiency is indiscernible. Infections may further worsen malnutrition through various mechanisms, which include decreased appetite induced by cytokines such as tumour necrosis factor alpha (TNFα) (Broadhurst and Wilson 2001) or decreased nutrient intake when swallowing is painful as occurs in some viral and fungal infections; while some intestinal viruses, bacteria and parasites cause diarrhoea, disrupt the intestinal mucosa lining and/or impede the absorption of nutrients (Amare *et al.* 2015; World Health Organization 2017b). This scenario increases the pressure on

the immune system's ability to efficiently eliminate the infectious agent. Additionally, both the HIV and intestinal helminth single infections are associated with anaemia. Thus the co-infection may have additive effect on the development of anaemia. This supports the notion that adequate intake of micro- and macronutrients is essential for the host to be able to mount efficient immune responses.

1.4 The importance of reliable assessment of nutritional status in adults

With all the complex interactions between infection, a compromised immune system and nutritional deficiency, reliable and objective indicators of malnutrition are necessary (Steyn *et al.* 2006), which are not influenced by disease processes and are able to depict changes in nutrient intake (Hedrick *et al.* 2012). Weight or anthropometric measurements and mid-upper arm circumference are commonly used for the evaluation of nutritional status, especially in children (Hall *et al.* 2008) and in emaciated hospitalised adults (Bharadwaj *et al.* 2016). However, measurement of weight and other anthropometric indices may be subjective, fraught with measurement errors (Himes 2009) and difficult to reproduce (Bhurosy and Jeewon 2013). Measurement of mid-upper arm circumference, weight, height for age and BMI for assessing nutritional risks such as overweight and thinness in children are reliable, sensitive, and specific and they correctly classify BMI into the appropriate categories (Knox *et al.* 2003; Crespi *et al.* 2012). In children, protein and carbohydrate malnutrition as well as an acute attack of diarrhoea easily manifest as weight loss. Anthropometric measurements, including mid-upper arm circumference detect fairly easily the wasting due to acute starvation and severe illness in children compared to adults (Corsi *et al.* 2011).

In adults, the assessment of malnutrition is complicated by the relative stability of weight even during malnutrition states, due to the fact that adults tolerate nutrient deficiencies better compared to children (Navarro-Colorado 2003). Obesity in adults masks micro- and macronutrient deficiency, making it difficult to measure malnutrition reliably using anthropometry in the group, except in severely emaciated adults. A German study (Damms-Machado *et al.* 2012) observed an association between inadequate dietary micronutrient supply (shown by low intake and low serum and intracellular levels of micronutrients) and obesity in adult participants. Also in other studies, obesity, viewed as a nutrition related disease on its own, has been associated with micronutrient malnutrition (Fekete and Kellems 2007; Garcia *et al.* 2009; Ngaruiya *et al.* 2017). Obesity impairs immunity, therefore further increasing the risk of infection (Andersen *et al.* 2016). In HIV infected South African individuals on ART, low abdominal adiposity was associated with better immune reconstitution, shown by an increase in

the CD4 counts in response to ART-mediated viral suppression compared to baseline pre-ART levels (Azzoni *et al.* 2011). This highlights the necessity of detecting malnutrition towards improving an immune system, especially in the presence of obesity. Fat accumulation has been associated with low grade inflammation due to persistent immune activation, which in turn may lead to lack of immune reconstitution (Mavigner *et al.* 2009; d'Ettorre *et al.* 2014; Fronczyk *et al.* 2014).

South Africa has an increasing prevalence of malnutrition with a predominant pattern of overweight and obesity among adults, shown as high body mass index (BMI) levels (Puoane *et al.* 2002; Cois and Day 2015). BMI is defined as the weight in kilograms (kg) divided by the square of the height in metres (m^2). Established cut-off points of between 25 and 29.9 kg/m^2 for overweight and greater or equal to 30 kg/m^2 for obesity for both males and females are used in the classification of BMI categories (World Health Organization 1995). The underweight category is when BMI is less than 18.5 kg/m^2 and normal weight category is between 18.5 and 24.9 kg/m^2. It is often difficult to obtain accurate measurement of current and previous weight in adults to allow for calculation of rate of weight loss or gain and BMI (Shenkin 2006; Simmons *et al.* 2009). Thus, weight gain in adults may be misleading and inaccurate when monitoring the effectiveness of nutritional replenishment (Garcia *et al.* 2009).

In addition to anthropometry, in clinical settings, serum biochemical markers such as total protein and albumin are commonly used to assess the nutritional status of patients, albeit albumin indicated as insensitive to acute changes in nutritional status (Banh 2006; Sathishbabu and Suresh 2012). This is due to albumin having a large body pool and a long half-life of twenty days (Andersen *et al.* 2014). Furthermore, albumin is decreased during inflammatory responses to infection, wherein protein synthesis is instead prioritized for the production of acute-phase proteins such as C-reactive protein (CRP), ferritin and complement at the expense of the former (Ahmed and Haboubi 2010). The inherent oedema during inflammation results in the redistribution of albumin from plasma into the interstitial compartment (Nicholson *et al.* 2000; Don and Kaysen 2004) thus resulting in artificial hypoalbuminaemia, and not necessarily protein malnutrition *per se*. Under these circumstances, the lower albumin levels may be interpreted as indicative of malnutrition in the event of albumin assayed on its own. A reliable indicator that would be able to differentiate between inflammation-induced hypoalbuminaemia and true malnutrition is thus essential.

HIV and intestinal helminth infections are documented to induce chronic inflammation through the persistent activation of the immune system (Deeks *et al.* 2013; Chachage *et al.* 2014; d'Ettorre *et al.* 2014). Furthermore, low grade chronic inflammatory response is also observed in obesity (Rodriguez-Hernandez *et al.* 2013; Castro *et al.* 2017), similar to the classical responses seen in the event of pathogenic infections (Lumeng and Saltiel 2011). Evidence shows that the obesity-induced chronic low-grade inflammation may elevate CRP levels, when the adipose tissues release IL-6 and TNF-α, resulting in the synthesis of CRP by the liver (Aronson *et al.* 2004; Eder *et al.* 2009; Fronczyk *et al.* 2014). Other studies have found an association between increased BMI and increased CRP levels, that was independent of inflammation and other factors that are known to increase CRP (Visser *et al.* 1999; Aronson *et al.* 2004). It is thus crucial that malnutrition is particularly detected with considerable caution in situations where an obese adult individual has nutritional deficiencies masked by obesity, wherein weight, BMI and anthropometric indices would not be reliable measures of nutritional status (Laky *et al.* 2008; Garcia *et al.* 2009). Hence, a reliable indicator or algorithm that would be able to detect malnutrition in inflammatory conditions is essential.

1.5 The use of pre-albumin as a reliable indicator of nutritional status in adults co-infected with HIV and intestinal helminths

Notwithstanding the fact that albumin and pre-albumin are affected by inflammation and infection (Bharadwaj *et al.* 2016), pre-albumin has nevertheless been suggested to be a more useful biochemical marker for monitoring nutritional status, due to its short half-life of two days and its sensitivity to changes in protein-energy status within four to eight days (Gaudiani *et al.* 2014). Pre-albumin reflects more recent protein intake as opposed to albumin, which reflects long term protein supply (Mosby *et al.* 2009; Blass *et al.* 2013). Moreover, pre-albumin has the highest ratios of essential to nonessential amino acids, it may then be a useful marker of protein energy nutritional status than albumin (Quadros *et al.* 2018). Therefore, an increase in pre-albumin in response to nutrient intake might be more specific to an improved nutritional status (Saka *et al.* 2011).

Low levels of both pre-albumin and albumin in the absence of inflammation, (depicted by normal CRP levels) are indicative of poor protein nutritional status (Bishop *et al.* 2005; Stearman and Tetlow 2008; Chen *et al.* 2014), as CRP is raised by pro-inflammatory cytokines associated with an acute phase response (Johnson *et al.* 2007). Significantly lower pre-albumin levels were found in non-metastatic cancer patients with evidence of malnutrition that developed after radiotherapy and not in those who had no malnutrition (Unal *et al.* 2013).

However, albumin levels were not significantly different between the two groups, implying that albumin could not distinguish between the presence and absence of malnutrition. Another study found that low pre-albumin levels were able to predict risk of mortality due to protein-energy wasting in patients on haemodialysis who otherwise had normal albumin levels (Rambod *et al.* 2008). Therefore, malnutrition should be assessed accurately, especially in the presence of infection with HIV and intestinal helminths, inflammation and obesity, which are factors that may confound the generation of reliable measurement of nutritional status. In light of the fact that pre-albumin is documented as a reliable predictor of malnutrition compared to albumin, the investigation of whether pre-albumin could be used as a reliable marker that would accurately determine nutritional status in HIV-intestinal helminth single and co-infections is crucial.

1.5.1 The use of food recall in the investigation of nutritional status in adults

The challenges of assessing malnutrition in adults have been cited, such as the unreliability of weight and anthropometric measurements in the presence of overweight and obesity. Although reliable and valid, biochemical markers of serum protein levels such as total protein and albumin are however not able to measure nutrient intake (Jacques *et al.* 1993; Shim *et al.* 2014). It is suggested that nutrient and energy intake be determined, especially when the risk or impact of disease is to be established (Yunsheng *et al.* 2009; Castell *et al.* 2015). The analysis of food intake data is the only method of evaluating energy intake (Cupisti *et al.* 2010). Biochemical micro- and macronutrient levels may not always correlate with food consumption (Moghames *et al.* 2016). Some food types may require absorption enhancers for adequate bioavailability, whilst others may have complex chemical compounds or consist of different forms (such as folic acid that has different glutamate residues) and this may impact on the accurate biochemical assessment of a nutrient (National Academy of Sciences 1986). This may then make it difficult to reliably correlate the data of the consumed food and nutrients with biochemical levels of micro- and macronutrients, where the biochemical markers may reflect on the nutrients that have already been metabolised.

Food recall is a retrospective quantitative technique of recording the food and beverages consumed in the previous 24 hours, by time periods starting from waking up time (Castell *et al.* 2015). The 24-hour food recall questionnaire is used to collect data on the types of food consumed, energy and micro- and macronutrient intake, their quantities and preparation, usually taken for a period spanning for two or more days from the same individual (Shim *et al.* 2014; Castell *et al.* 2015). Data collected for three days or more may be able to capture the daily variations of consumed food (Yunsheng *et al.* 2009; Streppel *et al.* 2013). The data is more

reliable when it is collected from the person who most likely prepared and/or cooked the food (Rose and Tschirley 2003). However, the reliance on memory and correct estimation of quantity is an inevitable limitation attached to the self-reported food recall data and the skill of a well-trained interviewer may minimise recall bias (Shim *et al.* 2014). Prompts are used to indicate the portion sizes and then the data is coded for analysis and reporting, as nutrient adequacy ratios (NARS). NARS is the ratio of a nutrient intake divided by the recommended daily requirement for that nutrient (Steyn *et al.* 2006).

1.6 The immune responses to HIV and intestinal helminth co-infection

Potent responses are essential in any infection. As has been elucidated, single infections with either HIV or intestinal helminths evoke dysregulated responses that result in the activation of effector cells, which are not completely protective to the host (Fevrier *et al.* 2011; Vijayan *et al.* 2017; Weisman *et al.* 2017). Moreover, these activated effector cells are further used by HIV as reservoirs and also as targets for entry and replication. In the activated $CD4^+$ cells, HIV subverts the host cell transcription factors, such as NFAT and NF-κB, using them for further replication within these cells, which increases the viral load (Jiang *et al.* 2017). Thus, the challenge to the host immune system is that the HIV-intestinal helminth co-infection may have an additive negative impact on the responses. The co-infection may inadvertently result in worsening of both diseases.

In addition, HIV infection may induce immune dysregulation of both innate and adaptive responses, further inhibiting control of viral replication. The activation of NK cells and the production of IFN-α or β by dendritic cells (the main antiviral responses from the innate arm of the immune system) may be dysfunctional, as a result of HIV-mediated signalling through CCR5, CXCR4 and the general immune activation (Boasso *et al.* 2009). Furthermore, attrition of $CD4^+$ and $CD8^+$ cells and failure to replenish them with naïve cells are some of the underlying HIV-induced mechanisms that cause dysregulated adaptive responses, which may result in further uncontrolled HIV replication (Borkow and Bentwich 2004). To make it worse, helminths may increase the expression of CCR5 and CXCR4 co-receptors in $CD4^+$ cells, which facilitates HIV entry more efficiently into these activated target cells, further promoting viral replication (Jaspan *et al.* 2011; Chachage *et al.* 2014; Woodham *et al.* 2016).

Helminths also induce Treg cells to secrete IL-10 and TGF-β cytokines so as to impede the production of aberrant adaptive host responses, to limit inflammation and defy immune exclusion, for their survival within the host (Weinstock and Elliott 2014). This helminth-

induced modulatory mechanism attenuates host responses, resulting in hyporesponsiveness which may consequently inhibit HIV control, since the required anti-HIV responses would be anergic (van Soelen *et al.* 2012; Salgame *et al.* 2013; Johnston *et al.* 2017). Potent Th1 responses are essential to control HIV and other intracellular pathogens, including *Mycobacterium tuberculosis* (Borkow and Bentwich 2004; Borkow *et al.* 2007; Diniz *et al.* 2010; George *et al.* 2014). Thus, this exploitation of the immunoregulatory power of the immune system by helminths may unfortunately, to the detriment of the host, also suppress the mounting of potent responses directed against third party antigens and vaccinations (Tristao-Sa *et al.* 2002; van Riet *et al.* 2007; Wang *et al.* 2008; Girgis *et al.* 2013).

It is therefore considered crucial that the helminth-induced activation of the immune system is reduced, through deworming interventions especially in adults in communities where such programmes are only provided to school-children. In addition, to ameliorate the impact of anti-helminthic interventions, other microbes should be cleared, so as to conserve the immune system required to control the incurable HIV infection and slow down its rapid progression. It is also recommended that algorithms which would ensure that intestinal helminth infection is detected accurately and timeously be investigated and instituted. This would aid in the effective elimination of these pathogens which may otherwise result in impaired responses against the co-infecting HIV and furthermore, deplete the essential micro- and macronutrients that may have reinforced the immune system against both the pathogens. It is therefore proposed that in HIV-intestinal helminth co-infection, the dysregulated responses may exacerbate the rapid progression of HIV infection to AIDS.

In the HIV-intestinal helminth co-infection, it is suggested that opposing Th1 and Th2 immune responses would be elicited. HIV stimulates predominance of Th1 response, whilst helminths stimulate a stronger Th2 response, which may significantly suppress a Th1 response directed against the HIV infection (Kaur *et al.* 2016; Maizels and McSorley 2016). Cytokines produced by Th1 cells such as IFN-γ down-regulate Th2 responses and those produced by Th2 cells such as IL-4 alone or with IL-10, block the expansion of Th1 cells and down-modulate cell mediated responses (Mitchell *et al.* 2017). High prevalence of intestinal helminth infections is suggested to promote increased transmission of HIV, and may result in accelerated progression of the HIV infection to AIDS due to impaired immune responses (Noblick *et al.* 2011).

1.7 The diagnosis of intestinal helminth parasite infections

The impact that intestinal helminth infection may have on the nutritional and immune status of affected individuals, especially those co-infected with HIV, makes it crucial that the diagnosis of intestinal helminth infections is accurate, more so in the situation where the infection is asymptomatic due to the modulatory effects of the parasite within the host. The host may not even be aware of the infection. Parasite infections are mostly expected and thus investigated in children, especially those that attend primary school. Generally, the sighting of worms in stools is what prompts adults to approach health care facilities for investigation. It is believed that early diagnosis and therefore early treatment of intestinal helminth infections would benefit the host that is co-infected with HIV, by limiting the additive effect of both infections, which may exacerbate the HIV disease progression.

1.7.1 Microscopic screening and diagnosis of intestinal helminth infections

Routine investigation and screening for intestinal helminth infection is commonly based on direct microscopic examination of a wet preparation of a single stool sample for parasite eggs and ova. The detection of the expelled parasite eggs or ova and/or parasite worms in stool samples is used as clinical and/or laboratory diagnostic indicators of parasite infection. Although this microscopic method is quick, cheap and convenient, accurate diagnosis is limited by several challenges: (i) the inconsistent egg production which may be caused by day to day variation in egg excretion (Hall *et al.* 2008); (ii) if individuals are infected by parasites in larval stages, by male worms only or non-fecund worms (Adams *et al.* 2006; Vlaminck *et al.* 2016); (iii) the method involves processing a wet preparation smear using saline with a small amount of the stool, usually picked off from the middle of the sample container. The stool may not have been mixed adequately in instances of light-intensity infections, or it could be formed and not loose, making it difficult to obtain a representative sample and (iv) microscopy may be subjective and the experience of microscopists may influence the ability to recognise eggs/ ova/worms versus artefacts. The above result in low sensitivity, thereby causing underestimation of true parasite infection and load (Hall *et al.* 2008).

Polymerase chain reaction (PCR) is the most sensitive technique that may be used for detecting parasite infection, however this molecular diagnostic test is costly especially for mass screening purposes and it requires resourced laboratories (Tavares *et al.* 2011; Ricciardi and Ndao 2015). Therefore, in the absence of a molecular test or technique that may be used as a gold standard in resource limited settings, attempts to increase the chances of egg detection using light microscopy include the suggestion of multiple examination of a single stool (Tarafder *et al.*

2010). This would however not address the day to day variation in egg excretion. In view of this challenge, others suggest the examination of up to three or more stool samples collected in three or more consecutive days, to mitigate the day to day variation in egg excretion challenge (Utzinger *et al.* 2001; Tarafder *et al.* 2010). These detailed investigations are crucial, however they can be labour intensive and time consuming. To increase the sensitivity, stool concentration methods such as zinc flotation and the original formol ether which was developed by Ritchie (1948) and its modifications such as the Mini Parasep® methods (Couturier *et al.* 2015) may be used. Also, the Kato Katz thick smear method may be used, which utilises a defined amount of stool sample, and has the ability to quantitate the number of eggs per gram of stool, thereby indicating the intensity of infection (Leuenberger *et al.* 2016).

In addition to the challenge of reliable diagnosis of intestinal helminth infections by the conventional microscopy method, the excretion of parasite eggs by the host may be influenced by genetic, immunity and environmental factors, especially in adults (Bethony *et al.* 2002; Adams *et al.* 2006). In light of this, the serological diagnosis of intestinal helminth infections using parasite-specific IgE and IgG4 may be useful in providing reliable data compared to only using the microscopic detection method.

1.7.2 Serological diagnosis of intestinal helminth infections

Other factors that may affect accurate parasite eggs detection besides genetic and environmental factors, include the fact that the excretion of parasite eggs is also dependent on immune responses against the parasite infection (Adams *et al.* 2006). A weakened immune response measured as low CD4 counts, observed in HIV-intestinal helminth infected, may reduce egg excretion and thus result in low or no eggs detected in stool samples (Karanja *et al.* 1997; Secor *et al.* 2003; Mkhize-Kwitshana *et al.* 2011). Adult female worms can release up to 300 eggs per day within the host's intestinal venules, some of which are not excreted (Secor *et al.* 1996). The host's venous blood that flows in the opposite direction of the excretory pathway may also prevent the eggs excretion to the environment (Colley and Secor 2014). Many of the eggs that are not excreted are permanently lodged in the intestines, liver, bladder and other organs, inducing granulomas that result in chronic inflammation and morbidity (Colley *et al.* 2014). Therefore, to avoid perpetuation of transmission of intestinal helminths and re-infection especially in asymptomatic individuals, and subsequent morbidity and mortality, efficient diagnosis of intestinal helminth infections is critical even in situations of no egg detection in stool samples.

In the quest of ensuring that diagnosis of intestinal helminth infections is accurate, it is recommended that supplementary diagnostic methods such as the determination of parasite-specific IgE serological levels are used (Adams *et al.* 2006; Mkhize-Kwitshana *et al.* 2011). For example, Adams *et al.* (2006) found higher proportions of adult individuals infected with intestinal helminths in Cape Town when parasite-specific IgE levels were used (51%) in addition to the microscopic detection of parasite eggs in stool samples compared to the diagnosis by microscopy only (26%). Furthermore, a study by Mkhize-Kwitshana *et al.* (2011) noted that some participants excreted helminth parasite eggs without increased *Ascaris*-specific IgE levels, and others had high *Ascaris*-specific IgE levels with no parasite eggs detected in their stool samples or both. Prevalence was 20–40% by egg detection only and 66% was detected by both methods. This way, intestinal helminth infection was not missed as both microscopic and parasite-specific serological methods supplemented each other. In contrast, a study conducted on children from Zimbabwe revealed a match between egg detection and serology positivity, however some egg-negative participants had positive serology results for anti-schistosome egg IgM antibodies (Imai *et al.* 2011). Absence of eggs in a stool sample should therefore not be interpreted as that there was no infection or no exposure to parasites (Vlaminck *et al.* 2016). Together these studies suggest that the use of stool samples as the only method of diagnosis of parasite infection may not give accurate results (Adams *et al.* 2006; Mkhize-Kwitshana *et al.* 2011). Notably, in the absence of stool samples, van Soelen *et al.* (2012) used high *Ascaris*-specific IgE levels as a proxy for intestinal helminth infection and cited that it is widely used as a standard indicator of *Ascaris lumbricoides* and/or *Trichuris trichiura* re-exposure or active infections. However, the limitation of this diagnostic modality is the fact that it may not distinguish between current and past infection (McKeand 1998; Smith *et al.* 2008). Another limitation in serodiagnosis is possible cross-reactivity between antigens of different intestinal helminth species which may reduce the sensitivity of the test (Khurana and Sethi 2017). In addition, the required use of an enzyme-linked immunosorbent assay (ELISA) technique associated with this modality as well as the need for the collection of blood samples may not be feasible in poor resource settings. Nevertheless, serological techniques have higher sensitivity compared to other conventional tests such as microscopy which is commonly used in resource-limited settings (Lamberton and Jourdan 2015).

1.7.3 Variations in responses to intestinal helminth infection: different response phenotypes

Th2 responses are elicited in helminth infection even before the parasite eggs are released, with the induction of Treg cells (de Oliveira Fraga *et al.* 2010; Webb *et al.* 2012). IL-4 and IL-13

then promote the production of IgE, thereafter the class switching to IgG4 occurs induced by IL-10 and TGF-β (McSorley and Maizels 2012; Filbey *et al.* 2014). Th2 responses are generally effective to eliminate and expel the intestinal helminths (Allen and Sutherland 2014), unless they become dysregulated or downregulated and immunopathology such as tissue damage may develop (Maizels *et al.* 2009; McSorley and Maizels 2012). Differences in immune responses to helminths within the Th2 compartment have been described. The Th2 responses elicited are different due to the fact that different species of helminths reportedly induce hugely different mechanisms of protection (Harris and Gause 2011). There are those mechanisms that lead to the expulsion of the helminths or those that result in the control of the helminth-induced inflammation, referred to as the 'regulated' helminth infection (Anthony *et al.* 2007).

The types of responses that are stimulated against the helminths include: (i) A balanced Th1/Th2 response phenotype, which occurs in individuals who generate a less skewed distribution of IgE and IgG4 and are thus resistant to helminth infection (Maizels and Yazdanbakhsh 2003). (ii) A modified Th2 cell phenotype, which is associated with individuals who generate predominantly Th2 responses with low levels of Th1 cells, expressing low levels of IgE, high levels of IgG4 and high levels of IL-10, showing susceptibility to parasitic infection (Maizels and Yazdanbakhsh 2003; Maizels *et al.* 2004). High parasite antigen loads are associated with this modified Th2 phenotype, wherein the infection is asymptomatic with long-lived metabolically active adult worms, found in peripheral tissues and organs (Borkow and Bentwich 2004). High levels of IgG4 in the modified phenotype are attributed to the IL-10-induced hyporesponsiveness to the high helminth load, the so called 'regulated' helminth infection (Maizels *et al.* 2004). (iii) High IgE and IgG4 antibody responses occur as a result of strong IL-4 and IL-5 cytokines, produced in response to the processing and presenting of the helminth antigens to B cells (Milner *et al.* 2010). When individuals who had mounted both IgE and IgG4 responses to a recombinant worm antigen had increased intensity of helminth re-infection, it was postulated that IgG4 was produced to attenuate the effect of IgE (Jiz *et al.* 2009). Others had found in participants who had active filarial worm infection and had reduced the IgG4 levels after supervised treatment with antihelminthic treatment, thereafter producing high IgG4 levels post-treatment, which were associated with re-infection (Terhell *et al.* 2003). It may be presumed that high IgG4 levels in intestinal helminths, including *Ascaris lumbricoides* and *Trichuris trichiura* indicate active infection.

The role of IgG4 in helminth infection is to regulate and dampen the anaphylactic reactions induced by IgE (Jiz *et al.* 2009; Guma and Firestein 2012; Rujeni *et al.* 2012; Sthoeger *et al.*

2012). It is therefore not surprising that IL-10 has been reported to be involved in the upregulation of IgG4 in helminth infection (van Riet *et al.* 2006; Filbey *et al.* 2014). In support of this view, Geiger *et al.* (2002) found high *Ascaris*- and *Trichuris*-specific IgE and IgG4 levels in infected individuals, which correlated with egg output, whereas uninfected individuals had low *Ascaris*- and *Trichuris*-specific IgG4 levels regardless of whether they were from endemic or non-endemic areas. Others had found that IgG4 levels reduced rapidly after treatment (McSorley and Maizels 2012). Furthermore, the authors found significant *Ascaris*- and *Trichuris*-specific IgE levels in controls from endemic areas, which suggested an association of IgE with past or continued exposure to helminths in the uninfected controls. In other words, it may be extrapolated from this data that high parasite-specific IgE levels may indicate both past and active infections, whilst high IgG4 levels indicate active infection. Also, Bhattacharyya *et al.* (2001) found 100% matching results between detection of eggs and highly elevated *Ascaris*-specific IgG4 levels, although independent of worm load, which was attributed to chronic exposure to parasites. This data therefore supports the clinical usefulness of both high parasite-specific IgE and IgG4 levels in the diagnosis of intestinal helminths, especially in situations of low infection rates as may be seen in adults. This may contribute to more reliable diagnosis of intestinal helminthiasis as opposed to using microscopic detection only, and may contribute towards early and effective elimination of intestinal helminths.

1.8 The benefit of antihelminthic treatment on HIV-intestinal helminth co-infection responses

Deworming may have an impact on the HIV epidemic by facilitating reinstatement of potent cell mediated (Th1) immune responses (Mulu *et al.* 2015). Others found evidence of ART immunological failure (shown as poor CD4 counts increases) in HIV-intestinal helminth co-infected patients (Efraim *et al.* 2013). Hence, the recommendation that infections with intestinal helminths and *Schistosoma species* be investigated and eradicated if present in HIV infected individuals is supported, as it may be an additional tool in the fight against the HIV epidemic (Chenine *et al.* 2008; Mulu *et al.* 2013; Kleppa *et al.* 2014). Deworming may reverse the persistent activation of the immune system by helminths and the stimulation of polarised Th2 responses, which suppress Th1 responses essential in the control of the HIV infection and the associated opportunistic infections. This approach may slow the rapid rate of progression of HIV infection.

Based on reviews of evidence, treatment of helminths in HIV co-infected individuals may decrease viral load (Modjarrad and Vermund 2010; Walson *et al.* 2010). Others observed

increased CD4 counts in HIV-intestinal helminth co-infected participants treated for helminthiasis after deworming, before starting ART (Abossie and Petros 2015). On the other hand, an HIV/AIDS Cochrane systematic review noted that antihelminthic treatment may have a short-term suppressive effect on viral load and small increases in CD4 counts of HIV infected adults (Means *et al.* 2016). Notably, high proportions of Treg cells decreased upon effective anti-schistosome treatment in patients infected with *S mansoni*, and the authors postulated that the decrease was due to the elimination of the chronic exposure to the parasites (Watanabe *et al.* 2007). Neglecting to treat parasites in developing countries is thought to contribute to the failure of ART, thereby increasing viral load and enabling the transmission of HIV (Stillwaggon 2009). Contrary to these opinions, Hosseinipour *et al.* (2007) found that deworming did not decrease viral load in HIV-intestinal helminth co-infected individuals. Furthermore, Brown *et al.* (2006) reported a significant transient increase in HIV viral load a month after deworming in an HIV-*Schistosoma* co-infection. Also, a retrospective study (Lankowski *et al.* 2014) found no benefit of deworming on CD4 count recovery among HIV infected individuals on ART.

In support of deworming, Rajamanickam *et al.* (2017) suggested that it may decrease microbial translocation and inflammation. This may benefit the host as microbial translocation is implicated in immune activation of effector cells and systemic inflammation. Weisman *et al.* (2017) observed decreased immune activation and normalisation of the immune profile in helminth infected individuals after the eradication of intestinal helminths. It is noted that advocacy for regular deworming is directed at school-children, as per the World Health Assembly Resolution 2001 (Uneke 2010).

Albeit the opposing views on the merits of deworming in HIV-intestinal helminth co-infection, our recommendation is that antihelminthic treatment be included in the management of HIV and intestinal helminth singly and co-infected individuals. We further recommend that the deworming programmes be extended to adults, in particular those co-infected with HIV. Decreasing helminth-induced activation of immune cells may reduce the rapid progression of the HIV infection or decrease the intensity of the infection that may be mediated by modulatory mechanisms that helminths use to survive the host immune system.

1.9 Rationale for the study

Previously, high intestinal helminth infections in KZN have been reported among primary school-children (Jinabhai *et al.* 2001). Later evidence suggested that in adults these infections generally range between 40-60% (Adams *et al.* 2006; Kwitshana *et al.* 2008). In eThekwini

particularly, where the current study is undertaken, intestinal helminthiasis was reported to be 20.4 % among adults, albeit such studies are very few. On the other hand, KZN has the highest HIV prevalence in South Africa (Department of Health South Africa 2017) with peak infection occurring in adults (25-49 years). Evidently, a significant overlap of HIV and intestinal helminth infections is expected in adults residing in poor communities, due to the typical geographical distribution of these two infections. Superimposed upon these two is poverty and malnutrition. Malnutrition also manifests as obesity, and in SA the latter has been reported to be increasing exponentially (Cois and Day 2015), with evidence of high prevalence of obesity in KZN (Wand and Ramjee 2013).

In spite of this milieu of HIV and intestinal helminth infections, poverty and malnutrition, studies that have determined the interactions in HIV-intestinal helminth co-infection are lacking, especially in the KZN adult population. Thus, this current work was aimed at determining the interaction between single or dual HIV and intestinal helminth infections with nutritional and immune status among KZN adults. Findings of the current study were intended to highlight and draw attention to the possible deleterious effects of HIV and intestinal helminth co-infections on the immune and nutritional status of adult participants. This current study, with the objective of diagnosing intestinal helminth infection accurately, in order to discern the interaction with HIV and also to determine the differences in immune responses associated with the different intestinal helminth infection phenotypes, included the use of high levels of *Ascaris*-specific IgE and IgG4, supplementary to the egg detection in stool samples.

Also, in the backdrop of the co-infection, obesity and malnutrition, reliable detection of nutritional status was essential in this current study. Hence, another objective of this current study was to investigate the use of pre-albumin as a reliable nutritional marker that would accurately determine nutritional status in KZN adults singly or dually infected with HIV and intestinal helminths, with or without inflammatory conditions in different BMI categories. It was also considered necessary to investigate nutrient intake to supplement the nutritional status data measured biochemically and with anthropometry. The 24-hour food recall analysis was thus included in the assessment of nutritional status, so as to correlate the data of the consumed food, energy and nutrients with the biochemical levels of micro- and macro-nutrients, whereby the biochemical markers reflect on the already metabolised nutrients. It was expected that the food intake analysis would indicate the food consumption patterns and elucidate the presence or absence of micro- and/or macro-nutrient deficiency, in overweight and obesity in particular.

Such a study may inform policy on the practical approaches to the future management of individuals living with HIV, who are co-infected with intestinal helminth parasites and may present with different immune response phenotypes. Helminth infection is one of the diseases that is neglected by policy makers globally, giving more attention and priority to HIV/AIDS, TB and malaria which are considered more clinically significant, probably since most helminth infected individuals remain asymptomatic (Hotez *et al.* 2006; Kwitshana *et al.* 2008; Hotez and Kamath 2009; Uneke 2010).

1.9.1 Hypothesis

Adults who are co-infected with HIV and intestinal helminths have lower levels of micro- and macronutrients, higher HIV viral load, increased immune activation and they express increased Th2 and decreased Th1 cytokine responses compared to those singly infected and uninfected.

1.9.2 Aims and objectives

Aim 1

To investigate the interaction between the nutritional status and co-infection with HIV and intestinal helminths in adults.

Objective 1

To investigate the effect of HIV and intestinal helminth single or co-infection on the nutritional status, measured by BMI and biochemical micro- and macronutrient markers against a 24-hour food recall nutrient intake levels, among adults singly or co-infected with HIV and intestinal helminths.

Aim 2

To evaluate an appropriate biochemical method for the assessment of nutritional status in adults.

Objective 2

To investigate the use of pre-albumin as a marker of nutritional status compared to albumin in adults singly or co-infected with HIV and intestinal helminths, with or without inflammatory conditions as indicated by CRP in different BMI categories.

Aim 3

To describe the immune profile of adult individuals with or without HIV and intestinal helminth co-infection.

Objective 3

1. To determine the different intestinal helminth infection phenotypes by the presence of parasite eggs, ova and/ or parasite-specific IgE and IgG4 serological phenotypes and classify single or dual infections.
2. To determine the immune profile as determined by levels of gene expression of IFN-γ and TNF-α (used as surrogate markers for T helper 1 responses); IL-4 (surrogate marker for T helper 2 responses); IL-10 (surrogate marker of T regulatory responses); and CD38 (as marker of immune activation) of the different intestinal helminth infection phenotypes of individuals (i) singly infected with intestinal helminths, as defined by the presence of parasite eggs, ova and/or parasite-IgE and IgG4 serological phenotypes; (ii) co-infected with HIV and intestinal helminths; (iii) singly infected with HIV and (iv) uninfected controls.

1.10 The outline of the thesis

The format of this thesis is presented as a thesis by manuscripts as per the recommended guidelines of the institution. The chapters therefore include published and in-preparation journal articles.

Chapter 1: This chapter gives the background, the research problem and the rationale of the study, highlighting the global burden of the HIV and intestinal helminth single and co-infections. It also includes the reviewed literature, which provides the body of work on the impact of the HIV-intestinal helminth co-infection on the immune and nutritional status as well as the use of pre-albumin as a marker of malnutrition. The chapter also outlines the challenges associated with the diagnosis of intestinal helminth infection using conventional microscopy only, and the merits of including serological diagnosis as supplementary. Furthermore, it provides a list of the three manuscripts that address the study objectives, presented as a chapter per objective.

Chapter 2: This chapter describes the methodology including the study design and all the laboratory methods and statistical tests used to analyse the data. The methodology and statistical analyses described in detail in the manuscripts will be mentioned briefly in this chapter.

Chapter 3: Paper 1

Title: The interaction between HIV and intestinal helminth parasites co-infection with nutrition among adults in KwaZulu-Natal, South Africa.

Mkhize, B.T., Mabaso, M., Mamba, T., Napier, C. E. and Mkhize-Kwitshana, Z. L. 2017. Published in Biomed Research International, 2017: 1-12.

Chapter 4: Paper 2

Title: The investigation of the use of pre-albumin as a tool for nutritional assessment in adults co-infected with HIV and intestinal helminth parasites in KwaZulu-Natal, South Africa

Mkhize, B.T., Mabaso, M., Madurai, S. and Mkhize-Kwitshana, Z. L. 2018.

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Chapter 5: Paper 3

Title: The immune profile and parasite-specific IgE and IgG4 serological phenotypes of adults co-infected with HIV and intestinal parasites in KwaZulu-Natal, South Africa

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In manuscript, planned to be submitted for publication at a later stage.

Chapter 6: Synthesis, conclusion and recommendations. A general discussion on the findings of the study and the recommendations for future interventions and further investigations.

2.0 CHAPTER 2

Materials and methods

2.1 Introduction

This chapter describes the methodology used in data collection and analysis approaches for this current work. It also includes in brief the methods used to conduct the work highlighted in the attached research papers. The overall aim of the present work was to investigate the interaction between nutrition, immunity and co-infections with HIV and intestinal helminths in an adult population in KZN, South Africa. Furthermore, the work was to investigate the use of pre-albumin compared to albumin in the assessment of malnutrition in uninfected controls, the singly or dually infected individuals with HIV and intestinal helminths, with or without inflammatory conditions in different BMI categories.

2.2 Study setting and population

The study was conducted in a peri-urban informal area, in the eThekwini Health District, which is in the north coast of KZN, South Africa. The area was selected randomly from the eThekwini enumeration areas. It is situated approximately twenty kilometres north-west of eThekwini city centre. It is governed by local government and municipal authority. The latest available census data indicate that the area comprises approximately 39,000 households with approximately 30% informal settlements (Statistics South Africa 2011). Poverty is widespread in this area, with low income households and approximately 52% of the population in the area reported to be living below the poverty line (eThekwini Municipality 2017). The population's livelihood is sustained through formal employment, informal trading and approximately 25% of them either have no income or are dependent on government grants (Statistics South Africa 2011). There is a substantial fraction of the population that has poor access to facilities in the area with about 22% households not having piped water inside the house approximately 25% not having access to flush toilet facility (Statistics South Africa 2012; eThekwini Municipality 2017). The study site was selected randomly from a list of six clinics which provide pre- and post- HIV counselling and testing (HCT) (KwaZulu Natal Health Department: The GCIS Unit 2009). The study site is a primary health care clinic (PHC), providing all essential health care services, including HCT, servicing about 100 attendees per day. The reason for recruiting from an HCT was to make use of the pre- and post- HIV test counselling services. The study population consisted mostly of females (91.6%) due to the fact that the majority of the clinic attendees were female.

2.3 Study design

A cross-sectional investigation of the immune response profile and nutritional status of individuals singly and co-infected with HIV and intestinal helminths was conducted between July 2015 and May 2016.

2.4 Sample size calculation

A sample size of 229 adults was calculated to detect an effect size of 0.4% of differences in nutritional and immune status among the HIV and helminth singly and dually infected study and control groups with 80% power and probability of 95%. The study sample was to include 160 adults not infected with parasites and 69 infected with parasites, assuming that 30% of adults in KZN are infected with parasites, based on the 20.4% - 59% prevalence reported on KZN adults (Kwitshana *et al.* 2008). Fifty percent of the study sample were to be co-infected with HIV and 50% not be infected with HIV, assuming that 50% of KZN adults are HIV infected, based on the HIV prevalence of 46.2% among antenatal women in KZN (Department of Health South Africa 2013a) and HIV prevalence of 38% in the eThekwini district (Department of Health South Africa 2012a).

2.5 Recruitment and selection of the study participants

Several education sessions on parasite infections, their impact on nutritional status were held with the clinic attendees. They were also made aware that adults may be infected with intestinal helminth parasites as well, not just children. Furthermore, on recruitment, they were informed of the possible impact of parasites on the nutritional status. Further one-on-one individual information sessions were held with those who were interested, who then gave written informed consent for participation in the study (appendix 6). Two hundred and ninety one potential participants were purposively recruited to allow for the enrolment of 20% more participants than the calculated sample size, in order to be able to adjust the sample in the event of withdrawals (Hanga *et al.* 2014) or when participants would not submit both the required stool samples, collected on a Friday and a Monday. The study site was a primary health care clinic, providing all essential health care services, including HCT. The reason for recruiting from an HCT was to make use of the pre- and post-test counselling services. A total of 263 eligible participants were enrolled into the study.

Adults who were 18 years of age and above, and if female, they had to be not pregnant. Potential participants who were willing to be screened for intestinal parasites were included. They were required to not have taken any antihelminthic treatment six months prior to enrolling

into the study. They had to be willing to donate blood and stool samples. Furthermore, they had to be willing to be tested for HIV status, for the purpose of allocating them into either the study group or the control group. HIV infected individuals had to be ART naïve.

The South African research guidelines recommend the protection of vulnerable individuals such as very sick or severely immunocompromised persons. At the time of study recruitment the country guidelines had set a threshold for ART initiation at 350 cells/μl. Thus, the HIV infected individuals who had CD4 counts below 350 cells/μl were referred to the HCT clinic and for ethical reasons were excluded from participating in the study (Figure 5). Likewise, for classifying helminth infection status, participants were screened for intestinal parasites. Those who were found to be infected were referred to the clinic for antihelminthic treatment.

If a participant visited the study site only on one day they were excluded from participating, since two analyses of both stool samples and food recall data was essential, for accuracy purposes.

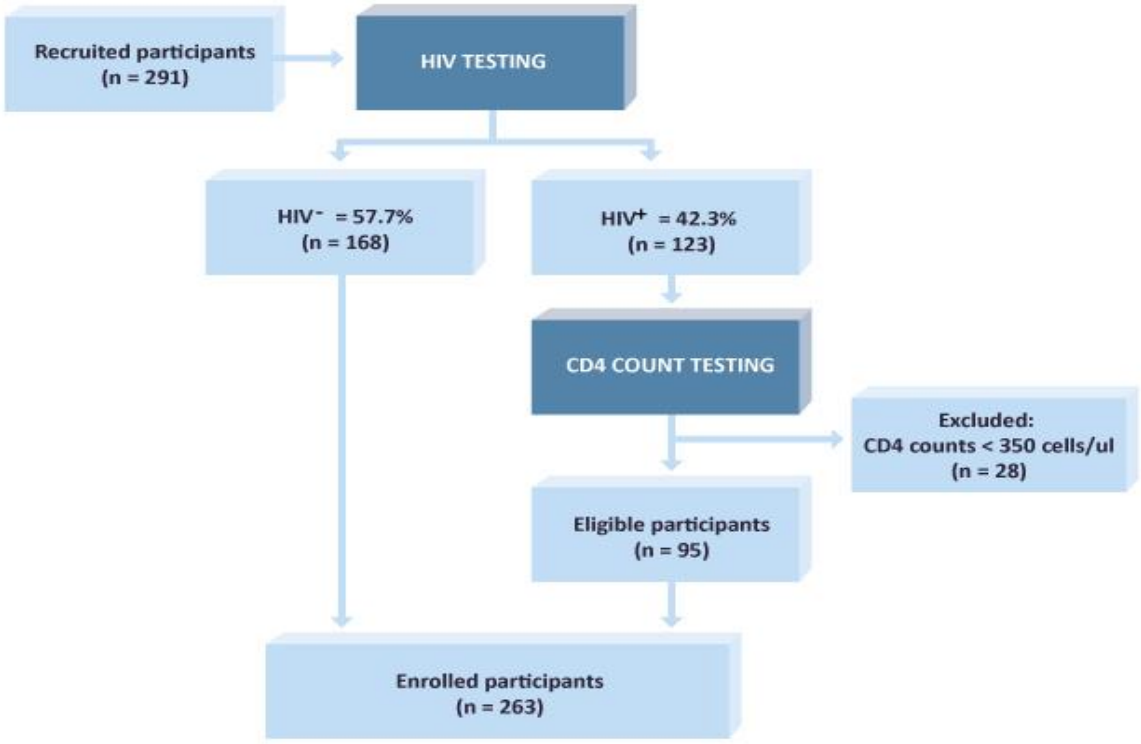

Figure 5: The recruitment and enrolment process of the study participants which shows that 263 participants were enrolled into the study after those with CD4 counts less than 350 cells/µl were excluded

2.6 Ethical considerations and gatekeeper permissions

2.6.1 Ethical approval

Ethical approval to conduct the PhD study was obtained from University of KwaZulu-Natal Biomedical Research Committee (BREC Ref: BE 230/14) (appendix 2). The current study was a sub-study of a main project (BREC Ref: BE193/11) (appendix 1.1), with some of the overlapping data that were collected for the main study used for the current study. The ethical approval for the main project had obtained recertification approval for the years 2013 - 2014 (Appendix 1.2) and 2014 - 2015 (Appendix 1.3). Permission was also granted by the KZN Provincial Department of Health (Ref NHRD: KZ_2015RP23_787) (appendix 3) and the Provincial and eThekwini Health District office (appendix 4) and as well as the local political authorities (appendix 5).

2.7 Data collection

Data on the socio-economic, nutritional status and the immune profile were collected from the 263 enrolled participants, who were stratified into four groups according to the infection status based on the HIV rapid (and enzyme-linked immunosorbent assay (ELISA) for indeterminate results), stool microscopy, IgE and IgG4 results. The groups were either singly infected, co-infected or uninfected with HIV and intestinal helminths. A sub-sample of 56 participants was selected for polymerase chain reaction (PCR) downstream work, according to the availability and quality of sample for analysis (Figure 6).

2.7.1 Socio-demographic data

A primary screening was done by a trained fieldworker, to exclude potential participants who had other infectious diseases. The fieldworker then administered a structured questionnaire to collect demographic, socio-economic and 24-hour food recall data (appendix 7) from enrolled participants. Anthropometric measurements (weight and height for the calculation of BMI) were measured by a fieldworker.

2.7.2 Laboratory analyses

2.7.2.1 Specimen collection

Blood and stool samples were collected from each participant, after enrolment in the study site. Blood samples were collected by a trained phlebotomist into the following blood tubes for the named tests/ assays:

- Ethylene-diamine-tetra-acetic acid (EDTA) tube for a full blood count, lymphocyte

phenotypes assay and the quantification of cytokine gene expression levels by polymerase chain reaction (PCR) analysis (5ml)

- EDTA tube for HIV viral load quantification (5ml)
- Serum separator tubes for confirmation of HIV status and for biochemical assays for the assessment of nutritional status (5ml)

Each participant also donated two stool samples (morning or first stool), collected on two different days (on a Friday and a Monday) in universal containers. They collected the samples at home and delivered each of the two samples at the collection room of the study site immediately after collection. The samples were then sent to the laboratory within four hours of collection in a cooler box to ensure good sample quality.

2.7.2.2 Testing for HIV and intestinal helminth infections

Determination of HIV status and screening for intestinal helminth parasites were undertaken in a biosafety level II laboratory in the Department of Medical Microbiology in University of KwaZulu-Natal (UKZN), in Durban.

Participants had been tested previously for HIV status in the HCT clinic which they were recruited from. However, they were tested again for confirmation. The HIV status was required for the purpose of allocating the participants into either a study group or a control group. Serum samples obtained after the centrifugation of blood samples in serum separator tubes were used to test for HIV status. The Alere Determine™ HIV-1/2 Ag/Ab Combo rapid test (Orgenics Ltd, Israel) was used, according to the manufacturer's instructions. When the Alere Determine™ HIV-1/2 Ag/Ab Combo rapid test results were negative, the participant was diagnosed as HIV uninfected. Positive and inconclusive results were confirmed using the Uni-Gold™ Recombigen® HIV-1/2 rapid test kit (Trinity Biotech, Ireland), following the manufacturer's instructions. In the very few cases where the Uni-Gold™ Recombigen® was discordant, the sample was sent to Global Clinical and Virology laboratory for confirmation using ELISA.

Upon arrival of the stool samples in the laboratory, the Kato Katz preparations were made on the same day of collection, between four to six hours after collection. The Kato Katz preparation for each stool sample were analysed according to the manufacturer's instructions. Thereafter, a proportion of each stool sample was preserved with 10% formol ether in the Mini Parasep tubes (Mini Parasep® Faecal Parasite Concentrator: Apacor Ltd, England), and processed for analysis the following day following the manufacturer's instructions. The stool samples were then

screened microscopically for intestinal helminth parasites eggs and ova using both the Kato Katz and modified formol ether (Mini Parasep) methods by two trained personnel. The study focused on the *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm species, which are the most common intestinal helminths in KZN.

Furthermore, serological diagnosis of intestinal helminth infections was undertaken, to supplement the conventional microscopic diagnosis. *Ascaris*-specific IgE and IgG4 antibodies show cross-reactivity between the antigens of different intestinal helminth parasites, including *Trichuris trichiura* and *Necator americanus* and other hookworm species (Pritchard *et al.* 1991; Chatterjee *et al.* 1996; Figueiredo *et al.* 2010). This implies that the high levels of *Ascaris*-specific antibodies could be denoting reactions with antigens of *Trichuris trichiura* and hookworm species as well. This cross-reactivity between different intestinal helminth antigens as well as other allergens may reduce the sensitivity of the serological test (da Costa Santiago *et al.* 2015) and thus result in high levels of parasite-specific antibody levels that are not specific to the intestinal helminths. The *Ascaris*-specific IgE and IgG4 analyses were undertaken in the Allergy Diagnostic and Clinical Research Unit, a South African National Accreditation System (SANAS) accredited laboratory in the University of Cape Town Lung Institute, using the Phadia® ImmunoCAP method.

Infection with intestinal helminths was then defined either by the presence of intestinal helminth eggs or ova in the stool samples and/or high levels of *Ascaris*-specific IgE and/or IgG4 in serum (Maizels and Yazdanbakhsh 2003; Adams *et al.* 2006; Mkhize-Kwitshana *et al.* 2011). Cut off values of *Ascaris*-specific IgE and *Ascaris*-specific IgG4 were 0.35 kUA/l and 0.15 mgA/l respectively, and any levels above the cut-off values were considered diagnostic of intestinal helminth infection.

2.7.2.3 Stratification of study participants

The participants were stratified into four groups, based on the HIV, stool, IgE and IgG4 results. The four participant groups were (1) co-infected with HIV and intestinal helminths, (2) singly infected with HIV, (3) singly infected with intestinal helminths, and (4) uninfected, a group that was used as a control (Figure 6). The design of the study required this stratification since interactions between HIV-intestinal helminth co-infection with the nutritional and the immune status were to be investigated in HIV and intestinal helminths single and co-infections. Furthermore, participants with one of the following helminth phenotypes were identified as being intestinal helminth infected: $Egg^{+}IgE^{hi}IgG4^{lo}$; $Egg^{+}IgE^{lo}IgG4^{hi}$; $Egg^{+}IgE^{hi}IgG4^{hi}$, Egg^{-}

$IgE^{hi}IgG4^{lo}$; $Egg^{-}IgE^{lo}IgG4^{hi}$; $Egg^{-}IgE^{hi}IgG4^{hi}$, $Egg^{+}IgE^{lo}IgG4^{lo}$; and $Egg^{-}IgE^{lo}IgG4^{lo}$ which was identified as uninfected with helminths.

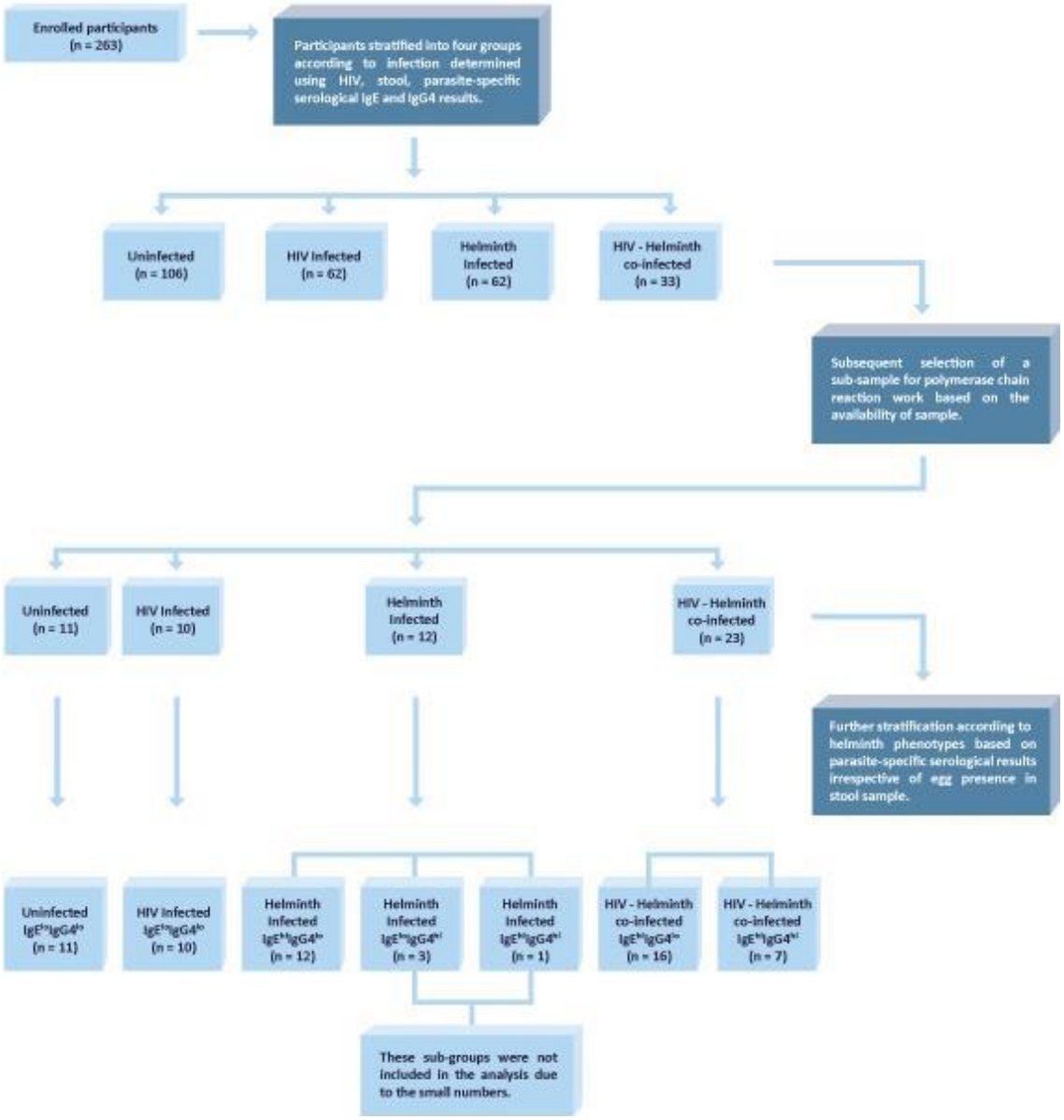

Figure 6: The stratification of participants into infection groups to enable the determination of the differences in nutritional status and immune profile among the participant groups. The infection groups were further stratified according to the serological intestinal helminth phenotypes

2.8 Assessment of nutritional status

2.8.1 Anthropometric measurements

Weight and height were measured using a calibrated Kern® MPE scale (Kern & Sohn, Germany). The participants were weighed with light clothing, without shoes. The scale

calculated and displayed the body mass index (BMI) after the weight and height were keyed into the scale. To determine the BMI (kg/m^2) of the participants, the cut-off points established by the World Health Organization (1995) were used to classify the participants into underweight (< 18.5), normal weight (18.5 - 24.9), overweight (25 - 29.9), and obese categories (≥ 30) for both males and females.

2.8.2 Biochemical and haematologic analysis of micronutrients and macronutrients

Biochemical and haematologic analyses were undertaken at Global Clinical and Virology laboratory, a SANAS accredited pathology laboratory in Durban. The following biochemical markers of nutritional status were analysed using the UniCel® DxC 600 Synchron System (Beckman Coulter, Inc.) spectrophotometric auto-analyser: (i) macronutrients: total protein, albumin, pre-albumin; (ii) micronutrients: calcium, magnesium, phosphate, zinc, iron and ferritin. (iii) The acute phase proteins: the C-reactive protein (CRP) was also measured by the spectrophotometric auto-analyser, to detect the presence of inflammatory conditions.

(iv) Haemoglobin, haematocrit, white cell count and differential count levels were assayed with the Sysmex XT-1800*i* (Roche Diagnostics) haematology auto-analyser that uses flow cytometry and sodium lauryl sulphate (SLS)-haemoglobin methods.

2.9 Determination of immune profile

2.9.1 Measurement of immune and virological markers

The immune status of all participants was determined by measuring the CD4/CD3/CD8/CD45 markers, using the Multitest kit on a FACS Calibur flow cytometer (Becton Dickinson, USA). The HIV viral load quantification was done for the HIV infected participants only, using the automated COBAS Amplicor HIV-1 Monitor Test V1.5 (Roche Diagnostics).

2.9.2 Cytokine gene expression using real-time PCR

The sample preparation for RNA isolation, quantification and analysis using real-time PCR was conducted at the Hasso Plattner Research Laboratory in HIV Pathogenesis Programme (HPP) Laboratory, in UKZN, Durban. The methods used in the quantification of the levels of cytokine gene expression of Th1, Th2, Treg and CD38 activation marker using quantitative PCR were developed and standardized in the laboratory (Singh *et al.* 2011). The levels of cytokine gene expression of interferon-gamma (IFN-γ) and tumour necrosis-alpha (TNF-α) (used as surrogate markers for Th1 immune responses); interleukin-4 (IL-4) (used as a surrogate marker for Th2 responses); IL-10, (a surrogate marker for Treg responses) and CD38, a marker of activation were compared among the study participants.

2.9.2.1 RNA isolation and analysis

For all samples, lysates were prepared using 1 X RBC lysis solution (Qiagen, Germany) and were then stored in RLT buffer from an RNA extraction kit (RNeasy kit; Qiagen, Germany) in a -80^{0}C. RNA was then extracted immediately after thawing of the samples. The total RNA concentration was quantified, and samples were used only if the optical density (OD_{260})/OD_{280} ratio was 1.90 or higher. All RNA samples were DNase treated (Thermo Fisher; USA). One microgram of total RNA from each sample was reverse transcribed by using the iScript cDNA synthesis kit (Bio-Rad, USA). The samples that were selected for analysis were those with optical density (OD_{260})/OD_{280} ratio of 1.90 or higher, using the Nanodrop (Thermo-Fisher; USA).

RNA quantification of cytokine gene expression by real-time PCR

For the quantification of RNA (cDNA) for cytokine gene expression by real-time PCR, the primers and cycling conditions for IFNα, IFN-γ, IL-4, IL-10 and CD38 real-time quantitative PCR were developed and validated in the laboratory (Table 2). The PCR product size of the primers that were used for the five cytokine gene expression quantification were between 150 and 250 base pairs long. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined to be the most suitable reference gene among the five genes based on PCR efficiency of 87% (Singh *et al.* 2011). The product size of the primers used for GAPDH was 225 base pairs long.

Table 2: PCR primers and cycling conditions used in the amplification and quantification of cytokine gene by real-time PCR

Gene	GenBank accession no	Sequence (5′-3′)*	Cycling conditions (denaturation, annealing and extension
IFN-γ	NM_000619	5′-TCGGTAACTGACTTGAATGTCCA-3′ (F) 5′-TCGCTTCCCTGTTTTAGCTGC-3′ (R)	95^{0}C for 30s, 60^{0}C for 30s, and 72^{0}C for 30s
TNF-α	NM_006291	5′-GGCCAATGTGAGGGAGTTGAT-3′ (F) 5′-CCCGCTTTATCTGTGACCC-3′ (R)	95^{0}C for 30s, 55^{0}C for 30s, and 72^{0}C for 30s
IL-4	NM_172374	5′-GCCAAGACCCCTTCGAGAAAT-3′ (F) 5′-CCGATCCTGTTATCTGCCTCC-3′ (R)	95^{0}C for 30s, 60^{0}C for 30s, and 72^{0}C for 30s
IL-10	NM_000572	5′-GACTTTAAGGGTTACCTGGGTTG-3′ (F) 5′-TCACATGCGCCTTGATGTCTG-3′ (R)	95^{0}C for 30s, 60^{0}C for 30s, and 72^{0}C for 30s
CD38	NM_001775	5′-CAACTCTGTCTTGGCGTCAGT-3′ (F) 5′-CCCATACACTTTGGCAGTCTACA-3′ (R)	95^{0}C for 30s, 60^{0}C for 30s, and 72^{0}C for 30s
GAPDH	NM_002046	5′-AAGGTCGGAGTCAACGGATT-3′ (F) 5′-CTCCTGGAAGATGGTGATGG-3′ (R)	95^{0}C for 30s, 65^{0}C for 30s, and 72^{0}C for 30s

*F: forward; R: reverse

Each PCR mixture was comprised of 0.5pmol/µl (for IFNα, IFN-γ, IL-4, IL-10 and CD38) or 0.25pmol/µl (for GAPDH) for each primer, 5µl SYBR green 1 master mix (2x) (Roche Diagnostics, Switzerland), 1µg cDNA, and nuclease-free water to 10µl. Reactions were run in duplicate on a Roche LightCycler 480 version 1.5 instrument, with 1 cycle at 95^0C (10 min) followed by 45 cycles consisting of denaturation, annealing, and extension steps (Table 2). Detection of the fluorescent products was carried out at the end of the 72^0C extension period. To confirm amplification specificity, the PCR products were subjected to a melting-curve analysis. Standard curves were generated using the Universal Human Reference RNA (Stratagene, USA) for quantitative analysis and also to determine the concentration of the unknown samples, reported as ratio of the unknown gene over GAPDH.

2.10 Statistical data analysis

Descriptive statistics (mean, frequencies and percentages) were used to summarize the immune profile data (lymphocyte subsets and cytokine levels) and intestinal helminth serology phenotypes (*Ascaris*-specific IgE and IgG4) and present the characteristics of the participants. All gene expression data (the messenger-ribonucleic acid (mRNA) levels) were log transformed to ensure normality. Values are expressed as medians. Groups singly or co-infected with HIV and intestinal helminth parasites, with or without helminth infection phenotypes, and those uninfected were compared using the Kruskal-Wallis test and paired observations were compared using Student's *t*-test for numeric variables and chi-square tests for categorical variables. Furthermore, median mRNA expression levels between HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected groups were compared and the differences between the study groups evaluated by using an unpaired *t* test. The differences with the p value of ≤ 0.05 and 95% confidence interval (CI) were considered statistically significant. Data was analysed using the statistics packages STATA version 13 (College Station, Texas: Stata Corporation, USA), SPSS version 25 (IBM Corporation., NY, USA) and GraphPad Prism version 5.01 (GraphPad Software, Inc., USA), which was also used to generate scatter plots and bar graphs.

Assessment of nutritional status

Differences between the infected and uninfected groups were assessed using the Kruskal Wallis test for categorical variables and the Wilcoxon signed rank sum test for continuous variables (p-value < 0.001). The outcome variable had four levels: uninfected, HIV singly infected, intestinal helminth singly infected and HIV-intestinal helminth co-infected, which is a multinomial outcome. Therefore, univariate and multivariate multinomial probit regression models were

used to assess nutritional factors associated with each group (HIV singly infected, intestinal helminth singly infected and HIV-intestinal helminth co-infected), and the uninfected group was used as a reference category. Regression coefficients with 95% confidence intervals (CI) were reported to indicate the strength and direction of association, and a p- value ≤ 0.05 to indicate the level of statistical significance.

Investigation of the use of pre-albumin compared to albumin in nutritional assessment

Four different multivariate multinomial logistic regression models were fitted to investigate the effect of pre-albumin versus albumin for nutritional assessment among HIV singly infected, intestinal helminth singly infected and HIV-intestinal helminth co-infected groups, using the uninfected group as a reference category. Models used to analyse the relationship included not specifying BMI; for normal weight, overweight and obese categories. Each model was fitted for individuals with no inflammation (CRP$\leq$5) and those with inflammation (CRP>5). The effect was estimated using relative risk ratios (RRR) with 95% CI significant at p-value ≤ 0.05.

Immune profile using levels of cytokine gene expression Th1, Th2, Treg and CD38

All gene expression data were log transformed to ensure normality. Groups singly or co-infected with HIV and intestinal helminth parasites and uninfected were compared using a *t*-test and analysis of variance (ANOVA) with Tukey's multiple comparison test for numeric variables and chi-square test for categorical variables. The differences with 95% CI and the p-value of ≤ 0.05 were considered statistically significant.

3.0 CHAPTER 3

The interaction between HIV and intestinal helminth parasites co-infection with nutrition among adults in KwaZulu-Natal

As part of the broad aim of the thesis to investigate the interaction between the coinfection of HIV and intestinal helminths with the immune profile and nutritional status, this chapter aims to analyse the latter among singly and co-infected adults in KZN.

Highly prevalent HIV and intestinal helminth single infections continue to plague a considerable proportion of the South African population (Adeleke *et al.* 2015; Kharsany and Karim 2016). The geographic overlap of these infections lands to the expectation that there may be high prevalence of dual infection with HIV and intestinal helminths. Furthermore, since HIV and intestinal helminth single infections may result in malnutrition, it is hypothesized that the HIV-intestinal helminth co-infection may result in worsened micro- and macronutrient deficiencies. These deficiencies are known to have negative impact on both the cell-mediated and humoral immune systems, resulting in rapid progression of HIV infection to AIDS (Duggal *et al.* 2012).

In light of this, the current study aimed to investigate the interaction of HIV and intestinal helminth co-infection with the nutritional status of infected individuals. Such studies that have determined the interaction of HIV-intestinal helminth co-infections with nutritional status are scarce, especially in the KZN adult population. To the best of our knowledge, there is no evidence of such work in South Africa that has interrogated the interaction between the HIV-intestinal helminth co-infection with malnutrition having been published. This is the first such study that has investigated the nutritional status in adults singly and co-infected with highly prevalent HIV and intestinal helminth infections in KZN.

The work was expected to test the hypothesis that the co-infection would have a more deleterious effect on the nutritional status. The co-infected individuals were expected to have worse off nutritional status compared to those who are singly infected and those uninfected with HIV and intestinal helminths. Part of this work was published as “The interaction between HIV and intestinal helminth parasites co-infection with nutrition among adults in KwaZulu-Natal” as follows:

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Research Article

The Interaction between HIV and Intestinal Helminth Parasites Coinfection with Nutrition among Adults in KwaZulu-Natal, South Africa

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In South Africa few studies have examined the effects of the overlap of HIV and helminth infections on nutritional status. This cross-sectional study investigated the interaction between HIV and intestinal helminths coinfection with nutritional status among KwaZulu-Natal adults. Participants were recruited from a comprehensive primary health care clinic and stratified based on their HIV, stool parasitology, IgE, and IgG4 results into four groups: the uninfected, HIV infected, helminth infected, and HIV-helminth coinfected groups. The nutritional status was assessed using body mass index, 24-hour food recall, micro-, and macronutrient biochemical markers. Univariate and multivariate multinomial probit regression models were used to assess nutritional factors associated with singly and dually infected groups using the uninfected group as a reference category. Biochemically, the HIV-helminth coinfected group was associated with a significantly higher total protein, higher percentage of transferrin saturation, and significantly lower ferritin. There was no significant association between single or dual infections with HIV and helminths with micro- and macronutrient deficiency; however general obesity and low micronutrient intake patterns, which may indicate a general predisposition to micronutrient and protein-energy deficiency, were observed and may need further investigations.

1. Background

Approximately 2 billion (24%) of the world's population is infected with intestinal helminth parasites, with high prevalence occurring in poor and deprived communities in tropical and subtropical regions, including sub-Saharan Africa [1]. Helminths may impair the nutritional status in these infected individuals [2]. In sub-Saharan Africa the geographic overlap between the human immunodeficiency virus (HIV), intestinal helminth parasites, and malnutrition may have an additive impact on the competency of the immune system in affected hosts [3, 4]. This triple burden may lead to accelerated HIV and helminth disease progression [5–7]. Potent immune responses and adequate nutrition are essential to resist infectious agents. Research suggests that individuals who are coinfected with HIV and helminths have lower biochemical levels of micronutrients [8], as well as carbohydrate and protein macronutrients [4, 9]. It has been reported that deficiencies of protein, energy, and micronutrients including iron, zinc, and vitamins impact on competent cell mediated and humoral immune responses, and the link to increased susceptibility to HIV and helminth coinfections in such cases has been demonstrated [10, 11]. Thus, micronutrient and macronutrient deficiencies may

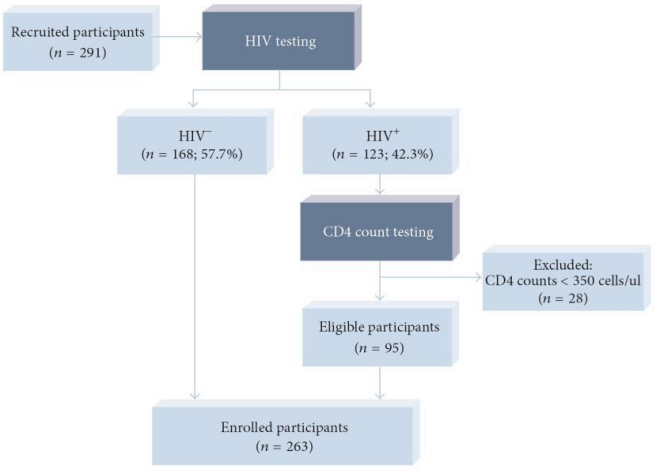

Figure 1: Participant recruitment and enrolment strategy.

predispose individuals to HIV and helminth coinfection as well as leading to exacerbated HIV progression, resulting in a vicious cycle of malnutrition, infection, and immune deficiency.

A significant proportion (approximately 54%) of South Africans live under conditions of poverty [12]. Furthermore, KwaZulu-Natal (KZN), a province of South Africa, has a significant proportion of the population living in environments where there is lack of adequate sanitation (22.7%) and safe water supplies (15.8%) [13]. In these areas, the standard of living is generally poor and intestinal helminth infections are highly prevalent [14]. Prevalence of intestinal helminths was found to range among adults from 11.2% in the inland region, 30.3% in the north coast region, and 29.2% in the south coast region [15]. KwaZulu-Natal also has the highest HIV prevalence in South Africa, reported to be 37.4% in 2014 compared to the national estimate of 10.2% [16]. However, despite these data, studies of the possible deleterious effects of HIV and helminth coinfection on nutritional status among adults in KZN are lacking. This study investigated the interaction of HIV and intestinal helminth coinfection with nutritional status as measured by body mass index (BMI) and biochemical micro- and macronutrient markers, against food intake levels, in a periurban informal setting in KZN.

2. Methods

2.1. Study Setting. The study was conducted in a periurban area, randomly selected from eThekwini enumeration areas under the eThekwini Health District in the KZN province of South Africa. It comprises approximately 39,000 households with approximately 30% informal settlements [17]. Poverty is widespread in this area, with low income households, and approximately 34% of the population in the area were not economically active [17]. There is generally poor access to facilities in the area [18] with about 60% households not having piped water inside the household [17].

The study site was a comprehensive primary health care clinic, providing all essential health care services, including HIV counselling and testing (HCT). Recruitment was therefore purposively conducted in this clinic. By default, the majority of clinic attendees were female.

2.2. Recruitment and Selection of Study Participants. Ethical approval to conduct the study was obtained from the University of KwaZulu-Natal Biomedical Research Committee (BREC Ref: BE 230/14). Permission to conduct the study was granted by the Provincial and eThekwini Health District office and the KZN Provincial Department of Health. The local political authorities granted permission to conduct the study in their area, after a series of meetings where the study objectives were explained and discussed.

During the recruitment process, information sessions were held in the reception area, to inform all the clinic attendees about the study. Those willing to participate were individually given further information. After ensuring that the potential participants fully understood the study, they were asked to give informed consent. They then underwent HIV pretest counselling at the HCT clinic. Eligible participants were adults who were 18 years of age and older, not on antiretroviral therapy, and not pregnant, if female. The enrolment process is outlined in Figure 1.

2.3. Ethical Considerations. The study commenced only after ethical approval and permissions from the relevant authorities were obtained. All eligible participants gave written

consent before enrolment into the study. Participants were tested for HIV status for the purpose of allocating them to either a study or a reference group. Pre- and post-HIV test counselling was provided. The HIV infected individuals who had CD4 counts below 350 cells/μl were referred to the HCT clinic and were excluded from participating in the study for ethical reasons. The country guidelines recommend the protection of vulnerable individuals such as very sick or severely immunocompromised persons. Likewise, for classifying helminth infection status, participants were screened for intestinal parasites. Those who were found to be infected were referred to the clinic for anthelminthic treatment.

2.4. Study Design and Sample Size. A cross-sectional survey of HIV and intestinal helminths prevalence including the investigation of nutritional status was conducted between June 2014 and May 2015 in the eThekwini Health District in KZN. The objective was to describe the nutritional status of individuals infected singly or dually with HIV and intestinal helminths in comparison with noninfected counterparts. A sample size of 229 adults was calculated to detect an effect size of 0.4 with 80% power and probability of 95% between the study groups. The study sample was to include 160 adults not infected with parasites and 69 infected with parasites, assuming that 30% of adults in KZN are infected with parasites, based on the 20.4% prevalence reported on KZN adults [15]. Fifty percent of the study sample would be coinfected with HIV and 50% not be infected with HIV, assuming that 50% of KZN adults are HIV infected, based on the 2011 HIV prevalence of 37.4% among antenatal women in KZN and 2011 HIV prevalence of 38% in the eThekwini district [19].

2.5. Measures. Participants were tested for HIV and were screened for intestinal helminth parasites. Demographic data and socioeconomic status data were collected using a structured questionnaire. Nutritional status was assessed using anthropometric measurements, micro- and macronutrient markers, and 24-hour food recall.

2.5.1. Diagnosis of HIV Status. Participants were tested for HIV status using the Alere Determine™ HIV-1/2 Ag/Ab Combo rapid test kit (Orgenics Ltd, Israel). Inconclusive results were confirmed using the Uni-Gold™ Recombigen® HIV-1/2 rapid test kit (Trinity Biotech, Ireland).

2.5.2. Screening for Intestinal Helminth Parasites. Each participant donated stool samples collected on two consecutive days. Upon arrival of the samples in the laboratory, the Kato Katz preparations were made on the same day. A proportion of the sample was then preserved in 10% formol ether in the Mini Parasep tubes (Mini Parasep® Faecal Parasite Concentrator: Apacor Ltd, England) for analysis the following day, by two trained personnel. The stool samples were screened microscopically for intestinal helminth parasites eggs and ova using both the Kato Katz and the modified formol ether (Mini Parasep) methods. However, diagnosis by egg count can be inaccurate [20], when a sample may not contain many eggs, which may be caused by light infections or by day to day variation in egg excretion [21]. Egg excretion depends on immune responses to the parasite infection and genetic and environmental factors [22]. Adams et al. [22] recommended that analyses on the interaction between HIV and helminths should not only be based on the detection or nondetection of eggs in stool samples, since individuals who are infected with parasites in larval stages or male worms only, which cannot produce eggs, may be excluded. Hence, serological diagnosis of intestinal helminths, using *Ascaris*-specific IgE and *Ascaris*-specific IgG4 levels, was done, which supplemented the conventional microscopic diagnosis of helminth infection [20, 22, 23]. Blood samples that were collected from each participant by a trained phlebotomist were assayed for *Ascaris*-specific IgE and *Ascaris*-specific IgG4 levels in a South African National Accreditation System (SANAS) accredited pathology laboratory, using the Phadia® ImmunoCAP method.

Ascaris-specific IgE and IgG4 antibodies show cross-reactivity between the antigens of different helminth parasites including *Trichuris trichiura* [24, 25]. Cut-off values of *Ascaris*-specific IgE and *Ascaris*-specific IgG4 were 0.35 kU/l and 0.15 kU/l, respectively, and any levels above the cut-off values were considered high. Infection with intestinal helminths was defined either by the presence of helminth eggs or ova in the stool samples and/or by high levels of *Ascaris*-specific IgE and/or IgG4 in serum.

The participants were stratified, based on the HIV, stool, IgE, and IgG4 results, into four groups: (1) coinfected with HIV and intestinal helminths, (2) infected with only HIV, (3) infected with intestinal helminths only, and (4) not infected.

2.5.3. Nutritional Status

Anthropometric Measurements. Weight and height were measured using a calibrated Kern® MPE scale (Kern & Sohn, Germany). The participants were weighed with light clothing, without shoes. The scale calculated and displayed the BMI after the weight and height were keyed into the scale. To determine the BMI (kg/m^2) of the participants, the cut-off points established by the World Health Organization [26] were used to classify the participants into underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9), and obese categories (≥30) for both males and females.

Nutrient Adequacy Ratios (NARS) Analysis for Micro- and Macronutrient Intake. Trained fieldworkers administered a structured questionnaire to collect 24-hour food recall data from the enrolled participants. Two food recall interviews were conducted to collect data on food items and their quantities, which were consumed the day before the day of the interview by each participant. The first questionnaire was for that which was consumed on a weekday and the second was for that consumed on the weekend. Beverages, regular and special meals, and between-meals snacks consumed, and how they were prepared, were recorded. Three-dimensional food models and a food model booklet were used to indicate food quantities and meal portions. Demographic data indicates that most of the interviewees were the main people in their households who were responsible for the preparing and

cooking of meals. Data for the two food recalls were then averaged and nutrient adequacy ratios (NARS) were calculated by a trained nutrition specialist. A nutrient adequacy ratio is the ratio of a nutrient intake divided by the recommended daily requirement for that nutrient [27].

Biochemical Analysis of Micro- and Macronutrients. Biochemical and haematologic analyses were conducted in a SANAS accredited pathology laboratory. The following biochemical markers of nutrition were analysed by a spectrophotometric autoanalyser: macronutrients: total protein, albumin, and prealbumin; micronutrients: calcium, magnesium, phosphate, zinc, iron, and ferritin. Haemoglobin, haematocrit, white cell count, and differential count levels were assayed with a haematology autoanalyser that uses flow cytometry and sodium lauryl sulphate- (SLS-) haemoglobin methods.

2.6. Statistical Analysis. Descriptive statistics was used to summarize the data. Differences between the infected and uninfected groups were assessed using the Kruskal Wallis test for categorical variables and the Wilcoxon signed rank sum test for continuous variables ($p < 0.001$). The outcome variable has four levels: uninfected, HIV singly infected, helminth singly infected, and HIV-helminth coinfected, which is a multinomial outcome. Therefore, univariate and multivariate multinomial probit regression models were used to assess nutritional factors associated with each group (HIV singly infected, helminth singly infected, and HIV-helminth coinfected), and the uninfected group was used as a reference category. Final multivariate models of effects of independent variables associated with each group are presented. Regression coefficients with 95% confidence intervals (CI) are reported to indicate the strength and direction of association and a p value ≤ 0.05 to indicate the level of statistical significance. Data was analysed using the statistics packages STATA 12.0 (College Station, Texas, Stata Corporation, USA), SPSS version 23 (IBM Corporation, NY, USA), and GraphPad Prism version 5.01 (GraphPad Software, Inc., USA).

3. Results

3.1. Characteristics of the Study Participants

3.1.1. Sociodemographic Profile of the Study Participants. Out of a total of 263 enrolled participants (Figure 1), the majority of the participants (91.6%; $n = 241$) were female. The average age of the study participants was 36 years, ranging from 18 to 83 years. The majority were generally poor and 91.3% ($n = 240$) were unemployed. Some relied on government grants, either pension ($n = 39$; 14.9%) or a child support grant ($n = 93$; 35.5%), as their main source of income and 31.2% ($n = 82$) were dependent on their parents for their livelihood. The education level of this population was low, only 3.3% had tertiary education and 67.7% ($n = 178$) had secondary level education, a few up to 12th Grade. About 33% were unable to access clean water; they reported having to share a public tap or use neighbours' taps or tanks and boreholes. Most of the population (54.8%) were using pit latrines while 7.6% reported not having any toilet facilities and some using public mobile toilets. Some participants (25.1%) were using flush toilets which were not connected to sewerage pipes (Table 1).

Table 1: Demographic and socioeconomic data of the study participants.

Variable	*n* (%)
N	263
Gender	
Male	22 (8.4)
Female	241 (91.6)
Age (years)	
18–24	88 (33.5)
25–49	116 (44.1)
≥50	58 (22.1)
Unknown	1 (0.4)
Marital status	
Single	219 (83.6)
Married	32 (12.2)
Widowed	11 (4.2)
Education level	
None	24 (9.2)
Primary	52 (19.8)
Secondary	178 (67.7)
Tertiary	8 (3.3)
Employment	
Unemployed	240 (91.3)
Employed	23 (8.7)
Source of income	
No income	8 (3.1)
Child support grant	93 (35.5)
Pension	39 (14.9)
Dependent	82 (31.2)
Self-employed	19 (7.3)
Salary	16 (6.1)
Salary and second income	5 (1.9)
Source of water	
Tap inside the house	144 (54.8)
Tap outside the house	32 (12.2)
Public tap	66 (25.1)
Tank	16 (6.1)
Neighbour's tap	4 (1.5)
Borehole	1 (0.4)
Toilet facility	
Pit	144 (54.8)
Flush, connected to sewage pipes	33 (12.5)
Flush, not connected*	66 (25.1)
Mobile	17 (6.5)
None	3 (1.1)

*Flush, not connected: toilets can flush; however, they dispose sewage into septic tanks and the waste is collected once in a while.

3.1.2. Nutrient Intake of the Study Population. Nutrient adequacy ratio (NARS) analysis from the 24-hour food recall

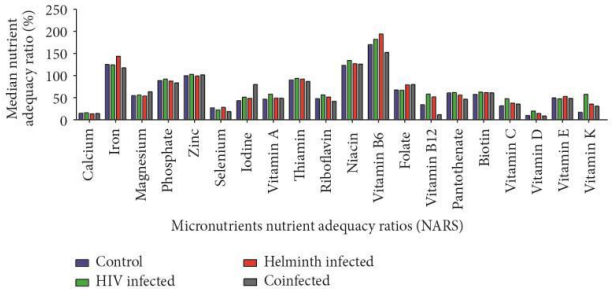

FIGURE 2: Median nutrient adequacy ratios (NARS) of micronutrients consumed by uninfected, HIV singly infected, helminth singly infected, and HIV-helminth coinfected participant groups.

showed that generally the intake of the micronutrients analysed was similar across all the groups, with the exception of iodine which was highest among the coinfected group and vitamin B12, which was lowest among this group (Figure 2). Further analysis showed that various micronutrient intake levels were lower than the required daily intake (100%) for all the groups, which included calcium, magnesium, selenium, iodine, vitamin A, riboflavin (vitamin B2), pantothenate (vitamin B5), folate, vitamin B12, biotin (vitamin H), vitamin C, vitamin D, vitamin E, and vitamin K. Phosphate, zinc, and thiamin (vitamin B1) were, however, close to the normal required intake. Intake levels for iron, niacin (vitamin B3), and vitamin B6 were higher than the 100% required daily intake for all the participant groups.

The macronutrient NARS analysis showed that the coinfected group did not differ from the other groups, where all groups had low levels in all the macronutrient intake levels except for carbohydrates. All the participant groups had a low mean intake (less than the 100% required daily intake) of energy, total protein, total fat, and total fibre (Figure 3). Notably, the intake of carbohydrates was higher than the daily required quantity in all the participant groups, way above 100%, and it was highest in the HIV infected and the coinfected groups (Figure 3).

The acceptable macronutrient distribution ranges (AMDR) (fat 15–30%, protein 10–15%, and carbohydrate and fibre 55–75%) to energy showed that all the participant groups had lower contributions of total fat and total protein, less than 30% and 15%, respectively. The contribution of carbohydrate and fibre to energy was within the acceptable range for all participant groups (Figure 4).

3.2. Prevalence of HIV and Intestinal Helminths. The overall prevalence of intestinal helminths was 36.1% (n = 95) and that of HIV was also 36.1% (n = 95). The participants who were singly infected with HIV were 23.6% (n = 62), those singly infected with helminths were also 23.6% (n = 62), those coinfected with both HIV and intestinal helminths were 12.5% (n = 33), and those uninfected with either HIV or helminths were 40.3% (n = 106).

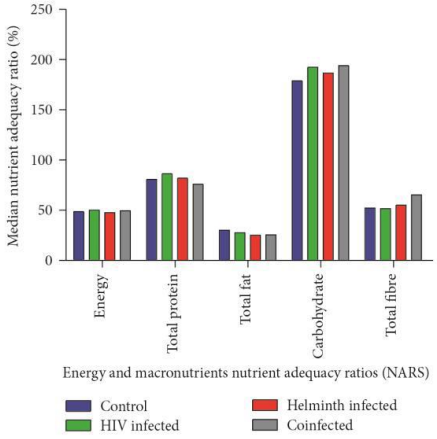

FIGURE 3: Comparison of median nutrient adequacy ratios (NARS) of macronutrients consumed by the different participant groups.

The prevalence of helminth infection, determined by microscopic screening for parasite eggs or ova was 11.8% (n = 31), where the predominant species was *Ascaris lumbricoides* (n = 25; 9.5%), followed by *Trichuris trichiura* (n = 6; 2.3%). Furthermore, the serological diagnosis of helminth infection, by high levels of *Ascaris*-specific IgE and IgG4, revealed a prevalence of 29.7% (n = 78).

3.3. Nutritional Status

3.3.1. Anthropometry. The body mass index (BMI) measures of nutritional status among HIV singly infected, helminth singly infected, HIV-helminth coinfected, and uninfected participants are described in Table 2, showing the differences between the participant groups, although not statistically significant (p = 0.089). In the uninfected group 39.0% of

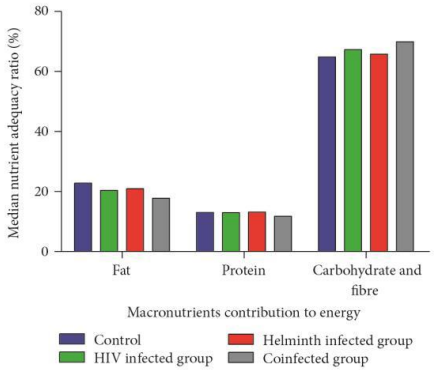

FIGURE 4: Macronutrient distribution ranges to energy in the different participant groups.

the participants were overweight and 51.3% were obese. In the HIV-helminth coinfected group 16.9% were overweight and 5.3% were obese. The helminth singly infected group had 26.3% of participants who were obese. The proportions of participants who were underweight was low ($n = 6$). Of this number, 50% were HIV infected, 33.3% were helminth infected, and only 16.7% were coinfected and none of the uninfected group were underweight.

3.3.2. Biochemical and Haematologic Analysis. The biochemical and haematologic measures of nutritional status among HIV singly infected, helminth singly infected, HIV-helminth coinfected, and uninfected participants are described in Table 2. Except for BMI and phosphate, there was a statistically significant difference in biochemical and haematologic measures across the groups ($p < 0.001$). The median micronutrient levels were varied among the groups although all were within the reference ranges, with transferrin and ferritin levels being lower in the coinfected group compared to the reference group. Percentage transferrin saturation levels were higher in the HIV infected and the coinfected groups compared to the other groups. C-reactive protein, a marker of inflammation, was within range for all the participant groups.

The median biochemical levels of macronutrients (total protein, albumin, and prealbumin) varied among the groups although all were within the reference ranges. Total protein levels were lowest in the uninfected group and highest in both the HIV infected and the HIV-helminth coinfected groups. Albumin levels were lowest in the HIV infected group and highest in the uninfected group. Prealbumin levels were lowest in the HIV infected group and highest in the helminth infected group.

The haematology parameters revealed levels that were within the reference ranges. However, the HIV and the coinfected groups had lower haemoglobin levels compared to the other groups. The absolute eosinophil count levels were highest in the helminth infected group compared to the other groups.

3.4. Associations between HIV and Helminth Coinfection and Single Infection with Nutritional Status. The estimated coefficients of the multivariate multinomial probit model are presented in Table 3. BMI was not statistically significant in all the infection groups. Relative to the uninfected group, the HIV-helminth coinfected group was associated with a significant increase in total protein [$\beta = 0.16$ (0.07–0.25), $p < 0.001$]; and percentage transferrin saturation was also significantly higher [$\beta = 0.34$ (0.02–0.67), $p = 0.040$] and ferritin significantly decreased [$\beta = -0.03$ (−0.06–0.01), $p = 0.006$]. The HIV singly infected group was associated with significant increase in total protein [$\beta = 0.16$ (0.08–0.24), $p < 0.001$] and a significant decrease in albumin [$\beta = -0.26$ (−0.45–0.08), $p = 0.005$]. The helminth infected group was associated with a significant increase in absolute eosinophils [$\beta = 5.07$ (1.52–8.63), $p = 0.005$].

4. Discussion

In many regions of developing countries, malnutrition is superimposed with endemic helminth and HIV infections. The findings of this study showed that the prevalence of HIV (36.1%) and helminths (36.1%) was high in this adult population (the majority of whom were females), with notable levels of HIV-helminth coinfection. This was against the backdrop of scant data on the prevalence of intestinal parasites in adults of KZN, where most of the prevalence studies have been conducted in schoolchildren. The only other study on prevalence of intestinal helminth parasites in KZN among adults found overall moderate levels of helminth prevalence (20.4%) in the eThekwini district [15]. The higher HIV prevalence in this study is to be expected given the fact that the study site was situated in eThekwini district which has one of the highest HIV prevalence rates in KZN, with a 38% HIV prevalence rate among antenatal women being reported in 2011 for this district [19].

The majority of participants who were obese and overweight (66.3%) were among the uninfected group. Nutrient adequacy ratios analysis revealed a significantly increased carbohydrate intake among all groups, much above the recommended dietary allowance [28]. Increased carbohydrate intake causes weight gain leading to obesity [29]. This may be expected as the general South African population is reported to have a significant proportion of adults who are overweight and obese. The South African National Health and Nutrition examination survey (SANHANES-1) established that 25% and 40.1% of women are overweight and obese, respectively, and 19.6% and 11.6% men are overweight and obese, respectively [30]. This could probably be due to the general consumption of diets that are rich in refined carbohydrates [29, 31] which would be in line with the current study which revealed excessive carbohydrate intake.

Despite the substantially elevated carbohydrate intake in this study population, the energy intake was low. This may be attributed to the fact that fat and protein intake were less than

Table 2: Anthropometric, biochemical, and haematologic measures of nutritional status among HIV singly infected, helminth singly infected, and HIV-helminth coinfected participants.

Variables	Reference ranges	Uninfected (n = 106)	HIV infected (n = 62)	Helminth infected (n = 62)	HIV-helminth coinfected (n = 33)	
Body mass index	kg/m^2	n (%)	n (%)	n (%)	n (%)	p value
Under-weight	<18.5	0 (0.0)	3 (50. 0)	2 (33.3)	1 (16.7)	0.089*
Normal weight	18.5–24.9	35 (34.7)	28 (27.7)	24 (23.8)	14 (13.9)	
Overweight	25.0–29.9	30 (39.0)	18 (23.4)	16 (20.8)	13 (16.9)	
Obese	≥30.0	39 (51.3)	13 (17.1)	20 (26.3)	4 (5.3)	
Nutritional status	Reference ranges	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	p value
Total protein (g/l)	60–85	73.0 (71.0–78.0)	78.0 (74.0–85.0)	75.0 (72.0–78.0)	78.0 (75.0–82.0)	<0.001
Albumin (g/l)	35–52	40.0 (37.0–42.0)	37.0 (36.0–40.0)	39.0 (37.0–42.0)	39.0 (37.0–41.0)	<0.001
Prealbumin (mg/dl)	18–38	21.8 (18.4–25.3)	20.9 (17.9–25.4)	23.5 (19.3–26.8)	21.1 (17.8–22.6)	<0.001
Iron (umol/l)	6.6–28.0	12.0 (8.0–16.0)	13.0 (9.0–17.0)	13.0 (9.0–18.0)	12.0 (8.0–17.0)	<0.001
Transferrin (g/l)	1.85–4.05	2.77 (2.45–3.11)	2.63 (2.38–2.96)	2.74 (2.48–3.07)	2.62 (2.20–2.94)	<0.001
% Transferrin saturation	20–55	20.0 (13.0–26.0)	23.5 (18.0–29.0)	21.0 (14.0–29.0)	22.0 (13.0–27.0)	<0.001
Ferritin (ng/ml)	30–400	48.9 (24.1–76.9)	38.5 (23.4–75.0)	44.4 (25.2–73.4)	40.5 (18.4–49.3)	<0.001
Calcium (mmol/l)	2.15–2.62	2.31 (2.25–2.37)	2.30 (2.22–2.36)	2.34 (2.28–2.40)	2.32 (2.23–2.38)	<0.001
Magnesium (mmol/l)	0.65–1.05	0.89 (0.84–0.95)	0.88 (0.83–0.92)	0.88 (0.81–0.94)	0.89 (0.81–0.96)	<0.001
Phosphate (mmol/l)	0.80–1.55	1.08 (0.94–1.22)	1.09 (0.97–1.20)	1.12 (0.97–1.28)	1.14 (0.99–1.27)	0.098*
Zinc (umol/l)	9.2–18.4	11.55 (9.1–13.7)	12.7 (10.5–16.3)	11.95 (9.80–14.50)	12.3 (9.6–14.8)	<0.001
C-reactive protein (mg/l)	0–5	3.85 (1.05–9.25)	2.60 (0.00–7.20)	3.10 (0.00–6.10)	3.00 (1.00–6.50)	<0.001
Haemoglobin (g/dl)	12.3–15.3	12.6 (11.9–13.5)	12.3 (11.7–13.3)	12.8 (12.0–13.5)	12.4 (11.8–13.4)	<0.001
Haematocrit (l/l)	0.4–0.56	0.39 (0.36–0.41)	0.38 (0.36–0.40)	0.39 (0.37–0.41)	0.39 (0.35–0.40)	<0.001
White cell count ($\times 10^9$/l)	4.0–10.0	5.99 (4.96–7.07)	5.19 (4.35–6.15)	6.46 (5.58–8.37)	6.69 (4.95–8.48)	<0.001
Absolute neutrophils ($\times 10^9$/l)	2.0–7.5	2.97 (2.29–3.89)	2.29 (1.71–3.03)	3.37 (2.44–4.69)	3.25 (2.37–4.50)	<0.001
Absolute lymphocytes ($\times 10^9$/l)	1.5–4.0	2.22 (1.87–2.58)	2.00 (1.71–2.40)	2.34 (1.85–2.79)	2.35 (1.85–2.74)	<0.001
Absolute eosinophils ($\times 10^9$/l)	0.04–0.4	0.12 (0.08–0.22)	0.12 (0.08–0.25)	0.24 (0.12–0.42)	0.23 (0.09–0.49)	<0.001
Absolute basophils ($\times 10^9$/l)	0.00–0.10	0.02 (0.01–0.02)	0.01 (0.01–0.02)	0.02 (0.02–0.03)	0.02 (0.01–0.03)	<0.001

* $p > 0.01$; IQR, interquartile range specifying first quantile (q25%) and third quantile (q75%).

the recommended daily intake. Protein and carbohydrates constitute a lower contribution to energy (16.8 kilojoules per gram each) compared to fat (37 kilojoules per gram) [32, 33], where protein, carbohydrates, fat, and fibre would all together contribute to the required 100%. The contribution of total protein to the daily energy intake was lower than the recommended 15% [34] for all the participant groups. This low protein-energy intake may predispose all the participant groups to protein-energy malnutrition.

The results of anthropometric measurements revealed that underweight was more common among the infected group: 50% among the HIV infected, 33% in the helminth infected group, and 17% in the coinfected group, while none of the uninfected group were underweight. This concurs with the fact that weight loss and wasting is associated with HIV infection and some helminth infections, through a variety of mechanisms including increased energy requirements and/or reduced dietary intake and absorption, reduced appetite, inflammatory cytokines, and diarrhoea [4, 35].

Further analysis showed that the HIV singly infected group was associated with higher total protein and lower albumin biochemical levels compared to the levels of the uninfected group. Similar observations were made in the HIV-helminth coinfected group with regard to significantly higher total protein accompanied by lower albumin levels. Total protein and albumin are serum proteins synthesized by the liver that are not only affected by nutritional status, but by inflammation and infection [36]. Total protein comprises albumin and globulin fractions. Albumin in healthy individuals is highest in concentration in serum, usually 60% of the total protein [37]. HIV infection induces a nonspecific expansion of the globulin fraction due to the polyclonal stimulation of B cells in response to the acute or chronic stages of the infection and associated opportunistic infections

Table 3: Multivariate multinomial probit model of nutritional factors associated with HIV singly infected, helminth singly infected, and HIV-helminth coinfected groups (base category: uninfected group).

Variables	HIV infected ($n = 62$)			Helminth infected ($n = 62$)			HIV-helminth coinfected ($n = 33$)		
	β	95% CI	p value	β	95% CI	p value	β	95% CI	p value
Body mass index									
Underweight	Ref			Ref			Ref		
Normal weight	15.90	1629.81–1598.01	0.985	15.02	1628.92–1598.89	0.985	16.59	1630.50–1597.31	0.984
Overweight	16.32	1630.23–1597.589	0.984	15.23	1629.17–1598.65	0.985	16.23	1630.14–1597.68	0.984
Obese	16.98	1630.89–1596.926	0.984	15.70	1629.61–1598.21	0.985	17.63	1631.54–1596.28	0.983
Nutritional status									
Total protein (g/l)	0.16	(0.08–0.24)	<0.001	−0.01	(−0.10–0.07)	0.747	0.16	(0.07–0.25)	<0.001
Albumin (g/l)	−0.26	(−0.45–0.08)	0.005	−0.16	(−0.33–0.01)	0.065	−0.12	(−0.35–0.10)	0.288
Prealbumin (mg/dl)	0.10	(−0.01–0.21)	0.078	0.08	(−0.02–0.18)	0.105	−0.03	(−0.17–0.11)	0.656
Iron (umol/l)	0.00	(−0.34–0.34)	0.992	0.06	(−0.19–0.31)	0.628	−0.49	(−1.03–0.05)	0.073
Transferrin (g/l)	−0.26	(−1.78–1.25)	0.735	−0.08	(−1.33–1.17)	0.895	0.18	(−1.91–2.27)	0.865
% Transferrin saturation	0.07	(−0.15–0.28)	0.531	−0.01	(−0.18–0.15)	0.879	0.34	(0.02–0.67)	0.040
Ferritin (ng/ml)	−0.01	(−0.02–0.00)	0.102	0.00	(−0.01–0.00)	0.399	−0.03	(−0.06–0.01)	0.006
Calcium (mmol/l)	0.13	(−4.97–5.22)	0.961	5.32	(−2.50–11.13)	0.073	0.75	(−6.25–7.75)	0.833
Magnesium (mmol/l)	−3.36	(−8.62–1.90)	0.210	−2.60	(−7.40–2.19)	0.287	0.11	(−6.05–6.27)	0.971
Phosphate (mmol/l)	1.28	(−0.99–3.55)	0.268	−0.14	(−2.15–1.87)	0.891	0.39	(−2.32–3.10)	0.779
Zinc (umol/l)	0.07	(−0.03–0.17)	0.149	0.05	(−0.04–0.14)	0.237	0.00	(−0.12–0.11)	0.934
Haemoglobin (g/dl)	−0.12	(−1.29–1.05)	0.841	−0.49	(−1.61–0.63)	0.390	0.23	(−1.28–1.74)	0.765
Haematocrit (l/l)	2.89	(−38.99–44.77)	0.892	21.55	(−18.19–61.29)	0.288	−0.38	(−55.10–54.33)	0.989
White cell count ($\times 10^9$/l)	0.92	(−2.07–3.92)	0.546	−2.27	(−5.22–0.68)	0.132	1.07	(−2.66–4.80)	0.572
Absolute neutrophils ($\times 10^9$/l)	−1.18	(−4.32–1.95)	0.459	2.63	(−0.48–5.73)	0.097	−0.74	(−4.65–3.18)	0.712
Absolute lymphocytes ($\times 10^9$/l)	−1.49	(−4.68–1.70)	0.359	2.42	(−0.77–5.61)	0.137	−0.99	(−4.96–2.97)	0.623
Absolute eosinophils ($\times 10^9$/l)	−1.53	(−5.68–2.62)	0.469	5.07	(1.52–8.63)	0.005	2.43	(−1.90–6.76)	0.272
Absolute basophils ($\times 10^9$/l)	−22.77	(−62.67–17.13)	0.263	0.53	(−32.59–33.65)	0.975	−24.12	(−66.90–18.67)	0.269

[38, 39]. Thus, the higher total protein seen in both the HIV singly infected group and the HIV-helminth infected group may have been as a result of prioritization in the formation of globulins and acute phase proteins in response to the HIV infection [37] and, proportionately, reduced albumin levels. On the other hand, lower albumin levels may have been due to the increased rate of transcapillary leak of albumin into the interstitial fluid associated with infection [40]. Both HIV and helminth infections have acute and chronic stages, resulting in chronic activation of the immune system. However, in this study, it was not possible to determine the stages of both HIV and helminths as this was not within the scope of the study objective. The low albumin finding in the current study is corroborated by a similar finding in the Kannangai et al. [41] study of HIV infected individuals, where albumin levels were low as well.

The food recall NARS analysis also revealed a general low micronutrient intake where the median intake of calcium, magnesium, selenium, iodine, vitamin A, vitamin B2, vitamin B5, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin H, vitamin K, and folate micronutrients was low for all the participant groups. The expected finding would be similarly low biochemical levels since intake levels were low [42]. It had been hypothesized that all the micronutrient biochemical levels would be low in the singly and coinfected infected with HIV and helminths study groups. HIV infection has been reported to predispose to micronutrient deficiency [43] and, likewise, helminth infections have been associated with deficiency of most of the micronutrients [44]. However, in this study, discrepant results were found where biochemical analyses showed that these micronutrients were within the reference range for all the participant groups. Biochemical markers as an indication of nutritional status are more reliable than food intake questionnaire data, and food intake data should be used as evidence of food variety rather than to indicate nutritional status [45, 46]. This discrepancy could have been due to underreported 24-hour food recall data as self-reported actual food intake may have been omitted consciously or by accident, leading to the discrepancy between infection status and biochemical micronutrient levels [47, 48]

or the participants may have been taking supplements and failed to declare these during the collection of data [49]. Food recall data collected over 3 days, accompanied by a food frequency questionnaire, may have given a more holistic indication of dietary consumption [50, 51].

Further analysis showed that the HIV-helminth coinfected group was associated with significantly lower ferritin levels, although percentage of transferrin saturation levels were higher with nonstatistically significant lower transferrin levels. Low ferritin levels are typical of iron deficiency anaemia [52]. However, iron intake levels were higher than the daily required quantity for all the participant groups. Intestinal parasitic helminths are associated with iron deficiency anaemia [9, 53] and HIV on its own is also associated with iron deficiency anaemia [54]. Intestinal helminths source nutrients from the host for their own growth, while the infection itself, either caused by HIV or helminths, may increase the host's need for nutrients [55]. Thus, the lower ferritin levels in the coinfected group may indicate subclinical iron deficiency. Subclinical iron deficiency, even though it may be mild, impacts on the physiological functions that drive the development of cells and their metabolic function, which would have an effect on the immune system action against the HIV-helminth coinfection [56]. Moreover, anaemia in the HIV-helminth coinfection may lead to increased HIV progression, increased mortality, and poor quality of life [57]. Mupfasoni et al. [58], in Rwanda, however, found no association of intestinal helminth parasite infection with anaemia, although the authors attributed this to the fact that anaemia was uncommon in their study area.

Although the eosinophil counts were within range for all the groups, the helminth infected and the dually infected groups had significantly higher levels compared to the other groups. These results are in keeping with the classic feature of helminthiasis. These infections are associated with increased production of eosinophils [59, 60], which are reported to decrease significantly after deworming [61, 62].

There was no significant association observed between HIV-helminth coinfection and single infections with micro- and macronutrient deficiency. However, the results highlighted the various micro- and macronutrient intake patterns in the population. Low intake levels of calcium, magnesium, selenium, iodine, vitamin A, vitamin B2, vitamin B5, folate, vitamin B12, biotin (vitamin H), vitamin C, vitamin D, vitamin E, vitamin K, total protein, and energy were noted in all the participant groups. This may indicate a general predisposition to micronutrient and protein-energy deficiency in the study participants and may need further nutritional investigations.

5. Limitations of This Study

The cross-sectional design in this study is limited to determining the associations only and cannot infer causality. A prospective cohort study design with randomised sampling would be recommended for such an investigation. The small sample size may have resulted in the inability to determine a significant association between macro- and micronutrient levels and the coinfection. Moreover, the use of self-reported food recall data collected over two days, which relies on memory and correct estimations of quantity, is a limitation, although the value of the data is recognised since it indicated the food intake patterns in the population. Energy intake of the study participants was only about 50% of the reference nutrient intake. However, the prevalence of overweight and obesity was almost two-thirds. Therefore the energy intake may be underestimated. The fat intake may be underestimated as the 24-hour dietary recall may not cover the cooking oil intake. This could result in inaccurate macronutrient contribution to energy. In addition, the study used biased sampling since recruitment was from individuals who attended the HCT clinic and thus the findings cannot be generalised to the population in the area where the study was conducted. Furthermore, the fact that the stool samples were screened microscopically for intestinal helminth parasites eggs and ova the following day is a limitation, although they were prepared and preserved on the same day of collection. This could have significantly affected the ability to detect hookworm eggs since these rapidly disintegrate upon storage of stools. Nevertheless, this study adds value to the less studied but growing research area of HIV-helminth infection impact on nutritional status in sub-Saharan Africa.

6. Concluding Remarks

Helminth infection is a neglected disease globally, with more attention and priority given to HIV/AIDS, TB, and malaria. The high prevalence of helminth infection observed in this adult population warrants attention, especially since HIV is endemic in the area. However, there was no significant association between single and dual HIV and helminth infections with micro- and macronutrient deficiency in this population. The frequent occurrence of obesity and overweight which is an additional health burden in South Africa, possibly due to excessive carbohydrate intake, and the general low intake of micro- and protein-energy macronutrients observed in this study require further nutritional investigations and the current South African Department of Health campaign on healthy lifestyle needs strengthening [63]. Future studies should investigate the nutritional, parasitic, and infectious conditions that may act as cofactors for rapid progression of HIV infection [64].

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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4.0 CHAPTER 4

The investigation of the use of pre-albumin as a tool for nutritional assessment in adults co-infected with HIV and intestinal helminth parasites in KwaZulu-Natal

The broader aim of this thesis focuses on the interaction between co-infection and nutritional status in adults. It therefore becomes imperative to investigate an appropriate marker of nutritional status in this population group. This chapter then addresses this need, so that the findings can inform even future work in the untapped area of impact of co-infection on nutritional status.

In view of the interactions between infection, compromised immunity and malnutrition, an appropriate indicator of true malnutrition within the milieu of infection, inflammation, overweight and obesity, is essential. Notably, the findings of the current study (previous chapter publication), where the majority of the participants were overweight and obese point to the importance of using reliable assessment methods that can detect nutrient deficiency, especially in overweight and obese adults. A biochemical marker for nutritional status in adults that is commonly used is albumin. However, albumin is not reliable as there are many possible confounders. For example, during inflammation, dehydration and renal disease, albumin is redistributed from the intravascular to the extravascular compartments which can be misinterpreted as malnutrition from blood assays of albumin. Since obesity causes low-grade inflammation (Fronczyk *et al.* 2014; Castro *et al.* 2017), it therefore means that albumin may not be a reliable marker in detecting malnutrition in obesity. Hence, the second objective of the current study aimed to investigate a reliable biochemical marker for nutritional status in adults with or without co-infection with HIV and intestinal helminths. Pre-albumin was investigated as an indicator that could be used compared to albumin in HIV-intestinal helminths co-infected KZN adults, with or without inflammation as indicated by C-reactive proteins (CRP) in different body mass index (BMI) categories. Pre-albumin was considered since it has been suggested as being more reliable due to its short half-life and its increased sensitivity to changes in nutritional status (Gaudiani *et al.* 2014). It was hypothesized that pre-albumin would be able to detect malnutrition in all inflammatory conditions which include infection and obesity.

To the best of our knowledge, this is the first such study, where CRP, albumin and pre-albumin biochemical markers were used in the investigation of nutritional status in the context of HIV-intestinal helminth co-infection in KZN adults. Part of this work was published as "The investigation of the use of prealbumin as a tool for nutritional assessment in adults co-infected with HIV and intestinal helminth parasites in KwaZulu-Natal, South Africa", as follows:

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Research Article

The Investigation of the Use of Prealbumin as a Tool for Nutritional Assessment in Adults Coinfected with HIV and Intestinal Helminth Parasites in KwaZulu-Natal, South Africa

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Serum prealbumin is considered to be as important as albumin in the nutritional status assessment. However, there is relatively little evidence of its advantage over the commonly used albumin. This study investigated the use of prealbumin compared to albumin as a marker of nutritional status in adults singly and dually infected with human immunodeficiency virus (HIV) and intestinal helminths, with or without inflammatory conditions, in different body mass index (BMI) categories. This cross-sectional study was conducted in a periurban setting in KwaZulu-Natal, South Africa. Multivariate multinomial logistic regression models were fitted to investigate the effect of prealbumin and albumin in nutritional assessment among HIV and helminth individuals with or without inflammation, indicated by elevated and normal C-reactive protein (CRP) levels. In normal CRP, albumin was significantly lower in unadjusted BMI [RRR = 0.8, $p = 0.001$] and in normal weight [RRR = 0.7, $p = 0.003$] and overweight [RRR = 0.5, $p = 0.001$] participants. In elevated CRP, albumin was significantly lower [RRR = 0.8, $p = 0.050$] and prealbumin was significantly higher in unadjusted BMI [RRR = 1.2, $p = 0.034$] and overweight [RRR = 1.4, $p = 0.052$] individuals. The current study found that prealbumin can differentiate between inflammation-induced reduction of albumin and true malnutrition in adults singly or coinfected with HIV and intestinal helminths in the presence or absence of inflammation in various BMI categories.

1. Background

Malnutrition is a major public health problem throughout the developing regions of the world, particularly in sub-Saharan Africa [1]. In addition to the widespread malnutrition, countries in sub-Saharan Africa including South Africa (SA) carry a heavy burden of infectious diseases such as the human immunodeficiency virus (HIV) epidemic and helminth infections that coexist and are compounded by poverty [2–5]. SA has the largest proportion of individuals living with HIV globally [6]. The country also has approximately 54% of its population living in poverty [7], where conditions of malnutrition overlap with high prevalence of HIV-ascariasis and/or trichuriasis coinfections [8, 9]. The KwaZulu-Natal province, which is the epicenter of the HIV epidemic in SA, is among the poorest provinces in the country [10]. In addition, 22.7% and 15.8% of the KwaZulu-Natal population live in conditions where there is lack of adequate sanitation and

safe water supplies, respectively [10], which predisposes to helminth infections [11].

While the two infections have been shown to have a deleterious effect on the immune system [12, 13], HIV and helminth infections can worsen malnutrition through various mechanisms. These include diarrhoea, disrupted intestinal mucosa lining and/or impeded absorption of nutrients, decreased nutrient intake when swallowing is painful as occurs in HIV induced candidiasis [5], and decreased appetite induced by cytokines such as tumour necrosis factor-alpha (TNF-α) [14, 15]. Under such conditions, malnutrition causes immune deficiency and further predisposition to infection [16], which may increase the pressure on the immune system's ability to efficiently eliminate the infectious agent. The importance of adequate nutrition on the potency of the immune system has been well established [17]. Malnutrition therefore may have an additive impact on the HIV-helminth disease progression in coinfected individuals and vice versa [4, 16, 18].

Given the complex interactions between infections, a compromised immune system, and nutritional deficiency, especially in the milieu of coexisting poverty, overweight, and obesity, reliable and objective indicators of malnutrition are essential [19], which respond to changes in nutrient intake and are not influenced by disease processes [20]. It is crucial that malnutrition is identified in obese individuals, especially in situations where an individual has nutritional deficiencies masked by obesity wherein weight, measured by body mass index (BMI), and anthropometric indices would not be reliable measures of nutritional status [21, 22]. SA has increasing prevalence of malnutrition with a predominant pattern of overweight and obesity among adults, shown as high BMI levels [23]. Obesity has been associated with malnutrition [21], which impairs immunity, further increasing the risk of infection [24]. Thus, weight gain may be misleading and inaccurate when monitoring the effectiveness of nutritional replenishment [21].

In addition to anthropometry in clinical settings, biochemical markers of serum protein levels such as total protein and albumin are commonly used to assess the nutritional status of patients, despite the fact that albumin was reported to be insensitive to acute changes in nutritional status [25], since it has a large body pool and its half-life is twenty days [20, 26]. Prealbumin has been suggested to be a more suitable biochemical marker for monitoring nutritional status, due to its short half-life of two days and its sensitivity to changes in protein-energy status within four to eight days, in both the presence and absence of inflammation [27, 28]. Prealbumin reflects more recent protein intake as opposed to albumin, which reflects long-term protein supply [29, 30]. The current study investigated the use of prealbumin as a marker of nutritional status compared to albumin in adults singly or dually infected with HIV and intestinal helminth parasites with or without inflammatory conditions as indicated by C-reactive protein (CRP) in different BMI categories in an adult population in KwaZulu-Natal, South Africa.

2. Methods

2.1. Study Setting. The study was conducted in a periurban area, randomly selected from eThekwini enumeration areas under the eThekwini Health District in the KwaZulu-Natal (KZN) province of South Africa. Currently available census data indicates that the area comprises approximately 39,000 households with approximately 30% informal settlements [31]. Poverty is widespread in this area, with low-income households, and approximately 34% of the population in the area were not economically active [31]. There is generally poor access to facilities in the area [32] with about 60% of households not having piped water inside the household [31]. The study site was a comprehensive primary healthcare clinic, providing all essential healthcare services, including HIV counselling and testing (HCT). The sociodemographic profile of the participants has been described in detail elsewhere [33].

2.2. Study Design and Sample Size. This study was based on a cross-sectional survey of HIV and intestinal helminths prevalence including the investigation of nutritional status, conducted between June 2014 and May 2015. A sample size of 229 adults was calculated to detect an effect size of 0.4 with 80% power and probability of 95% between the study groups.

2.3. Recruitment and Selection of the Study Participants. During the recruitment process, information sessions were held to inform all the clinic attendees about the study. Those willing to participate were individually given further information. After attending to any queries on the study, all those willing to participate were asked to give written informed consent before enrolment. The recruitment and enrolment process is detailed elsewhere [33]. 263 consenting adults (18 years of age and older, not on antiretroviral therapy (ART), and not pregnant if female) were enrolled in the study. Participants were then tested for HIV status for the purpose of allocating them to either a study or a reference group. Pre- and post-HIV test counselling was provided. Likewise, for classifying helminth infection status, the participants who were enrolled in the study (n = 263) were screened for intestinal parasites.

2.4. Ethical Considerations. Ethical approval to conduct the study was obtained from the University of KwaZulu-Natal Biomedical Research Committee (BREC Ref: BE 230/14). Permission to conduct the study was granted by the Provincial and eThekwini Health District office, the KwaZulu-Natal Provincial Department of Health, and the local political authorities.

The country guidelines at the time of study recruitment had set the threshold for ART initiation at 350 cells/μl. Thus, HIV-infected individuals who had cluster of differentiation-4 (CD4) counts below 350 cells/μl (n = 28) were referred to the HCT clinic and were excluded from participating in the study as per the ethical directive of protection of vulnerable individuals such as very sick or severely immunocompromised persons. Also, those who were found to be infected with intestinal parasites were referred to the clinic for anthelminthic treatment.

2.5. Measures

2.5.1. Testing for HIV-Helminth Infection. Enrolled participants were tested for HIV status using the Alere Determine™

HIV-1/2 Ag/Ab Combo rapid test kit (Orgenics Ltd., Israel). Inconclusive results were confirmed using the Uni-Gold™ Recombigen® HIV-1/2 rapid test kit (Trinity Biotech, Ireland). Participants were also screened for intestinal helminth parasites eggs and ova. The stool samples donated by each participant on two consecutive days were screened microscopically by two trained persons, after the Kato Katz and the Mini Parasep® Faecal Parasite Concentrator (Apacor Ltd., England) preparation methods were made. The stool preparation methods are described elsewhere [33]. Blood samples were collected from each participant by a trained phlebotomist. Screened participants were allocated into four groups: (1) the uninfected/controls, (2) HIV singly infected group, (3) helminth singly infected group, and (4) HIV-helminth coinfected group. The methods of screening for HIV status and the presence of intestinal helminth parasites for allocation into these groups have been described in detail elsewhere [33].

2.5.2. Anthropometry. Anthropometric measurement included weight and height, measured using a calibrated Kern® MPE scale (Kern & Sohn, Germany). The participants were weighed with light clothing and flat shoes. The scale calculated and displayed the BMI after the weight and height were keyed in. Participants were classified into the different BMI categories using World Health Organization [34] cut-off points: underweight (< 18.5), normal weight (18.5–24.9), overweight (25–29.9), and obese categories (≥ 30) for both males and females.

2.5.3. Biochemical Analysis. The C-reactive protein (CRP), prealbumin, and albumin biochemical markers were analysed by a spectrophotometric auto-analyser in a South African National Accreditation System (SANAS) accredited pathology laboratory. Participants were grouped into two subgroups: (1) those showing evidence of inflammation (with elevated levels, CRP > 5 mg/l) and (2) those with no inflammation (with normal levels, CRP ≤ 5 mg/l).

2.6. Statistical Analysis. Descriptive statistics were used to summarize the data using frequencies and percentages for categorical data and means and standard deviations (SD) for continuous data. Four different multivariate multinomial logistic regression models were fitted to investigate the effect of prealbumin versus albumin for nutritional assessment among HIV singly infected, helminth singly infected, and HIV-helminth coinfected groups, using the uninfected group as a reference category. Model A analysed the relationship without specifying BMI category; Model B was for normal weight; Model C was for overweight; and Model D was for the obese category. Each model was fitted for individuals with no inflammation (CRP ≤ 5) and those with inflammation (CRP > 5). The effect was estimated using relative risk ratios (RRR) with 95% confidence interval (CI) significant at p value ≤ 0.05. Data was analysed using the statistics package STATA version 13 (College Station, Texas: Stata Corporation, USA) and SPSS version 24 (IBM Corporation, USA).

3. Results

3.1. Characteristics of the Study Participants. The mean age of the study participants was 36 years, ranging from 18 to 83 years. The majority of the participants were female (91.6%). Out of 263 participants, 40.3% were uninfected, 23.6% were infected with HIV, 23.6% were infected with helminths, and 12.5% were coinfected with HIV and helminths. Overall, the proportion of participants who had evidence of inflammation was 36.1% and the proportion of those with no inflammation was 63.9%.

In the absence of inflammation, the majority of underweight individuals were HIV or helminth singly or dually infected, while the majority of uninfected individuals had normal weight or were overweight or obese (Figure 1). The majority of overweight and obese participants were uninfected and were mainly participants with inflammation. In the presence of inflammation (CRP > 5 mg/l), no underweight participants were recorded.

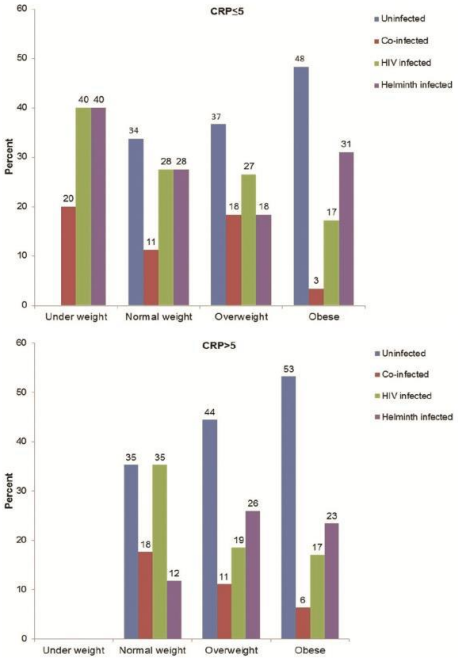

FIGURE 1: The percentage distribution of body mass index (BMI) levels among the uninfected, HIV singly infected, helminth singly infected, and HIV-helminth coinfected groups with normal (≤ 5) and high (>5) C-reactive protein (CRP) levels.

3.2. Biochemical Assessment of Prealbumin and Albumin Nutritional Status. Both prealbumin and albumin were within reference ranges among all subgroups; however, both

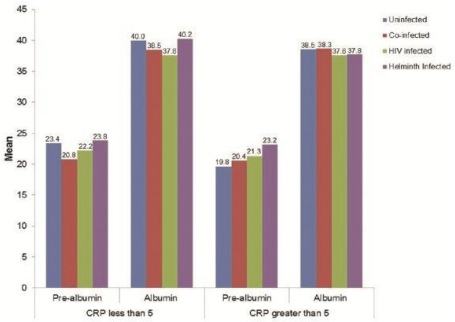

FIGURE 2: Biochemical measures of prealbumin and albumin nutritional status among the uninfected, HIV singly infected, helminth singly infected, and HIV-helminth coinfected participants with normal and high C-reactive protein (CRP) levels.

were lower in HIV singly infected and HIV-helminth coinfected groups with normal CRP levels. The mean prealbumin levels for the participants who had no inflammation (CRP $\leq$ 5 mg/l) were higher in the uninfected and the helminth-infected groups and lower in the HIV singly infected and the HIV-helminth coinfected groups (Figure 2). Similarly, the mean albumin levels were higher in the uninfected and helminth-infected groups and lower in the HIV-infected and HIV-helminth coinfected groups.

In participants with inflammation (CRP > 5 mg/l), the mean prealbumin levels were lower in the uninfected, HIV-infected, and HIV-helminth coinfected groups and higher in the helminth-infected group, while mean albumin was lower in the HIV-infected and helminth-infected groups and higher in the uninfected and HIV-helminth coinfected groups.

3.3. Models of the Effect of Prealbumin versus Albumin for Nutritional Assessment. The analysis revealed that HIV-helminth coinfection was associated with patterns of lower prealbumin and albumin levels across all BMI categories in the subgroup with normal CRP except in the obese, although not statistically significant. In the presence of inflammation, the pattern in the coinfected group was that of lower albumin and higher prealbumin levels in all BMI categories, except in the obese (Figure 3).

The HIV-infected group with normal CRP was associated with lower albumin and higher prealbumin across all BMI categories in both the presence and absence of inflammation, irrespective of statistical significance. Albumin was significantly lower in unadjusted BMI [RRR = 0.8 (95% CI: 0.7-0.9), p = 0.001] (Figure 3(a)), in normal weight [RRR = 0.7 (95% CI: 0.5-0.9), p = 0.003] (Figure 3(b)), and in overweight participants [RRR = 0.5 (95% CI: 0.3-0.7), p = 0.001] (Figure 3(c)). In the obese (Figure 3(d)), albumin was nonsignificantly lower. Prealbumin was nonsignificantly higher in all BMI categories.

In elevated CRP, albumin was significantly lower in unadjusted BMI [RRR = 0.8 (95% CI: 0.6-1.0), p = 0.050] and nonsignificantly lower in the normal weight, overweight, and obese. Prealbumin was significantly higher in unadjusted BMI [RRR = 1.2 (95% CI: 1.0-1.4), p = 0.034] and in overweight [RRR = 1.4 (95% CI: 1.0-1.9), p = 0.052] and nonsignificantly higher in normal weight and obese participants.

Helminth-infected group with inflammation was associated with lower albumin and higher prealbumin, irrespective of statistical significance. Albumin was significantly lower in unadjusted BMI [RRR = 0.7 (95% CI: 0.6-0.9), p = 0.012] and in overweight [RRR = 0.5 (95% CI: 0.3-1.0), p = 0.037] participants. Prealbumin was significantly higher in unadjusted BMI [RRR = 1.3 (95% CI: 1.1-1.5), p = 0.001], in overweight [RRR = 1.5 (95% CI: 1.1-2.1), p = 0.012], and in obese individuals [RRR = 1.3 (95% CI: 1.0-1.7), p = 0.042].

In the obese individuals, in both the presence and absence of inflammation, discordant results of both prealbumin and albumin were found in the HIV-helminth coinfected group compared to the rest of the BMI categories. The exception was in the HIV-infected group, where the pattern of lower albumin and higher prealbumin was observed in all the BMI groups, including the obese.

4. Discussion

In communities where single or dual infection with HIV and helminth and obesity coexist with malnutrition, it is essential that a marker that can reliably detect malnutrition in this milieu of conditions be used. The marker must ideally be unaffected by inflammation, which occurs in obesity [35], as well as in HIV and helminth infections [36, 37]. Evidence shows that obesity results in chronic low-grade inflammation, which may elevate CRP levels due to the adipose tissues releasing IL-6 and TNF-α and inducing the synthesis of CRP by the liver [35, 38]. Others also found an association between increased BMI and increased CRP levels, which was independent of inflammation and other factors that are known to increase CRP [39].

In the current study, despite the fact that some findings had no statistical significance, patterns were observed, which were suggestive of prealbumin being possibly useful in delineating between inflammation-induced hypoalbuminemia and true malnutrition. The key finding was that, in the absence of inflammation, participants with dual infection had lower levels of both prealbumin and albumin across all BMI categories except in the obese, which may be suggestive of malnutrition. Bishop et al. [40] and Chen et al. [41] state that low levels of both prealbumin and albumin in normal CRP are indicative of poor protein nutritional status. In the current study, it was further noted that a significant proportion of the dually infected participants with no inflammation were underweight, which is associated with malnutrition.

Furthermore, in the presence of inflammation, the pattern of lower albumin and higher prealbumin levels was observed in all the infected groups across all BMI categories, except in the coinfected participants. Similarly, this pattern was also observed in HIV-infected participants in both the

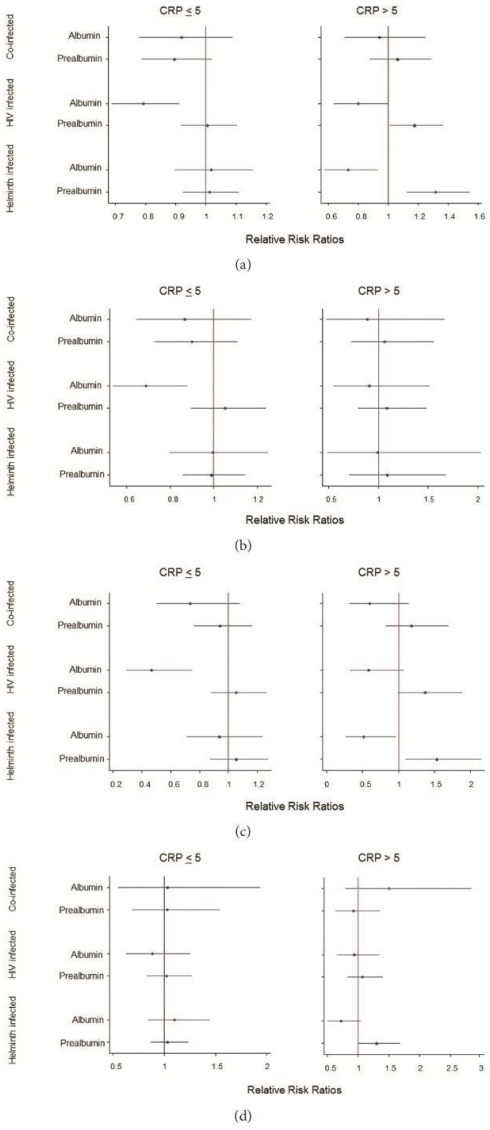

Figure 3: Coefficient plots for multinomial regression models of the effect of prealbumin and albumin for nutritional status assessment in (a) the body mass index (BMI) unadjusted model, (b) BMI normal weight model, (c) BMI overweight model, and (d) BMI obese model among HIV and helminth singly and dually infected groups relative to the uninfected group with C-reactive protein (CRP) levels less than 5 mg/l and greater than 5 mg/l.

absence and presence of inflammation, across all BMI categories including the obese. It is known that serum albumin levels are decreased in the presence of inflammation and infection due to the disproportionate distribution of protein between the albumin and globulin compartments [40, 42]. The assumption is that in the current study this is what caused the lower albumin in the participant groups with inflammation and/or HIV infection.

On the other hand, the higher prealbumin in these individuals may be suggestive of absence of malnutrition. Notably, the lower albumin levels in this instance may have been interpreted as indicative of malnutrition if albumin was assayed on its own and prealbumin was not included. This is a key finding that is suggestive of prealbumin being able to possibly differentiate between inflammation-induced hypoalbuminemia and true malnutrition. Saka et al. [43] found malnourished patients, with low BMI, prealbumin, and albumin levels and high CRP levels showing increased prealbumin after one week of nutritional support, which indicates prealbumin as a sensitive nutritional status marker. Others also found significantly lower prealbumin levels in patients with malnutrition compared to those who had no malnutrition; however, albumin levels were not significantly different between the two groups [44], implying that albumin was unable to distinguish between presence and absence of malnutrition, whereas prealbumin was able to indicate malnutrition.

Furthermore, in obese individuals in both the presence and absence of inflammation discordant prealbumin and albumin levels were found in the dually infected participants compared to the patterns observed in the rest of the BMI categories in the various infected groups. These study findings illustrate the difficulty in assessing malnutrition when there was dual infection and obesity in both the absence and presence of inflammation. Overweight and obesity were mostly prevalent in the participants who had evidence of inflammation, with a significant proportion mainly among the uninfected individuals. Therefore, assessing malnutrition when there is HIV and intestinal helminth coinfection and obesity requires further investigation in a longitudinal study with a large sample size.

5. Limitations

The cross-sectional nature of the study was limited in that it provided a "snapshot" and could not determine if malnutrition and inflammation preceded disease and changes in BMI and CRP levels. Another limitation was the small sample size, which may have resulted in the inability to determine significant associations between changes in BMI, prealbumin, and albumin in the presence and absence of inflammation in the different infections. For future studies, longitudinal cohorts with randomised sampling design and a large sample size would be recommended [45].

Furthermore, the single BMI measurements may not identify significant weight loss or gain [46]. In addition, the single CRP measurements may not accurately indicate long-term inflammation status. The fact that there was not any other measurement supporting the suggested malnutrition in the study participants was also a limitation. However, in another survey, we had observed a general low intake of micro- and macronutrients in the study participants [33].

6. Conclusion

The current study found prealbumin in HIV-helminth coinfection to be a possible delineator between inflammation-induced reduction and true malnutrition, since in all cases of elevated CRP it remained higher, whereas albumin was lower. To the best of our knowledge, this is the first study where CRP, prealbumin, and albumin biochemical markers were used in the investigation of malnutrition in the context of HIV-intestinal helminth coinfection in KwaZulu-Natal adults. It is recommended that CRP should essentially be included in any future investigations of malnutrition when prealbumin and albumin are used as indicators to detect malnutrition in order to delineate the inflammation-induced albumin reduction and true malnutrition in HIV and intestinal helminth singly and dually infected adults. The pattern of lower prealbumin and albumin in the coinfected group in the absence of inflammation may be suggestive of malnutrition and would require further investigation.

Furthermore, the study found that, in both the absence and presence of inflammation, obesity was associated with a discordant pattern of prealbumin and albumin which was contrary to the patterns seen in other BMI categories. This illustrates that assessing nutritional status in dual infection and obesity is a challenge, a key finding that requires further investigation in a large sample size.

Although not conclusive, these findings, however, add value to the growing research area on the investigation of the impact of HIV-helminth infection on nutritional status in South Africa, a country with increasing prevalence of obesity [23].

Data Availability

The data underlying the findings have been included in the manuscript. If requested, the authors agree to provide copies of the original data.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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5.0 CHAPTER 5

The immune profile and parasite-specific IgE and IgG4 serological phenotypes of adults co-infected with HIV and intestinal helminth parasites in KwaZulu-Natal

The last aspect of this work aims to analyse the interaction between co-infection and the immune status in adults, to discern whether co-infection with HIV and intestinal helminths results in higher HIV viral load, increased immune activation and increased expression of Th2 and decreased Th1 cytokine responses, compared to those singly infected and uninfected.

Both HIV and helminth infections lead to constant stimulation of the immune system, which triggers chronic immune activation and dysregulated host responses. Furthermore, plausible evidence supports the hypothesis that intestinal helminths induce a favourable environment for HIV to replicate and spread unabated (Weissman *et al.* 1996; Alemu *et al.* 2013; Mkhize-Kwitshana *et al.* 2017). High numbers of activated cells may increase the pool of cells available for HIV entry and replication, and HIV replicates better in activated than in naïve cells (Dai *et al.* 2009; Chavez *et al.* 2015). Moreover, chronic intestinal helminthiasis is associated with the activation of regulatory networks (Wammes *et al.* 2016), which augment a generalised immune suppression and hyporesponsiveness. This would mean that the co-infection with HIV and intestinal helminths may result in a more compromised immune system's ability to respond to HIV, which may lead to the accelerated progression of HIV disease to AIDS.

Despite the strong evidence emerging in helminth infection that demonstrates a characteristic strong Th2 and regulatory profile which may inadvertently suppress the essential Th1 HIV response (Brady *et al.* 1999; Figueiredo *et al.* 2010), others suggest that the regulatory network induced by helminths has a beneficial role in the affected host, whereby harmful inflammatory responses evoked in atopic and autoimmune diseases are controlled (Brown *et al.* 2006; Salgame *et al.* 2013). Moreover, helminths can benefit from these regulated responses and afford themselves an opportunity for survival within the host (Everts *et al.* 2010; Boyett and Hsieh 2014; Afifi *et al.* 2015),

In light of this, the current study thus aimed to describe the immune response profile of KZN adult individuals singly and dually infected with HIV and intestinal helminths. A manuscript preparation for submission for this work titled "The immune profile and parasite-specific IgE and IgG4 serological phenotypes of adults co-infected with HIV and intestinal helminth parasites in KwaZulu-Natal, South Africa", as follows:

The immune profile and parasite-specific IgE and IgG4 serological phenotypes of adults co-infected with HIV and intestinal helminth parasites in KwaZulu-Natal, South Africa

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ABSTRACT

Background: A significant proportion of the South African population lives in conditions of highly prevalent HIV and intestinal helminth infections, superimposed onto malnutrition and poverty. It is suggested that stronger T helper 2 (Th2) responses are elicited in helminthiasis, which may significantly suppress T helper 1 (Th1) responses, essential to control HIV infection.

Objective: The current study aims to describe the immune profile of a KZN adult population singly and co-infected with HIV and intestinal helminths.

Methods: The immune profile among HIV singly infected; intestinal helminth singly infected and HIV-intestinal helminth co-infected participants was described by the differences in the levels of immune markers including total lymphocytes, CD4 and CD8 counts, viral load and C-reactive protein. The differences in the levels of cytokine gene expression of the interferon-gamma and tumour necrosis-alpha (surrogate markers for Th1 responses); interleukin-4 (for Th2 responses); IL-10 (for Treg responses) and CD38 activation marker were determined using real time PCR. The effect of *Ascaris*-specific IgE and IgG4 responses on the immune profile among intestinal singly and co-infected participants was also described by the differences in the levels of the immune markers.

Results: The CD4 counts were lower ($p < 0.001$) and CD8 counts higher ($p < 0.001$) in the HIV-intestinal helminth co-infected participants compared to the uninfected group. The eosinophils were higher in the intestinal helminth singly infected and co-infected groups ($p = 0.015$) and the *Ascaris*-specific IgE ($p < 0.001$) and IgG4 ($p < 0.001$) levels were higher in the co-infected group. The co-infected participants had higher IFN-γ and TNF-α, higher IL-10 and lower IL-4 cytokine gene expression. Furthermore, the co-infected participants with the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype had significantly lower CD4 ($p = 0.003$) and higher CD8 counts ($p = 0.004$) compared to those with the $IgE^{hi}IgG4^{lo}$ phenotype.

Conclusion: The findings highlight the possible potent HIV responses that intestinal helminths may induce. This does not support the suggestion that the strong Th2 cytokine and immunoregulatory responses that may be induced by intestinal helminths may significantly down-regulate Th1 responses, required to control HIV.

1. Background

Sub-Saharan Africa carries a heavy burden of both human immunodeficiency virus (HIV) and intestinal helminth infections (World Health Organization 2016b; UNAIDS 2017a), superimposed onto malnutrition, which is an additional major public health problem (Food and Agriculture Organization 2015). Infections with either HIV or helminths lead to persistent activation of the immune system, resulting in progressive impairment of all arms of the host immune responses (Borkow and Bentwich 2004). Malnutrition worsens the situation since it reduces the competence of the immune system, further predisposing to infections (Katona and Katona-Apte 2008; Bourke *et al.* 2016). Thus, the milieu of HIV, intestinal helminth infections, malnutrition and poverty may have an additive impact on the competency of the host immune system, which may accelerate the HIV disease (Shey *et al.* 2015).

HIV infection activates the immune cells through various mechanisms which include the signalling through CD4 and the C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) co-receptors (Boasso *et al.* 2009; Paiardini and Muller-Trutwin 2013). Chronic helminth infection also increases the expression of CCR5 and CXCR4 co-receptors in HIV target cells (Hu *et al.* 2010). Increased expression of these co-receptors at the surface of activated $CD4^+$ cells confers increased susceptibility to HIV acquisition and more efficient HIV entry (Jaspan *et al.* 2011; Woodham *et al.* 2016). HIV also subverts host cell transcription factors, such as nuclear factor of activated T cells (NFAT) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Booiman *et al.* 2015; Jiang *et al.* 2017). These cell activation factors promote ease of virus entry and replication in the activated cells (Sokoya *et al.* 2017). Furthermore, both HIV and intestinal helminths compromise the integrity of the intestinal mucosa which may increase the translocation of microbial products; this further contributes to the persistent activation of the immune system and drives perpetual HIV pathogenesis (Klatt *et al.* 2013; Rajamanickam *et al.* 2017).

Moreover, HIV induces dysregulated host responses which result in depleted effector cells (Okoye and Picker 2013). This depletion may be caused by either the virus-induced cytolytic responses (Vijayan *et al.* 2017) or by the inability to effectively replenish naïve and memory effector cells due to clonal deletion, resulting from continuous programmed death -1 (PD-1) induced apoptosis (Boasso *et al.* 2009; Fevrier *et al.* 2011). Notably, an attempt to replenish the depleted $CD4^+$ immune cells inadvertently proliferates HIV target cells, which further increases the viral load (Paiardini and Muller-Trutwin 2013). On the other hand, helminth eggs and excretory/secretory (ES) products released daily persistently stimulate $CD4^+$ and other immune

cells (Walson and John-Stewart 2007; Hewitson *et al.* 2009), which presents a challenge of maintaining long-term $CD4^+$ T cell responses (Taylor *et al.* 2012) which may increase HIV viral load (Borkow and Bentwich 2004).

Helminths stimulate stronger T helper 2 (Th2) cytokine responses and they however induce complex immunomodulatory T regulatory (Treg) responses to attenuate these hostile Th2 host responses, to their benefit (Girgis *et al.* 2013; McSorley *et al.* 2013; Johnston *et al.* 2016). The helminth-induced regulatory mechanisms include the secretion of immunosuppressive IL-10 and transforming growth factor–β1 (TGF- β1) cytokines by activated Treg cells, which ensure that the parasites survive and live long within the host (Everts *et al.* 2010; Boyett and Hsieh 2014; Afifi *et al.* 2015). Other down-regulatory molecules which include cytotoxic T lymphocyte antigen 4 (CTLA-4) and apoptotic PD-1 (Wammes *et al.* 2016) also impede the production of aberrant adaptive host responses and limit inflammation, thus defy the immune exclusion of the intestinal helminths (Weinstock and Elliott 2014). The helminth-induced alternatively activated macrophages (AAMs) downregulate Th1 cells, decrease the secretion of IL-12 (which promotes strong Th1 responses) and inhibit pro-inflammatory IL-17 (Anthony *et al.* 2007; Cooke 2008; Wang *et al.* 2008). These modulatory mechanisms that result in anergic and hyporesponsive T lymphocyte and other cell-mediated responses, induced to protect the parasite, may inadvertently down-regulate Th1 responses, resulting in the dysregulation of cell-mediated responses required to limit HIV replication (Borkow and Bentwich 2004; Maizels and McSorley 2016). Notably, HIV itself induces the upregulation of IL-10, TGF- β cytokines and CTLA-4 molecules, which result in hyporesponsive and anergic anti-HIV effector cell responses (Borkow and Bentwich 2004; Okoye and Picker 2013; Shey *et al.* 2015), which may lead to rapid progression of HIV.

Since both HIV and helminth single infections persistently activate and modulate the immune system, it is thus anticipated that the HIV-intestinal helminth co-infection may result in hyporesponsive, dysregulated host responses with apoptosis, leading to continuous depletion of both naïve and resting memory $CD4^+$ and $CD8^+$ cells (Li *et al.* 2014; Prendergast *et al.* 2015; Sokoya *et al.* 2017). It is hypothesized that the HIV-intestinal helminth co-infection may inadvertently result in worsening of both diseases. There is however varying schools of thought on whether the typical Th2 cytokine and regulatory profile evoked by helminths have a detrimental effect on the HIV Th1 responses, wherein some investigators support this notion (Brady *et al.* 1999; Figueiredo *et al.* 2010). Others refute this, suggesting rather that the helminth-induced regulatory network has a beneficial role, whereby the host cell transcription

factors are down-regulated and therefore, may not promote HIV replication (Brown *et al.* 2006). This view suggests that the modulation of immune responses by helminths may slow down the HIV progression. Despite these opposing views, we however hypothesize that the chronic HIV-helminth co-infection may result in weakened immune responses which may increase the viral load in the HIV infection, based on the dysregulated host responses that HIV and helminth single infections induce.

In South Africa (SA), in spite of the highly prevalent HIV and intestinal helminth infections, and the impact each of these single infections have on the immune system, studies on the interactions between HIV and helminth single and co-infections with immunity are lacking (Mkhize-Kwitshana and Mabaso 2012; Adeleke *et al.* 2015). The study thus aimed to describe the immune profile of adults singly or co-infected with HIV and intestinal helminths in KwaZulu-Natal (KZN), a province that has approximately 45% of its population living in the milieu of the highest HIV epidemic (Department of Health South Africa 2017), compounded by highly prevalent intestinal helminth infections (Kwitshana *et al.* 2008; Molvik *et al.* 2017).

2. Methods

2.1 Study setting

The study was conducted in a peri-urban informal area, in the eThekwini Health District, which is in the north coast of KwaZulu-Natal (KZN), South Africa. It is situated approximately twenty kilometres north-west of eThekwini city centre. It is governed by local government and municipal authority. The latest available census data indicate that the area comprises approximately 39,000 households with approximately 30% informal settlements (Statistics South Africa 2011). Poverty is widespread in this area, with low income households and approximately 52% of the population in the area reported to be living below the poverty line (Statistics South Africa 2011; eThekwini Municipality 2017). The area has high prevalence of HIV, estimated to be 46.2% (Department of Health South Africa 2017). Also, the prevalence of intestinal helminths was found in the area to be 20.4 - 59%, with the most common species being *Ascaris lumbricoides* and *Trichuris trichiura* (Kwitshana *et al.* 2008; Tronnberg *et al.* 2010). There is a substantial fraction of the population that has poor access to facilities in the area, with about 22% households not having piped water inside the house and approximately 25% not having access to flush toilet facility (Statistics South Africa 2012; eThekwini Municipality 2017). Approximately 25% of the population either have no income or their livelihood depends on government grants with the rest sustained through formal employment, informal trading (Statistics South Africa 2011). These poor living conditions may be risk factors

to HIV and intestinal helminth infections (Smith Fawzi *et al.* 2010; Masaku *et al.* 2017). The study site was selected randomly from a list of six clinics (KwaZulu Natal Health Department: The GCIS Unit 2009). It is a primary health care clinic (PHC), providing all essential health care services, including HIV counselling and testing (HCT), servicing about 100 attendees per day. Two hundred and ninety one potential participants were purposively recruited from the study site. The reason for recruiting from an HCT was to make use of the pre- and post-test counselling services. The study population consisted mostly of females (91.6%) due to the fact that the majority of the clinic attendees were female. A total of 263 eligible participants were enrolled into the study.

2.2 Study design, sample size and selection of study participants

A cross-sectional survey of HIV and intestinal helminths prevalence including the investigation of immune profile was conducted between July 2015 and May 2016 in the eThekwini Health District. A sample of 263 adults was used to describe the immune profile of adults singly and co-infected with HIV and intestinal helminths, comparing with those uninfected. The sample size calculation was to detect an effect size of 0.4% of differences in immune status among the HIV and helminth singly and dually infected study and control groups with 80% power and probability of 95%. The calculation assumed that 30% of adults in KZN are infected with parasites, based on the 20.4% - 59% prevalence found on the KZN adults (Kwitshana *et al.* 2008). The sample thus included 168 adults uninfected and 95 infected with intestinal helminths.

Adults who were 18 years of age and older, not on antiretroviral therapy and not pregnant were purposively recruited from the randomly selected PHC. A series of education sessions on parasite infections and their impact on immunity and nutritional status were held with the clinic attendees. They were also made aware that adults may be infected with intestinal helminth parasites as well, not just children. A total of 263 eligible participants were enrolled in the study, after giving written informed consent.

2.3 Measures of the immune profile of the study participants

2.3.1 Diagnosis of HIV status and intestinal helminth infection

The overall design of the study required that participants be stratified according to their infection status, so that the interactions between HIV and intestinal helminth single and co-infection with immune status were investigated. Hence the participants were tested for HIV as well as screened for intestinal helminth parasites, for the purpose of allocating them to either a

study or a reference group. Thus the participants were stratified into four groups: 1) HIV singly infected, 2) intestinal helminth singly infected, 3) HIV-intestinal helminth co-infected and 4) the uninfected, who were a reference group.

HIV testing and diagnosis of intestinal helminth infection are described in detail elsewhere (Mkhize *et al.* 2017). Briefly, microscopy was used to detect the intestinal helminth eggs and/or ova. Two stool samples (morning or first stool), collected on two different days (on a Friday and a Monday) in universal containers were analysed by two trained personnel. The serological levels of *Ascaris*-specific immunoglobulin-E (IgE) and immunoglobulin-G subclass 4 (IgG4) were assayed in serum to supplement the conventional microscopic diagnosis. This was done to increase diagnostic sensitivity as well as to elucidate the effect of the different helminth infection phenotypes on the immune profile of the intestinal helminth singly and co-infected participants (Maizels and Yazdanbakhsh 2003; Adams *et al.* 2006; Mkhize-Kwitshana *et al.* 2011). Thus, in this study, infection with intestinal helminths was defined either by the presence of helminth eggs/ova in the stool samples and/or by high serological levels of *Ascaris*-specific IgE and/or IgG4.

2.3.2 Measurement of markers of immune status, viral load and CRP biochemical levels

The immune profile of the participant groups was assessed using levels of CD4/CD3/CD8 T cell sub-types immune markers using the Multitest kit on a four-parameter FACS Calibur flow cytometer (Becton Dickson, USA). Viral load was quantified only for those who were singly or co-infected with HIV, using the automated COBAS Amplicor HIV-1 Monitor Test V1.5 (Roche Molecular Systems, USA). C-reactive protein (CRP) biochemical levels were measured by a spectrophotometric auto-analyser, to describe the inflammatory status among the participants.

2.3.3 Levels of gene expression of Th1, Th2, Treg cytokines and CD38 activation marker

To further describe the immune profile of the participants, a sub-sample (n = 60) was selected, for real time polymerase chain reaction (PCR) work, to determine the levels of expression of cytokine genes among the HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected participants (Figure 1). The selection of this sub-sample was based on availability and quality of the samples for the PCR downstream preliminary analysis. Messenger- ribonucleic acid (mRNA) levels of the Th1, Th2, Treg and CD38 cytokine genes were determined. Interferon-gamma and tumour necrosis factor-alpha were used as surrogate markers for Th1 responses; interleukin-4, used as a marker for Th2 responses; IL-10 for Treg responses and CD38 used as a marker of activation.

Furthermore, of the sixty samples selected, a sub-sample of participants (n = 56) were stratified according to the serological helminth phenotypes, irrespective of whether helminth eggs were detected or not, into sub-groups: 1) $IgE^{lo}IgG4^{lo}$, 2) $IgE^{hi}IgG4^{lo}$, 3) $IgE^{hi}IgG4^{hi}$, and 4) $IgE^{lo}IgG4^{hi}$ (Figure 2). A small proportion of the intestinal helminth singly infected participants (n = 4) were excluded from the analysis, due to the small number of the phenotypes: ($IgE^{lo}IgG4^{hi}$: n = 3 and $IgE^{hi}IgG4^{hi}$: n = 1). Notably, there were no participants in the HIV-intestinal helminth co-infected group who had the $IgE^{lo}IgG4^{hi}$ helminth phenotype.

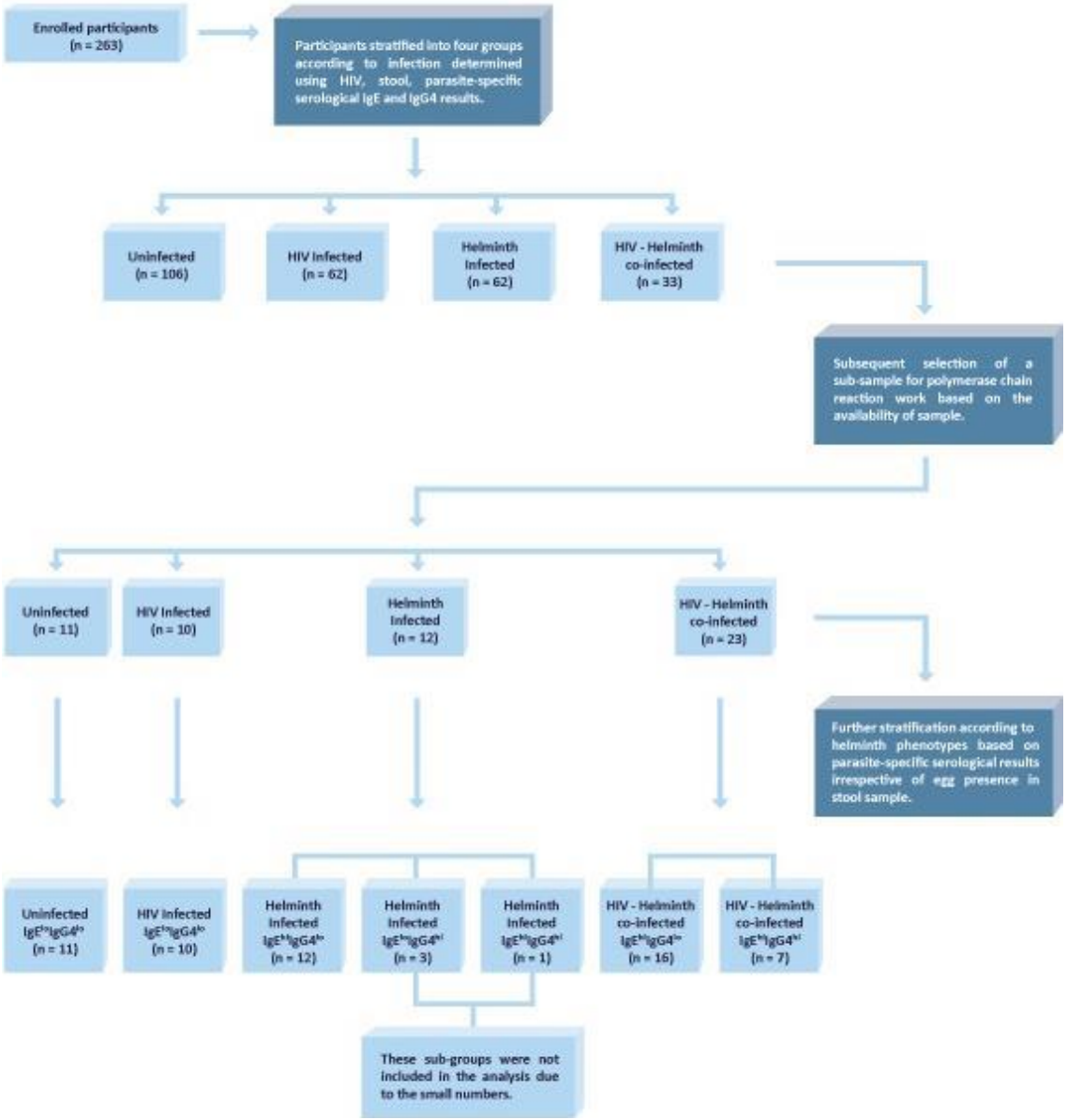

Figure 1: The stratification of HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected participants

The sample preparation for RNA isolation, quantification and analysis using real-time PCR was conducted in the HIV Pathogenesis Programme (HPP) Laboratory, at UKZN, Durban. The methods used in the quantification of the cytokine genes expression assays using quantitative PCR were standardized in the laboratory (Singh *et al.* 2011).

RNA isolation and analysis

Each of the selected samples were prepared by adding 1 X RBC lysis solution (Qiagen, Germany), and were stored in RLT buffer from an RNA extraction kit (RNeasy kit; Qiagen, Germany) in a -80^{0}C freezer. RNA was then extracted immediately after thawing of the samples, using the RNeasy kit according to the manufacturer's instructions. The total RNA concentration was quantified, and the samples were used only if the optical density (OD_{260}) /OD_{280} ratio was 1.90 or higher. All RNA samples were DNase treated (Thermo Fisher Scientific Inc., USA). One microgram of total RNA from each sample was reverse transcribed by using the iScript cDNA synthesis kit (Bio-Rad, USA) following the manufacturer's instructions. The samples that were selected for analysis were those with optical density (OD_{260})/ OD_{280} ratio of 1.90 or higher, which showed purity of the cDNA, using a Nanodrop.

RNA quantification of cytokine genes by real-time PCR

The PCR primers and cycling conditions used for IFN-γ, TNF-α, IL-4, IL-10 and CD38 cytokine genes real-time quantitative PCR were validated in the laboratory and are provided in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined to be the most suitable reference gene among the five genes based on PCR efficiency of 87% (Singh *et al.* 2011). Each PCR mixture, added into each tube was comprised of 0.5pmol/µl for primer 1 (forward template) and primer 2 (reverse template) for IFN-γ, TNF-α, IL-4, IL-10 and CD38 or 0.25pmol/ µl for each forward and reverse primer for GAPDH; 5µl SYBR green 1 master mix (2x) (Roche), 1µg cDNA, and RNAse-free water to 10µl. Positive and negative controls were included, which were the GAPDH housekeeping gene and PCR-grade water with no cDNA added, respectively. The samples in a PCR 96-well plate were run in duplicate on a Roche Light-Cycler 480 version 1.5 instrument (Roche Diagnostics), with 1 cycle at 95^{0}C for 10 minutes, followed by 45 cycles consisting of denaturation, annealing, and extension steps (Table 1). Detection of the fluorescent products was carried out at the end of the 72^{0}C extension period. To confirm amplification specificity, the PCR products were subjected to a melting-curve analysis. Standard curves were generated using the Universal Human Reference RNA (Stratagene, USA) for quantitative analysis and also to determine the concentration of the unknown samples, reported as ratio of the unknown gene over GAPDH.

Table 1: The PCR primers and cycling conditions used in the cytokine gene expression real-time quantitative PCR

Gene	GenBank accession no	Sequence (5′-3′)*	Cycling conditions (denaturation, annealing and extension)
IFN-γ	NM_000619	5′-TCGGTAACTGACTTGAATGTCCA-3′ (F) 5′-TCGCTTCCCTGTTTTAGCTGC-3′ (R)	95^0Cfor 30s, 60^0C for 30s, and 72^0C for 30s
TNF-α	NM_006291	5′-GGCCAATGTGAGGGAGTTGAT-3′ (F) 5′-CCCGCTTTATCTGTGACCC-3′ (R)	95^0Cfor 30s, 55^0C for 30s, and 72^0C for 30s
IL-4	NM_172374	5′-GCCAAGACCCCTTCGAGAAAT-3′ (F) 5′-CCGATCCTGTTATCTGCCTCC-3′ (R)	95^0Cfor 30s, 60^0C for 30s, and 72^0C for 30s
IL-10	NM_000572	5′-GACTTTAAGGGTTACCTGGGTTG-3′ (F) 5′-TCACATGCGCCTTGATGTCTG-3′ (R)	95^0Cfor 30s, 60^0C for 30s, and 72^0C for 30s
CD38	NM_001775	5′-CAACTCTGTCTTGGCGTCAGT-3′ (F) 5′-CCCATACACTTTGGCAGTCTACA-3′ (R)	95^0Cfor 30s, 60^0C for 30s, and 72^0C for 30s
GAPDH	NM_002046	5′-AAGGTCGGAGTCAACGGATT-3′ (F) 5′-CTCCTGGAAGATGGTGATGG-3′ (R)	95^0Cfor 30s, 65^0C for 30s, and 72^0C for 30s

*F: forward; R: reverse

2.4 Statistical data analysis

Summary statistics (mean, medians, frequencies and percentages) were used to describe the immune profile (lymphocyte subsets and cytokine levels) and intestinal helminth serology phenotypes (*Ascaris*-specific IgE and IgG4) of the participants. All gene expression data (the messenger-ribonucleic acid (mRNA) levels) were log transformed to ensure normality. The association between helminth infection phenotypes: (1) $IgE^{hi}IgG4^{lo}$ and (2) $IgE^{hi}IgG4^{hi}$ and the immune profile among the intestinal helminth singly infected and the HIV-intestinal helminth co-infected participants was also assessed. A small proportion of the intestinal helminth singly infected participants (n = 4) were excluded from the analysis, due to the small number of the phenotypes, $IgE^{lo}IgG4^{hi}$: n = 3 and $IgE^{hi}IgG4^{hi}$: n = 1. Groups singly or co-infected with HIV and intestinal helminth parasites, with or without helminth infection phenotypes, and those uninfected were compared using the Kruskal-Wallis test. Median mRNA expression levels between HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected groups were compared using unpaired *t* test. The differences with the p value of ≤ 0.05 and 95% confidence interval (CI) were considered statistically significant. Data was analysed using SPSS version 25 (IBM Corporation., USA) and GraphPad Prism version 5.01 (GraphPad Software, Inc., USA).

2.5 Ethical considerations

Ethical approval to conduct the study was obtained from University of KwaZulu-Natal Biomedical Research Committee (BREC Ref: BE 230/14). Permission to conduct the study was

granted by the Provincial and eThekwini Health District office and the KZN Provincial Department of Health as well as the local political authorities.

The HIV infected participants who had CD4 counts below 350 cells/µl (n = 28) were referred to the primary health care clinic (PHC), and were excluded from participating in the study as per the ethical directive of protection of vulnerable individuals such as very sick or severely immunocompromised. In addition, the participants who were found to be infected with helminths were referred to the clinic for antihelminthic treatment.

3. Results

3.1 Characteristics of the study sample

Of the 263 participants, the mean age was 36 years, and the majority of the study participants were female (91.6%). Overall, the prevalence of HIV was 36.1% with 23.6% HIV singly infected and 12.5% co-infected with intestinal helminths. Similarly, the prevalence of intestinal helminths was 36.1%, based on stool and *Ascaris*-specific IgE and IgG4 serology. The species detected in the participants who were diagnosed with helminthiasis by eggs/ova in their stool samples (n = 31), were *Ascaris lumbricoides* (80.6%) and *Trichuris trichiura* (12.9%) and 6.5% were infected with both intestinal helminth species. The serological diagnosis revealed a prevalence of 24.3% compared to that diagnosed by eggs/ova detection positivity only, which was 6.5%.

The HIV-intestinal helminth co-infected and the HIV infected participants were generally younger than the rest of the groups, with mean age of 30.5 and 32.9 years respectively. The intestinal helminth infected participants were older, with mean age of 41.6 years. Furthermore, a higher proportion of uninfected participants were observed to be overweight and obese (body mass index of ≥ 30 kg/m^2) compared to the other participant groups, although with no statistical significance ($p = 0.097$).

3.2 The immune profile of the study participants

Table 2 shows the comparison of the immune markers (total T lymphocytes, CD4, CD8, CD3 and eosinophil counts), *Ascaris*-specific antibody levels, viral load and CRP among HIV and intestinal helminth singly and co-infected individuals. Although within reference ranges, the mean levels of immune markers varied across the participant groups. However, the comparison may have been affected by the small sample size among the study groups. Overall, the CD4 counts were significantly lower ($p < 0.001$) in the HIV singly infected and HIV-intestinal

helminth co-infected participants [649.8 (242.8); 729.0 (283.5)] compared to those of the uninfected participants [973.4 (296.3)]. The CD8 counts were significantly higher ($p < 0.001$) in the HIV singly infected and HIV-intestinal helminth co-infected participants [1059.7 (451.2); 1127.9 (634.5)] compared to those of the uninfected participants [682.15 (369.4)]. The lymphocyte counts were significantly lower ($p = 0.025$) in the HIV infected group [2.12 (0.59)] compared to the uninfected participants [2.28 (0.64)] and the eosinophil counts were significantly higher ($p = 0.015$) in the intestinal helminth singly and co-infected groups [0.30 (0.28); 0.40 (0.50)] compared to the uninfected participants [0.18 (0.16)]. The *Ascaris*-specific IgE ($p < 0.001$) and IgG4 ($p < 0.001$) antibodies were significantly higher in the co-infected group [2.51 (5.15) and 0.787 (3.125)] compared to the intestinal helminth singly infected group [1.72 (3.51) and 0.096 (0.131)]. The HIV viral load was lower in the co-infected participants compared to those singly infected with HIV, although with no statistical significance ($p = 0.496$). The CRP levels were higher than the reference range in all participant groups, although with no statistical significance ($p = 0.290$).

Table 2: The profile of immunological markers indicating the differences among HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected participants (n = 263)

Immune markers mean (SD)	**Reference range**	**Uninfected (n=106)**	**HIV infected (n=62)**	**Intestinal helminth infected (n=62)**	**HIV-intestinal helminth co-infected (n=33)**	**p-value***
	mean (SD)	**mean (SD)**	**mean (SD)**	**mean (SD)**	**mean (SD)**	
CD4 count (cells/µl)	404 – 1612	973.4 (296.3)	649.8 (242.8)	1014.0 (322.2)	729.0 (283.5)	<0.001
CD8 count (cells/µl)	220 – 1129	682.15 (369.4)	1059.7 (451.2)	671.4 (294.1)	1127.9 (634.5)	<0.001
CD3 count (cells/µl)	723 – 2737	1714.7 (554.1)	1771.1 (538.4)	1746.3 (556.2)	1931.3 (754.4)	0.496
HIV viral load (copies/ml)[§]			58093.38 (243288.2)		12288.15 (28898.9)	0.496
Lymphocyte count (X 10^9/l)	1.5 – 4.0	2.28 (0.64)	2.12 (0.59)	2.46 (0.97)	2.46 (0.89)	0.025
Eosinophil count (X 10^9/l)	0.04 – 4.0	0.18 (0.16)	0.18 (0.15)	0.30 (0.28)	0.40 (0.50)	0.015
Ascaris-specific IgE (kU/l)	below 0.35	0.08 (0.08)	0.07 (0.09)	1.72 (3.51)	2.51 (5.15)	<0.001
Ascaris-specific IgG4 (mgA/l)	below 0.15	0.015 (0.018)	0.020 (0.032)	0.096 (0.131)	0.787 (3.125)	<0.001
C-reactive protein (mg/l)	0 – 5	7.1 (11.3)	5.2 (7.1)	5.7 (10.7)	6.8 (12.4)	0.290

*p-value significant at $p \leq 0.05$; [§]The HIV viral load was only assayed for participants who were singly or co-infected with HIV

Figure 3 A-E shows the comparison of immune cytokine profiles among HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected individuals. The results revealed that the HIV-intestinal helminth co-infected participants had significantly higher expression of IFN-γ and TNF-α cytokine genes (Th1 responses), lower IL-4 gene expression (Th2 response) and higher IL-10 expressed gene levels (Treg response). Notably, the intestinal helminth singly infected individuals showed lower levels of IL-4 gene expression. Furthermore, there was no significant difference in the levels of gene expression of the CD38 activation marker across all the participant groups.

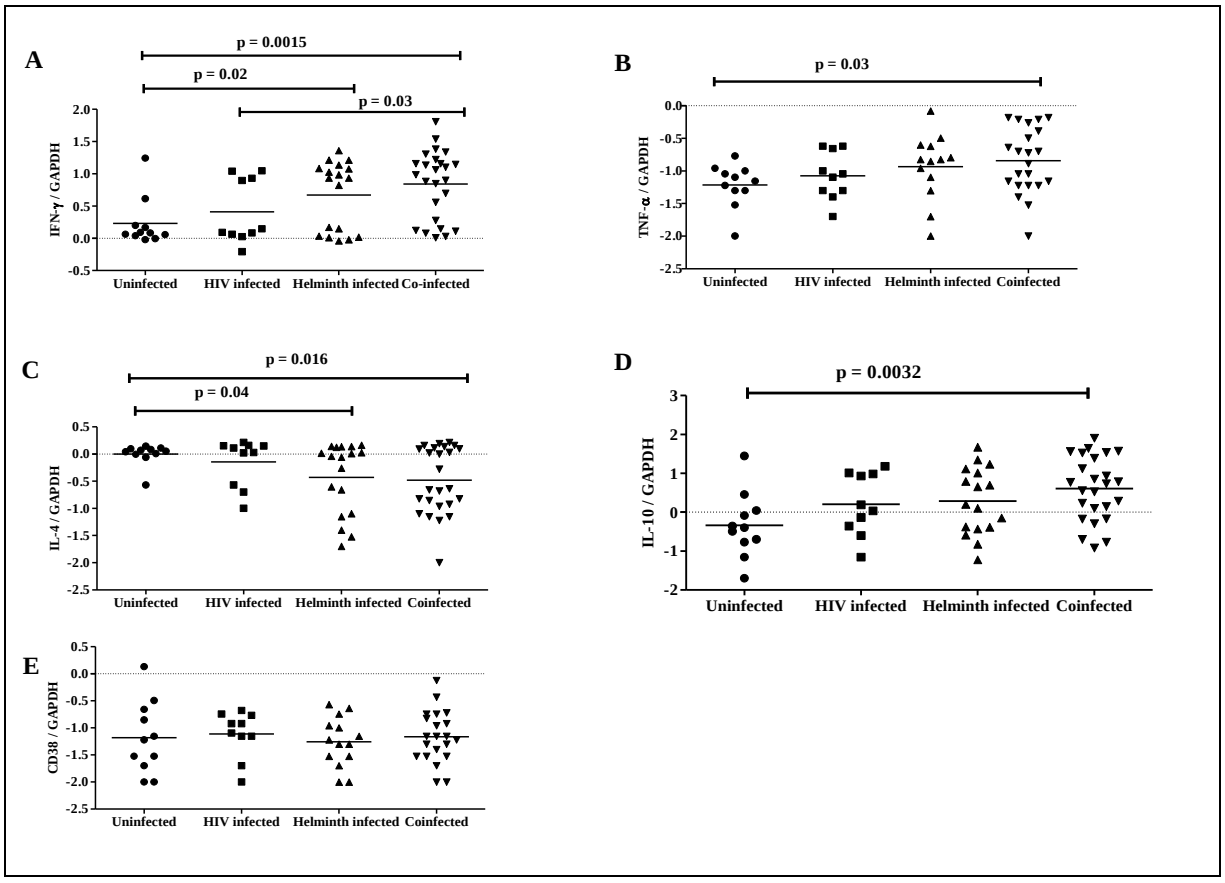

Figure 3 A-C: Comparison of the mRNA levels of cytokine genes for the Th1, Th2, Treg and CD38 responses among HIV singly infected, intestinal helminth singly infected, HIV-intestinal helminth co-infected and uninfected participants using unpaired *t*-test. (A) and (B) depicts expression of IFN-γ and TNF-α which were used as surrogate markers of Th1 responses, (C) expression of IL-4, used as surrogate marker of Th2 responses, (D) expression of IL-10, used as surrogate marker of Treg responses, and (E) expression of CD38, used as surrogate marker of activation. Data are depicted as the normalised ratios of IFN-γ, TNF-α, IL-4, IL-10 and CD38 to GAPDH.

Table 3 shows comparison of the immune markers and CRP among intestinal helminth singly and co-infected participant groups with different helminth infection phenotypes. Both the HIV-intestinal helminth co-infected sub-groups with $IgE^{hi}IgG4^{lo}$ and $IgE^{hi}IgG4^{hi}$ helminth infection phenotypes had significantly lower median CD4 counts compared to the uninfected group [759.5 (387-1151); 607.0 (438-1109)] vs 881 (657-1268)] ($p = 0.003$). This indicates that among the co-infected participants, the sub-group with the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype had lower CD4 counts compared to those with the $IgE^{hi}IgG4^{lo}$ phenotype. Furthermore, both the co-infected sub-groups had higher CD8 counts, with higher counts observed in the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype [1091.0 (606-2034)] compared to those with the $IgE^{hi}IgG4^{lo}$ phenotype [901.0 (265-3500)] ($p = 0.004$).

The eosinophil counts were higher in the intestinal helminth infected group compared to the co-infected sub-groups, although with no statistical significance ($p = 0.408$). The co-infected sub-group with the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype had a higher eosinophil count than the co-infected sub-group with the $IgE^{hi}IgG4^{lo}$ helminth infection phenotype. Although with no statistical significance, the co-infected participants with the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype had higher *Ascaris*-specific IgE ($p = 0.164$) and IgG4 levels ($p = 0.438$).

Table 3: Immune markers, biochemical and haematological indicators of immune status among the intestinal helminth singly infected and HIV-intestinal helminth co-infected with $IgE^{hi}IgG4^{lo}$ and with $IgE^{hi}IgG4^{hi}$ helminth infection phenotypes

Participant groups (n=46)	Reference range	Uninfected $IgE^{lo}IgG4^{lo}$ (n=11)	Intestinal helminth infected $IgE^{hi}IgG4^{lo}$ (n=12)	HIV-intestinal helminth co-infected $IgE^{hi}IgG4^{lo}$ (n=16)	HIV-intestinal helminth co-infected $IgE^{hi}IgG4^{hi}$ (n=7)	p-value*
Median (minimum – maximum)						
CD4 count (cells/µl)	404-1612	881 (657-1268)	1093.5 (353-1732)	759.5 (387-1151)	607.0 (438-1109)	0.003
CD8 count (cells/µl)	220-1129	464 (274-1381)	621.5 (223-1368)	901.0 (265-3500)	1091.0 (606-2034)	0.004
CD3 count (cells/µl)	723-2737	1388 (1118-2794)	1767 (606-2856)	1864.5 (782-4714)	1804.0 (1558-2501)	0.272
Lymphocytes (X 10^9/l)	1.5- 4.0	2.05 (1.25-3.43)	2.37 (1.69-3.67)	2.46 (1.02-5.35)	2.45 (2.05-3.07)	0.242
Eosinophils (X 10^9/l)	0.04- 4.0	0.13 (0.03-0.81)	0.44 (0.13-0.76)	0.23 (0.01-1.73)	0.37 (0.07-1.83)	0.408
Ascaris-specific IgE (kU/l)	below 0.35	0.04 (0.0-0.16)	0.735 (0.36-4.66)	1.055 (0.37-3.52)	2.87 (0.43-29.5)	0.164
Ascaris-specific IgG4 (mgA/l)	below 0.15	0.01 (0.0-0.06)	0.030 (0.0-0.14)	0.02 (0.01-0.05)	0.47 (0.16-17.7)	0.438
CRP (mg/l)	0 – 5	3.1 (0.0-12.6)	3.6 (0.0-13.8)	2.90 (0.0-23.0)	4.6 (1-63.5)	0.486

*p-value significant at $p \leq 0.05$

Further analysis which differentiated the medians of the immune markers was done, to elucidate the effect of the helminth infection phenotypes between both the HIV-intestinal helminth co-infected sub-groups with $IgE^{hi}IgG4^{lo}$ and $IgE^{hi}IgG4^{hi}$ helminth infection phenotypes compared to the uninfected group (Figure 4 A-G). Furthermore, the viral load was compared between both the co-infected sub-groups with $IgE^{hi}IgG4^{lo}$ and $IgE^{hi}IgG4^{hi}$ helminth infection phenotypes compared to the HIV singly infected group (Figure 4H). The analysis revealed that, compared to the intestinal helminth singly infected group with $IgE^{hi}IgG4^{lo}$ helminth infection phenotype, the co-infected group with $IgE^{hi}IgG4^{hi}$ helminth infection phenotype had lower CD4 counts ($p = 0.077$), higher CD8 counts ($p = 0.06$), significantly higher *Ascaris*-specific IgE ($p < 0.001$) and IgG4 levels ($p < 0.001$), higher CRP levels ($p = 0.470$) and higher viral load ($p = 0.974$).

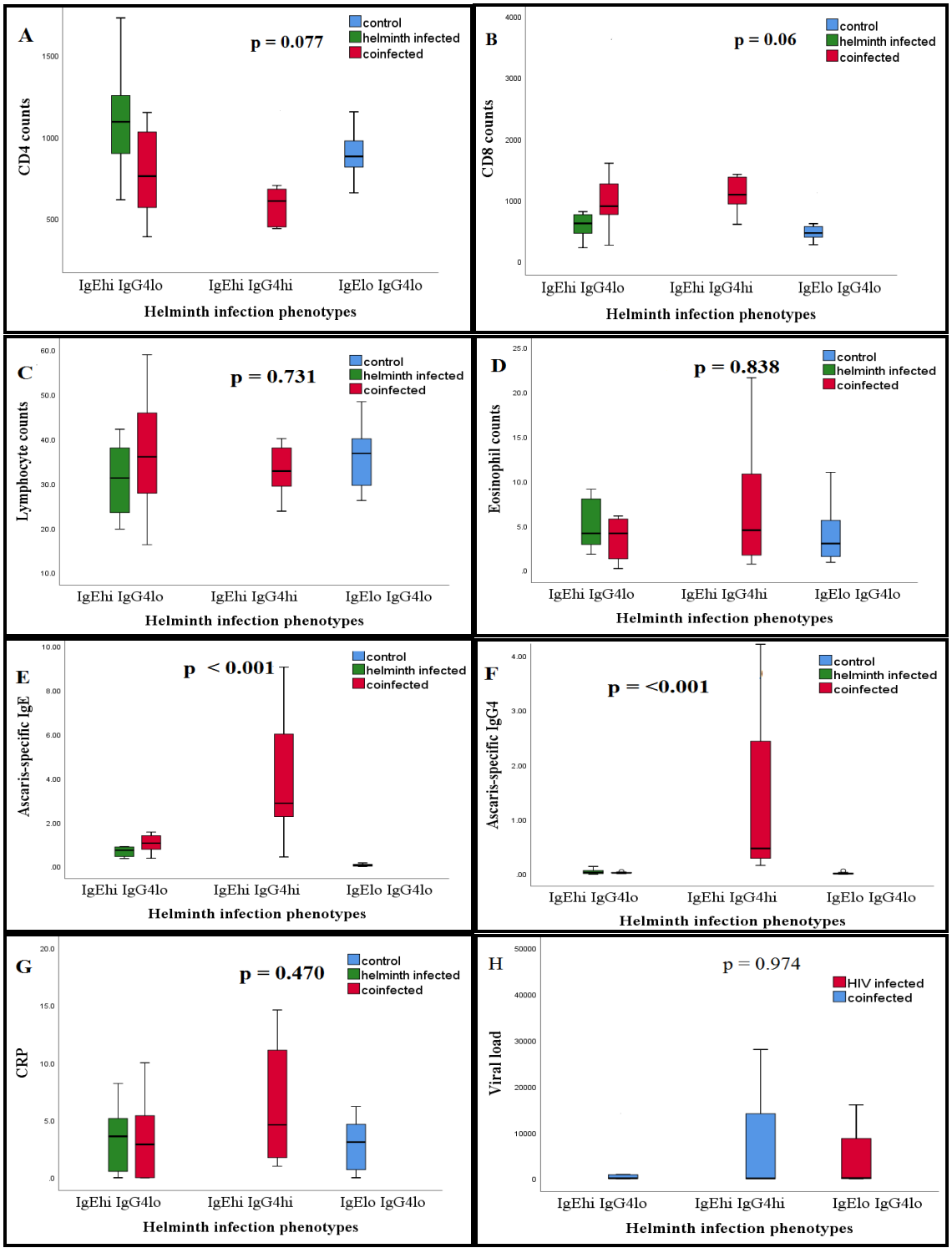

Figure 4: Boxplots indicating the differences in the immune profile of the HIV-intestinal helminth co-infected participants, comparing the medians of immune markers between the intestinal helminth singly infected and the co-infected participants with the $IgE^{hi}IgG4^{lo}$ with those co-infected with the $IgE^{hi}IgG4^{hi}$ helminth infection phenotypes, using the Kruskal Wallis ANOVA comparison of phenotypes against the uninfected group used as a control (A to G). The HIV singly infected group was used as a control for the differences in the viral load (H).

Discussion

In light of the cumulative effects that the HIV and intestinal helminth single infections has on the immune system of affected hosts, and in the backdrop of a high proportion of individuals predisposed to these infections in South Africa, the current study aimed to characterise the immune profile of the HIV and intestinal helminths co-infected adult population in KZN, South Africa. The results showed that in this adult population, a significant proportion of the intestinal helminth singly infected participants (36.1%) with a mean age of 42 years, were infected with *Ascaris lumbricoides* and *Trichuris trichiura* intestinal helminths. This highlights the fact that intestinal helminths may infect adults in higher proportions, contrary to the notion that they predominantly affect school-children (Arinola *et al.* 2015a). The results further showed that those participants singly or co-infected with HIV were generally younger, with a mean age of 30.5 and 32.9 years, respectively. This finding is relatively similar to the findings of the South African national survey on HIV where the highest HIV prevalence was observed in the 35 to 39 year old age group, especially among females (Simbayi *et al.* 2018).

The study characterised the immune profile of individuals singly and co-infected with HIV and intestinal helminths, and found that the co-infected participants had low CD4 and high CD8 counts compared to the uninfected group. Both HIV and helminth single infections have been associated with reduced CD4 and increased CD8 counts (Borkow and Bentwich 2004; Means *et al.* 2016; Morawski *et al.* 2017). HIV infected individuals were also observed with significantly lower CD4 counts, higher CD8 counts as well as a higher viral load, which is a documented hallmark of HIV infection (Ndumbi *et al.* 2014; McBride and Striker 2017; Trickey *et al.* 2017). The findings also showed that the intestinal helminth singly infected group had the highest CD4 counts compared to the rest of the infected groups, including those uninfected. This may be attributed to the observed eosinophilia in the intestinal helminth singly and dually infected participants. Helminths are classic inducers of eosinophils, and they express CD4 molecules upon activation (Hartnell *et al.* 1993).

Further analysis revealed that HIV-intestinal helminth co-infected participants had increased levels of expression for IFN-γ and TNF-α cytokines, reduced expression levels for IL -4 and increased IL-10 cytokine gene expression, which is a typical antiviral cytokine profile (Janeway *et al.* 2001). The reduced IL-4 levels would possibly promote antiviral Th1 responses, since Th2-derived IL-4 mediates the attenuation of inflammatory Th1 responses (Mitchell *et al.* 2017). The lower viral load in HIV-intestinal helminth co-infected individuals may explain this response compared to HIV singly infected individuals. This was contrary to Mulu *et al.* (2013)

who observed a higher viral load in co-infected individuals compared to those who were singly infected with HIV. However, in agreement with current findings, (Roberts *et al.* 2010) associated lower HIV viral load set-point with higher levels of IFN-γ cytokines.

Our observation of increased Th1 cytokine responses and reduced Th2 cytokine responses with increased Treg responses in co-infected participants may not fully support the hypothesis that helminth infection evokes stronger Th2 with immunoregulatory responses, which may suppress Th1 responses. It is documented that helminths induce strong Th2 responses that result in the production of cytokines such as IL-4, IL-5, IL-10 and IL-13, with increased activation of Th2 effector cells including eosinophils, mast cells, basophils and increased IgE and IgG4 antibody production (Anthony *et al.* 2007; Figueiredo *et al.* 2010; Abdoli and Pirestani 2014; Arinola *et al.* 2015a). Instead, in the current study the co-infection was associated with increased Th1 responses and reduced Th2 cytokine response, albeit increased Th2 effector responses which included eosinophilia and high IgE and IgG4 antibody levels. This discrepant result of low IL-4 gene expression and eosinophilia with high antibody levels cannot be explained.

Furthermore, when the effect of the helminth infection phenotypes on the immune profile of intestinal helminth singly and HIV-intestinal helminth co-infected participants was analysed, the key finding was significantly lower CD4 and higher CD8 counts in the co-infected participants with the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype compared to the co-infected group with the $IgE^{hi}IgG4^{lo}$ helminth infection phenotype. For the purpose of down-regulating the aberrant Th2 responses helminths induce the production of high IgG4 antibodies, which suppress the inflammatory activity of IgE antibodies (McSorley and Maizels 2012) and thus counteract the host-protective physiological responses of IgE (Maizels and Yazdanbakhsh 2003; Fitzsimmons *et al.* 2014). In support, Jiz *et al.* (2009) found that individuals who had both IgE and IgG4 responses had increased intensity of helminth re-infection as opposed to those who mounted sole IgE responses, who were able to resist re-infection. The majority of the participants in the current study had moderate intestinal helminth infections and only two participants had heavy infections (a grading of more than 400 eggs per gram of stool using the Kato Katz and MiniParasep methods (Ng'etich *et al.* 2016; Barenbold *et al.* 2017)). This could be explained by the fact that participants were adults and it is reported that generally adults usually present with moderate infections (Hall *et al.* 2008). Furthermore, the co-infected participants were observed with high levels of expressed IL-10 cytokine genes. The production of IgG4 while suppressing IgE in helminth infection is induced and regulated by IL-10 (Figueiredo *et al.* 2010; Girgis *et al.* 2013; Filbey *et al.* 2014; Maizels and McSorley 2016).

The current study did not find any evidence of immune activation or the presence of inflammation in HIV-intestinal helminth co-infected participants, as would be defined by high CD38 cytokine gene expression and/or elevated CRP levels respectively. Instead, CRP levels and expressed CD38 cytokine gene levels were not significantly different across all the participant groups, including those uninfected. This finding was not expected, as it was contrary to the findings of others, where untreated HIV infected individuals showed evidence of HIV-induced immune activation, shown by increased CD38 levels (Manjati *et al.* 2016). High expression of CD38 levels, even though assayed alone or in combination with HLA-DR have been reported to indicate an activated immune system (Meditz *et al.* 2011). Both HIV and helminth single infections are associated with immune activation and/or inflammation (Chachage *et al.* 2014; d'Ettorre *et al.* 2014; Sereti *et al.* 2016). The cause for this discrepant result cannot be explained.

Limitations

Due to the cross-sectional design, this study could not establish causality and was limited to determining the immune profile and the effect of the helminth infection phenotypes on the responses among HIV and intestinal helminth singly and co-infected individuals. The findings provided a "snapshot" of the interactions between HIV and intestinal helminth single and co-infections with immunity in the cohort investigated. The purposive recruitment of the participants could not allow to make findings generalizable. The majority of the study participants were female. This was due to the fact that by default the most of the clinic attendees were female, recruited from a single primary health care clinic. This is a limitation. Furthermore, it may have introduced bias due to the physiological variation of gene expression patterns and other immune markers within the different genders.

Although the sample size was calculated appropriately for power prior to the commencement of the investigation, the design of the study which stratified the participants by infection status resulted in small numbers in the infection groups and sub-groups. Furthermore, the small sample size, more so in the helminth infection phenotype sub-groups may have resulted in the inability to detect significant differences in CRP and viral load among the HIV-intestinal helminth co-infected sub-groups. In addition, the inability to analyse the effect of the $IgE^{lo}IgG4^{hi}$ helminth infection phenotype, referred to as the modified Th2 cell response phenotype on the immune profile (Maizels and Yazdanbakhsh 2003; Maizels *et al.* 2004) due to small numbers was another limitation. However, the observation of low CD4 counts in co-

infected individuals with $IgE^{hi}IgG4^{hi}$ helminth infection phenotype is an important finding that requires further exploration.

The inability to determine significant associations between HIV and intestinal helminths single and co-infection with CD38 gene expression and CRP, so as to indicate the levels of immune activation among the study groups was a limitation. It was hypothesized that the activation levels would be high based on the fact that both chronic HIV and helminths single infections induce immune activation. Another limitation was the inability to assay for the genetic level of expression for HLA-DR to validate the CD38 result due to budgetary limitations. The inability to determine the association between co-infection with HIV and intestinal helminths and the cytokine expression by assaying for intracellular or plasma cytokine levels using flow cytometry was a limitation. Due to costs of the analytical tests and the budgetary limitations, to only be able to analyse a limited number of samples for cytokine gene expression was another limitation. These limitations may have played a role in the study hypothesis not being completely supported by the current findings. In particular, the possible antiviral cytokine profile associated with co-infection was an important finding that may require further interrogation.

The study did not determine the infection intensity of the intestinal helminths, to be able to correlate the parasite-specific antibody levels and the helminth infection phenotypes. This was a limitation. The design of this investigation could not establish the stages of both HIV and intestinal helminth infections among the study participants, whether primary or chronic and thus the analysis included all in the same group. This resulted in the inability to decipher whether the responses were according to the disease stage. It was not feasible to determine the role played by the differences brought about by the stage of the disease, in particular the levels of expression of the cytokine genes, especially the lower IL-4 gene expression in the helminth singly infected individuals. The low levels of IL-4 cytokine gene expression in helminth singly infected participants cannot be explained.

Furthermore, a small proportion of the intestinal helminth singly infected participants (n = 4) was excluded from the analysis of the effect of helminth responses on the immune profile, due to the small numbers of the helminth infection phenotypes: ($IgE^{lo}IgG4^{hi}$: n = 3 and $IgE^{hi}IgG4^{hi}$: n = 1); this was a limitation and it may have introduced bias. The results therefore could not provide conclusive interpretations. In addition, the inability to analyse the effect of the presence or absence of eggs as well as the effect of the $IgE^{lo}IgG4^{hi}$ helminth infection phenotype on the

immune profile was a limitation. This may have indicated what the effect of helminth responses that trigger the sole production of high immunoregulatory IgG4 levels would have been on the immune profile of co-infected individuals.

Nevertheless, despite these mentioned limitations, this however is the first such study that has been conducted in a KZN adult population wherein the impact of the HIV and intestinal helminth single and co-infection and the effect of the helminth infection phenotypes on the immune profile of individuals singly and co-infected with HIV and intestinal helminths was assessed. This study highlights an area in research that requires more attention: the interaction of HIV-intestinal helminth co-infection with immunity and the effect the responses to intestinal helminths have in the face of competent immune responses. Future prospective studies should be longitudinal in design and community based, with randomised sampling and large sample sizes. Furthermore, longitudinal studies that may determine changes in immune responses after effective antihelminthic treatment are recommended.

Concluding remarks

Notwithstanding the limitations of the sample size, the gender and selection bias, however the findings from the study suggest possible potent HIV responses that intestinal helminths may induce. The study findings do not support the hypothesis that suggests that intestinal helminths induce a stronger Th2 response, which may significantly suppress Th1 responses that are essential to control HIV.

On the other hand, the study highlights the usefulness of including serological intestinal helminth infection phenotypes using parasite-specific IgE and IgG4 antibody levels when investigating the interactions of the HIV and intestinal helminth single and co-infection with immune status. The simultaneous increase in both *Ascaris*-specific IgE and IgG4 antibodies in the co-infection was associated with an immune profile of lower CD4 and higher CD8 counts, which as the disease progresses the effector cell responses required to limit HIV may weaken.

Future studies should interrogate the possible potent HIV responses that intestinal helminths may induce when they attenuate the Th2 cytokine responses. Furthermore, the influence of the intestinal helminth infection phenotypes on the levels of expression of Th1, Th2, and Treg cytokine genes must be explored, to elucidate the responses associated with HIV-intestinal helminth co-infection and the intensity of the intestinal helminth infection. These studies should be longitudinal in design, with large sample sizes.

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6.0 CHAPTER 6

Synthesis, conclusion and recommendations

6.1 Synthesis

Despite global efforts to eliminate the highly prevalent HIV epidemic, strategies to curb new infection and HIV-related deaths are still met with challenges especially in resource-limited settings, with no immediate availability of a cure or vaccine (Kharsany and Karim 2016). Notwithstanding the challenges of this global epidemic, great strides have been made regarding the control of HIV infection in particular, in the southern region of sub-Saharan Africa, which is the epicentre of this disease. These strategies include and are not limited to declines in new infections (UNAIDS 2017b), decreased mortality rates and widespread antiretroviral coverage (Mutevedzi and Newell 2014). The fact that there are factors that promote HIV transmission and entry into target cells, illustrates that HIV infection may be enabled. These factors include sexually transmitted infections (Ward and Ronn 2010) due to inadequate condom use for cultural and socio-economic reasons (Sarkar 2008); as well as cultural and poor socio-economic reasons including malnutrition (Muula 2008; Oramasionwu *et al.* 2011). Hence, it is recommended that the enablers of HIV transmission and acquisition should be investigated and addressed (Temah 2009).

The geographic overlap between HIV and intestinal helminth infections occurs, especially in poor communities, where malnutrition also co-exists (Amare *et al.* 2015) and thus a significant proportion of individuals is expected to be co-infected with HIV and intestinal helminths in these communities. Intestinal helminth infection is one of the promoters of HIV transmission due to the helminth-induced epithelial damage resulting in inflammation in the genital and gastrointestinal tracts, which persistently activates the immune system and also modulates the same immune responses (Mbabazi *et al.* 2011). The modulation of the immune system is to favour the chronic existence of the parasite within the host. The chronic activation of the Th2 immune responses may result in dysregulated and weakened Th1 responses (Salgame *et al.* 2013), whereas potent Th1 responses are required particularly to control HIV (Means *et al.* 2016). In addition, the constantly activated immune system facilitates HIV entry and replication more efficiently in the activated $CD4^+$ and other target cells, which further exacerbates HIV progression (Jaspan *et al.* 2011; Chachage *et al.* 2014; Woodham *et al.* 2016). To compound the disease challenge in the HIV-intestinal helminth co-infection, HIV infection is known to induce dysregulated responses as well (Sonnenberg and Artis 2015; Vijayan *et al.* 2017). However, much attention by policymakers and funders is afforded to the HIV epidemic, whilst the

infection with helminths remains largely a neglected disease. Notably, Farrell *et al.* (2018) recommends that the World Health Organization guidelines on antihelminthic therapy must ensure that it is community-wide and that adults are included in the treatment programmes as opposed to only targeting to school-children. Furthermore, neglecting to investigate the effect of intestinal helminth infection in promoting and perpetuating HIV transmission and acquisition is a concern and we are of a strong view that it should be given due consideration and attention. In lieu of the fact that both HIV and intestinal helminth single infections have a negative effect on the immune system and they induce hyporesponsiveness, therefore the thinking was that the HIV-intestinal helminth co-infection may have a more deleterious effect on the immune system, resulting in exacerbated HIV progression.

In addition, both HIV and intestinal helminths single infection have a negative effect on the nutritional status in affected individuals, which may worsen the already compromised immune system. HIV infection affects food intake, nutrient absorption and metabolic processes due to its damage of the gastrointestinal tract (GIT) (Katona and Katona-Apte 2008; Ivers *et al.* 2009; Gedle *et al.* 2015). Likewise intestinal helminth infection may result in malnutrition due to GIT injury when the parasite breaks into the intestinal epithelial barrier for either maturation or for feeding purposes, resulting in nutrient malabsorption (Gentile and King 2018). It is well known that malnutrition lowers the potency of immune response required for the control of HIV infection, which may result in its progression (Saeed *et al.* 2016). Malnutrition interferes with resistance to infectious disease by affecting both the cell mediated and humoral arms of the immune system (Krawinkel 2012), which may further propagate the immunodeficiency and worsen the HIV-intestinal helminth disease outcome. Hence, the current study aimed to investigate the interaction between HIV and intestinal helminth co-infection with nutrition and immunity in an adult population in KZN. The study expectations were that adults who are co-infected with HIV and intestinal helminths would have lower levels of micro- and macro-nutrients, higher HIV viral loads, increased immune activation, increased gene expression of Th2 and decreased Th1 cytokine responses compared to those singly infected and those uninfected with HIV and intestinal helminths.

The first objective of the study was expected to test the hypothesis that the HIV-intestinal helminth co-infection would have a deleterious effect on the nutritional status of infected individuals. The co-infected individuals were expected to have nutritional deficiency, with reduced micro- and macronutrient biochemical levels compared to those singly infected or uninfected with HIV and intestinal helminths. It is essential that malnutrition is detected as it

may compound the burden of the two debilitating infections, especially when the immune system is compromised as is expected in co-infected individuals. It is well established that competent immune responses require adequate nutrition (Saeed *et al.* 2016). In addition, infection increases energy demands, which necessitates that energy intake be increased so to prevent wasting in the affected individuals (World Health Organization 2003).

The work (**reported in paper 1; Chapter 3**) however showed no significant association between HIV and intestinal helminths single or co-infection with micro- and macronutrient deficiency in the cohort investigated. These findings did not support the hypothesis stated since the micro- and macronutrient levels were within reference range. The laboratory uses manufacturers' reference ranges, which were not established for the local population. This may explain the possible cause of the biochemical tests being within reference range. However, a general pattern of low intake of micro- and macronutrients was noted among the participants. Paired with that was an observation of significantly increased intake of carbohydrates, which may have contributed to the overweight and obesity observed in the majority of participants, with lower energy intake. These findings of low intake of micronutrients, macronutrients and energy may point out to a risk of developing micronutrient deficiency and protein-energy malnutrition (PEM) in the participants investigated, in particular those who were co-infected with HIV and intestinal helminths, possibly due to the interaction of both these infections with nutrition.

This work added value to the growing research area on the investigation of the impact of HIV-intestinal helminth co-infection on nutritional status in South Africa, a country with frequent occurrence of obesity and overweight, which are recognised as forms of malnutrition (Cois and Day 2015). It is indicated that obesity may mask malnutrition (Garcia *et al.* 2009). This may be observed especially in adults who take time to manifest malnutrition as weight loss. Hence, malnutrition should be detected reliably even in overweight and obese individuals.

In the investigation of the interaction between nutrition and the co-infection with HIV and intestinal helminths, it was therefore crucial that the assessment of nutritional status was reliable, considering the interaction between HIV and intestinal helminth single infections and nutrition. It is established that HIV and intestinal helminth single infections can worsen malnutrition in poor communities by various mechanisms, which include nutrient malabsorption as a result of the damage to the GIT caused by both these pathogens (Ramakrishna *et al.* 2006). Also, the effect of overweight and obesity in the measurement of nutritional status in the HIV-

intestinal helminth singly or co-infected cohort that was studied was essential. This was due to the fact that obesity may mask malnutrition, particularly in adults, where measurement of weight gain through anthropometric indices may be misleading when monitoring effective nutrient replenishment (Garcia *et al.* 2009). In any case, obesity is also a form of malnutrition (Ngaruiya *et al.* 2017). Moreover, obesity is associated with low-grade chronic inflammation (Eder *et al.* 2009; Fronczyk *et al.* 2014), as occurring in single or dual infection with HIV and intestinal helminths, since both infections induce chronic inflammation (Wang *et al.* 2008; Marchetti *et al.* 2013). This therefore means that albumin, a commonly used biochemical marker of malnutrition in adults may also not be a reliable marker in detecting malnutrition in obesity and inflammatory conditions, since it is affected by inflammation (Bharadwaj *et al.* 2016). Persistent inflammation may lead to hypoalbuminaemia, due to inflammation-induced catabolism of albumin and also its redistribution from the intravascular to the extravascular compartments (Bishop *et al.* 2005; Friedman and Fadem 2010; Alves *et al.* 2018).

In light of the impact of infection singly or dually with HIV and intestinal helminths on nutritional status in the presence of inflammation and obesity, the second objective of the study aimed at investigating a biochemical marker that would measure nutritional status accurately and objectively, in the milieu of HIV-intestinal helminth single or co-infection, with or without inflammatory conditions as indicated by CRP in different BMI categories in KZN adults. It was expected that pre-albumin would be shown as more reliable and stable in the assessment of nutritional status compared to albumin in these conditions. Notably, hypoalbuminaemia is closely correlated with inflammation rather than with malnutrition, as albumin is insensitive to changes in nutritional status during nutrient replenishment, due to its half-life of twenty days, thus it can be misinterpreted as indicating malnutrition (Sathishbabu and Suresh 2012; Ishida *et al.* 2014). Pre-albumin was considered more reliable due to its short half-life of two days and its increased sensitivity to changes in nutritional status (Gaudiani *et al.* 2014). It was hypothesized that pre-albumin would be able to detect malnutrition in all inflammatory conditions which include infection and obesity.

The work (**reported in paper 2; Chapter 4**) showed a general pattern of lower albumin and pre-albumin levels among HIV and intestinal helminth co-infected individuals, in all BMI categories in the absence of inflammation (as indicated by normal CRP levels), which was suggestive of malnutrition. In all cases when CRP was elevated, indicating presence of inflammation, pre-albumin levels were higher and albumin levels lower, which indicated that pre-albumin was able to delineate between inflammation-induced hypoalbuminaemia and true

malnutrition which would have been indicated by lower pre-albumin levels. The findings suggested that low albumin levels if assayed alone could be misinterpreted as indicating malnutrition, whereas if combined with increased pre-albumin and CRP levels it may point to inflammation-induced hypoalbuminaemia.

Furthermore, discordant albumin and pre-albumin results were observed in the obese HIV-intestinal helminth co-infected group in both the presence and absence of inflammation, which was contrary to the patterns observed in other BMI categories. This highlighted the challenge in the assessment of nutritional status in HIV and intestinal helminth co-infection and obesity, in both the absence and presence of inflammation.

The third objective was to characterise the immune profile between single infection and co-infection with HIV and intestinal helminths by describing the differences in the immune responses of the adult participants. This was to determine whether co-infection results in a more weakened immunity compared to single infections with HIV and intestinal helminths. The impact of the HIV and intestinal helminth single infections on the immune system have been documented, however investigations on the interaction between the HIV-intestinal helminth co-infection with immunity are limited. Thus, this study was addressing a broader aim of determining whether the co-infection with HIV and intestinal helminths results in higher HIV viral load, lower CD4 counts, increased immune activation and increased expression of Th2 and decreased Th1 cytokine responses, compared to those singly infected and uninfected. Furthermore, the variation in the Th2 responses to helminths have been documented (Maizels and Yazdanbakhsh 2003), thus this study also aimed to determine the effect of helminth infection phenotypes on the immune profile among the intestinal helminth singly infected and HIV-intestinal helminth co-infected individuals.

The work (**reported in manuscript 3; chapter 5**) showed that the HIV-intestinal helminth co-infection was associated with an immune profile of a reduced viral load and an antiviral cytokine response profile of highly expressed IFN-γ and TNF-α cytokine genes. The levels of the CD4 and CD8 immune markers in the co-infected participants were higher compared to those observed in HIV singly infected individuals. Moreover, the CD4 counts in intestinal helminth singly infected individuals were the highest compared to all the study groups, even higher than those of the uninfected participants. This was not expected, since we had hypothesized that infection with intestinal helminths, singly or dually would be associated with low CD4 counts that would be associated with high viral loads. However, the findings did not

support this hypothesis and further observed an association of co-infection with a stronger Th1 immune response profile. Others, however, have suggested that helminths promote the activation of regulatory networks (Wammes *et al.* 2016), which may have dampened the virus replication and by extension the immune competence.

The co-infected participants that elicited both IgE and IgG4 responses were associated with weak responses showed by reduced CD4 counts. This may have been as a result of the helminth-induced down-modulatory IgG4 antibodies, wherein Th2 CD4 responses were attenuated (de Moira *et al.* 2013; Fitzsimmons *et al.* 2014). This attenuation may possibly have resulted in potent HIV responses, depicted by the significantly higher expression of IFN-γ and TNF-α cytokine genes, which indicate Th1 responses required for HIV control. Efficient control of HIV infection requires competent Th1 responses (Kaur *et al.* 2016). Notably, these co-infected participants had a lower viral load. This immune profile that supports an antiviral response was not expected due to the fact that infection with helminths is associated with polarised Th2 responses and suppressed Th1 responses (Motran *et al.* 2018) and a high viral load (Mulu *et al.* 2013). Some authors support this thinking that the typical Th2 cytokine and regulatory profile evoked by helminths have a detrimental effect on the HIV Th1 responses (Brady *et al.* 1999; Figueiredo *et al.* 2010). There is however a different school of thought that rather suggests that the helminth-induced anti-inflammatory IL-10 and TGF-β cytokines that downregulate T and B cell responses (Hoffmann *et al.* 1999; van Riet *et al.* 2007; Wang *et al.* 2008) benefit the host, whereby the cell transcription factors are down-regulated and thus HIV replication and progression is reduced (Brown *et al.* 2006). Interestingly, the co-infected group expressed higher IL-10 cytokine gene levels.

6.2 Limitations of the study

6.2.1 Study design

Due to the cross-sectional design, this study was limited to determining the associations only and could not infer causality. The findings provided a "snapshot" of the interactions between HIV and intestinal helminth single and co-infections with immunity and nutrition in the cohort investigated. The purposive recruitment of the participants have not allowed to make findings generalizable. Future prospective studies should be longitudinal in design and community based, with randomised sampling and large sample sizes.

Due to costs of the analytical tests and the budgetary limitations, the inability to measure malnutrition and to only be able to analyse a limited number of samples for cytokine gene

expression was another limitation. Furthermore, the inability to determine the association between single and co-infection with HIV and intestinal helminths and the cytokine expression by assaying for intracellular or plasma cytokine levels using flow cytometry was a limitation. This may have validated the levels of expressed cytokine genes as well as to assay for the level of gene expression for HLA-DR to validate the CD38 result. Since the study was designed to determine the immune profile of the participants by using the levels of IL-4 gene expression as a surrogate marker for T helper 2 responses, thus it was expected that single and co-infection with helminths would be associated with increased IL-4 cytokine gene expression. The reduced levels of IL-4 cytokine gene expression in helminth singly and co-infected participants cannot be explained. It is however recognised that a higher gene expression does not always translate to higher levels of circulating cytokines (Vogel and Marcotte 2012). Future studies should include the measurement of IL-4, IL-5 and IL-13 cytokine cluster gene expression to establish an effective Th2 response, noting that Th2 inducing cytokines may not only be IL-4 as some responses could be induced by thymic stromal lymphopoietin (TSLP). Furthermore, it was indicated that some helminth infections would elicit strong Th2 responses even in the absence of TSLP receptors as long as the production of inflammatory cytokines such as IL-12 is inhibited through IL-10 (Maizels *et al.* 2009).

6.2.2 Sample size and sampling

Although the sample size was calculated appropriately for power prior to the commencement of the investigation, the design of the study which stratified the participants by infection status resulted in small numbers in the infection groups and sub-groups. Furthermore, a small proportion of the intestinal helminth singly infected participants (n = 4) was excluded from the analysis of the effect of helminth responses on the immune profile, due to the small numbers of the helminth infection phenotypes: ($IgE^{lo}IgG4^{hi}$: n = 3 and $IgE^{hi}IgG4^{hi}$: n = 1); this was a limitation and it may have introduced bias. The results therefore could not provide conclusive interpretations. In addition, the inability to analyse the effect of the presence or absence of eggs as well as the effect of the $IgE^{lo}IgG4^{hi}$ helminth infection phenotype on the immune profile was a limitation. This may have indicated what the effect of helminth responses that trigger the sole production of high immunoregulatory IgG4 levels would have been on the immune profile of co-infected individuals.

Furthermore, the small sample size may have resulted in the inability to determine significant associations between HIV and intestinal helminths single and co-infection with micro- and macro-nutrient levels, BMI (especially in obesity), CRP and viral load.

6.2.3 Cohort heterogeneity

The design of this investigation could not establish the stages of both HIV and intestinal helminth infections among the study participants, whether primary or chronic and thus the analysis included all in the same group. This resulted in the inability to decipher whether the immune responses were according to the disease stage. It was not feasible to determine the role played by the differences brought about by the stage of the disease, in particular the levels of expression of the cytokine genes, especially the lower IL-4 gene expression in the helminth singly infected individuals as well as whether the presence or absence of eggs had an effect on these responses, in particular the cytokine gene expression levels.

The majority of the study participants were female. This was due to the fact that by default the most of the clinic attendees were female, recruited from a single primary health care clinic. This is a limitation. Furthermore, it may have introduced bias due to the physiological variation of gene expression patterns and other biochemical analytes within the different genders (Whitney *et al.* 2003). Moreover, the fact that the study did not determine the use of contraceptives by the female participants and their effect on the immune responses was another limitation. It is established that selected progestins such as medroxyprogesterone suppress T cells and dendritic cell responses (Huijbregts *et al.* 2014; Zalenskaya *et al.* 2018).

Quality controls were included in all the analyses and all the instruments were controlled for analytical errors. However, the day to day variation of the biochemical analytes could not be determined such as CD4 counts, CD8 counts and HIV viral load (VL) since each parameter was assayed only once, which was a limitation. In addition, the counted $CD8^+$ T cells were not discerned whether they included $CD8^+$ T regulatory cells as well, another limitation.

These limitations may have played a role in the study hypothesis not being completely supported by the current findings. Importantly, the possible antiviral cytokine profile associated with co-infection was an important finding that may require further interrogation in community based studies, longitudinal in design, with randomised sampling and large sample sizes.

The interpretation of the findings in the current study was cognisant of the mentioned limitations, as well as those alluded to in detail in the individual chapters. These include the use of self-reported food recall data collected over two days, with reliance on memory and correct estimation of quantity which was a limitation. However, the use of the skill of a well-trained fieldworker for the food recall interviews was an attempt to minimise recall bias. This has been

mentioned in paper 1 (in Chapter 3). Furthermore, the inability to screen the stool samples microscopically immediately after collection for the diagnosis of intestinal helminth infection by the presence of parasite eggs or ova is a limitation. This may have significantly affected the ability to detect hookworm eggs since these disintegrate upon storage of stools or the eggs may have hatched to first stage larvae. This may explain the non-detection of hookworm in the study. This limitation has also been mentioned in paper 1 (Chapter 3). In any case, hookworm infections have not been commonly reported in the study area as they are common in the north and south coast of KZN (Mabaso *et al.* 2004).

Nevertheless, in spite of these limitations, this however is the first such study to the best of our knowledge that has been conducted in a KZN adult population, wherein the impact of the HIV and intestinal helminth single and co-infection on the nutritional status using biochemical, anthropometric and a 24 hour food recall analysis for nutrient adequacy ratios was assessed. This study also used CRP, pre-albumin and albumin biochemical markers in the investigation of malnutrition in the context of HIV and intestinal helminths single and co-infection. In addition, the study also determined the effect of helminth infection phenotypes on the immune profile of HIV and intestinal helminth singly and co-infected individuals by comparing the levels of immune markers, biochemical and haematological parameters and cytokine gene expression among the participants.

6.3 Conclusion

The current study confirms high prevalence of helminthiasis of 36.1% among adults. Furthermore, the study highlights the importance of reliable diagnosis of helminthiasis by including the serological diagnosis of helminth infection using the levels of parasite-specific IgE and IgG4 antibodies to supplement the conventional microscopic detection of helminth eggs and/or ova in stool samples, a method that may be fraught with challenges. The current study found a serological prevalence of intestinal helminths of 24.3% compared to that of 6.5% by helminth egg and/ova detection. Helminth infection is an area of research that is neglected and is also not included in the agenda that addresses the HIV epidemic.

Notwithstanding the fact that micro- and macronutrient deficiency was not detected in the investigated cohort using biochemical indicators, however risk of malnutrition was noted based on the general low micro- and protein-energy macro-nutrient intake patterns of the population in the face of obesity and overweight that may be associated with imbalanced diets, co-existing with single or co-infection with HIV and intestinal helminths. This was the first such study that

incorporated the investigation of nutrient adequacy ratios versus the biochemical levels of micro- and macronutrients in HIV and intestinal helminth single and co-infection. This was viewed as very useful particularly since both infectious agents may cause malnutrition and thus predisposition to nutrient deficiency must be investigated at population level in HIV-intestinal helminth endemic areas. This is viewed essential as it may contribute and strengthen the South African Department of Health campaign on healthy lifestyle.

The pre-albumin biochemical marker was found to be a reliable marker instead of the commonly used albumin in the detection of true malnutrition in HIV and intestinal helminth single and co-infection in adult participants. Pre-albumin was able to delineate between hypoalbuminaemia that was inflammation-induced and that which was indicating predisposition to malnutrition. This was also first such study, which investigated malnutrition taking into consideration the complex milieu of infection, inflammation as well as weight abnormalities, as defined by CRP levels and BMI respectively. A challenge in the assessment of nutritional status in HIV- intestinal helminth co-infection and obesity, whether in the presence of inflammation or not, was noted.

Furthermore, although established that higher gene expression does not always translate to higher levels of circulating cytokines (Vogel and Marcotte 2012), however, based on the cytokine gene expression levels, HIV-intestinal helminth co-infection in this cohort was associated with reduced Th2 cytokine responses, high Treg responses and increased Th1 responses, a typical antiviral responses profile. This is the first such study that has been conducted in a KZN population where the $IgE^{hi}IgG4^{hi}$ helminth infection phenotype was associated with a weak immune response profile of low CD4 counts, particularly associated with HIV-intestinal helminth co-infection.

6.4 Public health significance of the study findings

1. The current study confirms high prevalence of helminthiasis among adults (36%), which warrants attention in the backdrop of antihelminthic programmes that are provided largely to school-children. Adults are generally not included in deworming programmes. Bopda *et al.* (2016) suggests that intestinal helminth infected adults may constitute a reservoir and source of persistent dissemination of the soil transmitted parasites. Thus, health authorities should that provision of antihelminthic treatment is community-wide and includes adults particularly in HIV-intestinal helminth co-endemic areas. In addition, there should be supply of adequate

sanitation and potable water in areas that lack such, which are risk factors to intestinal helminth infection.

2. The high prevalence of HIV among the participants (36%) requires the strengthening of HIV prevention strategies. Community based campaigns must be deployed to curb this HIV epidemic. Antenatal and circumcision clinics should be some of the areas that must be targeted within the community.

3. Furthermore, the reduction of obesity linked to high intake of carbohydrates among the participants, requires the strengthening of healthy lifestyle campaigns in the community. Also, the community needs to be supported with nutritional interventions by the government of the country and other non-governmental institutions, as one of the mechanisms of reducing obesity. In addition, education on the choice of diets with nutritional value rather than those with high-carbohydrate and low-energy should be afforded to the communities where HIV and intestinal helminths are endemic. Reduced nutritional intake or clinical malnutrition may lead to a diversion of nutrients away from the immune system (Turner *et al.* 2003) which will result in immunodeficiency and infection in a cyclical process (Katona and Katona-Apte 2008).

4. The study also highlighted the possible regulatory influence of helminths, although the sample size was small. Co-infection in this cohort was associated with reduced Th2 cytokine responses, high Treg responses and increased Th1 responses, a typical antiviral responses profile. This is an important finding that requires further interrogation.

6.5 Recommendations

This study highlighted an area in research that requires more attention, the interaction of HIV-intestinal co-infection with nutrition and immunity and the effect that the responses to intestinal helminths have in the face of HIV and intestinal helminths co-infection. We recommend a rethinking of the current HIV management, wherein the reliable detection of intestinal helminth infections and antihelminthic interventions if needed are included.

Future studies must interrogate the interactions between the serological intestinal helminth infection phenotypes with the levels of expression of cytokine genes for Th1, Th2 and Treg responses in HIV-intestinal helminth coinfection so as to elucidate the responses associated with HIV-intestinal helminth co-infection and the intensity of the intestinal helminth infection. The simultaneous increase in both *Ascaris*-specific IgE and IgG4 antibodies in the co-infection was

associated with an immune profile of lower CD4 and higher CD8 counts, which as the disease progresses the effector cell responses required to limit HIV may weaken. Thus, the parasite-specific antibody responses to intestinal helminths in the milieu of HIV and intestinal helminth infection, inflammation and malnutrition must also be explored. These studies should be longitudinal in design, with large sample sizes.

The study highlighted the possible potent HIV responses that intestinal helminths may induce while they attenuate the Th2 cytokine responses. The study findings also do not support the hypothesis that suggests that intestinal helminths induce a stronger Th2 response, which may significantly suppress Th1 responses that are essential to control HIV. Future studies should interrogate the possible potent HIV responses that intestinal helminths may induce when they attenuate the Th2 cytokine responses. All efforts must be explored that will strengthen the immune system to mount potent responses against HIV, an incurable disease that can otherwise only be controlled by competent cell-mediated responses.

Although the current study associated the co-infection with an antiviral cytokine profile, however a proportion of the co-infected group had high levels of both parasite-specific IgE and IgG4 antibodies which were associated with a weak immune response profile. As has been alluded to as a limitation, this study was not able to decipher whether this antiviral cytokine profile was generally due to the primary stage of the HIV infection in the HIV- intestinal helminth co-infected individuals and whether it would be sustainable.

The co-infection is associated with a compromised immune system, which may worsened by malnutrition associated with the co-infection and/or overweight and obesity. Furthermore,

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APPENDICES

Appendix 1.1: UKZN BREC full ethics approval for the main study

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

06 September 2012

Dr. ZL Mkhize-Kwitshana
Department of Postgraduate and Research
5th Floor Room 546A Main Building
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Mkhize- Kwitshana

PROTOCOL:The impact of HIV and Intestinal parasite co-infection on nutritional and immune status of adults receiving nutritional supplements in KwaZulu-Natal. REF: BE193/11

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 30 September 2011.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 10 August 2012 to queries raised on 18 May 2012 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 06 September 2012.

This approval is valid for one year from **06 September 2012.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **09 October 2012.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor D.R Wassenaar
Chair: Biomedical Research Ethics Committee

Professor D Wassenaar (Chair)
Biomedical Research Ethics Committee
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban, 4000, South Africa
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Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix 1.2: UKZN BREC Recertification for the main study– Year 2013 - 2014

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
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Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

14 March 2014

Dr. ZL Mkhize-Kwitshana
Department of Postgraduate and Research
5th Floor Room 546A Main Building
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Mkhize-Kwitshana

PROTOCOL: The impact of HIV and intestinal parasite co-infection on nutritional and immune status of adults receiving nutritional supplements in KwaZulu-Natal.
REF: BE193/11

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	06 September 2013
Expiration of Ethical Approval:	05 September 2014

I wish to advise you that your application for Recertification received 13 February 2014 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be **ratified** by a full Committee at its next meeting to be held on **08 April 2014.**

Yours sincerely

Ms A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix 1.3: UKZN BREC Recertification for the main study – Year 2014 - 2015

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
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Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

02 December 2014

Dr. ZL Mkhize-Kwitshana
Department of Postgraduate and Research
5th Floor Room 546A Main Building
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

Dear Dr Mkhize-Kwitshana

PROTOCOL: The impact of HIV and intestinal parasite co-infection on nutritional and immune status of adults receiving nutritional supplements in KwaZulu-Natal.
REF: BE193/11

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 06 September 2014
Expiration of Ethical Approval: 05 September 2015

I wish to advise you that your application for Recertification received 26 November 2014 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

This approval will be **ratified** by a full Committee at its next meeting to be held on **10 February 2015.**

Yours sincerely

p.p. Ms A Marimuthu
Senior Administrator: Biomedical Research Ethics

Appendix 2: UKZN BREC full ethics approval for the PhD sub-study

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

25 June 2015

Mrs Brenda Mkhize
Department of Biomedical and Clinical Technology
Durban University of Technology
P.O Box 1334
Durban, 4000
mkhizebt@dut.ac.za

PROTOCOL: Evaluation of the interactions between nutrition, immunity and co-infections with human immunodeficiency virus and intestinal parasites: The use of per-Albumin as a tool for nutritional assessment in South African adults. REF: BE230/14

EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 16 April 2014.

The study was provisionally approved pending appropriate responses to queries raised. Your responses received on 19 May 2015 to queries raised on 30 March 2015 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval.

This approval is valid for one year from **25 June 2015.** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its meeting taking place on 14 July 2015.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor V Rambiritch
Deputy Chair: Biomedical Research Ethics Committee

Appendix 3: KZN Provincial Department of Health permission letter

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Postal Address: Private Bag X54318 Durban 4000
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Email. nan.hoosain@kznhealth.gov.za
www.kznhealth.gov.za

Enquiries: Ms Jabu Hlazo
Tel: 031 240 5303
Date: 17 October 2012

Attention: Dr Z.L Kwitshana: Kwitshana@ukzn.ac.za

REQUEST TO CONDUCT RESEARCH:

"THE IMPACT OF HIV AND INTESTINAL PARASITE CO-INFECTION ON NUTRITIONAL AND IMMUNE STATUS OF ADULTS RECEIVING NUTRITIONAL SUPPLEMENTS IN KWAZULU-NATAL."

Support is hereby granted to conduct research on the above topic.

Please note the following:

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

pp District Manager
eThekwini
Telephone: 031 2405303
Fax : 031 2405500
Email: jabulisiwe.hlazo@kznhealth.gov.za

Appendix 4: Provincial and eThekwini Health District office permission letter

health
Department:
Health
PROVINCE OF KWAZULU-NATAL

Health Research & Knowledge Management sub-component
10 – 103 Natalia Building, 330 Langalibalele Street
Private Bag x9051
Pietermaritzburg
3200
Tel.: 033 – 3953189
Fax.: 033 – 394 3782
Email.: hrkm@kznhealth.gov.za
www.kznhealth.gov.za

Reference : HRKM505/15
NHRD: KZ_2015RP23_787
Enquiries : Ms G Khumalo
Telephone : 033 – 395 3189

Dear Mrs B Mkhize

Subject: Approval of a Research Proposal

1. The research proposal titled **'Evaluation of the interactions between nutrition, immunity and co-infections with human immunodeficiency virus and intestinal parasites: the use of pre-Albumin as a tool for nutritional assessment in South African adults'** was reviewed by the KwaZulu-Natal Department of Health (KZN-DoH).

The proposal is hereby **approved** for research to be undertaken at Inanda Area and Inanda Clinic.

2. You are requested to take note of the following:
   a. Make the necessary arrangement with the identified facility before commencing with your research project.
   b. Provide an interim progress report and final report (electronic and hard copies) when your research is complete.

3. Your final report must be posted to **HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200** and e-mail an electronic copy to hrkm@kznhealth.gov.za

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

Yours Sincerely

Dr E Lutge
Chairperson, Health Research Committee
Date: 28/01/15

Appendix 5: Inanda study site permission letter

ETAFULENI DEVELOPMENT FORUM

EDF

193 INANDA MAIN ROAD
P.O. BOX 22
DURBAN
4000

Phone: 031 518 0343
Fax: 031 518 0343

15 AUGUST 2012

RE: PERMISSION TO ENGAGE WITH ETAFULENI COMMUNITY FOR AN INTESTINAL WORMS RESEARCH PROJECT

Dear Lungi

Our humble apology for the delays caused in responding to your request. We are happy to inform you that on our side as a community leadership we found your justice in your project and we had general consensus in granting you permission to engage with our community for its implementation phases.

We have trust in you that you will proceed with your programme with very great care and accuracy and you will never ever take any chances, risks and advantage with lives of the innocents, our precious community.

Kindly interact with Inanda C Clinic for a further discussion with utilization of their building structure and other logistics or necessities that you might also need in your operations.

Contact details of the Manager are: Mrs Gcabashe: 031 519 2571

We will be also available for our assistance should you still need it in this regard. We wish you all the best in your endeavours to improve quality health and give life to the nation, the less-fortunate in particular.

Thank you

With humble regards

.....

M. Nyaba: Chairperson, EDF

Appendix 6 (isiZulu version): Information letter and informed consent form

UKZN BIOMEDICAL RESEARCH ETHICS COMMITTEE

INCWADI YOLWAZI NEYOKUNIKA IMVUME YOKUHLANGANYELA KUCWANINGO

Usuku:

Sawubona

Singabacwaningi abavela kwi Nyuvesi yaKwaZulu-Natali. Ucwaningo esilwenzayo luzobheka umthelela ongenziwa izilwanyazana ezitholakala emathunjini (izikelemu), ikakhulukazi uma zihambisana negciwane lengculazi, ekubeni nomthelela emasosheni omzimba nakwizondla mzimba kubantu abadala.

Ucwaningo olwenziwa kwezinye izizwe zaphesheya lukhombisa ukuthi uma abantu benezikelemu, bengazilaphi, isikhathi eside, ukuhlala kwazo emzimbeni kuthikameza amasosha omzimba kanye nezondlamzimba. Uma umuntu edla, izikelemu zithatha umsoco kuqala bese onazo angatholi lezondlamzimba azidingayo. Ngoba uma izikelemu zingalashwa, zingahlala emzimbeni isikhathi eside (iminyaka), okushukuthi namasosha omzimba, nezondlamzimba kuhlala kuthikamezekile isikhathi eside, okwenza ukuthi kube lula-ke nokuthi uma lowomuntu ehlangabezana negciwane lengculazi, lisheshe limgulise ngoba vele amasosha omzimba asuke esethikamezekile. Yingakho-ke sifuna ukwenza lolucwaningo la eMzantsi Afrika, ukubheka ukuthi:

1. Bangakanani abantu abadala abanezikelemu ezindaweni zakithi
2. Bangakanani abanezikelemu kanye nesandulela ngculazi
3. Ngabe izondlamzimba kanye namasosha omzimba kumi kanjani kulabo abangenalutho uma beqhathaniswa nalabo abanezikelemu, kanye nezikelemu nesandulelangculazi.
4. Uma sithola ukuthi baningi abantu abanalezizinkinga, sesiyonxenxa uHulumeni ukuba engeze imizamo yokuba abantu abaningi belashelwe izikelemu, ukuze bahlale bondleke kahle futhi namasosha omzimba abo ahlale esezingeni elifunekayo ukuze bakwazi ukulwa namagciwane abahlangabezana nawo, kanye negciwane lesandulelangculazi.

Kulolucwaningo sifuna ukuthola ukuthi bangaki abantu abadala abahhaqwe izikelemu endaweni yangakini. Sizobheka futhi ukuthi bangaki abanazo izikelemu kanye nesandulelangculazi. Uma lezizifo zitholakala ndawonye negciwane lengculazi, sifuna ukuthi lenzani emasosheni omzimba nakwi zondlamzimba zomuntu nokubona nesisindo sizobangakanani.

Indawo yangakini itomuliwe ohlelweni lokutomula izindawo zaseThekwini (amagama afakwe esigqokweni, kwase kutonyulwa lendawo yangakini). Isizathu-ke sokuba sikumeme yingoba uhlala

endaweni etonyuliwe ukubamba iqhaza kulolucwaningo. Ngakho sikumema ukubamba iqhaza kuloluhlelo. Uyokwazi ukuba yingxenye kuloluhlelo uma usuwaziswe wavuma wasayina ifomu lokuvuma.

Okuzokwenzeka kuwe yilokhu: uyocelwa ukuthathwa igazi lokubheka isimo sokondleka kwakho, isimo samasosha omzimba kanye negciwane lengculazi ngenhloso yokwenza izigaba ezimbili eyocwaningo necontrol. Isigaba esinye siyoba nomuntu elitholakalile kuye leligciwane, esinye kube elingatholakalanga kuye. Ucwaningo luyo kwenziwa kubantu abangu 229. Uma litholakalile igciwane lengculazi kuyobhekwa nenani leCD4, uyokwazi ukuqhubeka nohlelo uma utholakale unenani elingaphezu kuka 350 wamacells/µl. Uma etholakala engaphansi ngeke ukwazi ukuqhubeka nohlelo, uyodluliselwa emtholampilo. Owesifazane oyotholakala ekhulelwe akavumelekile ukuqhubeka kuloluhlelo. Uyothathwa isisindo sakho kanye namasampula okudingekayo kuwe ukuze kuyohlolwa elebhu ukuhlola isimo samasosha omzimba kanye nezondlamzimba. Indle nomchamo kuzosetshenziswa ukubheka amaqanda ezikelemu.

Umuntu obamba iqhaza uyocelwa ukuba anikele ngegazi elingaba ngangamathispuni ayisithupha, ayofakwa kumashubhu okuhlola ayisithupha, isampula lomchamo kanye nelendle. Igazi liyothathwa unesi ogunyaziwe ukulikhipha. Isampula lendle liyothathwa nguwe ulifake esitsheni oyosinikwa. Ufanele uwabuyisele endaweni yocwaningo ukuze kuthathwe amanye amasampula omchamo nendle emva kwezinsuku ezintathu. Amasampula amabili omchamo nawendle ayosiza ekutholeni imiphumela esingayethemba. Uyocelwa futhi ukuba ubuzwe imibuzo emaqondana nendlela yakho yokuphila kanye nangokudla oyobe ukudle ngayizolo. Uyokuzwa ubuhlungwana obuncane ngenkathi kungena inaliti yokukhipha igazi kuwe. Kungenzeka ube nokungakhululeki ngenkathi uthatha isampula lomchamo nelendle kuwe. Nakanjani ngeke sifake impilo yakho ebucayini kanye nokulimala okuthile. Unesi oqokelwe kuloluhlelo uyokusiza uma kwenzeka ubanezinhlungu noma ungazizwa kahle ngenkathi uthatha amasampula.

Ngokubamba iqhaza kuloluhlelo uyohlolwa ukuthi unazo yini izikelemu, uma zitholakala uyodluliselwa kwabezempilo ukuze uthole imithi yokuzilapha. Siyobanomhlangano namalunga omphakathi ukuwafundisa ngazo izikelemu nokuthi ziwuthinta kanjani umphakathi nokunazisa ngemiphumela yocwaningo uma isitholakele.

Uma ubamba iqhaza uzobe uvolontiya ngakho awuphoqelekile ukubamba iqhaza uma ungathandi. Noma ungabambanga iqhaza uyobe usenalo ilungelo lokusebenzisa umtholampilo noma ngasiphi isikhathi. Futhi unalo ilungelo lokunqamula ukuba yingxenye nomanini uma ungasathandi, unganxusa ukuthi amasampula akho alahlwe, ngaphandle kokulahlekelwa amalungelo akho asemtholampilo. Angeke ukhokhe lutho ngokubamba iqhaza kulolucwaningo. Kuphela uyobuyiselwa imali engango R100 yokuhlangabezana nezindleko zakho zokugibela kulezizinsuku ezimbili ozokuza ngazo endaweni yocwaningo, okuwuR50 ngosuku.

Ukugcina imininingwane yakho kanye nemiphumela yakho kuyimfihlo kuyoqikelelwa ngaso sonke isikhathi. Wonke umuntu obamba iqhaza uyonikwa inombolo, bese konke okuphathelene naye kubhalwe ngalenombolo. Imininingwane yakho emaphepheni iyovela ngezinamba bese kugcinwa endaweni ekhiywayo iminyaka emihlanu, ukhiye uhlale kumphathi wocwaningo kanti futhi imininingwane ekwi computer inepassword ukuvikela ulwazi olukuyo lungaputshuki. Imiphumela yocwaningo iyohlanganiswa yonke ndawonye bese kubhalwa amarephothi, ngeke kubhalwe ngomuntu oyedwa, futhi imininingwane yabantu ababambe iqhaza ngeke idalulwe nanoma umuphi umuntu. Igama lakho nemininingwane yakho iyogcinwa iyimfihlo. Uyonikwa inamba echaza ngemiphumela yakho hhayi igama lakho ukugcina ongumnini engaziwa. Imiphumela yakho iyobonwa ngumphathi womcwaningo, nethimba asebenza nalo kanye nezimemba zeUKZN Biomedical Research Ethics Committee.Emva kweminyaka emihlanu, amaphepha anemininingwane ayodatshulwa bese amafayela ecomputer anemininigwnae acishwe. Amasampula egazi ayogcinwa ephephile iminyaka emihlanu kwenzelwa ucwaningo olungalandela bese elahlwa ngendlela esemthethweni yaselebhu. Amasampula egazi angeke adayiswe, ayiswe kumazwe aphesheya noma asetshenziswe noma imuphi umcwaningi ongekho kuloluhlelo.

Lolucwaningo lubukisisiwe ngokomthetho lwase lwaphasiswa yiUKZN's Biomedical Research Ethics Committee (inombolo yemvume yi _____).

Uma kwenzeka ubanenkinga noma unemibuzo edlulele, ungaxhumana nabacwaningi noma neUKZN Biomedical Research Ethics Committee, ungathinta abalandelayo:

Dr Zilungile Mkhize-Kwitshana	Mrs Brenda Mkhize
Tel: 031 260 1930	031 373 5297
Fax: 086 400 0407	086 674 0817
Mobile: 078 842 4574	082 879 4923
Email: kwitshana@ukzn.ac.za	mkhizebt@dut.ac.za

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus

Govan Mbeki Building

Private Bag X 54001 Durban, 4000

KwaZulu-Natal, SOUTH AFRICA

Tel: 031 260 4769

Fax: 031 260 4609

Email: BREC@ukzn.ac.za

IFOMU YOKUNIKA IMVUME

Mina……....ngazisiwe ngocwaningo “Umthelela ongenzeka kwizondlamzimba, kumasosha omzimba kubantu abaphethwe isandulela ngculazi nezikelemu ezitholakala emathunjini: nokusebenza kwe pre-Albumin njengethuluzi lokubhekisisa izakhamzimba kubantu abadala baKwaZulu-Natali” ngu ……..……..…..……..(igama lomcwaningi).

Ngiyaqonda inhloso yocwaningo kanye nenqubo mgomo yohlelo, okungukuthi ngiyonikela ngamabhodlela ayisithupha egazi kanye namabili omchamo namabili endle yami, elilodwa liyothathwa ngosuku lokuqala elesibili lithathwa emva kwezinsuku ezinthathu.

Nginikeziwe ithuba lokubuza imibuzo maqondana nohlelo futhi ngiphendulekile ngendlela egculisayo.

Ngiyaqinisekisa ukuthi ukuzinikela kwami ukubamba iqhaza kuloluhlelo kungukuvolontiya nokuthi futhi ngingahoxa noma inini ngaphandle kokuphazamiseka kwendlela yokuthola usizo noma ukuthola impatho ejwayelekile emtholampilo.

Ngazisiwe nokuthi kuyoba khona umhlengikazi obhekelele uhlelo, uma kuba nezinhlungu noma ukungaphatheki kahle ngenkathi kuthathwa amasampula.

Uma ngibanemibuzo noma imibono noma izinkinga eziqondene nohlelo ngiyaqonda ngingathintana abacwaningi ababhaliwe encwadini enikeza ulwazi.

Uma ngiba nemibuzo noma imibono maqondana namalungelo ami njengobamba iqhaza, noma nginensolo ngendlela okuqhutshwa ngayo uhlelo noma ngabo abacwaningi ngingathintana nalaba:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION:
Tel: 27 31 2604769 Fax: 27 31 2604609 Email: BREC@ukzn.ac.za

_____ _____

Ukusayina kozimbandakanyayo **Usuku**

_____ _____

Ukusayina kukafakazi **Usuku**
(uma kudingekile)

_____ _____

Ukusayina komhumushi **Usuku**
(uma kudingekile)

Appendix 6 (English version): Information letter and informed consent form

UKZN BIOMEDICAL RESEARCH ETHICS COMMITTEE

INFORMATION LETTER AND CONSENT TO PARTICIPATE IN RESEARCH

Date:

Greetings

We are researchers from University of KwaZulu-Natal. The research we are doing will investigate the parasites found in the intestines (worms), especially if they exist together with HIV, how they impact on the immune system and the nutritional status of adults.

Research that has been done in some countries overseas have shown that individuals infected with worms for long periods of time, not treated, have their immune system and their nutrition status affected by these worms. When an individual eats, the worms use the nutrients first, then a person infected with parasites will lack the nutrients needed by his/her body. If the worms are not treated, they remain in the body for long periods of time (for years), which leads to long term disturbance of the immune system and the nutritional status and this makes it easy that when the individual is infected with HIV, the disease progresses faster since the immune system is affected. That is why we want to do this study in South Africa, to investigate:

1. How many adults are infected with worms in our areas.
2. How many people are co-infected with parasites and HIV.
3. The immune status of those not infected compared to those who are infected with parasites and those co-infected with parasites and HIV.
4. If we find out that there are many people who are infected, we will ask the government to ensure that treatment programmes for parasite infections reach many people so that their nutritional status and their immune system are maintained at levels that will enable them to fight any diseases including HIV.

In this study we want to find out how many adults are infected with parasites in your area. We will also investigate how many people are co-infected with parasites and HIV. If parasite infections are found together with HIV, we wish to know how they impact on the immune system and on the nutritional status and how they affect their weight.

Your area was chosen randomly from a list of many areas in eThekwini (names of areas were put in a hat and the area was selected). Therefore we are inviting you to participate in this study since you live in this area that was selected. We are kindly inviting you to participate in this study. You will only be able to participate in this study when you fully understand and sign an informed consent form.

The following will happen to you: you will be asked to donate blood that will be used to assess the nutritional status your HIV status so that you will be allocated into one of two groups which is a study group and a control group. The study group will comprise of parasite infected individuals and a control group will comprise of individuals with no infection. The study will enrol 229 participants. If you are found to be infected, a CD4 count will be done and you will be able to enrol in the study if it is above 350 cells/ µl. If it is below, you will be referred to a clinic and you will not be able to participate in this study. Females who are found to be pregnant will not be able to participate. You will be weighed and then blood, stool and urine samples will be collected from you and sent to a laboratory for testing your immune status and nutritional status as well as the presence or absence of parasite eggs.

As a participant you will be asked to donate blood equal to 6 teaspoons taken into 6 blood tubes, a urine sample as well as stool sample. Blood samples will be collected by a qualified nurse. The stool sample will be collected by you in a stool jar you will be given. You will be required to return to the study site for the collection of another stool and urine samples three days later. Two urine and stool samples will assist in getting results that we will trust. You will be asked to fill in a questionnaire which will be asking about your living conditions and about the food you ate in the last 24 hours. You may experience mild discomfort when a needle is inserted during blood collection. You may also be uncomfortable during urine and stool sample collection. You will not however be put in any health risk and there will be no research related injuries. A study nurse will be available to help you if you experience any pain or discomfort during sample collection.

By participating in this study you will be investigated whether you are infected with parasites and if you are found to be infected you will be referred to health facilities for deworming treatment. Workshops will be held with the community to educate you on parasites and how they affect the community and to inform you of the study results once they are available.

Participation is entirely voluntary and you will not be forced into participating if you do not wish to do so. You will still be entitled to using the clinic facilities even if you do not participate in this study. You may discontinue participation at any time and you may request destruction of your samples without losing any of the benefits from the clinic. You will not pay anything for participating in this study. You will be given R100 as a refund for the money you would have used for transport to return to the study site for the two days, which will be R50 each day.

Your name, personal information and your results will be treated with confidentiality. You will be allocated a unique code which will be used to identify your results, and not by your name. All information linking your identity and the unique code will be stored separately in a locked cabinet for 5 years, the key will only be available to the principal investigator and electronic data will be stored in a password protected computer to ensure that your information is protected. Research results will be compiled and reports on all findings will be written and anonymity will be ensured at all times. Your results will only be available to the principal investigator, the study team and the UKZN's Biomedical Research Ethics Committee members. After 5 years, data will be disposed of by shredding hard copy documents and deleting all electronic files. Serum and buffy coat samples will be stored securely for 5 years for future research after which will be disposed of according to laboratory protocol. Samples will not be sold, exported or made available to any other researchers.

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee (approval number_____).

In the event of any problems or concerns/questions you may contact the researchers or the UKZN Biomedical Research Ethics Committee, contact details as follows:

Dr Lungi Mkhize-Kwitshana
Tel: 031 260 1930
Fax: 086 400 0407
Mobile: 078 842 4574
Email: kwitshana@ukzn.ac.za

Mrs Brenda Mkhize
031 373 5297
086 674 0817
082 879 4923
mkhizebt@dut.ac.za

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION

Research Office, Westville Campus
Govan Mbeki Building

Private Bag X 54001 Durban, 4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 031 260 4769
Fax: 031 260 4609
Email: BREC@ukzn.ac.za

INFORMED CONSENT FORM

I …….. have been informed about the study entitled "The evaluation of the interactions between nutrition, immunity and co-infections with human immunodeficiency virus and intestinal parasites: the use of pre-Albumin as a tool for nutritional assessment in South African adults by……..(researcher).

I understand the purpose of the research and the procedures of the study, that I will donate six tubes of blood and two urine and two stool samples, one taken on the first day and the second one three days later.

I have been given an opportunity to ask questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

I have been informed about the availability of a study nurse if I experience pain or discomfort during sample collection.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researchers listed in the information letter.

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION:

Tel: 27 31 2604769 Fax: 27 31 2604609 Email: BREC@ukzn.ac.za

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**
(where applicable)

_____ _____

Signature of Translator **Date**
(where applicable)

Appendix 7: Questionnaire

QUESTIONNAIRE

SECTION A:

Identification number .....: Lab Code…….

Date of interview

D	D	/	M	M	/	Y	Y	Y	Y
						2	0		

Name of Interviewer:

Area of residence

Weight (kg):

Height: (cm):

BMI

SECTION B: Demographic Information

Ethnic group

African		Coloured		Indian		White	

Gender

M ale		Female	

Marital status

Single	
Married	
Divorced	
Widowed	
Separated	
Living together	

Age of participant ……..years

Date of birth

D	D	/	M	M	/	Y	Y	Y	Y
		/			/				

Mobile number	
Phone number (Home)	

SECTION C: **Socio-Economic Status**

1.	Where do you live?				
2.	What would you classify the area as?	Rural	Urban	Per-urban	
3.	Are you employed?	Yes	No		
4.	If no, please specify source of income				
5.	If yes, what is your income per month?	<R1000	R1001–R5000	R5000–R10 000	>R10 000
6.	What is your level of education?	None	Primary	High school	Tertiary
7.	What is your occupation?				

SECTION D: **Household Information**

1. What type of house do you live in? ……

2. How many rooms does your house have? .....

3. How many people live in your household?

Babies/Preschool	
Primary school	
Adults	
TOTAL	

4. Where does the household usually get drinking water from?

River	
Own tap- inside the house	
Own tap-outside the house	
Public tap	
Neighbours' tap	
Borehole	
Other, specify……..... ……	

5. What kind of water activity do you do?

Swim	
Wash clothes	
Bathe	
Fish	
Farming	
Collect water for household use and cooking	
Cross the river	
Other, specify……. …….	

6. What toilet facilities do the household have?

Flush toilet, connected to public pipes	
Flush toilet, not connected to public pipes	
Pit toilet	
None	
Other, specify…… …….	

7. What is the main source of energy for cooking?

Electricity	
Wood, open fire outside dwelling	
Wood open fire inside dwelling	
Gas	
Paraffin	
Other, specify…… …….	

8. From where do you get your food?

Local shop(s)	
Shops in town	
Home garden	
Community garden	
Own livestock	
Food aids/welfare/NGO's	
Other, specify……. ……...	

SECTION E: Presence of other diseases

1.	Do you presently have any diseases that you are aware of?	Yes		No	
2.	Have you been ill in the past 30 days?	Yes		No	
3.	If yes, please list the disease/s				
4.	Have you suffered from any parasitic infection in the past?	Yes		No	
5.	Has any of your family members suffered from any parasitic infection?	Yes		No	Don't know
6.	Have you taken any deworming medication in the past 6 months?	Yes		No	

7. How often is deworming done in your household?

Never	
Once in 6 months	
Once a year	
Don't know	
Other, specify…….. ……	

8. Who gets deworming treatment in your household?

Children only	
Adults only	
Everyone in the house	

9.	Have you had an allergic reaction in the past 30 days?	Yes	No	
10.	Do you suffer from any chronic illness?	Yes	No	

11. Please list any medication you are taking

..…….

..……

……

……..

……..

SECTION F: 24 hour food recall

1. How often do you eat?
2. Are you constantly hungry?

		per day	
Yes		No	

Please indicate everything you ate or drank, including meals, snacks, sweets, beverages, alcohol in the past 24 hours

Time of day	What food and drink did you take	How was it prepared	What was added	How much was eaten
Waking up to about 9 o'clock (breakfast time)				
9 o'clock to 12 o'clock (mid-morning)				
12 o'clock to 2 o'clock (lunch time)				
2 o'clock to 5 o'clock (afternoon)				
5 o'clock to sunset (supper time)				
After supper at bedtime and through the night				

Thank you for your participation