Prevalence of Oral Candidiasis and Associated Risk Factors among HIV Positive Patients taking Anti –Retroviral Therapy at Ambo General Hospital, Oromia Regional State, Ethiopia

M.Sc. THESIS

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We hereby certify that We have read and evaluated this Thesis entitled "Prevalence of Oral Candidiasis and Associated Risk Factors among HIV Positive Patients taking Anti -Retroviral Therapy in Ambo General Hospital, West Shoa zone, Oromia Regional State, Ethiopia prepared under our guidance by Fikru Cherinet. We recommend that it can be submitted as fulfilling all the thesis requirements.

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DEDICATION

I dedicated this thesis manuscript to my dear family for their love, affection, encouragement and unrestricted support rendered to me during all stages of my education. They opened the doors of success for me.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this. Thesis any scholarly matter that is included in the Thesis has been given recognition through citation.

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ABBREVIATIONS AND ACRONYMS

ADRS	Adverse Drug Reactions
AGH	Ambo General Hospital
ART	Anti Retro Viral Therapy
CDC	Center for Disease Control
CSA	Central Statistical Agency
EDHS	Ethiopian Demographic and Healthy Survey
FHAPCO	Federal HIV /AIDS Prevention and Control Office
FMoH	Federal Ministry of Health
HAART	Highly Active Anti-Retro Therapy
HAPCO	HIV/AIDS Prevention and Control Office
NCCLS	National Committee on Clinical Laboratory Standard
OPI	Opportunistic Infection
UNAIDS	United Nations Joint Programme on HIV/AIDS
UNICEF	United Nations International Children Emergency Fund
WHO	World Health Organization

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ABSTRACT

Oral Candidiasis has been a worldwide health crises especially in immunocopromised patients particularly with HIV infections. Though the incidence and prevalence of Candida infections have been reduced due to the use of anti-retroviral therapy (ART), candidiasis remains the most frequent HIV associated health disorder. This study aimed at assessing the prevalence of canddiasis and associated risk factors among HIV positive participants taking anti-retro viral therapy. The study was conducted at Ambo General Hospital. Hospital based cross sectional design was used, to identify Candida infections among HIV positive patients who were taking ART treatment. By serial simple random sampling method, 374 HIV/AIDS patients were selected and examined for Candida species infection. Socio-demographic and clinical data was collected using questionnaire and structured format, respectively. The swaps from oral cavity were cultured on Sabouraud dextrose agar with choramphienicol followed by germ tube test for identification of Candida albicans in each HIV positive individual. Analyses of the results were done using SPSS version 20 statistical software. Chi-square tests, odds ratio generated by logistic regression, were used to measure the association risk factors among the study participants. P-value less than 0.05 were considered statistically significant for all tests. Out of 374 HIV positive participants involved in this research, 140(37.4%) were males and 234(62.6%) were females. The average age of the study subjects was 37.48 years old with the minimum and maximum age of 13 and 82 years old, respectively. The prevalence of oral candidiaisis among the study participants was 42.25% of which the prevalence of Oral Candidiasis for female HIV patients was 62.7% and 37.30% for male patients. The prevalence of Candida albicans and other candida species was 26.20% and 16.04% respectively. Marital status (being single), level of education and knowledge about candida diseases were significantly associated with prevalence of oral Candidiasis. Candida albicans was more prevalent than non albicans candidiasis among the study participants. Females HIV/AIDS patients were more infected in both Candida albicans and non-Candida albicans diseases. Being single, uneducated patients and, having poor knowledge about Candidiasis were factors. Thus from Federal Ministry of Health to hospitals stake holders (health professionals) are expected to provide proper treatment, create awareness about candidiasis. In the study area further study should be needed to identify the non-Candida albicans species.

Keywords: Ambo, Candidiasis, Prevalence

1. INTRODUCTION

Fungi cause a wide range of diseases, ranging from cutaneous dermatophyte infections to invasive infections in the severely immune-compromised patient (Facuc, 2011). They may have a yeast-like morphology or filamentous. Yeasts are eukaryotic micro-organisms classified in the kingdom fungi (Kurtzman and Fell, 2006). The most common mode of vegetative growth in yeast is asexual reproduction by budding (Balasubramanian *et al.,* 2004). Yeasts are unicellular fungi that cause a wide range of infections commonly called yeast infections in humans. Fungal (Yeast) infections are categorized into two groups; superficial and systemic. Superficial fungal infections can affect different parts of the human body including the skin, mouth, digestive tract, nails, vagina and oesophagus and can become chronic (Thevissen, 2005). Systemic fungal infections is often fatal and involve the spread of *Candida* species to the blood stream (candidemia) and to the major organs include brain, heart, eye, kidney, urinary tract and hepatic (Thevissen, 2005).

Candidiasis is the commonest fungal infections amongst HIV-positive patients worldwide. Infection can spread from the mouth through the pharynx to the oesophagus. A systematic infection of other parts of the body is not as common, but carries a high mortality of between 40% - 100% (Vazquez and Soble, 2003). *Candida* infection occur often via hypha formation and increasing adherence, invasion, tissue damage and form mycelia biofilms as well as a means of increasing proteolytic enzyme volume (Fidel *et al.*, 1999; Brown *et al.*, 2006). Though an increase in prevalence of *Candida* infections caused by non-albicans,*Candida albican*s remains the predominant *Candid*a species causing *Candida* infections accounting for over half of all cases in the world (Pfaller and Diekema, 2007). Likewise *Cryptococcus* species, *Cryptococcus neoformans* is the leading cause of infection mainly in less immunized patients while other *Cryptococcu*s species like *Cryptococcus albidus* and *Cryptococcus laurentii* can rarely cause infections (Kordossis *et al*., 1998).

The worldwide prevalence of candidiasis among HIV patients is 80% to 95% with *Candida albicans* being the most common causative agent (Hodgson and Rachanis 2002). Globally, there is a gradual trend toward change in the *Candida* species with non *Candida albican* being associated with HIV. Candidiasis is attributed to a reduction in host immune defenses

(Wabe *et al*., 2012). Many epidemiological studies have investigated the prevalence of *Candida* infections and their etiologic agents in different parts of the world. For example a study conducted in United States indicated that the most common type of *Candida* infection is vulvovaginitis (Sobel, 1992). This study shows that over 75% women suffer from at least one attack of *Candida* vulvovaginitis during their lifetime and nearly half of them suffer multiple episodes (Saporiti *et al.,* 2001; Ferrer, 2000). The prevalence and episodes of *Candida* infections depend on several factors such as age, locality and socio economic status (Ferrer, 2000; Enweani *et al.,* 2001).

Human immunodeficiency virus (HIV) infection is major global health concerns due to its continued spread in different parts of the world. Particularly, in the Sub Saharan African the spread of HIV is high. HIV positive patients carry more and a greater variety of yeasts than HIV negative patients (Morgan, 2005). Though the incidence and prevalence of opportunistic infection have been reduced due to use of anti-retroviral therapy (Diz Dios *et al.,* 2001), the occurrence of *Candida* infections in HIV patients has been increasing (Mousavi *et al*., 2012). However, in some studies, non- HIV infected and those infected with HIV with higher CD_4^+ T- cell counts have also been found infected with candidiasis (Katz *et al*., 1992). The spread of *Candida* species can indicate drug resistance or immunesuppression levels in a population. It could be a sensitive and specific indicator of a disease in the number of CD_4^+ cells and would show the onset of significant immune deficiency in people with HIV (Bodhade *et al*.2011).

Topics on HIV/AIDS such as its prevalence, its effects on socio-economy and several other issues have been well explored by researchers from different field of perspectives. In addition, the combined effect and association of HIV/AIDS with other diseases such as bacteria, protozoa, intestinal parasites, and others have been well assessed. However, only scant studies have been conducted that investigate *Candida albicans* and HIV/AIDS infections together (Moges and Kassa, 2014). Particularly, in the study area there was no evidence from previous studies that shows prevalence of candidiasis and associated factors among HIV infected patients. Hence, assessing the prevalence of candidiasis and associated factors among HIV infected patients taking ART therapy has become very important.

Therefore, the purpose of this study was to determine the prevalence of oral canddiasis and associated factors among HIV positive individuals taking anti-retro viral therapy.

Objective

The major purpose of this study was;

To assess the prevalence of oral canddiasis and associated factors among HIV positive individuals taking anti-retro viral therapy at Ambo General Hospital.

Specific objectives:

The specific objectives of the present study were:

1) To determine the prevalence of Oral candidiasis among the study populations.
2) To identify the major type of *Candida* species among the study population.
3) To correlate the major factors associated with oral candidiasis among the study population.

2. LITERATURE REVIEW

2.1. *Candida* Species

Fungi cause a wide range of diseases, ranging from cutaneous dermatophyte infections to invasive infections in the severely immune-compromised patient. They may have a yeast-like morphology or filamentous. Yeasts are eukaryotic micro-organisms classified in the kingdom fungi (Kurtzman and Fell, 2006). The most common mode of vegetative growth in yeast is asexual reproduction by budding with some yeast such *as Schizosaccharomyces pombe*, which reproduces by mitosis instead of budding (Balasubramanian *et al.*, 2004).

Candidiasis, commonly called yeast infection, is a fungal infection of any of the *Candida* species, of which *Candida albicans* is the most popular. Candidiasis thereby includes infections that range from superficial, such as oral thrush, vaginitis, to systemic and potentially life-threatening diseases. *Candida* infections of the latter category are also referred to as candidemia and are usually confined to severely immunocompromised persons, such as cancer, transplant, and HIV/AIDS patients, whereas superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort is common in many human populations Human Immunodeficiency Virus /Acquired Immune Deficiency Syndrome (HIV/AIDs) is among the most important influencing factors of fungal infections particularly candidiasis (Kourkoumpetis, 2010).

Candidiasis can be diagnosed via microscopic examination and culturing. In the microscope experiment, a sample of scraping or swab of the affected area is placed on a microscope slide and then a single drop of 10% potassium hydroxide (KOH) solution is added on the slide. Then hyphae and pseudo spores of *Candida* visible under microscope. Their existence in large numbers strongly suggests a yeast infection (Vazquez, 2003). On the other hand, for the culturing experiment to diagnose candidiasis, a sterile swab is rubbed on the infected skin surface and then swab is rubbed across a culture medium like sabouraudi's dextrose agar (SDA). The medium is incubated for 4 days at 37^0C, during which time colonies of *Candida* species develop. The features of the colonies provide a presumptive diagnosis of candidiasis (Vazquez, 2003).

Candida species are ubiquitous fungi (i.e., found in soil, inanimate objects, food, and hospital environments) representing the most common fungal pathogens that cause life threatening conditions. Candidiasis is caused by infection with species of the genus *Candida,* predominantly with *Candida albicans.* Similar to bacteria, *Candida* species are in the front list of the leading causes of nosocomial infections. Consequently, they are considered as true opportunistic pathogens that exploit recent scientific advances to gain access to the circulation and deep tissues. Moreover, the tendency of *Candida* species to produce oropharyngeal candidiasis in patients with HIV/AIDS has made it the leading fungal infection in immunosuppressed population.

2.1.1. Candida albicans

Candida albicans is a yeast-like fungus, thin-walled, microscopic morphology shows spherical to oval, 5 unit measures in diameter, much larger than bacteria in size, and reproduces by budding (Webb *et al*., 1998; Kayser *et al.*, 2005). The yeast form *Candida albicans* can take two forms, in a non-invasive, and the hyphal form which can penetrate the mucosa, and it is invasive (opportunistic agents). *Candida albicans* are normal flora and highly saturated in the human oral cavity and gastrointestinal tract, vaginal and the urinary environments with no harmful effects. They become pathogenic when the unicellular yeast-like form of *Candida albicans* reacts with environmental cues and switches in to insidious, multi-cellular filamentous forms causing infections (oral, and vaginal infections etc). Immunocompromised individuals such as HIV/AIDS patients are often vulnerable to any of these infections (Andrutis *et al.*, 2000; Wisplinghoff *et al.*, 2004).

Candida albicans can grow at a temperature range of 20 to 38°C, tolerates pH in the range of 2.5 to 7.5 (Rinaldi, 1993). Sabouraud dextrose agar containing chloramphenicol or gentamycin favored for *Candida* isolation (Ellis, 1994). On cornmeal agar following 72 hours incubation at 25°C, it forms abundant branched pseudohyphae and true hyphae with blastoconidia are present. The blastoconidia are formed in grape-like clusters along the length of the hyphae. Terminal chlamydoconidia may be formed with extended incubation (Sutton *et al.*, 1998). One of the more well known characteristics is the ability to ferment sugars and may ferment other carbohydrates for the production of ethanol (Bhavan *et al.*, 2010).

2.1.2. *Candida glabrata*

Candida glabrata is non dimorphic yeast that exists as small blastoconidia under all environmental conditions as a pathogen. It is the second most virulent yeast after *Candida albicans*. *Candida glabrata* is haploid yeast of the genus *Candida*. *Candida glabrata* has recently emerged as a highly opportunistic pathogen of the urogenital tract and cause of oral candidiasis. It is especially prevalent in elderly, cancer patients who are undergoing radiation treatment, patients undergoing bone marrow transplant and people who are HIV positive.

2.1.3. *Candida tropicalis*

Candida tropicalis is a species of yeast in the genus *Candida*. It is easily recognized as a common medical yeast pathogen, existing as part of the normal human flora. It most commonly occurs in digestive tract and can also be found on skin. When there is bacterial flora suppression due to antimicrobial use or gastro intestinal mucosa injury it can develop in to fungal infections. It has similar characteristics of identification to *Candida albicans* (Martin and White, 1981).

2.1.4. *Candida* krusei

Candida krusei is an emerging fungal nosocomial pathogen primarily found in the immunocompromised and those with hematological malignancies. It was considered as normal flora in female reproductive system, it can isolate from adult stool and it can cause pericardial inflammation (Emmons *et al.,* 1974). Mortality due to *Candida krusei* fungemia is much higher than the more common *Candida albicans*.

2.1.5. *Candida parapsilosis*

This is a type of *Candid*a become a significant causal agent for sepsis and tissue infections in immune compromised patients. It is strong resistance to anti-fungal treatments generate particular concern to hospital. It was difficult to be identified, due to its weakly growth, more branching on corn meal agar (Martin and White, 1981).

2.1.6. *Candida dubliniensis*

Candida dubliniensis is a fungal opportunistic pathogen originally isolated from HIV/AIDS patients. It is also rarely isolated from immunocompetent individuals. It is dimorphic yeast of the genus *Candida*, very closely related to *Candida albicans* but forming a distinct phylogenetic cluster in DNA fingerprinting. It is most commonly isolated from oral cavities (Gilfillan *et al.*, 1998).

2.2. Pathogenesis of *Candida* Infections

Candida albicans is normal flora, lives in 40- 80% of the human population with no harmful effects, it recognizes and destroys harmful bacteria. In a healthy person, it is found in low concentrations and is controlled by immune system. However, if the number of friendly bacteria is decreased and when the immune system is weakened, *Candida* overgrowth may occur and *Candida albicans* will shift from yeast form to mycelial fungal form and start to invade the body resulting in candidiasis (Salvo, 2009; Ferreira *et al.*, 2010). The infection caused by *Candida albicans* can be defined in three broad categories, superficial, mucocutaneous and systematic invasive.

2.2.1. Superficial infection

Superficial infection does not stimulate the immune system because the infection localized on stratum cornium and does not have the ability of penetrate to the body tissues. However, it can infect hair and nails then caused inflammation reactions due to keratinase and protinase enzymes which cause infection. Cutaneous candidiasis occurs particularly in warm and moist areas. The infection appeared to dark red vesicles with itch and pain, and then it will be as dandruff on the skin external layer (Negi *et al.*, 1948; Kobayashi, 1990). This result in inflammation around nail with redness, swelling and thickness nail plate, then the plate will split (Chandler *et al.*, 1989).

2.2.2. Mucocotaneous infection

It was discovered using electron microscope the fibril surface layer of *Candida albicans* that has important role in yeast adherence to the host mucous membranes and produce colonies

(Marrie and Costerton, 1981; Lee and King, 1983). As such oral candidiasis is the commonest infection seen in HIV infected person and known as thrush. It invades the mouth and throat tissues. The infections often seen inside the cheek, on the roof of the mouth, tongue, gums and lips. Esophageal thrush that spreads to the esophagus is known as esophagitis. This infection makes difficult mechanical food digestion in mouth (Webb, *et al*., 1998). Use of antibiotics for a long time causes reduction of the bacteria that do not allow bacteria to grow. Consequently, good bacterial population decreases, and the yeast turns into an opportunistic pathogen. Oral candidiasis infections can also be caused by tricycle antidepressants. Adults can also be infected with thrush specially those who suffering from HIV or periodical diseases (Aldred *et al.,* 1989; Koneman *et al.,* 1992).

2.2.3. Systemic infection

Systemic infection is acquired by the respiratory route. Severe disease is more likely in patients with reduced cell- mediated immunity. It involves the spread of *Candida albicans* to the blood stream and to the major organs including meningitis, endocarditis**,** pneumonia, urinary tract candidiasis, etc, and is often fatal (Ellis, 1994; Molero *et al.*, 1998).

2.3.Virulence factors of *Candida albicans*

Candida albicans has several known virulence factors and specific strategies that contribute to its ability to cause infection (Naglik *et al.*, 2003). There are different ways of pathogencity among *Candida* species isolates. Some properties related to *Candida albicans* cells that give them the capacity to cause disease are adherence to cell surface, germ tube formation with consequent development of the filamentous form, phenotypic variability, and production of toxins and extracellular enzymes constitute important factors for the emergence of infections by *Candida*, (Ferreria *et al*., 2010).

2.3.1. Hydrolytic enzymes

Extracellular hydrolytic enzymes include secreted aspartyl proteinases, phospholipase B1 and B2 and lipases 1 to 10. Hydrolytic enzymes damage membrane structure and results in tissue invasion (Nader and Zadeii, 1997). The Secreted aspartyl proteinases enables deep

colonization, adherence to epithelial cells and eventually to degrade the host barriers (Staib *et al.*, 2000). The phospholipase enzyme concentrates on the tip of mycelia and it is contributed to invasiveness, damage of cell envelopes and evasion of host response (Gacser *et al.*, 2007, Ferreria *et al*., 2010).

2.3.2. Adherence

Candida albicans posses many different adhesion that mediate binding to a variety of tissues, that allows it the colonization and infection of virtually all body locations (Staib *et al.*, 2000). Adhesion function is provided by agglutinin-like sequences (fibronectin, and laminin receptors, fibrinogen-binding proteins) and an outer surface mannoprotein adhesion is considered important means of adhesion to endothelial and epithelial cells (Fidel *et al.*, 1999). A further bimolecular important in adherence is a hyphal and germ-tube-specific protein and surface integrin -like molecules for binding extracellular matrix (Naglik *et al.*, 2003).

2.3.3. Adaptability

Candida albicans has high adaptability to many different host niches. A prerequisite for this adaptability is the capacity to respond to complex environmental signals representing the different host niches by the expression of an appropriate set of virulence-related and other genes (Staib *et al.*, 2000).

2.3.4. Cell surface hydrophobicity (CSH)

Candida albicans do have cell surface hydrophobicity (Vartivarian and Smith,1993). Those *Candida albicans* that do not have hydrophobicity can easily be killed by neutrophile than do have cell surface hydrophobicity. Cell surface is affected by environmental factors. It can also affect specific adherence based up on interaction of adhesion receptors (Fidel *et al.*, 1999).

2.3.5. Morphogenesis

Candida albicans has a character of a yeast-to-hyphal-phase transition (dimorphic transition); it enables virulence factor, its hyphal form is pathogenic (Lopez-ribot *et al.*, 1994). Hypha

formation enables increasing adherence, invasion, tissue damage and form mycelial biofilms as well as a means of increasing proteolytic enzyme elaboration and antigen modulation (Fidel *et al.*, 1999; Brown *et al.*, 2006). The morphogenesis in *Candida albicans* often induced by changes in temperature and pH, activate hyphal development (Molero *et al.*, 1998).

2.4. Identification Methods

2.4.1. Rapid test of *Candida*

One of the known test for *Candida* infection in the mouth is done simply at home with cup of water. During morning wake up before putting anything in the mouth a glass of water could be put in a clear glass that can be seen through saliva can be collected in the mouth and be spit it into the glass (Figure 1). After 15 minutes, according to advocates of this method if any of the following had seen, it indicates the presence of yeast colonies.

- The saliva stays at the top and thin strands that look like strings or spider legs extending downward.
- The saliva floats to the bottom and looks cloudy
- The saliva is suspended in mild air and looks like little specs are floating (Guarro *et al.*, 1999).

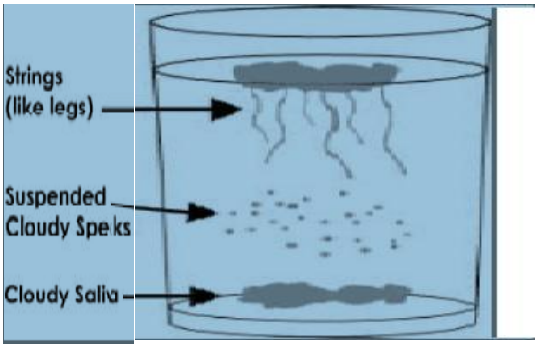

Figure 1. Rapid test of Candidiasis (Guarro *et al.*, 1999)

2.4.2. The body Itchiness test

The itch test is pretty simple and a lot of people with *Candida* skin infections have itch patches on their skin. If there are *Candida* over growth, though patients do not actually have to see a visible rash for it to cause itchiness. Most of patients are not very conscious of how often they reach their own hand to scratch and itch throughout the day. The itching will be noticeable anywhere, but is particularly common in places that are generally covered with clothing and kept warm (http://www:blog.probacto.com).

2.4.3. Body Odor test

There are patients who have a particular consistent body odor that simply does not go away no matter how clean their clothes or how long they have been out of the shower. This may be due to a lot of fermentation happening in the digestive system. Excess heat, sweat smells and odor can be produced due to *Candida albicans* infections. This can cause a different type of odor and a much stronger than usual odor around the armpits or feet (http://WWW.ericbakker.com).

2.4.4. The Tongue test

When a healthy pink tongue becomes coated with a white or yellowish coating, especially if accompanied by bad breath. One that is being impacted by some sort of bacterial infections or yeast or *Candid*a over growth. Having a discolored tongue or bad breath could simply be an indicator of gum disease or gingivitis. The further back on tongue becomes discolored; the more likely it is to be associated with digestive system, where most overgrowths begin. When the tongue can range from white to a yellow or brown shade, it shows an imbalance of digestive flora. (http://WWW.ericbakker.com).

2.5. HIV/AIDS

2.5.1. Human Immunodeficiency Virus (HIV) Infections

Human immunodeficiency Virus (HIV), the agent that causes acquired immune deficiency syndrome (AIDS), is classified as members of the lentivirus subfamily of retroviruses. Two

types: HIV type 1 (HIV-1): the most commonly distributed throughout the world. HIV type 2 (HIV-2) is prevalent in West Africa. Both can cause AIDS and their ways of spread are similar. However, relative to HIV-1, HIV-2 causes AIDS much less (Seoane *et al*., 2008). The disease is the result of reduced effectiveness of the immune system that can cause vulnerability to opportunistic diseases such as candidiasis (Weiss, 1993).

People at any status, rich, poor, old, young, male, female of all the races without any boarder and barrier can be affected by HIV/ AIDS. HIV epidemic is more distributed throughout the world in general. The vast majority of people living with HIV are located in low and middile income countries with estimated 25.5 million living in sub-Saharan Africa. Among this group 19.4 million are living in East and South Africa. According to estimates by WHO and UNAIDS, 36.7million people were living with HIV globally at the end of 2016. The same year including children 1.8 million became infected and 1 million died of HIV-related cause. Based on a single point estimate, there are nearly 1.2 million people living with HIV/AIDS in Ethiopia. Among adult prevalence 2.4 % where as urban 7.7% rural 0.9 %.gender prevalence for male 1.7% and female 2.6% (UNAIDS/UNICEF/WHO, 2017). The following series of pictures shows how the prevalence of HIV changed according to time and location.

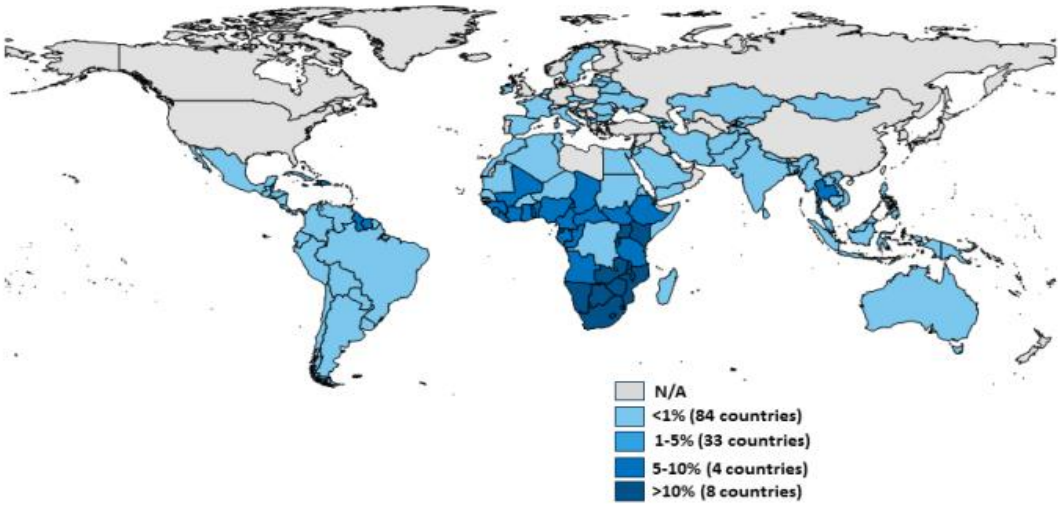

Figure 2 Spread of HIV in the world (Source: UNAIDS, 2017)

HIV mostly attacks the immune cells cluster determinant (CD_4^+) lymphocytes. The persons infected with HIV till the immune system becomes weak and get AIDS may look and feel

well for several years. At this stage the person can be said sero-positive. Therefore the best mechanism for someone to know his /her status is blood test in health center. If a patient has no symptoms and his/her CD_4^+T-cells count is greater than 350 cells/ mm^3 and viral load is less than 100,000, therapy is not recommended. If the CD_4^+T-cells count is between 200 and 350 cells/ mm^3 and the person has no symptoms, he/she and his doctor should decide whether to start treatment. If the CD_4^+T-cells count is less than 200 cells/ mm^3, it is recommended to start treatment. If the patient has severe symptoms or he/she has an AIDS-defining condition, it is recommended that he/she begins treatment (Kallings, 2008).

The knowledge of AIDS related to opportunistic is the appreciation of the association between the levels of the underlying immune dysfunction as measured clinically by CD_4^+T-cells count drops below 250 to 200 cells/mm^3. The reduction in the number of the CD_4^+T-cells indicates that the HIV related disorders (Taylor *et al.,* 1989). Therefore, the CD_4^+ T-Cells count clinically used as a gauge to decide the stage of HIV infections. It also assists in differential diagnosis and in making therapeutic decisions regarding antiretroviral treatment and prophylaxis against opportunistic infections (Quinn, 1997).

2.5.2. CD_4^+ T-Cells as Markers of Immune Status in HIV/AIDS Patients

CD_4^+ T-cells are the most responsible in protecting against pathogens. The antibody depletion of CD_4^+T-cells has shown that the CD_4^+T-cells are required for control of infection (Muller *et al.,* 1987). In humans, the remarkable susceptibility of patients with HIV/AIDS has demonstrated the critical role of CD_4^+ T-lymphocytes in protective immunity. The pathogenesis of HIV infection is largely attributable to the decrease in the number of T- cells (a specific type of lymphocytes) that bear the CD_4^+receptor. The immune status of a child or adult living with HIV can be assessed by measuring the absolute number (per mm^3) or percentage of CD_4^+T-cells, and this is regarded as the standard way to assess and characterize the severity of HIV-related immunodeficiency. Progressive depletion of CD_4^+T-cells is associated with progression of HIV disease and an increased likelihood of opportunistic infections (Vajpayee *et al.,* 2005).

In areas with adequate resources, laboratory measurements of CD_4^+T-cells and plasma HIV viral load are commonly used to establish a patients' degree of immunosuppression and the destruction of the immune system (Simon *et al.,* 2006). The WHO clinical staging system has been shown to be a practical and accurate way to manage HIV-infected patients, with international studies showing agreement between clinical manifestation included in the WHO staging system and laboratory markers including CD_4^+cells count and lymphocyte count(Kagaayi *et al.,* 2007). Advanced HIV/AIDS disease is defined for surveillance purposes as any clinical stage-3 or stage-4 disease or any clinical stage with a CD_4^+T-cells count greater than 350 per cubic mm, and this information can be used to calculate the burden of disease and the demand for antiretroviral therapy (WHO, 2005).

The WHO (2005) clinical staging system for adults sorts patients into one of four hierarchical clinical stages from stage I (asymptomatic) to stage IV (AIDS). Patients are assigned to a particular stage when they demonstrate at least one clinical condition in that stage's criteria. Patients remain at a higher stage after they recover from the clinical condition which placed them in that stage (Kagaayi *et al*., 2007).

Stage I patients who are asymptomatic or have persistent generalized lymphadnopathy for longer 6 months are categorized as being in stage I, where they remain for several years (WHO, 2005). Stage II (mildly asymptomatic stage) is unexplained weight loss of less than 10 percent of total body weight and recurrent respiratory infections and dermatological conditions including recurrent oral ulceration (WHO, 2005).

Stage III (Moderately symptomatic stage) are weight loss of greater than 10%of total ,prolonged (more than 1 month) unexplained diarrhea ,pulmonary TB and recurrent oral cndidiasis. Stage IV (Severely symptomatic stage) includes all of the AIDS defining illness such as, extrapulmonary tuberculosis, esophageal candidiasis (WHO, 2005).

2.5.3. Immunology of HIV infections

HIV pathogenesis is as result of depression of CD_4^+T-cell count. The CD_4^+T-cells are the primary targets of HIV that may become infected as they encounter HIV trapped on follicular

dendretic cells (FDC) which is considered as a significant reservoir of infectious HIV (Fauci and Lane, 2001). There are three ways of mechanisms the CD_4^+T-lymphocytes are being killed by HIV. These are direct virus-being mediated cytolysis, virus-induced apoptosis, and indirect killing through immune effecter mechanisms. Direct virus-mediated cytolysis has been demonstrated in vitro and syncytium formation may accelerate the catalytic process. Here infected cells are killed because of viral replication in these cells, disrupting the cell membrane (Fauci and Lane, 2001). The turnover rate of CD_4^+T-lymphocytes is correspondingly turbulent. Each day in an infected patient, up to 109 new versions are made and about 109 CD_4^+T-lymphocytes are killed and replaced (Hu *et al.,* 1996). Recent data shows that the destruction of billions of CD_4^+T-cells overwhelms the immune systems regenerative capacity and loss of CD_4^+T-cells in turn results in a loss of recognition of antigens that are presented on class II MHC molecules (Reynold *et al.,* 2006).

2.5.4. Opportunistic Disease in HIV/AIDS Patient

The various infectious agents that are defined by the Centers for Disease Control (CDC) as diagnostic of AIDS when present in persons infected with HIV can produce a host of clinical and pathologic conditions. There may be regional, racial, age, or gender-associated variations in the incidences of opportunistic infections seen with AIDS (Read, 2007). Opportunistic infections are late complications of HIV infection for the most part patients with less than 200 CD_4^+T-cells/mm^3. The causative agents are opportunistic organisms for instance, *Candida* species that cause illnesses in compromised immune systems of HIV/AIDS patients (Facuc , 2011).

Mucocutaneous candidiasis is usually one of the first signs of HIV infection and 90% of patients with AIDS will develop oropharyngeal candidiasis at some time during their illness (Vazquez, 2000). However, pulmonary candidiasis is an uncommon manifestation among HIV-infected patients. In 1987, data from the centers for disease control AIDS data base indicated a 50% prevalence of oropharyngeal *Candida* infection, a 10% rate of esophageal infection, and 5% rate of bronchopulmonary infection among AIDS patients (Selik *et al.,* 1997).

Oropharyngeal and esophageal candidiasis are common in HIV-infected patients, (Watanabe *et al.,* 1996). Most such infections are caused by *Candida albicans*. Oropharyngeal candidiasis is characterized by painless, creamy white, plaque-like lesions that can occur on the buccal surface, hard or soft palate, oropharyngeal mucosa, or tongue surface (Fichtenbaum *et al.,* 2000). It has been found that the high prevalence of esophageal candidiasis in patients with AIDS indicates the critical role of cell mediated immunity in normally protecting the esophagus from *Candid*a invasion. In addition, esophageal candidiasis in an HIV-positive patient can serve as a symptom of AIDS (Coleman *et al.,* 1993), typically occurring at lower CD_4^+T-cells counts, <100 cells/mm^3. In effect, all HIV-infected individuals with cutaneous candidiasis and it will harbor *Candida albicans* as the pathogen, with *Candida tropicalis* only rarely being diagnosed (Scott *et al.,* 1986).

Initiation of highly active antretroviral therapy (HAART) in HIV-infected populations has exerted a positive impact on the immunological recovery of such patients, thereby leading to decreased frequency of symptomatic *Candida* infections and an overall decline of some opportunistic infections (Mahgoub *et al.,* 1993). However, in a longitudinal study of the relationship between HAART therapy and recurrent oropharyngeal candidiasis in advanced HIV infected patients demonstrated that unless HAART is accompanied by significant decrease in the viral load and increase in the CD_4^+T-cell count, HAART alone may not lead to reduce recurrence rate of oropharyngeal candidiasis (Revankar *et al.,* 1984). With the introduction of the first HIV policy in 1998 and multi sectoral response on HIV preventation care and free treatment in 2005, new infections have declined by 90%. Even if new infections continue to decline by 90% 27,000 new infections were reported last year (HAPCO, 2017).

2.6. HIV/AIDS in Ethiopia

In Ethiopia, HIV prevalence is low compared to other Africans countries. In 2011, 1.5% of the population between the ages of 15-49 years were HIV positive (EDHS, 2011). However, taking into account the countries' large population, the absolute number of people infected with HIV is high and regional disparities remain. Despite many progress and achievement made regarding HIV/AIDS response, in 2014 Ethiopia accounted 10% of the 120,000 adolescent deaths reported globally (UNAIDS, 2013).While information in this group is

scanty nationally, available data shows that adolescent girls aged 15-19 are disproportionally affected by the epidemic. About 74% of the newly infected adolescents for that age group were girls (UNAIDS, 2013).

Currently, 718,000 are estimated people are living with HIV/AIDS across our country; 27,000 people are newly infected while 420,000 are estimated using ART. In addition, 9 million are estimated taking counselling service (HAPCO, 2017).

The length of time can vary widely between individuals .Left without treatment, the majority of people infected with HIV will develop signs of HIV related illness within 5-10 years, although this can be shorter. The time between acquiring HIV and an AIDS diagnosis is usually between 10-15 years, but sometimes longer. ART can slow the disease progression by preventing the virus replicating and therefore decreasing the amount in an infected person's blood (UNICEF, 2017).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted at Ambo General Hospital among HIV/AIDS patients who were attending ART. The hospital is used as a general hospital by a number of communities inhabiting the surrounding localities. It is found in Ambo town, western Shoa zone of Oromia region. Ambo town is located at 110 km west of Addis Ababa with a latitude and longitude of 8°59′N and 37°51′E, respectively and was an elevation of 2101 meters above sea level. The 2007 national census reported total populations for this town was 48,171 of which 24,634 were men and 23,537 were women. According to census results latest projections, total populations were 70,900 (CSAE, 2007; CSAE 2015).

3.2. Study Design

Hospital based cross sectional design was used to identify *Candida* species among HIV positive patients who were talking ART treatment. It was conducted between August and September, 2017. However, the study did not include those who had taken treatments or drugs for any opportunistic fungi infections, specifically for *Candidia* diseases during the study period. A serial random sampling was used for participants involving. In addition, interview questioners were administered to the same individuals of HIV positive patients in order to assess the demographic features of the participants.

3.3. The Study Population

Patients with both HIV/AIDS and *Candida* infections at Ambo General Hospital were the target population from which sample patients were taken at serial simple random sampling. In the Hospital, about 8573 patients have been ever enrolled of which 5,638 started the ART treatments at Ambo hospital and other referral health care institutions. About 2,897 patients (1156 males and 1741 females) were attending their treatments at Ambo General Hospital, while the rest of them sent to different health care centers in order to get treatments and follow up for their health condition. This study focused only on those who were admitted and treated at the target hospital.

3.4.Sample Size Determination and Sampling Techniques

In practice, the most common problem in sample size determination is the tradeoff between the desire to have large sample size and the feasibility of dealing with only small sample size (Hassan, 1991). Ideally, the sample size must be large enough to represent the corresponding population for which generalization is made based on the sample data. Of several factors that could potentially determine the sample size, resources such as study cost, time, and level of precision are few to mention. There is no fixed number or percentage of total subjects that determined as the sample size of an adequate sample (Hassan, 1991). However, it is important to make sure that enough sample size is used in scientific study when valid generalization about the population is of interest.

For this purpose, researcher may specify the level of significance and margin of error to determine adequate sample size. In addition, variance of the target population is needed to determine the sample size. If there was no previous study conducted on the same research problem from which variance can be accessed, one can take the maximum possible variance ensure adequate sample size to be used in the study. Accordingly, since there was no previous investigation conducted on the same issue in this study area, a proportion P equals to 0.5 can be taken to ensure the sample size large enough to satisfy the precision and confidence constraints. By taking this in to consideration, the sample size n for single population is calculated based on the 95% confidence limits and 5% sampling error by using a formula described in Hassan (1991) as shown below.

$n = \frac{Z_{\frac{\alpha}{2}}^2 P(1-P)}{d^2}$, where $Z_{\frac{\alpha}{2}}^2$ the critical value at the given level of significance is α, P is the expected prevalence or proportion and d is the marginal error. In this study, the level of significance α is fixed at 5% to get the corresponding critical value $Z_{\alpha/2} = 1.96$ with the marginal error $d = 5.07\%$. Using the expected prevalence $P = 0.5$ in the above equation a minimum sample size of 374 should be used to make sound generalization about the population.

3.5. Data Collection Methods

Combination of different methods such as laboratory experiments, interview and clinical records were used to generate information about the patients who gave their consents to be included in the study. Laboratory examination was conducted using swabs samples taken from the oral cavities rolled upon areas in the entire mouth of HIV Patients receiving ARVT to test for oral *Candida albicans* infection. Accordingly, using sterile applicator stick with cotton used to swab the mouth of patients. Then sample was transported to Ambo University Micro Biology Laboratory for microscopic examination and culturing process using Sabouraud dextrose agar with chloramphenicol.

3.6.Laboratory Procedure

The first step in testing for *Candida albicans* was to identify patients infected with yeasts. Then, experimenting to check for *Candida albicans* was followed.

Smears of the swabs were made on heat sterilized glass slides. The slides are then flooded with crystal violet solution for up to one minute. Then it was washed off briefly with tap water (5 seconds) and drained. Then it was flooded with Iodine solution and allowed to act (as a mordant) for about one minute. After being washed with tap water it was drained. Then after flooded with 95% alcohol for 10 seconds, it was washed off with tap water. Finally, the drained slides were flooded with safranin solution and allowed to counter stain for 30 seconds. Then it was examined under the oil immersion lens at x100 (David-Rollins and Joseph, 2000, Srikumar and Nagaraja, 2010). Budding cells were regarded as positive for yeast.

3.6.1. Culture and Isolation Procedures

The identified yeast cells were allowed to grow in order to observe the colony of *Candida* species. Although there were many *Candida* species that could be observed, lack of laboratory facilities forced this study to be confined to *Candida albica*n's and other non *albicans* species identification.

For this purpose, the sample swabs taken from the oral cavity of participants were inoculated the plates by streak method to be incubated in Sabouraud's dextrose (SAD) agar with chloramphienicol at 35^0C for 24 to 48 hours under aerobic conditions for the growth of *Candida*. The soft moist white/cream colonies on the plates were considered positive (Bhavan *et al.*, 2010).

3.6.2. Observation of germ tubes

Of the *Candida* colonies (grown yeasts), germ tube tests for the *Candida albicans* were conducted. Yeasts grown on the plate of SDA were transferred to clean test tube containing three drops of human blood serum for further growth. The grown yeasts colony of the tests was touched and gently emulsified in the serum which was incubated at 35^0C for 3 hours. Then after, a drop of the incubated suspension was transferred to a slide for microscopic examination at x 40 and confirmed under oil immersion lens at x100. *Candida*s that produce a short one piece germ tube under that condition were considered as *Candida albicans* (Sheppared *et al.*, 2008).

3.7. Questionnaire

Questionnaire survey used to collect data on related variables from patients via interview. Patients who were regularly visiting the Ambo hospital for their treatments on average, fifty patients per day visit their doctor. The investigator waited for these patients coming to the hospital to collect all types of data used in this study complying with the specified inclusion criteria. Questionnaires were devised to collect various demographic characteristics of the patients. Moreover, hospital medical records on HIV/AIDs such as current and baseline CD_4^+count, WHO stage and ART adherence for each individual were reviewed. Each level of this study is abided by the research ethics; hence each participant was assigned code and the collected data were recorded properly and were kept confidential.

- **Inclusion criteria**: were previous positive diagnoses of HIV taking ART and no history of antifungal therapy within two weeks prior to the attendance. The specimens must be yeast positive.

- **Exclusion criteria**: All specimens that are not from HIV patients , patients do not have full information in their clinical record file and age of participants less than 13 participants.

3.8. Methods of Data Analysis

In order to understand the prevalence of *Candida albicans* and other species the proportions and percentages were calculated. Other descriptive and comparative type analyses were also done using different statistical methods(Odds ratio and confidence interval). The association between *Candida albicans* infections and other demographic factors were assessed using statistical chi-square method. P-value less than 0.05 were considered statistically significant for test. The statistical package for social science, SPSS version 20 was employed to perform data analysis.

3.9. Data Quality Control

To ensure quality control, all the laboratory procedures including collection and handling of swab samples were carried out in accordance with standard protocols. To ensure general safety, disposable gloves were worn and universal bio-safety precautions (NCCLS, 2002) were followed at all times.

3.10. Ethical Consideration

Ethical permission were obtained from the Ethical Review Committee of Haramaya University, a formal letter that have been submitted to all concerned bodies of Ambo General hospital to obtain their formal cooperation. Then officials at different levels in the study area had been communicated through letters. Permission was obtained from Ambo General Hospital. Accordingly, confidentiality of the information was maintained.

4. RESULTS AND DISCUSION

4.1.Socio Demographic Characteristics, Immunological Profile and ART Adherence of the Study Participants

The socio-demographic characteristics of the study participants are summarized and presented in Table 1. As the result shown in table 1, a total of 374 HIV positive partcipants were participated in the present study. Of these, 140(37.7%) and 234(62.6%) were males and females, respectively.

Table 1: Socio-demographic Features of the Study Participants (№=374) at Ambo General Hospital during the August-September, 2017

	Frequency(%)	Character	Frequency(%)
Sex		Latrine Facility	
Male	140(37.7)	Present	295(78.9)
Female	234(62.6)	Absent	79(21.1)
Age(Years)		Education status	
≤30	102(27.3)	Illiterate	91(24.3)
31-45	202(54)	Primary	179(47.9)
≥46	70(18.7)	Secondary	67(17.9)
Religion		Diploma and above	37(9.9)
Orthodox	234(62.6)	Urban	254(67.9)
Protestant	127(34)	Rural	120(32.1)
Muslim	2(0.5)	Family size	
Catholic	1(0.3)	<5	220(58.8)
Wakefata	7(1.9)	≥6	154(41.2)
Kalu	3(0.8)	Monthly income(Birr)	
Occupation		< 1000	180(48.13)
Student	15(4)	1001-2000	95(25.40)
Government employee	56(15)	2001-3000	50(13.37)
		3001-4000	40(10.70)
Private	158(42.2)	≥4001	9(2.41)
Unemployed	145(38.8)	Alcohol Use	
Marital status		Yes	20(5.35)
Single	40(10.7)	No	354(94.65)
Married	208(55.6)		
Divorced	126(33.7)		

The study participants were divided into three age groups: ≤30, 31-45 and ≥46 years. The mean age of the study participants was 37.48 years old, and the minimum and maximum age of the study participants was 13 and 82 years, respectively. A total of 102 (27.3%) of the study

participants were ≤30 years old, 202 (54%) were 31-45 years old and 70 (18.7%) were ≥46 years old. More than 50% of the study participants were in the age group of 31-45 years old (Table 1).The study done in Nigeria showed that the highest numbers of HIV patients age were between the age ranges 26-35 which was younger than the present participants (Okonkowo E., *etal*,2013).

As shown in Table 1, 91(24.3%), 179 (47.9%), 67(17.9%) and 37(9.9%) of the study participants were illiterate, completed primary school education, completed secondary school education and had diploma and above, respectively. Regarding their occupation, 158 (42.2%) of the patients worked privately, followed by unemployed 145(38.8%), government employed 56 (15%) and student 15(4%). Majority (55.6%) of the study participants were married, while 126 (33.7%) and 40 (10.7%) were divorced, and single, respectively. A total of 295 (78.9%) of the study participants had latrines in close vicinity of their homes. The remaining 79(21.1%) study participants did not have latrines at their homes. A 220 (58.8%) and 154 (41.2%) of the study participants had family size of < 5 and ≥ 6 persons per house, respectively (Table1).

Out of 374 study participants, 292(78.1%), 44(11.8%) and 38(10.2%), were knew their HIV status before 3 years, before 2 years and before 1 year, respectively (Table 2). Regarding ART status of the study participants, majority of the patients, 362(96.8%) were adhered to ART while only 12(3.2%) of the study participants were failed to adhere to ART regularly (Table 2). Regarding level of CD_4^+ T-cell counts, 187(50%), 156(41.7%) and 31(8.3%) of the study participants had CD_4^+ T-cell counts of 501-≥1000, 201-500 and ≤200 cells/mm^3, respectively (Table 2). In addition 174(46.5%), 163(43.6%) and 34(9.9%) participants had the baseline CD_4^+ counts of in the category ≤ 200, 201-500 and -≥500, respectively (Table 2). In this study it was observed that the $CD4^+$counts were higher among the HIV positive patients who adhered to ART than those HIV Positive patients who did not adhered to ART (Table 2). Therefore, taking ART has been improved CD_4^+counts number and hence immunological improvement.

On the other hand, concerning current WHO clinical stages of HIV sero-positive individuals 341(91.2%), 22(5.9%), and 11(2.9%) of the study participants were WHO stage I, WHO

stage II and WHO III stage, respectively (Table 2). The baseline WHO clinical stages of HIV patients were included in this study were 74(19.8%) stage I, 122(32.6%) stage II, 158(42.2%) stage III and 20(5.3%) of stage IV (Table 2). The frequent difference of current stage and base line stage at each level could be due to patients started ARV medication.

Table 2: Clinical Stage, Immunological Profile and ART Adherence of the Study Participants at Ambo General Hospital.

Factors	Frequency	Percent
HIV detection time		
≤1 year	38	10.2
> 2 years	44	11.8
≥3years	292	78.1
Current CD_4^+cells/mm^3		
501-≥1000	187	50
201-500	156	41.7
≤200	31	8.3
Baseline CD_4^+cells/mm^3		
501-≥1000	37	9.9
201-500	163	43.6
≤200	174	46.5
Current WHO Clinical Stage		
Stage I	340	91.2
Stage II	22	5.9
Stage III	11	2.9
Stage IV	-	-
Baseline WHO Clinical Stage		
Stage I	74	19.8
Stage II	122	32.6
Stage III	158	42.2
Stage IV	20	5.3
ART Adherence		
Good	362	96.8
Poor	12	3.2

4.2 Prevalence of Oral Candidiasis in the Study Population

The number and percent of occurrence of oral candidiaisis among HIV patients participants who were attending at Ambo General Hospital are summarized and presented in (Table 3). According to this study, the prevalence of oral candidiaisis was 42.25%. Among 374 study

participants, the prevalence of oral candidiasis of patients in age groups, ≤30, 31-45 and ≥46 years were 45.10%, 28.71% and 37.14%, respectively (Table 3). In all age groups, the prevalence of oral candidiasis for female and male HIV patients was about 62.66% and 37.34%, respectively. Except for the ≥46 age group, the oral candidiasis prevalence of HIV patients was higher in women than in men (Table 3). However, the same table reveals that disparity in gender prevalence was not age-dependent. That is, gender of the study participants had no any significant relation p-value 0.840 ($P > 0.05$) with the prevalence of oral candidiasis. The study conducted in India also supports this result (Kolar, *etal*. 2015).

Literature reported that oral *Candida* species are common in individuals with HIV patients with the prevalence of 62%-67% (Gugnani., 2003). In Nigeria (Esebelahie *et al.*, 2013) reported that 44.0% of HIV patients were infected with oral *Candida* infection. The results of this study based on samples collected from the mouth cavity of patients with HIV show that the prevalence of oral candidiasis was 42.06% of which 26.02% were *Candida albicans.* This prevalence *of Candidia albicans* was below relative to reports from other literatures 32%-62% (Thompson, *et al*, 2010).

The use of ART in HIV patients is believed to decrease the prevalence of oral candidiasis (Schmidt *et al.*, 2000; Dunic *et al.*, 2004). This study indicates that about 362(96.79%) of the study participants were adhered to the ART intervention, (Table 2) of which about (42.25%) infected by oral candidiasis. This result is very odd in a sense that one expects that patients who are adhered to ART treatment should have less infected by oral *candidia.* However, it can also be asked that how does ART intervention impact the oral candidiasis? In other words, it might be the baseline of oral *Candidia* reflected in the HIV patients. Similar to the findings, a study in Nigeria (Esebelahie *et al.*, 2013) reported that 44.0% of patients who are on ART were infected with oral *Candida* infection. But the study suspected that the reason for this high prevalence of oral *Candida* infection among patients on ART was probably due to lack of ART adherence (Owotade *et al.*, 2013; Moges and Kassa., 2014). This cannot be the case in this study since all patients included in the study are all taking ART treatment and about 97% of them were adhered to it.

Table 3: Prevalence of Oral Candidiasis among HIV Positive Study Participants (№=374) at Ambo General Hospital during August-September, 2017

Age(Year) and sex	№ Examined	№ Positive	Percentage	Chi-square	P-value
≤30					
Male	20	10	9.61		
Female	82	36	35.29	0.241	0.623
Sub-total	102	46	45.10		
31-45					
Male	78	33	16.34		
Female	124	53	26.24	0.004	0.952
Sub-total	202	58	28.71		
≥46					
Male	42	16	22.86		
Female	28	10	14.35	0.41	0.840
Sub-total	70	26	37.14		
All age groups					
Male	140	59	37.34		
Female	234	99	62.66		
Total	374	158	42.25		

4.3 Major *Candida*sis Identified in Oral Swab Samples

The number and percent of occurrence of *Candida albicans* and other *Candida* species among HIV sero-positive individuals who were attending at Ambo General Hospital are summarized and displayed in Table 4. According to this study, the prevalence of *Candida albicans* and other *Candida* species were 26.20% and 16.04%, respectively. This shows that HIV/AIDS patients are more susceptible to *Candida albicans* disease than other *Candida* species. Results given in Table 4 shows that both *Candida albicans* and other *Candida* species were more prevalent in 31-45 age group 58(28.71%) and 28(13.86%), respectively.

Different authors reported that Oral *Candida* species are common in individuals with HIV patients with the prevalence of 62%-67% (Gugnani, 2003). The study conducted in Nigeria showed that the numbers of females in each age groups greater than age groups of males. In that study the numbers of females were greater than males (Okonkwo, *etal,* 2013). Contrarily, the study conducted in Tanzania (Gwakisa and, Maseke 2016) did not find any difference

between the prevalence of *Candida* infection between males and females. Study in India indicated that more males than females were infected with oral *Candida* (Ranganathan *et al.*, 2004). In Nigeria, Okonkwo *et al.* (2013) reported that more females than males are infected with oral *Candid*a. The results of this study based on samples collected from the mouth cavity of patients with HIV show that the prevalence of oral candidiasis was 42.06% of which 26.02% were *Candida albicans.* This prevalence *of Candida albicans* is under estimated relatively reports from other literature 32%-62% (Thompson, *et al*, 2010).

Generally, both *Candida albicans* and non-*albicans Candida* diseases were more detected among female HIV patients than those male patients. This might be due to the fact that significant disparity in immunity (current CD_4^+) between men and women patients (chi-square = 18.522 with p-value = 0). Table 4 also depicted that only younger patients (of age group < 30 years) showed significant association p-value 0.033 (p < 5%) of sex of the study participants with the prevalence. Related findings in Tanzania also showed HIV patients young age groups were more infected by oral *Candida* infection than the other age groups (Gwakisa and Maseke, 2016). Nevertheless, the prevalence was not significantly associated in all age groups of the study HIV sero-positive patients (Table 4).

Table 4: Major *Candida* Species detected in Oral Swab Samples among HIV Positive Study Participant (№=374) at Ambo General Hospital

Age(Years) and sex	№ Examined	*Candida albicans*	Other *Candida* species	Chi-square	P-value
		№ Positive (%)	№ Positive(%)		
≤30					
Male	20	9(8.82)	1(0.98)		
Female	82	19(18.63)	17(16.67)	4.552	0.033
Sub-total	102	28(27.45)	18(17.65)		
31-45					
Male	78	22(10.89)	11(5.45)		
Female	124	36(17.82)	17(8.42)	0.015	0.904
Sub-total	202	58(28.71)	28(13.86)		
≥46					
Male	42	7(10.00)	9(4.46)		
Female	28	5(7.14)	5(2.48)	0.097	0.756
Sub-total	70	12(17.14)	14(20.00)		
All age groups					
Male	140	38(38.76)	21(35.00)		
Female	234	60(61.22)	39(65.00)	0.227	0.634
Total	374	98(26.02)	60(16.04)		

4.4 Factors Associated with Oral Candidiasis in the Study population

The association of prevalence of oral candidiasis with socio-demographic characters were presented in Table 5. The result showed that none of the job category is significantly related to the prevalence of oral candidiasis relative to unemployed study participants. Similarly, the study patients residence area, use of latrine facilities, alcohol use and khat use were unrelated to the oral candidiasis. The study conducted in Debremarkos, North West Ethiopia alcohol use and cigarette smoking was not found to be associated with occurrence of opportunistic disease such as *candida* (Moges and Kassa, 2014).

However, marital status was significantly associated with the prevalence of oral candidiasis. The odds of the prevalence of oral candidiasis of single HIV patients were 3.044 times that of divorced HIV patients. In addition, study participants' education level (being illiterate) was considerably correlated with oral candidiasis among HIV patients. That is, the odds of illiterate HIV patients were about 4 times the odds of patients with diploma and above education level. The results in Table 5 also revealed that knowledge about candidiasis has considerable association with prevalence of oral candiadiasis. The odds of study participants who lack pre-knowledge about candidiasis was seen to be 5 times the odds of patients who had knowledge about the candidiasis. The reason could be the more educated and well awareness individuals keep hygiene properly and see their doctor appropriately.

It has been reported that patients who use khat along with ART medications are at risk to develop oral *candidia* by 4.733 than those who do not use Khat (Moges and Kassa, 2014). However, the result of this study indicated that there is no association between Khat use and oral *candidia*(both *candidia albicans* and non-*albica*n candidiasis).To resolve the conflict in reports of results of this type, further studies will be needed. The results of this study show that alcohol use and cigarette smoking were not found to be associated with occurrence of oral candidiasis among HIV patients taking ART and similar results reported by (Moges and Kassa,2014). Similar study conducted in Nigeria which found no association between alcohol consumption and smoking with the occurrence of oral candidiasis among HIV patients taking ART (Iroezindu, *et al*., 2013).

On the other hand, as indicated in Table 6, WHO clinical stages, both baseline and current CD_4^+counts, and adherence to ART were insignificantly related (p-value of stage I=0.281,stage II p-value=0.115, for both baseline and current CD_4^+counts p-value =0.256 and p-value= 0.891, p-value=0.209 and P-value 0.309; for ART adherence p-value=0.945) to the oral candidiasis. However, the numbers of individual in advanced current clinical Stage III were less than II and I. However, in stage IV individuals were none. The reason for such difference could be due to the majority of individuals was adhered to ART medication properly.

Previous study revealed that low count of CD_4^+cells is an important contributor to an increase the probability of oral *Candida*. Specifically, a CD_4^+cell count of less than 200 cells/mm^3 is considered as a predisposing factor for candidiasis (Delgado., *et a*l., 2009). However, the studies indicate that only a low CD_4^+cell count in patients on ART is not associated to high risk of oral *Candida* (Campisi, *et a*l, 2002; Sánchez, *et al.*, 2005). But neither low CD_4^+count nor high CD_4^+count in our study is associated with the risk of oral candidiasis. This result is not supporting the results reported in some literature (Campisi, *et a*l.,2002;Sánchez ,*et al.*, 2005; Delgado *et al.*,2009; Bodhade *et al.*, 2011; Patil and Ganapathy, 2011). *Candida* infections can be present with different levels of CD_4^+reduction, even with normal CD_4^+counts($\geq$500cells/mm^3) and in early cases of AIDS (Nermin et al., 2015)

Table 5: Association of Oral Candidiasis with Demographic Features and Habits of HIV Positive Study Participant (№=374) in Ambo General Hospital during August-September, 2017

Character	№ Examined	Oral Candidiasis		OR	CI	P-value
		№(%) Positive	№(%) Negative			
Occupation						
Student	15	5(1.34)	10(2.67)	0.288	(0.07,1.178)	0.083
Government employee	56	24(6.42)	32(8.56)	1.451	(.665,3.165)	0.349
Private	158	67(17.91)	91(24.33)	1.060	(.656,1.713)	0.813
Unemployed	145	62(16.58)	83(22.19)			
Marital Status						
Single	40	21(5.61)	19(5.08)	3.044	(1.206,7.683)	0.018
Married	208	84(22.46)	124(33.15)	.950	(.592,1.525)	0.833
Divorced	126	53(14.17)	73(19.52)			
Educational Status						
Illiterate	91	47(12.57)	44(11.76)	3.947	(1.430,10.890)	0.008
Primary	179	72(19.25)	107(28.61)	2.436	(0.956,6.204)	0.062
Secondary	67	28(7.49)	39(10.43)	2.295	(0.855,6.162)	0.099
Diploma and above	37	11(2.94)	26(6.95)			
Residence area						
Urban	254	100(26.74)	154(41.18)	0.644	(0.404,1.025)	0.064
Rural	120	58(15.51)	62(16.58)			
Latrine facility						
Present	295	125(33.42)	170(45.45)	1.210	(0.708,2.068)	0.486
Absent	79	33(8.82)	46(12.29)			
Knowledge about Candidiasis						
Yes	13	10(2.67)	3(0.80)	5.074	(1.301,19.784)	0.019
No	361	148(39.57)	213(56.95)			
Alchol use						
Yes	24	10(2.67)	14(3.74)	0.977	(0.386,2.475)	0.961
No	350	148(39.57)	202(54.01)			
Khat use						
Yes	7	5(1.34)	2(0.53)	4.567	(0.744,28.134)	0.101
No	367	153(40.91)	214(57.22)			
Cigarette use						
Yes	26	8(2.14)	18(4.81)	0.379	(0.136,1.056)	0.063
No	348	150(40.11)	198(52.94)			

Table 6: Association of Oral Candidiasis with Clinical Status, ART Adherence and Immunological profile of HIV positive study participants (№ =374) in Ambo General Hospital during August-September, 2017

Character	№ Examined	Oral Candidiasis	OR	CI	P-value
Current WHO Clinical					
Stage I	341	143	2.128	(0.540,8.386)	0.281
Stage II	22	12	3.634	(0.73,18.07)	0.115
Stage III	11	3			
Stage IV	-				
Current CD4+cells/mm3					
501-≥1000	187	77	1.617	(0.706,3.706)	0.256
201-500	156	65	0.969	0.618,1.519	0.891
≤200	31	16			
Baseline CD4+cells/mm3					
501-≥1000	37	12	1.653	(0.755,3.622)	0.209
200-500	163	67	1.496	(0.689,3.251)	0.309
≤200	174	78			
`ART adherence					
Good	362	153	1.046	(0.293,3.729)	0.945
Poor	12	5			

5. SUMMARY, CONCLUSIONS AND RECOMENDATIONS

5.1. Summary

Candidiasis is the main health problem associated with HIV/AIDS patients in many developing countries including Ethiopia. There are several *Candida* species and only scant studies conducted to assess the prevalence of oral candidiasis and associated factors among HIV positive patients taking ART. So, a cross-sectional study was employed to determine the prevalence and correlate the major factors of oral *Candida albicans* and non-*albicans* candidiasis in HIV positive patients at Ambo General Hospital, Ethiopia. To assess the prevalence of candidiasis and associated risk factors among HIV patients taking ART, for the clinical record and demographic characters structured format and questionnaire survey was used, respectively. Moreover, in laboratory experiment the collected samples were observed through microscopic examination. Culturing on Sabouraud's dextrose with choramphienicol and Germ tube test techniques was also used for identification of *Candida albicans* among HIV positive individuals during the study period. Analyses of the results were done using SPSS version 20 statistical software. Chi-square test, odds ratio generated by logistic regression, was used as the measure of association between the prevalence of the *Candida* diseases and various factors of HIV positive participants.

Out of 374 HIV positive participants involved in this research, 140 (37.7%) were males and 234 (62.6%) were females. The average age of the study subjects was 37.48 years old with the minimum and maximum age of 13 and 82 years old, respectively. The prevalence of oral candidiaisis among the study participants was 42.25% of which the prevalence of Oral candidiasis for female HIV positive patients was 62.66% and 37.34% for male patients. The prevalence of *Candida albicans* and other *candida* species were 26.20% and 16.04% respectively. Marital status, level of education and knowledge about *candida* diseases are significantly(associated with prevalence of oral candidiasis.

5.2. Conclusion

- *Candida albicans* are more prevalent than non-*albicans* candidiasis among the study HIV positive patients.
- Female HIV/AIDS study patients were seen to be more vulnerable to both *Candida albicans* and non-*albicans* candidiasis.
- The CD4+ count was higher for the study patients who adhered to ART as compared to those who did not adhered to ART.
- Being single, being illiterate and lack of knowledge about candidiasis were identified as factors significantly associated with prevalence of oral candidiasis.

5.3. Recommendations

Candida infections received less clinical attention among HIV patients. However, an increased prevalence of invasive oral *Candida* infections has becoming a major challenge in HIV/AIDS patients who are taking ART. Therefore, in view of the findings in this study, the following recommendations have been made.

- This study revealed considerable prevalence of oral *Candida* infections among HIV patients on ART. Due attention of all stakeholders (health professionals of the hospital) is required to treat *Candida* disease in addition to the principal HIV/AIDS treatments. Particularly, proper treatments should be administered for HIV positive patients to treat *Candida albicans* which is the most prevalent candidiasis .
- The awareness and education about oral *Candida* infections should be well introduced to particularly illiterate HIV positive patients by health professionals.
- Further study should be administered to identify the non-*Candida albicans* species.
- Appropriate laboratory setup for candidiasis test should be established in the hospital.

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7. APPENDICES

A. Consent Form (English Version)

I was conducting a Thesis to investigate the Prevalence of Candidiasis and Associated risk Factors among HIV Positive Patients taking Anti–Retroviral Therapy in Ambo General Hospital,Oromia Regional State ,Ethiopia to fulfill the requirement for my MSc. graduation. The required primary, laboratory experiment and secondary data were collected only from participants that satisfy the inclusion criteria as well as willing to participate in this study. Data were collected on the basis of hospital and laboratory experiment. For the laboratory experiment sample of swab (oral-scrapings) were taken from participants, which would be used to detect the presence of candidiasis in addition to HIV/AIDS. The investigator would like to assure participants that there was no risk of any type in participating in this study. Participation in this study is completely voluntary and you can refuse to participate or free to withdraw yourself from the study at any time. The information in your records is strictly confidential and individual's data were not be disseminated. If additional explanation is required, participants can ask for further explanation.

I participant, whose name/Id is given above, hear this agreement/ read to me in my own language, and I understand the content and I am voluntary consent to participate in the study.

Study code no----------

Name----------

Signature----------Date----------

Witness Name----------Signature----------Date----------

Investigator Name----------Signature----------Date----------

B. Consent Form (Amharic Version)

የተዘጋጀው የስምምነት ቅፅ በአማርኛ

የኤች.አይ.ቪ/ኤድስ ህሙማን የተጓዳኝ በሽታ ከንዲድያ ዝሪያ ጋር ያለዉን ጥምረት ፥ ስርጭትና ተፅዕኖዉን በተመለከተ ጥናትና ምርምር በማድረግ ላይ ነኝ፡፡ በጥናቱ ላይ ተሳታፊ ከሆኑ ከእርስዎ ለምርምር የሚያገለግል አስፈላጊ ናሙናዎቸን በመስጠት እንዲተባበሩኝ በትህተና እጠይቆታለሁ፡፡ ናሙናዎቹን ለመስጠት መሳተፍዎ እርስዎ ላይ ምንም አይነት የጤና ችግር አያስከትልም፡፡

የእርስዎ በዚህ ጥናት ላይ መካተት የእርስዎ ሙሉ ፈቃድ ሲሆን በጥናቱ ላለመካተትም ሆነ ከተካተቱም በኋላ የለምንም ቅድመ ሁኔታ ፈቃደኝነትዎን የማንሳት መብትዎ ሙሉ በሙሉ የተጠበቀ ነው፡፡

እርስዎ በጥናቱ ላለመካተት መወሰንዎ እርስዎም ሆነ ቤተሰብዎ በሆስፒታሉ ያገኙት የነበረ የህክምና ተጠቃሚነትንም ሆነ ሌላ ችግር ፈፅሞ ሊያስከትልብዎ አይችልም፡፡ ለእርስዎ የተባለውን ነገር በትክክል ተረድተውታል ? ጥያቄ ካለዎት መጠየቅ እና ማብራሪያ የማግኘት መብት አለዎት፡፡

ስለ ጥናቱ ዓላማ ምርመራ እና ሂደት ተገልፆልኛል፡፡ ምክንያት ሳያስፈልገኝ ከጥናቱ ለማቋረጥ እነደምችል ተረድቻለሁ፡፡ ይህ የስምምነት ቅፅ በትክክል ተረድቼ በራሴ ፈቃደኝነት በጥናቱ ለመሳተፍ ተስማምቻለሁ፡፡ ለዚህም በፊርማዬ አረጋግጣለው፡፡

መለያ ቁጥር_____ የጥናቱ ስፍራ_____ ቀን_____

ኮድ _____ ፊርማ _____ቀን_____

የምስክር ስም_____ ፊርማ _____ ቀን_____

የጥናቱ ባለሙያ ስም_____ ፊርማ_____ ቀን_____

C. Structured Questionnaire (English Version)

The following questionnaires were used to collect some demographic characteristics of study participants.

Dear participants, the main purpose of this study is to Investigate prevalence of *Candidiasis* and associated risk Factors among HIV Positive Patients taking Anti –Retroviral Therapy in Ambo General Hospital, Oromia regional State, Ethiopia. You are kindly requested to respond the following questions. Sincerely as your sincere response will have great contribution to the study. Thank you.

Patient's ID _____ Sex _____ Age_____

1. Marital status A . Single B. Married C. Divorced
2. Educational Status A. Illiterate B. Primary C. Secondary D. Tertiary
3. Occupation A. Student B. Gov't employee C. Private D. Unemployed
4. Latrine facility at home A. present B. Absent
5. Family size A. $\leq$ 5 B. $\geq$ 6
6. Knowledge and awareness about Candida infection A. Good B. Poor
7. Place of Residence A. Rural B. Urban
8. Alchol use A. Yes B. No
9. Khat use A. Yes B. No
10. Condom use A. Yes B. No
11. Cigarete smoking A. Yes B. No
12. When did you know HIV in your blood ? A. Before five year B. Five years ago
13. Do you wash your hand during toilet use ? A. Always C. Some times B. No

D. Structured Questionnaire (Amharic Version)

የተዘጋጁት መጠይቆች በአማርኛ

ዉድ ተሳታፊዎች የዚህ ጥናት ዋና አላማ የኤች.አይ.ቪ/ኤድስ ህሙማን የተጓዳኝ በሽታ ከንዲድያ ዝሪያ ጋር ያለዉን ጥምረት ፥ ስርጭትና ተፅዕኖዉን በተመለከተ ጥናትና ምርምር ለማድረግ ነዉ፡፡ በመሆኑም እርስዎ ለጥያቄዎቹ የሚሰጡት መልሶች ይህ ጥናት የታቀደለትን አላማ ለማሳካት በከፍተኛ ደረጃ የሚረዳና ወሳኝ በመሆኑ ጥያቄዎቹን በቀረቡት ሁኔታ በመመለስ አንዲተባበሩን በአክብሮት ይጠየቃሉ፡፡ የኤች.አይ.ቪ/ኤድስ ህሙማን የስነ-ሕዝብ ማህበራዊ ሁኔታዎች እና የተጓዳኝ አስጊ በሽታ በተመለከተ ቀጥሎ ያሉትን መጠይቆች ይመልሱ::

የበሽተኛዉ መለያ ቁጥር---------- ዕድሜ---------- ጾታ----------

1. የትምህርት ደረጃዎ
   ሀ. ያልተማረ ለ. የመጀመሪያ ደረጃ የትምህርት
   ሐ. የሁለተኛ ደረጃ የትምህርት መ. ዲፕሎማ እና ከዚያ በላይ
2. የስራ ምድብ
   ሀ. ተማሪ ለ. የመንግስት ሰራተኛ
   ለ. የግል ስራ ሐ. ስራ ያሌለው
3. የጋብቻ ሁኔታ
   ሀ. ያገባ ለ. አግብቶ የፈታ
   ለ. የትዳር ጓደኛዉን በሞት ያጣ መ. ያላገባ
4. የመፀዳጃ ቤት ሁኔታ
   ሀ. አለ ለ. የለም
5. የቤተሰብ ብዛት
   ሀ. ≤ 5 ለ. ≥ 6
6. ስለ ተጓዳኝ በሽታ ፈንገስ/ካንዲድያ ያልዎት እውቀት እና ግንዛቤ
   ሀ. አነስተኛ ለ. ጥሩ
7. አልኮል ይጠጣሉ ሀ. አዎ ለ. አይደለም
8. መቼ ነዉ በ HIV እንደተያዙ ያወቁት ሀ. ከ5ዓመት ወድህ ለ. ከ 5 ዓመት በፊት
9. ጫት ከ ተጠቀሙ ሀ. አዎ ለ. አይደለም
10. ኮንደም በ አግባቡ ይጠቀማሉ ሀ. አዎ ለ. አይደለም

E. Structured Format

Structured questionnaire were used to collect medical record result of the study participant.

Patient ID. No_____ code_____ Sex____ Age _____

1	HIV/AIDS combined with	Candidiasis	Present	☐	Absent ☐
2	ART Status ; Starts receiving		yes	☐	No ☐
3	Mouth lesions		Present	☐	Absent ☐
4	Wight loss		yes	☐	Absent ☐
5	Oral whitish sputum patches/lesions		Present	☐	Absent ☐
6	CD4+T-cell count in No/mm^3	_____			
7	Level of CD4+T-cell count	≤ 200	☐		
		201-500	☐		
		501-≥1000	☐		
8	Base line CD+4	≤ 200	☐		
		201-500	☐		
		501-≥1000	☐		
9	Clinical Sage of WHO	Stage I	☐		
		Stage II	☐		
		Stage III	☐		
		Stage IV	☐		
10	Clinical Sage base line of WHO	Stage I	☐		
		Stage II	☐		

Stage III

Stage IV