THE RELATIONSHIP BETWEEN ORAL CANDIDIASIS AND MICRONUTRIENT DEFICIENCY IN AN ADULT TB COHORT IN ALEXANDRA, JOHANNESBURG

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A Research Report submitted to the Faculty of Health Science, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of
Master of Community Dentistry

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DECLARATION

I, **Maphefo Desiree Thekiso,** declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Community Dentistry in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

_____ [Signature of Candidate]

Monday of 23 January, 2012

DEDICATION

I dedicate this work to:

God, My Lord and Saviour, and Moipone Thekiso (Mama wam), for making this episode a possibility in my life.

ABSTRACT

Tuberculosis (TB) is a major public health problem worldwide, and particularly in South Africa. Micronutrient deficiency (malnutrition) is caused by insufficient supply of vitamins and minerals for normal cell function in the body. Nutritional deficiencies in minerals, vitamins and diets rich in carbohydrates have been implicated in the pathogenesis of oral candidal infections. Malnutrition and wasting are associated with TB, and HIV/TB co-infection may potentially worsen the wasting that occurs in TB or HIV infection alone. **Aim** of the study was to investigate the relationship between micronutrient deficiency and Oral Candidiasis (OC) in adult TB patients. The prevalence of OC and its association with malnutrition in terms of Zinc (Zn), Iron (Fe), Albumin, Selenium (Se),Vitamin A (Vit A) and Vitamin D (Vit D) deficiencies were evaluated in a cross sectional study among eighty eight (n=88) TB adult patients. Patients underwent a complete oral examination for presence and type of OC and blood collection was done for serum nutritional assessment for levels of the micronutrients (Zn, Fe, Albumin, Se, Vit A and Vit D). **Results:** The mean age was 36 years of age, with the majority being females (60%) and HIV positive (69.3%). The prevalence of OC was 60% with pseudomembranous OC (48%) being the most common. Serum concentrations for Zn deficiency (< 8.2 µmol/L) was 69.4%, Vit A deficiency (< 1.05 µmol/L) was 52.3%, Albumin deficiency (< 35 g/L) was 69%, Se deficiency (< 46 µg/L) was 93.2%, Fe deficiency (< 9 µmol/L) was 53.7% and Vit D deficiency (< 49 nmol/L) was 45.1%. OC was prevalent in 40% (Zn deficiency), 25% (Vit A deficiency), 32% (Albumin deficiency), 33% (Se deficiency), 31% (Fe deficiency) and 36% (Vit D deficiency). However, there was no significant association between micronutrient deficiency and OC ($p > 0.05$). **Conclusion:** These data demonstrate that OC in TB adult patients is not associated with micronutrient malnutrition. Longitudinal studies are required to investigate the relationship between micronutrient deficiency and OC in adult TB patients further.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
BMI	Body Mass Index
CD	Chronic Diarrhoea
CMV	Cytomegalovirus
CRP	C-reactive proteins
Fe	Iron
GDHSD	Gauteng Department of Health and Social Development
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
JHB	Johannesburg
K	Potassium
MDR TB	Multidrug resistant tuberculosis
MTB	Mycobacterium tuberculosis
Ng/ul	Nanogram per microlitre
NHLS	National Health Laboratory Services
NICUS	Nutrition Information Centre of the University of Stellenbosch
NIRS	Nutritional Indicator Research Survey
OC	Oral Candidiasis
OHL	Oral Hairy Leukoplakia
PHC	Primary health care
PLWA	People Living With HIV/AIDS
SA	South Africa
SANTA	South Africa National Tuberculosis Association
Se	Selenium
TB	Tuberculosis
UNAIDS	Joint United Nations Programme on HIV/AIDS

UNGASS	United Nations General Assembly Special Session
Vit	Vitamin
Vit A	Vitamin A
Vit B	Vitamin B
Vit D	Vitamin D
WHO	World Health Organization
XDR TB	Extreme drug resistant tuberculosis

CHAPTER 1: INTRODUCTION

1.1 Background

Mycobacterium tuberculosis (*M. tuberculosis*) is the most widespread bacterial pathogen in the world, infecting close to one-third of the human population (Raviglione, 2003). Approximately two million people across the world succumb to tuberculosis (TB) every year (Smith, 2003).

Tuberculosis (TB) is a major public health problem in South Africa (SA). South Africa is ranked fifth on the list of 22 high burden TB countries in the world. According to the World Health Organization (WHO, 2009), there were an estimated 460 000 new TB cases in SA in 2007, with the incidence rate of 948 cases per 100 000 population. The control of TB is jeopardised by the Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) as one-third of 40 million people living with HIV/AIDS (PLWA) are also infected with *M. tuberculosis* (Tovaru et al., 2008).

Despite SA's investments in TB control, progress toward reaching programme objectives has been slow. Treatment increased from 65% in 2001 to 74% in 2006, but is still lower than other African countries that have high HIV/AIDS prevalence rates with fewer resources. Multidrug resistant (MDR) TB, as a result of non-adherence to drug regimens and/or inappropriate drug regimens, further worsens the epidemic. The prevalence of MDR TB has tripled, from 2 000 cases in 2005 to 7 350 in 2007. From 2007 there has also been an increase of extreme drug resistant (XDR) TB cases, from 74 in 2004 to 536 in 2007 (SA National Department of Health Report, 2009).

In SA, TB is associated with poverty, overcrowding, alcoholism, stress, drug addiction and malnutrition (Nutrition Information Centre of the University of Stellenbosch [NICUS, 2007]). An important component of malnutrition is "micronutrient deficiency", which refers to the lack of sufficient micronutrients such as vitamins, iron and zinc in an individual. Lack of micronutrients in foods can lead to low energy levels, low immunity

and higher rates of disability and chronic illness among affected populations. Those most at risk are children, pregnant and breastfeeding mothers, and PLWA and/or TB (Woods et al., 1999).

Patients who have HIV/AIDS, TB and pneumonia have opportunistic infections, such as oral candidiasis (OC) [Saha et al., 2011]. Oral candidiasis is one of the common opportunistic infections of the oral cavity that can involve the hard and soft palate, tongue, buccal mucosa and floor of the mouth. It is caused by the overgrowth or infection of the oral cavity by yeast-like fungus from the *candida* species (Scully, 1990). The main species are *C albicans* (most common), *C tropicalis, C glabrata, C pseudotropicalis, C guillierimondii, C krusei, C lusitaniae, C parapsilosis, and C stellatoidea.* The most common OC oral lesions are pseudomembranous, eythematous and angular cheilitis (Reichart, 2003). Previous studies have shown an association between OC and micronutrient deficiency in individuals that have been infected with TB. However, the prevalence of OC in TB and TB/HIV co-infected cases is worthy of further investigation and research, particularly regarding micronutrient deficiency in these patients, since there are very few studies demonstrating this association.

CHAPTER 2: LITERATURE REVIEW

Multiple bibliographic databases, including EBCOhost, PUBMED, WEB OF SCIENCE and Goggle Scholar, were used to develop a comprehensive review of the literature on OC and micronutrient deficiency in TB patients. The databases were searched for publications related to this topic until August 2011. Selected keywords included *TB, HIV/AIDS and TB patients, micronutrient deficiency, social habits, oral candidiasis*.

2.1 Tuberculosis

Tuberculosis is an infectious disease caused by the bacilli micro-organism called *Mycobacterium tuberculosis (M. tuberculosis)*, which usually enters the body by inhalation through the lungs. It spreads from the lungs to other parts of the body via the blood stream, the lymphatic system, the airways, or by direct extension to other organs. The most common form of TB is pulmonary TB, but it can also occur in the lymph nodes, kidneys, bones and oral cavity. The symptoms of pulmonary TB are chest pain and severe cough with blood in the sputum, exhaustion, night sweats, fever, loss of appetite and weight loss (NICUS, 2007).

M. tuberculosis has the ability to enter into a dormant state, leading to an asymptomatic infection that persists for years. Approximately 95% of individuals that are exposed to *M. tuberculosis* remain asymptomatic; the remaining 5% develop TB which is localised in the lungs (Tovaru et al., 2008). In addition to the weakening of the immune system due to a debilitating disease, malnutrition or advancing age may result in the reactivation of the latent bacilli.

M. tuberculosis infects all parts of the mouth (soft and hard palate, uvula, buccal mucosa, gingivae, lips, tongue, maxilla, and mandible) more often in men than in women, appearing mainly in the form of ulcerative lesions (Kakisis et al., 2010).

2.2 HIV in SA

By 2009 an estimated 5, 6 million people were living with HIV in SA (more than in any other country) and 310 000 people in the country were reported to have died of AIDS

(UNAIDS, 2010). In the same year almost one-in-three women aged 25 to 29, and over a quarter of men aged 30 to 34 were living with HIV (Shisana et al., 2009). The TB and HIV epidemics stimulate each other in several ways. HIV-infected patients have the greatest risk of developing TB and if this is left untreated it leads to death within months in 90% of those co-infected with TB/HIV (Corbett et al., 2003). People living with HIV are particularly vulnerable to XDR TB because of their increased susceptibility to infection through nosocominal transmission, malabsorption of TB medication, acquired Rifampicin resistance and poor response to TB therapy (Dye et al., 2003).

2.3 Malnutrition, TB and HIV Co-infection

Micronutrients are substances (vitamins and minerals) required for normal cell function in the body. Micronutrient deficiency (malnutrition) occurs because the supply of vitamins and minerals to cells in the body is insufficient to satisfy physiological requirements. There is a complex three-way relationship between micronutrient deficiency, the immune system and infection. Malnutrition elicits immune system dysfunctions, which in turn promote increased vulnerability of the host to infection, with the latter intensifying the severity of malnutrition (Enwonwu, 2006). Malnutrition alters all defence mechanisms, including anatomic barriers, cell-mediated immune responses, phagocytic cell/microbial functions and humoral immunity function (antibody and complement responses) among many others (Biesel, 1996; Chandra, 1999). Malnutrition and wasting are associated with TB, and co-infection with HIV/TB may potentially exacerbate the wasting that occurs in TB or HIV infection alone (Niyongabo et al., 1999).

In the developing world, the impact of TB on nutritional status is worse. A number of studies have demonstrated the effect of TB on nutritional status; for example, nutritional status was significantly lower in patients with TB than in healthy controls in studies conducted in Indonesia, India and Japan (Karyadi et al., 2002). A study conducted in Malawi reported a significant reduction in weight, muscle mass, functional subcutaneous fat and serum albumin in TB patients (Harries et al., 1988). Tuberculosis/Human Immunodeficiency Virus co-infection introduces a new dimension on the pathogenesis of nutritional status. Paton et al. (1997) compared wasting in HIV-positive individuals with or without TB co-infection in Brazil, and reported that the severity of fat and lean

depletion was striking, although the severity of muscle loss in the HIV group was underestimated owing to the tendency of extracellular water increase in HIV-infected individuals. A study found that wasting and high viral load in pulmonary TB cases is indicated by low Body Mass Index (BMI) and plasma micronutrient concentrations (van Lettow et al., 2004).

Micronutrient deficiencies have been reported in individuals with TB (Karyadi et al., 2002) and in those with HIV infection alone (Semba et al., 1999). Nutritional deficiencies of vitamin (Vit) B12, Zinc (Zn) and Selenium (Se) in malnourished people living with HIV/AIDS are associated with decreased immune indices and higher risk for disease progression (UNAIDS, 2010). Malnutrition weakens immunity, increasing the chance that latent TB will develop into active disease. Reduced micronutrient intake (Vit A, C & E and Se & Zn) has been associated with impaired immunity (Health System Trust, 2009). Evidence from several cross-sectional studies suggests that patients with TB are at high risk for deficiencies of Vit A, Thiamine, Vit B6, folate, Vit E, and Zn (van Lettow et al., 2004). Evidence suggests that Vit D deficiency causes deficient monocyte-macrophage function and may predispose towards vulnerability to TB (Chan, 1997). A higher incidence of TB during the winter or early spring was seen in the former Ciskei (Shennan, 1993), South Africa (Schaaf, 1996) and Bashkortostan in Russia (Iakovlev, 1994), suggesting an increased transmission of TB infection in autumn and winter.

Selenium (Se) deficiency has been shown to increase the risk of developing mycobacterial disease among HIV-infected drug users as Se plays an important role in the selenoenzyme glutathione peroxidise that protects cells against free radical damage and oxidase stress (Shor-Posner et al., 2002). Koyanagi et al. (2004) reported that patients with pulmonary TB had low serum concentrations of Vit A, Zn and Se compared to non-TB patients.

The influence of HIV on nutritional status results in a complex series of reactions, i.e. fever, production of specific acute–phase proteins, release of inflammatory mediators, anorexia, proliferation of the immune cells, endothelial cell activation and other metabolic changes (Baumann, 1994). Consequently nutrients (e.g. Fe, copper, Se and Zn) are simultaneously compartmentalised to the tissues, lost from the body or blocked from

cellular utilisation (Gabbay and Kushner, 1999). HIV infection affects the nutritional status through a reduction in food intake, resulting in the loss of appetite, an increase in the side effects of medication, mouth lesions and depression (Enwonwu, 2006). Other causes of malnutrition in HIV-infected individuals are nutrient malabsorption (e.g. malabsorption of fats, fat-soluble vitamins and carbohydrates) and the metabolic changes which promote increased utilisation of nutrients, particularly micronutrients and antioxidants (Friis et al., 1998; Semba et al., 1999).

2.4 Nutritional Deficiencies and Oral Mucosa Diseases

Oral manifestations of nutritional deficiency include nonspecific signs and symptoms that involve the mucous membranes, the teeth, the periodontal tissues, the salivary glands and the perioral skin (Thomas et al., 2010). Activities such as eating, drinking and breathing affect mucous membrane that is weakened by deficiencies. In addition, the local microenvironment of the mouth is non-sterile to commensal and pathological microorganisms that may further stress the weakened mucous membrane (Boyd, 2007).

Not all vitamins and minerals affect the oral mucosa. Water-soluble vitamins that have oral mucosal involvement include vitamins B2, B3, B6, B12, folic acid and Vit C. Fat-soluble vitamins that affect the mucosa include Vit A, D, E, and minerals relevant to the oral mucosa are calcium, fluoride, Fe and Zn (Thomas et al., 2010). Vit A is responsible for producing photosensitive pigments, maintaining epithelial tissue, preventing infectious diseases and growth, and modelling bones and tissues (Boyd, 2007; Krall et al., 2007). The oral manifestations of Vit A deficiency include xerostomia, reduced resistance to infections and impaired growth of the teeth. Vit A can be stored in the body reaching toxic levels, with oral manifestations such as cheilitis, gingivitis, carotenemia and impaired healing (Palmer and Boyd, 2009). Vit D deficiency may increase the likelihood of periodontitis (Boyd, 2007) and Vit E deficiency may be associated with oral cancer (Thomas et al., 2010)

Iron is important for different normal functions of the body, i.e. as part of haemoglobin, which plays an important role in oxygen transport, adesone triphosphate production and normal immune function, and is a cofactor with Vit C in collagen production (Boyd, 2007; Palmer and Boyd., 2009). The oral manifestation of Fe deficiency includes atrophy

of the lingual papillae, burning and redness of the tongue, angular cheilitis, dysphagia, recurrent aphthous ulcers and pallor of the oral tissues due to underlying anaemia (Palmer and Boyd, 2009).

Zinc plays a variety of critical roles in the cell, particularly acting as an enzymatic cofactor critical for cell growth and reproduction, normal immune function, metabolism, and as a stabiliser of DNA and RNA and collagen synthesis (Boyd, 2007). Pregnant women, diabetics, those infected with HIV/AIDS, the elderly, those with sickle cell anaemia (Thomas et al., 2010) and those with TB (Lin et al., 2000) are at high risk of Zn deficiency. Oral manifestations of Zn deficiencies include changes to the epithelium of the tongue, flattened filiform papillae, ulcers and xerostomia (Boyd et al., 2001; Palmer and Boyd, 2009). It also decreases the taste sensation, which can cyclically contribute to the malnutrition problem (Krall et al., 2007; Palmer and Boyd, 2009).

2.5 The Oral Cavity and Oral Candidiasis

The oral cavity is a habitat of potential pathogens, i.e. fungal, bacterial and viral, which can multiply and cause disease in immunosuppressed patients. Most of the organisms associated with oral opportunistic infections are either part of the normal flora or common in the environment and likely to pass through the mouth. Oral pathogens and their toxins can spread to other tissues and organs directly (e.g. necrotising gingivitis and periodontitis spreading to cause some degree of necrosis of the soft tissue (Ibeziako et al., 2003) and OC to pharyngeal and oesophageal candidiasis) and indirectly via lymphatics and the blood stream and possibly even to the central nervous system (Johnson et al., 2006).

Candida spp. resides as commensal organisms in the normal oral, vaginal and stomach flora. If the balance in the normal flora is disrupted or the immune responses are compromised, the *Candida spp*. becomes pathogenic, causing mucosal disease (Naglik et al., 2011).

OC has been has been identified as one of the oral lesions strongly associated with HIV/AIDS (EC-Clearinghouse, 1993) and it is a marker of immune suppression (Miziara et al., 2006). OC associated with HIV infection occurs frequently and could be considered as an initial manifestation of the disease (Pienaar et al., 2010). OC has been

reported in 50 to95% of all HIV-positive persons at some point during their progression to full-blown AIDS (Gupta et al., 2006). Opportunistic infections and co-infections are common among HIV-infected individuals, especially in individuals with a low CD4 count. A cohort study conducted by Holmes et al. (2006) in Cape Town found that opportunistic infections among HIV-infected individuals increased significantly at lower CD4 cell (50 cells/ µL or less). These opportunistic infections included conditions such as TB, bacterial infections, chronic diarrhoea, OC and oesophageal candidiasis, wasting syndrome, and cryptococcal disease. Also in this study, TB and OC occurred commonly with CD4 counts of more than 200 cells/ µL and were the only opportunistic infections to occur in CD4 counts of more than 500 cells/ µL.

A longitudinal study conducted in Kolkata (India) by Saha et al. (2011) found that opportunistic infections and co-infections that were common among HIV-infected individuals were TB, chronic diarrhoea, OC, herpes simplex virus-2 (HSV-2), cytomegalovirus (CMV), hepatitis B virus (HBV) and hepatitis C virus (HCV). The most common dual infections were chronic diarrhoea (CD) and OC (28.29%), OC and TB (25.94%), CD with TB (21.08%), HSV-2 and OC (19.11%), HSV-2 and CMV (14.21%), HBV and HSV-2 (3.92%), HBV and CMV (2.94%), the least common being HCV and HBV(0.49%). The most common triple infections were OC, TB and CD (8.34%) and HSV-2, CMV and CD (8.34%).

2.6 OC and Micronutrient Deficiencies

Micronutrient deficiencies of Fe, folic acid, Vit A, B, C, K and Zn, and a carbohydrate-rich diet have a significant impact on the pathogenesis of OC infection (Samaranayake, 1986). Studies in relation to OC associated with micronutrient deficiencies in adult TB cases are limited. A study conducted by Paillaud et al. (2004) found that OC is related to malnutrition and that mucosal lesions have a negative impact on energy intake, which subsequently worsens the nutritional status in institutionalised elderly people. In individuals with nutritional Vit B12 deficiency, the oral signs and symptoms include glossitis, angular cheilitis, recurrent oral ulcers, OC, diffuse erythematous mucositis and pale oral mucosa, which offer the dentist an opportunity to participate in the diagnosis of the condition (Pontes et al., 2009).

There is a paucity of studies that have investigated the relationship between OC and micronutrient malnutrition in adults with TB and TB/HIV co-infection. The findings of this study will be helpful in identifying subgroups that will benefit from nutritional interventions for reducing the prevalence of OC and micronutrient malnutrition in adult TB patients.

Aims and Objectives of the Study

AIM

The aim of the study was to investigate the relationship between micronutrient deficiency and Oral Candidiasis (OC) in adult TB patients, in Alexandra, Johannesburg (JHB).

OBJECTIVES

1. To describe the demographics and HIV status among a cohort of adult TB patients between 18 and 60 years of age.
2. To determine the prevalence of OC among the TB cohort.
3. To investigate the association between OC and micronutrient status among TB patients.

HYPOTHESIS

There is a significant association between OC and micronutrient deficiency amongst adult TB patients.

CHAPTER 3: METHODOLOGY

This chapter describes the research methodology used to conduct this study. The methods, ethical considerations and analytic techniques pertaining to this study are also dealt with in this chapter.

3.1 Study Design

The study adopted a cross-sectional, analytical study design. A questionnaire was designed to establish the age, gender and HIV status of the sample population. Clinical oral examination was carried out to assess the presence of OC, and blood samples were collected for serum analysis of micronutrient levels of Se, Albumin, Vit A, Vit D, Fe and Zn.

3.2 Study Population

This research was part of a bigger study called the "Nutritional Indicator Research Survey" (NIRS) (Ethics clearance certificate: M090955) that evaluated the impact of fortified food (e-pap) on the nutritional status of adult TB patients over a three-month period. The methodology adopted in the current research and described below was derived from the NIRS study (see Appendix G).

3.3 Study Sample

A convenient sampling technique was employed to recruit patients into this study. The NIRS researchers contacted South African National Tuberculosis Association (SANTA) and requested them to identify Johannesburg (Joburg) clinics in Alexandra that provided TB outpatients with treatment and support. Permission was obtained from the Joburg City Department of Health and the Gauteng Department of Health and Social Development (GDHSD). A copy of the permission letter is attached as Appendix F.

Four JHB clinics were identified and two with the highest number of TB patients were selected. Thus , two clinics were selected as research sites. The total sample size screened and recruited for the NIRS was 120. Oral examination and blood specimens were

collected in only 88 participants as 32 of the recruited participants were not present on the day of examination and blood sample collection. Thus, this study is based on a sample size of 88 adult TB patients.

3.4 Study Instruments

The study instruments consisted of the following components.

3.4.1 Demographics

A questionnaire referred to as a "personal record sheet" was developed to obtain the demographic data, such as age, race and gender, of the participants (see Appendix A). A primary health care (PHC) nurse completed the personal record sheet with the patient and recorded the HIV status of each participant from their clinical records.

3.4.2 Two clinical instruments

3.4.2.1 Oral examination for OC

Oral examination was conducted by a calibrated dentist and dental therapist. The inter-examiners' agreement was assessed using the kappa statistic, with an overall value of 0.80 – 0.95 for the diagnosis of OC. The patients were examined at the research site, seated on mobile dental chairs and under head lamp and light, using disposable hand mirrors. The diagnosis of the clinical variants of OC was made in accordance with the criteria developed by Sharon and Fazel (2010) and is described below:

a. Pseudomembranous Candidiasis was characterised by the presence of extensive white pseudo-membranes consisting of desquamated epithelial cells, fibrin and fungal hyphae.

b. Erythematous Candidiasis was characterised as erythematous patches that appear bright red and that can be found intraorally.

c. Angular Cheilitis was characterised as erythematous fissuring at one or both corners of the mouth, associated with an intraoral candidial infection.

Patients were also asked if they experienced any burning mouth or sore tongue or lip. The presence of lower and upper dentures was also recorded.

3.4.2.2 Laboratory component: serum biochemical analysis for micronutrient levels

The micronutrient analysis for the serum levels of Se, Zn, Fe, Vit A, Vit D and Albumin were conducted by the National Health Laboratory Services (NHLS), under the supervision of staff from Department of Biochemical Pathology. These nutrients were selected because of their key roles in supporting immune function and quenching free radicals (Rudolph et al., 2011). Patients were considered to be micronutrient deficient if one or more of the following micronutrients' serum levels were below the normal range as shown in Table 1. The normal micronutrient levels' criteria outlined in Table 1 are used by the NHLS, based on the clinical guide developed by Alan (1995).

Table 1: Individual micronutrient serum analysis (*Alan, 1995)

Micronutrient	***Normal Range**	**Cut Off Levels**
Zinc	8.2-23 µmol/L	< 8.2 µmol/L
Vitamin A	1.05-2.80 µmol/L	< 1.05 µmol/L
Albumin	35-52 g/L	< 35 g/L
Selenium	46-143 µg/L	< 46 µg/L
Iron	9-31 µmol/L (Male: 11.6-31.3 µmol/L) (Female: 9-30.4 µmol/L)	< 9 µmol/L (male and female)
Vitamin D	49 -172 nmol/L	< 49 nmol/L

The micronutrient levels were analysed individually, as outlined in Table 1. The micronutrient levels were reported as group prevalences (percentage normal versus percentage below normal) in this cohort and the relationship between each individual micronutrient deficiency and the prevalence of OC was analysed to determine if there was a significant association ($p < 0.05$).

3.5 Data and Statistical Analysis

STATA 11 software was used for all statistical analysis. All statistical significance was calculated at the 5% significance level.

3.5.1 Data checking and cleaning

Range checks for outliers were carried out using histograms, and box and whisker plots for continuous variables. Consistency checks to identify inconsistencies in the data were undertaken using scatter plots.

3.5.2 Independent variables

Micronutrient deficiency and demographic profile (Age and gender)

3.5.3 Dependent variables

Oral Candidiasis

3.5.4 Analysis by objective

Objective 1: To describe the demographics social and HIV status of adult TB patients.

The study population variables were described using frequency tables for categorical variables and continuous variables, and were summarised in terms of mean and standard deviation, median and inter-quartile range.

Objective 2: To determine the prevalence of OC amongst the TB cohort.

The proportion of patients with oral candidiasis was determined using frequency tables and the prevalence of different types of oral candidiasis was reported using pie charts.

Objective 3: To investigate the association between OC and micronutrient status among TB patients

The associations between categorical variables and OC were tested using Pearson's Chi-squared test of proportions. The Fischer exact test was used for variables that had an expected frequency of five or less. Continuous variables were described using histograms and normal quantile plots. Normally distributed continuous variables were tested for their association with OC using the Student's t test. Non-normal continuous variables were tested using the Wilcoxon rank-sum test. Logistic regression was used to identify factors which were associated with a presence of OC. The odd ratios were used to determine the

strength of the association. The statistical significance was calculated at the 5% significance level and estimates were reported at the 95% confidence interval.

3.6 Ethics

The NIRS received ethical clearance from the University of Witwatersrand Ethics Committee (Ethics clearance certificate: M090955) to conduct the study and collect blood samples from the TB patients. Permission to undertake the current study was also granted by the University of Witwatersrand Ethics Committee (Ethics clearance certificate: M10733, see Appendix E). Each participant was given an information sheet (see Appendix C) and a written consent form for obtaining their permission to participate in the study (see Appendix D), which they had to sign. For illiterate participants the researcher explained the purpose of the study to obtain their consent. All adults diagnosed with oral candidiasis or any oral lesions were referred to their local dental clinic in Alexandra for treatment.

CHAPTER 4: RESULTS

4.1 Demographics

Table 2 below shows that the mean age of the total sample (n=88) was 36.7 years (S.D. 9.97). The majority of TB patients were women (60.2%) and most were unemployed (74%). Almost 70% of TB patients in this cohort were HIV positive.

Table 2: Overall characteristics of TB patients (n=88)

Demographics		
Age in years Median (IQR) 36 (28 – 42) Mean (Std Deviation) 36.66 (9.97)		
	N	**%**
Sex		
Female	53	60.2
Male	35	39.8
Employment		
Yes	23	26.1
No	65	73.9
HIV Status		
Negative	27	30.7
Positive	61	69.3

4.2 Micronutrient Deficiency Levels

Table 3 provides information about the mean blood serum levels of the micronutrients investigated in this study. Although the sample size was 88, not all blood results were available for the micronutrient analysis, hence the number's per micronutrient is different

often less than 88. The mean values of most of the micronutrient (Zn, Vit A, Se, Fe, and Albumin) levels were above the normal ranges for these micronutrients except for Vit D, which had a mean level that was below the normal range (47.96 nmol/L; S.D. 17.91). This was also indicated by 54.9% of the sample who had Vit D deficiency.

Table 3: Individual micronutrient deficiency of TB patients (n=88)

Micronutrients	**N**	**%**	**Median(IQR)**	**Mean (Std Deviation)**
Zn n = 49				
No (> 8.2 µmol/L)	34	69.39		
Yes (< 8.2 µmol/L)	15	30.61	10.05 (7.4 – 11.1)	10.60 (4.66)
Vit A n = 65				
No (> 1.05 µmol/L)	34	52.31	1.05 (0.74 – 1.46)	1.13 (0.43)
Yes (< 1.05 µmol/L)	31	47.69		
Se n = 44				
No **(> 46 µg/L)**	41	93.18	68 (58 – 83)	69.12 (17.41)
Yes **(< 46 µg/L)**	3	6.82		

Micronutrients	**N**	**%**	**Median(IQR)**	**Mean (Std Deviation)**
Fe n = 67			10.1 (6.6 – 15.8)	11.19 (6.59)
No **(> 9 µmol/L)**	36	53.73		
Yes (< 9 µmol/L)	31	46.27		
Albumin n= 67				
No (> 35 g/L)	46	68.66	38 (33 – 41)	37.28 (5.55)
Yes (< 35 g/L)	21	31.34		
Vit D n= 51				
No (>49 nmol/L)	23	45.10	46.35 (36.13 – 60.23)	47.96 (17.91)
Yes (< 49 nmol/L)	28	54.90		

Table 4 provides information of the mean blood serum levels of the micronutrients investigated in the TB/HIV co-infected cases (n=61; 70%). The mean values of all the micronutrient (Zn, Vit A, Se, Fe, Albumin and Vit D) levels were above the normal ranges of these micronutrients.

Table 4: Individual micronutrient deficiency of TB/HIV co-infected patients (n=61)

Micronutrients	**N**	**%**	**Median(IQR)**	**Mean (Std Deviation)**
Zn n = 35				
No (> 8.2 µmol/L)	15	43		
Yes (< 8.2 µmol/L)	20	57	10.00 (6.2–11.00)	9.60 (5.82)
Vit A n = 28				
No (> 1.05 µmol/L)	17	60.71	1.00 (0.82 – 1.32)	1.08 (0.68)
Yes (< 1.05 µmol/L)	11	39.28		
Se n = 36				
No **(> 46 µg/L)**	23	64		
Yes **(< 46 µg/L)**	13	36	62 (55-73)	67.15 (13.28)

Micronutrients	**N**	**%**	**Median(IQR)**	**Mean (Std Deviation)**
Fe n = 36			9.63 (7.8 – 13.8)	10.6 (7.2)
No **(> 9 µmol/L)**	15	41.66		
Yes (< 9 µmol/L)	21	58		
Albumin n= 37				
No (> 35 g/L)	23	62	39 (31-39)	38.42 (4.62)
Yes (< 35 g/L)	14	38		
Vit D n= 46				
No (> 49 nmol/L)	17	37	44.28 (34.22 – 61.48)	49.46 (19.84)
Yes (< 49 nmol/L)	29	63		

4.3 Prevalence of OC among TB patients (n=88)

Of the 88 TB patients, 40% (n=35) had OC and 60% (n=57) did not have OC present (Figure 1). In terms of prevalence of OC by clinical type, Figure 2 shows that 48% (17) of the TB patients had pseudomembranous, 28% (10) had erythematous, 14% (5) had angular cheilitis and 10% (3) had hyperplastic.

Figure 1: Overall Oral Candidiasis prevalence (n=88)

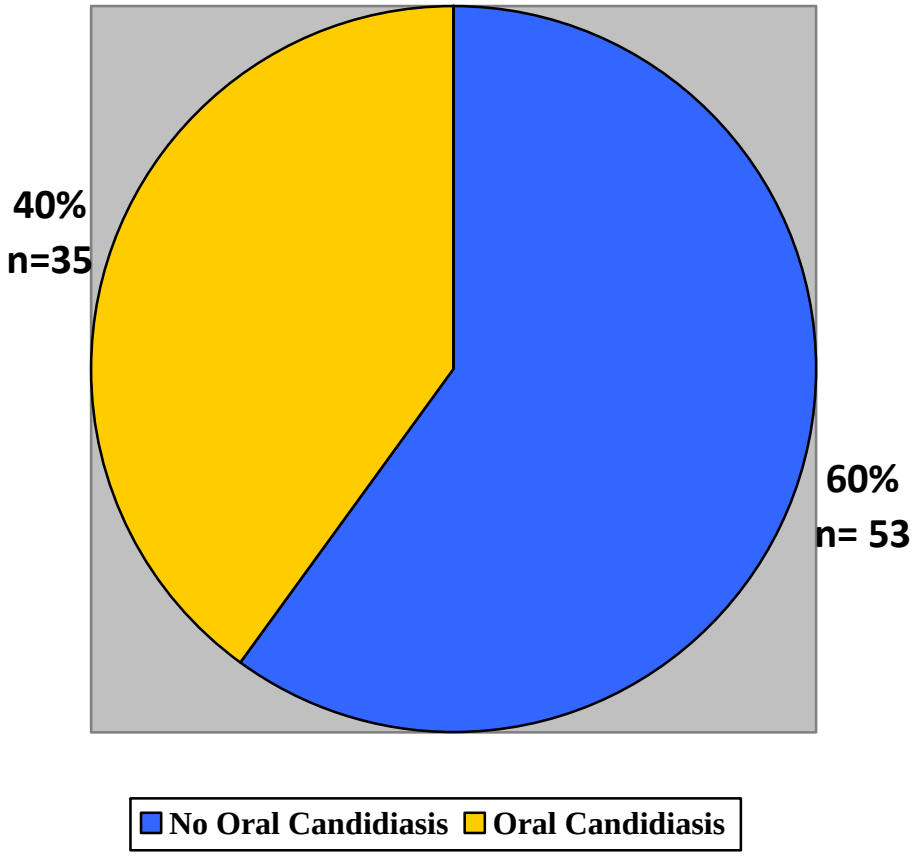
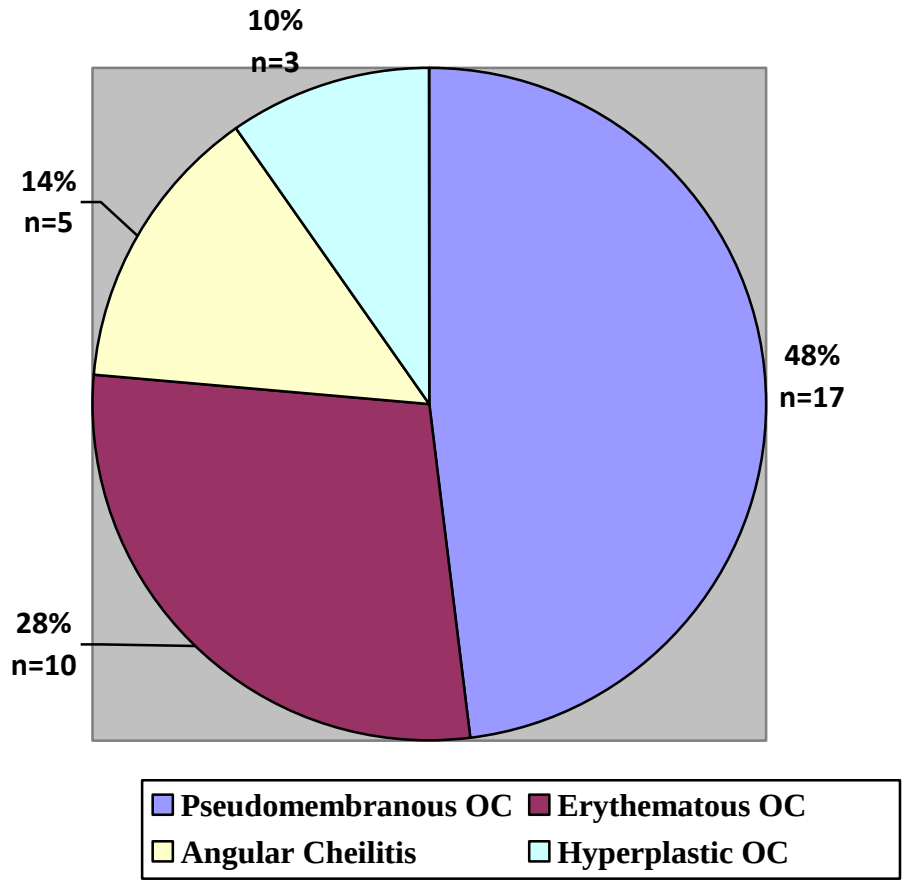


Figure 2: Prevalence of Oral Candidiasis by clinical type (n=35)



4.4 Association between OC and Demographics, HIV Status and Micronutrient Deficiency

The relationship between OC, demographics, HIV status and micronutrient deficiency was determined by the Pearson chi-squared (Fisher exact) test for categorical variables. The Wilcoxon rank-sum test for non-normal continuous variables and Student's t test for normally distributed continuous variables (e.g. age) were used.

4.4.1 Demographics and OC

Table 5 below shows the median age for patients with OC was 38 and the mean age was 37; there was no statistically significant relationship between OC and age. The prevalence of OC was higher among the females (39.62%) than among males (28.57%), although this was not statistically significant (p=0.288). Employed subjects had a slightly higher prevalence of OC (37.70%) than those unemployed (34.78%; p=0.805). Thirty-five per cent of TB/HIV co-infected patients had OC compared to 37% of TB patients. There was no statistically significant relationship between OC and any of the demographics discussed below.

Table 5: Associations between oral candidiasis and demographics

Demographics	**No OC**		**OC Present**		***p value**
Age in years					
Median(IQR)	35.5 (28 – 42)		38 (31 – 44)		
Mean (Std Deviation)	36.5 (10.21)		37.35 (9.47)		0.702
Sex (n, %)					
Female	32	60.38	21	39.62	
Male	25	71.43	10	28.57	0.288
Employment (n, %)					
No	15	65.22	8	34.78	
Yes	38	62.30	24	27.27	0.805
HIV Status (n, %)					0.524
Negative (TB only)	17	19.36	10	11.4	
Positive (TB/HIV co-infection)	37	42.0	24	27.3	

*p value for Student's t test for normally distributed continuous variables (e.g. Age), Wilcoxon rank-sum test for non-normal continuous variables and Pearson's Chi squared test for categorical variables, Fisher's exact test for categorical variables with n < 5 per cell.

4.4.2 Association between OC and micronutrient deficiency

For subjects with OC micronutrient levels below the normal range were present among 40% (6) of Zn, 25% (8) of Vit A, 33% (1) of Se, 32% (10) of Fe, 32% (7) of Albumin and 36% (10) of Vit D respectively. For TB patients with OC micronutrient levels within the normal range were present among 23% (8) for Zn, 47% (16) for Vit A, 26% (11) for Se, 38% (14) for Fe, 36% (17) for Albumin and 17% (4) for Vit D respectively. OC was not significantly associated with lower levels of micronutrients when compared to normal micronutrient levels in TB patients who had no OC (Table 6).

Table 6: Associations between oral candidiasis and micronutrient deficiency

Micronutrient Deficiency	**No OC**		**OC Present**		***p value**
Zinc	N	%	N	%	
No (> 8.2 µmol/L)	27	77	8	23	0.216
Yes (< 8.2 µmol/L)	9	60	6	40	
Median (IQR)	10.05 (8.2-11.1)		9.6 (6.8-12.9)		0.705
Mean (Std Deviation)	10.33 (3.25)		11.3 (7.24)		
Vitamin A					
No (> 1.05 µmol/L)	18	53	16	47	
Yes(< 1.05 µmol/L)	24	75	8	25	0.063
Median (IQR)	0.99 (0.69-1.32)		1.21 (0.99-1.58)		0.182
Mean (Std Deviation)	1.07 (0.45)		1.22 (0.39)		
Selenium					

No(> 46 µg/L)	31	74	11	26	1.000
Yes(< 46 µg/L)	2	67	1	33	
Median (IQR)	68 (58-83)		65 (58.5-78.5)		0.739
Mean (Std Deviation)	69.65 (17.94)		67.67 (16.57)		
Iron Deficiency					
No (> 9 µmol/L)	23	62	14	38	0.567
Yes (< 9 µmol/L)	22	69	10	31	
Median (IQR)	9.5 (6.9-15.2)		10.1(6.05-17)		0.816
Mean(Std Deviation)	11.33 (6.96)		10.94(5.98)		
Albumin					0.728
No (> 35 g/L)	30	64	14	36	
Yes (< 35 g/L)	15	68	7	32	0.728
Median (IQR)	37 (34-41)		39 (31.5-40.5)		0.978
Mean (Std Deviation)	37.29 (5.69)		37.25 (5.37)		
Vitamin D					0.210
No (> 49 nmol/L)	19	83	4	17	0.210
Yes	18	64	10	36	

(< 49 nmol/L)					
Median (IQR)	48.83 (36.13-60.23)		43.39 (35.81-64.58)		0.739
Mean (Std Deviation)	48.45 (17.47)		46.57 (19.67)		

*p value for Wilcoxon rank-sum test for non-normal continuous variables and Pearson's Chi squared test for categorical variables, Fisher's exact test for categorical variables with n < 5 per cell.

4.5 Predictor Variables for OC among TB patients

There was no significant association between gender, employment status, HIV status, micronutrient level and the presence of OC (Table 7). The strength of these associations was weak ($p > 0.05$) and suggests that the demographic variables and micronutrient levels are not good predictors of the presence of OC in TB patients.

Table 7: The odds ratio between demographics, micronutrient levels and OC

Demographics	**Odds Ratio**	**95% Confidence Interval**	***p value**
Age in years	1.01	0.97 – 1.05	0.698
Sex			
Female	1		
Male	0.61	0.24 – 1.52	0.284
Employment			
No	1		
Yes	1.13	0.42 – 3.09	0.804
HIV Status			
Negative	1	0.44 – 3.44	0.845
Positive	1.12		
Zinc Deficiency			
No **(> 8.2 µmol/L)**	1		
Yes **(< 8.2 µmol/L)**	2.25	0.61 – 8.25	0.221
Vitamin A Deficiency			
No **(> 1.05 µmol/L)**	1		

Yes **(< 1.05 µmol/L)**	0.37	0.13 – 1.07	0.066
Selenium Deficiency			
No **(> 46 µg/L)**	1		
Yes **(< 46 µg/L)**	1 1.41	0.12 – 17.11	0.788
Iron Deficiency			
No **(> 9 µmol/L)**	1		
Yes **(< 9 µmol/L)**	0.75	0.27 – 2.03	0.567
Albumin Deficiency			
No **(> 35 g/L)**	1		
Yes **(< 35 g/L)**	1 0.82	0.28 – 2.41	0.724
Vitamin D Deficiency			
No (> 49 nmol/L)	1		
Yes (< 49 nmol/L)	2.25	0.61 – 8.25	0.221

There was no significant association between TB/HIV co-infection, micronutrient level and the presence of OC. The strength of these associations was weak ($p > 0.05$) and suggests that micronutrient levels are not good predictors of the presence of OC in TB/HIV co-infected patients (Table 8).

Table 8: The odds ratio between micronutrient deficiency and OC in TB/HIV co-infected (n=61)

Micronutrient levels	**Odds Ratio**	**95% Confidence Interval**	***p value**
Zinc (< 8.2 µmol/L) & OC	2.25	1.9-2.4	0.22
Vitamin A (< 1.05 µmol/L) & OC	1.5	1.2-1.8	1.02
Selenium (< 46 µg/L) & OC	1.4	0.95-1.5	1.00
Iron (< 9 µmol/L) & OC	0.75	0.55-1.2	0.57
Albumin (< 35 g/L) & OC)	0.8	0.45-1.3	0.73
Vitamin D (< 49 nmol/L) & OC	2.63	2.45-3.00	0.20

CHAPTER 5: Discussion and Conclusion

This study aimed to determine the relationship between micronutrient deficiency and OC among adults with TB. The demographic profile of the TB cohorts was determined. In addition an oral examination was conducted to determine the presence of OC and blood serum levels of the micronutrients (i.e. Zn, Vit A, Se, Fe, Albumin and Vit D), which were analysed by the NHLS.

5.1 Demographic Profile

The mean age in this TB cohort was 36, 7 years; the majority of whom were females (60%) and unemployed (73, 9%). This is in contrast to general population statistics, which show that approximately twice as many males as females are diagnosed with TB in SA and developing countries each year (Dolin, 1998; Holmes et al., 1998). Austin et al.'s (2004) retrospective study of secondary data analysis from the laboratory register of the SA Institute of Medical Research also reported that male TB patients outnumbered females TB patients at a ratio of 2.08:1 in the Western Cape. The higher percentage of females in the TB cohort might be due to the fact that more females are HIV positive and more likely to be compliant in attending clinics for TB treatment and recall visits than males (Lertmaharit et al., 2005). Brennan et al. (2010) reported that females tend to utilise public health services more often than males do. Although the study sample is not representative of all TB patients in SA, it is indeed worrying that fewer males (who are the majority of people infected with TB) than females were regular attendees at these clinics as indicated by the clinic register (Rudolph et al., 2011).

Approximately 70% of the TB cohort in the current study was HIV positive. However, John et al. (2007) reported a 90% rate of TB/HIV co-infected patients admitted to the medical ward in Helen Joseph Hospital. This finding could be due to Helen Joseph in-patients having more co-existing conditions than outpatients do.

The present study did not explore HIV prevalence by gender. However, the NIRS (2011) using the same sample reported that more females (76%) were HIV positive than males

24% (Rudolph et al., 2011). In SA (SA NDoH, 2009) and sub-Saharan Africa nearly 60% of those living with HIV/AIDS are females (Prah, 2004). This higher percentage is not unexpected because females are generally at higher risk for HIV infection as they are biologically, socially and culturally more HIV susceptible than men (Darnton–Hill et al., 2005).

5.2 TB or HIV/TB Co-infection and Micronutrient Deficiency Levels

The link between TB and malnutrition consists of two interactions: the effect of TB upon nutritional state and the effect of malnutrition on the occurrence and clinical manifestations of TB (Macallan, 1999). Consequently in TB cases, nutrients (e.g. Fe, Copper, Se and Zn) are simultaneously compartmentalised to the tissues, lost from the body or blocked from cellular utilisation (Enwonwu, 2006).

5.2.1 Zinc

Several studies have demonstrated that the serum levels of Zn decreased significantly during active TB and increased following recovery after institution of antitubercular therapy and improvement of nutritional status (Wiid et al., 2004; Milano et al., 2004). In the present study, 30% of the sample had mean serum levels of 10.60 (S.D. 4.66) µmol/L for Zn deficiency. This prevalence was significantly lower than that reported by Koyanagi et al. (2004), who reported Zn deficiency in 61% of the TB cases [10.01 (S.D. 1.88) µmol/L]. A study investigating the relationship between wasting and micronutrient malnutrition in 222 TB patients and 579 TB/HIV co-infected patients conducted by van Lettow et al. (2004) also reported that TB patients are at a higher risk of Zn deficiency with prevalence as high as 85%. The large percentage differences in the findings between this TB cohort and the reported studies is due to the differences in the cut off levels of defining Zn deficiency [current study < 8.2 µmol/L; Koyanagi et al., (2004) < 10.7 µmol/L; van Lettow et al., (2004) <11.5 µmol/L].

Tuberculosis/Human Immunodeficiency co-infection worsens Zn deficiency in patients as it is shown in the current study that 57% of the TB/HIV co-infected patients had Zn deficiency at a mean value of 9.60 (S.D. 5.82) µmol/L. Studies have shown Zn deficiency to be more prevalent in TB/HIV co-infected cases versus TB only controls (van Lettow et

al., 2004). A study conducted by Kassu et al. (2006) to evaluate the serum levels of copper, Zn, Se and Fe in TB patients with or without HIV reported that the concentration of Zn in 74 of the TB/HIV co-infected patients (mean 73.65, S.D. 37.66 µg/dl) was significantly lower ($p<0.05$) than that in 81 of the TB patients without HIV co-infection (mean 81.14, S.D. 14.16 µg/dl) and (mean 88.85, S.D. 34.16 µg/dl) in 31 of the healthy controls (no TB or HIV infection) [$p<0.001$]. The difference between the TB cohort in this study and the cohort of the reported study by Kassu et al. (2006) is related to the cut off levels and units defining Zn deficiency; however, it is worth noting that the level of Zn in patients that are TB/HIV co-infected is very low, indicating strong association of hypozincaemia with impaired immune response and degree of malnutrition as reported previously from HIV-positive and AIDS patients (Graham et al., 1991).

5.2.2 Vitamin A

Slightly more than half of this sample (52%) had Vit A deficiency (< 1.05 µmol/L), with a mean of 1.13 (S.D. 0.43) µmol/L. This compares favourably with Koyanagi et al. (2004), who found 52% of 46 pulmonary TB cases and 10 controls (non-TB cases) who had a serum concentration of Vit A below 1.05 µmol/L. Koyanagi et al. (2004) also reported that there was a significantly lower mean Vit A in the TB cases [1.16 (S.D. 0.65) µmol/L] versus in the non-TB controls [2.80 (S.D. 0.83) µmol/L]. However, Karyadi et al. (2002), in a randomised control trial investigating whether Vit A and Zn supplementation increases the efficacy of anti-tuberculosis treatment with respect to clinical response and nutritional status, reported that at baseline 32% of newly diagnosed TB patients (n=80) had Vit A deficiency [(<0.70 µmol/L), with a mean of 0.82 (S.D. 0.04) µmol/L]. The lower percentage of Vit A deficiency in Karyadi et al.'s (2002) study is probably because, firstly, the sample consists of newly diagnosed TB patients who have never received any anti-tuberculosis intervention and, secondly, that the cut off levels defining Vit A deficiency were different from the one used in the current study and that of Koyanagi et al. (2004) [current study and Koyanagi et al. (2004) cut off levels (< 1.05 µmol/L) and Karyadi et al. (2002) (< 0.70 µmol/L)].

In this TB cohort, 61% of the TB/HIV co-infected patients had Vit A deficiency [mean 1.08 (S.D. 0.43) µmol/L] as compared to the 52% of the TB only infected patients. The

high proportion of individuals with low Vit A levels in the TB/HIV co-infected subgroup cannot be explained by nutritional deficiencies alone. Studies have also shown that low Vit A levels are common in patients with TB and those infected with HIV and are even more severe among patients with HIV/TB co-infected (Semba, 1997). Mugusi et al., (2003) conducted a study that sought to determine serum Vit A levels of 100 HIV-positive and -negative TB patients and 144 healthy controls and reported that serum Vit A levels were lower among HIV-infected individuals [mean 18.8, S.D. 5.7 µg/dl] and lowest among TB/HIV co-infected patients [mean 13.1, S.D. 5.6 µg/dl] than controls [mean 26.6, S.D. 5.4 µg/dl]. Low levels of Vit A were found to be more common among TB patients with a significantly high prevalence among those HIV positive (64, 4%) than healthy controls (9.1%) ($p< 0.0001$). Although this current study did not show any significant association between Vit A deficiency and TB or TB/HIV co-infected subgroups, it seems that TB/HIV co-infection may potentially worsen Vit A deficiency than TB or HIV alone because Vit A deficiency is associated with TB/HIV co-infection and more pronounced with HIV disease progression (Baum et al., 2003).

5.2.3 Selenium

Selenium has immunopotentiating effects and its deficiency appears to result in immunosuppression (Roy et al., 1994). Thus, it reduces the virulence of HIV and slows the progression of HIV-related disease (Rayman, 2000). Approximately 7% of the TB cohort had Se deficiency (< 46) µg/L) with a mean of 69.12 (S.D. 17.41) µg/L. Koyanagi et al. (2004) conducted a study that compared the serum concentrations of Zn, copper, Se, Vit A and Vit E among 46 pulmonary TB cases with of 10 healthy controls (non-TB cases). This study reported a significantly lower Se concentration in pulmonary TB cases than in the healthy controls [1.81 (S.D. 0.25) versus 1.99 (S.D. 0.23) µmol/L]. The difference in the current study and Koyanagi et al.'s (2004) findings is due to the difference in the cut off levels and units defining Se deficiency [current study (46 µg/L) and Koyanagi et al. (2004) (1.81 µmol/L)].

Koyanagi et al. (2004) also reported only three HIV/TB cases 6.52% as compared to an estimated 70% of the current study sample being co-infected with TB/HIV. In the 70% of the TB/HIV co-infected cases in this study, 36% had Se deficiency [mean 67.15 (S.D.

13.28) µg/L], compared to the only 7% of TB only infected cases with Se deficiency. An inverse correlation between Se and HIV infection was reported by Dworkin (1994), who conducted a study to determine plasma Se concentration in 12 patients with AIDS compared to five healthy controls. He reported that plasma Se in AIDS patients was significantly lower than in the healthy controls [mean 0.043 (S.D. 0.01) microgram/ml versus mean 0.095 (S.D. 0.016) microgram/ml ($P < 0.001$)] and that patients with AIDS tend to have more severe Se deficiency than those with earlier stages of HIV infection. There is a lack of a standardised definition of micronutrient deficiency for some micronutrients such as such as Se (cut off levels and units), making it difficult to achieve a valid comparison between the current study and the reported studies as illustrated above [current study Se deficiency < 46 µg/L, Koyanagi et al. (2004) < 1.81 µmol/L and Dworkin (1994) < 0.53 micrograms/g. Never the less , the current study and other reported studies indicate that those with TB/HIV co-infection are susceptible to lower concentrations of Se as compared to TB only infected cases or healthy controls (non-TB). Furthermore, Kassu et al.'s (2006) study aimed to determine serum levels of micronutrient (Zn, copper, Se, calcium and magnesium) in 375 pregnant (42 HIV positive) and 76 non-pregnant women (20 HIV positive) who visited the University of Gondar Hospital in Ethiopia. Kassu et al. (2006) reported that the mean serum level of Se was significantly lower in pregnant women with HIV co-infection compared to HIV-negative pregnant women ($p < 0.05$). This study differs from the current study in terms of study sample size (n=88 versus n=375) and the type of sample (HIV-positive and -negative pregnant women, as pregnant women were excluded from the current study).

5.2.4 Iron

Iron deficiency prevalence varies according to age, gender, physiological, pathological and socioeconomic conditions (WHO, 2001) and it is more prevalent in infants, children, pregnant women and people of low socioeconomic status (Gibson, 2005). There was an Fe deficiency (< 9 µmol/L) in 46.3% of this TB cohort [mean 11.19 (S.D. 6.59) µmol/L] and in 58% of the TB/HIV co-infected cohort, with no significant association between Fe deficiency and TB or TB/HIV co-infection [mean 10.6 (S.D. 7.2) µmol/L]. However, Kassu et al.'s (2006) study, which compared serum levels of Fe, copper, Zn and Se

among 81 TB only infected cases, 74 TB/HIV co-infected cases and 31 healthy controls (non-TB and HIV negative) and investigated changes in serum levels of these micronutrients before and after an intensive phase of anti-TB chemotherapy in the TB only infected cases and TB/HIV co-infected cases, reported a significant difference of Fe deficiency (<60 µg/dl) between TB/HIV co-infected patients [mean 14 (S.D 18.9)] µg/dl and healthy controls [mean 1 (S.D. 3.2)] µg/dl ($p < 0.01$). There was also Fe deficiency (< 60 µg/dl) in the TB patients without HIV co-infection [mean 9 (S.D. 11.1)] µg/dl versus healthy controls [mean 1(S.D. 3.2)] µg/dl, but the difference was not statistically significant ($p < 0.07$). The difference between the current study and that of Kassu et al. (2006) is that different cut off levels and units to define Fe deficiency were used in the two studies. Taha and Thanoon (2010) also reported that serum Fe values were significantly lower in pulmonary TB cases in comparison with controls. Although the current study did not find a significant association between Fe deficiency and TB only infected cases and TB/HIV co-infected cases, studies have shown that severe Fe deficiency in TB/HIV co-infected cases is directly linked to HIV infection, as HIV-positive cases experience reduction in food intake (Enwonwu, 2006) and nutrient malabsorption (Friss et al., 1998), making those individuals co-infected with TB/HIV more vulnerable to Fe deficiency.

5.2.5 Albumin

Albumin deficiency was found in 31% of the TB cases, with a mean value of 37.28 (S.D. 5.55) g/L, and in 38% of the TB/HIV co-infected cases, with a mean value of 38.42 (S.D. 4.62), with no significant association between Albumin deficiency and TB. Although Ramakrishnan et al. (2008) reported significantly lower serum levels of Albumin in TB/HIV co-infected cases (mean 2.9, S.D. 0.4 g/dL) than in TB cases (mean 3.6, S.D. 0.7 g/dL). In their study participants had to fast before blood collection for micronutrient analysis, while in the current study patients were provided with food during the data and blood collection phase. The units are different between the two studies but the means are similar, as 3.6 g/dL in the TB cases is equivalent to 36 g/L, although there is a slight difference in the S.D. between the two studies. Mugusi et al. (2003) also reported that the mean serum Albumin was significantly lower in HIV-positive [mean 29.7, S.D. 7.6

µmol/l] than in HIV-negative patients [mean 31.4, S.D. 8.1 µmol/l]. However, Okamura et al. (2011) further investigated the relationship between hypoalbuminemia and computed tomography findings of the lungs, and reported that hypoalbuminemia [mean value of 3.3 (S.D. 0.71) g/dL] was significantly related to the presence of typical radiographic findings of TB in patients of over 70 years of age.

5.2.6 Vitamin D

Fifty-five per cent of the TB cohort in the current study had Vit D deficiency, with a mean of 47,96 (S.D. 17.91) nmol/L; however, a case control study by Ho-Pham et al. (2010) that sought to determine the association between Vit D deficiency (< 30 ng/mL), parathyroid hormone and the risk of TB in a Vietnamese population of 166 TB cases (113 men and 53 women) and 219 non-TB healthy controls (113 men and 106 women) reported that the prevalence of Vit D insufficiency was 35.4% in men with TB and 19.5% in controls ($p = 0.01$). In women, there were no significant differences in Vit D between TB patients (n=53) and controls (n=106). The prevalence of Vit D insufficiency in women with TB (45.3%) was not significantly different from those without TB (47.6%; $p = 0.91$).

The difference between the findings of the current study and that of Ho-Pham et al. (2010) could be due to differences in the TB sample size (n=166) versus the 88 TB sample size of the current study. There is also a difference in the cut off levels that define Vit D deficiency (current study 49 nmol/L and Ho-Pham et al. (2010) 30 ng/mL). The prevalence of Vit D deficiency was higher in the TB/HIV co-infected (63%), with a mean value of 49.46 (S.D. 19.84), with no significant association between TB/HIV co-infection and Vit D deficiency. Nansera et al.'s (2011) study, which measured Vit D and calcium levels in 50 HIV-negative, 50 HIV-infected and 50 TB/HIV co-infected Ugandan adults, also reported mean and standard deviation Vit D levels of 26 (S.D.7 ng/ml) in HIV-negative, 28 (S.D. 11 ng/ml) in HIV-infected and 24 (S.D. 11 ng/ml) in TB/HIV co-infected adults ($p > 0.05$ all comparisons). Also Vit D deficiency (<12 ng/ml) was absent in the HIV-negative controls and present in 10% of the HIV-positive and in 12% of those TB/HIV co-infected ($p = 0.03$ HIV negative versus $p > 0.05$ TB/HIV co-infected cases). Suboptimal Vit D levels (< 20 ng/ml) were noted in 20% of the healthy controls, 22% of

the HIV-positive subjects and 38% of the TB/HIV co-infected subjects (p = 0.047 healthy vs TB/HIV co-infected cases p > 0.05). Individuals with TB/HIV co-infection usually have severe micronutrient malnutrition due to a combination of TB infection and high viral load.

5.3 OC and Demographics

A bivariate analysis was conducted using the Pearson Chi-squared and Fischer exact tests to determine the association between OC, demographics, HIV status and micronutrient deficiency.

5.3.1 OC, demographics and HIV status

Forty per cent (n=35) of the TB cohort had OC, with pseudomembranous OC being the most prevalent (48%). OC was more prevalent in females and in those above the mean age of 37, with no significant association between age, gender and OC. Ikebe et al. (2006) also reported that candidal activity was not significantly associated with age or gender in the relatively healthy individuals of above 60 years of age, as certain systemic conditions (e.g. diabetes mellitus), defects of the immune system (Zaremba et al., 2003; Coleman et al., 1993; Jacob et al., 1998), and /or some medications (e.g. antibiotics, corticosteroids) may predispose the transformation of colonisation of species such as *Candida* into opportunistic pathogens (Webb et al., 1998) .

In addition OC was more prevalent in the HIV-positive (27, 3%) cases than in HIV-negative individuals. This finding is in line with the findings of Akpan et al. (2002), who reported that OC was more prevalent in HIV-positive individuals, as 95% of HIV-positive individuals had OC when compared to HIV-negative individuals in the reported study. In the majority of HIV-positive patients OC is often present as an initial manifestation of HIV infection (Pienaar et al., 2010), and is a useful clinical marker of patients with high viral load (Campro et al., 2002). Therefore, even though only 27.3% of the HIV-positive cases in the current study had OC, evidence has shown that OC is one of the key opportunistic infections that occur commonly and that it recurs frequently in HIV-positive cases.

5.3.2 OC and micronutrient deficiency

The oral cavity is a reservoir of different potential pathogens, bacterial, viral and fungal, which can multiply and cause disease in malnourished and immunocompromised patients. Only a few studies have investigated factors such as Zn, Albumin, Se, Fe, Vit A and D and their possible role in the alteration of oral mucosal integrity (e.g. Samaranayake, 1986). Thus, it was also difficult to make a comparison of the current findings with other studies for the association between micronutrient deficiency and OC as illustrated below (especially in relation to Vit A, Albumin and Vit D) .

5.3.2.1 Zinc

In the present study, 40% of the TB patients had OC and Zn deficiency (< 8.2 µmol/L) and 23% had OC within Zn levels the normal range (> 8.2 µmol/L) .However, there was no significant association between OC and Zn deficiency. In contrast , Paillaud et al.'s (2004) case control study comparing OC cases and no OC controls reported that 44% of hospitalised elderly patients (> 70 years of age) had Zn deficiency (< 12·5 µmol/L), with a significant association between OC and Zn deficiency. An estimated 70% of the current sample was co-infected with TB/HIV and Baum et al. (2003) reported Zn deficiency (< 0.73 µg/mL) in 56% of HIV-positive drug users, with a mean of 0.73µg/mL, and fasting blood samples were collected from the participants. Baum et al.'s (2003) sample (drug users) and Zn deficiency definition are different from those used in the current study, resulting in differences in the results. However, it is important to note that Zn deficiency has been linked to a declining CD4 count in HIV-positive individuals because it reduces the generation of T cells and depresses humoral and cell-mediated immunity; thus, Zn supplements are important in Zn-deficient HIV/AIDS patients even among those being administered HAART (Beach et al., 1992).

5.3.2.2 Iron

OC was present in both, patients with Fe deficiency (31% at < 9 µmol/L) and with Fe levels within the normal range (38% at > 9 µmol/L). There was no significant difference between those with high Fe and OC compared to those with low Fe levels. Walker et al. (1972) also could not find any differences in the frequency of OC or oral carriage rate of

Candida in Fe-deficient subjects and controls, and there was no significant change in the disease process observed after Fe replacement therapy. Similarly, Samaranayake and Macflare (1981) identified five Fe-deficient patients with chronic atrophic OC in whom adequate restoration of serum Fe levels had no effect on the recurrence of OC. In addition, Jenkins et al. (1977) could not establish a relationship between Fe deficiency and chronic atrophic or hyperplasic variety of OC. Recurrent OC infection in normal Fe levels could be due to systemic diseases, such as diabetes mellitus, and dentures.

However, Paillaud et al. (2004) reported OC in hospitalised patients of above 70 years of age with an Fe level of below < 40 µmol/L, with 13% experiencing OC and 24% not experiencing OC. In addition Fletcher et al. (1975) reported a high prevalence of OC in Fe-deficient patients who had angular cheilitis and atrophic glossitis. The difference in the findings may be due to the difference in the demographics of the population (age and hospitalised) and the study design. Also institutionalisation per se may render elderly individuals vulnerable to nutritional deficiency (Schorah et al., 1979; Marazzi et al., 1990) because the storage of foods and the cooking procedures used in institutions may contribute to the loss of vitamins from the food. Hospitalised elderly TB patients also have limited food choice, which ultimately affects their food intake and micronutrient serum levels as compared to TB outpatients. There is also a high prevalence of edentulousness in elderly patients, which limits their food choice and impacts negatively on their micronutrient status (Sheiham et al., 2001) as compared to the TB cohort dentate sample (100% of the population had more than 20 teeth in their mouth). Also none of the sample for the current study had dentures while the sample used in Sheiham study had dentures, as dentures and old age are a high risk to OC, which probably explains Paillaud et al.'s (2004) findings.

5.3.2.3 Selenium

OC was present both in patients with Se deficiency (33% at < 46 µg/L) and in patients with Se levels within the normal range (26% at < 46 µg/L), with no significant difference between OC and Se levels. Even though the current study did not analyse the viral loads in the TB/HIV co-infected cases, Dworkin (1994) reported significantly lower Se levels in AIDS cases (mean 0.043, S.D. 0.01 microgram/ml vs mean 0.095, S.D. 0.016

microgram/ml) than in controls. This occurred in both homosexuals and drug users with AIDS and irrespective of the presence or absence of diarrhoea or gastrointestinal malabsorption. Selenium deficiency is common in HIV-positive patients, indicated by low plasma and red blood cell levels of Se, and diminished activity of glutathione peroxidase, and AIDS patients tend to have more severe deficits than those with earlier stages of HIV infection (Dworkin, 1994). The differences in the findings between Dworkin's study and the current study could be the result of Dworkin's (1994) homosexual and drug user sample, which had a high viral load as compared to the TB outpatient sample.

5.3.2.4 Vit A, Albumin and Vit D

In the present study, OC was present both in patients with micronutrients below the normal range [Vit A (25%), Albumin (32%) and Vit D (36%)] and in patients with micronutrient levels within the normal range [Vit A (47%), Albumin (36%) and Vit D (17%)]. However, there was no significant difference between OC and these micronutrient levels, and there is a lack of studies that have investigated the relationship between these micronutrients (Vit A, Albumin and Vit D) and OC in a TB cohort. However, Steenkamp et al.'s (2009) study conducted in Mangaung in children (one to 10 years of age) reported a median Albumin level of 32 g/l (IQR 28; 35) in 78% of the sample. An estimated 63% of the children had Vit A deficiency at a median of 18.15 (IQR 15.3; 22.35) µg/dl and 44% for Vit D deficiency at a median of 20 (IQR 16; 24.5) ng/ml. Children with abnormally low Vit A levels presented with significantly higher viral loads and lower CD4 cell counts.

There are differences between the current study and Steenkamp et al.'s (2009) study in terms of the sample, recording and analysis of the viral load and CD 4 cell count, and analysis of the micronutrients in combination. However, Steenkamp et al. (2009) indicated that the negative impact HIV has on these micronutrient deficiencies and OC has been identified as one of the oral lesions strongly associated with HIV/AIDS (EC-Clearinghouse, 1993) and is a marker of immune suppression (Miziara et al., 2006). OC associated with HIV infection occurs frequently and could be considered as an initial manifestation of the disease (Pienaar et al., 2010). Although the current study shows no

significant association between OC and Vit D, Vit A and Albumin deficiency, there is evidence that 70% HIV-positive patients are likely to have experienced OC and/or Vit D, Vit A and Albumin deficiency (Kassu et al., 2006).

It is important to note that there are other different host factors which are independently implicated in the development of OC; these factors include old age, treatment with antibiotics, denture wearing and neglected oral hygiene, all of which predispose patients to the occurrence of OC (Shay et al., 1997).

5.4 Limitations and Future Directions

This study had a few limitations worthy of mentioning, namely the small sample size of TB patients and the lack of a control group, which restricted the ability to draw conclusions about the associations between the independent and dependent variables. Future studies will benefit from much larger sample sizes to draw more convincing conclusions. This study is, however, a useful starting point for understanding the association between micronutrient deficiency and OC in TB patients. More case control studies are needed to investigate the association of micronutrient deficiency and OC in TB cases versus non-TB cases.

5.5 Conclusion

The present study shows that there is a high prevalence of OC, especially pseudomembranous OC as well as high prevalence of several micronutrient deficiencies among adult TB patients. Yet there was no association between micronutrient deficiency and OC in adult TB patients. Further longitudinal investigations with larger samples are needed for determining whether micronutrient deficiencies are an independent risk factor for OC.

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APPENDIX A: Personal Record Sheet

Reference #

Date (dd/mm/yy)	
Gender	
Age	
Race	
HIV Status (0= HIV-,1=HIV+)	

APPENDIX B: Oral Candidiasis Status Data Capture Sheet

1. **Refer no.**……..

Oral Candidiais *(tick appropriate box)***:**

Present ☐ **Absent** ☐

Type of Oral Candidiasis (Clinical diagnosis):

1. Pseudomembranous ☐
2. Erythematous ☐
3. Angular Cheilitis ☐

Symptoms: *(tick appropriate box)***:**

a. Burning oral sensation ☐

b. Sore Tongue ☐

Denture *(tick appropriate box)***:**

a. Present ☐

b. Absent ☐

Type of denture *(tick appropriate box)***:**

a. Partial ☐

b. Full/Full ☐

Location of denture *(tick appropriate box)***:**

a. Lower ☐

b. Upper ☐

Location *(tick appropriate box)***:**

1. upper lip	
2. lower lip	
3. mucosa of the upper lip	
4. mucosa of the lower lip	
5. mucosa around the corner on R side	
6. mucosa around the corner on L side	

7. cheek mucosa on R side of patient	
8. cheek mucosa on L side of patient	
9. mucosa of upper jaw, bet lip/cheek & gums	
10. mucosa of lower jaw, bet lip/cheek & gums	
11. mucosa of gums of upper teeth	
12. mucosa of gums of lower teeth	
13. top surface of tongue	
14. sides of tongue	
15. under surface of tongue	
16. mucosa between undersurface of tongue & gums of L teeth	
17. mucosa of hard palate	
18. mucosa of soft palate	
19. mucosa behind last molar of U & L jaws	

APPENDIX C: Participant Information Sheet

Good day

My name is Dr Maphefo Thekiso. I am a Registrar in the Public Oral Health Division at the School of Public Health at the University of Witwatersrand (Wits). I would like to invite you to participate in a study I'm conducting entitled **"The relationship between micronutrient deficiency and Oral Candidiasis among Tuberculosis patients in Alexandra, Johannesburg".**

What is the purpose of the Study?

The main aim is to investigate the association between certain vitamins and minerals, and oral sores - "oral thrush" among TB patients. The information gathered may assist the Wits Public Oral Health Division and the South African National Tuberculosis Association (SANTA) to understand the relationship between micronutrient deficiency and oral candidiasis among Tuberculosis (TB) patients and recommend appropriate intervention.

What the study entails?

The research process includes the following.

- Questions about your personal details (i.e. age, gender and social habits).
- Viewing of clinical records in relation to your HIV status – in full confidentiality.
- Examination of the mouth will be done using a mirror and light.
- Blood sample of 10ml will be collected from each participant to measure nutrients levels.
- Blood will be discarded after nutrient analysis and the findings will be kept confidential.
- The collection of blood samples may involve mild discomfort or pain.
- The blood sample and health status information will be kept confidential as every participant will be allocated a reference number.
- The full examination will take about 15 minutes.

If there are any questions you do not wish to answer, you are free not to answer these questions.

Confidentiality:

All information obtained from you will remain confidential. Only the researcher will have access to the data. The information collected will be kept in a secure and locked office. You will be given a reference number to maintain anonymity.

Participation is voluntary:

Participation is voluntary and you are free not to participate or to respond to any questions. Refusal to participate or discontinue will not disadvantage you in any way or your treatment.

APPENDIX D: Consent Form

Date: ……

Reference no:.....

I have read the information sheet about the study and had it explained to me. I understood the purpose of the study.

I also understand that I have the right to cease participation at any time and to refuse answering certain questions. I am told that there will be no risks for me to take part or not to take part in the study.

I understand that my confidentiality will be carefully guarded and no one outside the research team will be able to know about my answers, health and oral status. I understand that a reference number will be allocated to me to maintain anonymity.

Signature of respondent (If yes)…….

Initials of investigator…….

Contact details of main researcher:

Dr Maphefo Thekiso
Wits School of Public Health
Division of Public Oral Health,
Tel: (011)717-2005,
Email: maphefo.thekiso@wits.ac.za

APPENDIX E: Ethical Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Desiree Thekiso

CLEARANCE CERTIFICATE	M10733
PROJECT	The Relationship betwee Oral Cadidiasis and Micronutrient deficiency in an Adult TB Cohort in Alexandra, Johannesburg (Revised Title)
INVESTIGATORS	Desiree Thekiso.
DEPARTMENT	School of Public Health
DATE CONSIDERED	30/07/2010
M100DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 20/01/2012 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr V Yengopal

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX F: Johannesburg City and the Gauteng Department of Health and Social Development

Joburg
a world class African city

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13 August 2010

Dear Professor Rudolph

APPROVAL TO CONDUCT RESEARCH WITHIN HEALTH IN THE CITY OF JOHANNESBURG

Permission has been granted to you to conduct research in the Health Department within the City of Johannesburg.

Topic: A Longitudinal Pilot Study to evaluate the efficacy of a Fortified Food Supplement

Please contact the following person(s) before you commence with your project and to gain access to the clinics:

Region E: Regional Health Manager: Mr Vusi Mazibuko
011 582 1504/082 464 9547

Should you have any queries please do not hesitate to contact our department.

We look forward to your Final Research Report.

Thank you

13/8/10

DR. R. BISMILLA
Executive Director
City of Johannesburg
Health Department

Appendix G: Executive Summary

Nutritional Indicator Research Study (NIRS)

Introduction

Vitamin and nutrient enriched foods can be vital for individuals suffering from illnesses such as TB or HIV, but there is still little evidence demonstrating their efficacy. This pilot study sought to begin close this gap. This study will assess the nutritional effects of a fortified food product on adults with TB and children in Alexandra, South Africa. The study will evaluate the sensitivity and validity of measurement tools to identify alternatives to blood analysis to accurately measure individuals' nutritional status in a real world clinical setting.

The Objectives of this study are:

- To record and describe the socio-economic status and the nutritional concerns (including food security) of two selected groups recruited from the population of Alexandra.
- To generate baseline data on the nutritional status of these groups; 3-6 year old children and adult TB populations in Alexandra, using the following indicators:
  - (1) nutrient intakes
  - (2) blood inflammatory markers and key micronutrient serum levels
  - (3) anthropometric measures of height, weight, waist and hip circumference
  - (4) clinical signs of micro-nutrient deficiency,
  - (5) bio-electrical impedance analysis,
  - (6) skin elasticity,
  - (7) hand-grip strength, and
- To evaluate the sensitivity and validity of non-invasive indicators of nutritional status by correlating these with biochemical serum tests.
- To evaluate the process and practicality of proposed research instruments and procedures.

- To develop data-collection skills and build capacity among support staff within the community at the research location.
- To evaluate the impact of a fortified food (e'Pap) on the nutritional status of children and adult TB patients over a three (3) month period.

The Investigational Product

The investigational product (e'Pap) is a fortified food product which includes minerals in a chelated, highly bio-available form, and uses processes which preserve essential fatty acids and fibre. The formulation is a pre-cooked, multivitamin and mineral fortified, wholegrain maize and soya food. It is specially formulated to ensure bio-availability and absorption of nutrients. It is widely distributed in South Africa and other African countries.

Methodology

A convenient sample of participants fulfilling the selection criteria listed below will drawn from the different site populations. Two sample groups will be selected: adult TB patients receiving treatment at local clinics, and children attending creches in Alexandra. A total of 120 adults and 65 children will be enrolled in the study. A broad range of nutritional state indicators (Table 1) will be measured on three distinct measurement events and recorded on a dedicated set of record sheets. Each participant will be monitored at approximately weeks 1, 5, 10 using the full set of evaluation instruments. Record sheets will be encoded to ensure anonymity.

After baseline measurements are recorded, all participants will receive a course of de-worming medication, then receive the nutritional supplement and will be instructed on how to use it. Data will be captured in a database and analysed statistically to determine aggregate trends, to detect significant changes in nutritional state, and to evaluate the sensitivity and validity of measurement tools.

Table 1 (Alan and NHLS , 1995)

Micronutrient	Normal Range
Zinc	8.2-23 µmol/L
Vitamin A	1.05-2.800 µmol/L
Albumin	35-52 g/L
Selenium	46-143 µmol/L
Iron	9-31 µmol/L (Male:11.6-31.3 µmol/L) (Female: 9-30.4 µmol/L)
Vitamin D	49-172 nmol/L

Patient Selection Criteria

1. Adults

TB patients receiving treatment from health clinics in Alex will be selected and invited to participate based on the following criteria:

- Inclusion: Adult male and female patients receiving care and TB treatment from a clinic in Alexandra.
- Inclusion: Patients BMI > 16
- Inclusion: Patients between ages of 18-60
- Inclusion: Patients consented to participate in the study for its full duration.
- Inclusion: Patients were outpatients with a history of at least 3 regular visits to the clinic.
- Inclusion: Patients were not regularly consuming e'Pap or a similar fortified food supplement.
- Exclusion: Patients have known food allergies to maize and soya products.
- Exclusion: Known conditions for which de-worming medication is contra-indicated.
- Exclusion: Candidates are below critical body weight for blood sample collection (BMI < 16)
- Exclusion: Patients being treated for Multidrug-Resistant TB.

2. Children

Children from creches in Alexandra will be selected according to their fulfilment of selection criteria below. Their parents and or guardians will be invited to provide consent to allow their children to participate in the research.

- Inclusion: Children between ages of 3-6 at the time of enrolment
- Inclusion: Written consent of children's parents and or legal guardians
- Inclusion: Regular attendance at selected creches
- Inclusion: Patients were not regularly consuming e'Pap fortified food supplement.
- Exclusion: Known food allergies to maize and soya products.
- Exclusion: Known conditions for which de-worming medication is contra-indicated.
- Exclusion: Candidates are below critical growth reference for blood sample collection (children: MUAC < 110mm)