ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCE
SCHOOL OF MEDICINE DEPARTMENT OF PATHOLOGY

Tuberculosis Lymphadenitis among HIV-positive individuals and its correlation to CD4 count, cytomorphological pattern and clinical Diagnosis in Mekell University Ayder Comprehensive Specialized Referral Hospital

By: Kesete Alemseged (BSc)
Advisor: Dr. Yonas Girma (MD, Pathologist)
Co-Advisor: Habtamu Hilekiros (Ass.Professor)

A thesis submitted to, Addis Ababa University, College of Health Sciences, School of medicine, Department of Pathology, in partial fulfillment of the requirements for Masters of Science in Histotechnology.

May.2020
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Acknowledgments

First and foremost my deepest blessing goes to my Almighty of GOD for his generosity of my healthy and wisdom. My heart full gratitude also goes to my advisor, Dr. yonas Girma (MD) pathologist for his guidance, supervision, critical evaluation of the thesis draft and immediate feedback with valuable comments and suggestions throughout the course of my work and his considerable follow-up and was on my side from the very beginning to this , I would also like to express my gratitude to my co-advisors Habtamu hailekiros (Ass.Professor) for their valuable inputs concerning proposal development, thesis analysis and encouragement throughout the study.

I also would like to express my thanks to the Department of pathology and laboratory staff members for their helpful and mature decision concerning the cooperation of sample processing and handling and material support. My thanks also go to Addis Ababa university for the financial support from the level of proposal development as well as to all staffs of Ayder comprehensive specialized referral hospital (ACSRH) Especially ART staff for the access they friendly provide me and kind cooperation during Data collection, sample receiving and processing in their department (unit) for their great and respectful help to me concerning the TBLN test in HIV patients service and to all my department staff members.

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List of abbreviations and acronyms

ACSRH – Ayder Comprehensive Specialized Referral Hospital

AIDS – Acquired immunodeficiency syndrome

ART – Antiretroviral therapy

BD FACSCalibur – Becton Dickenson fluorescence-activated cell sorter

CD4 – human T helper cells expressing CD4 antigen (T helper cell)

EPTB – extrapulmonary tuberculosis

FNA – Fine needle aspiration

FNAC – Fine needle aspiration cytology

HAART – Highly activated antiretroviral treatment

HIV – human immune deficiency virus

LAP –lymphadenopathy

MDR-TB – multidrug resistance tuberculosis

MTB – mycobacterium tuberculosis

PI – principal investigator

PLWHA – people living with HIV/AIDS

PTB – pulmonary tuberculosis

SERO – scientific and ethics research office

SPSS – statistical package for social sciences

TB – Tuberculosis

TBLN (LNTB) – lymph node tuberculosis

WHO – World Health Organization

PCR – polymerase chain reaction

Abstract

Background: Tuberculosis is the second leading cause of death from infectious disease globally with its impact more dramatic in resource limited settings. Individuals with human immunodeficiency virus infection who also develop tuberculosis represent a significant challenge to TB control. HIV not only increases the number of TB cases, but also alters the clinical course of TB disease. As HIV related immunosuppression increases, the clinical pattern of TB changes, with increasing numbers of smear-negative and extrapulmonary cases. Lymphadenopathy is one of the most common manifestations at any stage of HIV/AIDS with different underlying pathogenesis. Tuberculous Lymphadenitis is the most common form of extrapulmonary TB and typically presents with asymmetric involvement of multiple nodes 1 cm or larger in size. Cervical and axillary nodes are most frequently affected.CD4 T cells play central roles in the function of the immune system and are important mediators of immunologic memory,

Objective: the objective of this study is to determine the prevalence of tuberculosis lymphadenitis infection among HIV/AIDS positive patients and its correlation to CD4-count, morphological patterns and clinical Diagnosis.

Method: A cross sectional study design was conducted from January 10/2019 to July 10/2019. A total of 90 HIV positive patients who develop lymph node were enrolled into the study by contacting the patients during their visit to the ART clinic. Socio -demographic characteristics and other variables were collected using a pretested semi-structured questionnaire. Detailed clinical history and laboratory investigations were considered in all patients. The statistical software and methods including the level of statistical significance were explained and employed for data analysis and interpretation. The results were then analyzed statistically using the statistical package for social sciences (SPSS) version 23 for Windows.

Result: Maximum number of tuberculosis lymphadenitis is seen in the age group of 21-30 years followed by age group of 31-42 and with a decreasing trend in elderly. There was a female preponderance of cases. Most cases of Tuberculosis lymphadenitis emerged as the 2nd most frequent Cytomorphological pattern of diagnosis next to reactive lymphoid hyperplasia to be associated with HIV infection With the typical caseous necrosis and Epitheloid granuloma.CD4 count <200/mm3 and WHO Severe clinical stage of the disease were found to be predictors of tuberculosis lymphadenitis and cervical and axillary group of lymph node were found to be the

most involved site. Finally Tuberculosis lymphadenitis is over diagnosed clinically compared to Cyto-morphological Diagnosis.

Conclusion; Caseous necrosis and Epitheloid granuloma were leading causes of Tuberculosis lymphadenitis .CD4 count <200/mm3 and Sever clinical stage (stage IV) of the disease were found to be predictors of tuberculosis lymphadenitis. So initiation of Anti-retroviral treatment before the CD4 count drops below 350 /mm3 should be encouraged and intervention aimed at preventing and treating HIV Associated tuberculosis lymphadenitis is crucial.

The cervical and auxiliary region was found to be the most involved site respectively and Tuberculosis lymphadenitis is over diagnosed in clinical Diagnosis compare to Cyto-morphological Diagnosis suggesting the need of diagnostic test(s) like cytomorphological examination to supplement the clinical examinations.

Recommendation: cytologic examination of fine needle aspirates should be included in the diagnostic work-up of any patient suspected for TBLN in health institutions with limited laboratory capacity like regional or district Hospitals. Intervention aimed at preventing and treating HIV associated Tuberculosis lymphadenitis are crucial. High quality services should provide by incorporating an efficient quality control system and a molecular technique that detects DNA sequences specific for Mycobacterium tuberculosis by polymerase chain reaction can be used as screening test. In addition since culture is considered as golden standard emphasis should be given in detection of mycobacterium tuberculosis from lymph node aspiration.

1. INTRODUCTION

Tuberculosis (TB) has remained a major health problem globally, affecting millions of people every year. It has been ranked as the second leading cause of death from an infectious disease worldwide. Tuberculosis and infection with HIV constitute the major burden of infectious diseases in resource-limited regions such as African and Asian countries. The World Health Organization (WHO) in 2014 estimated that there are over 9 million new active cases of tuberculosis resulting in 1.5 million TB deaths per year. Over one quarter of these deaths are found in human immunodeficiency virus (HIV) positive individuals *(1).*

Tuberculosis (TB) is the second leading cause of death from infectious disease globally with its impact more dramatic in resource limited settings. Individuals with human immunodeficiency virus (HIV) infection who also develop tuberculosis represent a significant challenge to TB control. When both infections occur in the same individual, this coinfection can result in diagnostic and therapeutic challenges due to the peculiar clinical picture that is seen with TB–HIV coinfection. TB–HIV coinfection is prevalent in Africa as the WHO report of 2014 shows that about 80% of the 1.1 million TB patients who were HIV-positive were from the African region *(1).*

Tuberculosis is the most common opportunistic infection leading killer of people living with HIV/AIDS. The risk of developing tuberculosis (TB) is estimated to be between 26 and 31 times greater in people living with HIV than among those without HIV infection. HIV not only increases the number of TB cases, but also alters the clinical course of TB disease. As HIV related immunosuppression increases, the clinical pattern of TB changes, with increasing numbers of smear-negative and extrapulmonary cases. Delayed diagnosis may be an important cause of excess mortality in people living with HIV who have smear-negative pulmonary and extrapulmonary tuberculosis. EPTB constituted about 15 to 20 percent of all cases of TB in general practice. EPTB can account for up to 53 to 62 percent of cases of TB in HIV-positive individuals *(2).*

The guideline of the World Health Organization to initiate HAART at CD4 T-cell count ≤ 350/μL should therefore be followed strictly to prevent TB or other opportunistic infections *(2).*

Qualitative and quantitative defects of CD4+ T-lymphocytes explain the inability of HIV-infected individuals to contain mycobacterial proliferation. (14)The risks of disseminated disease become higher as the CD4+ count falls. A clear association has been reported between low CD4+ count and an increased frequency of extrapulmonary TB (EPTB), positive *(2).*

Extrapulmonary manifestations of TB are more common in the setting of HIV coinfection and have been shown to increase with progressive immune deficiency. With advanced immune suppression (CD4 cell count < 100/μL) extrapulmonary TB occurs in up to 70% of patients and often involves multiple sites. Extrapulmonary sites are more often involved among patients with immunosuppression *(6).*

Extrapulmonary tuberculosis (EPTB) refers to infection of Mycobacterium tuberculosis at body sites other than the lungs. Lymph node TB (LNTB) is one of the most common manifestations of EPTB that affects, most frequently, the peripheral and cervical lymph nodes. EPTB is reported to occur in isolation or along with the more frequent clinical presentation of TB, pulmonary TB (PTB). EPTB prevalence is reported from 15 to 20% of all cases of TB in endemic countries and accounts for more than 50% cases in immune compromised individuals. In 2012, 0.8 million out of total 6.1 million notified TB cases had EPTB *(7).*

The prevalence of EPTB among notified TB cases in African region in 2017 was 16%, which is the second highest next to Eastern Mediterranean region (24%), which is more than global prevalence of EPTB (15%). The lowest prevalence was reported from Western Pacific region (13%), (37). The proportional rise in EPTB cases has been associated with the human immunodeficiency virus (HIV)/AIDS epidemic because there is increased susceptibility for reactivation and dissemination of tuberculosis in these patients*(9).*

Lymphadenitis is the most common form of extrapulmonary TB and typically presents with asymmetric involvement of multiple nodes 1 cm or larger in size. Cervical and axillary nodes are most frequently affected, occurring in 99% and 82%, respectively. Inguinal and epitrochlear nodes are also commonly involved (11). Tubercular lymphadenitis is seen in nearly 35% of extra pulmonary TB (Extra Pulmonary TB constitutes 15-20% of all cases of TB). Further in patients with human immunodeficiency virus (HIV) infection extra pulmonary TB constitutes almost 53-62% of TB. Common extrapulmonary sites include lymph nodes (superficial) and pleura; less commonly, the brain, pericardium, meninges, and abdomen are affected *(12).*

The CD4 cell count is an important investigation in the clinical evaluation of any patient with HIV infection, as it helps to decide the stage of HIV disease. It also assists in differential diagnosis and in making therapeutic decisions regarding antiretroviral treatment and prophylaxis against opportunistic infections." Serial CD4 counts have a prognostic significance and are used as markers for assessing progression from HIV infection to AIDS *(14).*

HIV infection among patients with tuberculosis ranges from 50% to 80% in many settings in sub Saharan Africa followed by the Southeast Asia region (mainly India), which had 11% of total cases *(10).* The World Health Organization (WHO) has classified Ethiopia 7th among the 22 high burden countries with TB and HIV infection in the world *(17).*

Cytomorphological diagnoses of tuberculous Lymphadenitis were grouped into four categories: Epitheloid granulomas with caseous necrosis, Epitheloid granulomas without necrosis, necrosis only without Epitheloid granulomas and polymorphs with necrosis with or without Epitheloid granulomas *(28).*

2. LITERATURE REVIEW

Tuberculosis (TB) is the second leading cause of death from infectious disease globally with its impact more dramatic in resource limited settings. Individuals with human immunodeficiency virus (HIV) infection who also develop tuberculosis represent a significant challenge to TB control. This study was carried out to determine the prevalence of TB–HIV coinfection and pattern of infection among TB patients. We also compared treatment outcome among coinfected patients with those not coinfected. A six-year retrospective review of records of patients in Nigeria from January 2009 to December 2014 was carried out. One hundred and five (26.3%) of the 399 TB patients seen in the study period were coinfected with HIV. About 10% of the subjects had extrapulmonary tuberculosis. Treatment failure was significantly worse among patients who had both HIV and TB compared with those who had TB only (49.5% vs. 32%, p = 0.001). Death rate was also higher in the coinfected individuals implying a poorer clinical outcome. High prevalence of TB–HIV coinfection and poor treatment outcome in this group of individuals, though predictable, calls for a more concerted effort in the management of TB–HIV coinfection *(1).*

TB is the most common opportunistic infection and a leading killer of people living with HIV/AIDS (PLWHA). HIV not only increases the number of TB cases, but also alters the

clinical course of TB disease. As HIV related immunosuppression increases, the clinical pattern of TB changes, with increasing numbers of smear-negative and extrapulmonary cases. Common presenting symptom in this study was fever (91%), cough (65%), anorexia (62%), weight loss (61%), diarrhea (58%) and breathlessness (36%). Out of total 100 study participants, 54 were having pulmonary tuberculosis and 46 were having extrapulmonary tuberculosis. In extrapulmonary TB Spleen (41%) was most commonly involved followed by Lymph node (39%), tuberculous pleuricy (12%) and meningitis (8%). Among pulmonary TB patients maximum number of patients 24 (44.44%) were having CD4 count 100 - 199 per mm3. Among Extrapulmonary TB patients, maximum number of patients 28 (60.87%) were having CD4 count 100 -199 per mm3 *(2).*

Tuberculosis (TB) is a cause of 1.2-1.5 million deaths worldwide, including deaths from TB among HIV positive people. Determining the extent of immune cells belonging to cell mediated immunity and hematological parameters is critical to maximize the potential benefit of anti-tubercular treatment and case management. From consecutively enrolled 108 TB patients, pulmonary TB (PTB) accounted for 48(44.4%), TB lymphadenitis accounted for 48(44.4%), and disseminated/miliary TB accounted for 12(11.1%). Analysis of variance revealed that mean + SD of CD4 count of male TB patients (650 + 224cells/µl) was significantly lower than male control group (883 + 256 cells/µl) (p= 0.001). In a similar manner, the mean CD4 count of female TB patients (793 + 332cells/µl) was lower than female control group (975 + 300 cells/µl) (p=0.001). There was no statistically significant difference in CD8 counts between cases and controls for both genders. Forty (37.0%) CD4 lymphopenia was significant among TB patients. Granulocyte count was increased. Mild anemia was found major hematological abnormality among newly diagnosed TB patients *(3).*

The human immunodeficiency virus (HIV) epidemic has led to an increase in the incidence of tuberculosis globally, particularly in sub-Saharan Africa. Coinfection with HIV leads to difficulties in both the diagnosis and treatment of tuberculosis. Because of the poor performance of sputum smear microscopy in HIV-infected patients, more sensitive tests such as liquid culture systems, nucleic acid amplification assays, and detection of mycobacterial products in various body fluids are being investigated. The treatment of coinfected patients requires anti tuberculosis and antiretroviral drugs to be administered concomitantly; challenges include pill burden and patient compliance, drug interactions, overlapping toxic effects, and immune reconstitution

syndrome. Both multidrug-resistant and extensively drug-resistant tuberculosis can spread rapidly among an immune compromised population, with resulting high mortality rates. Current guidelines recommend starting antiretroviral treatment within a few weeks of anti-tuberculosis therapy for patients with CD4 cell counts<350 cells/mL; however, important questions about the drug regimens and timing of antiretroviral therapy remain. Ongoing trials may answer many of these unresolved questions *(10).*

The sub-Saharan Africa bears the brunt of being the region with the highest burden of both Human Immunodefiency Virus (HIV) infection and of tuberculosis (TB). While only 10% of immunocompetent individuals infected with Mycobacterium tuberculosis go on to develop active disease in their lifetime, 50% of those co-infected with HIV develop active TB. Qualitative and quantitative defects of CD4+ T-lymphocytes explain the inability of HIV-infected individuals to contain mycobacterial proliferation. Similarly, TB also accelerates the progression of HIV infection. The clinical and radiological presentation of TB in patients infected with the HIV virus may be different and atypical posing significant diagnostic challenges. This is compounded by the dearth of diagnostic facilities in sub-Saharan Africa. The initiation of antiretroviral therapy (ART) during anti-TB therapy significantly improves survival of TB/HIV co-infected persons. There are challenges in treatment of HIV associated TB because of overlapping drug toxicities, pill burden and suboptimal adherence, drug-drug interactions between ART and anti-TB therapy as well as timing of ART. Of particular importance are the immune reconstitution inflammatory syndrome (IRIS) and the emergence of multi-drug resistant and extensively drug resistant TB. Centre's for tuberculosis diagnosis and treatment and for HIV care and treatment need to be integrated. This has not been so successful in sub-Saharan Africa. In spite of sustained support by donor organizations, a substantial number of HIV-TB co-infected individuals remain undiagnosed and are poorly managed. This review focuses on the epidemiology and pathogenesis of HIV-TB co-infection and the special areas of difficulty in the diagnosis and treatment of the dual infection. Emphasis is placed on the peculiarities of management in sub-Saharan Africa, the region with the highest burden of both infections *(14).*

Fine-needle aspiration cytology (FNAC) of 32 HIV-positive cases presenting with lymphadenopathy was performed to evaluate its role in this group of patients. For each case air-dried smears were stained with MGG, and Ziehl-Neelsen stains for acid fast bacilli (AFB). The results were tuberculous (TB) lymphadenopathy (15), reactive lymphadenopathy (10), acute

lymphadenitis/abscess (5), and suspected malignancy (2). In seven cases of TB lymphadenitis findings were suggestive of TB since no AFB was demonstrable on the cytology smears. In TB lymphadenitis, two additional cytomorphologic patterns besides necrotizing granulomatous (4) and granulomatous (2) were observed. These were necrotizing (6) and necrotizing suppurative (3) patterns. FNAC is a simple, accurate, inexpensive, rapid and safe investigative procedure which can reduce surgical excisions and provide definite guidelines about further management.

A total of 32 HIV-positive cases presenting with lymphadenopathy were studied. There were 18 males and 14 females in the age range 18–50 yr. Lymphadenopathy sites aspirated were cervical (17), axillary (8), submandibular (3), inguinal (2), and intra-abdominal (2). The diagnosis made on FNAC is shown in Table I and the details of the 15 cases of TB lymphadenitis which formed 47% of the total cases is shown in Table II. In three of the four cytomorphologic patterns used in the diagnosis of TB lymphadenitis caseous necrosis was evident with demonstration of the bacilli on AFB (eight cases). In neither of the two cases of granulomatous lymphadenitis (GL) the bacilli was not identified on AFB stain. In these two cases of GL and also in the AFB negative cases (five) of the other patterns, a diagnosis suggestive of TB lymphadenitis was offered. There were 10 (31.2%) cases of reactive lymphoid hyperplasia. In eight cases the smears showed a heterogenous population of small and large lymphocytes along with a few tingible body macrophages and plasma cells. In two cases cellularity was scanty and there was a predominance of small lymphocytes. In five (15.6%) cases where smears showed a large number of polymorphs with minimal necrosis, and with absence of AFB the diagnosis given was acute lymphadenitis/ pyogenic abscess. Malignancy was suspected in two cases and biopsy was advised *(29).*

A study in Butajira, Southern Ethiopia A total of 136/161 (84.5%) patients were diagnosed with TBLN by histology. TBLN was culture confirmed in 107/156 (68.6%) patients. The sensitivity, specificity, positive and negative predictive values of histology was respectively 92.5%, 49%, 79.8% and 75% when compared to culture as gold standard. Patient's positive for TBLN by cytology and Ziehl-Neelsen (ZN) were also positive by histology and culture. Among the 143 confirmed TBLN patients, nine (6.3%) were HIV positive. Of the 1608 healthy individuals, 77 (4.8%) were HIV-positive. Younger age (P = 0.0001), female sex (P = 0.016), not being married (P = 0.0001) and illiteracy (P = 0.016) showed a strong association with HIV in healthy individuals. Clinical criteria alone over-diagnosed TBLN by 15.4% compared to histological and/or bacteriological results *(30).*

TBLN (Mycobacterial lymphadenitis), also known as scrofula, has been recognized for at least 3000 years. Hippocrates described scrofula in ancient Greece. In medieval Europe, scrofula was known as the ‘‘King’s Evil’’ because certain monarchs would touch patients to ‘‘cure’’ the disease. In the 19th century, after Koch demonstrated mycobacteria in lymph nodes, scrofula was finally recognized to be a consequence of tuberculosis (TB). For most of the 20th century, lymphadenitis was treated with wide excision and prolonged anti tuberculous therapy. Only in the modern era, with the introduction of effective short course rifampin-containing chemotherapy has tuberculous lymphadenitis become an easily treatable medical disease *(34).*

TBLN is the most common form of extrapulmonary TB Lymph node tuberculosis (LNTB), also known as tuberculous. Its epidemiology and diagnostic aspects vary according to the patient’s geographic origin and the burden of TB and human immunodeficiency virus (HIV) infection in the country of origin. In sub-Saharan African countries, with generalized HIV epidemics and high TB incidence, all types of active TB have increased tremendously in the last 15 years *(35).*

Mycobacterial culture is generally considered to have the highest sensitivity for detection of Mycobacterium tuberculosis (MTB), and is generally used for reference in evaluation of alternative techniques it is also the only method that can give information on antibiotic sensitivity patterns. However, wide- spread use of culture is compromised by protracted result delivery high upfront cost and strict requirement of advanced biosafety precautions. Furthermore, culture results may be affected by antibiotic therapy, as well as storage and transport conditions. In resource-limited settings, mycobacterial culture therefore mainly has a role for surveillance and for cases with suspected drug resistance (45).

In most developing countries culture, isolation, identification and susceptibility testing of M. tuberculosis are usually carried out in a Reference Tuberculosis Laboratory to manage treatment failures and patients that have relapsed, to monitor multi-drug resistance, and to identify M. tuberculosis variants and strains, e.g. M. bovis, for epidemiological purposes. As more rapid and less expensive culture and other techniques are developed for detecting, identifying, and susceptibility testing of M. tuberculosis, it may be possible to detect tuberculosis at an earlier stage of infection when AFB are too few to be detected in direct sputum smears. Culture is considerably more sensitive than microscopy, detecting 10–100 viable organisms/ml of sputum.

M. tuberculosis will grow aerobically on a protein enriched medium, e.g. Lowenstein Jensen egg

Medium. The optimal temperature for growth is 35–37 ºC. The organism is slow-growing. When cultured on Lowenstein Jensen medium at 35–37 ºC, M. tuberculosis produces raised, dry, cream (buff) coloured colonies. Visible colonies are usually produced 2–3 weeks after incubation (49).

2.1. Epidemiology

The World Health Organization (WHO) in 2014 estimated that there are over 9 million new active cases of tuberculosis resulting in 1.5 million TB deaths per year. Over one quarter of these deaths are found in human immune deficiency virus (HIV) positive individuals worldwide. The prevalence of TB– HIV coinfection globally A report which revealed the case fatality rate for TB in South Africa to be twice that of Brazil attributed it mainly to the high HIV infection rate in South Africa compared to Brazil, proposing that high death rates are associated with TB–HIV coinfections is about 13% but much higher in African countries which have the highest burden of HIV infections. TB–HIV coinfection is prevalent in Africa as the WHO report of 2014 shows that about 80% of the 1.1 million TB patients who were HIV-positive were from the African region. Although pulmonary tuberculosis is the commonest TB manifestation, those who are HIV positive are more likely to have extrapulmonary TB. Extrapulmonary TB is seen in 40–80% of TB–HIV infection but in only 10–20% of patients without co infection *(1).*

A six-year retrospective review of records of patients managed at the Tuberculosis Treatment Center of the LAUTECH Teaching Hospital, South-Western Nigeria from January 2009 to December 2014 was carried out. One hundred and five (26.3%) of the 399 TB patients seen in the study period were coinfected with HIV. About 10% of the subjects had extrapulmonary tuberculosis (1).

Hospital data of the Ministry of health in Ethiopia show that TB is the leading cause of morbidity, the third cause of hospital admission, and the first cause of hospital death in Ethiopia. According to WHO Global Report of 2011, Ethiopia ranked 7th among the high TB burden countries in the world, with an estimated incidence of all forms of TB of 261/100,000 population/year. The estimated prevalence of all forms of TB reported was 394/100,000 population (3).

Globally, an estimated 33.2 million (30.6 million to 36.1 million) people were living with human immunodeficiency virus (HIV) infection in 2008. Southern Africa continued to bear a disproportionate share of the global burden of HIV: 35% of HIV infections and 38% of AIDS

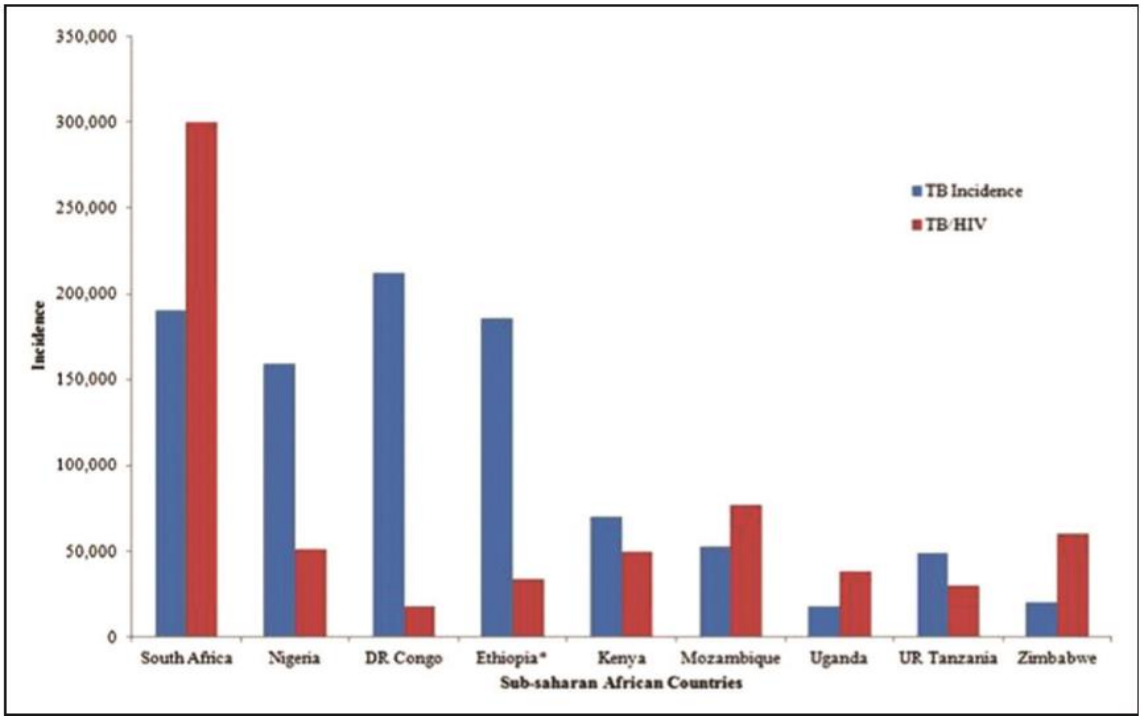
deaths in 2007 occurred in this sub region. The African region accounted for most HIV-positive tuberculosis cases (79%), followed by the Southeast Asia region (mainly India), which had 11% of total cases. Although the prevalence of HIV infection among patients with tuberculosis ranges from 50% to 80% in many settings in sub Saharan Africa, in other parts of the world it varies from 2% to 15% (10).

Sub-Saharan Africa remains the region most heavily affected by HIV. In 2010, about 68% of all people living with HIV resided in sub-Saharan Africa, a region with only 12% of the global population. South Africa's HIV epidemic remains the largest in the world and with an estimated 5.6 million people living with HIV while Nigeria continues to have the second largest number of people living with HIV in sub-Saharan Africa. Both countries are listed among the 22 high TB burden countries (14).

Although the total number of cases of TB continues to decline within the United States, the number of exclusively extrapulmonary cases of TB is declining much less rapidly and currently accounts for approximately 20% of all cases. Thirty to 50% of all extrapulmonary cases of TB involve the peripheral lymph nodes. Lymphadenopathy occurred most frequently in the neck (57%) or supraclavicular area (26%) and involved 1–3 nodes. (22).

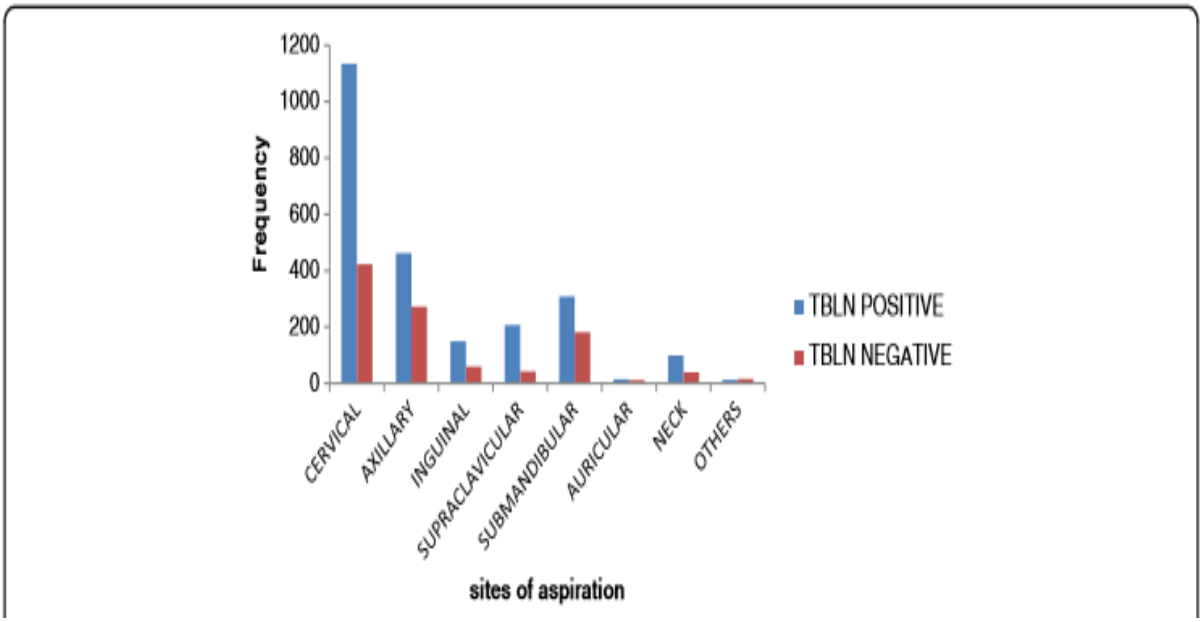
The prevalence of HIV seropositivity in Butajira, Southern Ethiopia study subjects and in the laboratory-confirmed TBLN cases was respectively 10/171 (5.8%) and 9/143 (6.3%). This prevalence is lower than previously reported in Ethiopia. A study from Southern Ethiopia reported a prevalence of HIV of respectively 15%, 30% and 11% in rural and urban areas and among EPTB cases (30).

Figure1; Burden of HIV-associated tuberculosis in 9 sub-Saharan African countries.



(14)

Figure 2; Prevalence of tuberculous lymphadenitis by Site of aspiration



(47)

2.2. HIV

Natural history of HIV infection

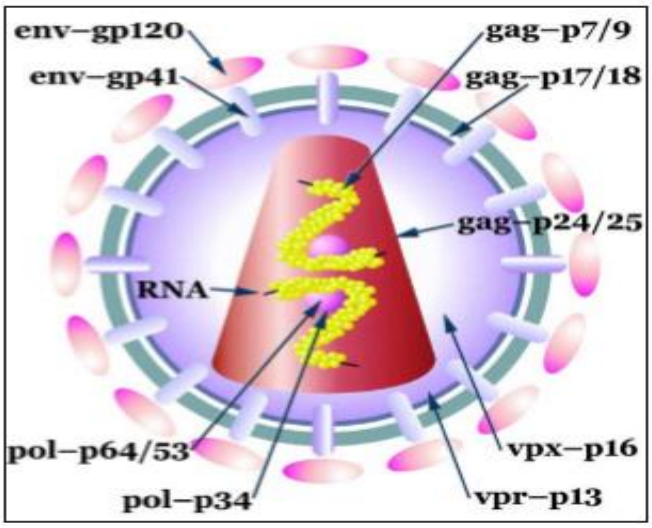
Magnitude of problem

The HIV/AIDS pandemic has severely affected health development and eroded improvements in life expectancy, particularly in countries with the highest prevalence of infection. Since the first cases of acquired immunodeficiency syndrome (AIDS) were reported in 1981, infection with human immunodeficiency virus (HIV) has grown to pandemic proportions, resulting in more than 65 million infections and 25 million deaths. At the end of 2006, an estimated 39.5 million people were living with HIV, with Sub-Saharan Africa carrying the highest burden *(31).*

In 2006, an estimated 4.3 million people became newly infected with HIV. More than 95% of these new infections are in low and middle income countries. Each day 11,000 persons become newly infected with the virus; of these, half are women and 40% are young people (15-24 years old). Of the estimated 37 million adults living with HIV worldwide, nearly 18 million are women.

At the end of 2006, there were an estimated 2.3 million children living with HIV. The increasing number of child deaths due to AIDS threatens to reverse many of the recent gains of child survival programmes. Moreover, the socio-economic impact of HIV/AIDS on children is profound. As their parents fall sick and die of AIDS, children undergo a long trail of painful experiences such as: economic hardship, withdrawal from school; lack of love, attention and affection; psychological distress, stigma, discrimination and isolation, and malnutrition and illness. Cumulatively, there are a total of 16 million children worldwide who have become orphans because their parents died of AIDS *(31).*

Figure 3: Internal Structure of HIV

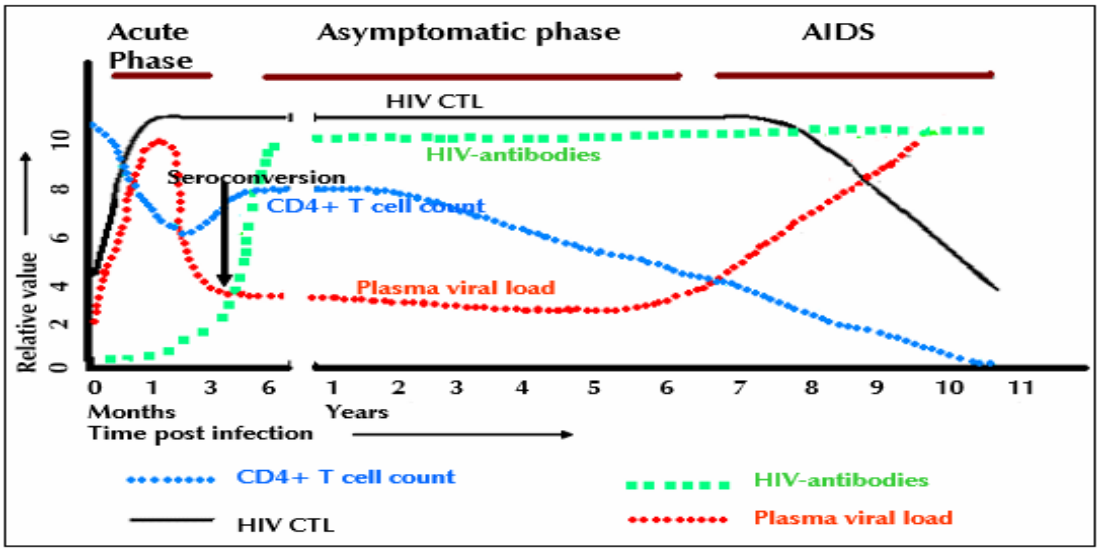


(48)

Pathogenesis of HIV

Natural history of HIV-1 infection encompasses an acute/ primary phase that lasts for months, followed by a clinically latent phase that typically lasts for few years and ultimately by the collapse of immune system that characterized AIDS. Pattern of commonly detected HIV-specific immune responses and plasma viral RNA level is shown in Fig. 4. (31) .

Figure 4: Immunological and virological events in natural course of HIV infection



(48)

AIDS Stage

The gradual decrease in CD4 T lymphocytes cells (below 200 cells/mm3) ultimately results in loss of control over the immune response and various opportunistic infections start appearing. This is the terminal stage of HIV infection and called as AIDS. Common opportunistic infections include Pneumocystis carinii1 pneumonia, cryptococcal meningitis, and oral and oesaphageal candidiasis. Recurrent activation of Herpes zoster and malignancies such as non-Hodgkin's lymphoma are more commonly seen during this phase. Symptoms such as night sweats, fever, diarrhea, profound weight loss and fatigue are commonly seen during this period *(31).*

Factors influencing HIV disease progression

The rate of progression of HIV disease is usually influenced by a number of factors. Prominent among those are age at acquisition of HIV infection, genetic factors, other diseases such as tuberculosis and hepatitis, nutrition etc.

Genetic factors also influence the disease progression. One of the prominent example is mutation in CCR5 gene. Heterozygosity for A32 mutation in CCR5 gene is associated with slow disease progression. HLA alleles such as B27 and B57 are associated with slower progression of the disease. The influence of genetic factors on the disease progression has not yet been fully understood.

Besides genetic factors coinfections such as tuberculosis, Hepatitis B and helminthic infections also accelerate the disease progression. Malnutrition and vitamins and micronutrient deficiencies may also be associated with rapid progression to AIDS *(13).*

WHO clinical staging of HIV-related disease in adults and adolescents aged 15 years or more

The revised WHO clinical classification of HIV-associated disease is designed to be used in patients with confirmed HIV infection. Along with measurement of the CD4 T lymphocytes count, where available, the staging system is used to guide decisions on when to start prophylaxis for opportunistic infections (OI) and when to start and switch antiretroviral therapy (ART) (31).

Table 1 . WHO clinical Staging of established HIV infection

HIV-associated symptoms	WHO clinical stage
Asymptomatic	1
Mild symptoms	2
Advanced symptoms	3
Severe symptoms	4

(46)

Table 2 . Revised WHO Clinical staging of HIV related disease in adults and adolescents aged 15 years or more

Clinical stage 1
Asymptomatic Persistent generalized lymphadenopathy
Clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)[1] Recurrent respiratory tract infections sinusitis, tonsillitis, otitis media and pharyngitis) Herpes zoster Angular cheilitis Recurrent oral ulceration Papular pruritic eruptions Seborrhoeic dermatitis Fungal nail infections

Clinical stage 3
Unexplained[i] severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than one month
Unexplained persistent fever (above 37.6°C intermittent or constant, for longer than one month)
Persistent oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis (current)
Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis or bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis
Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 10^9 per litre) or chronic thrombocytopaenia (<50 × 10^9 per litre)
Clinical stage 4[ii]
HIV wasting syndrome
Pneumocystis pneumonia
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis
Kaposi's sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extrapulmonary cryptococcosis including meningitis
Disseminated non-tuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis (with diarrhoed)
Chronic isosporiasis
Disseminated mycosis (coccidiomycosis or histoplasmosis)
Recurrent non-typhoidal Salmonella bacteraemia
Lymphoma (cerebral or B-cell non-Hodgkin) or other solid HIV-associated tumours
Invasive cervical carcinoma
Atypical disseminated leishmaniasis
Symptomatic HIV-associated nephropathy or symptomatic HIV-associated cardiomyopathy

(46)

2.3. CD4 T lymphocytes in disease progression

The CD4 T lymphocytes; a subpopulation of the lymphocytes also known as T helper cells, are coordinators of the body's immune response, e.g., providing help to B cells in the production of antibody, as well as in augmenting cellular immune response to antigens.

The "CD" or cluster of differentiation is a protein expressed on the surface of the cells of the hematopoietic system. The expression of these proteins is used in lymphocyte nomenclature. Over 300 CD molecules have been reported so far. These proteins are often associated with the specific function of the cells. Cells with different functions express different CD molecules (for eg: CD3+ cells are total T lymphocytes, CD4+ cells are T helper cells, CD8+ cells are cytotoxic T lymphocytes and CD19+ are B lymphocytes) *(31).*

CD4 T lymphocytes occupy the central position in regulating immune functions. CD4 T lymphocytes are the primary targets of HIV. The relentless destruction of CD4 T lymphocytes by HIV, either directly or indirectly, results in the loss of HIV-specific immune response, recall antibody response and, finally, non-specific immune response in the AIDS stage *(31).*

CD4 T lymphocytes and HIV

Within hours of exposure to HIV, CD4 T lymphocytes are found to be infected showing active viral replication. The infected CD4 cells release virions by budding through the cell membrane or by lysis of the infected cells. The released virus particles then infect uninfected CD4 T lymphocytes. CD4 T lymphocytes also serve as important reservoirs of HIV: a small proportion of these cells carry HIV provirus integrated in the host DNA without active virus multiplication.

During the primary HIV infection, the number of CD4 T lymphocytes in the bloodstream decreases by 20% to 40%. HIV brings about the lysis of HIV infected cells *(31).*

Laboratory guidelines for enumerating CD4 T lymphocytes in the context of HIV/AIDS

Infected cells as well as bystander uninfected cells enumerate using various mechanisms such as lysis of the cells infected with HIV. Billions of CD4 T lymphocytes may be destroyed every day, eventually overwhelming the immune system's regenerative capacity. In acute HIV-1 infection, in addition to the decline in CD4 T lymphocytes counts, qualitative impairments of CD4 T lymphocytes function are detected. The impairment of HIV-1-specific CD4 T lymphocytes function occurs very early in acute infection. Following acute primary HIV infection, one may remain free of HIV-related illnesses, often for years, despite ongoing replication of HIV in the lymphoid organs and relentless destruction of the immune system. However, during the period, the immune system remains sufficiently competent to provide immune surveillance and to prevent most infections. Although the decrease in the total number of T lymphocytes marks the decrease in immune competence, sometimes the quantitative loss of CD4 T lymphocytes may not be matched by the qualitative functions. A number of assays such as cytokine induction, antigen induced proliferation, measurement of activation markers etc. can assess the functions of lymphocytes. However, the total CD4 T lymphocytes number still remains the most robust marker of immune competence *(31).*

The progressive loss of CD4 T lymphocytes eventually results in the loss of an ability to mount desirable immune response to any pathogen and vulnerability to opportunistic pathogens

characteristic of AIDS. The estimation of peripheral CD4 T lymphocytes counts is relied upon for taking a decision on initiation of ART (31).

Figure 5: FACS Count micro bead-based system

(48)

The estimation of peripheral CD4 T lymphocytes counts has also been used as a tool for monitoring disease progression and the effectiveness of antiretroviral treatment (ART). The changes in the CD4 T lymphocytes counts are important indicators of the response to ART. HIV plasma virus load is a sensitive indicator of the progression of HIV disease. However, due to the relatively high cost of virus load estimation, the CD4 T lymphocytes count remains the most important key indicator for initiation and monitoring of ART and a measure of the effectiveness of the treatment in clinical trial evaluations.

Table 3. CD4 levels in relation to the severity of immunosuppression

Immune status	Age		
	Up to 12 months	13-59 months	5 years or over
Not significant immunosuppression	>35%	>25%	>500/mm^3
Mild immunosuppression	25–34%	20–24%	350–499/mm^3
Advanced immunosuppression	20–24%	15–19%	200–349/mm^3
Severe immunosuppression	<20%	<15%	<200/mm^3

(39)

Table 4: Starting antiretroviral therapy by CD4T lymphocytes count

WHO clinical staging	CD4 count available
1	Treat if CD4 count <200 cells/mm^3
2	
3	General principles • Consider treatment if CD4 count <350 cells/mm^3 but initiate before CD4 count drops below 200 cells/mm^3 In the case of pregnancy or TB • Start ART in all HIV-infected pregnant women with WHO stage 3 disease and CD4 count <350 cells/mm^3 • Start ART in all HIV-infected patients with CD4 count <350 cells/mm^3 and pulmonary TB (WHO stage 3) or severe bacterial disease
4	Treat irrespective of CD4 count (extrapulmonary TB is WHO stage 4 disease)

(48)

2.4. Lymphadenopathy the main symptom of HIV infection

Lymphadenopathy (LA) is usually translated as a 'lymph gland disease'. Typically the affected lymph nodes are swollen so the meaning of this definition has transformed to 'the enlarged lymph nodes'. So LA is an enlargement of the LNs of more than 2 cm and this symptom must be present for at least 3 months in two or more LNs. However the term LA refers to the lymph nodes that are abnormal not only in size, but also in consistency, location and mobility *(9).*

A simple and clinically useful system of classification of LA is to identify localized LA and the generalized LA. The localized LA is when lymph nodes are abnormal only in one region of the body. The generalized LA can be stated when the lymph nodes are enlarged or abnormal in two or more noncontiguous areas. The generalized LA is an indication that a systemic disease is present, so further investigation is therefore necessary. Distinguishing between the localized and the generalized lymphadenopathy is a very important step in the evaluation of a patient and helps to determine the differential diagnosis.

Lymphadenopathy is frequently a symptom deriving from an obvious cause, but in some cases, when the root of the abnormal lymph node or lymph node group is less clear further diagnostic investigation should take place. It is a diagnostical challenge to be able to distinguish between a self-limiting LA and a LA that is caused by a serious disease *(9).*

The majority of localized lymph nodes are seen in the head and neck region, less in inguinal region and seldom in axillary lymph node groups. A supraclavicular localized LA is frequently identified as a malignant enlargement so further investigation of this type of localized LA is essential. Basically when a localized LA is identified further physical examination becomes crucial not to miss a generalized LA and to be able to diagnose the abnormal lymph nodes in other regions of the body. Due to the fact that the generalized LA is an indication of a systemic disease a biopsy is an absolute necessity when the reason for the LA is not clear after the initial evaluation. The biopsy should be made on the most abnormal and preferably the largest LN and not on the LN that is most accessible. Patients with unexplained localized LA who have constitutional symptoms or signs, risk factors for malignancy or LA that persists for 3 or 4 weeks should also undergo a biopsy.

Lymphadenopathy is one of the profound and persistent signs during the progression of the HIV infection. The HIV virus infects primarily the CD4 lymphocytes therefore the lymph nodes are commonly involved during all the stages of the infection. LA is also one of the first, earliest

symptoms and signs of the HIV infection. It is seen among other general symptoms typical for a retrovirus infection and associated with a change in peripheral blood counts, a lymphocytosis *(9).* The syndrome of 'persistent generalized lymphadenopathy' was described as one of the first symptoms of HIV infections. This type of LA is present throughout the life of the patient. The swelling in atypical areas is regularly present such as supracondylar and submandibular regions. This is usually accompanied with symptoms of fever with night sweats, malaise, weight loss of more than 10% in 6 months and diarrhea. Therefore LA is the most consistent symptom of HIV infection throughout the progression of the clinical course.

There are various types of LN abnormalities in HIV positive patients: reactive lymphadenitis, granulomatous lymphadenitis, with a further division to tuberculous and non tuberculous subtype, pyogenic lymphadenitis and fungal lymphadenitis, also malignancies are regularly seen such as Kaposi sarcoma Hodgkin, non-Hodgkin lymphomas, carcinomas and metastasizes *(31).*

Physical examination of the patient with lymphadenopathy: how to examine and what to look for?

A clinical practitioner that encounters a lymphadenopathy should make a detailed anamnesis of the patient's medical history and interrogate him/her about present symptoms, complaints and the Progression of LA *(9).*

Physical examination

After a detailed anamnesis and medical history of the patient a careful physical examination should follow. Special attention should be given to the lymph nodes. Characteristics of the enlarged lymph node have to be carefully examined. It is very important to estimate the size and the consistency of the nodes. When an evaluation of a localized LA takes place a careful examination of the region from which the lymph drains to this particular LN has to be made. Attention should be given to identify the presence of skin lesions, evidence of infection or inflammation and the tumors. Careful examination of the whole body has to be made to be able to exclude the generalized LA with the presence of abnormal lymph nodes in the other regions of the body. Each examination should include a careful palpation of anterior and posterior cervical LN, submandibular, axillary and inguinal lymph nodes. When a lymph node is detected, the following characteristics have to be estimated: size, pain/tenderness, consistency, matting and location *(9).*

Lymphadenopathy in HIV-positive patients as a result of opportunistic infections

During the progression of HIV infection weakening and deterioration of the immune system slowly takes place. The number of CD4 cells declines making the patient vulnerable and susceptible to various infections, especially opportunistic infections.

When a patient still has the amount of 600 or more per µl CD4 cells, the immune system remains Protective enough against opportunistic infections. But when the counts of CD4 further decline and reach a number of fewer than 350 cells per µl the immune system is becoming to be impaired and risk for associated infections increases. When the AIDS phase is reached there is an imminent risk and the infection can lead to serious complications.

In such cases the relation between HIV and the following infections can be described: Tuberculosis (TB), Nontuberculous mycobacteriosis, Cytomegalovirus, Pneumocystis jirovecii, Fungi, Toxoplasmosis, Mycoplasmosis, Cryptococcosis and Clastridiosis *(9).*

2.5. FNA

Fine needle aspiration (FNA) cytology is an important technique done to identify cause of LAP. This method is cost-effective, with a fairly low risk and provides a rapid diagnosis. Studies have shown that FNA cytology has an established role in the differential diagnosis of infection, persistent generalized lymphadenopathy and malignancy in HIV-infected patients *(9).*

Fine needle aspiration cytology (FNAC) is an accepted first line investigation for mass lesions, which may be targeted by palpation or a variety of imaging methods. Although FNA cytology has been shown to be a cost-effective, reliable technique its accurate interpretation depends on obtaining adequately cellular samples prepared to a high standard. Its accuracy and cost-effectiveness can be seriously compromised by inadequate samples. Although cytopathologists, radiologists, nurses or clinicians may take FNAs, they must be adequately trained, experienced and subject to regular audit. The best results are obtained when a pathologist or an experienced and trained biomedical scientist (cytotechnologist) provides immediate on-site assessment of sample adequacy whether or not the FNA requires image-guidance. In our country FNA is done and interpreted by anatomic pathologists. Ayder there are three anatomic pathologists and one has subspecialized in cytopathology who can do FNAC and interpret results much better than others.

Lowe and colleagues describe a study on 62 HIV-positive patients. The total amount of FNA cytology procedures was 73 and different LN groups were investigated: axillary (17%), cervical and supraclavicular (29%), inguinal (15%) and other (2%). The most common diagnosis was persistent generalized LA (50%) followed by infection (22%) and malignancy (18%); inflammation (10%) was also observed *(9)*.

An Indian study also performed a study on FNA cytology of the LN. Their goal of the research was to evaluate FNA as a guiding tool to diagnose the etiology of the LA. They also tried to find out if this method was useful in finding undiagnosed HIV positive cases with already a present symptom of persisting LA. The research was made on 1082 LA cases and the incidence discovered and previously Undiagnosed HIV positivity was 23%. People with an undiagnosed HIV are unaware of their infection and are transmitting the virus further. So a diagnosis of HIV is crucial and clinicians should always keep a possibility of HIV positivity in mind when they encounter patients with unexplained and persistent LA *(9)*.

Organization of service

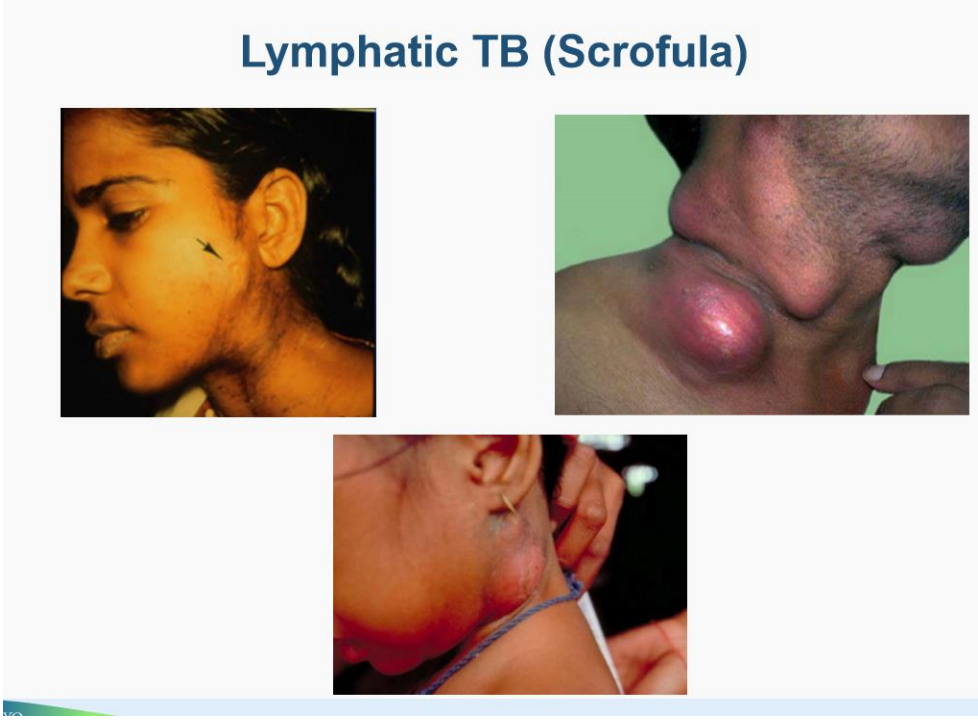
The provision of a comprehensive FNA service should be under the direction of a named pathologist with responsibility for overseeing all its aspects, working in conjunction with the clinicians involved in the service. The pathologist is usually the head of the cytopathology section of a cellular pathology department, which in the UK refers to combined histopathology and cytopathology departments. In large departments the responsibility may be delegated to another cytopathologist in the same department who is directly involved in FNA cytology *(31)*.

Who should take the specimen?

The responsibility for taking FNAs should lie in the hands of individuals who have a sufficient FNA workload to gain and maintain the necessary expertise and who are subject to clinical audit. In many centers cytopathologists take specimens directly from patients with palpable lesions, but in others radiologists and clinicians take most of the aspirates. There are three levels of skill required in providing a safe and reliable FNA service; taking the sample, preparing it, and interpreting the findings. The interpretation is made in the context of the clinical findings and the results of other investigations and is dependent on obtaining adequate samples and preparing them to maximize their diagnostically relevant information. Cytopathologists should be able to acquire all these skills and are well placed to provide a high quality FNA service. Cytopathologists have been shown to perform well in terms of taking adequate FNAs and it has

long been known that training and experience are important. It has been shown that clinicians carrying out relatively few aspirates with a high turnover of trainees may perform poorly in terms of specimen adequacy even when given prior training by cytopathologists. Good results may be achieved in combined services where cytopathologists take FNAs from palpable lumps alongside clinicians or radiologists using US-guidance when necessary. An important ingredient seems to be the presence of a cytopathologist to assess sample adequacy. Deep-seated or impalpable lesions in which ultrasound or radiological guidance is required to target the lesion are usually carried out by radiologists or, in the case of endoscopic and endobronchial US-guided FNAs, by gastroenterologists, chest physicians or specialist nurses as will be considered in a later section. Providing the aspirator is well trained, has sufficient practice to maintain his or her skills, has a close working relationship with the cellular pathology department and is subject to clinical audit, there should be no difficulty in their achieving a high quality service. FNAs should not be taken by unsupervised staffs who have no training in the technique, staff who do not take such aspirates regularly, or who are unaware of the risks and complications *(31).*

Figure 6; lymphadenopathy



Extrapulmonary Tuberculosis Diagnostic Approaches John W. Wilson, MD Associate Professor of Medicine Division of Infectious Diseases Mayo Clinic, Rochester MN Mayo Clinic Center for Tuberculosis

3. STATEMENT OF THE PROBLEM

Tuberculosis and infection with HIV constitute the major burden of infectious diseases in resource-limited regions such as African and Asian countries. When both infections occur in the same individual, this coinfection can result in diagnostic and therapeutic challenges due to the peculiar clinical picture that is seen with TB–HIV coinfection. Tuberculosis is one of the earliest opportunistic infections seen in HIV-infected individuals and is the leading cause of death in these patients. The prevalence of TB– HIV coinfection globally is about 13% but much higher in African countries which have the highest burden of HIV infections.

Mycobacterium tuberculosis (MTB) and Human Immunodeficiency virus / Acquired Immunodeficiency remain the most common infectious disease in resource limited countries. Mycobacterium tuberculosis – HIV co infection causes therapeutic and diagnostic challenges and in turn poses immense pressure on health care agencies in such countries where the populations of coinfected individuals are ever increasing. At least one-third of the 33.3 million people living with HIV worldwide are infected with TB. Persons co-infected with TB and HIV is 20-30 times more likely to develop active TB disease than persons without HIV. In patients dually infected with Mycobacterium tuberculosis and HIV, both the organisms together will accelerate the deterioration of the immune system especially CD4 cells resulting in early death if untreated. Diagnosis of TB in HIV infected patients may be delayed because of atypical clinical presentations and involvement of inaccessible sites (EPTB) and low sputum smear positivity.

The co-infection of HIV and tuberculosis is characterized by the appearance of multidrug-resistant tuberculosis (MDRTB) and which are very difficult to treat and increase the mortality rate. And the most frequent forms of tuberculosis affect lymph nodes (lymphadenitis) predominantly in the neck region.

In developing countries, TB has remained an important public health problem, exacerbated in the last decade by poverty, war, demographic changes and the rapid spread of HIV with case rates increasing by an average of 7% per year after 1985 in 20 sub-Saharan African countries.'

4. SIGNIFICANCE OF THE STUDY

TB is the most frequent opportunistic infection in HIV infected patients and more than 25% of TB patient are co infected with HIV, HIV infected patients often presents with un usual clinical manifestations particularly extrapulmonary TB accounts for approximately 20% of all TB cases and the lymph node are the most frequent extrapulmonary site. HIV changes the pathogenesis of tuberculosis causing a progressive loss of immunity at the cellular level which leads to a significant increase in the risk of tuberculosis among HIV positive patients.

The gap identified is that the clinical course of TB disease is altering due to HIV infection with increasing number of smear negative and EPTB, so that TB disease is more likely to be disseminated and more difficult to diagnose and treat as the immunosuppression progress .

The significance of this study is firstly that the real burden of TBLN among HIV patient related to their CD4 count and cytomorphological pattern is not done previously and well known in Mekelle ACSH. Secondly it can improve diagnosis and treatment of TBLN among PLHIV and finally it will minimize risk of progression of morbidity, mortality and delay in the diagnosis and treatment of HIV-TB co infected patients.

5. OBJECTIVES

5.1. General objectives

Tuberculosis Lymphadenities among people living with HIV/AIDS and its correlation to CD4 count, cytomorphological pattern and clinical Diagnosis, In Mekell University Ayder Comprehensive Specialized Referral Hospital

5.2. Specific objectives

- To determine prevalence of tuberculosis lymphadenitis among people living with HIV/AIDS
- To assess the patterns of CD4 count among HIV positive patients with TBLN
- To observe cytomorphological pattern of TBLN in HIV positive individuals
- To assess correlation of clinical diagnosis and morphologic diagnosis

6. METHODS AND MATERIALS

6.1. Study design and period

Hospital based cross sectional study design was conducted among HIV-positive patients who develop lymph node during their visit on ART who have all the data needed for the purpose of higher research at Mekelle University Ayder Referral Hospital from January 2019 to June 2019.

6.2. Study area

The study was conducted at Mekelle University Ayder comprehensive specialized Hospital in Mekelle city located at a distance of 760 Km away from Addis Ababa to the northern part of Ethiopia with a total population of > 400,000. The hospital is a referral hospital in Tigray region which has a primary role on the identifying and solving of the regional health problems. It has a total of 400 beds with specialty service of internal medicine, surgery, guyni & obstetrics, pediatrics, dermatology& STD, orthopedics, radiology, psychiatry, dentistry, forensic medicine and pathology. In the department of pathology there are three well experienced anatomic pathologists one of which is a cytopathologist in subspecialty who is mainly engaged in doing FNAc, body fluids and interpreting the microscopic pictures.

6.3. Source population

The source population was all HIV-infected patients visiting on ART at Mekelle University Ayder comprehensive specialized Hospital located in Mekelle city.

6. 4. Study population

From the HIV-infected patients visiting ART at Mekelle University Ayder Referral Hospital those who develop lymph node were selected statistically by a cross sectional convenience sampling and used as study population. Study participants will be screened for development of lymphadenopathy once during the study period.

6.5. Inclusion and exclusion criteria

6.5.1. Exclusion criteria

HIV positive patients with no lymphadenopathy, patients on HAART and patients card files with incomplete data of one of the variables were excluded from this study.

6.5.2. Inclusion criteria

HIV positive patients who develop lymph node, patient's card files with complete data of the variables and patient's not on HAART were included in this study.

6.6. Study Variables

Socio demographic characteristics, CD4 count cytomorphological pattern and clinical diagnosis were considered as explanatory variables; Tuberculosis lymphadenitis status of HIV-infected patients was considered as outcome variables of this study.

6.6.1. Dependent variables

Tuberculosis lymphadenitis characteristics in patients living with HIV/AIDS

- Site of LAP
- Duration of LAP
- appearance of Lymph Node
- Size of Lymph Node
- Aspiration appearance
- Consistency of lymph node
- mobility of Lymph node

6.6.2. Independent variables

- Age
- sex
- CD4- count
- HIV stage of patient
-
- morphological (microscopically) diagnosis
- religion
- Clinical Diagnosis
- educational status
- Patient type

6.7. Sample size and sampling techniques

Sample size was calculated using single population proportion formula. A total of 90 HIV-infected patients and develop lymphadenopathy Will be included in the study considering, 95 % of confidence interval and 5% degree of accuracy required. That is, the allowed deviation from the true proportion in the population. Study participants will be selected through cross sectional study method among HIV infected patient during the study period.

The sample size was determined using single population formula for estimating single population proportion from the infinite population. The formula for calculating the sample size (n) would be:

$$n^* = \frac{(z\alpha/2)^2 \, P \, (1-P)}{d^2}$$

Where

N= sample size from the population

P= the lowest expected prevalence of TBLN among HIV positive patients in southern part of Ethiopia, Butajira (6.3%). (30)

Z= standard normal distribution at 95% confidence limit =1.96

D= allowable error = (5% = 0.05)

$$n^* = \frac{(1.96)2x0.063(1-0.063)}{(0.05)2}$$

= (1.96)2 x 0.063 (1-0.063) / (0.05)2

= 3.842 x 0.063 (0.937) / 0.0025

= 3.842 x 0.0590 / 0.0025

= 90

6.7.1. Data collectors and data collection tools

Pretested semi-structured questionnaire was used for data collection. ART Department senior staffs and technicians will participate in the assessment of the files and documents with reports. With the help of the professionals, first the documents was pre-assessed for their reports then the variables specific to HIV will be selected by the professionals using the data's found from ART data's each data will be cross matched if there is assessment bias. Convenience sampling method will be used as sampling method among the HIV positive patients on ART who have developed lymphadenopathy for the purpose of our variable comparison.

6.7.2. Quality assurance

The data collector's staffs and Technicians were expected to have the knowledge and additional short training was given about the reviewing of this documentation. In analytical case; most of the processes was performed by the laboratory professionals(use SOP's and control) even though the nurses or technicians will be participated that is; the files in the computer as well as hard copy files will be checked for all the results to insure data quality.

6.7.3. Data processing and analysis

Cross-sectional data for Tuberculosis lymphadenities in HIV patients was entered in to computer, Analysis and interpretation was done using spss-version-23, statistical package, and Chi-square test was also done to determine associations.

Patients were contacted during their routine visit to ART center. Informed written consent was obtained from all patients after explaining them nature of the study. Data was collected from patient card files of Mekelle university Ayder Comprehensive specialized Hospital ART clinic after checking the completeness of patient's necessary information like age, sex and fine needle aspiration cytology results. Data was entered and analyzed using SPSS version 23. Chi-square test was done to determine associations and to correlate cytomorphological pattern clinical diagnosis and CD4 count with. A 2×2 analysis was done to test the degree of correlation. A $P < 0.05$ was considered as significant.

6.7.4. Ethical consideration

This study will obtain an ethical clearance from Scientific and Ethics Research Office (SERO), Addis Ababa University college of health Science school of Medicine Department of pathology There was a formal letter from director of Mekelle university Ayder comprehensive specialized referral hospital and this was expected to fulfill the formality and the confidentiality of the data was maintained and will be analyze.

7. RESULT AND DISCUSSION

7.1. Result

Socio-demographic and other factors

In this study A total of 90 HIV positive patients who referred to ART unit for follow up and those who develop lymph node were included ; of whom 52(57.8%) were female patients and 38(42.2%) were male patients. The female to male ratio is 1.36:1 .the minimum age found was 4 and maximum age was 58.the majority of patient were in age group 19-30 (40/90,44.4%) followed by 31-42(26/90,28.2%).the mean age the study subject was 30.6 ranging from 4-58.

Table 5: Demographic characteristics of patients

CHARACTERISTIC	FREQUENCY	PERCENT (%)	Gender	
Gender				
Male	**38**	**42.2%**	**Male**	**Female**
Female	**52**	**57.8%**		
Age group				
<18	**9**	**0.1%**	**5**	**4**
19-30	**40**	**44.4%**	**19**	**21**
31-42	**26**	**28.9%**	**10**	**16**
>42	**15**	**16.7%**	**4**	**11**
Educational Status				
No formal Education	**16**	**17.8%**	**5**	**6**
primary school	**32**	**35.6%**	**15**	**11**
2secondary school	**28**	**31.1%**	**9**	**12**
College	**14**	**15.6%**	**9**	**23**
Religion				
Orthodox	**58**	**64.4 %**	**25**	**33**
Muslim	**23**	**25 %**	**9**	**14**
Catholic	**6**	**6.5 %**	**4**	**2**
Protestant and others	**3**	**3.2 %**	**0**	**3**
Patient Type				
Out patient	**85**	**94.4 %**	**36**	**49**
In patient	**5**	**5.6 %**	**2**	**3**

Prevalence of Tuberculosis lymphadenitis and pattern of cytomorphological Diagnosis

The overall prevalence of Tuberculosis lymphadenitis was found to be (n = 17/90, 18.9 %). Tuberculosis lymphadenitis emerged as the 2nd most frequent infection among Cytomorphological pattern of lymphadenopathy to be associated with HIV infected patients across the total range of CD4+ next to reactive hyperplasia(n=62/90,68.9%), followed by pyogenic lymphadenitis (n=7/92,7.6%),and Hodgkin lymphoma(n=4/90,4.4%). So Tuberculosis lymphadenitis was found to be prevalent among (n=17/90, 18.9%) of patients who are HIV positive and develop lymphadenopathy Of which (n=3/17, 17.6%) caseous necrosis, ,(n=4/17,23.5%) Epitheloid granuloma,(n=8/17,47%) caseous necrosis and Epitheloid granuloma and finally (n=2/17,11.7%) polymorphs and macrophages without granuloma.

Table 6: General Characteristics of Lymphadenopathy based on Cytomorphological pattern

	Frequency	percent	Valid percent	Commutative %
Caseous necrosis (TBLN)	3	3.3	3.3	3.3
Epitheloid granuloma (TBLN)	4	4.4	4.4	7.8
Caseous necrosis and Epitheloid granuloma (TBLN)	8	8.9	8.9	16.8
Reactive hyperplasia	62	68.9	68.9	85.6
Pyogenic lymphadenitis	7	7.8	7.8	93.3
Hodgkin lymphoma	4	4.4	4.4	97.8
Polymorphs and macrophages	2	2.2	2.2	100
	90	100	100	

Figure 7. General characteristics of lymphadenopathy based on cytomorphological pattern.

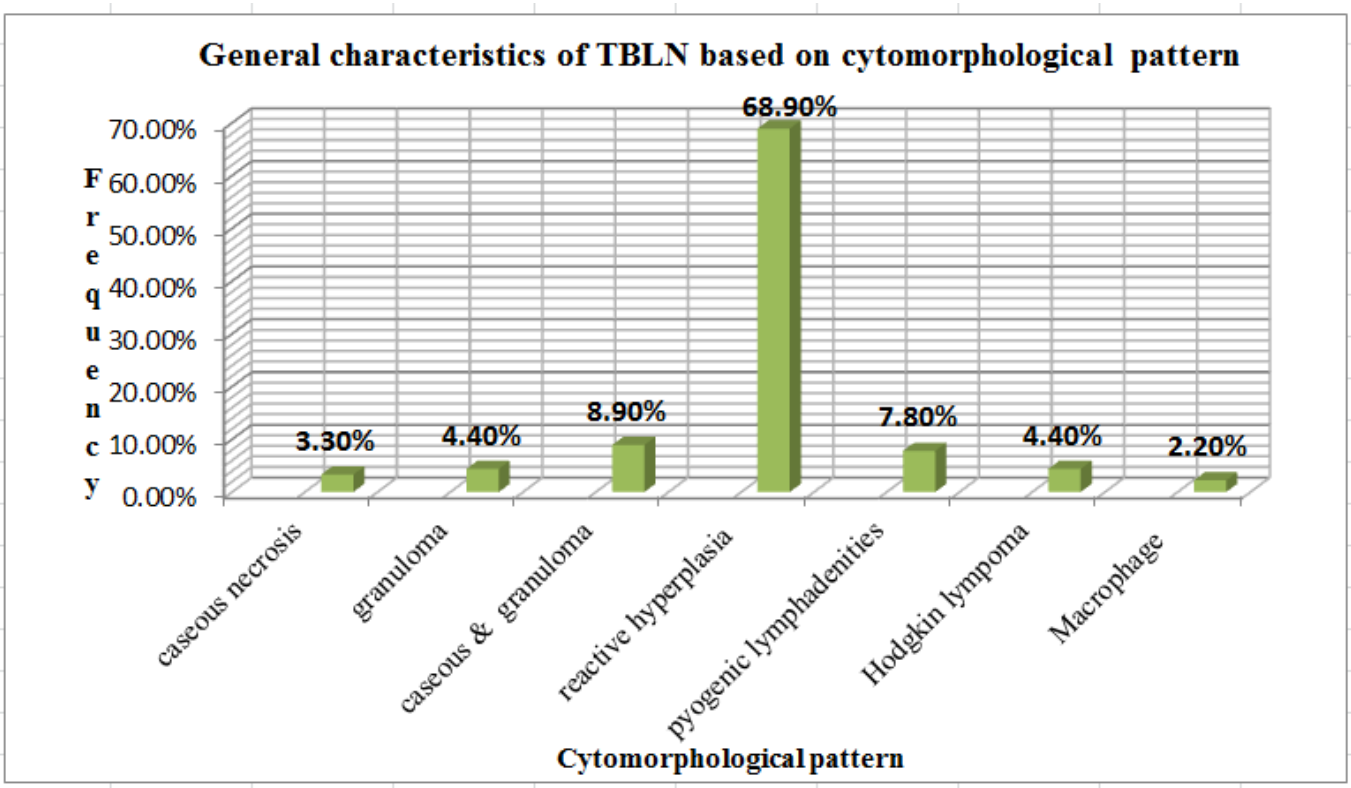
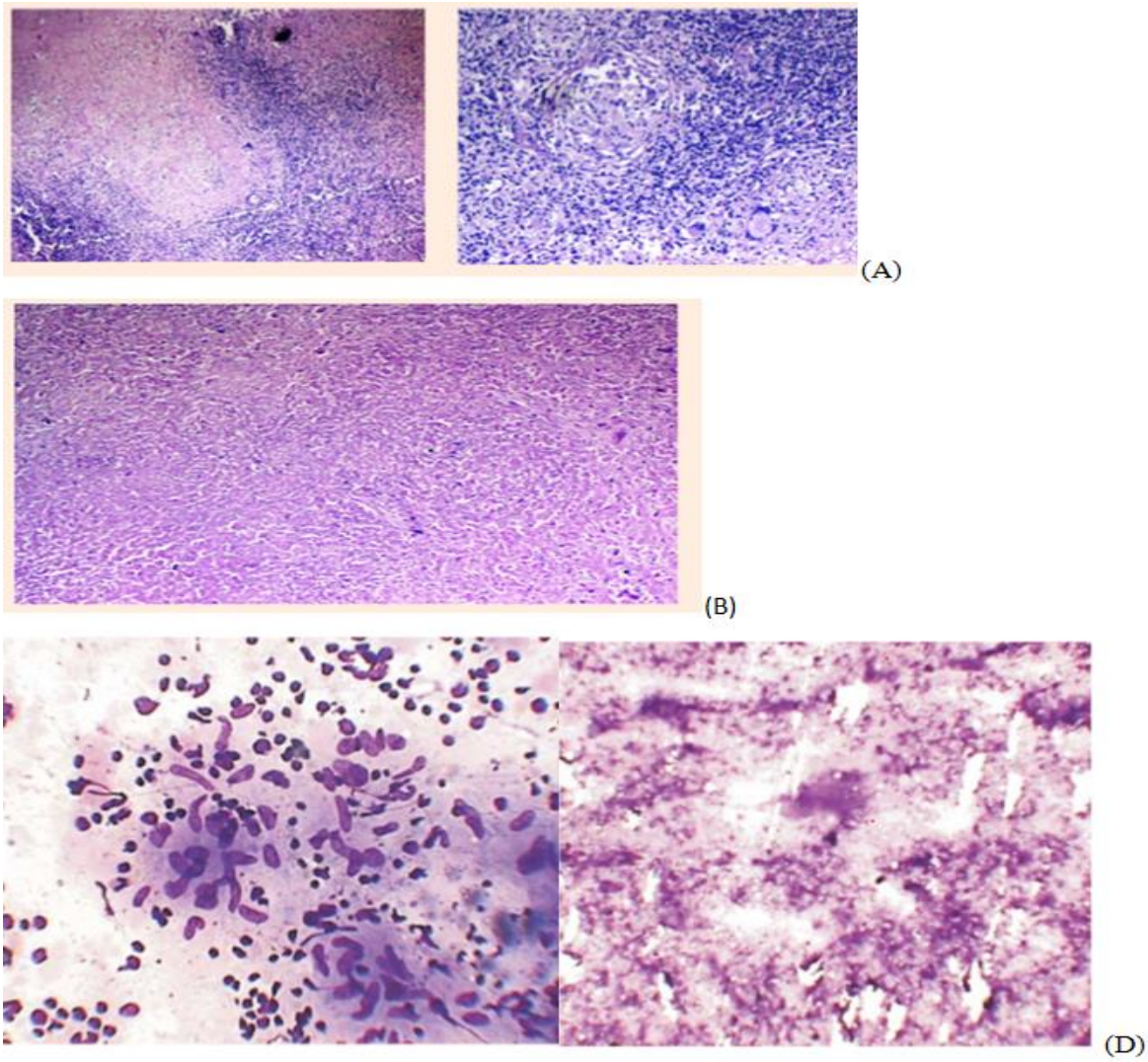


Table 6.1; General characteristics of lymphadenitis based on cytomorphological pattern

	Frequency	Percent
Caseous necrosis only	3	17.6
Epitheloid granuloma only	4	23.5
Caseous necrosis and Epitheloid granuloma	8	47.0
Macrophages without granuloma	2	11.7
Total	17	100.0

Figure 8. Microscopically cytomorphological pattern



A = Granulomas(H&E10X).& (40X), B = Caseous necrosis(H&E10X), C = FNAC lymph node smea granuloma (MGG stain, 40X), D =FNAC lymph node smear showing caseation (MGG stain, 40X)

General characteristics Tuberculosis lymphadenitis positive patients and their CD4+ count

The CD4 count of Tuberculosis lymphadenitis among Cytomorphological pattern of lymphadenitis who were HIV positive with CD4+ count less than 200/mm3 found to be highest (n=13/17,76.5%) followed by,200-349/mm3(n=2/17,11.8%),350-499/mm3 (n=1/17,5.9%) and similarly>500/mm3 (n=1/17,5.9%). As to pyogenic lymphadenitis(n=6/8,75%)are in the range of CD4 count between 200-500/mm3 and (n=2/8, 5.9%) are less than 200/mm3. the others including reactive hyperplasia scores(n=5/61,8.1%) <200/mm3.(n=56/61,91.8%) between 200-

500/mm3 finally Hodgkin lymphoma score (n=1/4,25%,) <200/mm3 and (n=3/4,75%) between 200-500/mm3 of the total morphological pattern .

Of the tuberculosis lymphadenitis among Cytomorphological pattern of lymphadenitis who were HIV positive with CD4 count less than 200/mm3, caseous necrosis only (3/3,100%), Epitheloid granuloma (3/4,75%), caseous necrosis and Epitheloid granuloma(6/8,75%) and macrophage and polymorphs without nuclei (1/2,50%).

Table 7; characteristics of Morphological pattern and CD4+ count category of HIV patient cross tabulation

		CD4 category of study participant				Total
		>500	350-349	200-349	< 200	
Morphological pattern of LAP	Caseous necrosis only (TBLN)	0	0	0	3 (100%)	3(100%)
	Epitheloid granuloma only (TBLN)	0	1	0	3(75%)	4(100%)
	Caseous necrosis and Epitheloid granuloma(TBLN)	1 12.5%	0	1 12.5%	6(75%)	8(100%)
	Reactive hyperplasia	26(42.6%)	13(21%)	17 27.9%	5(8.2%)	61(100%)
	Pyogenic lymphadenitis	3(37.5%)	1(12.5%	2(25%)	2(25%)	8(100%)
	Hodgkin lymphoma	0	2(50%)	1(50%)	1(25%)	4(100%)
	Polymorphs and macrophage with out granuloma(TBLN)	0	0	1(50%)	1(50%)	2(100%)
Total		30 (33.35)	17 (18.9%)	22 (24%)	21 (23%)	90 (100%)

Figure 9. Tuberculosis lymphadenitis positive patients and their CD4+ count pattern

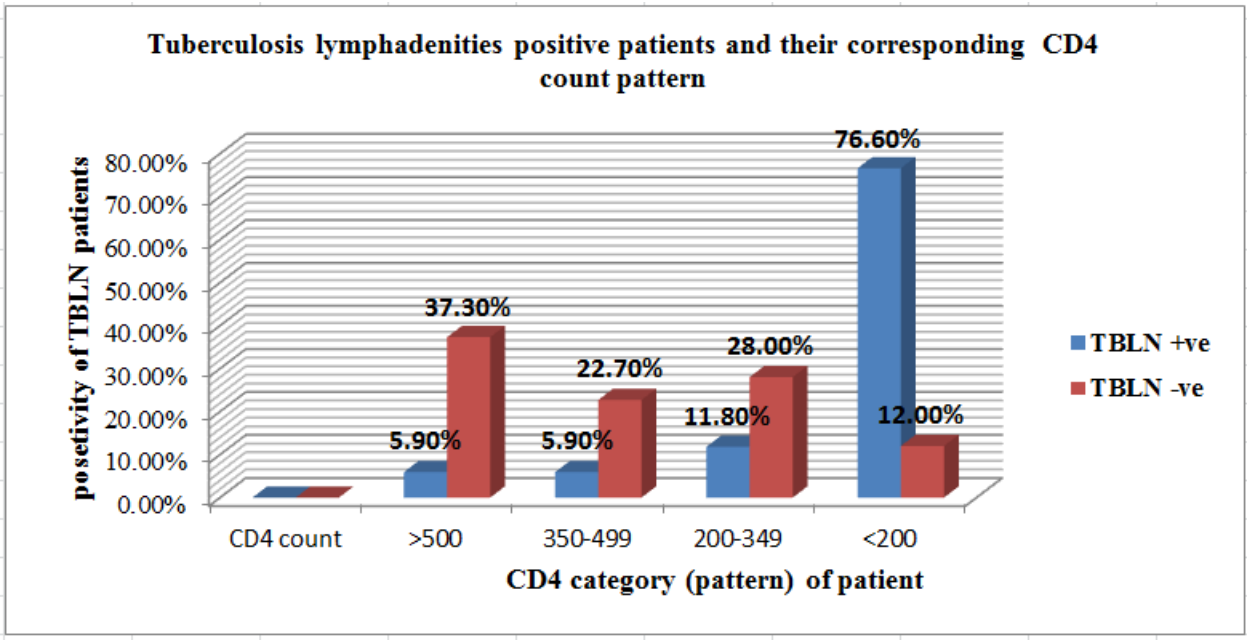


Table 8; Tuberculosis lymphadenitis positive patients and their CD4+ count category(cross tabulation)

		CD4 category of study participant				Total
		>500	350-499	200-349	<200	
TBLN ŧve	**Yes**	1 (5.9%)	1 (5.9%)	2 (11.8%)	13 76.5%	17 100%
	No	29 (39.7%)	16 (21.9%)	20 (27.4%)	8 (11%)	73 (100%)
Total		30 33.3%	17 (18.9%)	22 (24.4%)	21 (23.3%)	90 (100%)

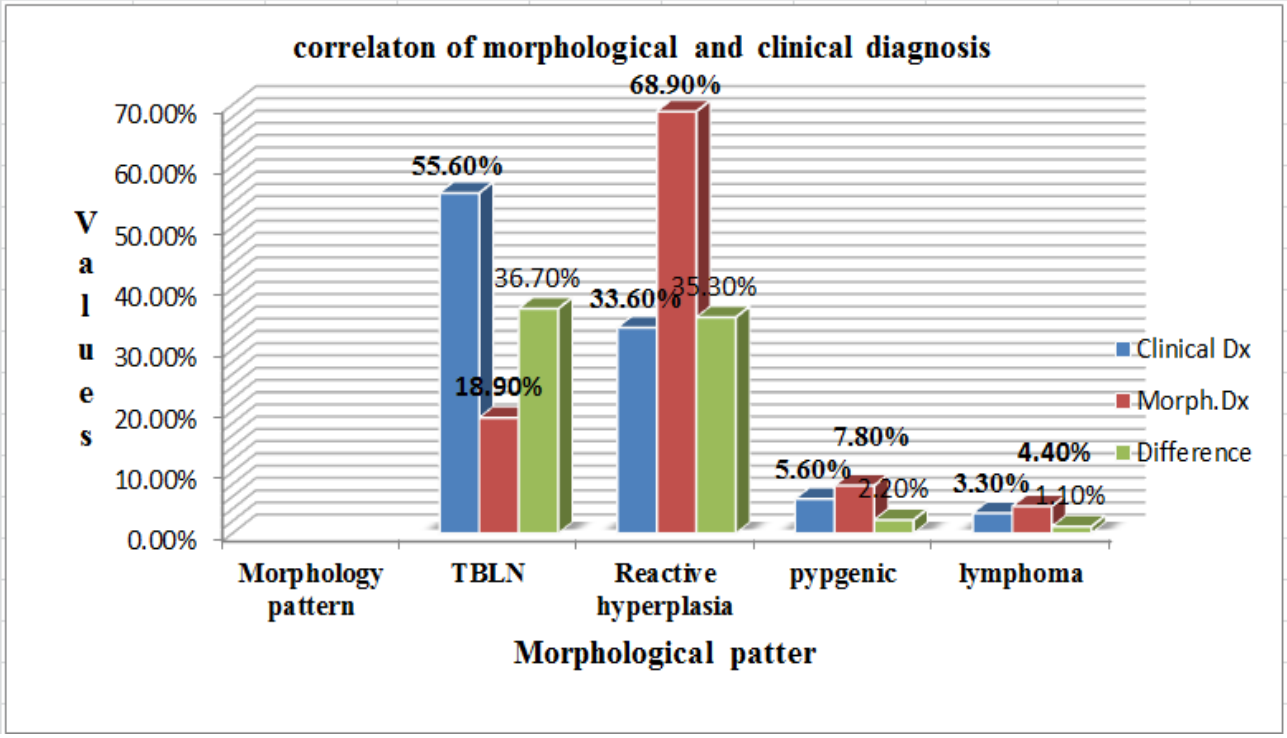
Characteristics of Clinical diagnosis versus morphological diagnosis of Tuberculosis lymphadenitis among HIV positive patients.

Cytomorphological pattern of Tuberculosis lymphadenitis was found to be prevalent among (n=17/90, 18.9%) of patients who are HIV positive and develop lymphadenopathy. The remaining (n=83/90, 92.2 %) include pyogenic lymphadenitis (7.8%),reactive hyperplasia(68.9%) and Hodgkin lymphoma(4.4%). As to the clinical diagnosis (n=50/90,55.6%) of the patients was diagnosed clinically as Tuberculosis lymphadenitis The remaining also diagnosed clinically as (n=32/90,35.6%) reactive hyperplasia(n=5/90,5.6%)pyogenic lymphadenities and (n=3/90,3.3%) lymphoma .So Tuberculosis lymphadenitis is over diagnosed by 36.7%,reactive hyperplasia under diagnosed by(33.3%), pyogenic lymphadenities under diagnosed by (2.2%) and finally lymphoma under diagnosed by (1.1%) in clinical Diagnosis compare to morphological Diagnosis.

Table; 9 cross tabulation of clinical diagnosis and morphological diagnosis characteristics of Tuberculosis lymphadenitis HIV positive patients

		CD4 category of study participant				Total
		>500	350-349	200-349	< 200	
Morphological Diagnosis	Caseous necrosis only (TBLN)	3	0	0	0	3
	Epitheloid granuloma (TBLN)	4	0	0	0	4
	Caseous necrosis and Epitheloid granuloma(TBLN)	5	3	0	0	8
	Reactive hyperplasia	29	25	3	4	61
	Pyogenic lymphadenitis	5	2	0	1	8
	Hodgkin lymphoma	2	2	0	0	4
	Polymorphs and macrophage without granuloma(TBLN)	**2**	0	0	0	2
Total		50	32	3	5	**90**

Figure 10. Correlation of morphological and clinical Diagnosis



Stage of HIV and morphological diagnosis of Tuberculosis lymphadenitis HIV positive patients

Among All patients who are positive for HIV and develop lymphadenopathy those with morphologic pattern of Tuberculosis lymphadenitis (n=13/17,76.5% were categorized under stage IV followed by pyogenic lymphadenitis(n=1/7,14.3% stage IV, n=6/7,85.7% stage I,II and III). Majority of the remaining cytomorphological pattern like reactive Tuberculosis lymphadenitis (n=7/62,11.3%) stage IV the others are belong to , I,II and III) finally Hodgkin lymphoma lies at stage I and stage III.

Table 10; Stage of HIV and morphological diagnosis of Tuberculosis lymphadenopathy

		Stage of HIV				
		Stage I	Stage II	Stage III	Stage IV	Total
Morphological Diagnosis	Caseous necrosis only (TBLN)	3	0	0	0	3
	Epitheloid granuloma (TBLN)	4	0	0	0	4
	Caseous necrosis and Epitheloid granuloma(TBLN)	5	3	0	0	8
	Reactive hyperplasia	29	25	3	4	61
	Pyogenic lymphadenitis	5	2	0	1	8
	Hodgkin lymphoma	2	2	0	0	4
	Polymorphs and macrophage without granuloma(TBLN)	**2**	0	0	0	2
Total		50	32	3	5	**90**

Different status of Tuberculosis lymphadenitis and their major findings among HIV positive patients

Among 90 HIV positive patients (n=85/90,94.4%) are out patient with site of the LAP of TBLN at (n=9/17,52.9)cervical and (n=5/17,29.4) axiliary, duration of the LAP with in 1month up to 5 months(n=8/17,47.1%) followed by 6 month up to 1 year (n=5/17,29.4%) ,1-4 weeks (n=3/17,17.6),as to the appearance of the LAP(n=11/17,64.7%)matted followed by (n=6/17,35.5%) discrete, with regard to consistency (n=10/17,58.8%)was firm and (n=5/17,29.4%) soft. the mobility of the LAP shows (n=13/17,76.5%) fixed and (n=4/17,23.5%0 was mobile,. The size of the LAP was (n=7/17, 41.2%) 1-2 cm followed by (n=5/17,29.4%) less than 1 cm .The aspiration type of the lymphadenopathy was cellular(n=5/17,29.4%) and pus (n=4/17,23.5%) finally the aspiration type was (n=3/17.17.6%)hemorrhagic and (n=3/17.17.6)was caseous

Correlation of Tuberculosis lymphadenitis, CD4+ count, clinical diagnosis and HIV staging

The morphological Diagnosis of HIV positive Tuberculosis lymphadenitis patients found to be strongly correlated (Pearson's correlation, 2- tailed) and statistically significance association with the CD4 pattern, clinical diagnosis and clinical staging of HIV with p- values of 0.000, 0.022 and 0.000 respectively. The CD4 counts of all these patients ranged from 80-850/mm3. The CD4 count of Tuberculosis lymphadenitis among Cytomorphological pattern of lymphadenitis who were HIV positive with CD4+ count less than 200/mm3 found to be highest (n=13/76.5%).

As to the clinical diagnosis (n=50/90,55.6%) of the patients was diagnosed clinically as Tuberculosis lymphadenitis, So Tuberculosis lymphadenitis is over diagnosed by 36.7%, and Among All patients who are positive for HIV and develop lymphadenopathy those with morphologic pattern of Tuberculosis lymphadenitis (n=13/17,76.5% were categorized under stage IV .

Table 12; Correlation of Tuberculosis lymphadenitis, CD4+ count, clinical diagnosis and HIV staging

Gender and age category		TBLN		CD4+ category				P-value	Clinical Diagnosis 0f patient		p-value	HIV stage				p-value
		Yes	No	>500	350-499	200-349	<200		yes	N0		I	2	3	4	
< 18	M	1	4		1			0.000		1	0.022	1				0.000
	F	1	3				1	0.000		1	0.022				1	0.000
19-30	M	5	17	1		1	3	0.000	1	2	0.022	1		1	4	0.000
	F	5	20			1	4	0.000		1	0.022			1	3	0.000
31-42	M	0	9								0.022					0.000
	F	3	15				4	0.000	1		0.022				4	0.000
>42	M	0	1						3		0.022				1	0.000
	F	2	4				1	0.000	7		0.022					0.000
Total		**17 18.9%**	**73 81.1%**	**1 5.9%**	**1 5.9%**	**2 11.8%**	**13 76.5%**		**12 71%**	**5 29. 4%**		**2 12 %**		**2 12 %**	**13 77 %**	

8. DISCUSSION

Ninety patients living with HIV AIDS and who develop lymph node were seen during the study period. The age range was 4-58 years with a mean age of 30.6+_11.7years. The female to male ratio was 1.36:1 of whom 52(57.8%) were female patients and 38(42.2%) were male patients. the majority of patient were in age group 19-30 (42/90, 46.7 %) followed by 31-42(26/90, 28.9%).

In our study the overall prevalence of tuberculous lymphadenitis among HIV positive patients who develop lymph node was found to be (n=17/90, 18.9%). Similar study findings showed that (11%), (14.3%) , (40.5%)and ((42.19%) at ART clinic of Gondar University Hospital, Bayero University Teaching Hospital Kano Nigeria, Tehran Iran, Manipur India respectively(8),(30), (41),(13), and (12).

The prevalence of our study was higher among studies conducted in other parts of Ethiopia and this was firstly due to difference in selection of study population and difference in techniques (histology, culture). In addition this study was also lower compare to study conducted in India and Tehran, Iran again due to study subjects (admitted patient) and even though same study unit used as in India the techniques, quality service and reagents used like May-Grundwald Giemsa (MGG) used which was not common in our country.

In addition Histological Features of Tubercular Lymphadenitis in HIV Positive Patients studied by Department of surgical oncology, Pushpadi cancer care center, India (44) % were diagnosis as Tuberculosis lymphadenitis and were the second most common causes of lymphadenopathy after reactive hyperplasia (42).

Although lymphadenopathy is one of the most common manifestations at any stage of HIV/AIDS, it reflects a serious underlying pathology that requires immediate attention. Caseous necrosis only, malignant lymphoma, Epitheloid granuloma, reactive hyperplasia, pyogenic lymphadenities and polymorphs and macrophage with or without granuloma was among the many pattern causes resulting in lymphadenopathy in HIV/AIDS (80) .

This study showed that caseous necrosis and Epitheloid granuloma accounts together (n=8/17, 47.1%), Epitheloid granuloma only (n=4/17, 23.5%), caseous necrosis only (n=3/17, 17.7%) and macrophages (n=2/17, 11.8%) was the most encountered cause of lymphadenopathy of patients who was HIV positive and develop lymphadenopathy. Other findings were also observed in

study conducted at Departments of Pathology Sri Deva raj URS Medical College, India reveals caseous necrosis and Epitheloid granuloma accounts (n= 46/84 (55%) ,Epitheloid granuloma (n= 6/29 ,21%),caseous necrosis (n= 25/34,73.5%) and macrophages (n= 4/34 11.8(15).other studies in Manipur India show caseous necrosis and Epitheloid granuloma accounts (n= 8/27 (29.6%) ,Epitheloid granuloma (n= 5/27 ,18.5%),caseous necrosis (n= 9/27,33.3%) and suppurative with viable neutrophil and macrophages (n= 5/27, 18.5%),

Study in tertiary level referral center India smears revealed Epitheloid granulomas with caseous necrosis in 16.4% of cases, Epitheloid granulomas without necrosis in 14.3% of cases, necrosis only without Epitheloid granulomas in 39.2% of cases and polymorphs with necrosis with or without Epitheloid granulomas in 30.1% of cases. (43).Though our study and studies conducted apart from our study shows slight similarity it lacks consistency on morphologic pattern of cases even among those made in India alone this shows morphologic diagnostic challenge that need to be improved by introducing or incorporation of quality control system and using screening test such as molecular techniques to detect the specific gene like mycobacterium tuberculosis by polymerase chain reaction.

CD4 testing is useful for determining the degree of immune compromise, and where CD4 facilities are available they should be used to support and reinforce clinical decision-making and can be used to monitor responses to treatment. CD4 levels in relation to the severity of immunosuppression can be expressed as Not significant immunosuppression (>500/mm3), Mild immunosuppression (350−499/mm3) Advanced immunosuppression 200−349/mm3 and Severe immunosuppression (<200/mm3), (39).

In this study The CD4 count of Tuberculosis lymphadenitis among Cytomorphological pattern of lymphadenitis who were HIV positive and with lymphadenopathy accounts (n=13/17,76.5%) less than 200/mm3,(n=2/17,11.8%)200-349/mm3,(n=1/17,5.9%)350-499/mm3 and finally (n=1/17,5.9%) >500/mm3 .similar study show < 200 (58%), (61.11%),(84.8) and (60.87%) at ART clinic of Gondar University Hospital, Tehran Iran, Department of surgical oncology India and Gujarat India respectively (8), (13), (42), (3).

The prevalence of tuberculous lymphadenitis among HIV positive patients who develop lymph node was found to be (n=17/90, 18.9%),However ,The major clinical conditions reported by HIV-infected persons with lymphadenopathy attending our clinic were (n=50/90,55.6%) of the patients was diagnosed clinically as Tuberculosis lymphadenitis The remaining also diagnosed

clinically as (n=32/90,35.6%) reactive hyperplasia,(n=5/90,5.6%)pyogenic lymphadenities and (n=3/90,3.3%) lymphoma .This study showed that Tuberculosis lymphadenitis is over diagnosed by 36.7%, other study conducted at Butajira, Southern Ethiopia. Clinical criteria alone over-diagnosed TBLN by 15.4% compared to histological and/or bacteriological results (30) and study from 4 sites in different parts of Ethiopia diagnosed as having LNTB suggesting that TBLN is over-diagnosed in up to 15% of cases.

This result show that studies made in Butajira and other health facilities, situated in districts part of our country was slightly lower than ours due to study population who were selected suspected of TBLN and develop lymph node alone and using histology diagnosis and additional confirmatory tests like culture.

Among All patients who are positive for HIV and develop lymphadenopathy those with morphologic pattern of Tuberculosis lymphadenitis (n=13/17, 76.5% were categorized under stage IV which indicate that they are severely immunosuppressed in which TB not limited to lungs. Systemic illness usually with prolonged fever, night sweats, weight loss. Clinical features of organs involved, e.g. focal lymphadenopathy, cold abscess, sterile pyuria, pericarditis, ascites, pleural effusion, meningitis, arthritis, orchitis, lupus vulgaris. Responds to standard anti-TB therapy.(39) study in Aminu Kano Teaching Hospital, Northern Nigeria shows (33.6%) of the patients were in WHO stage IV.(41)The higher percentage of our study compare to others may indicate delayed of patients in visiting our hospital and delayed in treatment and lack of early diagnosis .

Among 90 HIV positive patients (n=85/90,94.4%) are out patient with site of the LAP of TBLN at (n=9/17,52.9)cervical and (n=5/17,29.4) auxiliary, which was very cloth to almost all studies conducted like in Tehran Iran, The cervical region (63%)and axillary (22.2%), URS Medical College India cervical region (81.3%)and axillary (14%), tertiary level referral center India The cervical region was involved in 90% cases, followed by axillary (6.4%),Gondar university cervical region (47.5%)and axillary (19.4%).

9. LIMITATION

Though Histological diagnosis considered as golden standard test of histopathology examination this study was done on cytopathology diagnosis of Tubercular Lymphadenitis among HIV positive who develop lymph node. As per previous study conducted in India the expected prevalence was 44% which indicate that it needs at least 376 patient sample (biopsy) to be collected over a period of more than three years.

Even if Biopsy is a medical procedure that involves taking a small sample of body tissue so it can be examined under a microscope it is an invasive procedure that can create fear in patient and may receive local anesthetics to numb the area of the biopsy. Some surgical biopsy procedures require general anesthetics to make you unconscious during the procedure. You may also be required to stay in the hospital for observation after the procedure and in most cases; the results of your biopsy are available in a few days. Some samples may need more time to be analyzed we prefer cytology examination of lymph node.

10. CONCLUSION

Tuberculosis and infection with HIV constitute the major burden of infectious diseases in resource-limited regions such as African and Asian countries. This coinfection can result in diagnostic and therapeutic challenges due to the peculiar clinical picture that is seen with TB–HIV coinfection.

A morphological diagnosis is essential to start anti tuberculous treatment in cases of tuberculous lymphadenopathy and The CD4T-lymphocyte is one of the most important determining factors for the occurrence of various opportunistic infections, and has a central role in human defenses against mycobacteria, which plays the key roles in protective immunity in tuberculosis.

In our study the overall prevalence of tuberculous lymphadenitis among HIV positive patients who develop lymph node was found to be (n=17/90, 18.9%).Maximum number of tuberculosis lymphadenitis is seen in the age group of 21-30 years followed with a 31-42 by decreasing trend in elderly. There was a female preponderance of cases. Tuberculosis lymphadenitis emerged as the 2nd most frequent infection among Cytomorphological pattern of lymphadenitis next to reactive hyperplasia to be associated with HIV infection. Of which caseous necrosis and

Epitheloid granuloma were the predominance.CD4 count <200/mm3 and WHO Sever clinical stage of the disease were found to be predictors of tuberculosis lymphadenitis.

The cervical and auxiliary region was found to be the most involved site respectively and Tuberculosis lymphadenitis is over diagnosed in clinical Diagnosis compare to Cyto-morphological Diagnosis suggesting the need of diagnostic test(s) like cytomorphological examination to supplement the clinical examinations.

11.RECOMMENDATION

Control of both TB and HIV is likely to be most successful if programs collaborate whenever possible and are closely integrated with other healthcare providers by taking measures like early recognition, diagnosis, and treatment of TB suspects, particularly those with lymphadenitis TB and giving emphasis to HIV positive patients with Tuberculosis lymphadenitis who develop lymph node. Commencement of ART should be encouraged before the patient's CD4 count drops below 350/mm3 as to the guideline of the World Health Organization to initiate HAART at CD4 T-cell count ≤ 350/μL should therefore be followed strictly to prevent TB or other opportunistic infections especially in HIV positive patients. Finally cytologic examination of fine needle aspirates should be included in the diagnostic work-up of any patient suspected for TBLN in health institutions with limited laboratory capacity like regional or district Hospitals. Intervention aimed at preventing and treating HIV associated Tuberculosis lymphadenitis are crucial.

High quality services should provide by incorporating an efficient quality control system and a molecular technique has to be incorporate that detects DNA sequences specific for Mycobacterium tuberculosis by polymerase chain reaction that can be used as screening test. Finally since culture is considered as golden standard emphasis should be given in detection of mycobacterium tuberculosis from lymph node aspiration as combination test.

12.ANNEXES

12.1. Annex-I Consent form (English Tigrigna and Amharic)

Title*: tuberculosis lymphadenities among HIV-positive individuals and its correlation to CD4 count, cytomorphology pattern in Mekelle University Ayder comprehensive specialized hospital*

I am post graduate histotechnology student at Addis Ababa University School of medicine department of pathology and am doing a cross-sectional research study to determine the prevalence of tubercular lymphadenities among peoples living with HIV and to correlate with their CD4 count. After finishing the research the study aims to provide information that will create a better awareness of tuberculosis lymphadenities in HIV patients for health professionals and patients as well as improve treatment outcome. Especially in the interview will take approximately 25-30 minutes. The information collected will be Confidential and no individual name will be mentioned throughout the process. This study will be reported and some expressions might be used word by word without mentioning the Identity of speaker. Participants have the right to leave form the study at any time throughout Audio recording and interviewing.
If you require any additional information or have any further questions relating to the Study please contact me at +251-914-51-53-94 or email me at kesii2002@gmail.com

I have read this consent form, fully understand and voluntarily consent to participate in this Study.

Participant signature _____ Date _____

ለቃለ-መጠይቅ ተሳታፊዎች የጥናቱ መረጃና ስምምነት መግለጫ

የጥናቱ ርዕስ:- በመቀለ ዩኒቨርስቲ አይደር ኮምፕርሄንሲቭ ስፔሺያላይዝድ ሆስፒታል በ ኤችአይቪ ኤድስ በሽተኞች ላይ የሚፈጠር የእጢ እብጠት ነቀርሳና CD4 ቁጥር ተያያዥነት በተመለከተ ያለው ተጨባጭ ሁኔታ

እኔ በአዲስ አበባ ዩኒቨርስቲ የድህረ ምረቃ ተማሪ ስሆን በ ኤችአይቪ ኤድስ በሽተኞች ላይ የሚፈጠር የእጢ እብጠት ነቀርሳና CD4 ቁጥር ተያያዥነት በተመለከተ ያለው ተጨባጭ ሁኔታ ና ያላቸውን አመለካከት በማጥናት ለይ እገኛለሁ። ጥናቱ ከተጠናቀቀ ቡኃላ ጥናቱን ተመርኩዞ በጤና ባለሙያዎች እና በህመምተኞች መካከል ስለ በሽታዉ የተሻለ ግንዛቤና እዉቀት ለመፍጠር እንዲሁም በቂ ህክምና ለማግኘት የሚያስችል መረጃ ያስገኛል ተብሎ ይታሰባል፡

በዚህ ጥናት ላይ ለመሳተፍ የሚያስፈልጉ መስፈርቶችን ማሟላቶን ለማወቅ አንዳንድ ጥያቄዎችን እጠይቆታለሁ። በጥናቱ ላይ ለመሳተፍ ላሳዩት ፍላጎትም ላመሰግኖት እወዳለሁ።

በዚህ ጥናት ውስጥ እንዲሳተፉ የተጋበዙት ኤችአይቪ ኤድስ በሽታያለብዎት በመሆኑ፣ በተጨማሪ የእጢ እብጠት ምልክት በሰዉነትዎ በመታየቱ እድሜዎ ከ18 ዓመት በላይ በመሆኑና በአማርኛ ቋንቋ መግባባት በመቻልዎ ነው። በምናደርገው ቃለ መጠይቅ ህመምዎንና ህክምናውን በተመለከተ እንወያያለን። በጥናቱ ተሳታፊ ለመሆን ከፈቀዱ ከ20-30 ደቂቃ የሚቆይ ቃለ መጠይቅ አመቺ በሆነ ቦታ እናደርጋለን:: ። በጥናቱም መሳተፍ ከጀመሩም በኋላ በማናኛውም ጊዜ ማቋረጥ ይችላሉ። አንዳንድ ጥያቄዎች ካልተመችዎት የግድ መመለስ የለብዎትም፤ ቃለ መጠይቁ ረጅምና አድካሚ ከሆነብዎት አባክዎ ይንገሩኝ፣ እረፍት መውሰድ ወይም ቀሪውን ለሌላ ጊዜ ማስተላለፍ እንችላለን። የርስዎን ህክምና የበለጠ ለመረዳት በሆስፒታሉ ያልዎትን የህክምና ካርድ እመለከታለሁ፣ አንዳንድ መረጃዎችንም እወስዳለሁ።

ከዚህ ጥናት ጋር በተያያዘ የርስዎን ማንኛውም የግል ጉዳይ እንደምጠብቅ፤ ስምዎ፣ ማንነትዎ እንዲሁም ማንኛውንም መረጃ በምስጢር እንደምይዝ እንዲያውቁ አፈልጋለሁ። በመጀመሪያ ስምዎን ከኔ በስተቀር ሌላ ሰው እንዲያውቀው አይደረግም። ስምዎን በምናደርጋቸው ቃለ መጠይቆች፣ ተከትለው በሚወጡ ጽሁፎች ላይ አንጠቀምም። ከዚህ ጥናት ውስጥ አንዳንድ አባባሎች ቃል በቃል ሊወሰዱ ይችላሉ ነገር ግን የተናገረው ሰው ስም አይገለፅም። የጥናቱ አጠቃላይ ውጤት ፍላጎት ላላቸው የጥናቱ ተሳታፊዎች ይሰጣል።

ማንኛውም አይነት ጥያቄ ወይም መረጃ ከፈለጉ በ0914515394 በመደወል ወይም በኢሜይል አድራሻ kesii2002@gmail.com መጠየቅ ይችላለ::

ይህን የስምምነት ውል በሚገባ አንብቤዋለሁ;በጥናቱ ላይ ለመሳተፍ ዝግጁ ነኝ::

ፊርማ---------- ቀን_____

መግለፂ ስምምዕነትን መረዳእታን ንተሳተፍቲ ቃለ መሕትት ናይዚ መፅናዕታዊ ፅሑፍ

ርእሲ መፅናዕታዊ ፅሑፍ ፡ ኣብ መቀለ ዩኒቨርስቲ ዓይደር ኮምፕርሄንሲቭ ስፔሺያላይዝድ ሆስፒታል ምስ ኤችኣይቪ ኤድስ ዝነብሩ ሕሙማት ኣብኣቶም ዝፍጠር ናይ ነቀርሳ ፅኪ ሕብጠትን CD4 ቁፅሪን ተተሓሓዝነት ብዝምልከት ዘለዎም ጭቡጥ ኩነታት

ኣነ ኣብ አዲስ አበባ ዩኒቨርስቲ ናይ ድህረ ምረቃ ተማሃሪ እንትኾን ምስ ኤችኣይቪ ኤድስ ዝነብሩ ሑሙማትን ኣብኣቶም ዝፍጠር ናይ ነቀርሳ ፅኪ ሕብጠትን CD4 ቁፅሪን ተተሓሓዝነት ብዝምልከት ዘለዎም ጭቡጥ ኩነታትን ዘለዎም አመለኻኽታን ኣብ ምፅናዕ ይርከብ ። እቲ ፅንዓት ምስ ተጠናቐቐ አፍቲ ፅንዓት ተመርኩዝካ ኣብ ጥዕና ሰብ ሞያን አብ ሕሙማትን ማእኸል ብዛዕባ እቲ ሕማም ዝሓሸ ግንዛቤን ፍልጠትን ንምፍጣር ከምኡዉን እኹል ሕክምና ንምርካብ ዘኽእል መረዳእታ ክርከብ ይኽእል ተባሂሉ ይሕሰብ.

አብዚ ፅንዓት ውሽጢ ንክሳተፉ ዝተጋበዝሉ ምክንያት ኤችኣይቪ ኤድስ ሕማም ዘለዎም ብሙኻኑ፣ ብምቅፃል ፅኪ ሕብጠት ምልክት አብ ሰዉነቶ ም ብምርአዩ ዕድሜኦም ካብ18 ዓመት ንላዕሊ ብምኻኑን ብትግርኛ ቓንቓ ምርድዳእ ምኽአሎምን እዩ ። አፍቲ ንገብሮ ቃለ መሕተት ብዛዕባ እቲ ሕማምን ምልክታቱን ክንዘራረብ ኢና ። አብዚ ፅንዓት ውሽጢ ንክሳተፉ ፍቓደኛ እንተኾይኖም ካብ 20-30 ደቂቃ ዝፀንሕ ቃለ መሕተት ምችዉ አብ ዝኾነ ቦታ ክንገብር ኢና :: ። አብዚ ፅንዓት እዚ ምስታፉ ምስጀመሩ ኣብዝኾነ ሰዓት ከቓርፁ ይኽእሉ እዮም። ሐደሐደ ጥያቄታት እንድሕር ዘይተመችይዎም ናይግድን ክምልስዎ አይግባእን፤ እቲ ቃለ መሕተት ነዊሕን አድካምን እንተኮይኑ በይዛኹም ንገሩኒ ፣ ዕረፍቲ ምዉሳድ ወይከዓ እቲ ዝተረፈ ንካሊእ ጊዜ ምምሕላፍ ይከአል እዩ ። ናትኩም ሕማምን ምልክታትን ብዝበለፀ ንምርዳእ ኣፍቲ ሆስፒታል ዘሎ ካርድኹም ክርእይ እየ። ሐደሐደ መረዳእታታት ዉን ክወስድ እየ ።

ዝኾነ ይኹን ናይ ግሊ ጉዳይ ምስዚ ፅሑፍ ዝተተሓሓዘ ከምዝሕሉን፤ ሽሞም፣ መንነቶም ከምኡዉን ዝኾነ ዓይነት መረዳእታ ብምሸጥር ከምዝሕዝ ክፈልጡ ይግባእ ። ብመጀመሪያ ሽሞም ብዘይካእነ ካሊእ ሰብ ክፈልጦ አይግበርን። አብዚ ፅንዓት ውሽጢ ሐደሐደ አበሃህላታት ቃል ብቃል ከዉሰዱ ይክእሉ እዮም ኮይኑ ግን ናይ ተሓታቲ ሽም አየግለፀን ። ናይቲ ፅንዓት ኣጠቓላሊ ዉፅኢት ድሌት ንዘለዎም ናይቲ ፅንዓት ተሳተፍቲ ከወሃብ ይከአል::

ዝኾነ ዓይነት ጥያቄ ወይከዓ መረዳእታ እንድሕር ዝደልዩ ብ 0914515394 ብምድዋል ወይከዓ ብኢሜይል አድራሻ kesii2002@gmail.com ምጥያቕ ይከአል::

እዚ ናይ ስምምዕነት ዉዕሊ ብዝግባእ ዘንበብክዎ እንትኾን አፍቲ ፅንዓት ንምስታፍ ዝግጁ እየ::

ፌርማ---------- ዕለት_____

12.2. Annex II: Interview questions (English Tigrigna and Amharic)

TUBERCULOSIS LYMPHADENITIES AMONG HIV-POSITIVE INDIVIDUALS AND ITS CORRELATION WITH CD4 COUNT IN MEKELL UNIVERSITY AYDER COPRHENSIVE SPECIALIZED REFERAL HOSPITAL, ETHIOPIA TO BE COMPLETED BY PERSONAL INVESTIGATOR

1. Age:_____ sex:_____
2. Educational Status : A, - no formal education B,- primary C, - Secondary
   D, - Diploma E, - Degree F,- Above Degree G, pre school
3. Religions : A, - Orthodox B.- Muslim
   C, - Catholic D, - protestant
4. Patient type: A, - outpatient B,- inpatient
5. Clinical diagnosis: A,- TB lymphadenities B, reactive lymphadenities
   C, - lymphoma D, - kaposis sarcoma
   F, - pyogenic lymphadenities E, - other
6. Microscopic finding A,- caseous necrosis only B, Epitheloid granuloma only
   C,- caseous necrosis and Epitheloid granuloma E, reactive hyperplasia
7. Site of LAP: A,-cervical B,- Auxiliary
   C, - inguinal D,-supraclavicular E, submandibular
8. Duration of the LAP: A,- less than one week B,-two week
   C, three weeks D, one month E, - more than 1 week
9. Appearance of LAP A,- discrete B,- matted
10. Consistency of lymph node A, soft B, Firm C, hard
11. Mobility of lymph node A, mobile B, Fixed
12. Size of lymph node A,- less than 1 cm B,- two cm C, three cm D,- above three
13. Clinical sign and symptoms A,- fever B,- night sweeting C, loss of appetite
    D,-weight loss E,-cough
14. FNAC- Aspirate type: A,-cellular B,- fluidic C,- turbid D,-hemorrhagic E, pus
15. Slide numbers: A, 1x1 B,1x2 C, 2x2 D, 2x3 E,-above
16. Patient HIV/AIDS stage: A,- 1 B,- 2 C,- 3 D,- 4
17. Level of CD4 count A, 1-100 B, 100-200 C, 200-300 D, 300-400 E, 400-500

ከህሙማን ጋር ህመማቸውንና ምልክቶችን በተመለከተ ለሚደረገው ቃለ-መጠይቅ የሚያገለግሉ ጥያቄዎች

የጥናቱ ርዕስ:- በመቀለ ዩኒቨርስቲ አይደር ኮምፕርሄንሲቭ ስፔሺያላይዝድ ሆስፒታል በ ኤችኣይቪ ኤድስ በሽተኞች ላይ የሚፈጠር የእጢ እብጠት ነቀርሳና CD4 ቁጥር ተያያዥነት በተመለከተ ያለው ተጨባጭ ሁኔታ

የቃለ-መጠይቁ ጥያቄዎች የሚከተሉት ናቸው፤

1. ዕድሜ:_____ ፃታ:_____
2. የትምህርት ሁኔታ ሀ፣ ምንም አይነት ትምህርት የለኝም ለ፤ 1ኛ ደረጃ ሐ፤ 2ኛ ደረጃ
   መ፤ ዲፕሎማ ሰ፤ ዲግሪ ረ፤ ከ ዲግሪ በላይ ሸ፤ አፀደ ህፃናት
3. ሀይማኖት ሀ፤ኦርቶዶክስ ለ፤ ሙሰሊም ሐ፤ ካቶሊክ መ፤ ሌላ
4. የበሽተኛዉ አይነት ሀ፤ ተመላላሽ ለ፤ ኣስተኝቶ ማከም
5. የበሽታው ምልክት ምርመራ ሀ፤ የቲቢ የእጢ እብጠት ለ፤የ በሽታ አምጪ ህዋስ እጢ እብጠት
   ሐ፤ የ ቱቦ እጢ ካንሰር መ፤ የአጥንትና ጡንቻ እጢ ካንሰር
   በ፤ በ ባክተርያ ምክንያት የሚፈጠር መግል ያለው እብጠት ረ፤ ሌላ
6. የበሽታው ማይክሮስኮፕ ምርመራ ሀ፤ ኬዚዮስ ኔክሮሲሰ ለ፤ ኢፕተሎይድ ግራኑሎማ
   ሐ፤ ኬዚዮስ ኔክሮሲሰ ና ኢፕተሎይድ ግራኑሎማ
7. የእጢዉ ቦታ ሀ፤ አንገት አከባቢ ለ፤ብብት አከባቢ
   ሐ፤ መንጋጋ አከባቢ መ፤ ብልት አከባቢ
8. የእጢዉ እብጠት ግዜ መጠን ሀ፤ ከ 1ሳምንት በታች ለ፤ 2 ሳምንት ሐ፤ 3 ሳምንት መ፤ 1 ወር መ፤ 1 ወር በላይ
9. የእጢዉ እብጠት ሁኔታ ሀ፤ የተያያዘ ለ፤በአንድ ቦታ የተወሰነ
10. የእጢዉ ይዘት ሀ፤ ጥብቅ መ፤ ጠንካራ ሰ፤ ለስላሳ
11. የእጢዉ አይነት ሀ፤ ተንቀሳቃሽ ለ፤ የተወሰነ
12. የእጢዉ እብጠት መጠን ሀ፤ ከ 1 ሴ.ሜ በታች ለ፤ 2 ሴ.ሜ ሐ፤ 3 ሴ.ሜ መ፤ ከ 3 ሴ.ሜ በላይ
13. የበሽታዉ አይነትና ምልክቶች ሀ ፤ ትኩሳት ለ፤ የምግብ ፍላጎት መቀነስ ሐ፤ ሳል
    መ፤ ክብደት መቀነስ ሰ፤ ሌሊት ማስላብ
14. የተመጠጠዉ የእጢዉ ይዘት ሀ፤ ሴል የበዛበት ለ፤ ፈሳሻማሐ ፤ ድፍርስ መ፤ ደም የተቀላቀለበት
15. የተሰራ ስላይድ ብዛት ሀ፤ 1x1 ለ፤ 1x2 ሐ፤ 2x2 መ፤ 2x3 ሰ፤ ከዛም በላይ
16. የበሽተኛዉ ኤችኣይቪ ኤድስ ደረጃዉ ሀ፤ 1 ለ፤ 2 ሐ፤ 3 መ፤ 4
17. የበሽተኛዉ CD4 መጠን ሀ፤ 1-100 ለ፤ 100-200 ሐ፤ 200-300 መ፤ 300-400 ሰ፤ 400-500

ምስ ሕሙማት ብዛዕባ ሕማሞምን ምልክታቱን ብዝምልከት ዝግበር ቃለ-መሕተት ዘገልግሉ ሕቶታት

ርዕሲ እቲ ፅንዓታዊ ፅሑፍ :- ኣብ መቐለ ዩኒቨርስቲ ዓይደር ኮምፕርሄንሲቭ ስፔሺያላይዝድ ሆስፒታል ምስ ኤችኣይቪ ኤድስ ዝነብሩ ሕሙማት ዝፍጠር ናይ ሳንባ ነቀርሳ ፅኪ ሕብጠትን CD4 ቁፅሪ ተተሓሓዝነትን ብዝምልከት ዘለዎ ጭቡጥ ኩነታት

እቶም ናይ ቃለ-መሕተት ጥያቄታት ከምዝስዕብ ክቕረቡ እዮም

1. ዕድመ :_____ ፃታ:_____
2. ናይ ትምህርቲ ኩነታት ሀ፣ ምንም ዓይነት ትምህርቲ የብለይን ለ፤ 1ይ ደረጃ ሐ፤ 2ይ ደረጃ
   መ፤ ዲፕሎማ ሰ፤ ዲግሪ ረ፤ ካብ ዲግሪ ንላዕሊ ሸ፤ አፀደ ህፃናት
3. ሀይማኖት ሀ፤ኦርቶዶክስ ለ፤ ሙሰሊም ሐ፤ ካቶሊክ መ፤ ካብኡ ወፃኢ
4. ናይቲ ተሓካሚ ዓይነት ሀ፤ ተመላላሲ ተሓካሚ ለ፤ ኣደቂስካ ዝሕከም
5. ናይቲ ሕማም አይነት ሀ፤ ናይ ቲቢ ፅኪ ሕብጠት ለ፤ናይ ሕማም አምፃኢ ህዋስ ፅኪ ሕብጠት
   ሐ፤ ናይ ቱቦ ፅኪ ካንሰር መ፤ ናይ አዕፅምትን ጡንቻን ፅኪ ካንሰር
   በ፤ ብ ባክተርያ ምክንያት ዝፍጠር መግሊ ዘለዎ ሕብጠት ረ፤ ካሊእ
6. ናይቲ ሕማም ማይክሮስኮፕ ምርመራ ሀ፤ ኬዚዮስ ኔክሮሲሰ ለ፤ ኢፕተሎይድ ግራኑሎማ
   ሐ፤ኬዚዮስ ኔክሮሲሰ ና ኢፕተሎይድ ግራኑሎማ
7. እቲ ናይ ፅኪ ሕብጠት ቦታ ሀ፤ አብ ክሳድ አከባቢ ለ፤መንኮራርዕቲ አከባቢ
   ሐ፤ መንጋጋ አከባቢ መ፤ ብልዕቲ አከባቢ
8. እቲ ናይ ፅኪ ሕብጠት ግዜ መጠን ሀ፤ 1 ሰሙን ለ፤ 2 ሰሙን ሐ፤ 3 ሰሙን መ፤ 1 ወርሒ መ፤ ልዕሊ ሓደ ወርሒ
9. እቲ ናይ ፅኪ ሕብጠት ምስሊ ሀ፤ ዝተተሓሓዘ ለ፤ኣብ ሓደ ቦታ ዝተወሰነ
10. ናይ ፅኪ ትሕዝቶ ሀ፤ ልስሉስ ለ፤ ጠንካራ ሐ፤ ጥቡቕ
11. እቲ ናይ ፅኪ ሕብጠት ዓይነት ሀ፤ ተንቀሳቃሲ ለ፤ ዝተወሰነ
12. እቲ ናይ ፅኪ ሕብጠት ዓቕን ሀ፤ ትሕቲ 1 ሴ.ሜ ለ፤ 2 ሴ.ሜ ሐ፤ 3 ሴ.ሜ መ፤ ካብ 3 ሴ.ሜ ንላዕሊ
13. ናይቲ ሕማም ዓይነትን ምልክታትን ሀ ፤ ትኩሳት ለ፤ ናይ ምግብ ድሌት ምቅናስ ሐ፤ ሰዓል
   መ፤ ክብደት ምቅናስ ሰ፤ ለይቲ ምርሀፅ
14. ናይቲ ዝተመጠጠ ፅኪ ሕብጠት ትሕዝቶ ሀ፤ ሴላት ዝበዝሕዎ ለ፤ ፈሳሲ ዓይነት
   ሐ ፤ ድፍርስ መ፤ ደም ዝተሓወሶ
15. ዝተሰርሐ በዝሒ ስላይድ ሀ፤ 1x1 ለ፤ 1x2 ሐ፤ 2x2 መ፤ 2x3 ሰ፤ ካብኡ ንላዕሊ
16. ናይቲ ሕሙም ኤችኣይቪ ኤድስ ደረጃ ሀ፤ 1 ለ፤ 2 ሐ፤ 3 መ፤ 4
17. ናይቲ ሕሙም CD4 መጠን ሀ፤ 1-100 ለ፤ 100-200 ሐ፤ 200-300 መ፤ 300-400 ሰ፤ 400-500

12.3. Annex III Guidelines on Standard Operating Procedures for Fine needle aspiration cytology and CD4 count

12.3.1. Standard Operating Procedures for Fine needle aspiration cytology

Introduction

Fine needle aspiration (FNA) cytology is widely accepted as a first line of investigation in any patient with a mass lesion. Superficial lesions can be aspirated by palpation. Radiological imaging such as ultrasound, fluoroscopy, and computed tomography (CT) can guide fine needles to impalpable or deeply located lesions. In common with many cytopathological investigations, the procedure can be quick, inexpensive (apart from when certain complex imaging techniques are required) and reliable. FNA requires readily available equipment, takes very little time to perform, causes minimal trauma, and may be repeated if required. As an investigative technique it is acceptable to most patients and it rapidly provides a diagnosis on which to base further management. There are three levels of skill required in providing a safe and reliable FNA service; taking the sample, preparing it, and interpreting the findings. The interpretation is made in the context of the clinical findings and the results of other investigations and is dependent on obtaining adequate samples and preparing them to maximize their diagnostically relevant information.(31)

FNA has become the primary investigative procedure for mass lesions in HIV-positive patients, particularly in the assessment of lymphadenopathy. The procedure is rapid, easily performed and in many cases obviates excision while guiding therapy or observation. (31)

Equipment

Most of the equipment needed for a high quality FNA service is inexpensive and readily available but a good quality microscope, preferably with a double-head, should be available for rapid on-site assessment.

1. **Needles**

Needle gauge is based on external diameter. Fine needles should be 23 gauge (external diameter 0.6 mm) or less (external diameter 0.7 mm). It is important to use smaller needles for the following reasons: i) it is less painful ii) it causes less bleeding and iii) the risk, albeit rare, of tumor seeding is considerably reduced.24 Thicker needles (G18, external diameter 1.2 mm, or wider) carry an ever-increasing risk of complications including significant hemorrhage.

2. **Syringes and syringe holders**

   Either a 3,5 or a 10 ml sterile disposable plastic syringe can be used, depending on personal preference.

3. **Sharps container**

   A sharps container for safe disposal of needles and unused slides should be available at any site where FNAs are being carried out. In known high-risk patients, needles should not be separated from the syringe at the time of disposal into the sharps container. (31)

4. **Slides, fixative and collection**

   fluid Clean slides with frosted ends are required if direct smears are to be prepared at the time an aspirate is taken. Direct smears can be either wet-fixed by alcohol spray or, preferably, by immersion in 95% alcohol, or rapidly air-dried

5. **Local anesthetic**

   Local anesthesia of the overlying skin and proposed needle tract is usually only needed in especially sensitive locations such as nipple, lip or eyelid, for small children or in situations where multiple passes of the needle are planned

   The FNA procedure

1. **Request form**

   The request form serves several functions. It identifies the patients' name, address, age and gender, the person requesting the test, the type of test required. It provides clinical information about the anatomical site of the procedure and date of collection

2. **Talk to the patient**

   The aspirator should put the patient at ease before taking as full a history as necessary. The technique of FNA should be briefly explained, emphasizing the purpose of the test, its limitations, its potential complications and that it may be necessary to repeat

3. **Consent**

   The technique of FNA has a clear purpose as well as certain limitations and complications, which should be discussed honestly and fully with the patient before he or she is asked to consent to aspiration

4. **Take the aspirate**

   The skin or mucosal surface over the lesion should be cleaned with an alcohol wipe (70% alcohol). If local anesthetic is to be used, time should be taken to allow it to achieve its full

numbing effect. If required, an FNA needle, syringe and holder can be assembled during this time. When ready, one hand should be used to feel and steady the lump; the other hand is free to hold and manipulate the FNA syringe holder.

The FNA syringe holder is held in one hand; the thumb and first two fingers of the free hand can be used to stretch the skin over the lesion and to immobilize it (acting like a bridge for the syringe as if it were snooker or pool cue). The needle should be pushed quickly through the skin at a right angle to it and immediately into the lump. The syringe may then be drawn back using the holder to create negative pressure as required. Cells are collected by the cutting edge at the tip of the needle as it is pushed repeatedly through the lesion. *(*31*)*

5. **Stop the bleeding**

As soon as the needle is withdrawn, the aspirator or assistant should press carefully and firmly against the aspiration site for about 1 minute to reduce bruising

6. **Direct smears**

Direct smears permit rapid staining and assessment at the time an aspirate is taken and are very helpful in reducing the number of unsatisfactory FNAs

7. **Health and safety**

Because all fresh body fluids may harbor unexpected biohazards, such as mycobacteria or hepatitis B, C or human immunodeficiency virus (HIV), it is essential that appropriate precautions are taken to prevent infection of staff and patients both during aspiration and slide preparation. If surgical gloves are used, latex-free gloves should be available for sensitized staff or patients. In high-risk cases, masks, eye protection and an apron or gown should be used. Patients with active infections should themselves wear masks. There are no absolute contraindications to making direct smears, which are essential for rapid assessment, but appropriate care must be taken to minimize risk. FNA procedure does not need to be carried out in premises specially designed for handling hazardous materials. It can be carried out in any standard consulting room. However, the use of special procedures room is preferable since this provides better conditions for health and safety, such as space to access the patient and the equipment easily. *(28)*

Staining procedure

Giemsa or wright stain

1. Make a uniform smear (aspirate specimen) on a clean grease-free glass slide.

2. Flood the slide with methyl alcohol and leave for 3-5 minutes for fixation.(if Giemsa stain is to be used)

3. Cover the slide with freshly filtered Wright stain (it is important to cover the entire slide) and leave for 1-3 minutes.

4. without removing the stain, pour on buffer solution (pH 6.4); surface tension will not allow the buffer to run off. Some workers prefer using tap water instead of the buffer solution.

5. Blow gently over the surface of the fluid to mix the buffer and the stain. Upon proper mixing metallic green sheen (green scum) rises to the surface of the fluid. Leave for three minutes or more (e.g. bone marrow takes longer to stain).

6. Wash the slide gently with flowing tap water, and wipe the bottom of the slide with a clean tissue.

7. Air dry the slide, and observe under the microscope. *(29).*

Precautions

(1) Since Wright stain is prepared in methanol, a separate fixation step is not required.

(2) The timings of each step should be standardized in the laboratory for optimal coloration.

(3) The staining of different components in a smear is dependent on the pH of the medium, which has to be maintained. Excess alkaline or acidic conditions may cause the colour to be too blue or red to be seen properly.

(4) Unfiltered Wright stain may leave granular particles on the preparation.

Observation

Preparation of Wright stain

Wright stain is a Romanowsky type stain which contains methylene blue as the active ingredient. At the proper pH, the methyl groups are activated and react with charged components of the cell to produce coloration.

Powdered Wright's stain 9 g (0.3% w/v) Powdered Giemsa stain

1 g (0.033% w/v) Glycerin

90 ml (3% v/v) Absolute acetone-free methanol

2910 ml (to make up the volume)

The components have to be mixed in a brown bottle and let stand for one month before use. The stain must be stored at 40C; otherwise the components may degrade. *(29)*

Preparation of Giemsa staining

To prepare Giemsa stock solution, dissolve 0.5 gm of Giemsa powder in 33 ml of glycerine by placing the mixture in a water bath (55o-60oC) for 90 minutes. When the powder is dissolved, add 33 ml of absolute methanol. The stock solution is stored at room temperature.

The scraping prepared onto clean glass slide is fixed in absolute methanol for ten minutes. The slides are then stained with Giemsa stain (freshly prepared by diluting the stock solution 1:20 in water) for two hours. The stained slides are decolorized in 95% ethanol for a few seconds and rinsed in tap water. *(29).*

Microscope examination

In granulomas associated with certain infectious organisms (most classically Mycobacterium tuberculosis), a combination of hypoxia and free radical–mediated injury leads to a central zone of necrosis. Grossly, this has a granular, cheesy appearance and is therefore called caseous necrosis. Microscopically, this necrotic material appears as amorphous, structure less, eosinophilic granular debris, with complete loss of cellular details. The granulomas in Crohn disease, sarcoidosis, and foreign body reactions tend to not have necrotic centers and are said to be noncaseating. Healing of granulomas is accompanied by fibrosis that may be extensive.

Reporting of results

The laboratory management should be responsible for formatting reports. The laboratory director or manager should systematically review, evaluate and authorize the results of testing. The report shall be clear and unambiguous and include date, time, procedure used, identification and location of the patient. It should also include identification of the person authorizing the release of the report with signature. The laboratory should retain all primary files, worksheets, and report forms for two years or in accordance with the approved policy. (27).

12.3.1. Guidelines on Standard Operating Procedures for CD4 T cell count

Introduction

Immunofluorescence analysis by flow cytometry is the gold standard for CD4 T lymphocytes measurements and also the method of choice if a large throughput of samples is required.. The flow cytometric assays work on the principle of scattering of light due to different sizes, granularity of the cells passing thorough the laser beam, and also by the fluorescence emitted by

the cells after staining with the specific monoclonal antibodies to cell surface markers that are tagged with different fluorescence dyes. The population of interest can be thus identified and gated for further analysis within the population of interest. The monoclonal antibodies specifically bind different surface receptors like CD4 for T helper cells. Relative percentages of the cells expressing the specific receptor (e.g. CD4) on its surface are obtained from the flow cytometer and the absolute counts can be calculated with the help of absolute lymphocyte count obtained from hematology analyzer.

Collection transport and report of specimens

Quality laboratory results begin with proper collection and handling of the specimen submitted for analysis. Correct patient preparation, specimen collection, specimen packaging and transportation are of vital importance. All necessary biosafety precautions should be followed during collection and transport of the blood samples. The Standard Operating Procedures (SOPs) should be available and should be followed strictly. Errors during these procedures can lead to serious consequences and affect patient and on subsequent patient management.

The collection procedures may vary depending upon the methodology used for the enumeration of absolute CD4 T lymphocytes, although Ethylenediamine tetra-acetate (EDTA) is generally recommended by most of the manufacturers. For dual platform approach the whole blood collected in EDTA should reach and be processed in the laboratory for lymphocyte counts within six hours.

Blood collection procedures

- Ensure that the specimen, collection tube is labeled with the date, time of collection, and a unique patient identifier/name. Labeling should be done legibly with a ballpoint pen or permanent marker.
- Record the age and sex of the patient and the collector information (name and initials) in the requisition form/work sheet, which should accompany the specimen being transported to the testing laboratory
- Collect blood specimens by venipuncture using evacuated tubes (e.g. BD Vacutainer blood collection tube) containing an appropriate anticoagulant, completely expending the vacuum in the tubes. Do not use tubes after the expiry date.
- Draw blood as per the standard procedure for venipuncture blood collection. The plastic collection tubes should be preferred due to the risk of breakage and possibility of cuts due to

sharp edges with the glass tubes. Partial draws (less volume in higher capacity tube) are not recommended, as the anticoagulant is hypotonic.

- Mix the blood well with the anticoagulant (invert the tube at least eight times) soon after the collection to prevent clotting.
- Collect all specimens from individual patients, wherever possible, at the same time of day (either morning or evening) to minimize diurnal variation
- Examine the tubes for sample integrity before transporting to the testing laboratory. Repeat specimens may be requested, if there is evidence of gross hemolysis or clots. Keep the specimen at room temperature (preferably below 28 oC) and do not refrigerate or freeze. Do not remove the stopper at any time. (27).

Transport of whole blood specimen

Packaging and transportation of HIV-infected material should be in accordance with the local regulations of the postal and/or courier service used. It is important to maintain and transport specimens at room temperature (approximately18-22 °C). Avoid extreme temperatures. Temperatures greater than 37 °C) may cause cellular destruction and affect both haematology and flow cytometry results. If the external temperature is high, the samples should be shipped with a cool (not refrigerated) pack.

The insulated container should be used for packing of samples keeping the cool packs separately. Use overnight carriers with an established record of consistent overnight delivery to ensure arrival the following day so that the specimens reach the testing laboratory within 2430 hours. Check the equipment specifications to determine the acceptable time interval between collection and processing of the samples. During the transportation, the mechanical shaking should be avoided, as this may damage the cells integrity.

Reporting of results

The laboratory management should be responsible for formatting reports. The laboratory director or manager should systematically review, evaluate and authorize the results of testing. The report shall be clear and unambiguous and include date, time, procedure used, identification and location of the patient. It should also include identification of the person authorizing the release of the report with signature. The laboratory should retain all primary files, worksheets, and report forms for two years or in accordance with the approved policy. (27).

General bio-safety guidelines

- Wear laboratory coats and gloves when processing and analyzing specimens.
- Never recap, bend, or break used needles – but recapping may be done with single hand method.
- Dispose needles and syringes in puncture-proof containers designed for this purpose.
- Handle and manipulate infectious fluid specimens with protective Equipments such as a face shield, mask, goggles etc.
- Wash hands with soap and water after working with specimens in the laboratory and when leaving the laboratory.
- For stream-in-air flow cytometers, follow the manufacturer's recommended procedures to eliminate the operator's exposure to any aerosols or droplets of sample material.
- Disinfect flow cytometer wastes. Add a volume of undiluted household bleach (1% sodium hypochlorite) to the waste container before adding waste materials so that the final concentration of bleach will be 10% (0.1% sodium hypochlorite) when the container is full (e.g., add 100 ml undiluted bleach to an empty 1000-mL container). (27).

- Disinfect the flow cytometer as recommended by the manufacturer. One method is to flush the flow cytometer fluidics with a 1% sodium hypochlorite solution for 5-10 minutes at the end of the day, and then flush with water or saline for at least 10 minutes to remove excess bleach, which is corrosive.
- Disinfect the blood spills with 10% sodium hypochlorite.
- In case of a large number of samples, the use of a flow cytometer equipped with an auto sample loader/biosampler is recommended to minimize sample handling.
- Specimen preparation should be performed in a designated area, which should be thoroughly decontaminated after use.
- Management of exposure: In case of needle prick injury or contact of skin lesions with infected sample: • Report it to Biosafety committee. • Allow the site to bleed. • Wash it with plenty of soap and running water. • Determine HBsAg and HIV status of the source.
- Accordingly, HBsAg/ vaccine against HBV (if not previously immunized) / PEP for HIV should be started.

13. REFERENCES

1. Olowe OA, Makanjuola OB, Adekanmi AS, Adefioye OJ, Olowe RA. Epidemiological characteristics and clinical outcome of HIV-related tuberculosis in a population of TB patients in South-Western Nigeria. European Journal of Microbiology and Immunology. 2017 Jun; 7(2):127-32.

2. Sutariya SB, Shah HM, Patel DA, Dandge VA. Tuberculosis in Patients Living with HIV/AIDS: Types and Its relation to CD4 count. National Journal of Medical Research. 2015; 5(1):75-8.

3. Atomsa D, Abebe G, Sewunet T. Immunological markers and hematological parameters among newly diagnosed tuberculosis patients at Jimma University Specialized Hospital. Ethiopian journal of health sciences. 2014; 24(4):311-8.

4. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian Journal of Medical Research. 2004 Oct 1; 120(4):316.

5. Mehta JB, Dutt A, Harvill L, Mathews KM. Epidemiology of extrapulmonary tuberculosis: a comparative analysis with pre-AIDS era. Chest. 1991 May 1; 99(5):1134-8.

6. Badri M, Ehrlich R, Wood R, Pulerwitz T, Maartens G. Association between tuberculosis and HIV disease progression in a high tuberculosis prevalence area. The International Journal of Tuberculosis and Lung Disease. 2001 Mar 1; 5(3):225-32.

7. Laishram RS, Devi RT, Khuraijam S, Devi KR, Khuraijam S, Sharma LD. Fine needle aspiration cytology of HIV-related lymphadenopathy in Manipur. Journal, Indian Academy of Clinical Medicine. 2014 Apr; 15(2).

8.Mohammed H, Assefa N, Mengistie B. Prevalence of extrapulmonary tuberculosis among people living with HIV/AIDS in sub-Saharan Africa: a systemic review and meta-analysis. HIV/AIDS (Auckland, NZ). 2018; 10:225.

9. World Health Organization. Interim WHO clinical staging of HVI/AIDS and HIV/AIDS case definitions for surveillance: African Region. Geneva: World Health Organization; 2005.

10. Swaminathan S, Padmapriyadarsini C, Narendran G. HIV-associated tuberculosis: clinical update. Clinical Infectious Diseases. 2010 May 15; 50(10):1377-86.

11. JacobM.Gray, MD, David L. Cohn, MD.Tuberculosis and HIV Coinfection. Division of Infectious Diseases, Department of Medicine, University of Colorado School of Medicine, 12700 E. 19th Ave. B168, Aurora, CO 80045.

12. Hemalatha A, Shruti PS, Kumar MU, Bhaskaran A. Cytomorphological patterns of tubercular lymphadenitis revisited. Annals of medical and health sciences research. 2014; 4(3):393-6.

13. Nidhi P, Sapna T, Shalini M, Kumud G. FNAC in tuberculous lymphadenitis: Experience from a tertiary level referral centre. Indian J Tuberc. 2011 Jul; 58(3):102-7.

14. Adeiza MA, Abba AA, Okpapi JU. HIV-Associated tuberculosis: A sub-Saharan African perspective. Sub-Saharan African Journal of Medicine. 2014 Jan 1; 1(1):1.

15. Ghate MV, Mehendale SM, Mahajan BA, Yadav R, Brahme RG, Divekar AD, Paranjape RS. Relationship between clinical conditions and CD4 counts in HIV-infected persons in Pune, Maharashtra, India. National Medical Journal of India. 2000 Jul 1; 13(4):183-7.

16. Jaryal A, Raina R, Sarkar M, Sharma A. Manifestations of tuberculosis in HIV/AIDS patients and its relationship with CD4 count. Lung India: official organ of Indian Chest Society. 2011 Oct; 28(4):263.

17.Deribew A, Tesfaye M, Hailmichael Y, Negussu N, Daba S, Wogi A, Belachew T, Apers L, Colebunders R. Tuberculosis and HIV co-infection: its impact on quality of life. Health and quality of life outcomes. 2009 Dec; 7(1):105.

18.Krasic D, Trajkovic M, Mihailovic D, Stojanovic M, Arandjelovic S. Extrapulmonary manifestations of tuberculosis in the neck in HIV positive patient. OA Case Reports 2014 Apr 19; 3(4):34.

19. Dr.harapriya kar, Dr. Chitra (bhat) pai, Dr. Revati sharma, Ms. Minakshi bhattacharjee and Dr. Samir pachpute. A profile of tuberculosis cases among HIV positive patients in navi mumbai. Vol 5/ issue 3/July 2015.

20. Vajpayee M, Kanswal S, Seth P, and Wig N. Spectrum of opportunistic infections and profile of CD4+ counts among AIDS patients in North India. Infection. 2003 Oct 1; 31(5):336-40.

21. Hudson CP, Wood R, Maartens G. Diagnosing HIV-associated tuberculosis: reducing costs and diagnostic delay. The International Journal of Tuberculosis and Lung Disease. 2000 Mar 1; 4(3):240-5.

22. Polesky A, Grove W, Bhatia G. Peripheral tuberculous lymphadenitis: epidemiology, diagnosis, treatment, and outcome. Medicine. 2005 Nov 1; 84(6):350-62.

23. Huda MM, Taufiq M, Yusuf MA, Rahman MR, Begum F, Kamal M. Histopathological Features of Lymph Nodes of Tuberculous Lymphadenitis Patients: Experience of 50 cases in Bangladesh. Bangladesh Journal of Infectious Diseases. 2016; 3(2):40-2.

24. Alves F, Baptista A, Brito H, Mendonça I. Necrotising granulomatous lymphadenitis. Case Reports. 2011 Jan 1; 2011:bcr1120103548.

25. Steinbrook R. Tuberculosis and HIV in India. New England Journal of Medicine. 2007 Mar 22; 356(12):1198-9.

26. Moore D, Liechty C, Ekwaru P, Were W, Mwima G, Solberg P, Rutherford G, Mermin J. Prevalence, incidence and mortality associated with tuberculosis in HIV-infected patients initiating antiretroviral therapy in rural Uganda. Aids. 2007 Mar 30; 21(6):713-9.

27. World Health Organization (WHO). Laboratory guidelines for enumerating CD4 T Lymphocytes in the context of HIV/AIDS. Regional Office for South. East Asia. 2007.

28. Hadadi A, Jafari S, Jebeli ZH, Hamidian R. Frequncy and etiology of lymphadenopathy in Iranian HIV/AIDS patients. Asian Pacific journal of tropical biomedicine. 2014 May 1; 4:S171-6.

29. Shenoy R, Kapadi SN, Pai KP, Kini H, Mallya S, Khadilkar UN, Prabha A. Fine needle aspiration diagnosis in HIV-related lymphadenopathy in Mangalore, India. Acta cytologica. 2002; 46(1):35-9.

30. Beyene D, Ashenafi S, Yamuah L, Aseffa A, Wiker H, Engers H, Klatser P, Sviland L. Diagnosis of tuberculous lymphadenitis in Ethiopia: correlation with culture, histology and HIV status. The International Journal of Tuberculosis and Lung Disease. 2008 Sep 1; 12(9):1030-6.

31.Kocjan G, Chandra A, Cross P, Denton K, Giles T, Herbert A, Smith P, Remedios D, Wilson P. BSCC Code of Practice–fine needle aspiration cytology. Cytopathology. 2009 Oct; 20(5):283-96.

32. Bonnet MM, Pinoges LL, Varaine FF, Oberhauser BB, DO O'Brien D, Kebede YY, Hewison CC, Zachariah RR, Ferradini LL. Tuberculosis after HAART initiation in HIV-positive patients from five countries with a high tuberculosis burden. Aids. 2006 Jun 12; 20(9):1275-9.

33. Abhijit Maji, Richa Misra, Anupam Kumar Mondal, Dhirendra Kumar, Divya Bajaj, Anshika Singhal.et al., Expression profiling of lymph nodes in tuberculosis patients reveal inflammatory milieu at site of infection. Cluster Innovation Centre, University of Delhi, Delhi, India. 15 October 2015.

34. Andrea Polesky, MD, PhD, William Grove, MD, and Gulshan Bhatia. Peripheral Tuberculous Lymphadenitis Epidemiology, Diagnosis, Treatment, and Outcome. Medicine 2005; 84:350–362

35. Magnier, Jean-dominique Bergmann, Jean-françois. Lymph node tuberculosis in patients from regions with varying burdens of tuberculosis and human immunodeficiency virus (HIV) infection. La Presse Medical 2018; 30(19):223-230.

36. Kwan CK, Ernst JD. HIV and tuberculosis: a deadly human syndemic. Clinical microbiology reviews. 2011 Apr 1; 24(2):351-76.

37. Damtie D, Yismaw G, Woldeyohannes D, Anagaw B. Common opportunistic infections and their CD4 cell correlates among HIV-infected patients attending at antiretroviral therapy clinic of Gondar University Hospital, Northwest Ethiopia. BMC research notes. 2013 Dec; 6(1):534.

38. Fekadu S, Teshome W, Alemu G. Prevalence and determinants of Tuberculosis among HIV infected patients in south Ethiopia. The Journal of Infection in Developing Countries. 2015 Aug 29; 9(08):898-904.

39. Byzov AB. Lymphadenopathies in HIV. Maaster in de Geneeskunde. Universiteit Feint. Academiejaar. 2009; 2010:1-49.

40. Leeds IL, Magee MJ, Kurbatova EV, Del Rio C, Blumberg HM, Leonard MK, Kraft CS. Site of extrapulmonary tuberculosis is associated with HIV infection. Clinical Infectious Diseases. 2012 Mar 15; 55(1):75-81.

41. Iliyasu Z, Babashani M. Prevalence and predictors of tuberculosis coinfection among HIV-seropositive patients attending the Aminu Kano Teaching Hospital, northern Nigeria. Journal of epidemiology. 2009 Mar 5:0903030070-.

42. Agarwal L, Agrawal M, Geetha V, Saxena R (2017) Histological Features of Tubercular Lymphadenitis in HIV Positive Patients. Adv Cytol Pathol 2(5): 00039. DOI: 10.15406/acp.2017.02

43. Nidhi P, Sapna T, Shalini M, Kumud G. FNAC in tuberculous lymphadenitis: Experience from a tertiary level referral centre. Indian J Tuberc. 2011 Jul; 58(3):102-7.

44. Muluye D, Biadgo B, Woldegerima E, Ambachew A. Prevalence of tuberculous lymphadenitis in Gondar University Hospital, Northwest Ethiopia. BMC Public Health. 2013 Dec; 13(1):435.

45. Balcha,Taye ,T.Sturegård, Erik,Winqvist, Niclas,Skogmar, StenReepalu, AntonJemal, Zelalem Habtamu, Tibesso, Gudeta,Schön, Thomas,Björkman, Per. Intensified Tuberculosis Case-Finding in HIV-Positive Adults Managed at Ethiopian Health Centers: Diagnostic Yield of Xpert MTB/RIF Compared with Smear Microscopy and Liquid Culture. PLoS ONE.2014; 9(1)

46. World Health Organization. "WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children." (2007).

47. Denkinger, Claudia M. Schumacher, Samuel G. Boehme, Catharina C. Dendukuri, Nandini Pai, Madhukar Steingart, Karen R. Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: A systematic review and meta-analysis. European Respiratory Journal. 2014; 44(2): 435-446.

48. World Health Organization. Laboratory guidelines for enumeration CD4 T lymphocytes in the context of HIV/AIDS (revised version 2009). WHO Regional Office for South-East Asia; 2009.

49. Iwnetu R, Van Den Hombergh J, Woldeamanuel Y, Asfaw M, Gebrekirstos C, Negussie Y, Bekele T, Ashenafi S, Seyoum B, Melaku K, Yamuah L. Is tuberculous lymphadenitis over-diagnosed in Ethiopia? Comparative performance of diagnostic tests for mycobacterial lymphadenitis in a high-burden country. Scandinavian journal of infectious diseases. 2009 Jan 1; 41(6-7):462-8.