ADDIS ABABA UNIVERSITY COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE,DEPARTMENT OF ZOOLOGICAL SCIENCECE

PREVALENCE OF DIARRHEAL DISEASE AMONG HIV- PATIENTS FROM DEBRE MARKOS REFERAL HOSPITAL, DEBRE MARKOS.

BY

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LIST OF ABBREVIATIONS

- AIDS- Acquired immune deficiency syndrome
- ART – Antiretroviral therapy
- CDC – Center for disease control
- CSA – Central statistical agency
- EDHS – Ethiopia Demographic and health survey
- HIV - Human immunodeficiency virus
- NIAD – National institute of Allergy infections Disease
- SPSS - Statistical package for social science
- UNICEF – United Nations international children Educational fund.
- U.S.GHPFS- United States Global Health Policy Fact Sheet.
- GHIV/AIDSR- Global HIV/AIDS programme.
- WHO – World Health Organization.
- FHIV/AIDSPCO- Federal HIV/AIDS Prevention and Control Office.
- JUNPHIV/AIDS –Joint United Nations Program on HIV/AIDS.

Abstract

Diarrhea disease is defined as passage of three or more loss of watery stool in 24 hours period. It is caused by both opportunistic and non-opportunistic parasites. Diarrhea can affect all people all over the world. However, a lot of people in low-income countries are affected due to lack of sanitation, contaminated food and drinks. Diarrhea has been aggravated due to human immunodeficiency virus infection. The objective of this study was to determine the occurrence of diarrhea and detected pathogens in human immune deficiency infected patients. Using purposive and random sampling 384 study subjects were recruited, of which 127 were diarrheal-patients..The participants were selected each day by simple random sampling when they attended in Antiretroviral therapy clinic of the hospital. The dependent variable was measured in terms of diarrhea at the time of clinical examination. Stool samples were collected from all 127 dirrheal patients immediately after they were examined by the physician in the clinic and examined by direct microscopy by the researcher and pre-oriented laboratory technicians for type of diarrhea and pathogens to be identified. The number of females and males were almost equal, 68 and 59 respectively. 90 of them were from urban and 37 of them were from rural area of both sexes. Other data were collected through semi-structured questionnaire and interview and by trained oriented data collectors with the support of the researcher from January,2016 up to end of April,2016. All participants were recruited by their identification card and accordingly were given the questionnaire to fill and were interviewed at the same time. The major pathogens of diarrhea detected from cases were E. histolytica/dipar 32.3 %, Taenia spp., 23.6%, and Bacteria spp.17.32 %. In chi-square analysis, the risk factors associated with diarrhea, such as low level of education /OR=2,at 95% level of confidence/ economic status, irregular hand washing before meal (OR=4.7 at 95% level of confidence), irregular hand washing after toilet (OR =2,at 95% level of confidence), use stored cooked food for later use (OR=8.9,at 95% level of confidence), eating raw meat and vegetable (OR=8.5, at 95 % level of confidence), lack of enough food, untreated water, not knowing of activities exposing for diarrhea and preventive methods/OR=3.1&2 at 95% level of confidence), as well as low CD_4-counts/ µl were associated as risk factors for diarrhea on diarrheal- patients in Debre Markos Referral Hospital.

Key words: Diarrhea, pathogens, risk factors.

Table of Contents

Contents **Page**

List of Tables

Page

1. Introduction

Diarrhea is defined as a passage of three or more loss of watery stool in 24 hours period. It can affect all people in the world in general, and that of in developing countries in particular, due to economic, social and other related factors. It affects more people infected with HIV infection and usually tends to be chronic (Kosek. et al, 2003). Diarrheal disease is also the second leading cause of death in children under five years old, and is responsible for killing around 760 000 children every year. Diarrhea can last several days, and can leave the body without the water and salts that are necessary for survival. Most people who die from diarrhea actually die from severe dehydration and fluid loss.

Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhea. Globally, there are nearly 1.7 billion cases of diarrheal disease every year (WHO, 2013). Of the estimated total 10.6 million deaths among children younger than five years of age worldwide, 42 percent occur in African region (Bryce, et al, 2005). The under-five morbidity rate in Africa region is seven times higher than that in European region. In 1980 this difference was equal to 4.3 times (WHO. 2005). A factor that may contribute to this situation is the HIV/AIDS epidemic and weakness of the implementation capacity of health system in the region likely to balance (Walker, et al, 2002).

HIV infection has added considerably to the burden of diarrheal diseases among adults and children. According to the latest estimates in 2011 from UNAID, globally an approximate of 34 million people were living with HIV (U.S. GHPFS, 2011). Although HIV infections have declined by more than 21% since their peak in 1997 (GHIV/AIDS R 2011). There were about 2.2 million new infections in 2011, 500,000 fewer than the 2001(JUNP, 2012).

Since the beginning of epidemic, nearly 30 million people have died of AIDS - related causes (UNAIDS, 2012). In 2011 the annual number of people dying from AIDS – related causes worldwide was estimated 1.7 million (JUNP HIV/AIDS 2012). At the end of 2010 about 22.9 million which is 67% of those living with HIV/AIDS globally are in Africa though only about 12% of the world population lives in the region (GHIV/AIDS R, 2011). Africa region represents about 79% of AIDS mortality globally (Edward .et al, 2006), the estimated mortality from AIDS-related illnesses at end of 2010 are 1.2 million (G HIV/AIDS, 2011).

Ethiopia is one of the African countries of with high HIV prevalence. According to the recent Ethiopia Demographic and Health Survey (EDHS), the number of people living with HIV in the country was estimated at 769,000 of whom 458,100 (59.5%) 311,500(40.5%) were females and males respectively (FHIV/AIDS PCO, 2014). People living with HIV in the country (2%) more lower than some African countries such as U ganda (4%), Kenya (5%), Nigeria (9%) and South Africa (18%) (UNAIDS, 2013). Amongst the region the prevalence of HIV in Amhara region was 2.2% (CSA, 2011).

Diarrhea is one of the most common AIDS-related illness causing a significant morbidity and mortality in HIV-infected patients (Siddiqui, et al, 2012). Reports indicated that diarrhea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in developing countries (Framm and Soave, 1997).

The etiologic spectrum of enteric pathogens causing diarrhea includes bacteria, parasites, fungi and viruses (Mitra et al, 2001), though that of parasitic origin is prominent in patients with AIDS in developing countries (Cimerman, et al., 1999). However, with the progressive introduction of Highly Active Antiretroviral Therapy (HAART), modifications have been observed in the morbidity and mortality profile among HIV/AIDS patients; reflecting the reduced occurrence of opportunistic infections, including those caused by enteric parasites (Willemot, et al, 2004).

According to Amhara national region state, Health Bureau of East Gojjam Health Department, people who were recorded affected by diarrheal disease in the zone were 75,400 in the year 2015.

Though Hiwotie (2014) conducted a study on the prevalence of intestinal parasitic infections among people with and without HIV/AIDS infections of their association with diarrhea, the work on co-infection of diarrheal diseases and HIV is limited.

The present study is therefore, aimed to determine the prevalece of diarrheal disease in HIV-infected individuals and risk factors among HIV-patients in ART Clinic of Debre Markos Hospital.

1.1. Statement of the problem

Diarrhea, one of the most prevalent diseases in children under five years and top ten diseases for most young and adult people in the study area. Specially, on HIV/AIDS-infected people, diarrhea is a serious health problem. Knowledge of the specific pathogens that cause diarrheal diseases in HIV-infected individuals and their magnitude in the area is critical for the implementation of specific intervention strategies. The type of diarrheal diseases and the pathogens as well as associated risk factors should be identified in order to apply the proper intervention to prevent such infectious diseases. Therefore, to achieve this possibility it is required to know the magnitude of diarrhea and associated risk factors in HIV-patients.

1.2. General objective

The research is aimed to identify and describe the prevalence and type of diarrheal diseases and associated risk factors that affect HIV-infected individuals, in particular and assessment of socio-demographic characteristics and other risk factors related to such diseases.

1.3. Specific objective of this study

- To determine the prevalence and type of diarrhea disease in HIV-infected individuals attending in the antiretroviral therapy clinic of Debre Markos Referral Hospital until the end of April 2016.

  To characterize socio-demographic characters of the study subjects
- To identify associated risk factors related to diarrheal disease in HIV infected individuals in ART Clinic of Debre Markos Referal Hospital until the end of April 2016.

2. Literature Review

2.1. Definition of diarrhea

Diarrhea is defined by the World Health Organization (WHO, 2009) as three or more watery or loose bowel movements in a 24 hour period .It is caused by bacterial, viral, and parasitic organisms and is usually a symptom of gastrointestinal infection (*WHO , 2013).* However, the population may claim they have diarrhea when they, in fact, have other problems, such as fecal impaction (Schiller, (2009). Therefore when evaluating a person with complaints of diarrhea, it is imperative to closely question the patient about associated signs and Symptoms (Schiller, (2009).

Almost everyone has become ill of, or would be affected by diarrhea at some point in their lives. Diarrhea can occur as a symptom of many different illnesses, as a side effect of some drugs or may be due to anxiety among other things. Diarrhea results from an imbalance in the absorption and secretion properties of the intestinal tract; if absorption decreases or secretion increases beyond normal, diarrhea results. It can range in severity from an acute, self-limited annoyance to a severe, life-threatening illness. .

The definition of diarrhea depends on what is normal for the individual. For some, diarrhea can be as little as one loose stool per day. Others may have three daily bowel movements normally and not be having what they consider diarrhea. According to K. Armon, diarrhea is defined as a change in bowel habit for an individual resulting in substantially more frequent and/or looser stools (Armon, 2001). Although changes in frequency of bowel movements and looseness of stools can vary independently of each other, changes usually occur in both.

Clinical features vary greatly depending on the cause, duration, and severity of the diarrhea, on the area of bowel affected, and on the patient's general health. Diarrhea is the most serious public health problem connected to water and sanitation and can be both ―waterborne" and ―water-washed". In recent decades, a consensus developed that the key factors for the prevention of diarrhea are sanitation, personal hygiene, availability of water and good quality drinking water; and that the quantity of water that people have available for hygiene is of equal or greater

Importance for the prevention of diarrhea as the bacteriological water quality (Jensen, et al, 2004).

2.2. Type of Diarrhea

Diarrhea may be classified into four general types, based on the mechanism, including osmotic diarrhea, secretory diarrhea, exudative diarrhea, and motility disorder diarrhea (Brooks, et al.2003). According to(WHO,1988),(Vesikari ,et al,1994), based on clinical syndromes, diarrhea could be classified into four types, each reflecting a different pathogenesis, including acute watery diarrhea, dysentery, persistent or prolonged diarrhea and chronic diarrhea

2.2.1 Acute watery diarrhea

This term refers to diarrhea characterized by abrupt onset of frequent, watery, loose stools without visible blood, lasting less than two weeks. Usually, acute watery diarrhea episodes subside within 72 hours of onset. It may be accompanied by flatulence, malaise and abdominal pain. Nausea, vomiting may occur and also fever may be present. The common causes of acute watery diarrhea are viral, bacterial, and parasitic infections. Bacteria also can cause acute food poisoning. The enteric pathogens causing this diarrhea in developing countries are largely the same that are encountered in developed countries, but their proportions are different (Jay, 2013).

In general, bacterial pathogens are more important in countries with poor hygienic conditions. The most important causes of this diarrhea in developing countries are Rotavirus, Shigellae, enterotoxigenic E.coli (ETEC), Vibriocholerae, *Campylobacter jejuni*, *enteropathogenic* E. coli (EPEC), *Salmonella spp*. and *Cryptosporidium* (Vesikari, et al, 1994).

Diarrhea can be classified by several methods with duration of the symptom being foremost. Diarrhea lasting less than 2 weeks is considered acute (Hall, 2010). This phenomenon is most likely caused by an infectious agent, such as bacterial, parasitic or viral invasion, or by a non-infectious agent such as dietary indiscretion or a new medication (Amerine,et al, 2006). Acute diarrhea is typically self limiting and resolves quickly with no lasting sequelae (Amerine,et al, 2006). Infectious agents are one of the factors associated with acute diarrhea. Some of these pathogens can cause an inflammatory response in the gut where the epithelial lining is damaged either by a toxin produced by the organism or by an organism invading the mucosa (Bliss, et.al 2006).Some organisms that cause an inflammatory response are –Cytomegalovirus, Herpes simplex virus, *Shigella, Salmonella, Chlamydia, Nisseria gonorrheae, Campylobacter jejuni,*

Clostridium difficile, Escherichia coli O157:H5, *Entamboeba histolytic"*(Bliss, et.al 2006). Symptoms of acute inflammatory diarrhea include fever (higher than 38.5^{C}), lethargy, and a stool that contains pus, blood, leukocytes and/or mucus (Bliss, et.al 2006).

There are organisms that cause acute diarrhea that do not produce an inflammatory response although the person may have a low grade fever, malaise, nausea and vomiting as well as diarrhea. These causative organisms include –Norwalk virus, Rotavirus, *Staphylococcus aurous, Clostridium perfringens, Vibrio cholera* and enterotoxigenic *Escherichia coli …"* (Bliss, et.al 2006).Less commonly, protozoa such *Guardia* or *Cryptosporidium* may be the causative factor (Amerine, et al, 2006).

The most dangerous complication is dehydration that occurs when there is excessive loss of fluids and minerals (electrolytes) from the body. With vomiting, dehydration becomes more severe. Dehydration is especially dangerous in infants and young children due to rapid body water turnover, high body water content and relatively larger body surface (Molbak, 2000). Patients with mild dehydration may experience only thirst and dry mouth. Moderate to severe dehydration may cause orthostatic hypotension with syncope (fainting upon standing due to a reduced volume of blood, which causes a drop in blood pressure upon standing), a diminished urine output, severe weakness, shock, kidney failure, confusion, acidosis (too much acid in the blood), and coma.

2.2.2. Dysentery

May simply be defined as diarrhea containing blood and mucus in feces. The illness also includes abdominal cramps, fever and rectal pain. The most important cause of bloody diarrhea is Shigella (Abraham Haileamlak, 2005).

2.2.3. Persistent diarrhea

Diarrhea lasting longer than two weeks but resolving within a month is known as persistent diarrhea (Bushen, et al,2003). This is typically a slower to resolve infection or continuing use of an offending agent (Amerine, et al, 2006). Persistent diarrhea is defined as diarrheal episodes of presumed infectious a etiology that have an unusually long duration and last at least 14 days (Jay, 2013). The episode may begin acutely either as watery diarrhea or dysentery. This diarrhea

causes substantial weight loss in most patients. It may be responsible for about one-third to half of all diarrhea-related deaths. Since persistent diarrhea is a major cause of malnutrition in the developing countries, even the milder, non-fatal episodes contribute to the overall high mortality rates that are frequently associated with malnutrition in these countries. The pathogenesis of persistent diarrhea is not fully known. Several causes, probably in combination, include: infections with entero aggregative E. coli (EAggEC), EPEC and Cryptosporidium; intolerance to foods; delayed recovery of intestinal mucosal damage due to protein-energy malnutrition or Vitamin A or zinc15 deficiency; immunodeficiency (with the exception of Acquired Immune Deficiency Syndrome - AIDS causing chronic diarrhea); and inappropriate use of antibiotics (Vesikari, et al,1994).

Diarrhea lasting longer than two weeks but resolving within a month is known as persistent diarrhea (Bushen, et al,2003) . This is typically a slower to resolve infection or continuing use of an offending agent (Amerine, et al, 2006).

2.2.4. Chronic diarrhea

On the other hand, lasts longer than four weeks (Bliss, et.al 2006). .Approximately 3%-5% of the American population is thought to suffer from chronic diarrhea during any given period of time (Schiller, 2009). Chronic diarrhea can be the result of disease processes, medication, genetic abnormalities, or a variety of other causes (Marchiondo, 2009). The term chronic diarrhea refers to diarrhea which is recurrent or long lasting due to mainly non-infectious causes. Chronic diarrhea may be caused by gastrointestinal disease, may be secondary to systemic disease, and may be psychogenic in nature (Jay, 2013). Path physiologically, chronic diarrhea may be categorized as inflammatory diarrhea (caused by regional enteritis, ulcerative colitis), osmotic or mal-absorptive diarrhea (resulted from lactose intolerance, tropical spruce, celiac disease, Whipple's disease, chronic pancreatitis, bile duct obstruction), secretary diarrhea is caused by medications, bowel resection, mucosal disease), dysmotility diarrhea (caused by conditions such as diabetic neuropathy or irritable bowel syndrome) and factitious (self-induced, e.g., from laxative abuse) diarrhea (Armon, et al,2001).

Secretory diarrhea occurs when there is an increase in the amount of fluid being drawn into the lumen of the bowel such that the ability of the intestines to reabsorb is overwhelmed (Bliss, et al

2006).Typically, infectious agents are the cause of secretory diarrhea but any substance (secretagogue) that causes fluid to be pulled into the bowel can be the culprit (Strasinger, et al,(2008). Infectious secretagogues include *Vibrio cholerae, E. coli, Camylobacter jejuni, Salmonella, Shigella,* and *Clostridium difficile* (Farthing, 2006).

These pathogens secrete toxins that bind with the structures within the gut, altering, sometimes irreversibly, the amount of fluid secreted into the bowel (Bliss, et.al 2006). As an example, the toxin excreted by the pathogen cholera causes massive secretory diarrhea which, during its acute phase, can be as much as 24 liters in 24 hours(Farthing,2006).Non-infectious secretagogues include chemicals produced by certain types of cancer, prostaglandins produced in patients with bowel inflammation and substances not well absorbed such as fatty acids and bile acid(Bliss, et.al 2006). Persons with secretory diarrhea typically had stool volume of more than one litter daily, with neutral pH and have no change in the amount of stool produced with fasting (Bliss, et.al 2006).

2.3. HIV/AIDS - related diarrheal disease and pathogens

HIV is the virus that causes AIDS by weakening the natural immune system. HIV causes progressive depletion of the CD4 T cells, which leads to life threatening opportunistic infection or malignancies during the natural course of disease (Miller, et al,1999). More than 90% of opportunistic infections are responsible for the development of AIDS morbidity and mortalities (Federal HIV/AIDS Prevention and Control Office, 2014).

The risk for the development of opportunistic infections in HIV- infected individuals depends on exposure to potential pathogens, virulence of pathogens, the degree of host immunity and the use of antimicrobial prophylaxis (Chaisson, et al, 1997).

Diarrhea, which means two or more loose or unformed stools per day, can last for a few days or can be chronic lasting for weeks or months. Many people with AIDS have diarrhea that persists for more than a month or is so severe they expel several gallons of fluid a day (NIAID, 1995). The diarrhea may be accompanied by abdominal cramps, nausea, vomiting, fever, and blood in the stool or weakness (NIAID, 1995).

Diarrhea disease in HIV-infected patient population has been demonstrated to be associated with a significant decrease in quality of life, whatever the etiology of the diarrheal illness might be (Watson, et al, 1996). Multiple infectious pathogens including bacteria, mycobacterium, viruses, and parasites have been implicated as causes of diarrheal illness in patients infected with HIV (Mayer et al., 1994). The etiology of diarrheal disease appears to vary by geographical region (Malebranche, et al, 1983). In patients with HIV in Africa, diarrheal disease and wasting are a more common complication of HIV infection than they are in United States and Western Europe. A study in Uganda reported the commonest causes of diarrhea to be helminthic infections (24.5%), bacterial infections (19.2%) and protozoal infections (9.2%) (Binka,et al, 2002) . Enteric viruses have also been reported associated with diarrhea (Grohmannp, et al, 1993).

Soil transmitted helminthes are endemic in sub-Saharan Africa. A few studies showed significant association between HIV and geohelminthic infections though this association was not consistent for all helminthes. A study in Honduras found a strong association between Strongloides sterocalis and HIV infection and lower risk for infections of Giardia lamblia. Ascaris lumbricoides and Trichuris trichiurias (Lindo,et al, 1998). Another study found a high prevalence

Of Giardiasis and strongloidias is among HIV-positive patients in Brazil (Feitosa, et al, 2001) . A similar study in Ethiopia also found higher prevalence of strongyloidiasis among HIV-patients with CD4 count less than 200 cell/ µL (Assefa, et al, 2009).

The most common infectious organisms causing AIDS-related diarrhea include cytomegalovirus (CMU), the parasites cryptosporidium, microsporidia and Giardia lamblia and the bacterium mycobacterium avium-intracellulare (MAC). Other bacteria and parasites that cause diarrheal symptoms in otherwise healthy people may cause more severe, prolonged or recurrent diarrheal disease in people with HIV or AIDS (NIAID, 1995).

Diarrheal diseases also have non-infectious causes. These include antibiotics or other drugs that can destroy beneficial bacteria in the intestines, HIV- entropath, which is disease caused by HIV itself, cancers such as non-Hodgkin's lymphoma or kaposi's sarcoma, and lactose intolerance, irritable bowel or other gastrointestinal disorders. Sometimes diarrhea can stem from a lack of stomach acids, a condition that is common people with AIDS (NIAID, 1995).

According to Health Bureau of East Gojjam Health Department report in the year 2015, people with diarrheal disease and diarrhea with blood were reported from the woredas. Accordingly, in Debre Markos woreda (town), people above 5 years old who were affected by diarrhea have been reported. The report, for non-bloody diarrhea, males 1407, females 702, diarrhea with watery diarrhea, males 100, females 126, diarrhea with blood (dysentery), males 299 and females, 222 which totally make up males 1806 and females 1050 patients. Therefore, the prevalence of diarrhea disease in the town was high in which a total of 2856 diarrheal disease patients were recorded in which HIV infected individuals were included.

Though some diarrheas are due to errors of metabolism, chemical irritation or organic disturbance, the vast majority are caused by infectious pathogens (Gracey ,1985).

2.3.1 Bacterial infections:

Diarrhea caused by enteric bacterial infections is very important worldwide, especially in tropical and developing countries, and is a serious problem among older children and adults as well as in infants and young children. The range of causative micro organisms is very large; they include E. coli, Salmonella, Shigella, Campylobacter, Yersinia, vibrios, and *Clostridium difficile (Gracey, 1996).*

2.3.2. Viral infections:

Rotavirus is one of the most common causes of severe diarrhea. Other viruses may be important causes of diarrheal disease in human, includingNorwalk virus, Norwalk-like viruses, enteric adenoviruses, caliciviruses, and astroviruses (Gracey, 1996).

2.3.3 Parasites

Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhea include Giardia lamblia, Entamoeba histolytica, Cyclosporacayetanensis and Cryptosporidium (WHO. 2005).

A high prevalence of intestinal infectious has been reported in HIV-positive patients, in the form of diarrhea associated with parasitosis (Wafa.2010). The intestinal parasites frequently

Encountered in Ethiopia include: *Cryptosporidium* species, *Isospora belli*, *Microsporidia* spp, Entamoeba histolytica *Giardia lamblia,* Tri*churistrichiura*, Ascarislumbricodies, *Stongyloides stercoralis*, hookworms and *Teania* spp.(Hailemeriam,et al,2004). The main clinical manifestation of the parasitic infections is diarrhea. The decline of mucosal immunologic defense mechanisms predisposes patients to acute, intermediate, or late gastrointestinal manifestations such as diarrhea (Haileyesus Adamu and Beyene Petors. 2009).

Diarrhea has been reported to be associated with HIV- infected individuals caused by opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non- opportunistic agents that usually cause acute treatable diarrhea illness (Marissal,et al,2007). In HIV- infected patients, in addition to microbes, diarrhea may be caused by other factors such as medication, immune deregulation, autonomic dysfunction and nutritional supplementation (Awole et al., 2003). Studies on human parasitic infections have demonstrated a common relationship between parasitic infections and lower socio-economic status (Kia, et al., 2008).

The effect of socio-economic status on risk of infectious diseases in general, and parasitic infections in particular, is complex in nature and could be attributed to several other factors such as lack of access to clean water, poor hygienic environment, lack of access to education due to financial constraints and overcrowded condition(Mehraj et al, 2008). Diarrhea is one of the most common complications of HIV and has many possible causes. First, diarrhea can be an early sign of the HIV disease itself, also known as acute HIV infection. HIV produces flu-like symptoms within two months of the initial infection. Afterward, you may continue to experience the symptoms, including diarrhea, for a few weeks. Other symptoms of an acute HIV infection include: Poor nutrition can worsen diarrhea when it's due to HIV or an HIV medication.

Diarrhea, the most common complications of HIV has many causes including intestinal parasites such as protozoan and helminthes. Reports from many regions of the world where HIV/AIDS is endemic have acknowledged that intestinal parasitism is widespread among these populations (Assefa et.al. 2009).Several intestinal parasitic pathogens which have been reported in HIV patients include *Cryptosporidium* parvum, Isospora belli, microsporidia (Enterocytozoon bieneusi, Encephalitozoon intestinalis), Giardia duodenalis, Entamoeba histolytica/dispar,

Cyclospora cayetanensis, Ascaris lumbricoides, Trichuris trichuria, hookworms *and* Strongyloidis stercoralis (Farthing et al., 2009).

2.3.3.1. Intestinal protozoan parasites:

The protozoa are an extremely diverse group of unicellular Organisms occurring in almost all of the ecological niches known to humans, including the bottom of hot springs and the edges of ice flows. Even though the majority of protozoa occur as free living organisms in the soil, moist, marine or fresh water environments, a substantial number also exist as mutualists, commensals or parasites (Mehlhorn, 1988, Katz,et al, 1989). Protozoan parasites are known to affect all species of vertebrates and many invertebrates. They are able to adapt to life in virtually all body sites of their hosts. Their characteristic high infectivity enhances their pathogencity within the host (Katz,et al, 1989).Numerous protozoa inhibit the gastrointestinal tract of humans. This list includes representatives from many diverse protozoan groups.

The majority of these organisms can cause severe disease under certain circumstances (Stenzel, et al, 2004). Some can cause serious diseases of the respiratory tract (air passages from the nose to lungs), and the central nervous system (brain, cranial nerves and spinal cord), while others live in our intestines and cause symptoms like diarrhea and are not deadly. It is hard to protect ourselves from protozoan parasites that are in the environment, because they produce cysts. A cyst has very resistant walls that surround and protect the protozoan parasite and make it able to survive extreme environmental conditions, such as big changes in temperature and too much or too little water. This means that once a protozoan parasite cyst is in the environment it can wait a long time for the perfect chance to get inside our bodies to infect us.

2.3.3.1.1. Entamoeba histolytica /Entamoeba Infection

The genus *Entamoeba*, amoeboid protozoanparasite, includes six species (*Entamoebahistolytica*, *E. dispar*, *E. moshkovskii*, *E.polecki*, *E. coli*, and *E. hartmanni*). *E.histolytica*, *E. dispar*, and *E.moshkovskii*arecapable of infecting the intestinal lumen ofhumans, are morphologically identical, butgenetically different (Parija, 2013). Entamoebahistolytica infection is common in most developing countries. It is also becoming more frequent in the United States and other

developed countries as the result of increasing tourism abroad and arising number of refuges and other immigrants and nonimmigrants originating in endemic countries (Petri, et al, 1999).

The two *species Entamoeba histolytica and* Entamoeba dispar are morphologically identical but pathologically distinct (WHO, 1997, Petri, et al, 1999). However, only E.histolytica is capable of causing disease (WHO, 1997). Colonization with E. dispar is said to be three times more common than E. histolytica in developing countries while it is ten times more common in developed nations (Petri, et al, 1999). Entamoeba histolytica is reported to be responsible for approximately 50 million cases of invasive amoebiasis (Petri, et al, 1999).And upwards of 100,000deaths/year(WHO,1997).

Thus, it is ranked second only to malaria as the cause of mortality due to a protozoan infection (WHO, 1997). The parasite normally inhabits the large intestine but is also capable of invading other organs such as the liver, brain and spleen (Petri,et al, 1999). The majority of amoebic infections are reported to occur in Central America, South America, Africa and Asia. These are often associated with poor water and food hygiene and sanitation practices (Petri,et al, 1999). Humans are the most significant reservoir of infection even though morphologically identical amoebae have been isolated from certain domestic and wild animals (Katz,et al, 1989).

2.3.3.1.2. Giardia lamblia /Giardia lamblia Infection

Guardia intestinal is, flagellated protozoan parasite, worldwide distributed, burden of disease remains high in developing countries, in immuno-compromised individuals, especially in children < 10 years of age (Parija, 2013). With progressive reduction in CD4 counts, symptomatic, Guardia infection is increased (Gazzard,2009). Complications include steatorrhea leading to malabsorption and weight loss. Almost 80% of sexually active gay men carry E. dispar, E. coli, E.hartmanni, Iodamoeb abutschlii, Dientamoeba fragilis, and G. intestinalis, as compared to its incidence in the general population of 13% (Parija, 2013). G.lamblia is appearing shaped, flagellated protozoan that causes a wide variety of gastrointestinal complaints. Guardia is arguably the most common parasite infection of humans worldwide and the second most common in the United States after pinworm (Katz, et al,2001). Between 1992 and 1997, the Centers for Disease Control and Prevention (CDC) estimated that more than 2.5 million cases of Giardiasis occurs annually (Furness, et al, 2000).Because Giardiasis is spread by fecal-oral

contamination, the prevalence is higher in populations with poor sanitation, close contact, and oral-anal sexual practices.

The disease is commonly water-borne because Giardiais resistant to the chlorine levels in normal tap water and survives well in cold mountain streams. Because giardiasis frequently infects persons who spend a lot of time camping, back packing, or hunting, it has gained the nick names of ―backpacker's diarrhea" and ―beaver fever"(Dupont,et al,, 1995). Food borne transmission is rare but can occur with ingestion of raw or under cooked foods. Giardiasis is a zoonosis, and cross infectivity among beaver, cattle dogs, rodents, and big horn sheep ensures a constant reservoir (Glaser,et al, 2000).

2.3.3.1.3. Cryptosporidium / Cryptosporidium Infection

Cryptosporidiosis is a parasitic disease caused by Cryptosporidium, a protozoan parasite in the phylum Apicomplexa. It affects the intestines and is typically an acute short- term infection. It is spread through the fecal-oral route, often through contaminated water (Centers for Disease Control and Prevention, 2009); the main symptom is self-limiting diarrhea in people within tact immune systems. In immunocompromised individuals, such as AIDS patients, the symptoms are particularly severe and often fatal Chen, et al 2007).

Cryptosporidium is the organism most commonly isolated in HIV positive patients presenting with diarrhea. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Infection is through contaminated material, water, uncooked or cross-contaminated food that has been in contact with the feces of an infected individual or animal Contact must then be transferred to the mouth and swallowed. It is especially prevalent amongst those in regular contact with bodies of fresh water including recreational water such as swimming pools. Other potential sources include insufficiently treated water supplies, contaminated food, or exposure to feces (Centers for Disease Control and Prevention, 2009). The high resistance of Cryptosporidium cysts to disinfectants such as chlorine bleach enables them to survive for long periods and still remain infective (Carpenter, et al, 1999).

2.3.3.2 Intestinal helminth parasites:

Parasitic worms, often referred to as helminthes, are a division of eukaryotic parasites (Maizels,et al, 2003). They are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts 'nutrient absorption, causing weakness and disease .

2.3.3.2.1 Ascaris / Ascaris Infection

Ascariasis is infection with the parasitic roundworm Ascaris lumbricoides. Ascariasis is caused by consuming food or drink contaminated with roundworm eggs. Ascariasis is the most common intestinal worm infection. It is found in association with poor personal hygiene, poor sanitation, and in places where human feces are used as fertilizer (Kazura, 2007).

Once consumed, the eggs hatch and release immature round worms called larvae within the small intestine. Within a few days, the larvae then move through the blood stream to the lungs, exit up through the large air ways of the lungs, and are swallowed back in to the stomach and reach the small intestine .During movement through the lungs the larvae may produce an uncommon form of pneumonia called eosinophilic pneumonia. Once they are back in the small intestine, the larvae mature in to adult round worms. Adult worms live in the small intestine where they lay eggs that a represent in feces. They can live 10–24 months (Kazura, 2007). It is estimated that 1 billion people are infected worldwide .Ascariasis occurs in people of all ages though patients area affected more severely than adults.

Most of the time, Ascariasis causes no symptoms. If there are symptoms ,they may include: bloody sputum, cough, low-grade fever, passing worms in stool, shortness of breath, skin rash, stomach pain, vomiting worms, wheezing and worms released through the nose or mouth(Kazura, 2007).Most people recover from symptoms of the infection, even without treatment, although they may continue to carry the worms in their body. Complications may be caused by adult worms that move to certain organs such as the bile duct, pancreas, or appendix, or multiply and cause a blockage in the intestine. Improved sanitation and hygiene in developing countries would reduce the risk in those areas. In areas where this disorder is

common, routine or preventive (prophylactic) treatment with deworming medications may be advised (Kazura, 2007).

2.3.3.2.2. Hookworm / Hookworm infection

Hook worms (a type of round worm) are another common intestinal parasite. The U.S. Centers for Disease Control (CDC) and Prevention estimates that 1 billion people worldwide have hook worm infestations, although improved sanitation has reduced the number of cases. Human hookworm infection is caused by blood-feeding nematode parasites of the genus *Ancylostoma* and the species *Necator americanus*. Worldwide, *N. americanus* is the predominant etiology of human hookworm infection, whereas *A. duodenale* occurs in more scattered focal environments (Hotez, et al.2004).

These two hookworms, together with the roundworm, *Ascaris lumbricoides*, and the whipworm, *Trichuristrichiura*, are often referred to collectively as soil-transmitted helminthes (STHs).No international surveillance mechanisms are in place to determine the prevalence and global distribution of hookworm infection. However, based on an extensive search of the literature since 1990, the worldwide number of cases of hookworm was recently estimated to be 740million people (De Silva, et al, 2003). The highest prevalence of hook worm occurs in sub-Saharan Africa and eastern Asia .High transmission also occurs in other areas of rural poverty in the tropics, including southern China (Hotez, 2002), the Indian sub-continent (Yadla,et al,2003), and the Americas(Hotez ,2003). In all regions, there is a striking relationship between hookworm prevalence and low socioeconomic status (De Silva, et al, 2003) Hookworm's neglected status partly reflects its concentration among the world's poorest 2.7 billion people who live on less than $2 a day.

2.3.3.2.3. Pinworms/Enterobius vermicularis;

Pinworms are small, white, thread-like worms that sometimes live in the colon and rectum of humans. Pinworms are about one-half inch long. While the infected person sleeps, female pinworms lay their eggs on the skin around the anus. This can cause intense itching in this area. *E. vermicularis* is a common helminthic parasite that affects people worldwide from all socioeconomic classes. Humans are the only natural host for the parasite. A high prevalence is

noted among children (typically aged 5–10 yr), institutional populations, homosexual men, and family contacts (Cook, 1994; Goldmann,et al ,1997). Infection commonly occurs by transfer of highly infective eggs from the perianal area to the mouth. The human gastrointestinal tract is the primary site of habitat, mainly in the cecum and appendix. Symptoms range from an asymptomatic presentation to perianal pruritus, insomnia, irritability, restlessness, and rarely, impetigo of scratched skin, vulvovaginitisor enuresis (Cook, 1994).

2.3.3.2.4 Strongyloides stercoralis / Strongyloidiasis;

Strongyloidiasis is a human parasitic disease caused by the nematode (roundworm) *Strongyloides stercoralis*, or sometimes Strongyloides fülleborni. S.stercoralis, also called threadworms, is a nematode helminth parasite that causes strongyloidiasis. There are an Estimated 100 million to 200 million people infected with *S stercoralis* residing in 70 different countries. (Vadlamudi, 2006).

The true prevalence of an *S stercoralis* infection is underestimated because a majority of the cases are sub-clinical (Rose, 2008). There are 53 species of the organism, and the most common infection is due to the species *S stercoralis*. Species such *as S fuelleborni* and *S kellyi* are frequently found in humans living in Africa. *S stercoralis* is most prevalent in warm climates but has the ability to survive in colder climates (Rose, 2008).

There is a high prevalence of S.stercoralis in Brazil, Central America, and Australia. It is endemic in Africa South and Southeast Asia, South America, rural parts of Italy, Papua New Guinea, and the Pacific Islands such as Fiji (Vadlamudi, 2006).Endemic areas in the United States of America are Kentucky, West Virginia, and eastern Tennessee (Rose, 2008).It can cause a number of symptoms in people, principally skin symptoms, abdominal pain, diarrhea and weight loss. In some people, particularly those who require corticosteroids or other immunosuppressive medication, Strongyloides can cause a hyper infection syndrome that can lead to death if untreated.

2.4 Transmission of diarrhea

Diarrhea is caused by infectious organisms, including viruses, bacteria, protozoa, and helminthes transmitted from the stool of one individual to the mouth of another, termed fecal-oral

transmission. Some are well known, others are recently discovered or emerging new agents, and presumably many remain to be identified. Infectious diarrhea is acquired by fecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with fecal matter. With regard to water-borne-diarrhea, transmission patterns occur when in-house water storage facilities or/and water sources are contaminated (corresponding to domestic domain and public domain contamination (Black, 2008).Most of transmission of diarrhea occurs in the domestic domain (Atherly) ,stool to the mouth and in the number of organisms needed to cause infection and illness .According to Curtis V.,Most of, there are four transmission routes that the major infectious agents use to reach human hosts, namely human-to human via the environment; human-to-human multiplying in the environment; human-to-animal-tohuman via the environment; and animal-to-human via the environment. In situations, where faecal contamination of the domestic environment is high, majority of cases of endemic disease probably occur either by human-to-human transmission, or from the human-to-Human transmission of pathogenic agents which have multiplied in the environment (Molbak, 2000).

2.5. Risk Factors for Diarrhea

2.5.1. Environmental and related factors

Broadly recognized risk factors for diarrheal diseases include little or no access to safe water and sanitation, as well as poor hygiene and feces disposal practices at home (Daniels, *et al,* 1990). These and many other factors, such as poor housing and crowding, are intrinsically associated with poverty. Furthermore, poverty usually limits access to health care and restricts appropriate and balanced diets (Daniels, *et al,* 1990).

Diarrhea disease affects rich and poor, old and young, and those in developed and developing countries alike , yet a strong relationship exists between poverty, an unhygienic environment as well as demographic factors. The relationship between environmental factors and the occurrence of diarrhea in households have been addressed in a number of studies. Environmental factors include access to improved water sources, availability of toilet facilities, compound hygiene housing condition and refuse disposal (Woldemichael, 2001).

Some studies have shown that the association between socioeconomic factors such as poor housing, crowded conditions (Woldemichael, 2001), a low income (Molbak, 2000). .and higher rate of diarrhea was statistically significant. As diarrhea is acquired via contaminated water and foods, water related factors are very important determinants of diarrhea occurrence. Poor storage of drinking water (Marjatta, 1994).use of unsafe water sources (Tumwine ,2002)' has been found to be risk factors for more diarrhea occurrence among people.

Sanitation obviously plays a key role in reducing diarrhea (morbidity). Some sanitation factors like, improper disposal household garbage (Aulia, et al, 1994) no existence of latrine or unhygienic toilet (Wijewardene,et.al,1992) increased the risk of diarrhea. Some studies revealed that people not washing hands before meals or after defecation (Curtis, et al, 2000).

Unsafe food storage is associated with risk of diarrhea morbidity human transmission of pathogenic agents which have multiplied in the environment (Molbak, 2000). The association between diarrhea and malnutrition is common in low income societies that the concept of a vicious circle is appealing, with diarrhea leading to malnutrition and malnutrition predisposing to diarrhea

Individuals whose immune system has been weakened by malnutrition are the most vulnerable to diarrhea. Diarrhea can cause significant morbidity in HIV-infected patients and can be due to a multitude of etiologies from infectious pathogens to malignancy to medications. Diarrhea is also an independent predictor of reduced quality of life (Siddiqui ,et al,2012).

2.5.2. The level of CD4 T cells related to diarrheal disease

Immunodeficiency is not only a cause of persistent or chronic diarrhea but also a risk factor for diarrhea. Chronic diarrhea is the major cause of morbidity and death among adults with HIV (Cohen,et al,2001) . Diarrhea is reported in up to 60% of patients with AIDS (National Medical Society, USA). HIV causes progressive depletion of CD4 T cells, which leads to life threatening opportunistic infections during the natural course of the disease (Hogg, et al1999).

Opportunistic infections can occur in up to 40% of people living with CD4 T cells count less than 250 mm (Gallant,et al,1994) Non infectious diarrhea, for the majority of patients, is limited to a period of one to two months (Niyogi, et al, 1994) On the other hand, there are those who

require greater medical attention since low CD4 T cell count (<200 cells/ mm^3) cause greater susceptibility to the infectious forms (De Olvera-silva,et al,2007). In the developing world, HAART is not routinely available for HIV-infected individuals, and diarrheal disease remains highly endemic even for those without HIV (Wanke,et al1999).Chronic diarrheal disease in adults in Africa has been used as a predictor of HIV-seropositivity (Colebunders ,et al,1987) . HIV-infected children are more likely to die with diarrhea than children with diarrhea that is not infected with HIV (Thea ,et al,1993).

In contrast, in resource-rich nations where HAART is widely available, the incidence of infectious causes of diarrhea in HIV-infected patients with low CD4 counts (<200 cells/microL) has declined (Ledergerber,et al,1999). Experience at a single center between 1995 and 1997 found that, while the incidence of diarrhea remained constant, infectious etiologies declined from 53 to 13 percent (Call, 2000).

2.6. Definition of key terms

- Magnitude: - the occurrence of diarrheal disease in HIV-infected individuals due to the related infectious disease.
- Malnutrition: - lack of adequate nourishment on HIV/AIDS patients.
- Hygiene: - cleanliness of personal and environmental hygiene.
- Complication: - health condition of the patients caused by infectious disease including diarrhea.
- Risk factor: - condition (socio-demographic characteristics, environment, behavior) that expose HIV/AIDS patient to be affected by diarrheal disease.
- Socio-demographic factors: - sex, age, occupation, income and education of studied subjects.
- Knowledge about diarrhea disease: - ability to remember and can answer about, preventions, related factors diarrhea disease by answering the interview questions.
- Prevalence: - The number of diarrheal cases at the time of physical examination and interview divided by the total number of patients attending during the study period.
- Occurrence: - the existence/presence of diarrhea and causative agents in the study subjects.

3. Methodology

3.1. Study area and period

The study was conducted in Debre Markos Referral Hospital at Debre Markos town on HIV/AIDS-patient co-infected with diarrheal infectious agents from January 2016 up to the end of April 2016. Debre Markos town is the capital city of East Gojjam Zone, Northwest Ethiopia. It is located 300 km away from Addis Ababa, the capital city of Ethiopia, and 265 km from Bahir Dar, the capital city of Amhara regional state.

3.2. Study design

The design of the study was purposive sampling to determine the magnitude of diarrheal disease with HIV/AIDS infected individuals. Hence, the research basically focused on HIV/AIDS infected individuals and attending in the ART clinic of the Hospital.

3.3. Sampling size and Sampling method

To determine the sample size of this study, 95% confidence interval was used preferably; P from the studies with similar study design and study population from the most recent studies could be most preferable (Cochran, 1977). Because of the lack of similar study a sample was made as P equal to 0.5 to yield the maximum sample size. The following simple formula, (Daniel, 1999).can be used.

$$n = \frac{Z^2 * p(1-p)}{d^2}$$

Where, n= sample siz

Z is statistic for a level of confidence

P is expected prevalence or proportion of one; if 50%, p=0.5)

d is precision (in proportion of one; if 5%, d=0.05)

Z statistic (z) for the level of confidence of 95% which is conventional, z value is 1.96.

Therefore,

$$n = \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2} = 384$$

Hence, the total sample size were become 384.

The samples were taken from the maximum number of 4,000 HIV/AIDS patients who have been taking antiretroviral therapy. From this large numbers of HIV-infected outpatients a minimum of 15 attended in the clinic with opportunistic infections, including diarrheal cases. From the start of the experiment patients with complications were randomly selected until the sampling size reached the required number (384).The whole information was collected during the examination time at the clinic, from the laboratory result and other data through questionnaire and interview by assigned trained health officers and the researcher.

3.4 Methods of data collection

3.4.1. Collection of stool samples

Stool samples were collected using disposable plastic cups and sticks from the study participants along with brief instruction on how to collect the stool. They were instructed to bring

Sizable fresh stool sample of their own. Each plastic cups was labeled with codes of patients. The stool samples were carried to the laboratory for parasitic examination.

3.4.2. Laboratory diagnosis of stool samples

Fresh stool was collected from diarrheal cases(127 patients) using disposable screw cup .A direct wet mount of stool in normal saline (0.85% Nacl solution) was prepared and was examined for the presence of motile intestinal parasites and trophozoites under light microscope (10x and 40 x magnification). The uninfected normal saline was used for routine examination of stools as it is isotonic with living organisms in order to observe blood cells, motile forms of micro organisms. Lugo's iodine staining was used to detect ova and cyst of intestinal parasites.

3.4. 3 Socio demographic data collection

Among diarrheal cases, 36(28.35%) in January, 38(29.92%) cases in February, 33(25.98 %) in March and 20(15.75%) were recruited in April Among 127 cases, 36(28.35%) in January, 38(29.92%) cases in February, 33(25.98 %) in March and 20(15.75%) were recruited in April respectively. Relevant parasite infections data were collected by inspecting the patient's health record and analyzing individual patient cards. This analysis was used to collect appropriate information about characteristics of the study patients, such as age, status of diarrhea, residence and economical status and result of stool examination.

3.4.4. Questionnaire and interview

After diarrheal and non-diarrheal study groups were identified, questionnaire and interview was conducted on both groups in order to assess the potential risk factors for diarrhea. The questionnaire interviews were taken from Senait Esayas(2014) and modified .

3.5. Data analysis method

Data entry and analysis was performed using SPSS software. Statistical analysis was done using Chi-square to evaluate any association between risk factors for diarrhea. Bivariate and multivariate analyses were used to see the relationship of risk factors to diarrhea.

The magnitude and related risk factors for diarrhea were calculated from the outpatients attending the ART clinic of the hospital. Whether the factors affect the patients significantly or not the researcher used chi-square to assess which factors predominantly affect the patients in the study area. The other data (demographic data) were analyzed with descriptive statistics with percentage whereas data obtained from interview were qualitatively analyzed.

3.6. Ethical clearance

The ethical clearance was received from the ethical committee of the college of Natural Science, AAU. The health workers were oriented about the confidentiality of the data and all data were used only for the purpose of this research.

3.7. Significance of the study

This study would provide medical practitioners and other stakeholders a better understanding about the magnitude of diarrheal disease in HIV-infected patients still who are taking ART.

4. Results and Discussion

4.1 Laboratory diagnosis of faecal samples (diarrhea)

A total of 384 HIV/AIDS infected patients were selected in to the study after meeting the inclusion criteria. Interviews based on questionnaire were conducted with 384 sample population.

Patients with diarrhea (127 cases) stool samples were collected and examined with direct microscope laboratory to identify patients' diarrhea and causative agents. The others with different complaints were non-diarrheal study groups (257).

Table 4.1. Types of diarrhea detected on diarrhearic-patients.

Types of diarrhea	Male	Female	Total
Bloody	8	12	20(16%)
Non bloody(loose bowel) diarrhea	30	27	57(45%)
Watery diarrhea	21	29	50(39%)

As stool examination indicated that of the sample characterized by 50(39%)watery diarrhea followed by non-bloody(loose bowel) 57(45%) and bloody diarrhea(10%).

Diarrhea which was bloody and watery was caused by bacteria, Giardia and amoeba spp. Although the bacteria species were not identified, the bacteria that cause such bloody diarrhea might be due to shigella and E.coli or other Bacteria spp.

The pathogens of the diarrhea were detected after the examinations of faecal samples of the diaharreal patients. (Table 4.2.). Accordingly, 41(32.3%) of the diarrhea showed high prevalence of E*, histolytica / dispar* followed by Taenia saginata, 30(23.62%) and Bacteria species 22 (17.32%), *A. lumbricoides*, 14(11.02%), and the others *Strongloides stercoralis*

8(6.3%), *Giardia. Lamblia* 5(3.93%), pinworm (*Enterobius vermecularis,* 5(3.93%) and hookworm (Ancylostoma duodenale/N. Americans) 2 (1.57%).

Table 4.2.Diagnosis of the pathogens from stool samples of HIV-infected outpatients from Debre Markos Referal Hospital, ART clinic, taken from January up to end of April, 2016,

	Sex		Total
Pathogens	**Male**	**Female**	
E, histolytica/dispar	20	21	41(32.3%)
Taenia saginata	20	10	30(23.62%)
Bacteria species	7	15	22(17.32%)
S.stercoralis	5	3	8 (6.3%)
G. lamblia	1	4	5(3.93%)
A. lumbricoides	3	11	14(11.02%)
Pinworm/ E.vermicuralis	1	4	5(3.93%)
Hookworm/**Ancylostoma duodenale**/*N.americanus*	2	0	2(1.57%)
Total	**59**	**68**	**127**

Table 4.2. showed the prevalence of diarrheal diseases in relation to their causative agents. Accordingly, the most frequently encountered causative agent of diarrhea in this study was E. histolytica/dispar 41(32.3%).The prevalence of this causative agent in this study was higher than all previous studies from Debre Markos, (14.3%) (Hiwotie, 2014), Southeast of Lake Langano (12.7%)(Mengistu and Berhanu, 2004), from Jimma (17.1%) (Amare et al 2007), but much lower than from the study in Gondar (1.6%) (Afewerk et al, 2008).

The prevalence of *Taenia saginata* (23.62%) in this study was higher than the prevalence 13.9% reported from rural areas close to south east lake Langano ,Ethiopia, (Mengistu and Berhanu, 2004), and .the prevalence (1.1%)from Nigeria (Akinbo et al, 2010).

Similarly, the prevalence of *Strongloides .stercoralis* (6.3%) was higher than the study reported from north west of Ethiopia (1.6%) by Abebe et al. (2011), previous study from Debre markos(2.6%) (Howotie, 2014), Gondar (5.5%) (Afework et al, 2008), South east langano(5.8%) (Mengistu and Berhanu, 2004), but much lower than reported from Jimma (17.5%) by Amare et al (2007).

With regard to the prevalence of giardiasis, 3.93% of the stool samples contained *Girdia lamblia* which was much lower than the previous work from Debre markos (11.7%),Hiwotie,2014), Jimma (13.9%) by Amare,et al,2004, and Southeast Langano (6.2%), but higher than the prevalence(0.8%) in Gondar by Afework,et al (2008).(Table 4.3).

In this study hook worm spp. were detected (1.57%) of the patients which was higher than the prevalence in Gondar (0.8%), Jimma (1.1%) and much lower than the previous work from Debre markos (16.1%)(Hiwotie,2014),and Southeast Langano (60.2%) (Table 4.3). The prevalence of *Enterobius vermicularis* in this study was (3.93% which was higher than reported from South east Langano (2.7%) (Mengisru and Berhanu, 2004).

The data also showed 17.32% of the faecal samples of Bacteria spp. Which is higher than reported from Gondar (3.1%) by Afework et al(2008). This indicted that one or more Bacteria growth could be implicated with the disease.

With regard to prevalence of A.lumbricoides(11.02%) in this study lower than reported from Gondar (18.11%) by Afework et al (2008), but higher than reported from Southeast of Langano(6.2%) by mengistu and Berhanu(2004) (Table 4.3).

Higher or lower prevalence of different causative agents of this study in comparison with other studies might be due to different environmental conditions or study subjects.

Table - 4.3. Pathogens of diarrhea from studies from different study sites.

Disease	Pathogens	Study Area	Site	Prevalence	Reference
Amoebiasis	*E. histolytica/dispar*	Ethiopia	Debre markos	32.3%)	This study
		Ethiopia	Gondar	1.6%	Afework et al, 2008
			D. Markos	14.3%	Hiwotie, 2014
			Jimma	17.1%	Amare et al, 2007
Bacterial	*Bacteria spp.*	Ethiopia	Debre Markos	17.32	This study
		Ethiopia	Gondar	3.1%	Afework et al,2008
Taeniasis	*T. saginata*	Ethiopia	D /markos	23.62%	This study
		Ethiopia	SE Langano	13.9	Mengistu and Birhanu, 2004
		Nigeria	Benin City	1.1%	Akinbo et al, 2010
Strogylodiasis	*S. stercoralis*		D/Markos	6.3%	This study
		Ethiopia	D. Markos	2.6%	Hiwotie, 2014
			Gondar	5.5%	Afework et al, 2008
			Jimma	17.5%	Amare et al, 2007
Giardiasis	*Giardsia lamblia*	Ethiopia	This study	3.93%)	This study
			D. Markos	11.7%	Hiwotie, 2014
			Jimma	13.9%	Amare et al, 2007
Pinworm	*E. vermicularis*	Ethiopia	Debre Markos	3.93%	This study
			SE of Langano	2.7%	**Mengistu &Berhanu**
Hook worm	*A. duodenale/N. americanus*	Ethiopia	D/ Markos	1.57%)	This study
			Gondar	0.8%	Afework et al, 2008
			D. Markos	16.1%	Hiwotie, 2014
			SE of Langano	60.2%	Mengistu & Berhanu, 2004
Ascariasis	A.lumbricodes	Ethiopia	Debre Markos	11.02%	This study
			Gondar	18.11%	Afework et al, 2008

Table 4.4- The Prevalence of Intestinal parasites in Relation to Diarrhea with respect to Sex and Age in the HIV –diarrheal-patients, ART clinic, Debre marckos Hospital.

Sex	Age Groups			
	5-14	15-24	25-34	≥35
Male	8(6.3%)	13(10.23%)	18(14.17%)	20(15.75%)
Female	9(7.1%)	23(18.11%)	19(14.96%)	17(13.38%)
Total	17(13.4%)	36(28.34%)	37(29.13%)	37(29.13%)

The prevalence of diarrhea in these HIV-patients was 33.1% which is lower than reported (59.4%) by Hiwotie(2014) in the same study site. Amongst these patients 46.6% were males and 53.4% were females. This showed that females were more prevalent than males.

The prevalence of intestinal infectious agents in relation to diarrhea amongst HIV-patients in the age group 5-14 years old was 13.4%, in the age group 15-24 years was 28.34%.in the age group 25-34 and 35 and above was 29.13% . This showed that the prevalence of intestinal parasites in relation to diarrhea was higher in the age groups 25-34yeras old and 35 and above than other age groups. The reason might be due to higher number patients aged from 25-34 and 35 and above 74(58.26%).

In addition to the other risk factors associated with diarrhea low CD_4 cell/µl counts was another risk factor to be considered. In this study the CD4 T cells/µl count was identified from diarrhea and non-diarrheal patients

As indicated from table 4.5, the CD_4 cell/µl counts of the diarrheal cases 250 cell/µl and below were 60.62% and from non-diarrheal patients was 18.68%. This result indicated that the CD_4 Cells/µl counts 250 and below in diarrheal patients was higher than the non-diarrheal patients. This showed that low CD_4 cell/µl counts was a risk factor associated with diarrhea.

Table- 4.5- The CD4 cells/µl counts identified from diarrhea and non-diarrheal patients.

CD4 T cells count/µl	Diarrheal Patients		Total	Female: male ratio	Non-diarrheal Patients		Female male ratio
	Male	Female			Male	Female	
≤100	12 (9.45%)	15 (11.81%)	21.26 %	1.25	6 (2.3%)	5 (1.95)	0.8
101-250	22 (17.32%)	28 (22.04%)	39.36 %	1.27	16 (6.22%)	21 (8.17%)	1.3
≥250	25 (19.68%)	25 (19.68%)	39.36 %	1	63 (24.51%)	146 (56.80%)	2.31

Table 4.5.:- Shows that 39.37%) of the diarrheal patients had CD4 count more than 250; whereas 39.37% and 21.26% had CD4 counts of 101-250 and <100, respectively. The female: male CD4 count ratio showed 1.27 and 1 within the higher CD4 counts of 101-250; and 250, except that the female –male ratio was 1.25 (higher counts in female than male) within the CD4 count of <100 (Table-4.5). HIV causes progressive depletion of CD4 T cells, which leads to life threatening opportunistic infections during the natural course of the disease (Hogg, et al 1999). Opportunistic infections can occur in up to 40% of people living with CD4 T cells count less than 250 mm (Gallant, et al, 1994).

4.2 Demographic and social characteristics

Geographical distribution of patients with diarrhea (Urban and Rural), 90 (70.87%) were living in urban and 37(29.13%) were living in rural areas. Table 4,6. Showed that of the total 127diarrheal cases 59(46.46 %) and 68 (53.54%) were male and female patients, respectively.

Concerning age of the participants 13.38% of diarrheal-patients and 3.5% of the non-diarrheal-patients were 5-14 years old ,and 16.53% of diarrheal-patients ,and 11.67% of the non-diarrheal-patients were 15-24 years old ,24.4% of diarrheal-patients and 12.84% non-diarrheal-patients were 25-34 years old and the rest 45.66% of diarrheal-patients and 60.31% 0f the non-diarrheal-patients were 35 and above years old.

From 384 patients selected to the study 47(37.00%) of diarrheal-patients 76(29.57%) of non-diarrheal patients were illiterate. 20(15.74%) ofdiarrhealpatients and 25(9.73% of the non-

diarrheal ones had primary education. The rest 60(42.24%) of the diarrheal-patients and 156(60.7%) of non-diarrheal patients had secondary and above education.

76(29.57 %) of non-diarrheal patients and 47(37. %) of diarrhearic-cases were illiterate. 20(15.74%) of diarrheal-cases and 25(9.73%) in non-diarrheal patients had primary education. The rest 60(42.24%) in diarrheal-cases and 156(60.7. %) in non-diarrheal patients had secondary and above education.

With regard to economic status, monthly income of diarrheal-patients was 48 (37.8%) ≤500 birr. 47(37.01%) 501-1000 birr. 21(16.54%) 1001-1500 and 14(11.02%) 1500 birr and above respectively, whereas monthly income of non-diarrheal patients was 16(6.23) ≤500 birr, 85. (33.1%) 501-1000 birr, 34(13.3.23%) 1001-1500 birr and 122(47.47%) 1500 birr. (table 4.6)

Table 4.6. Demographic and social characteristics of study subjects.

Characteristics	Study groups			
Residence	Diarrheal		Non-diarrheal	
Rural	37(29.13%)		22(8.56)	
Urban	90(70.87%)		235(91.44%)	
Total	127 (30.1)		257 (%)	
Age and Sex	M	F	M	F
5-14	8(6.30 %)	9 (7.09%)	3 (1.17 %)	6(2.34%)
15 -24	7(5.51%)	14(11.02 %)	13(5.06%)	17(6.61%)
25-34	16(12.6%)	15(11.81 %)	18(7.00%)	45(17.51 %)
35 and above	28(22.05%)	30(23.62%)	51(19.84%)	104(40.47 %)
Education				
Illiterate	47 (37.00%)		76(29.57%)	
Primary	20(15.74%)		25(9.73%)	
Secondary and above	60(42.24%)		156(60.7%)	
Monthly income				
≤500	48(37.8%)		16(6.23%)	
501-1000	47(37.00%)		85(33.07%)	
1001-1500	21(16.00%)		34(13.23%)	
1501-and above	11(8.66%)		122(47.47%)	

The data also showed potential risk factors associated with diarrhea, such as education, residence, monthly income (economic status) would be analyzed and discussed in sections of bivariate analyses

4.3. Chi-square analysis of potential risk factors associated with diarrhea.

The Chi-square analysis based on findings that were presented with matched odds ratio (MOR), at 95% level of confidence) it was that discussed factors below table were significantly associated with diarrhea on HIV/AIDS and its complications (Table 4.7) .

Table-4.7.Chi square analysis of risk factors associated with diarrhea among diarrheal-cases and non-diarrheal patients.

Potential risk factors	Diarrhearic (n=127)	Non-diarrhearic (n=257)	Matched odds ratio	(95%ci)	P value
Levels of patients education					
.Illiterate	47(37.00%)	76(29.57%)	2	(0.193-0.275)	<0.001
Illiterate	47	76	ref.		
Primary	20(15.74%)	25(9.73%)	1.3	(0.010-0.007)	<0.001
Secondary and Above	60(42.24%)	156(60.7%)	0.4	(0.993-1.000)	0.5
Poor economic status					<0.001
≤500	48(37.79%)	16(6.23%)	ref.		<0.001
≤500	48	16	9.5	(0.220-0.580)	<0.021
501-1000 1001-1500 1500above	47(37.00%) 21(16.00%) 11(8.66%)	85(33.07%) 34(13.23%) 122(47.47)		(3.140-7.930) (1.060-3.150) (1.106-4.420)	<0.001 0.210 0.326
Having Hand Washing facility					
NO	90(70.86%)	63(24.51%)	7.5	(0.000-0.007)	<0.001
No	90	63	ref.		
Yes	37(29.14%)	194(75.49%)	0.1	(0.467-0.562)	0.2
Do you wash your hands regularly before meal?					
No	60(47.24%)	41(15.95%)	4.7	(0.220-0.580)	<0.001
No	60	41	ref.		
Yes	67(52.75%)	216(84.05%)	0.2	(0.0467-0.562)	0.5
Do you wash your hands regularly after toilet?					
No	97(76.38%)	47(18.29%)	2	(0.221-0.581)	<0.001
No	97	47	ref.		
Yes	30(23.62%)	210(81.71%)	0.1	(0.993-1.000)	0.2

Potential risk factors	Diarrhearic (n=127)	Non-diarrhearic (n=257)	Matched odds ratio	(95%ci)	P value
Use Stored coked food for later					
Yes	75(59.05%)	36(14.00%)	8.9	(0.192-0.275)	<0.001
Yes	75	36	ref.		
No	52(40.95%)	221(86%)	0.1	(0.660-0.745)	0.4
.Eating raw meat ,vegetables					
Yes	80(63%)	43(16.73%)	8.5	(0.000-0.007)	<0.001
Yes	80	43	ref.		
No	47(37%)	214(83.27%)	0.5	(0.660-0.747)	0.1
Lack of enough food					
Yes	83(65.4%)	40(15.56%)	10	(0.994-1.006)	<0.001
Yes	83	43	ref.		
No	44(34.6%)	217(84.44%)	0.1	(0.465-0.562)	0.3
Lack of balanced diet					
Yes	78(61.4%)	39(15.18%)	8.9	(0.661-0.748)	<0.001
Yes	78	39	ref.		
No	49(38.6%)	218(84.82%)	0.1	(0.993-1.000)	0.4
Using unsafe water					
River	22(17.3%)	11(4.28%)	8.1	(0.000-0.007)	<0.001
River	22	11	ref.		
Well	26(20.5%)	7(2.72%)	2	(0.660-0.747)	0.5
Pipe	79(62.2%)	239(92.99%)	0.33	(1.060-3.150)	
Infrequent cleaning /emptying /of container					
Sometimes	86(67.71%)	42(16.34%)	2	(0.001-0.002)	<0.001
Sometimes	86	42	ref.		
Usually	41(32.9 %.)	215(83.66%)	0.1	(0.995-1.000)	0.3
Use Untreated water					
Yes	77(60.63%)	37(14.40%)	9	(0.006-0.032)	<0.001
Yes	77	37	ref.		
No	50(39.37) %	220(85.60%)	0.1	(0.465-0.562)	0.5
Do you know what exposes you for diarrhea ?					
No	74(58.27%)	80(31.13%)	3.1	(0.221-0.580)	<0.001
No	74	80	ref.		
Yes	53(41.73%)	177(68.87%)	0.3	(0.662-0.747)	0.4
Do you know the preventive methods of diarrhea?					
No	72(56.69%)	102(39.7%)	2	(0.062-0.047)	<0.001
No	72	102	ref.		
Yes	55(43.31%)	155(60.3%)	0.5	(0.993-1.000)	0.3

4.3.1. Socio demographic and economic factors

Although, the study area referral hospital is found in urban area there are sub-urban and rural areas around it. Some of cases were from rural areas and the others were from sub urban areas near Debre Markos Referal Hospital as observed in their cards during data collection.

The data showed that 47(37.00%) of the diarrheal-patients and 76(29.57%) of non-diarrheal patients were illiterate, whereas 20(15.74%) of diarrheal-patients and 25(9.73%) of the non-diarrheal ones had primary education. Likewise, 60(42.24%) of the diarrheal-patients and 156(60.7%) of non-diarrheal patients had secondary and above education. Consequently, Comparing with education those illiterate 47(37.00%) in diarrheal-patients had association to diarrhea and the magnitude of diarrhea was high with matched odds ratio 2, at 95% level of confidence and the p –value $\leq$ 0.001.This shows that the risk of diarrhea on those illiterate cases was 2 times greater than cases with primary education, but almost similar (MOR 0.6) with that of Patients with secondary and above education (table 4.3)

Patients should be educated to protect them from diarrhea diseases and other intestinal parasites. If they are illiterate they can't identify correctly the factors that cause diarrhea and unable to protect themselves (Hiwotie Mengstie ,2014).

A study in India amongst HIV-patients, lower education, primary education in diarrheal-patients (71.2%) and in non-diarrheal-patients(37.9%) was significantly associated with diarrhea. This likely due to lower education serving as a proxy for lower socioeconomic status (Marissa,et al,2007).

4.3.2. . Hygiene related factors

In this study irregular hand washing practice 60(47.24.%) and 41(15.95%) was in diarrheal-patients and non-diarrheal-patients respectively . Similarly, Irregulr hand washing practice after toilet 97(76.38) and 47(18.29) in diarrheal-patients and non-diarrheal-patients respectively.This result of irregular hand washing showed higher percentage reported (6.5%) in diarrheal-patients and (8.7%) in non-diarrheal-patients as well as irregular hand washing practice after toilet(7.9%) in diarrheal-patients and (11.5%) in non-diarrheal patients from Karnataka, India(Marissa et al,2007) respectively. This difference may be due to location and environmental conditions.

In this study, the risk of diarrhea among diarrheal patients who had poor hand washing practices before meal was 4.7 times greater than the non diarrheal patients who had paid attention to washing their hands than the diarrhearic ones. Similarly, concerning hand washing practice after toilet patients with diarrhea were 2 times greater than by practicing poor hand washing (table-4.3). Consequently, irregular hand washing before meal showed a risk factor of diaharrea with MOR 4.7 at 95% level of confidence, P≤ 0.001 followed by irregular hand washing after toilet with MOR 2. at 95% level of confidence, P<0.001) (table-4.3).

The study showed that poor hand washing practice before meal and after toilet as well as absence of hand washing facility were associated with risk factors of diarrhea are compatible with some studies so far (Daniels, *et al* ,1990; Amy, et al,2010; Tumwine et al,2002).

4.3.3.. Eating raw meat, vegetables

In Ethiopia, the widespread habit of raw beef consumption is a potential cause for food borne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene (Siddiqui,et al, 2006) . By eating raw meat people can be exposed to intestinal parasitic disease. Concerning eating raw meat and raw vegetables, 80 (63%) in diarrheal-cases and 43 (16.73%) in non-diarrheal patients had the habit of eating raw meat and vegetables. This might be the factor that increased the risk of diarrhea (MOR 8.5; at 95% level of confidence ,P<0.001)(table-4.3).

4.3.4. Food related factors

Concerning food related factors, 75 (59.05%) in diarrheal- cases- and 36(14%) in non- diarrheal-cases were associated with unsafe food storage as risk factors (MOR 8.5; at 95% level of confidence, P<0.001) (table-4.3). with regard to lack of enough food. 83(65.35) in diarrheal-cases 40(15.56%) in non-diarrheal patients did not get enough food (OR=10 at 95% level of confidence, P<0.001). The last food related factor found to increase the risk of diarrheal and magnitude of diarrheal disease was unbalanced diet 78(61.42%) in diarrheal-cases and 39(15.56%) in non-diarrheal patients did not get balanced diet (OR=8.at 95% level of confidence, P<0.001). This shows that diarrheal-cases were associated for diarrhea 8.9 times higher than the non-diarrheal patients. In addition to this, monthly income of most patients with

diarrhea was very low (74.8%) between 500 and 1001 birr. Due to these reasons, it is possiple to realize that most of them lead poor quality of life.

Diarrhea is an independent predictor of reduced quality of life (Siddiqui ,et al,2012). Unsafe food storage is associated with risk of diarrhea morbidity human transmission of pathogenic agents which have multiplied in the environment (Molbak, 2000). The association between diarrhea and malnutrition is common in low income societies that the concept of a vicious circle is appealing, with diarrhea leading to malnutrition and malnutrition predisposing to diarrhea (Molbak, 2000).

4.3.5. Infrequent cleaning (emptying) of container before filling with fresh water.

With regard to cleaning of storage of water container, most 86(67.71. %) diarrheal- cases and 42(16.34%) non-diarrheal-patient cleaned storage of drinking water sometimes. (OR=2, P≤ 0.001). This shows that diarrheal-cases were 2 times higher than non-diarrhearic by infrequent cleaning (emptying) of water container before filling with fresh water (table-4.3).

4.3.6. Water related factors

In the surrounding urban study areas river and well water are available. Water from rivers and wells for drinking and other domestic needs. With respect to using Water from river 22(17.3%), from well 26(20.5%), from pipe 79(62.2%) in dirrheal-patients and from river11 (4.28%), from well 7(2, 72), from pipe 239(92.99%) in non-diarrheal-patients respectively. As a result diarrheal-patients using river water more prevalent 2 times and 3 times than non-diarrheal-patients respectively (table-4.3). This showed that this increased the risk of diarrhea and magnitude of diarrheal disease (OR 2, 95% level of confidence p≤ 0.001) .In this study ,well source for water 20.5% in dirrheal-patients is lower (29.6%) and 2.72% in non-diarrheal patients is lower (21.2%) than in non-diarrheal patients reported from India respectively (Marissa,et al,2007). Likewise, 77(60.62%) in diarreal-cases and 37(14.39%) in non-diarrheal patients used untreated drinking water (OR= at 95% level of confidence, P≤0.001). This showed that patients used untreated water was exposed to diarrhea 9 times higher than the non-diarrheal patients (table-4.7).

4.3.7. Knowledge of factors that expose for diarrhea and preventive methods in diarrheal-cases and non-diarrheal- patients.

From 384 HIV/AIDS patients 74(58.27%) in diarrheal-cases and 80(31.13%) in non-diarrheal patients were linked with lack of knowledge that the activities them expose for diarrhea (MOR=3.1, at 95% confidence interval, p≤0.001). In addition to this 72(56.69%) of diarrheal-cases and 102 (39.70%) of non-diarrheal patients had no knowledge on diarrhea preventive method. (Table-4, 3). Concerning the knowledge of preventive ways and knowing exposing things for diarrhea, non-diarrheal patients had better knowledge in comparison with diarrhearic patients (MOR=2, at 95% confidence interval, p ≤0.001).

5. Conclusion and Recommendations

5.1 Conclusion

In this study 33, 07% of the total 384 outpatients infected with HIV were also affected with diarrhea. After examining 127 stool samples, the most frequent pathogens that were detected, E. histolytic/dipar were high 41(32.3%) followed by Taenia spp 30(23.62%) Bacterial spp, 22(17.32%) and A. lumbricoides 14(11.02%).

The result study showed that the factors, namely low level of education, poor economic status, absence of hand washing facility, irregular hand washing, use stored cooked for later use, eating raw meat and vegetables, lack of balanced diet, unsafe water resource, infrequent cleaning of container, untreated water and poor knowledge of Factors exposing for diarrhea and preventive methods were significantly associated as risk factors of diarrhea on diarrheal cases in the study area. In addition to this CD_4- cell/µl counts of both diarrheal-case (60.62%) and non-diarrheal-patients (18.68%) showed ≤250 CD_4- cell/µl counts respectively. This indicted that low CD_4- cell/µl counts was more associated as risk factor in diarrheal-patients than non-diarrheal-patients.

5.2 Recommendations

Based on the present findings the prevalence of diarrheal disease, pathogens and associated risk factors of HIV/AIDS patients the researcher suggested the following recommendations.

- Measures including education on personal and environmental hygiene should be taken in to account to aware patients about diarrhea causes and preventive methods and to reduce the prevalence.
- Encourage patients to wash their hands before meal and after toilet
- Advice people (patients) to use safe and treated water to avoid water borne diseases.
- Recommend patients to use fresh and cooked food.
- Due to absence of some laboratory materials at the time of this study period some laboratory tests were note performed in study area. Therefore, further study on the prevalence of diarrheal disease and causative agents in HIV-patients is recommended to be done.

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Appendix I

Questionnaires and interviews

The questionnaires and interviews are prepared to conduct a research on a topic magnitude and risk factors of diarrheal diseases among HIV/AIDS patients who have complications attending in D/M/R Hospital. You are selected randomly since you have complication and going to be examined by the doctor to be interviewed and fill the questionnaires.

I received permission from Addis Ababa University College of natural science, Debre Markos Hospital administration and respective clinic of the Hospital.

I would very much appreciate your participation in this interview and response for questionnaires. This research outcome will help for the government and NGO to interfere and solve the problem of HIV/AIDS patients vulnerable to diarrheal diseases and related issues.

I hope you will be participating voluntarily since you are one are one of the victims of HIV.

I assure that the interview and the questionnaire will not bring any harm to you, but advantages what even information you provide will be kept strictly confidential, except to be used for this research. No need to write your name.

- Write "X" for your agreement for the questionnaires.
- Give short and precise answer for the interviews

THANK YOU!!

Personal and related information

1. Sex male ☐ female ☐
2. Age 5-14 ☐ 15-24 ☐ 25-34 ☐ 35 and above ☐
3. Residence urban ☐ rural ☐
4. Educational status illiterate ☐ primary ☐ secondary and above ☐
5. Your family's income per month $\leq$500 ☐ 500-1000 ☐ 1000-1500 ☐ 1500 an above

Hygienic related factors

6. Do you wash your hands before meal? Yes ☐ No☐
7. How often you wash your hands before meal?
   Usually ☐ Sometimes ☐ Never ☐
8. Do you wash your hands after going to latrine? Yes ☐ No☐
9. How often do you wash your hands after going to latrine?
   Usually ☐ Sometimes ☐ Never ☐

Water related factor

10. From where do you get water for drinking? Well☐ pipe☐ river☐ other ☐
11. Do you treat your drinking water? Yes ☐ No ☐
12. How often do you clean (empty) the storage container?
    Usually ☐ sometimes ☐ not at all ☐

Food related factors

13. Do you store cooked food for later use? Yes ☐ No ☐
14. Do you eat raw meat? Yes ☐ No ☐
15. Do you eat raw vegetables such as cabbage, lettuce? yes ☐ No ☐
16. Do you get enough food daily? Yes ☐ No ☐
17. Is it balanced diet? Yes ☐ No ☐

Knowledge of diarrhea

18. Do you know what expose you for diarrheal disease? Yes ☐ No ☐
19. Do you know the preventive methods of diarrhea? Yes ☐ No ☐

I. Data collection sheet after laboratory result of case group

1. Diarrhea- present ☐ no diarrhea (but other disease) ☐
2. Type of diarrhea- bloody ☐ non bloody ☐ Watery diarrhea ☐
3. Causative agent of the diarrhea _____
4. Level of CD4 T-cells _____

የጽሁፍ መጠይቅ /Amharic version/

በአዲስ አበባ ዩኒቨርስቲ ዞሎጂካል ሳይንስ ዲፓርትመንት
በምስራቅ ጎጃም ዞን በደብረ ማረቆስ ከተማ በደብረ ማርቆስ ሪፈራል ሆስፒታል ለሚታከሙ
የአ፵ አይ ቪ ቫይረስ ህሙማን የተዘጋጀ መጠይቅ

ውድ ታካሚዎች

ህሙማን ስለህመም መነሻ ምክንያቶችና ስለህመማቸው ምልክቶች የሚሰጧቸው መረጃዎች የበሽታቸውን መንስኤና የተጋላጭነት ደረጃ ለመለየት ጠቃሚ ናቸው። የዚህ መጠይቅ ዋና ዓላማ በአ፵ አይ ቪ ቫይረስ ተጠቅተውና ህሙማን ሆነው ሌሎች ተጨማሪ የጤና ችግሮች ያሉባቸው ታማሚዎች ለተቅማጥ በሽታ ያላቸው ተጋላጭነትና ለተቅማጥ የሚያጋልጡ መንስአ፵ችን በመለየት የታማሚዎችን የጤና ሁኔታ ለማሻሻል ነው። የጥናቱ ውጤታማነት የሚወሰነው እርስዎ በሚሰጡት መረጃ ትክክለኛ መሆኑን ተገንዘበው ትክክለኛ መረጃ እንዲሰጡ እያስገነዘብኩ የሚሰጡት መልስ ሚስጢራዊነቱ የተጠበቀ ስለሆነ ያለምንም ስጋት መልስዎን እንዲሰጡ እጠይቃለሁ። ውድ ጊዜዎን መስዋት በማድረግ ለዚህ መጠይቅ ስለተባበሩኝ ከልብ አመሰግናለሁ።

አጥኝው

የአሞላል መመሪያ

1. **መጠይቁ ላይ ስም መፃፍ አያስፈልግም**
2. **ለቀረቡት ጥያቄዎች የ " x " ምልክት በመጠቀም መልስዎን ይሰጡ**
3. **አስተያየት ለሚጠይቁ ጥያቄዎች በተሰጠው ክፍት (ባዶ) ቦታ ላይ ሀሳብዎትን ያስፍሩ።**

I. ክፍል አንድ - ግላዊ መረጃ

1. **ፆታ - ወንድ ☐ ሴት ☐**
2. **እድሜ - 5-14 ☐ 15 እና በላይ ☐**
3. **የመኖሪያ ቦታ - ከተማ ☐ ገጠር ☐**
4. **የትምህርት ደረጃ - ማንበብና መፃፍ የማይችል ☐ 1ኛ ደረጃ ያጠናቀቀ/ች ☐**
   2ኛ ደረጃና ከዚያ በላይ ☐

5. የወር ገቢ መጠን - ከ500 ብር በታች ☐ ከ500-1000 ብር ☐ ከ1000-1500 ☐ ከ1500 ብር በላይ ☐

II. <u>ክፍል ሁለት የጤና አጠባበቅ ሁኔታና ሌሎች ተዛማጅ ልምዶች</u>

6. ከምግብ በፊት እጅዎን ይታጠባሉ? አዎ ☐ የለም ☐
7. "አዎ" ከሆነ መልስዎ የመታጠብ ልምድዎት ምን ያህል ነው? ሁል ጊዜ ☐ አብዛኛውን ጊዜ ☐ አልፎ አልፎ ☐
8. ከመፀዳጃ ቤት መልስ እጅዎን ይታጠባሉ? አዎ ☐ አልታጠብም ☐
9. "አዎ" ከሆነ መልስዎ የመታጠብ ልምድዎ እንዴት ነው? ሁል ጊዜ ☐ አብዛኛውንጊዜ ☐አልፎ አልፎ ☐

III. ከውሃ ጋር ተዛማጅነት ያላቸው መጠይቆች

10. ለመጠጥ ውሃ የሚያገኙት ከየት ነው? ከጉድጓድ ☐ከቧንቧ ☐ ከወንዝ ☐
11. ለመጠጥ የሚጠቀሙበትን ውሃ ያክማሉ? አዎ ☐የለም ☐
12. የመጠጥ ውሃ ማጠራቀሚያ እቃዎችን ለምን ያህል ጊዜ ያፀዳሉ (ያጥባሉ)? በሳምንት አንዴ ☐ አልአልፍ ☐ አይፀዳም ☐

IV. ከምግብ ጋር ተዛማጅነት ያላቸው መጠይቆች

13. የተረፈዎትን ምግብ በማስቀመጥ ይጠቀማሉ? አዎ ☐ አልጠቀምም ☐
14. ጥሬ ስጋ ይመገባሉ? አዎ ☐ አልመገብም ☐
15. አትክልቶች /እንደ ጎመን ፣ ሰላጣ/ በጥሬው ይመገባሉ? አዎ ☐ አልመገብም ☐
16. በየቀኑ በቂ ምግብ ያገኛሉ አዎ ☐ የለም ☐
17. "አዎ" ከሆነ መልስዎ የተመጣጠነ ምግብ ነውን? አዎ ☐ አይደለም ☐

ቃለ መጠይቅ /interview/

18. ለተቅማጥ በሽታ የሚያጋልጥ ሁኔታ (መንስኤ) ያውቃሉ?_____

19. የተቅማጥ በሽታን መከላከያ ዘዴዎችን ያውቃሉ?_____

የሰገራ ምርመራ ውጤት:- ካርድ ቁጥር_____

1. ታማሚው ተቅማጥ አለው ☐ የለውም ☐

2. የተቅማጡ ደረጃ ደም የተቀላቀለበት (bloody) ☐

ደም የሌለው (non-bloody) ☐

ቀጭን ተቅማጥ (water diarrhea) ☐

3. ተቅማጡን ያመጣው ተውሳስ _____

_____

4. የCD_4 መጠን_____

1. Introduction

Diarrhea is defined as a passage of three or more loss of watery stool in 24 hours period. It can affect all people in the world in general, and that of in developing countries in particular, due to economic, social and other related factors. It affects more people infected with HIV infection and usually tends to be chronic (Kosek. et al, 2003). Diarrheal disease is also the second leading cause of death in children under five years old, and is responsible for killing around 760 000 children every year. Diarrhea can last several days, and can leave the body without the water and salts that are necessary for survival. Most people who die from diarrhea actually die from severe dehydration and fluid loss.

Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhea. Globally, there are nearly 1.7 billion cases of diarrheal disease every year (WHO, 2013). Of the estimated total 10.6 million deaths among children younger than five years of age worldwide, 42 percent occur in African region (Bryce, et al, 2005). The under-five morbidity rate in Africa region is seven times higher than that in European region. In 1980 this difference was equal to 4.3 times (WHO. 2005). A factor that may contribute to this situation is the HIV/AIDS epidemic and weakness of the implementation capacity of health system in the region likely to balance (Walker, et al, 2002).

HIV infection has added considerably to the burden of diarrheal diseases among adults and children. According to the latest estimates in 2011 from UNAID, globally an approximate of 34 million people were living with HIV (U.S. GHPFS, 2011). Although HIV infections have declined by more than 21% since their peak in 1997 (GHIV/AIDS R 2011). There were about 2.2 million new infections in 2011, 500,000 fewer than the 2001(JUNP, 2012).

Since the beginning of epidemic, nearly 30 million people have died of AIDS - related causes (UNAIDS, 2012). In 2011 the annual number of people dying from AIDS – related causes worldwide was estimated 1.7 million (JUNP HIV/AIDS 2012). At the end of 2010 about 22.9 million which is 67% of those living with HIV/AIDS globally are in Africa though only about 12% of the world population lives in the region (GHIV/AIDS R, 2011). Africa region represents about 79% of AIDS mortality globally (Edward .et al, 2006), the estimated mortality from AIDS-related illnesses at end of 2010 are 1.2 million (G HIV/AIDS, 2011).

Ethiopia is one of the African countries of with high HIV prevalence. According to the recent Ethiopia Demographic and Health Survey (EDHS), the number of people living with HIV in the country was estimated at 769,000 of whom 458,100 (59.5%) 311,500(40.5%) were females and males respectively (FHIV/AIDS PCO, 2014). People living with HIV in the country (2%) more lower than some African countries such as U ganda (4%), Kenya (5%), Nigeria (9%) and South Africa (18%) (UNAIDS, 2013). Amongst the region the prevalence of HIV in Amhara region was 2.2% (CSA, 2011).

Diarrhea is one of the most common AIDS-related illness causing a significant morbidity and mortality in HIV-infected patients (Siddiqui, et al, 2012). Reports indicated that diarrhea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in developing countries (Framm and Soave, 1997).

The etiologic spectrum of enteric pathogens causing diarrhea includes bacteria, parasites, fungi and viruses (Mitra et al, 2001), though that of parasitic origin is prominent in patients with AIDS in developing countries (Cimerman, et al., 1999). However, with the progressive introduction of Highly Active Antiretroviral Therapy (HAART), modifications have been observed in the morbidity and mortality profile among HIV/AIDS patients; reflecting the reduced occurrence of opportunistic infections, including those caused by enteric parasites (Willemot, et al, 2004).

According to Amhara national region state, Health Bureau of East Gojjam Health Department, people who were recorded affected by diarrheal disease in the zone were 75,400 in the year 2015.

Though Hiwotie (2014) conducted a study on the prevalence of intestinal parasitic infections among people with and without HIV/AIDS infections of their association with diarrhea, the work on co-infection of diarrheal diseases and HIV is limited.

The present study is therefore, aimed to determine the prevalece of diarrheal disease in HIV-infected individuals and risk factors among HIV-patients in ART Clinic of Debre Markos Hospital.

1.1. Statement of the problem

Diarrhea, one of the most prevalent diseases in children under five years and top ten diseases for most young and adult people in the study area. Specially, on HIV/AIDS-infected people, diarrhea is a serious health problem. Knowledge of the specific pathogens that cause diarrheal diseases in HIV-infected individuals and their magnitude in the area is critical for the implementation of specific intervention strategies. The type of diarrheal diseases and the pathogens as well as associated risk factors should be identified in order to apply the proper intervention to prevent such infectious diseases. Therefore, to achieve this possibility it is required to know the magnitude of diarrhea and associated risk factors in HIV-patients.

1.2. General objective

The research is aimed to identify and describe the prevalence and type of diarrheal diseases and associated risk factors that affect HIV-infected individuals, in particular and assessment of socio-demographic characteristics and other risk factors related to such diseases.

1.3. Specific objective of this study

- To determine the prevalence and type of diarrhea disease in HIV-infected individuals attending in the antiretroviral therapy clinic of Debre Markos Referral Hospital until the end of April 2016.

  To characterize socio-demographic characters of the study subjects
- To identify associated risk factors related to diarrheal disease in HIV infected individuals in ART Clinic of Debre Markos Referal Hospital until the end of April 2016.

2. Literature Review

2.1. Definition of diarrhea

Diarrhea is defined by the World Health Organization (WHO, 2009) as three or more watery or loose bowel movements in a 24 hour period .It is caused by bacterial, viral, and parasitic organisms and is usually a symptom of gastrointestinal infection (*WHO , 2013).* However, the population may claim they have diarrhea when they, in fact, have other problems, such as fecal impaction (Schiller, (2009). Therefore when evaluating a person with complaints of diarrhea, it is imperative to closely question the patient about associated signs and Symptoms (Schiller, (2009).

Almost everyone has become ill of, or would be affected by diarrhea at some point in their lives. Diarrhea can occur as a symptom of many different illnesses, as a side effect of some drugs or may be due to anxiety among other things. Diarrhea results from an imbalance in the absorption and secretion properties of the intestinal tract; if absorption decreases or secretion increases beyond normal, diarrhea results. It can range in severity from an acute, self-limited annoyance to a severe, life-threatening illness. .

The definition of diarrhea depends on what is normal for the individual. For some, diarrhea can be as little as one loose stool per day. Others may have three daily bowel movements normally and not be having what they consider diarrhea. According to K. Armon, diarrhea is defined as a change in bowel habit for an individual resulting in substantially more frequent and/or looser stools (Armon, 2001). Although changes in frequency of bowel movements and looseness of stools can vary independently of each other, changes usually occur in both.

Clinical features vary greatly depending on the cause, duration, and severity of the diarrhea, on the area of bowel affected, and on the patient's general health. Diarrhea is the most serious public health problem connected to water and sanitation and can be both ―waterborne" and ―water-washed". In recent decades, a consensus developed that the key factors for the prevention of diarrhea are sanitation, personal hygiene, availability of water and good quality drinking water; and that the quantity of water that people have available for hygiene is of equal or greater

Importance for the prevention of diarrhea as the bacteriological water quality (Jensen, et al, 2004).

2.2. Type of Diarrhea

Diarrhea may be classified into four general types, based on the mechanism, including osmotic diarrhea, secretory diarrhea, exudative diarrhea, and motility disorder diarrhea (Brooks, et al.2003). According to(WHO,1988),(Vesikari ,et al,1994), based on clinical syndromes, diarrhea could be classified into four types, each reflecting a different pathogenesis, including acute watery diarrhea, dysentery, persistent or prolonged diarrhea and chronic diarrhea

2.2.1 Acute watery diarrhea

This term refers to diarrhea characterized by abrupt onset of frequent, watery, loose stools without visible blood, lasting less than two weeks. Usually, acute watery diarrhea episodes subside within 72 hours of onset. It may be accompanied by flatulence, malaise and abdominal pain. Nausea, vomiting may occur and also fever may be present. The common causes of acute watery diarrhea are viral, bacterial, and parasitic infections. Bacteria also can cause acute food poisoning. The enteric pathogens causing this diarrhea in developing countries are largely the same that are encountered in developed countries, but their proportions are different (Jay, 2013).

In general, bacterial pathogens are more important in countries with poor hygienic conditions. The most important causes of this diarrhea in developing countries are Rotavirus, Shigellae, enterotoxigenic E.coli (ETEC), Vibriocholerae, *Campylobacter jejuni*, *enteropathogenic* E. coli (EPEC), *Salmonella spp*. and *Cryptosporidium* (Vesikari, et al, 1994).

Diarrhea can be classified by several methods with duration of the symptom being foremost. Diarrhea lasting less than 2 weeks is considered acute (Hall, 2010). This phenomenon is most likely caused by an infectious agent, such as bacterial, parasitic or viral invasion, or by a non-infectious agent such as dietary indiscretion or a new medication (Amerine,et al, 2006). Acute diarrhea is typically self limiting and resolves quickly with no lasting sequelae (Amerine,et al, 2006). Infectious agents are one of the factors associated with acute diarrhea. Some of these pathogens can cause an inflammatory response in the gut where the epithelial lining is damaged either by a toxin produced by the organism or by an organism invading the mucosa (Bliss, et.al 2006).Some organisms that cause an inflammatory response are –Cytomegalovirus, Herpes simplex virus, *Shigella, Salmonella, Chlamydia, Nisseria gonorrheae, Campylobacter jejuni,*

Clostridium difficile, Escherichia coli O157:H5, *Entamboeba histolytic"*(Bliss, et.al 2006). Symptoms of acute inflammatory diarrhea include fever (higher than 38.5^{C}), lethargy, and a stool that contains pus, blood, leukocytes and/or mucus (Bliss, et.al 2006).

There are organisms that cause acute diarrhea that do not produce an inflammatory response although the person may have a low grade fever, malaise, nausea and vomiting as well as diarrhea. These causative organisms include –Norwalk virus, Rotavirus, *Staphylococcus aurous, Clostridium perfringens, Vibrio cholera* and enterotoxigenic *Escherichia coli …"* (Bliss, et.al 2006).Less commonly, protozoa such *Guardia* or *Cryptosporidium* may be the causative factor (Amerine, et al, 2006).

The most dangerous complication is dehydration that occurs when there is excessive loss of fluids and minerals (electrolytes) from the body. With vomiting, dehydration becomes more severe. Dehydration is especially dangerous in infants and young children due to rapid body water turnover, high body water content and relatively larger body surface (Molbak, 2000). Patients with mild dehydration may experience only thirst and dry mouth. Moderate to severe dehydration may cause orthostatic hypotension with syncope (fainting upon standing due to a reduced volume of blood, which causes a drop in blood pressure upon standing), a diminished urine output, severe weakness, shock, kidney failure, confusion, acidosis (too much acid in the blood), and coma.

2.2.2. Dysentery

May simply be defined as diarrhea containing blood and mucus in feces. The illness also includes abdominal cramps, fever and rectal pain. The most important cause of bloody diarrhea is Shigella (Abraham Haileamlak, 2005).

2.2.3. Persistent diarrhea

Diarrhea lasting longer than two weeks but resolving within a month is known as persistent diarrhea (Bushen, et al,2003). This is typically a slower to resolve infection or continuing use of an offending agent (Amerine, et al, 2006). Persistent diarrhea is defined as diarrheal episodes of presumed infectious a etiology that have an unusually long duration and last at least 14 days (Jay, 2013). The episode may begin acutely either as watery diarrhea or dysentery. This diarrhea

causes substantial weight loss in most patients. It may be responsible for about one-third to half of all diarrhea-related deaths. Since persistent diarrhea is a major cause of malnutrition in the developing countries, even the milder, non-fatal episodes contribute to the overall high mortality rates that are frequently associated with malnutrition in these countries. The pathogenesis of persistent diarrhea is not fully known. Several causes, probably in combination, include: infections with entero aggregative E. coli (EAggEC), EPEC and Cryptosporidium; intolerance to foods; delayed recovery of intestinal mucosal damage due to protein-energy malnutrition or Vitamin A or zinc15 deficiency; immunodeficiency (with the exception of Acquired Immune Deficiency Syndrome - AIDS causing chronic diarrhea); and inappropriate use of antibiotics (Vesikari, et al,1994).

Diarrhea lasting longer than two weeks but resolving within a month is known as persistent diarrhea (Bushen, et al,2003) . This is typically a slower to resolve infection or continuing use of an offending agent (Amerine, et al, 2006).

2.2.4. Chronic diarrhea

On the other hand, lasts longer than four weeks (Bliss, et.al 2006). .Approximately 3%-5% of the American population is thought to suffer from chronic diarrhea during any given period of time (Schiller, 2009). Chronic diarrhea can be the result of disease processes, medication, genetic abnormalities, or a variety of other causes (Marchiondo, 2009). The term chronic diarrhea refers to diarrhea which is recurrent or long lasting due to mainly non-infectious causes. Chronic diarrhea may be caused by gastrointestinal disease, may be secondary to systemic disease, and may be psychogenic in nature (Jay, 2013). Path physiologically, chronic diarrhea may be categorized as inflammatory diarrhea (caused by regional enteritis, ulcerative colitis), osmotic or mal-absorptive diarrhea (resulted from lactose intolerance, tropical spruce, celiac disease, Whipple's disease, chronic pancreatitis, bile duct obstruction), secretary diarrhea is caused by medications, bowel resection, mucosal disease), dysmotility diarrhea (caused by conditions such as diabetic neuropathy or irritable bowel syndrome) and factitious (self-induced, e.g., from laxative abuse) diarrhea (Armon, et al,2001).

Secretory diarrhea occurs when there is an increase in the amount of fluid being drawn into the lumen of the bowel such that the ability of the intestines to reabsorb is overwhelmed (Bliss, et al

2006).Typically, infectious agents are the cause of secretory diarrhea but any substance (secretagogue) that causes fluid to be pulled into the bowel can be the culprit (Strasinger, et al,(2008). Infectious secretagogues include *Vibrio cholerae, E. coli, Camylobacter jejuni, Salmonella, Shigella,* and *Clostridium difficile* (Farthing, 2006).

These pathogens secrete toxins that bind with the structures within the gut, altering, sometimes irreversibly, the amount of fluid secreted into the bowel (Bliss, et.al 2006). As an example, the toxin excreted by the pathogen cholera causes massive secretory diarrhea which, during its acute phase, can be as much as 24 liters in 24 hours(Farthing,2006).Non-infectious secretagogues include chemicals produced by certain types of cancer, prostaglandins produced in patients with bowel inflammation and substances not well absorbed such as fatty acids and bile acid(Bliss, et.al 2006). Persons with secretory diarrhea typically had stool volume of more than one litter daily, with neutral pH and have no change in the amount of stool produced with fasting (Bliss, et.al 2006).

2.3. HIV/AIDS - related diarrheal disease and pathogens

HIV is the virus that causes AIDS by weakening the natural immune system. HIV causes progressive depletion of the CD4 T cells, which leads to life threatening opportunistic infection or malignancies during the natural course of disease (Miller, et al,1999). More than 90% of opportunistic infections are responsible for the development of AIDS morbidity and mortalities (Federal HIV/AIDS Prevention and Control Office, 2014).

The risk for the development of opportunistic infections in HIV- infected individuals depends on exposure to potential pathogens, virulence of pathogens, the degree of host immunity and the use of antimicrobial prophylaxis (Chaisson, et al, 1997).

Diarrhea, which means two or more loose or unformed stools per day, can last for a few days or can be chronic lasting for weeks or months. Many people with AIDS have diarrhea that persists for more than a month or is so severe they expel several gallons of fluid a day (NIAID, 1995). The diarrhea may be accompanied by abdominal cramps, nausea, vomiting, fever, and blood in the stool or weakness (NIAID, 1995).

Diarrhea disease in HIV-infected patient population has been demonstrated to be associated with a significant decrease in quality of life, whatever the etiology of the diarrheal illness might be (Watson, et al, 1996). Multiple infectious pathogens including bacteria, mycobacterium, viruses, and parasites have been implicated as causes of diarrheal illness in patients infected with HIV (Mayer et al., 1994). The etiology of diarrheal disease appears to vary by geographical region (Malebranche, et al, 1983). In patients with HIV in Africa, diarrheal disease and wasting are a more common complication of HIV infection than they are in United States and Western Europe. A study in Uganda reported the commonest causes of diarrhea to be helminthic infections (24.5%), bacterial infections (19.2%) and protozoal infections (9.2%) (Binka,et al, 2002) . Enteric viruses have also been reported associated with diarrhea (Grohmannp, et al, 1993).

Soil transmitted helminthes are endemic in sub-Saharan Africa. A few studies showed significant association between HIV and geohelminthic infections though this association was not consistent for all helminthes. A study in Honduras found a strong association between Strongloides sterocalis and HIV infection and lower risk for infections of Giardia lamblia. Ascaris lumbricoides and Trichuris trichiurias (Lindo,et al, 1998). Another study found a high prevalence

Of Giardiasis and strongloidias is among HIV-positive patients in Brazil (Feitosa, et al, 2001) . A similar study in Ethiopia also found higher prevalence of strongyloidiasis among HIV-patients with CD4 count less than 200 cell/ µL (Assefa, et al, 2009).

The most common infectious organisms causing AIDS-related diarrhea include cytomegalovirus (CMU), the parasites cryptosporidium, microsporidia and Giardia lamblia and the bacterium mycobacterium avium-intracellulare (MAC). Other bacteria and parasites that cause diarrheal symptoms in otherwise healthy people may cause more severe, prolonged or recurrent diarrheal disease in people with HIV or AIDS (NIAID, 1995).

Diarrheal diseases also have non-infectious causes. These include antibiotics or other drugs that can destroy beneficial bacteria in the intestines, HIV- entropath, which is disease caused by HIV itself, cancers such as non-Hodgkin's lymphoma or kaposi's sarcoma, and lactose intolerance, irritable bowel or other gastrointestinal disorders. Sometimes diarrhea can stem from a lack of stomach acids, a condition that is common people with AIDS (NIAID, 1995).

According to Health Bureau of East Gojjam Health Department report in the year 2015, people with diarrheal disease and diarrhea with blood were reported from the woredas. Accordingly, in Debre Markos woreda (town), people above 5 years old who were affected by diarrhea have been reported. The report, for non-bloody diarrhea, males 1407, females 702, diarrhea with watery diarrhea, males 100, females 126, diarrhea with blood (dysentery), males 299 and females, 222 which totally make up males 1806 and females 1050 patients. Therefore, the prevalence of diarrhea disease in the town was high in which a total of 2856 diarrheal disease patients were recorded in which HIV infected individuals were included.

Though some diarrheas are due to errors of metabolism, chemical irritation or organic disturbance, the vast majority are caused by infectious pathogens (Gracey ,1985).

2.3.1 Bacterial infections:

Diarrhea caused by enteric bacterial infections is very important worldwide, especially in tropical and developing countries, and is a serious problem among older children and adults as well as in infants and young children. The range of causative micro organisms is very large; they include E. coli, Salmonella, Shigella, Campylobacter, Yersinia, vibrios, and *Clostridium difficile (Gracey, 1996).*

2.3.2. Viral infections:

Rotavirus is one of the most common causes of severe diarrhea. Other viruses may be important causes of diarrheal disease in human, includingNorwalk virus, Norwalk-like viruses, enteric adenoviruses, caliciviruses, and astroviruses (Gracey, 1996).

2.3.3 Parasites

Parasites can enter the body through food or water and settle in the digestive system. Parasites that cause diarrhea include Giardia lamblia, Entamoeba histolytica, Cyclosporacayetanensis and Cryptosporidium (WHO. 2005).

A high prevalence of intestinal infectious has been reported in HIV-positive patients, in the form of diarrhea associated with parasitosis (Wafa.2010). The intestinal parasites frequently

Encountered in Ethiopia include: *Cryptosporidium* species, *Isospora belli*, *Microsporidia* spp, Entamoeba histolytica *Giardia lamblia,* Tri*churistrichiura*, Ascarislumbricodies, *Stongyloides stercoralis*, hookworms and *Teania* spp.(Hailemeriam,et al,2004). The main clinical manifestation of the parasitic infections is diarrhea. The decline of mucosal immunologic defense mechanisms predisposes patients to acute, intermediate, or late gastrointestinal manifestations such as diarrhea (Haileyesus Adamu and Beyene Petors. 2009).

Diarrhea has been reported to be associated with HIV- infected individuals caused by opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non- opportunistic agents that usually cause acute treatable diarrhea illness (Marissal,et al,2007). In HIV- infected patients, in addition to microbes, diarrhea may be caused by other factors such as medication, immune deregulation, autonomic dysfunction and nutritional supplementation (Awole et al., 2003). Studies on human parasitic infections have demonstrated a common relationship between parasitic infections and lower socio-economic status (Kia, et al., 2008).

The effect of socio-economic status on risk of infectious diseases in general, and parasitic infections in particular, is complex in nature and could be attributed to several other factors such as lack of access to clean water, poor hygienic environment, lack of access to education due to financial constraints and overcrowded condition(Mehraj et al, 2008). Diarrhea is one of the most common complications of HIV and has many possible causes. First, diarrhea can be an early sign of the HIV disease itself, also known as acute HIV infection. HIV produces flu-like symptoms within two months of the initial infection. Afterward, you may continue to experience the symptoms, including diarrhea, for a few weeks. Other symptoms of an acute HIV infection include: Poor nutrition can worsen diarrhea when it's due to HIV or an HIV medication.

Diarrhea, the most common complications of HIV has many causes including intestinal parasites such as protozoan and helminthes. Reports from many regions of the world where HIV/AIDS is endemic have acknowledged that intestinal parasitism is widespread among these populations (Assefa et.al. 2009).Several intestinal parasitic pathogens which have been reported in HIV patients include *Cryptosporidium* parvum, Isospora belli, microsporidia (Enterocytozoon bieneusi, Encephalitozoon intestinalis), Giardia duodenalis, Entamoeba histolytica/dispar,

Cyclospora cayetanensis, Ascaris lumbricoides, Trichuris trichuria, hookworms *and* Strongyloidis stercoralis (Farthing et al., 2009).

2.3.3.1. Intestinal protozoan parasites:

The protozoa are an extremely diverse group of unicellular Organisms occurring in almost all of the ecological niches known to humans, including the bottom of hot springs and the edges of ice flows. Even though the majority of protozoa occur as free living organisms in the soil, moist, marine or fresh water environments, a substantial number also exist as mutualists, commensals or parasites (Mehlhorn, 1988, Katz,et al, 1989). Protozoan parasites are known to affect all species of vertebrates and many invertebrates. They are able to adapt to life in virtually all body sites of their hosts. Their characteristic high infectivity enhances their pathogencity within the host (Katz,et al, 1989).Numerous protozoa inhibit the gastrointestinal tract of humans. This list includes representatives from many diverse protozoan groups.

The majority of these organisms can cause severe disease under certain circumstances (Stenzel, et al, 2004). Some can cause serious diseases of the respiratory tract (air passages from the nose to lungs), and the central nervous system (brain, cranial nerves and spinal cord), while others live in our intestines and cause symptoms like diarrhea and are not deadly. It is hard to protect ourselves from protozoan parasites that are in the environment, because they produce cysts. A cyst has very resistant walls that surround and protect the protozoan parasite and make it able to survive extreme environmental conditions, such as big changes in temperature and too much or too little water. This means that once a protozoan parasite cyst is in the environment it can wait a long time for the perfect chance to get inside our bodies to infect us.

2.3.3.1.1. Entamoeba histolytica /Entamoeba Infection

The genus *Entamoeba*, amoeboid protozoanparasite, includes six species (*Entamoebahistolytica*, *E. dispar*, *E. moshkovskii*, *E.polecki*, *E. coli*, and *E. hartmanni*). *E.histolytica*, *E. dispar*, and *E.moshkovskii*arecapable of infecting the intestinal lumen ofhumans, are morphologically identical, butgenetically different (Parija, 2013). Entamoebahistolytica infection is common in most developing countries. It is also becoming more frequent in the United States and other

developed countries as the result of increasing tourism abroad and arising number of refuges and other immigrants and nonimmigrants originating in endemic countries (Petri, et al, 1999).

The two *species Entamoeba histolytica and* Entamoeba dispar are morphologically identical but pathologically distinct (WHO, 1997, Petri, et al, 1999). However, only E.histolytica is capable of causing disease (WHO, 1997). Colonization with E. dispar is said to be three times more common than E. histolytica in developing countries while it is ten times more common in developed nations (Petri, et al, 1999). Entamoeba histolytica is reported to be responsible for approximately 50 million cases of invasive amoebiasis (Petri, et al, 1999).And upwards of 100,000deaths/year(WHO,1997).

Thus, it is ranked second only to malaria as the cause of mortality due to a protozoan infection (WHO, 1997). The parasite normally inhabits the large intestine but is also capable of invading other organs such as the liver, brain and spleen (Petri,et al, 1999). The majority of amoebic infections are reported to occur in Central America, South America, Africa and Asia. These are often associated with poor water and food hygiene and sanitation practices (Petri,et al, 1999). Humans are the most significant reservoir of infection even though morphologically identical amoebae have been isolated from certain domestic and wild animals (Katz,et al, 1989).

2.3.3.1.2. Giardia lamblia /Giardia lamblia Infection

Guardia intestinal is, flagellated protozoan parasite, worldwide distributed, burden of disease remains high in developing countries, in immuno-compromised individuals, especially in children < 10 years of age (Parija, 2013). With progressive reduction in CD4 counts, symptomatic, Guardia infection is increased (Gazzard,2009). Complications include steatorrhea leading to malabsorption and weight loss. Almost 80% of sexually active gay men carry E. dispar, E. coli, E.hartmanni, Iodamoeb abutschlii, Dientamoeba fragilis, and G. intestinalis, as compared to its incidence in the general population of 13% (Parija, 2013). G.lamblia is appearing shaped, flagellated protozoan that causes a wide variety of gastrointestinal complaints. Guardia is arguably the most common parasite infection of humans worldwide and the second most common in the United States after pinworm (Katz, et al,2001). Between 1992 and 1997, the Centers for Disease Control and Prevention (CDC) estimated that more than 2.5 million cases of Giardiasis occurs annually (Furness, et al, 2000).Because Giardiasis is spread by fecal-oral

contamination, the prevalence is higher in populations with poor sanitation, close contact, and oral-anal sexual practices.

The disease is commonly water-borne because Giardiais resistant to the chlorine levels in normal tap water and survives well in cold mountain streams. Because giardiasis frequently infects persons who spend a lot of time camping, back packing, or hunting, it has gained the nick names of ―backpacker's diarrhea" and ―beaver fever"(Dupont,et al,, 1995). Food borne transmission is rare but can occur with ingestion of raw or under cooked foods. Giardiasis is a zoonosis, and cross infectivity among beaver, cattle dogs, rodents, and big horn sheep ensures a constant reservoir (Glaser,et al, 2000).

2.3.3.1.3. Cryptosporidium / Cryptosporidium Infection

Cryptosporidiosis is a parasitic disease caused by Cryptosporidium, a protozoan parasite in the phylum Apicomplexa. It affects the intestines and is typically an acute short- term infection. It is spread through the fecal-oral route, often through contaminated water (Centers for Disease Control and Prevention, 2009); the main symptom is self-limiting diarrhea in people within tact immune systems. In immunocompromised individuals, such as AIDS patients, the symptoms are particularly severe and often fatal Chen, et al 2007).

Cryptosporidium is the organism most commonly isolated in HIV positive patients presenting with diarrhea. The parasite is transmitted by environmentally hardy microbial cysts (oocysts) that, once ingested, exist in the small intestine and result in an infection of intestinal epithelial tissue. Infection is through contaminated material, water, uncooked or cross-contaminated food that has been in contact with the feces of an infected individual or animal Contact must then be transferred to the mouth and swallowed. It is especially prevalent amongst those in regular contact with bodies of fresh water including recreational water such as swimming pools. Other potential sources include insufficiently treated water supplies, contaminated food, or exposure to feces (Centers for Disease Control and Prevention, 2009). The high resistance of Cryptosporidium cysts to disinfectants such as chlorine bleach enables them to survive for long periods and still remain infective (Carpenter, et al, 1999).

2.3.3.2 Intestinal helminth parasites:

Parasitic worms, often referred to as helminthes, are a division of eukaryotic parasites (Maizels,et al, 2003). They are worm-like organisms that live and feed off living hosts, receiving nourishment and protection while disrupting their hosts 'nutrient absorption, causing weakness and disease .

2.3.3.2.1 Ascaris / Ascaris Infection

Ascariasis is infection with the parasitic roundworm Ascaris lumbricoides. Ascariasis is caused by consuming food or drink contaminated with roundworm eggs. Ascariasis is the most common intestinal worm infection. It is found in association with poor personal hygiene, poor sanitation, and in places where human feces are used as fertilizer (Kazura, 2007).

Once consumed, the eggs hatch and release immature round worms called larvae within the small intestine. Within a few days, the larvae then move through the blood stream to the lungs, exit up through the large air ways of the lungs, and are swallowed back in to the stomach and reach the small intestine .During movement through the lungs the larvae may produce an uncommon form of pneumonia called eosinophilic pneumonia. Once they are back in the small intestine, the larvae mature in to adult round worms. Adult worms live in the small intestine where they lay eggs that a represent in feces. They can live 10–24 months (Kazura, 2007). It is estimated that 1 billion people are infected worldwide .Ascariasis occurs in people of all ages though patients area affected more severely than adults.

Most of the time, Ascariasis causes no symptoms. If there are symptoms ,they may include: bloody sputum, cough, low-grade fever, passing worms in stool, shortness of breath, skin rash, stomach pain, vomiting worms, wheezing and worms released through the nose or mouth(Kazura, 2007).Most people recover from symptoms of the infection, even without treatment, although they may continue to carry the worms in their body. Complications may be caused by adult worms that move to certain organs such as the bile duct, pancreas, or appendix, or multiply and cause a blockage in the intestine. Improved sanitation and hygiene in developing countries would reduce the risk in those areas. In areas where this disorder is

common, routine or preventive (prophylactic) treatment with deworming medications may be advised (Kazura, 2007).

2.3.3.2.2. Hookworm / Hookworm infection

Hook worms (a type of round worm) are another common intestinal parasite. The U.S. Centers for Disease Control (CDC) and Prevention estimates that 1 billion people worldwide have hook worm infestations, although improved sanitation has reduced the number of cases. Human hookworm infection is caused by blood-feeding nematode parasites of the genus *Ancylostoma* and the species *Necator americanus*. Worldwide, *N. americanus* is the predominant etiology of human hookworm infection, whereas *A. duodenale* occurs in more scattered focal environments (Hotez, et al.2004).

These two hookworms, together with the roundworm, *Ascaris lumbricoides*, and the whipworm, *Trichuristrichiura*, are often referred to collectively as soil-transmitted helminthes (STHs).No international surveillance mechanisms are in place to determine the prevalence and global distribution of hookworm infection. However, based on an extensive search of the literature since 1990, the worldwide number of cases of hookworm was recently estimated to be 740million people (De Silva, et al, 2003). The highest prevalence of hook worm occurs in sub-Saharan Africa and eastern Asia .High transmission also occurs in other areas of rural poverty in the tropics, including southern China (Hotez, 2002), the Indian sub-continent (Yadla,et al,2003), and the Americas(Hotez ,2003). In all regions, there is a striking relationship between hookworm prevalence and low socioeconomic status (De Silva, et al, 2003) Hookworm's neglected status partly reflects its concentration among the world's poorest 2.7 billion people who live on less than $2 a day.

2.3.3.2.3. Pinworms/Enterobius vermicularis;

Pinworms are small, white, thread-like worms that sometimes live in the colon and rectum of humans. Pinworms are about one-half inch long. While the infected person sleeps, female pinworms lay their eggs on the skin around the anus. This can cause intense itching in this area. *E. vermicularis* is a common helminthic parasite that affects people worldwide from all socioeconomic classes. Humans are the only natural host for the parasite. A high prevalence is

noted among children (typically aged 5–10 yr), institutional populations, homosexual men, and family contacts (Cook, 1994; Goldmann,et al ,1997). Infection commonly occurs by transfer of highly infective eggs from the perianal area to the mouth. The human gastrointestinal tract is the primary site of habitat, mainly in the cecum and appendix. Symptoms range from an asymptomatic presentation to perianal pruritus, insomnia, irritability, restlessness, and rarely, impetigo of scratched skin, vulvovaginitisor enuresis (Cook, 1994).

2.3.3.2.4 Strongyloides stercoralis / Strongyloidiasis;

Strongyloidiasis is a human parasitic disease caused by the nematode (roundworm) *Strongyloides stercoralis*, or sometimes Strongyloides fülleborni. S.stercoralis, also called threadworms, is a nematode helminth parasite that causes strongyloidiasis. There are an Estimated 100 million to 200 million people infected with *S stercoralis* residing in 70 different countries. (Vadlamudi, 2006).

The true prevalence of an *S stercoralis* infection is underestimated because a majority of the cases are sub-clinical (Rose, 2008). There are 53 species of the organism, and the most common infection is due to the species *S stercoralis*. Species such *as S fuelleborni* and *S kellyi* are frequently found in humans living in Africa. *S stercoralis* is most prevalent in warm climates but has the ability to survive in colder climates (Rose, 2008).

There is a high prevalence of S.stercoralis in Brazil, Central America, and Australia. It is endemic in Africa South and Southeast Asia, South America, rural parts of Italy, Papua New Guinea, and the Pacific Islands such as Fiji (Vadlamudi, 2006).Endemic areas in the United States of America are Kentucky, West Virginia, and eastern Tennessee (Rose, 2008).It can cause a number of symptoms in people, principally skin symptoms, abdominal pain, diarrhea and weight loss. In some people, particularly those who require corticosteroids or other immunosuppressive medication, Strongyloides can cause a hyper infection syndrome that can lead to death if untreated.

2.4 Transmission of diarrhea

Diarrhea is caused by infectious organisms, including viruses, bacteria, protozoa, and helminthes transmitted from the stool of one individual to the mouth of another, termed fecal-oral

transmission. Some are well known, others are recently discovered or emerging new agents, and presumably many remain to be identified. Infectious diarrhea is acquired by fecal-oral transmission that includes consumption of contaminated food or water, person-to-person contact, or direct contact with fecal matter. With regard to water-borne-diarrhea, transmission patterns occur when in-house water storage facilities or/and water sources are contaminated (corresponding to domestic domain and public domain contamination (Black, 2008).Most of transmission of diarrhea occurs in the domestic domain (Atherly) ,stool to the mouth and in the number of organisms needed to cause infection and illness .According to Curtis V.,Most of, there are four transmission routes that the major infectious agents use to reach human hosts, namely human-to human via the environment; human-to-human multiplying in the environment; human-to-animal-tohuman via the environment; and animal-to-human via the environment. In situations, where faecal contamination of the domestic environment is high, majority of cases of endemic disease probably occur either by human-to-human transmission, or from the human-to-Human transmission of pathogenic agents which have multiplied in the environment (Molbak, 2000).

2.5. Risk Factors for Diarrhea

2.5.1. Environmental and related factors

Broadly recognized risk factors for diarrheal diseases include little or no access to safe water and sanitation, as well as poor hygiene and feces disposal practices at home (Daniels, *et al,* 1990). These and many other factors, such as poor housing and crowding, are intrinsically associated with poverty. Furthermore, poverty usually limits access to health care and restricts appropriate and balanced diets (Daniels, *et al,* 1990).

Diarrhea disease affects rich and poor, old and young, and those in developed and developing countries alike , yet a strong relationship exists between poverty, an unhygienic environment as well as demographic factors. The relationship between environmental factors and the occurrence of diarrhea in households have been addressed in a number of studies. Environmental factors include access to improved water sources, availability of toilet facilities, compound hygiene housing condition and refuse disposal (Woldemichael, 2001).

Some studies have shown that the association between socioeconomic factors such as poor housing, crowded conditions (Woldemichael, 2001), a low income (Molbak, 2000). .and higher rate of diarrhea was statistically significant. As diarrhea is acquired via contaminated water and foods, water related factors are very important determinants of diarrhea occurrence. Poor storage of drinking water (Marjatta, 1994).use of unsafe water sources (Tumwine ,2002)' has been found to be risk factors for more diarrhea occurrence among people.

Sanitation obviously plays a key role in reducing diarrhea (morbidity). Some sanitation factors like, improper disposal household garbage (Aulia, et al, 1994) no existence of latrine or unhygienic toilet (Wijewardene,et.al,1992) increased the risk of diarrhea. Some studies revealed that people not washing hands before meals or after defecation (Curtis, et al, 2000).

Unsafe food storage is associated with risk of diarrhea morbidity human transmission of pathogenic agents which have multiplied in the environment (Molbak, 2000). The association between diarrhea and malnutrition is common in low income societies that the concept of a vicious circle is appealing, with diarrhea leading to malnutrition and malnutrition predisposing to diarrhea

Individuals whose immune system has been weakened by malnutrition are the most vulnerable to diarrhea. Diarrhea can cause significant morbidity in HIV-infected patients and can be due to a multitude of etiologies from infectious pathogens to malignancy to medications. Diarrhea is also an independent predictor of reduced quality of life (Siddiqui ,et al,2012).

2.5.2. The level of CD4 T cells related to diarrheal disease

Immunodeficiency is not only a cause of persistent or chronic diarrhea but also a risk factor for diarrhea. Chronic diarrhea is the major cause of morbidity and death among adults with HIV (Cohen,et al,2001) . Diarrhea is reported in up to 60% of patients with AIDS (National Medical Society, USA). HIV causes progressive depletion of CD4 T cells, which leads to life threatening opportunistic infections during the natural course of the disease (Hogg, et al1999).

Opportunistic infections can occur in up to 40% of people living with CD4 T cells count less than 250 mm (Gallant,et al,1994) Non infectious diarrhea, for the majority of patients, is limited to a period of one to two months (Niyogi, et al, 1994) On the other hand, there are those who

require greater medical attention since low CD4 T cell count (<200 cells/ mm^3) cause greater susceptibility to the infectious forms (De Olvera-silva,et al,2007). In the developing world, HAART is not routinely available for HIV-infected individuals, and diarrheal disease remains highly endemic even for those without HIV (Wanke,et al1999).Chronic diarrheal disease in adults in Africa has been used as a predictor of HIV-seropositivity (Colebunders ,et al,1987) . HIV-infected children are more likely to die with diarrhea than children with diarrhea that is not infected with HIV (Thea ,et al,1993).

In contrast, in resource-rich nations where HAART is widely available, the incidence of infectious causes of diarrhea in HIV-infected patients with low CD4 counts (<200 cells/microL) has declined (Ledergerber,et al,1999). Experience at a single center between 1995 and 1997 found that, while the incidence of diarrhea remained constant, infectious etiologies declined from 53 to 13 percent (Call, 2000).

2.6. Definition of key terms

- Magnitude: - the occurrence of diarrheal disease in HIV-infected individuals due to the related infectious disease.
- Malnutrition: - lack of adequate nourishment on HIV/AIDS patients.
- Hygiene: - cleanliness of personal and environmental hygiene.
- Complication: - health condition of the patients caused by infectious disease including diarrhea.
- Risk factor: - condition (socio-demographic characteristics, environment, behavior) that expose HIV/AIDS patient to be affected by diarrheal disease.
- Socio-demographic factors: - sex, age, occupation, income and education of studied subjects.
- Knowledge about diarrhea disease: - ability to remember and can answer about, preventions, related factors diarrhea disease by answering the interview questions.
- Prevalence: - The number of diarrheal cases at the time of physical examination and interview divided by the total number of patients attending during the study period.
- Occurrence: - the existence/presence of diarrhea and causative agents in the study subjects.

3. Methodology

3.1. Study area and period

The study was conducted in Debre Markos Referral Hospital at Debre Markos town on HIV/AIDS-patient co-infected with diarrheal infectious agents from January 2016 up to the end of April 2016. Debre Markos town is the capital city of East Gojjam Zone, Northwest Ethiopia. It is located 300 km away from Addis Ababa, the capital city of Ethiopia, and 265 km from Bahir Dar, the capital city of Amhara regional state.

3.2. Study design

The design of the study was purposive sampling to determine the magnitude of diarrheal disease with HIV/AIDS infected individuals. Hence, the research basically focused on HIV/AIDS infected individuals and attending in the ART clinic of the Hospital.

3.3. Sampling size and Sampling method

To determine the sample size of this study, 95% confidence interval was used preferably; P from the studies with similar study design and study population from the most recent studies could be most preferable (Cochran, 1977). Because of the lack of similar study a sample was made as P equal to 0.5 to yield the maximum sample size. The following simple formula, (Daniel, 1999).can be used.

$$n = \frac{Z^2 * p(1-p)}{d^2}$$

Where, n= sample siz

Z is statistic for a level of confidence

P is expected prevalence or proportion of one; if 50%, p=0.5)

d is precision (in proportion of one; if 5%, d=0.05)

Z statistic (z) for the level of confidence of 95% which is conventional, z value is 1.96.

Therefore,

$$n = \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2} = 384$$

Hence, the total sample size were become 384.

The samples were taken from the maximum number of 4,000 HIV/AIDS patients who have been taking antiretroviral therapy. From this large numbers of HIV-infected outpatients a minimum of 15 attended in the clinic with opportunistic infections, including diarrheal cases. From the start of the experiment patients with complications were randomly selected until the sampling size reached the required number (384).The whole information was collected during the examination time at the clinic, from the laboratory result and other data through questionnaire and interview by assigned trained health officers and the researcher.

3.4 Methods of data collection

3.4.1. Collection of stool samples

Stool samples were collected using disposable plastic cups and sticks from the study participants along with brief instruction on how to collect the stool. They were instructed to bring

Sizable fresh stool sample of their own. Each plastic cups was labeled with codes of patients. The stool samples were carried to the laboratory for parasitic examination.

3.4.2. Laboratory diagnosis of stool samples

Fresh stool was collected from diarrheal cases(127 patients) using disposable screw cup .A direct wet mount of stool in normal saline (0.85% Nacl solution) was prepared and was examined for the presence of motile intestinal parasites and trophozoites under light microscope (10x and 40 x magnification). The uninfected normal saline was used for routine examination of stools as it is isotonic with living organisms in order to observe blood cells, motile forms of micro organisms. Lugo's iodine staining was used to detect ova and cyst of intestinal parasites.

3.4. 3 Socio demographic data collection

Among diarrheal cases, 36(28.35%) in January, 38(29.92%) cases in February, 33(25.98 %) in March and 20(15.75%) were recruited in April Among 127 cases, 36(28.35%) in January, 38(29.92%) cases in February, 33(25.98 %) in March and 20(15.75%) were recruited in April respectively. Relevant parasite infections data were collected by inspecting the patient's health record and analyzing individual patient cards. This analysis was used to collect appropriate information about characteristics of the study patients, such as age, status of diarrhea, residence and economical status and result of stool examination.

3.4.4. Questionnaire and interview

After diarrheal and non-diarrheal study groups were identified, questionnaire and interview was conducted on both groups in order to assess the potential risk factors for diarrhea. The questionnaire interviews were taken from Senait Esayas(2014) and modified .

3.5. Data analysis method

Data entry and analysis was performed using SPSS software. Statistical analysis was done using Chi-square to evaluate any association between risk factors for diarrhea. Bivariate and multivariate analyses were used to see the relationship of risk factors to diarrhea.

The magnitude and related risk factors for diarrhea were calculated from the outpatients attending the ART clinic of the hospital. Whether the factors affect the patients significantly or not the researcher used chi-square to assess which factors predominantly affect the patients in the study area. The other data (demographic data) were analyzed with descriptive statistics with percentage whereas data obtained from interview were qualitatively analyzed.

3.6. Ethical clearance

The ethical clearance was received from the ethical committee of the college of Natural Science, AAU. The health workers were oriented about the confidentiality of the data and all data were used only for the purpose of this research.

3.7. Significance of the study

This study would provide medical practitioners and other stakeholders a better understanding about the magnitude of diarrheal disease in HIV-infected patients still who are taking ART.

4. Results and Discussion

4.1 Laboratory diagnosis of faecal samples (diarrhea)

A total of 384 HIV/AIDS infected patients were selected in to the study after meeting the inclusion criteria. Interviews based on questionnaire were conducted with 384 sample population.

Patients with diarrhea (127 cases) stool samples were collected and examined with direct microscope laboratory to identify patients' diarrhea and causative agents. The others with different complaints were non-diarrheal study groups (257).

Table 4.1. Types of diarrhea detected on diarrhearic-patients.

Types of diarrhea	Male	Female	Total
Bloody	8	12	20(16%)
Non bloody(loose bowel) diarrhea	30	27	57(45%)
Watery diarrhea	21	29	50(39%)

As stool examination indicated that of the sample characterized by 50(39%)watery diarrhea followed by non-bloody(loose bowel) 57(45%) and bloody diarrhea(10%).

Diarrhea which was bloody and watery was caused by bacteria, Giardia and amoeba spp. Although the bacteria species were not identified, the bacteria that cause such bloody diarrhea might be due to shigella and E.coli or other Bacteria spp.

The pathogens of the diarrhea were detected after the examinations of faecal samples of the diaharreal patients. (Table 4.2.). Accordingly, 41(32.3%) of the diarrhea showed high prevalence of E*, histolytica / dispar* followed by Taenia saginata, 30(23.62%) and Bacteria species 22 (17.32%), *A. lumbricoides*, 14(11.02%), and the others *Strongloides stercoralis*

8(6.3%), *Giardia. Lamblia* 5(3.93%), pinworm (*Enterobius vermecularis,* 5(3.93%) and hookworm (Ancylostoma duodenale/N. Americans) 2 (1.57%).

Table 4.2.Diagnosis of the pathogens from stool samples of HIV-infected outpatients from Debre Markos Referal Hospital, ART clinic, taken from January up to end of April, 2016,

	Sex		Total
Pathogens	**Male**	**Female**	
E, histolytica/dispar	20	21	41(32.3%)
Taenia saginata	20	10	30(23.62%)
Bacteria species	7	15	22(17.32%)
S.stercoralis	5	3	8 (6.3%)
G. lamblia	1	4	5(3.93%)
A. lumbricoides	3	11	14(11.02%)
Pinworm/ E.vermicuralis	1	4	5(3.93%)
Hookworm/**Ancylostoma duodenale**/*N.americanus*	2	0	2(1.57%)
Total	**59**	**68**	**127**

Table 4.2. showed the prevalence of diarrheal diseases in relation to their causative agents. Accordingly, the most frequently encountered causative agent of diarrhea in this study was E. histolytica/dispar 41(32.3%).The prevalence of this causative agent in this study was higher than all previous studies from Debre Markos, (14.3%) (Hiwotie, 2014), Southeast of Lake Langano (12.7%)(Mengistu and Berhanu, 2004), from Jimma (17.1%) (Amare et al 2007), but much lower than from the study in Gondar (1.6%) (Afewerk et al, 2008).

The prevalence of *Taenia saginata* (23.62%) in this study was higher than the prevalence 13.9% reported from rural areas close to south east lake Langano ,Ethiopia, (Mengistu and Berhanu, 2004), and .the prevalence (1.1%)from Nigeria (Akinbo et al, 2010).

Similarly, the prevalence of *Strongloides .stercoralis* (6.3%) was higher than the study reported from north west of Ethiopia (1.6%) by Abebe et al. (2011), previous study from Debre markos(2.6%) (Howotie, 2014), Gondar (5.5%) (Afework et al, 2008), South east langano(5.8%) (Mengistu and Berhanu, 2004), but much lower than reported from Jimma (17.5%) by Amare et al (2007).

With regard to the prevalence of giardiasis, 3.93% of the stool samples contained *Girdia lamblia* which was much lower than the previous work from Debre markos (11.7%),Hiwotie,2014), Jimma (13.9%) by Amare,et al,2004, and Southeast Langano (6.2%), but higher than the prevalence(0.8%) in Gondar by Afework,et al (2008).(Table 4.3).

In this study hook worm spp. were detected (1.57%) of the patients which was higher than the prevalence in Gondar (0.8%), Jimma (1.1%) and much lower than the previous work from Debre markos (16.1%)(Hiwotie,2014),and Southeast Langano (60.2%) (Table 4.3). The prevalence of *Enterobius vermicularis* in this study was (3.93% which was higher than reported from South east Langano (2.7%) (Mengisru and Berhanu, 2004).

The data also showed 17.32% of the faecal samples of Bacteria spp. Which is higher than reported from Gondar (3.1%) by Afework et al(2008). This indicted that one or more Bacteria growth could be implicated with the disease.

With regard to prevalence of A.lumbricoides(11.02%) in this study lower than reported from Gondar (18.11%) by Afework et al (2008), but higher than reported from Southeast of Langano(6.2%) by mengistu and Berhanu(2004) (Table 4.3).

Higher or lower prevalence of different causative agents of this study in comparison with other studies might be due to different environmental conditions or study subjects.

Table - 4.3. Pathogens of diarrhea from studies from different study sites.

Disease	Pathogens	Study Area	Site	Prevalence	Reference
Amoebiasis	*E. histolytica/dispar*	Ethiopia	Debre markos	32.3%)	This study
		Ethiopia	Gondar	1.6%	Afework et al, 2008
			D. Markos	14.3%	Hiwotie, 2014
			Jimma	17.1%	Amare et al, 2007
Bacterial	*Bacteria spp.*	Ethiopia	Debre Markos	17.32	This study
		Ethiopia	Gondar	3.1%	Afework et al,2008
Taeniasis	*T. saginata*	Ethiopia	D /markos	23.62%	This study
		Ethiopia	SE Langano	13.9	Mengistu and Birhanu, 2004
		Nigeria	Benin City	1.1%	Akinbo et al, 2010
Strogylodiasis	*S. stercoralis*		D/Markos	6.3%	This study
		Ethiopia	D. Markos	2.6%	Hiwotie, 2014
			Gondar	5.5%	Afework et al, 2008
			Jimma	17.5%	Amare et al, 2007
Giardiasis	*Giardsia lamblia*	Ethiopia	This study	3.93%)	This study
			D. Markos	11.7%	Hiwotie, 2014
			Jimma	13.9%	Amare et al, 2007
Pinworm	*E. vermicularis*	Ethiopia	Debre Markos	3.93%	This study
			SE of Langano	2.7%	**Mengistu &Berhanu**
Hook worm	*A. duodenale/N. americanus*	Ethiopia	D/ Markos	1.57%)	This study
			Gondar	0.8%	Afework et al, 2008
			D. Markos	16.1%	Hiwotie, 2014
			SE of Langano	60.2%	Mengistu & Berhanu, 2004
Ascariasis	A.lumbricodes	Ethiopia	Debre Markos	11.02%	This study
			Gondar	18.11%	Afework et al, 2008

Table 4.4- The Prevalence of Intestinal parasites in Relation to Diarrhea with respect to Sex and Age in the HIV –diarrheal-patients, ART clinic, Debre marckos Hospital.

Sex	Age Groups			
	5-14	15-24	25-34	≥35
Male	8(6.3%)	13(10.23%)	18(14.17%)	20(15.75%)
Female	9(7.1%)	23(18.11%)	19(14.96%)	17(13.38%)
Total	17(13.4%)	36(28.34%)	37(29.13%)	37(29.13%)

The prevalence of diarrhea in these HIV-patients was 33.1% which is lower than reported (59.4%) by Hiwotie(2014) in the same study site. Amongst these patients 46.6% were males and 53.4% were females. This showed that females were more prevalent than males.

The prevalence of intestinal infectious agents in relation to diarrhea amongst HIV-patients in the age group 5-14 years old was 13.4%, in the age group 15-24 years was 28.34%.in the age group 25-34 and 35 and above was 29.13% . This showed that the prevalence of intestinal parasites in relation to diarrhea was higher in the age groups 25-34yeras old and 35 and above than other age groups. The reason might be due to higher number patients aged from 25-34 and 35 and above 74(58.26%).

In addition to the other risk factors associated with diarrhea low CD_4 cell/µl counts was another risk factor to be considered. In this study the CD4 T cells/µl count was identified from diarrhea and non-diarrheal patients

As indicated from table 4.5, the CD_4 cell/µl counts of the diarrheal cases 250 cell/µl and below were 60.62% and from non-diarrheal patients was 18.68%. This result indicated that the CD_4 Cells/µl counts 250 and below in diarrheal patients was higher than the non-diarrheal patients. This showed that low CD_4 cell/µl counts was a risk factor associated with diarrhea.

Table- 4.5- The CD4 cells/µl counts identified from diarrhea and non-diarrheal patients.

CD4 T cells count/µl	Diarrheal Patients		Total	Female: male ratio	Non-diarrheal Patients		Female male ratio
	Male	Female			Male	Female	
≤100	12 (9.45%)	15 (11.81%)	21.26 %	1.25	6 (2.3%)	5 (1.95)	0.8
101-250	22 (17.32%)	28 (22.04%)	39.36 %	1.27	16 (6.22%)	21 (8.17%)	1.3
≥250	25 (19.68%)	25 (19.68%)	39.36 %	1	63 (24.51%)	146 (56.80%)	2.31

Table 4.5.:- Shows that 39.37%) of the diarrheal patients had CD4 count more than 250; whereas 39.37% and 21.26% had CD4 counts of 101-250 and <100, respectively. The female: male CD4 count ratio showed 1.27 and 1 within the higher CD4 counts of 101-250; and 250, except that the female –male ratio was 1.25 (higher counts in female than male) within the CD4 count of <100 (Table-4.5). HIV causes progressive depletion of CD4 T cells, which leads to life threatening opportunistic infections during the natural course of the disease (Hogg, et al 1999). Opportunistic infections can occur in up to 40% of people living with CD4 T cells count less than 250 mm (Gallant, et al, 1994).

4.2 Demographic and social characteristics

Geographical distribution of patients with diarrhea (Urban and Rural), 90 (70.87%) were living in urban and 37(29.13%) were living in rural areas. Table 4,6. Showed that of the total 127diarrheal cases 59(46.46 %) and 68 (53.54%) were male and female patients, respectively.

Concerning age of the participants 13.38% of diarrheal-patients and 3.5% of the non-diarrheal-patients were 5-14 years old ,and 16.53% of diarrheal-patients ,and 11.67% of the non-diarrheal-patients were 15-24 years old ,24.4% of diarrheal-patients and 12.84% non-diarrheal-patients were 25-34 years old and the rest 45.66% of diarrheal-patients and 60.31% 0f the non-diarrheal-patients were 35 and above years old.

From 384 patients selected to the study 47(37.00%) of diarrheal-patients 76(29.57%) of non-diarrheal patients were illiterate. 20(15.74%) ofdiarrhealpatients and 25(9.73% of the non-

diarrheal ones had primary education. The rest 60(42.24%) of the diarrheal-patients and 156(60.7%) of non-diarrheal patients had secondary and above education.

76(29.57 %) of non-diarrheal patients and 47(37. %) of diarrhearic-cases were illiterate. 20(15.74%) of diarrheal-cases and 25(9.73%) in non-diarrheal patients had primary education. The rest 60(42.24%) in diarrheal-cases and 156(60.7. %) in non-diarrheal patients had secondary and above education.

With regard to economic status, monthly income of diarrheal-patients was 48 (37.8%) ≤500 birr. 47(37.01%) 501-1000 birr. 21(16.54%) 1001-1500 and 14(11.02%) 1500 birr and above respectively, whereas monthly income of non-diarrheal patients was 16(6.23) ≤500 birr, 85. (33.1%) 501-1000 birr, 34(13.3.23%) 1001-1500 birr and 122(47.47%) 1500 birr. (table 4.6)

Table 4.6. Demographic and social characteristics of study subjects.

Characteristics	Study groups			
Residence	Diarrheal		Non-diarrheal	
Rural	37(29.13%)		22(8.56)	
Urban	90(70.87%)		235(91.44%)	
Total	127 (30.1)		257 (%)	
Age and Sex	M	F	M	F
5-14	8(6.30 %)	9 (7.09%)	3 (1.17 %)	6(2.34%)
15 -24	7(5.51%)	14(11.02 %)	13(5.06%)	17(6.61%)
25-34	16(12.6%)	15(11.81 %)	18(7.00%)	45(17.51 %)
35 and above	28(22.05%)	30(23.62%)	51(19.84%)	104(40.47 %)
Education				
Illiterate	47 (37.00%)		76(29.57%)	
Primary	20(15.74%)		25(9.73%)	
Secondary and above	60(42.24%)		156(60.7%)	
Monthly income				
≤500	48(37.8%)		16(6.23%)	
501-1000	47(37.00%)		85(33.07%)	
1001-1500	21(16.00%)		34(13.23%)	
1501-and above	11(8.66%)		122(47.47%)	

The data also showed potential risk factors associated with diarrhea, such as education, residence, monthly income (economic status) would be analyzed and discussed in sections of bivariate analyses

4.3. Chi-square analysis of potential risk factors associated with diarrhea.

The Chi-square analysis based on findings that were presented with matched odds ratio (MOR), at 95% level of confidence) it was that discussed factors below table were significantly associated with diarrhea on HIV/AIDS and its complications (Table 4.7) .

Table-4.7.Chi square analysis of risk factors associated with diarrhea among diarrheal-cases and non-diarrheal patients.

Potential risk factors	Diarrhearic (n=127)	Non-diarrhearic (n=257)	Matched odds ratio	(95%ci)	P value
Levels of patients education					
.Illiterate	47(37.00%)	76(29.57%)	2	(0.193-0.275)	<0.001
Illiterate	47	76	ref.		
Primary	20(15.74%)	25(9.73%)	1.3	(0.010-0.007)	<0.001
Secondary and Above	60(42.24%)	156(60.7%)	0.4	(0.993-1.000)	0.5
Poor economic status					<0.001
≤500	48(37.79%)	16(6.23%)	ref.		<0.001
≤500	48	16	9.5	(0.220-0.580)	<0.021
501-1000 1001-1500 1500above	47(37.00%) 21(16.00%) 11(8.66%)	85(33.07%) 34(13.23%) 122(47.47)		(3.140-7.930) (1.060-3.150) (1.106-4.420)	<0.001 0.210 0.326
Having Hand Washing facility					
NO	90(70.86%)	63(24.51%)	7.5	(0.000-0.007)	<0.001
No	90	63	ref.		
Yes	37(29.14%)	194(75.49%)	0.1	(0.467-0.562)	0.2
Do you wash your hands regularly before meal?					
No	60(47.24%)	41(15.95%)	4.7	(0.220-0.580)	<0.001
No	60	41	ref.		
Yes	67(52.75%)	216(84.05%)	0.2	(0.0467-0.562)	0.5
Do you wash your hands regularly after toilet?					
No	97(76.38%)	47(18.29%)	2	(0.221-0.581)	<0.001
No	97	47	ref.		
Yes	30(23.62%)	210(81.71%)	0.1	(0.993-1.000)	0.2

Potential risk factors	Diarrhearic (n=127)	Non-diarrhearic (n=257)	Matched odds ratio	(95%ci)	P value
Use Stored coked food for later					
Yes	75(59.05%)	36(14.00%)	8.9	(0.192-0.275)	<0.001
Yes	75	36	ref.		
No	52(40.95%)	221(86%)	0.1	(0.660-0.745)	0.4
.Eating raw meat ,vegetables					
Yes	80(63%)	43(16.73%)	8.5	(0.000-0.007)	<0.001
Yes	80	43	ref.		
No	47(37%)	214(83.27%)	0.5	(0.660-0.747)	0.1
Lack of enough food					
Yes	83(65.4%)	40(15.56%)	10	(0.994-1.006)	<0.001
Yes	83	43	ref.		
No	44(34.6%)	217(84.44%)	0.1	(0.465-0.562)	0.3
Lack of balanced diet					
Yes	78(61.4%)	39(15.18%)	8.9	(0.661-0.748)	<0.001
Yes	78	39	ref.		
No	49(38.6%)	218(84.82%)	0.1	(0.993-1.000)	0.4
Using unsafe water					
River	22(17.3%)	11(4.28%)	8.1	(0.000-0.007)	<0.001
River	22	11	ref.		
Well	26(20.5%)	7(2.72%)	2	(0.660-0.747)	0.5
Pipe	79(62.2%)	239(92.99%)	0.33	(1.060-3.150)	
Infrequent cleaning /emptying /of container					
Sometimes	86(67.71%)	42(16.34%)	2	(0.001-0.002)	<0.001
Sometimes	86	42	ref.		
Usually	41(32.9 %.)	215(83.66%)	0.1	(0.995-1.000)	0.3
Use Untreated water					
Yes	77(60.63%)	37(14.40%)	9	(0.006-0.032)	<0.001
Yes	77	37	ref.		
No	50(39.37) %	220(85.60%)	0.1	(0.465-0.562)	0.5
Do you know what exposes you for diarrhea ?					
No	74(58.27%)	80(31.13%)	3.1	(0.221-0.580)	<0.001
No	74	80	ref.		
Yes	53(41.73%)	177(68.87%)	0.3	(0.662-0.747)	0.4
Do you know the preventive methods of diarrhea?					
No	72(56.69%)	102(39.7%)	2	(0.062-0.047)	<0.001
No	72	102	ref.		
Yes	55(43.31%)	155(60.3%)	0.5	(0.993-1.000)	0.3

4.3.1. Socio demographic and economic factors

Although, the study area referral hospital is found in urban area there are sub-urban and rural areas around it. Some of cases were from rural areas and the others were from sub urban areas near Debre Markos Referal Hospital as observed in their cards during data collection.

The data showed that 47(37.00%) of the diarrheal-patients and 76(29.57%) of non-diarrheal patients were illiterate, whereas 20(15.74%) of diarrheal-patients and 25(9.73%) of the non-diarrheal ones had primary education. Likewise, 60(42.24%) of the diarrheal-patients and 156(60.7%) of non-diarrheal patients had secondary and above education. Consequently, Comparing with education those illiterate 47(37.00%) in diarrheal-patients had association to diarrhea and the magnitude of diarrhea was high with matched odds ratio 2, at 95% level of confidence and the p –value $\leq$ 0.001.This shows that the risk of diarrhea on those illiterate cases was 2 times greater than cases with primary education, but almost similar (MOR 0.6) with that of Patients with secondary and above education (table 4.3)

Patients should be educated to protect them from diarrhea diseases and other intestinal parasites. If they are illiterate they can't identify correctly the factors that cause diarrhea and unable to protect themselves (Hiwotie Mengstie ,2014).

A study in India amongst HIV-patients, lower education, primary education in diarrheal-patients (71.2%) and in non-diarrheal-patients(37.9%) was significantly associated with diarrhea. This likely due to lower education serving as a proxy for lower socioeconomic status (Marissa,et al,2007).

4.3.2. . Hygiene related factors

In this study irregular hand washing practice 60(47.24.%) and 41(15.95%) was in diarrheal-patients and non-diarrheal-patients respectively . Similarly, Irregulr hand washing practice after toilet 97(76.38) and 47(18.29) in diarrheal-patients and non-diarrheal-patients respectively.This result of irregular hand washing showed higher percentage reported (6.5%) in diarrheal-patients and (8.7%) in non-diarrheal-patients as well as irregular hand washing practice after toilet(7.9%) in diarrheal-patients and (11.5%) in non-diarrheal patients from Karnataka, India(Marissa et al,2007) respectively. This difference may be due to location and environmental conditions.

In this study, the risk of diarrhea among diarrheal patients who had poor hand washing practices before meal was 4.7 times greater than the non diarrheal patients who had paid attention to washing their hands than the diarrhearic ones. Similarly, concerning hand washing practice after toilet patients with diarrhea were 2 times greater than by practicing poor hand washing (table-4.3). Consequently, irregular hand washing before meal showed a risk factor of diaharrea with MOR 4.7 at 95% level of confidence, P≤ 0.001 followed by irregular hand washing after toilet with MOR 2. at 95% level of confidence, P<0.001) (table-4.3).

The study showed that poor hand washing practice before meal and after toilet as well as absence of hand washing facility were associated with risk factors of diarrhea are compatible with some studies so far (Daniels, *et al* ,1990; Amy, et al,2010; Tumwine et al,2002).

4.3.3.. Eating raw meat, vegetables

In Ethiopia, the widespread habit of raw beef consumption is a potential cause for food borne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene (Siddiqui,et al, 2006) . By eating raw meat people can be exposed to intestinal parasitic disease. Concerning eating raw meat and raw vegetables, 80 (63%) in diarrheal-cases and 43 (16.73%) in non-diarrheal patients had the habit of eating raw meat and vegetables. This might be the factor that increased the risk of diarrhea (MOR 8.5; at 95% level of confidence ,P<0.001)(table-4.3).

4.3.4. Food related factors

Concerning food related factors, 75 (59.05%) in diarrheal- cases- and 36(14%) in non- diarrheal-cases were associated with unsafe food storage as risk factors (MOR 8.5; at 95% level of confidence, P<0.001) (table-4.3). with regard to lack of enough food. 83(65.35) in diarrheal-cases 40(15.56%) in non-diarrheal patients did not get enough food (OR=10 at 95% level of confidence, P<0.001). The last food related factor found to increase the risk of diarrheal and magnitude of diarrheal disease was unbalanced diet 78(61.42%) in diarrheal-cases and 39(15.56%) in non-diarrheal patients did not get balanced diet (OR=8.at 95% level of confidence, P<0.001). This shows that diarrheal-cases were associated for diarrhea 8.9 times higher than the non-diarrheal patients. In addition to this, monthly income of most patients with

diarrhea was very low (74.8%) between 500 and 1001 birr. Due to these reasons, it is possiple to realize that most of them lead poor quality of life.

Diarrhea is an independent predictor of reduced quality of life (Siddiqui ,et al,2012). Unsafe food storage is associated with risk of diarrhea morbidity human transmission of pathogenic agents which have multiplied in the environment (Molbak, 2000). The association between diarrhea and malnutrition is common in low income societies that the concept of a vicious circle is appealing, with diarrhea leading to malnutrition and malnutrition predisposing to diarrhea (Molbak, 2000).

4.3.5. Infrequent cleaning (emptying) of container before filling with fresh water.

With regard to cleaning of storage of water container, most 86(67.71. %) diarrheal- cases and 42(16.34%) non-diarrheal-patient cleaned storage of drinking water sometimes. (OR=2, P≤ 0.001). This shows that diarrheal-cases were 2 times higher than non-diarrhearic by infrequent cleaning (emptying) of water container before filling with fresh water (table-4.3).

4.3.6. Water related factors

In the surrounding urban study areas river and well water are available. Water from rivers and wells for drinking and other domestic needs. With respect to using Water from river 22(17.3%), from well 26(20.5%), from pipe 79(62.2%) in dirrheal-patients and from river11 (4.28%), from well 7(2, 72), from pipe 239(92.99%) in non-diarrheal-patients respectively. As a result diarrheal-patients using river water more prevalent 2 times and 3 times than non-diarrheal-patients respectively (table-4.3). This showed that this increased the risk of diarrhea and magnitude of diarrheal disease (OR 2, 95% level of confidence p≤ 0.001) .In this study ,well source for water 20.5% in dirrheal-patients is lower (29.6%) and 2.72% in non-diarrheal patients is lower (21.2%) than in non-diarrheal patients reported from India respectively (Marissa,et al,2007). Likewise, 77(60.62%) in diarreal-cases and 37(14.39%) in non-diarrheal patients used untreated drinking water (OR= at 95% level of confidence, P≤0.001). This showed that patients used untreated water was exposed to diarrhea 9 times higher than the non-diarrheal patients (table-4.7).

4.3.7. Knowledge of factors that expose for diarrhea and preventive methods in diarrheal-cases and non-diarrheal- patients.

From 384 HIV/AIDS patients 74(58.27%) in diarrheal-cases and 80(31.13%) in non-diarrheal patients were linked with lack of knowledge that the activities them expose for diarrhea (MOR=3.1, at 95% confidence interval, p≤0.001). In addition to this 72(56.69%) of diarrheal-cases and 102 (39.70%) of non-diarrheal patients had no knowledge on diarrhea preventive method. (Table-4, 3). Concerning the knowledge of preventive ways and knowing exposing things for diarrhea, non-diarrheal patients had better knowledge in comparison with diarrhearic patients (MOR=2, at 95% confidence interval, p ≤0.001).

5. Conclusion and Recommendations

5.1 Conclusion

In this study 33, 07% of the total 384 outpatients infected with HIV were also affected with diarrhea. After examining 127 stool samples, the most frequent pathogens that were detected, E. histolytic/dipar were high 41(32.3%) followed by Taenia spp 30(23.62%) Bacterial spp, 22(17.32%) and A. lumbricoides 14(11.02%).

The result study showed that the factors, namely low level of education, poor economic status, absence of hand washing facility, irregular hand washing, use stored cooked for later use, eating raw meat and vegetables, lack of balanced diet, unsafe water resource, infrequent cleaning of container, untreated water and poor knowledge of Factors exposing for diarrhea and preventive methods were significantly associated as risk factors of diarrhea on diarrheal cases in the study area. In addition to this CD_4- cell/µl counts of both diarrheal-case (60.62%) and non-diarrheal-patients (18.68%) showed ≤250 CD_4- cell/µl counts respectively. This indicted that low CD_4- cell/µl counts was more associated as risk factor in diarrheal-patients than non-diarrheal-patients.

5.2 Recommendations

Based on the present findings the prevalence of diarrheal disease, pathogens and associated risk factors of HIV/AIDS patients the researcher suggested the following recommendations.

- Measures including education on personal and environmental hygiene should be taken in to account to aware patients about diarrhea causes and preventive methods and to reduce the prevalence.
- Encourage patients to wash their hands before meal and after toilet
- Advice people (patients) to use safe and treated water to avoid water borne diseases.
- Recommend patients to use fresh and cooked food.
- Due to absence of some laboratory materials at the time of this study period some laboratory tests were note performed in study area. Therefore, further study on the prevalence of diarrheal disease and causative agents in HIV-patients is recommended to be done.

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Appendix I

Questionnaires and interviews

The questionnaires and interviews are prepared to conduct a research on a topic magnitude and risk factors of diarrheal diseases among HIV/AIDS patients who have complications attending in D/M/R Hospital. You are selected randomly since you have complication and going to be examined by the doctor to be interviewed and fill the questionnaires.

I received permission from Addis Ababa University College of natural science, Debre Markos Hospital administration and respective clinic of the Hospital.

I would very much appreciate your participation in this interview and response for questionnaires. This research outcome will help for the government and NGO to interfere and solve the problem of HIV/AIDS patients vulnerable to diarrheal diseases and related issues.

I hope you will be participating voluntarily since you are one are one of the victims of HIV.

I assure that the interview and the questionnaire will not bring any harm to you, but advantages what even information you provide will be kept strictly confidential, except to be used for this research. No need to write your name.

- Write –X" for your agreement for the questionnaires.
- Give short and precise answer for the interviews

THANK YOU!!

Personal and related information

1. Sex male ☐ female ☐
2. Age 5-14 ☐ 15-24 ☐ 25-34 ☐ 35 and above ☐
3. Residence urban ☐ rural ☐
4. Educational status illiterate ☐ primary ☐ secondary and above ☐
5. Your family's income per month $\leq$500 ☐ 500-1000 ☐ 1000-1500 ☐ 1500 an above

Hygienic related factors

6. Do you wash your hands before meal? Yes ☐ No☐
7. How often you wash your hands before meal?
   Usually ☐ Sometimes ☐ Never ☐
8. Do you wash your hands after going to latrine? Yes ☐ No☐
9. How often do you wash your hands after going to latrine?
   Usually ☐ Sometimes ☐ Never ☐

Water related factor

10. From where do you get water for drinking? Well☐ pipe☐ river☐ other ☐
11. Do you treat your drinking water? Yes ☐ No ☐
12. How often do you clean (empty) the storage container?
    Usually ☐ sometimes ☐ not at all ☐

Food related factors

13. Do you store cooked food for later use? Yes ☐ No ☐
14. Do you eat raw meat? Yes ☐ No ☐
15. Do you eat raw vegetables such as cabbage, lettuce? yes ☐ No ☐
16. Do you get enough food daily? Yes ☐ No ☐
17. Is it balanced diet? Yes ☐ No ☐

Knowledge of diarrhea

18. Do you know what expose you for diarrheal disease? Yes ☐ No ☐
19. Do you know the preventive methods of diarrhea? Yes ☐ No ☐

I. Data collection sheet after laboratory result of case group

1. Diarrhea- present ☐ no diarrhea (but other disease) ☐
2. Type of diarrhea- bloody ☐ non bloody ☐ Watery diarrhea ☐
3. Causative agent of the diarrhea _____
4. Level of CD4 T-cells _____

የጽሁፍ መጠይቅ /Amharic version/

በአዲስ አበባ ዩኒቨርስቲ ዞሎጂካል ሳይንስ ዲፓርትመንት
በምስራቅ ጎጃም ዞን በደብረ ማረቆስ ከተማ በደብረ ማርቆስ ሪፈራል ሆስፒታል ለሚታከሙ
የአ቎ አይ ቪ ቫይረስ ህሙማን የተዘጋጀ መጠይቅ

ውድ ታካሚዎች

ህሙማን ስለህመም መነሻ ምክንያቶችና ስለህመማቸው ምልክቶች የሚሰጧቸው መረጃዎች የበሽታቸውን መንስኤና የተጋላጭነት ደረጃ ለመለየት ጠቃሚ ናቸው። የዚህ መጠይቅ ዋና ዓላማ በአ቎ አይ ቪ ቫይረስ ተጠቅተውና ህሙማን ሆነው ሌሎች ተጨማሪ የጤና ችግሮች ያሉባቸው ታማሚዎች ለተቅማጥ በሽታ ያላቸው ተጋላጭነትና ለተቅማጥ የሚያጋልጡ መንስአ቏ችን በመለየት የታማሚዎችን የጤና ሁኔታ ለማሻሻል ነው። የጥናቱ ውጤታማነት የሚወሰነው እርስዎ በሚሰጡት መረጃ ትክክለኛ መሆኑን ተገንዘበው ትክክለኛ መረጃ እንዲሰጡ እያስገነዘብኩ የሚሰጡት መልስ ሚስጢራዊነቱ የተጠበቀ ስለሆነ ያለምንም ስጋት መልስዎን እንዲሰጡ እጠይቃለሁ። ውድ ጊዜዎን መስዋት በማድረግ ለዚህ መጠይቅ ስለተባበሩኝ ከልብ አመሰግናለሁ።

አጥኝው

<u>የአሞላል መመሪያ</u>

1. **መጠይቁ ላይ ስም መፃፍ አያስፈልግም**
2. **ለቀረቡት ጥያቄዎች የ " x " ምልክት በመጠቀም መልስዎን ይሰጡ**
3. **አስተያየት ለሚጠይቁ ጥያቄዎች በተሰጠው ክፍት (ባዶ) ቦታ ላይ ሀሳብዎትን ያስፍሩ።**

I. <u>ክፍል አንድ - ግላዊ መረጃ</u>

1. **ፆታ - ወንድ ☐ ሴት ☐**
2. **እድሜ - 5-14 ☐ 15 እና በላይ ☐**
3. **የመኖሪያ ቦታ - ከተማ ☐ ገጠር ☐**
4. **የትምህርት ደረጃ - ማንበብና መፃፍ የማይችል ☐ 1ኛ ደረጃ ያጠናቀቀ/ች ☐**
   2ኛ ደረጃና ከዚያ በላይ ☐

5. የወር ገቢ መጠን - ከ500 ብር በታች ☐ ከ500-1000 ብር ☐ ከ1000-1500 ☐ ከ1500 ብር በላይ ☐

II. <u>ክፍል ሁለት የጤና አጠባበቅ ሁኔታና ሌሎች ተዛማጅ ልምዶች</u>

6. ከምግብ በፊት እጅዎን ይታጠባሉ? አዎ ☐ የለም ☐
7. "አዎ" ከሆነ መልስዎ የመታጠብ ልምድዎት ምን ያህል ነው? ሁል ጊዜ ☐ አብዛኛውን ጊዜ ☐ አልፎ አልፎ ☐
8. ከመፀዳጃ ቤት መልስ እጅዎን ይታጠባሉ? አዎ ☐ አልታጠብም ☐
9. "አዎ" ከሆነ መልስዎ የመታጠብ ልምድዎ እንዴት ነው? ሁል ጊዜ ☐ አብዛኛውንጊዜ ☐አልፎ አልፎ ☐

III. ከውሃ ጋር ተዛማጅነት ያላቸው መጠይቆች

10. ለመጠጥ ውሃ የሚያገኙት ከየት ነው? ከጉድጓድ ☐ከቧንቧ ☐ ከወንዝ ☐
11. ለመጠጥ የሚጠቀሙበትን ውሃ ያክማሉ? አዎ ☐የለም ☐
12. የመጠጥ ውሃ ማጠራቀሚያ እቃዎችን ለምን ያህል ጊዜ ያፀዳሉ (ያጥባሉ)? በሳምንት አንዴ ☐ አልአልፍ ☐ አይፀዳም ☐

IV. ከምግብ ጋር ተዛማጅነት ያላቸው መጠይቆች

13. የተረፈዎትን ምግብ በማስቀመጥ ይጠቀማሉ? አዎ ☐ አልጠቀምም ☐
14. ጥሬ ስጋ ይመገባሉ? አዎ ☐ አልመገብም ☐
15. አትክልቶች /እንደ ጎመን ፣ ሰላጣ/ በጥሬው ይመገባሉ? አዎ ☐ አልመገብም ☐
16. በየቀኑ በቂ ምግብ ያገኛሉ አዎ ☐ የለም ☐
17. "አዎ" ከሆነ መልስዎ የተመጣጠነ ምግብ ነውን? አዎ ☐ አይደለም ☐

ቃለ መጠይቅ /interview/

18. ለተቅማጥ በሽታ የሚያጋልጥ ሁኔታ (መንስኤ) ያውቃሉ?_____

19. የተቅማጥ በሽታን መከላከያ ዘዴዎችን ያውቃሉ?_____

የሰገራ ምርመራ ውጤት:- ካርድ ቁጥር_____

1. ታማሚው ተቅማጥ አለው ☐ የለውም ☐

2. የተቅማጡ ደረጃ ደም የተቀላቀለበት (bloody) ☐

ደም የሌለው (non-bloody) ☐

ቀጭን ተቅማጥ (water diarrhea) ☐

3. ተቅማጡን ያመጣው ተውሳስ _____

_____

4. የCD_4 መጠን_____