"THE STUDY OF PREVALENCE OF IDIOPATHIC CD4 T CELL LYMPHOCYTOPENIA IN HIV NEGATIVE PATIENTS WITH DISSEMINATED INFECTIONS"

THESIS

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DECLARATION

I hereby declare that the thesis titled **"THE STUDY OF PREVALENCE OF IDIOPATHIC CD4 T CELL LYMPHOCYTOPENIA IN HIV NEGATIVE PATIENTS WITH DISSEMINATED INFECTIONS"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

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CERTIFICATE

This is to certify that the thesis titled **"THE STUDY OF PREVALENCE OF IDIOPATHIC CD4 T CELL LYMPHOCYTOPENIA IN HIV NEGATIVE PATIENTS WITH DISSEMINATED INFECTIONS"** is the bonafide work of Dr. Tejaswee Banavathu carried out under our guidance and supervision in the Department of General Medicine, All India Institute of Medical Sciences, Jodhpur.

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"*Alone we can do so little; together we can do so much*"

-Helen Keller

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Dedicated to my family

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LIST OF ABBREVIATION

CD	**CLUSTER OF DIFFERENTIATION**
HIV	**HUMAN IMMUNODEFICIENCY VIRUS**
CDC	**CENTERS FOR DISEASE CONTROL AND PREVENTION**
ICL	**IDIOPATHIC CD4 LYMPHOCYTOPENIA**
Th CELLS	**T HELPER CELLS**
IL	**INTERLEUKIN**
IFN	**INTERFERON**
EAE	**EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS**
TCR	**T-CELL RECEPTOR**
ROR γ	**RETINOIC ACID-RELATED ORPHAN RECEPTOR GAMMA**
JAK/STAT	**JANUS KINASE/SIGNAL TRANSDUCERS AND ACTIVATORS OF TRANSCRIPTION**
T-bet	**T-BOX EXPRESSED IN T CELLS**
GATA1	**GATA-BINDING FACTOR 1**
Foxp3	**FORKHEAD BOX P3**
MHC	**MAJOR HISTOCOMPATIBILITY COMPLEX**
APC	**ANTIGEN-PRESENTING CELL**
IBD	**INFLAMMATORY BOWEL DISEASE**
Fas	**FAS CELL SURFACE DEATH RECEPTOR**

RAG1	**RECOMBINATION ACTIVATING GENE 1**
NAGT1	**SODIUM-DEPENDENT GLUCOSE TRANSPORTER 1**
UNC119	**UNC-119 LIPID BINDING CHAPERONE**
CXCR4	**C-X-C MOTIF CHEMOKINE RECEPTOR 4.**
HTLV	**HUMAN T-CELL LEUKEMIA VIRUS.**
NK	**NATURAL KILLER CELLS**
VZV	**VARICELLA ZOSTER VIRUS**
JC	**JOHN CUNNINGHAM VIRUS**
CMV	**CYTOMEGALOVIRUS**
EBV	**EPSTEIN-BARR VIRUS**
TB	**TUBERCULOSIS**
HLA DR	**HUMAN LEUKOCYTE ANTIGEN – DR ISOTYPE**
ATT	**ANTITUBERCULOSIS TREATMENT**
EDTA	**ETHYLENEDIAMINETETRAACETIC ACID**
LCA	**LEUKOCYTE COMMON ANTIGEN**
CKD	**CHRONIC KIDNEY DISEASE**
CVA	**CEREBROVASCULAR ACCIDENT**
LPS	**LIPOPOLYSACCHARIDE**
CBC	**COMPLETE BLOOD COUNT**
KFT	**KIDNEY FUNCTION TEST**
LFT	**LIVER FUNCTION TEST**

Hs CRP	**HIGH-SENSITIVITY C-REACTIVE PROTEIN**
ESR	**ERYTHROCYTE SEDIMENTATION RATE**
CSF	**CEREBROSPINAL FLUID**
MRI	**MAGNETIC RESONANCE IMAGING SCAN**
CT	**COMPUTED TOMOGRAPHY**
USG	**ULTRASOUND SONOGRAPHY**
HBsAg	**HEPATITIS B SURFACE ANTIGEN**
HCV	**HEPATITIS C INFECTION**
ECG	**ELECTROCARDIOGRAM**
SD	**STANDARD DEVIATION**
CPE	**CYTOPATHOGENIC EFFECT**
PBMC	**PERIPHERAL BLOOD MONONUCLEAR CELLS**
MDR	**MULTI DRUG RESISTANT**
MRSA	**METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS**
TMP-SMX	**TRIMETHOPRIM/SULFAMETHOXAZOLE**

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SUMMARY OF THE PROJECT

Background: Idiopathic CD4 lymphocytopenia (ICL) is defined as low CD4 counts (< 300 cells/µl or < 20% of the total lymphocyte counts) without evidence of any immunodeficiency. ICL is considered a rare disease, and research is ongoing to establish the clinical significance. Disseminated infections in immunocompetent individuals are not uncommon, and their association with ICL is studied to a lesser extent. This study was aimed to find the occurrence of ICL in patients presented with disseminated infections without known immunodeficiency.

Aims and objectives:

1. To study the prevalence of idiopathic CD4 lymphocytopenia in disseminated bacterial, fungal, tubercular, viral, and parasitic infections in HIV-negative individuals
2. To study the correlation of CD4 counts with treatment response and mortality in disseminated bacterial, fungal, tubercular, viral, and parasitic infections.

Methods: This prospective observational study was conducted between January 2020 and July 2021 in a tertiary care center in western India. HIV-negative patients with age ≥ 18 yearsand satisfying the standard definition of disseminated disease (either viral, bacterial, fungal, or tubercular) were included in the study. Patients with a known case of immunodeficiency, malignancy, autoimmune disease, and moribund patients were excluded. All patients were investigated for CD4 counts (flow cytometry) and HIV (ELISA) at the time of hospitalization and after three months.

Results: A total of 110 patients with a mean age of 42.7 ± 19.2 years were enrolled. Common disseminated infections were bacterial sepsis (47.3%), tuberculosis (32.7%), mucormycosis (8.2%), viral (7.3%) and nocardiosis (4.5%). Low CD4 counts (< 300 cells/µl or < 20% of the total lymphocyte counts) were found in 41% of cases at the time of diagnosis of infection. On follow-up, 30% of patients died within three months. Among those who survived, ICL

criteria were satisfied in 9.1% of patients on measuring CD4 counts at three months (Table 1). On Multivariate logistic regression, Higher age (> 55 years), (HR 1.03, CI 1.01 – 1.06; p 0.004), low CD4 counts (< 200 cells/µl) (HR 3.21, CI 1.17 – 8.78; p 0.023) and high C-reactive protein level (HR 1.01, CI 1.01 – 1.02; p 0.003) at the time of diagnosis of infection, were found independent predictor of mortality in disseminated infections.

Conclusion: This study emphasized the usefulness of CD4 counts measurement in disseminated infections. Incorporation of routine CD4 counts measurement in patients with disseminated infections, especially in which T cell immunity is essential, could further resolve the misty of ICL.

Table 1: Occurrence of ICL in disseminated Infections in immunocompetent patients

S. No.	Disseminated Infections	Low CD4 count (at the time of diagnosis)	Low CD4 counts (at three months follow-up) (ICL criteria met)
1	Bacterial Sepsis (n=51)	15 (33%)	1 (10%)
2	Tuberculosis (n=33)	20 (45%)	5 (50%)
3	Fungal (n=9)	2 (4%)	0 (0%)
4	Viral (CMV and VZV) (n=8)	5 (7%)	3 (30%)
5	Nocardiosis (n=5)	3 (11%)	1 (10%)

Abbreviations: ICL: Idiopathic CD4 lymphocytopenia; CMV: Cytomegalovirus; VZV: Varicella Zoster virus; CD4: Cluster of differentiation

INTRODUCTION

INTRODUCTION

Cluster of differentiation-4 (CD4) T helper cells are an integral part of the acquired immune system and it plays a vital role in the control of various infections. CD4 is a glycoprotein found on the surface of immune cells as T helper, monocytes, macrophages, and dendritic cells[1]. In humans, CD4 protein is encoded by the CD4 gene. They play an important role in the immune system through their capacity to help B cells make antibodies, to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation and through their production of cytokinesand chemokines, to orchestrate the full panoply of immune responses[2].

A normal CD4 T cell count in an adult is usually between 500 and 1500 cells/mm³ (3,4). Depletion of CD4 count in HIV infection is an integral part of the pathogenesis. CD4 counts less than 200 in HIV infection are associated with an increased risk of opportunistic infections and mortality. CD4 count can be low in HIV-negative individuals[5]. Idiopathic CD4 lymphocytopenia is defined by CDC in 1992 as CD4 counts less than 300 or less than 20% of total lymphocyte count on two occasions in HIV-negative individuals. In the literature around 200 cases of idiopathic CD4 lymphocytopenia have been reported[6]. Although most ICL cases were reported to have low a CD4/CD8 ratio, the CDC definition forICL diagnosis does not exclude patients with pan lymphopenia (low CD4 and CD8) and normal CD4/CD8 ratio[7].

There are four 4 types of populations of CD4 T-cell namely Th1, Th2, Th17, and induced regulatory T(iTreg)cells. Mossman and Coffman recognized the Th1 and Th2 phenotypes among the set of long-term T-cell lines that they studied and the early history of this field was devoted to understanding these 2 cell populations, with Th1 cells being regarded as critical for immunity to intracellular microorganisms and Th2 cells for immunity to many extracellular pathogens, including helminths[8]. Abnormal activation of Th1 cells was seen as the critical event in most organ-specific autoimmune diseases while Th2 cells were responsible for allergic inflammatory diseases and asthma. Th17 cells have been recognized much more recently but there is now a growing body of work indicating not only that these cells exist but that they play a critical function in protection against microbial challenges, particularly extracellular bacteria and fungi[9].

Several cases were reported since 1989 with patients having opportunistic infections in the setting of persistent low CD4 counts without HIV infection. Following this, CDC in 1992 launched a survey in an attempt to characterize this newly evolving entity. The CDC Idiopathic CD4 Lymphocytopenia Task Force reviewed 230,129 cases in the Centers for CDC AIDS Reporting System and found 47 patients to meet the CDC criteria of ICL. Amongthese 47 patients, 40% had AIDS-defining illnesses, 53% had conditions that were not AIDS defining, and 6% were asymptomatic. This indicated that ICL is a rare entity with no evidence of transmissible agent since the cases were not clustered and the contacts of the patients had no immunodeficiency[10].

In the literature, several infections causing T cell lymphocytopenia in HIV-negative individuals were described. Most studies of ICL related to infections have been described with Cryptococcus, Mycobacterial infections, and some viral infections. Both opportunistic and non-opportunistic infections were reported. Multiorgan involvement has been reported in infections due to Cryptococcus, CMV, VZV, Blastomyces, and MAC. The role of CD4 counts in HIV-negative patients with disseminated infections is poorly understood. Few studies have been done to find the role of CD4 counts in predicting treatment response and mortality in ICU settings[11]. We plan this study to find the role of CD4 counts in disseminated infections with a focus on predicting the treatment response and mortality in disseminated infections among the ICL population.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORY AND EVOLUTION

LYMPHOCYTES

Lymphocytes arise from their precursors in Lymphoid organs (Bone marrow, thymus) from which mature lymphocytes enter the peripheral organs, where they respond to foreign antigens and subsequently recirculate in blood and lymph. (Fig.1)

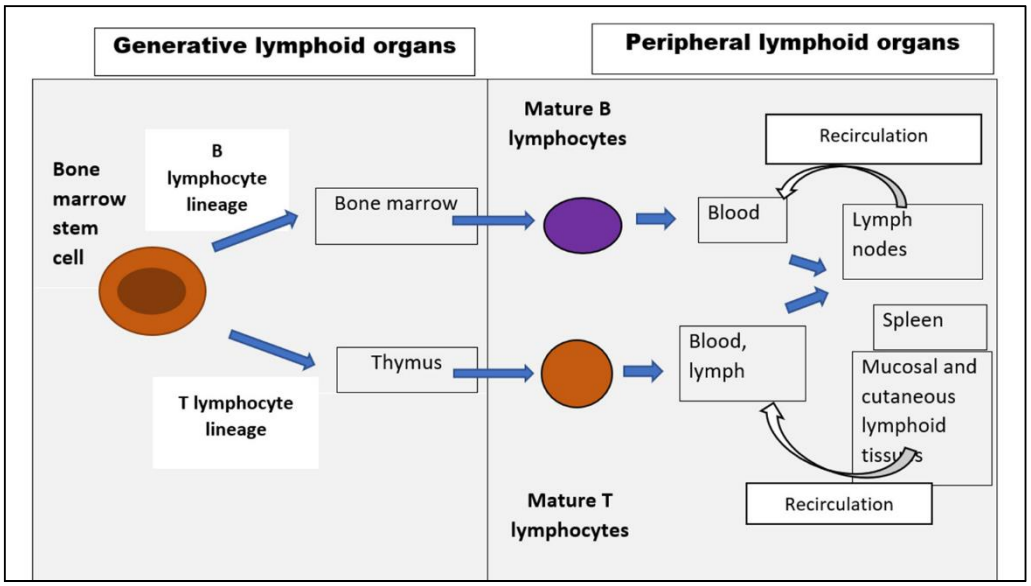

Figure 1: Maturation of Lymphocyte

The process through which the naive cells differentiate into these distinct states shows several similar features. TCR engagement is an essential step. A major product of the differentiated cells is a principal stimulant, providing potent positive feedback that can enforce the development of a high degree of polarization. Essential pathways for differentiation are the Jak/Stat pathways and a specific Stat in association with one of 4 master regulators, T-bet, GATA-3, RORγt, and Foxp3.

The study of this process has illuminated how central cytokines are to the mounting of effective immune responses and, through the commonalities in their pathway of differentiation which also support the assertion that cytokine biology is more than a collection of isolated facts but rather involves a set of principles in which knowledge about any of the pathways points the way to a deeper understanding of the others. The analysis of the effects of mutations in key players in the differentiation process has also provided a

deeper understanding of the true biologic function of this set of cells that are so central to the mounting of effective and regulated immune responses.

CD4 T CELLS AND THEIR FUNCTIONS:

CD4 T cells represent a remarkable cell population. They are central to protection against a wide range of pathogens and do so through the adoption of a series of distinct differentiated states, each evolved under the pressure of a particular set of pathogens. They play a central role in immune protection. They do so through their capacity to help B cells make antibodies,to induce macrophages to develop enhanced microbicidal activity, to recruit neutrophils, eosinophils, and basophils to sites of infection and inflammation, and, through their production of cytokines and chemokines, to orchestrate the full panoply of immune responses[(2)].

The first clinical description was done by Mossman and Coffman in 1986 showing that long-term CD4 T-cell lines could be subdivided into 2 groups, those that made IFNγ as their signature cytokine and those that produced IL-4, it has been realized that CD4 T cells are not a unitary set of cells but represent a series of distinct cell populations with different functions[(12)]. Kim Bottomly also worked on this subject; she and her colleagues subdivided CD4 T-cell lines based on functional criteria, distinguishing inflammatory and helper CD4 T cells, with the latter being IL-4 producers[(13)].

The earliest description of in vitro differentiation was reported in 1990 by Susan Swain, demonstrating first that naive CD4 T cells failed to make IL-4 (or most other effector cytokines) and that these cells could be induced to develop into vigorous IL-4 producers if they were stimulated both with T-cell receptor ligands and IL-4, itself[(14,15)].

Ken Murphy, Anne O'Garra, and their colleagues showed that naive CD4 T cells could acquire the capacity to produce IFNγ in vitro (16). They stimulated T-cell receptor transgenic naive CD4 T cells and antigen-presenting cells with cognate antigen and heat-killed Listeria monocytogenes organisms; the heat-killed Listeria caused cells in the culture to produce IL-12, which was critical for Th1 differentiation.

In 2006, Stockinger, Weaver, Kuchroo, and their colleagues each showed that Th17 cells could be induced in vitro from naïve mouse CD4 T cells by stimulation through their T-cell receptor (TCR) in the presence of IL-6 and TGF-beta (17–19). RORγt was identified as the master regulator gene for Th17 cells.

CD4+T cells recognize peptides presented on MHC class II molecules, which are found on antigen-presenting cells (APCs). As a whole, they play a major role in instigating and shaping adaptive immune responses. (Fig 2)

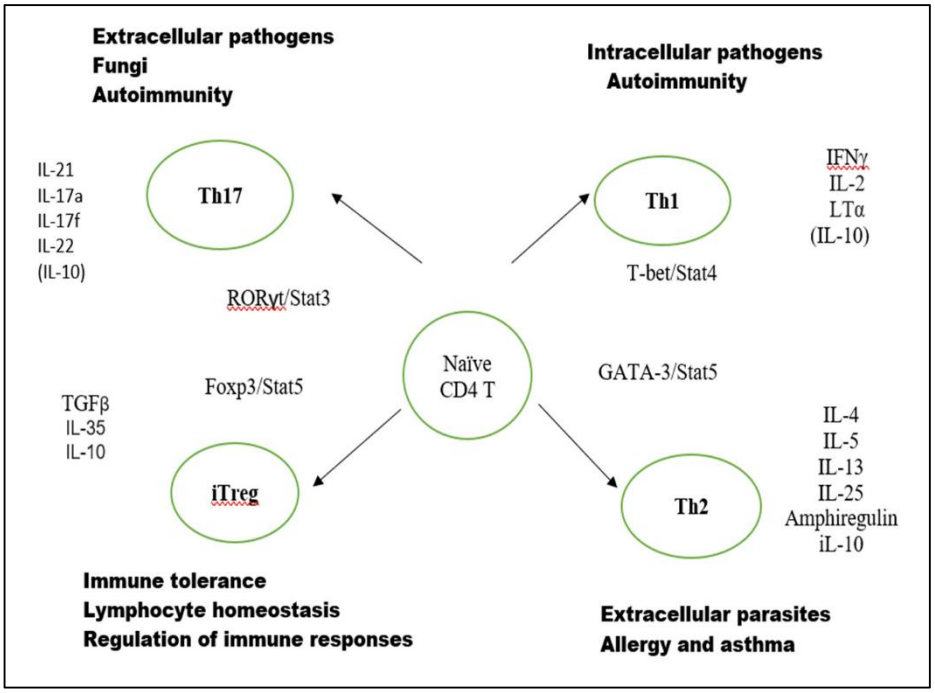

Figure 2: Types of CD4 T helper cells and their function

Th1/Th2 cells

Th1-polarised cells are responsible for the control of intracellular pathogens such as viruses and some bacteria. IL-12 and IFN-γ are important cytokines involved in Th1 responses, and the intracellular transcription factors T-bet and STAT-4 are essential for Th1 cell differentiation and function. Th2 polarized cells are important in the defense against large extracellular organisms such as helminths, utilizing cytokines such as IL-4, IL-5, and IL-13, promoting eosinophilia, mastocytosis, and goblet cell hyperplasia. Gata-3 and STAT-6 are essential for Th2 cell differentiation and function.

Allergy/Autoimmunity

If the Th1/Th2 balance is disturbed there can be severe consequences. Asthma and allergy are Th2-driven and some autoimmune diseases, such as type 1 diabetes and multiple sclerosis are Th1-driven.

Th17 cells

Recently discovered T helper cell subset, characterized by its production of IL-17. IL-23 promotes the expansion of these cells and Th17 cells have been linked to several inflammatory conditions such as arthritis and IBD.

Treg cells

Regulatory T cells are a subpopulation of cells that maintain homeostasis and tolerance within the immune system. Subsets include inducible Tregs, CD25+CD45RBlo Tregs, etc[20].

IDIOPATHIC CD4 LYMPHOCYTOPENIA

Idiopathic CD4 lymphocytopenia (ICL) was defined in 1992 by the US Centre for Disease Control and Prevention (CDC) as the repeated presence of a CD4+ T lymphocyte count of fewer than 300 cells per cubic millimeter or less than 20% of total T cells with no evidence of human immunodeficiency virus (HIV) infection and no condition that might cause depressed CD4 counts.

The diagnosis of ICL requires the presence of the following criteria:

1. Low CD4 cell counts (in peripheral blood) < 300 cells/µl or < 20% of the total lymphocyte counts in two separate tests (at least 6 weeks apart)
2. No evidence of HIV-1 or HIV-2 infections
3. The absence of any defined immunodeficiency or therapy associated with depressed levels of CD4[21–23].

Prevalence:

ICL is a rare disease; fewer than 300 cases have been reported but the true prevalence of the disease is unknown[24]. Only a few familial cases have been reported[21,22,25].

Mechanism of CD4 lymphocytopenia:

Decreased CD4 cell production and differentiation and increased destruction with tissue sequestration (such as spleen and lymph nodes) are most probably involved in the pathogenesis of ICL.

Two factors related to CD4+ lymphocyte function play a role in developing ICL. First, increased activation of CD4, which may result from stimulation by an unidentified pathogen, resulting in a persistent decrease in the number of CD4+ lymphocytes[23]. Lee et al. found

increased levels of serum lipopolysaccharide (LPS) and markers of CD4+ lymphocyte activation in patients with ICL. Therefore, they hypothesized that abnormally increased microbial translocation through the intestinal wall may be an underlying etiology[26]. Second, apoptosis of CD4+ lymphocytes may be associated with enhanced expression of Fas and Fas ligand. Roger et al. demonstrated that a patient with ICL and disseminated Mycobacterium xenopi infection had overexpression of Fas/CD95c and spontaneous and Fas-induced apoptosis[27]. However, patients with stable, physiologic, CD4 cell lymphopenia without opportunistic infections did not demonstrate accelerated apoptosis, suggesting that infection may be a necessary initial stimulus for this phenomenon.

Various other immune defects have been described in some ICL patients as well. Disseminated thymic cell maturation and expression of Fas or Fas ligand (enhanced apoptosis) were reported. T cell differentiation may be disturbed due to T cell expansion withdefective production of IFN α and γ. High interleukin (IL)-7 may be caused by decreased IL- 7 receptor levels. Mutations in various genetic factors like (RAG1, NAGT1, and UNC119) were found associated with low CD4 count. However, according to the CDC diagnostic criteria, the defined genetic etiology excluded the diagnosis of ICL.

Low CD8+ T cells counts were noticed in some patients. Patients with CD8 counts <180 cells/mm^3 in a study of 39 patients were found to have a higher risk of serious opportunistic infections and death. This subgroup of patients may represent a more severe variant of ICL. The complete absence of specific CD8+ cells (CD8+ 28+) has been reported in a small number of patients with ICL[6]. Defective expression of CXCR4, which binds the chemokine stromal cell-derived factor 1, on the surface of CD4+ cells, was noticed in six patients with ICL. The interaction of the receptor/ligand pair is critical for multiple aspects of normal T cell differentiation and trafficking. The alpha/ beta and gamma/delta T cell repertoires of ICL patients are highly restricted, which may suggest a problem in maturation or differentiation during T cell development[28]. Biochemical defects of the T cell receptor transduction pathway have been noticed, possibly due to an abnormality of the tyrosine kinase activity of p56 (Lck). Defects in this kinase appear to affect CD4 cell function and maintenance of adequate counts of cells. ICL has been associated with increases in immature or transitional Bcells and increased serum levels of IL-7[29]. Low B cell numbers or even a complete absence of B cells has been noticed in some patients. Isgrò et al. suggest that ICL may be due to

decreased bone marrow clonogenic capability, or the inability of bone marrow stem cells to mature successfully[30].

Genetic factors might be involved in the pathogenesis of ICL as well. Zonios et al. found higher proportions of HLA- DR+ CD4 cells in ICL patients compared to controls, which suggests that there could be a genetic predisposition to ICL, or that ICL is more common in certain populations[2]. Hypomorphic mutations in the recombination activating gene 1 (RAG1) were identified in a patient with Varicella infection and recurrent pneumonia[10].

Etiology of ICL:

To make the diagnosis of ICL in patients with low CD4 cell counts one must exclude all known or possible causes of CD4 lymphopenia (in addition to HIV). In those cases of secondary CD4 lymphopenia, the CD4 cell count may be severely low (< 300 cells/µl) but itis usually transient (improved following treatment of the primary pathogenic cause, or following the withdrawal of the causative immunosuppressive drug)[23].

The main causes of secondary lymphopenia include mycobacterial infections (mainly tuberculosis), viral infections (HIV, cytomegalovirus, Epstein-Barr virus, hepatitis B, and HTLV1), malignancy (non-Hodgkin's lymphoma, myelodysplastic syndrome), autoimmune disorders (Sjogren syndrome, systemic lupus erythematosus) and drugs such as corticosteroids, chemotherapy and cytotoxic agents (Table 2, 3).

Table 2: Secondary causes of ICL

Mycobacterial	Viral	Fungal	Bacterial
	Hepatitis B EBV CMV VZV, Shingles HPV HSV 1 and 2 JC virus Mollusca contagious HHV 8 Parvovirus B19	Candida Cryptococcus Pneumocystis Histoplasmosis Aspergillosis Blastomycosis	Salmonella Nocardia Cerebral abscess Hepatic abscess Actinomycosis Perianal abscess Corynebacterium Shigella Enteritis Mycoplasma Protozoal Toxoplasmosis Cryptosporidiosis Leishmaniosis Giardiasis

Abbreviations: EBV: Ebstein barr virus; CMV: Cytomegalovirus; VZV: Varicella Zoster virus; HSV: Herpes simplex virus; JC: John Cunningham virus, HHV: Human herpes virus, DAG: diacyl glycerol, MCR-2: Mineralocorticoid receptor-2.

Table 3: Secondary causes of ICL

Malignancies			Autoimmune disorders
Hematological	Solid organ	Others	Sjogren disease Sarcoidosis Psoriasis Auto immune hemolytic anemia Idiopathic thrombocytopenic purpura Systemic lupus erythematosus Vasculitis Raynaud's disease Thrombotic thrombocytopenic purpura Hashimoto thyroiditis Bechet's-like syndrome Anti-phospholipid Antibody syndrome Alopecia aerate Vitiligo
Lymphoma NHL DLBCL ALL CLL	Testicular cancer Prostate cancer Gastric cancer Prickle cell carcinoma NSCLC Primary leptomeningeal lymphoma Anaplastic astrocytoma Spinocellar carcinoma Bladder tumor CIN	SCC of Skin Kaposi's sarcoma BCC of skin Bowen's disease Vulvar intraepithelial neoplasia Primary effusion lymphoma Mycosis fungicides	

Abbreviations: NHL: Non-Hodgkin lymphoma; DLBCL: Diffuse large B cell lymphoma; ALL: Acute Lymphocytic leukemia; CLL: Chronic Lymphocytic leukemia; NSCLC: non-small cell lung cancer, CIN: Cervical intraepithelial neoplasia, SCC: Squamous cell carcinoma, BCC: Basal cell carcinoma.

The clinical spectrum of ICL varies. ICL patients may be asymptomatic for many years. In those cases, the CD4 lymphopenia is discovered accidentally. However, most of the patients have opportunistic infections similar to those observed in HIV patients with low CD4 cell counts[26]. Indeed, most ICL patients are diagnosed following an opportunistic infection or after recurrent opportunistic infections.

The main opportunistic infections reported in ICL patients include Mycobacterium tuberculosis, Mycobacterium avium intracellular, Salmonella typhimurium, cytomegalovirus, John Cunningham virus (JC virus), human papillomavirus, varicella-zoster virus, herpes simplex virus, human herpes virus-8, Aspergillus spp, Candida albicans, Cryptococcus neoformans, Pneumocystis jirovecii and toxoplasmosis.

Cryptococcosis is the most common opportunistic infection reported in ICL patients followed by M. tuberculosis and herpes zoster infections[11]. Because of the high risk for herpes virus-8 and human papillomavirus infections in ICL patients, disseminated Kaposi sarcoma and human papillomavirus-related malignancies should be looked for in ICL patients[26,27]. The main differential diagnosis in a patient with suspected ICL is HIV infection, but patients with ICL have negative HIV serology and HIV polymerase chain reaction[25,31].

Investigation toward alternative diagnoses at disease presentation should always include lymphoproliferative diseases or lymphomas and other forms of immunodeficiency, such as common variable immunodeficiency. There appear to be at least 2 subtypes of ICL in terms of the presence or absence of CD8 T lymphocytopenia. This observation precludes the use of the CD4/CD8 ratio for diagnostic purposes in ICL and supports that it is a heterogeneous syndrome that could be further accompanied by B-cell and/or NK-cell lymphocytopenia[28].

During the search for literature about trends of CD4 lymphocytes in various infectious disorders among ICL patients, the following were found:

Regent et al (2014) performed a study by recruiting 40 ICL patients (24 female) of mean age 44.2±12.2 (19-70) years by T-lymphocyte phenotyping and lymph proliferation assay at diagnosis, and experiments related to thymic function and interferon (IFN)-γ release by natural killer (NK) cell were performed and concluded that 25 patients had opportunistic infections (12 with human papillomavirus infection), 14 had autoimmune symptoms, 5 had malignancies, and 8 had mild or no symptoms.

Yarmohammadi et al (2017) evaluated 24 ICL patients (14 female [58%] and 10 males [42%]). Seventeen patients (71%) had opportunistic infections, 4 (17%) had malignancies, and 3 (13%) had the unexplained demyelinating disease and neurologic problems. Most patients had normal levels of immunoglobulins. Thirteen patients had abnormally low to absent response to phytohemagglutinin, concanavalin A, and antigens (candida and tetanus). Three patients had resolution of warts and 1 had mycobacterial lung infection on interleukin-

2 with increases in CD4 count. The 11 patients on trimethoprim and sulfamethoxazole had no further hospital admissions for infections.

Smith et al (1993) interviewed 31 out of 47 identified people with Idiopathic CD4 lymphocytopenia and their 23 contacts and reported that 29 patients have no identifiable risk factors for HIV. Blood from 28 patients showed CD4 count less than <300 cells/ cumm and 6 had CD8 lymphocyte count <250/cumm and concluded that association of ICL with opportunistic infections are rare and represents various clinical and immunological states.

Sabhapandit et al (2000) studied newly diagnosed pulmonary TB patients (N=30) with negative HIV status. They were subjected for estimation of CD4, CD8 counts and ratio for prediction of HIV coinfection and highlights the importance of estimation of CD4+ and CD8+ T cell counts and ratio in newly diagnosed pulmonary TB patients with negative HIV status They found significantly lower CD4 and CD8 counts among Pulmonary TB infected HIV negative patients as compared with controls.

Al-aska et al (2011) studied patients with disseminated disease and found significantly lower CD4 cells (but not lower CD8 cells) compared to study patients with localized disease, both at baseline and after treatment and concluded that tuberculosis may be associated with CD4 and CD8 lymphopenia even in patients without human immunodeficiency virus infection, there was the tendency of recovery towards normality especially of the CD4 and CD8 counts after treatment, and that disseminated disease is associated specifically with profound CD4 lymphopenia.

Ahmad et al (2013) collected data about age, sex, pathogens, site of infections, CD4 count, CD8 count, CD4:CD8 ratio, presence of HIV risk factors, malignancies, autoimmune diseases, and whether the patients survived or died of ICL patients from the database.The mean age at diagnosis of first opportunistic infection (or ICL if no opportunistic infection is reported) was 40.7 ± 19.2 years (range 1-85 years) . The majority of patients 226 (87.6%) hadat least one infection. Cryptococcal infections were the most prevalent infections in ICL patients (26.6%), followed by mycobacterial infections (17%), candidal infections (16.2%), and VZV infections (13.1%). Malignancies were reported in 47 (18.1%) patients. Autoimmune diseases were reported in 37 (14.2%) patients.

Asher et al (2016), taken the mean age of the patients at the time of ICL diagnosis is 41 ± 19 years (range 1–85) with a slightly higher male predominance (60%). The main causes of

secondary lymphopenia include mycobacterial infections (mainly tuberculosis), viralinfections (HIV, cytomegalovirus, Epstein-Barr virus, hepatitis B, and HTLV1), malignancy (non-Hodgkin's lymphoma, myelodysplastic syndrome), autoimmune disorders (Sjogren syndrome, systemic lupus erythematosus), and drugs such as corticosteroids, chemotherapy, and cytotoxic agents.

Zonios et al (2008) evaluated thirty-nine patients (17 men, 22 women) 25 to 85 years old with ICL between 1992 and 2006, and 36 were followed for a median of 49.5 months. Cryptococcal and nontuberculous mycobacterial infections were the major presenting opportunistic infections. Seven patients presented with no infection. In 32, CD4 T-cell countsremained less than 300/mm3 throughout the study period and in 7 normalized after an average of 31 months. Overall, 15 (41.6%) developed an opportunistic infection in follow-up,
5 (13.8%) of which were AIDS-defining clinical conditions, and 4 (11.1%) developed autoimmune diseases. Seven patients died, 4 from ICL-related opportunistic infections, within 42 months after diagnosis. Immunologic analyses revealed increased activation and turnover in CD4 but not CD8 T lymphocytes. CD8 T lymphocytopenia (< 180/mm3) and the degree of CD4 T cell activation (measured by HLA DR expression) at presentation were associated with adverse outcomes.

Skogmar et al (2013) determined CD4 cell levels longitudinally during anti-tuberculosis treatment (ATT) in patients, with and without HIV co-infection, and their associations with clinical variables. Among 809 HIV-negative patients, 200 (25%) had subnormal CD4 cell counts (<500 cells/mm3), with <350 cells/mm3 in 82 (10%) individuals. CD4 cell levels increased significantly during the course of ATT in both HIV+ and HIV- TB patients but did not reach the levels in healthy subjects (median 896 cells/mm3). Sputum smear status, signs of wasting (low mid-upper arm circumference (MUAC)), and bedridden state were significantly associated with low CD4 cell counts.

The management of ICL patients is aimed at treating and/or preventing opportunistic infections and increasing the number of CD4 lymphocytes. Opportunistic infection should be treated according to the specific infection. There are no accepted guidelines for primary and secondary infection prevention in ICL patients. It has been suggested to follow the current primary and secondary prevention recommendations used in HIV patients which is based on the number of CD4 cell counts[28]. There is no way of predicting the clinical course of a particular ICL patient (based on his or her laboratory results, e.g., the number of CD4

cells)[11]. However, a patient with recurrent opportunistic infections should be treated aggressively (including primary and secondary prophylaxis)[25].

The efficacy of vaccination in ICL patients is unknown. In HIV patients CD4 cell counts >400 cells/µl is associated with a good (normal) response to vaccination, whereas in patients with lower CD4 cell counts a poor efficacy was reported[29]. Live vaccines are contraindicated and should be avoided in ICL patients. All other types of vaccines (dead, recombinant) may be given but their efficacy and protective effects are not known and are unpredictable[25].

A few case reports suggested the efficacy of IL-2 therapy in patients with ICL. IL-2 infusion in ICL patients was shown to increase CD4 cell counts[32] and prevent the reoccurrence of cryptococcal meningitis[33] and relapsing herpes zoster virus infection in patients with ICL[34]. Treatment with IFNγ was also shown to increase CD4 cell counts in four ICL patients, leading to sustained clinical amelioration of cryptococcal meningitis and non-tuberculous mycobacterial infection[35]. In animal models (Rhesus masques), recombinant IL-7 was shown to induce CD4 cell proliferation (36). Currently, there are no human studies with IL-7 in ICL patients. Allogeneic bone marrow transplantation was successfully performed in one ICL patient[37]. Taken together, cytokine treatments for ICL may be beneficial, but since the clinical experience with such treatments is limited, its real efficacy remains undefined.

To conclude, ICL is a rare immunological disorder characterized by persistent low levels of CD4 cell counts (< 300 cells/µl). The etiology of ICL is unknown. Most patients are diagnosed following an opportunistic infection. HIV diagnosis must be excluded in all ICL patients. The guidelines for primary and secondary infectious prevention of ICL are not yet established but we recommend following the prevention policy used in HIV patients. Novel biological treatments aimed at increasing CD4 cell counts are of great interest but their real efficacy is still undetermined.

METHODOLOGY

METHODOLOGY

OBJECTIVES

PRIMARY OBJECTIVE:

1. To study the prevalence of idiopathic CD4 lymphocytopenia in disseminated infections in HIV-negative individuals.

SECONDARY OBJECTIVE:

1. To study the correlation of CD4 counts with treatment response and mortality in disseminated infections.

STUDY SETTING: Patients attending the in-patient services of the Department of Internal Medicine of All India Institute of Medical Sciences, Jodhpur, and Rajasthan.

STUDY DESIGN: Prospective observational study

STUDY PARTICIPANTS

Inclusion criteria

1. Hospitalized patients with disseminated fungal, bacterial, viral, tubercular, and parasitic infections diagnosed by standard protocol.
2. HIV negative patients
3. Age more and equal to 18 years

Exclusion criteria

1. HIV negative at the initiation of study but positive after 2 months of follow-up.
2. Know case or diagnosed as Malignancies and Autoimmune disorders along with Idiopathic CD4 lymphocytopenia.

DATA COLLECTION: We had included all consecutive patients with the diagnosis of disseminated fungal, bacterial, viral, tubercular, and parasitic infections.

The study was conducted after seeking written informed consent from the study participants.

On the first visit to the hospital baseline assessment was done which included:

1. Socio-demographic: Name, age, and gender.

2. Clinical Examination

3. Investigations: All patients underwent the following investigations.

a. Baseline haematological and biochemical assessment as per routine clinical care including CBC, serum electrolytes, ESR, Hs CRP, KFT, LFT Viral markers (HIV, HBsAg, HCV rapid).

b. Cultures as per patient's clinical profile (Blood, Urine, CSF, and other samples)

c. Radiological investigations based on the requirement (USG, CT/MRI, Other Imaging)

d. Serum samples were analysed for investigational biomarkers– CD4 at the time of diagnosis.

On the follow-up visit at 2 months, the following investigations were repeated

a. HIV testing

b. Serum samples were analysed for repeat CD4 for patients with Initial CD4 ≤ 300 cells/cu mm and Negative HIV status at Initial and follow up visit.

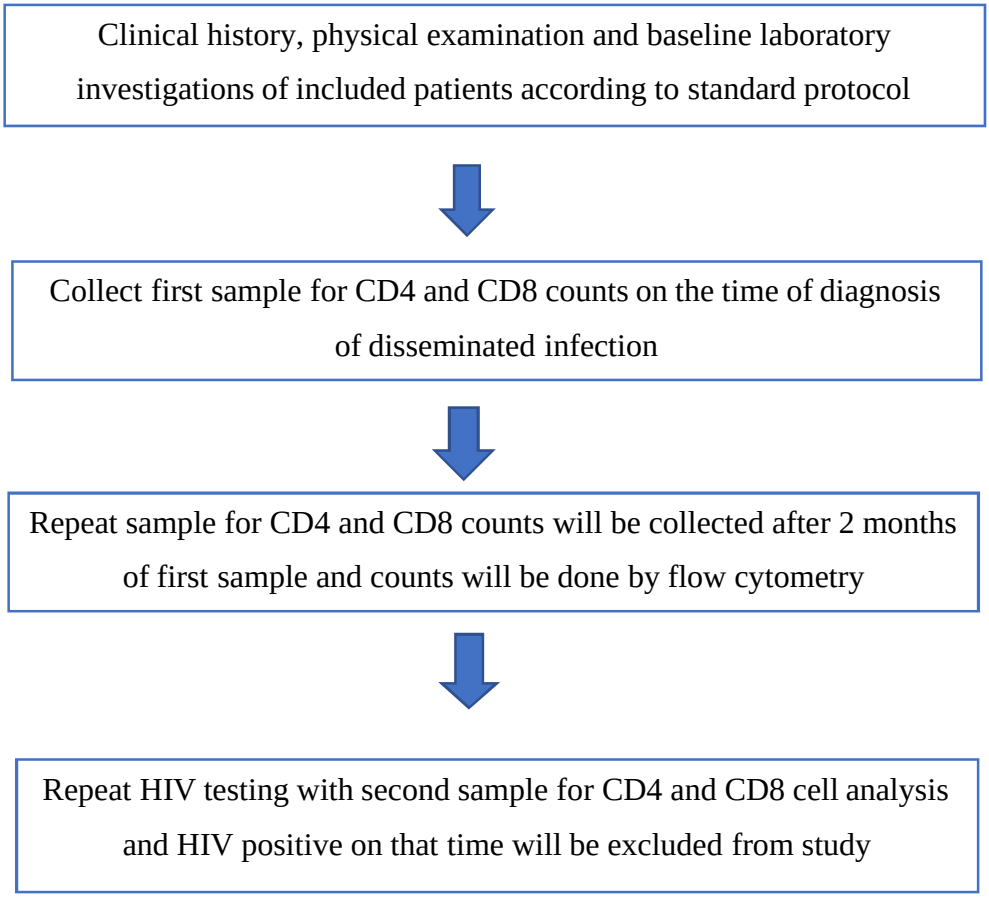

Figure 3: Flowchart showing methodology for the study

CD4: For measurement of serum CD4, the Coulter Sandwich ELISA kit was used. (Catalog # tetraCHROME CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5 Antibody Cocktail**)**

Principle: The basic principle of flow cytometry is the passage of cells in a single file in front of a laser so they can be detected, counted, and sorted. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths. The fundamental basis of CD4 counting and identification relies on the specific use of electronic gating approaches

Specimen collection: Peripheral blood samples of patients were drawn in EDTA blood collection tubes (BD vacutainers). Samples were stored and transported to the laboratory at 2-6°C. All samples were processed within 24 hours of blood collection.

Reagent preparation:

Standard preparation: For the preparation of standard, 120µl of concentrate standard (std1) was added with 120 µl of standard diluent to prepare standard2 solution. This process was repeated from std 2 to 5 to produce a dilution series. Standard diluent served as the zero standards and was used as blank in the assay.

Wash buffer: Working wash buffer solution (600ml) was prepared by diluting 30X wash buffer concentrate with deionized or distilled water.

Assay Procedure:

- CD4 positive lymphocytes were detected by flowcytometric immunophenotyping using a panel of monoclonal antibodies-kit which comprised CD45-FITC, CD3-PC5, CD4-RD1, and CD8-ECD.
- The optimal working antibody concentrations were determined by titration experiments using whole blood samples.
- In the optimized staining procedure, the whole blood sample (50 µL) was taken in test tubes and five microlitres (5 µL) of antibody combination was added to the respective tube and tubes were vortexed and incubated in dark at room temperature for 20 minutes.
- After 20 minutes, 500 µL of optilyse C was added to all the tubes and vortexed.
- Tubes were again incubated in dark at room temperature for 15 minutes. Then tubes were centrifuged at 1700 rpm for five minutes and the supernatant was discarded.
- Two ml of sheath fluid was added to all the tubes and vortexed. This washing step was repeated by adding sheath fluid, centrifugation, and discarding the supernatant.

- Then 600 µL of sheath fluid was added to all the tubes.
- The acquisition was done using Navious flow cytometer (Beckman Coultre) equipped with three lasers and 50,000 events were acquired.

Gating and analysis:

- Flow cytometric data analysis was done on Navious software.
- After excluding doublets, cells with low forward scatter and low side scatter were gated.
- With the help of CD45-side scatter plots, mononuclear cells were gated.
- Further, sequential gating was done with the help of CD3-CD4 plot, CD3-CD8 plot, and enumeration of CD4-positive and CD8-positive cells was done.

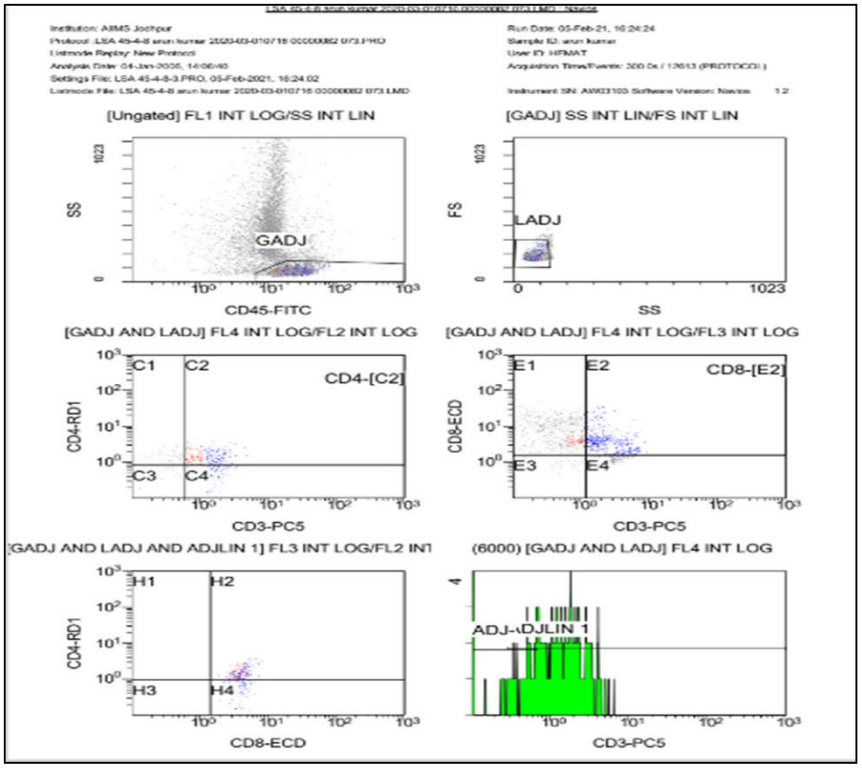

Figure4: Flowcytometric scatter plots revealing sequential gating done for enumeration of CD4-positive and CD8-positive lymphocytes

Calculation of results

- A standard curve was plotted using the mean OD value on the Y-axis and concentration on the X-axis. A best-fit curve was drawn through the points on the graph.

- According to the OD value on the graph, its corresponding concentration of CD4 was calculated.

STUDY DURATION: From January 2020- July 2021

STATISTICAL ANALYSIS

Statistical analysis was done using a statistical package -SPSS 20.0. Descriptive statistics were presented as mean with standard deviation or median with interquartile range in case of continuous variables and percentage were used for categorial variables. Tests of Normality, Kolmogorov-Smirnov and Shapiro-Wilk were used for numerical data. Student t test were used to calculate difference of mean for normality distributed variables and Kruskal-wallis test was applied for skewed data. Chi square test was used for calculation of difference in categorial variables. Prognostic indicators of outcome were calculated by using multivariate analysis in general linear model. P value < 0.05 was considered as statistically significant.

RESULTS

RESULTS

Population characteristics

This study was conducted in a tertiary care center in western Rajasthan. Total 120 patients admitted with the diagnosis of Disseminated infections were screened for enrolment, out of which 110 were enrolled in the study after written informed consent.

Age and gender distribution

The mean age of the study population was 42.7 ± 19.2 years (range 18-90 years) with age distribution as given in Table 4. Out of 110 patients, 64% were males and 36% were females (Fig 5) and most of the study subjects belonged to the Urban population (69%) (Fig 6).

Table 4: Demographical profile of study population (N=110)

Demographic Details	**Number of Patients(N=110)**	**Percentage (%)**
Mean age(years) ± SD	42.73±19.238	
25 Percentile	26.00	
75 Percentile	58.25	
100 Percentile	90.00	
Age Group		
18-30	41	37%
30-45	28	26%
45-60	19	17%
60-90	22	20%
Address		
Rural	34	31%
Urban	76	69%
Sex		
Male	70	64%
Female	40	36%

Comorbidities		
Diabetes	23	21%
Hypertension	17	15%
CKD	9	8%
Previous infection	25	22%
Others	38	34%

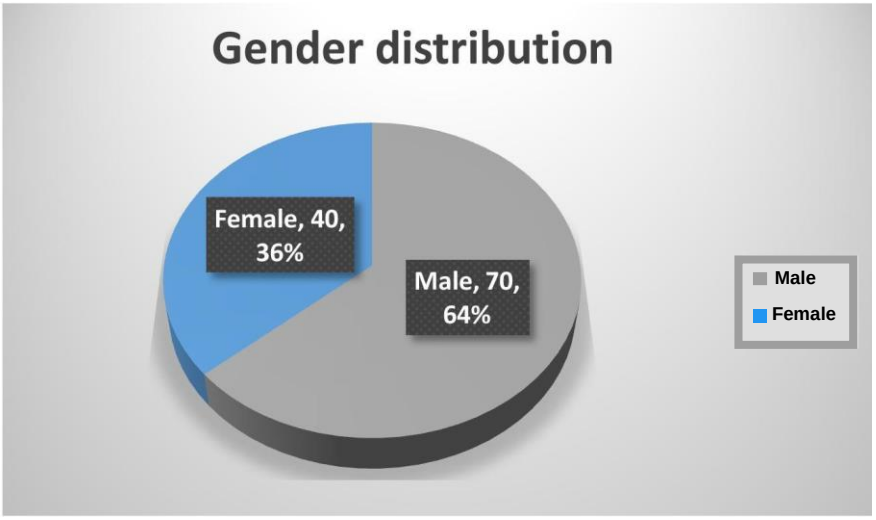

Figure 5: Gender distribution in study population

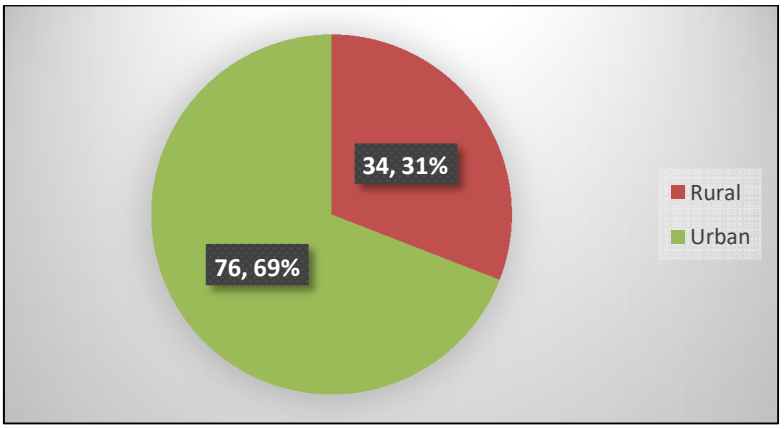

Figure 6: Address distribution in study population

Among 110 patients, 25 (22 %) had a history of significant previous infection and 23 (21%) were known cases of Diabetes mellitus. Hypertension, CKD, cardiovascular disease

(Coronary artery disease, Valvular heart disease), CVA, Bronchial Asthma, Bronchiectasis, and Coeliac disease were other comorbidities as depicted in Figure 7.

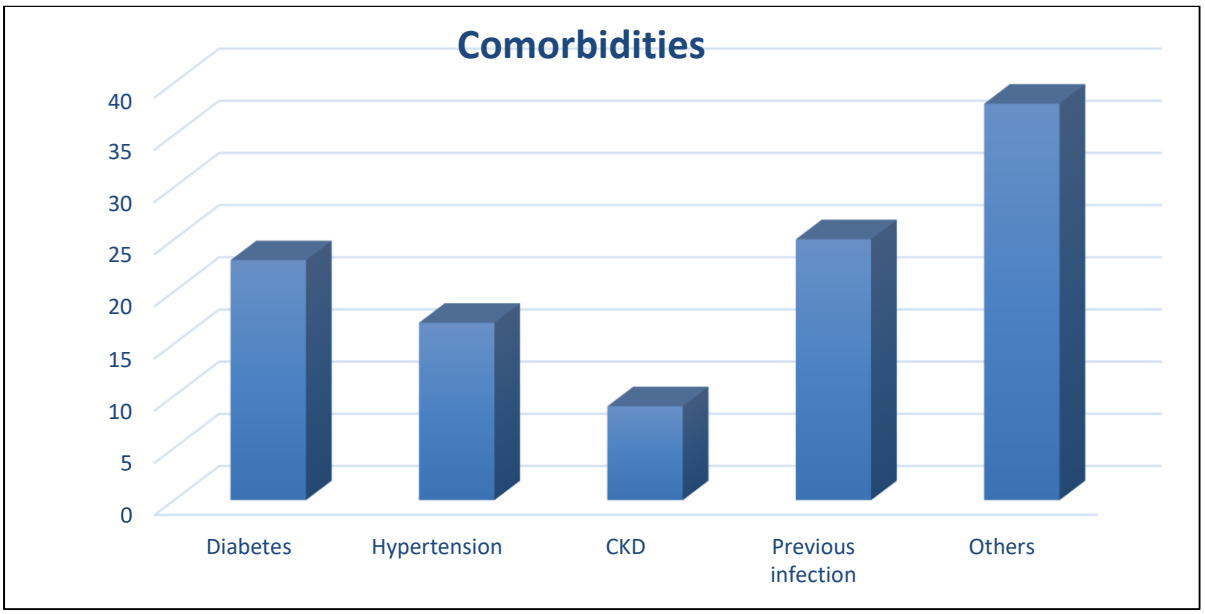

Figure 7: Comorbidities distribution in study population

Disease characteristics

Bacterial sepsis (47%) was the most common disseminated clinical syndrome encountered followed by disseminated TB (33%). Other disseminated infections among the study subjects were fungal, nocardiosis, and viral infections as shown in Figure 8.

Table 5 is showing the mean age distribution among different disseminated infections included in the study.

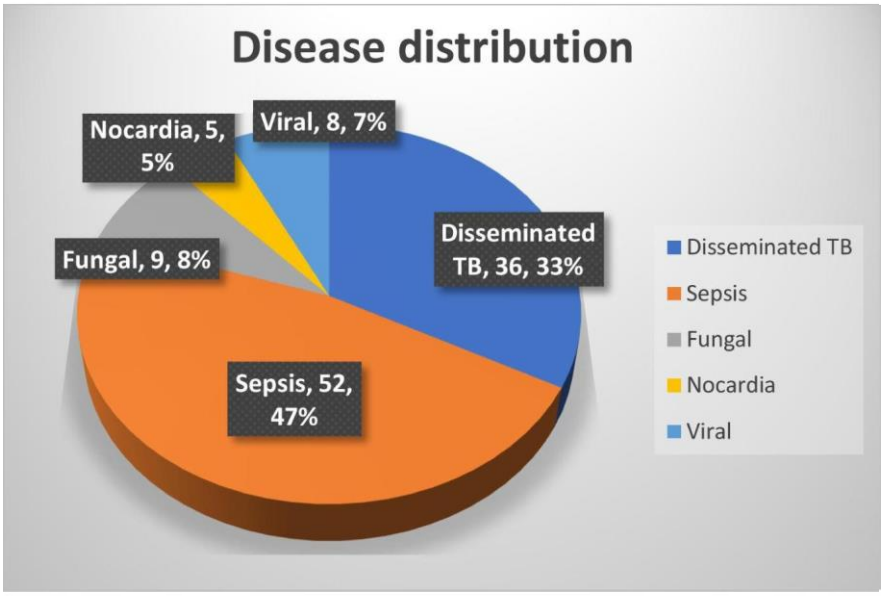

Figure 8: Disease distribution among study population

Table 5: Age distribution among disease groups

Disease	**AGE (years)**			
	N	**MEAN ± SD**	**MIN**	**MAX**
Disseminated TB	36	37.39 ± 17.127	18	79
Sepsis	52	46.21 ± 21.055	18	90
Fungal	9	46.11 ± 15.980	20	64
Nocardia	5	56.40 ± 16.920	38	81
Viral	8	31.75 ± 9.407	18	45
	110	42.73 ± 19.238	18	90

The most common symptomatology among the study population was fever (73%) followed by dyspnoea (42%). Others Symptoms include cough, dyspnoea, weight loss, altered sensorium, abdominal symptoms, Urological symptoms, and others as given in Figure 9.

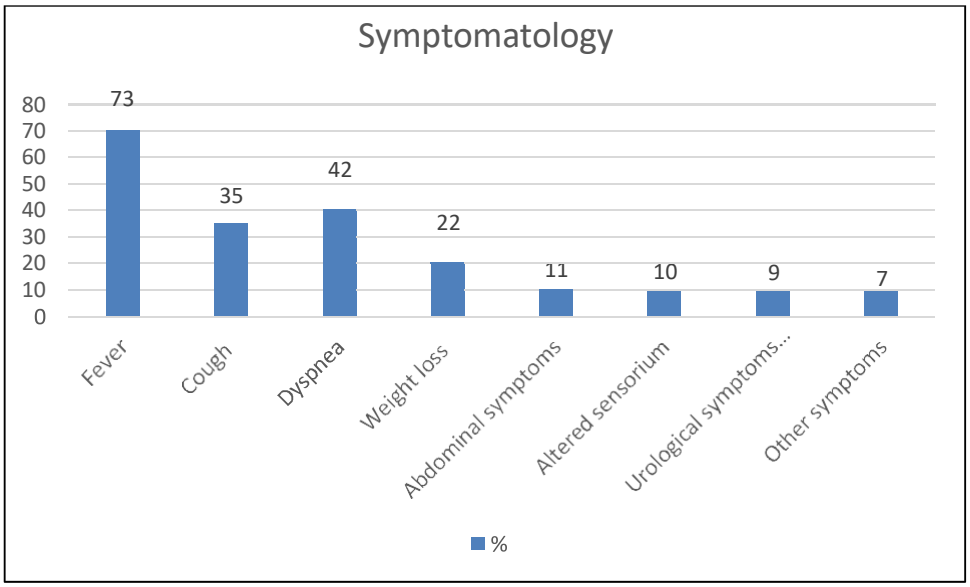

Figure 9: Symptomatology among study population (%)

Most common Examination finding encountered was Pallor (53%) followed by Pedal edema (21%) and Lymphadenopathy(10%). Other findings include Icterus, Clubbing, skin changes as given in Figure 10.

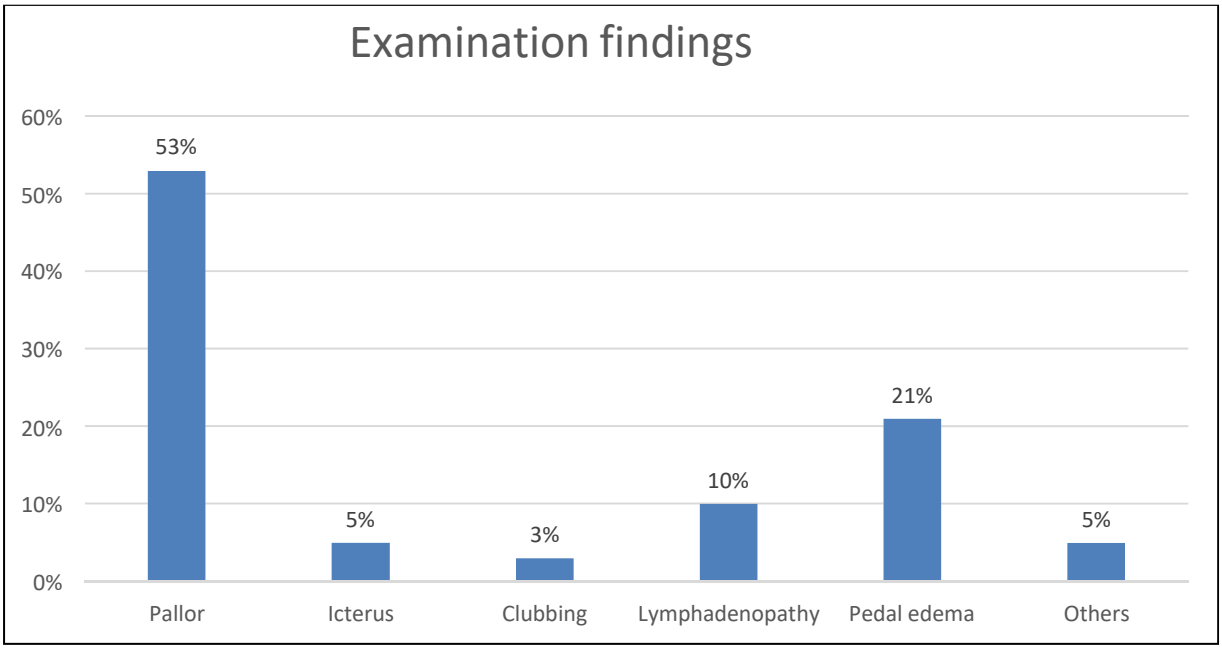

Figure 10: Examination findings among study population

Laboratory features

Tables 6 and 7 were showing the mean values of laboratory investigations of study subjects on the day of diagnosis and fifteen days of follow-up.

Table 6: Laboratory values of the study population at Diagnosis

Parameters	Disseminated TB	Sepsis	Fungal	Nocardia	Viral
MEAN ± SD					
Hb (g/dl)	11.36 ± 2.125	10.46 ± 2.38	10.27 ± 2.20	10.44 ± 2.54	10.05 ± 2.94
WBC (cells/mm^3)	11.00 ± 6.09	17.38 ± 9.10	243.65 ± 703.65	13.37 ± 7.28	9.56 ± 8.31
Platelet(10^3/mm^3)	339.56 ± 159.16	258.46±170.64	263.89 ± 126.18	372.8 ± 147.08	193.25 ± 174.06
SGOT (IU/L)	40.83 ± 46.78	64.73 ± 70.78	70.90 ± 71.45	33.60 ± 22.72	117.75 ± 182.89
SGPT (IU/L)	38.87 ± 44.94	62.85 ± 84.69	59.87 ± 53.21	23.80 ± 19.99	59.65 ± 69.41
Bilirubin (mg/dl)	0.90 ± 1.13	1.37 ± 1.61	0.85 ± 0.65	0.51 ± 0.13	0.76 ± 0.42
Albumin (g/dl)	3.02 ± 0.86	2.82 ± 0.85	3.36 ± 0.75	2.70 ± 0.65	2.92 ± 0.52
Urea (mg/dl)	40.00 ± 40.97	60.23 ± 53.56	32.22 ± 18.88	28.60 ± 8.08	22.25 ± 8.84
Creatinine(mg/dl)	1.28 ± 1.60	1.93 ± 2.82	0.95 ± 0.47	0.83 ± 0.27	0.79 ± 0.27

Table 7: Laboratory values of the study population at Follow up

Parameters	Disseminated TB	Sepsis	Fungal	Nocardia	Viral
	MEAN ± SD				
Hb (g/dl)	10.72 ± 1.97	9.98 ± 2.60	9.17 ± 1.18	10.28 ± 2.43	9.27 ± 3.30
WBC (cells/mm^3)	9.18 ± 4.75	11.31 ± 5.66	532.34 ± 1577.87	11.34 ± 7.92	8.19 ± 4.08
Platelet(10^3/mm^3)	324.31 ± 166.07	308.88 ± 152.44	301.33 ± 245.39	296.60 ± 208.53	339.38 ± 214.0
SGOT (IU/L)	48.61 ± 66.69	62.81 ± 102.89	42.73 ± 42.87	48.36 ± 34.33	55.62 ± 49.97
SGPT (IU/L)	107.35 ± 403.41	48.30 ± 55.19	45.55 ± 44.86	37.90 ± 22.07	46.75 ± 39.46
Bilirubin (mg/dl)	1.03 ± 1.05	1.33 ± 3.58	0.67 ± 0.31	0.65 ± 0.42	0.51 ± 0.24
Albumin (g/dl)	2.90 ± 0.82	2.76 ± 0.68	2.88 ± 0.55	2.77 ± 0.55	2.76 ± 0.30
Urea (mg/dl)	34.08 ± 30.36	44.42 ± 42.34	31.89 ± 16.22	19.60 ± 7.70	24.00 ± 9.68
Creatinine(mg/dl)	0.99 ± 0.78	1.56 ± 2.21	0.97 ± 0.29	0.82 ± 0.25	0.68 ± 0.26

The distribution of the mean of the inflammatory markers among study subjects (CRP and ESR) at the time of diagnosis and follow-up visit in different disease subgroups are depicted in Table 8.

Table 8: Inflammatory markers of the study population at Diagnosis and Follow up visit

Disease	Initial visit		Follow up	
	HSCRP	ESR	HSCRP	ESR
	MEAN ± SD			
Disseminated TB	80.97 ± 69.79	45.59 ± 32.37	57.55 ± 49.73	37.22 ± 21.47
Sepsis	125.28 ± 73.74	55.42 ± 31.69	75.86 ± 65.77	44.35 ± 27.43
Fungal	63.37 ± 57.23	31.44 ± 21.45	47.13 ± 44.13	33.59 ± 18.43
Nocardia	150.75 ± 60.71	64.00 ± 26.85	95.39 ± 81.62	52.60 ± 25.24
Viral	106.43 ± 81.43	42.00 ± 21.33	53.39 ± 71.30	36.75 ± 22.89

Positive findings of imaging in different disease subgroups are depicted in Table 9. Most common Chest X ray findings were consolidation, fibrosis, tree in bud appearance, and ground-glass opacities. ECG findings encountered were LVH, ST-T wave changes, arrhythmias, and ischemic changes.USG whole abdomen mostly depicted lymphadenopathy and organ enlargement. CT and MRI Brain changes were the most common changes consistent with the disease which mainly depicted meningitis, brain parenchymal changes.

Table 9: Imaging findings of the study population

Disease	USG	CT and MRI	Chest XRAY	ECG
Disseminated TB	15	33	27	5
Sepsis	29	39	24	10
Fungal	3	8	5	2
Nocardia	0	4	4	1
Viral	2	7	6	1
Total	49(45%)	91(83%)	66(60%)	19(17%)
P Value	0.267	0.757	0.057	0.947

Only blood cultures proven sepsis were included in the study population and *Escherichia coli* was the most common organism encountered followed by *Klebsiella*. Most common organism in Urine cultures was *Escherichia Coli*. Other cultures were CSF, pus, BAL fluid, and synovial fluids, among these MRSA being the most common organism (Table 10).

Table 10: Culture findings of the study population

Disease	**Blood culture**	**Urine culture**	**Other cultures**
Disseminated TB	1	1	1
Sepsis	16	6	0
Fungal	2	0	0
Nocardia	0	0	0
Viral	2	0	0
Total	21(19%)	7 (6%)	1(1%)
P Value	**0.016**	0.286	0.922

CD4 Function

The mean CD4 counts of the study population at the time of diagnosis were 690.54 ± 703.08 cells/cumm with a median of 412.38 cells/cumm. Minimum count was found to be 16 cells/cumm and the maximum was 3428 cells/cumm. Detailed mean, median, minimum, and maximum CD4 counts in each disease group were detailed in Table 11 and Figure 11.

Table 11: CD4 counts of the study population at Diagnosis (Initial visit)

Disease	N	Mean± SD	Median	MIN	MAX
Disseminated TB	33	472.83 ± 529.93	264	33	2368
Sepsis	51	881.39 ± 794.87	709	16	3428
Fungal	9	642.71 ± 558.60	397.83	66	1764
Nocardia	5	562.37 ± 645.41	184	160	1657
Viral	8	505.86 ± 693.47	275.72	124	2200
Total	106	690.54±703.08	412.38	16	3428

The Mean CD4 counts of the study population at Follow up visit were 601.94±626.70 cells/cumm with a median of 442.19 cells/cumm. Minimum count was found to be 63 cells/cumm and the maximum was 3011 cells/cumm. Detailed mean, median, minimum, and maximum CD4 counts in each disease group were detailed in Table 12 and Figure 11.

Table 12: CD4 counts of the study population at Follow up

Disease	N	Mean± SD	Median	MIN	MAX
Disseminated TB	13	435.49±248.54	347	111	901
Sepsis	7	1082.28±1020.56	890	63	3011
Fungal	2	1005.00±718.42	1005	497	1513
Nocardia	2	258.50±260.92	258.50	74	443
Viral	4	272.50±140.89	216.50	177	480
Total	28	601.94±626.70	442.19	63	3011

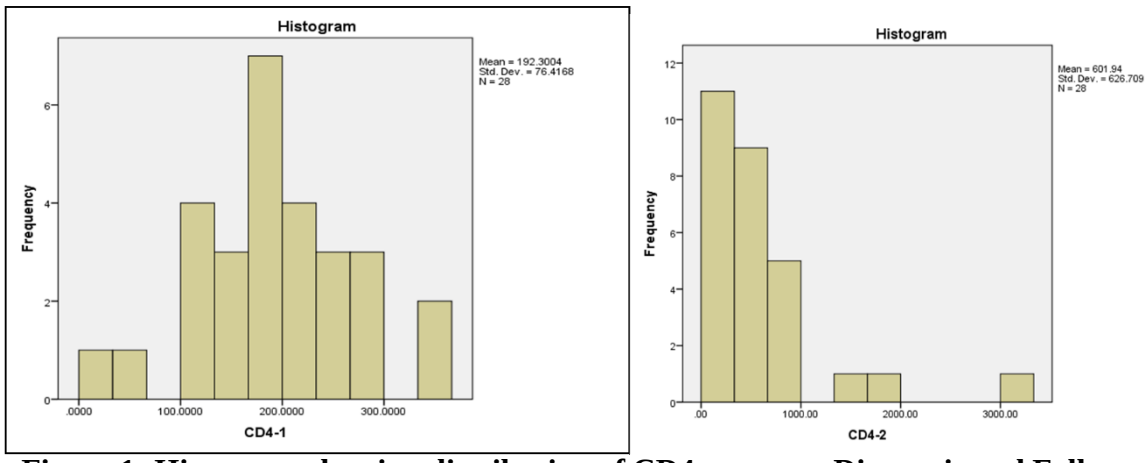

Figure 1: Histogram showing distribution of CD4 counts at Diagnosis and Follow up

CD4 Lymphocytopenia

Out of 110 study populations, 27% were found to have CD4 count less than 200 cells/cumm and 41% were found to have CD4 count less than 300 cells/cumm at the time of diagnosis of disseminated infections. Individual distribution in each disease subgroup is detailed in Table 13 and Fig 12.

Table 13: Percentage of population with Low CD4 counts at Diagnosis (Initial Visit)

	CD4 AT DIAGNOSIS	
Disease	**Low CD4 ≤ 200 cells/cumm**	**Low CD4 ≤ 300 cells/cumm**
Disseminated TB	13(43%)	20(45%)
Sepsis	11(37%)	15(33%)
Fungal	1(3%)	2(4%)
Nocardia	3(10%)	3(7%)
Viral	2(7%)	5(11%)
Total	**30(27%)**	**45(41%)**

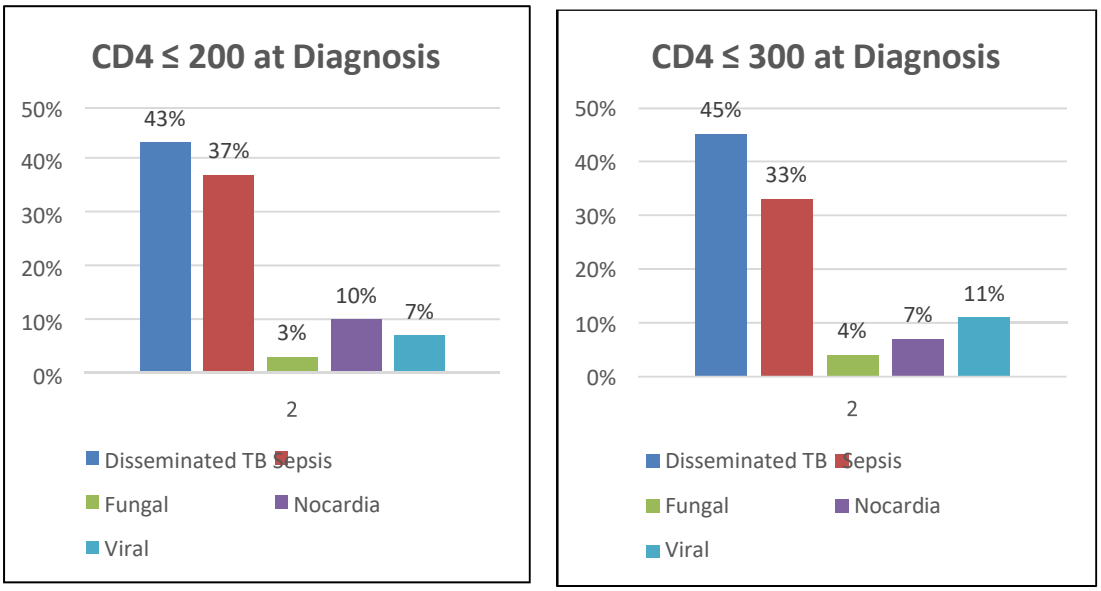

Figure 12: Figure showing percentage of Low CD4 distribution in subgroups

ICL Population

A total of 9% of the study population were found to have Idiopathic CD4 Lymphocytopenia with CD4 count less than 300 cells/cumm on two occasions at 3 months apart. Maximum of the ICL patients were found to be in Disseminated TB Group followed by Viral infections with the distribution as depicted in Table 14 and Fig 13.

Table 14: Percentage of population with ICL

Disease (n=10)	**ICL-Number (%)**
Disseminated TB	5(50%)
Viral	3(30%)
Sepsis	1(10%)
Nocardia	1(10%)
Fungal	0(0%)
Total	**10(9%)**

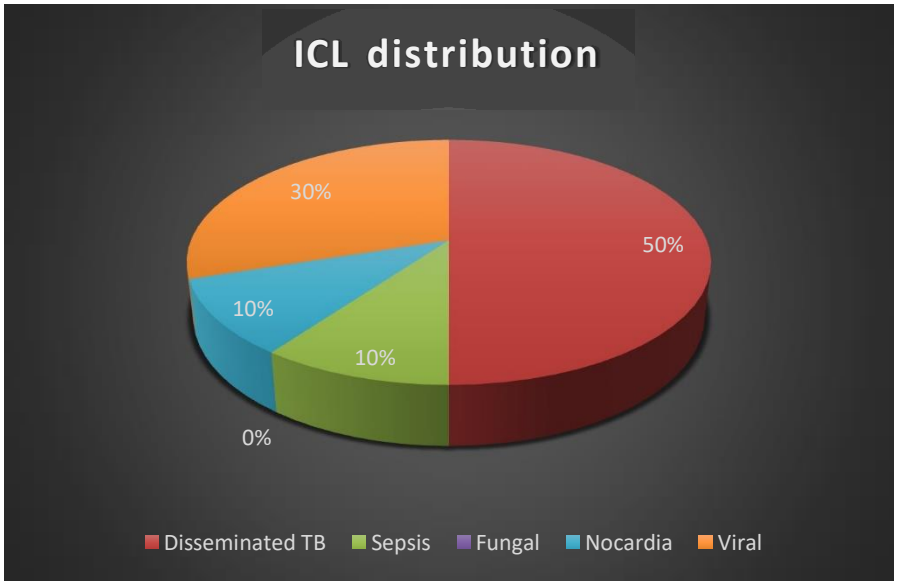

Figure 13: Distribution of ICL in disseminated infections

Majority of the ICL population belong to the age group of 18-30 (50%) with 80 % belonging to Urban backgrounds and most of them were females (60%) as depicted in Table 15.

Table 15: Demographical profile of ICL population (N=10)

Demographic Details	**Number of ICL Patients (N=10)**	**Percentage (%)**
Age Group		
18-30	5	50%
30-45	2	20%
45-60	2	20%
60-80	1	10%
Address		
Rural	2	20%
Urban	8	80%
Sex		
Male	4	40%
Female	6	60%

The distribution of the mean of the various laboratory parameters among the ICL population were as shown in table 16. Mean CD4 count among the ICL population was 178.52 ± 85.26 cells/cu mm. Inflammatory markers (ESR and CRP) were significantly raised (Table 16).

Table 16: Laboratory profile of ICL population (N=10)

Lab Parameters	**ICL (MEAN±SD)**
Diagnosis	
Age (years)	37.20 ± 16.732
Hb (g/dl)	9.88 ± 2.53
TLC (cells/ mm3)	8.73 ± 3.88
PLT (10^3/mm3)	293.10 ± 164.89
CRP (mg/L)	43.02 ± 33.07
ESR (mm 1st hr)	31.40 ± 24.75
Urea (mg/dl)	28.60 ± 23.35
Creatinine (mg/dl)	0.85 ± 0.47
CD4 at diagnosis (cells/ mm3)	178.52 ± 85.26
Follow up	
Hb (g/dl)	9.32 ± 2.20
TLC (cells/ mm3)	8.03 ± 2.76
PLT (10^3/mm3)	354.30 ± 162.89
CRP (mg/L)	26.19 ± 34.77
ESR (mm 1st hr)	31.10 ± 24.27
Urea (mg/dl)	22.30 ± 14.81
Creatinine (mg/dl)	0.70 ± 0.27

Outcome

Out of 110 study population, 30% had mortality at 3 months with the highest being among the Sepsis group (49%) followed by Disseminated TB (30%). Survival and mortality data in other disease subgroups are as detailed in Table 17.

Table 17: Outcome of the study population

	OUTCOME AT 3 MONTHS	
Disseminated Infections (n=110)	**Alive (n=77)**	**Death (n=33)**
Disseminated TB	26 (34%)	10 (30%)
Sepsis	36 (46%)	16 (49%)
Fungal	6 (8%)	3 (9%)
Nocardia	3 (4%)	2 (6%)
Viral	6 (8%)	2 (6%)
Total	**77 (70%)**	**33 (30%)**

Mortality was significant higher, 47% (P-value-0.03) in population with CD4 ≤ 200 cells/cumm as described in Table 18. The mean duration of death after admission was 23.3 ± 8.5 days in patients with CD4≤ 200 cells/cumm and 24.1 ± 8.3 in patients with CD4≤ 300 cells/cumm (Table 19).

Table 18: Percentage of the mortality in population with Low CD4 count at Diagnosis

	Mortality	**P value**
Initial CD4 ≤ 200	14/30 (47%)	**0.03**
Initial CD4 ≤ 300	18/45 (40%)	0.153

Table 19: The mean duration of death from admission in population with Low CD4 count

	DAY OF MORTALITY
	MEAN ± SD
Initial CD4 ≤ 200	23.3 ± 8.5
Initial CD4 ≤ 300	24.1 ± 8.3

Univariate analysis was applied to find the predictors of mortality. Age, respiratory rate, Hs-CRP, blood urea, and serum creatinine at follow-up were found to have a statistically significant association in predicting mortality at 3 months (Table 20).

Table 20: Univariate analysis showing the predictors of Outcome at 3 months

VARIABLE	P-VALUE
Age	**0.002**
Temperature	0.364
RR	**0.002**
TLC at diagnosis	0.667
TLC at 15 days	0.419
Neutrophil count at diagnosis	0.922
Lymphocyte count at diagnosis	0.966
Hs CRP at diagnosis	0.125
Hs CRP at 15 days	**0.002**
Urea at diagnosis	0.062
Creatinine at diagnosis	0.059
Urea at 15 days	**0.000**
Creatinine at 15 days	**0.005**

Multivariable analysis was done to study the predictors of outcome at 3 months. It was found that higher age, high respiratory rate, raised Hs-CRP, raised blood urea, and serum creatinine at 15 days follow-up were found to be significantly associated with mortality at 3 months (Table 21). Presence of pedal edema, abnormal Chest Xray and ECG findings, and CD4 count ≤ 200 cells/cu mm at the time of diagnosis of disseminated infections were also significantly associated with mortality at 3 months (Table 21).

Table 21:Multivariate analysis showing the predictors of Outcome at 3 months

Variable	P-value	Hazard Ratio	95% C.I.	
			Lower	Upper
Age	**0.002**	1.03	1.01	1.06
RR	**0.002**	1.28	1.07	1.52
Hs CRP at 15 days	**0.001**	1.01	1.01	1.02
Urea at 15 days	**0.000**	1.03	1.01	1.04
Creatinine at 15 days	**0.022**	1.51	1.01	2.29
Pedal edema	**0.000**	5.58	2.79	11.12
Abnormal Chest Xray	**0.030**	2.31	1.04	5.13
Abnormal ECG	**0.001**	3.52	1.73	7.18
Low CD4 ≤ 200 cell/mm3	**0.030**	2.26	1.13	4.53

Hazard ratio of mortality was almost twice higher among patients with CD4 count ≤ 200 cells/cumm at Diagnosis as compared to patients with CD4 count > 200 cells/cumm as represented by Kaplan Meyer curve in Fig 14. with HR 2.26; CI 1.13-4.53 and a statistically significant p-value (0.02).

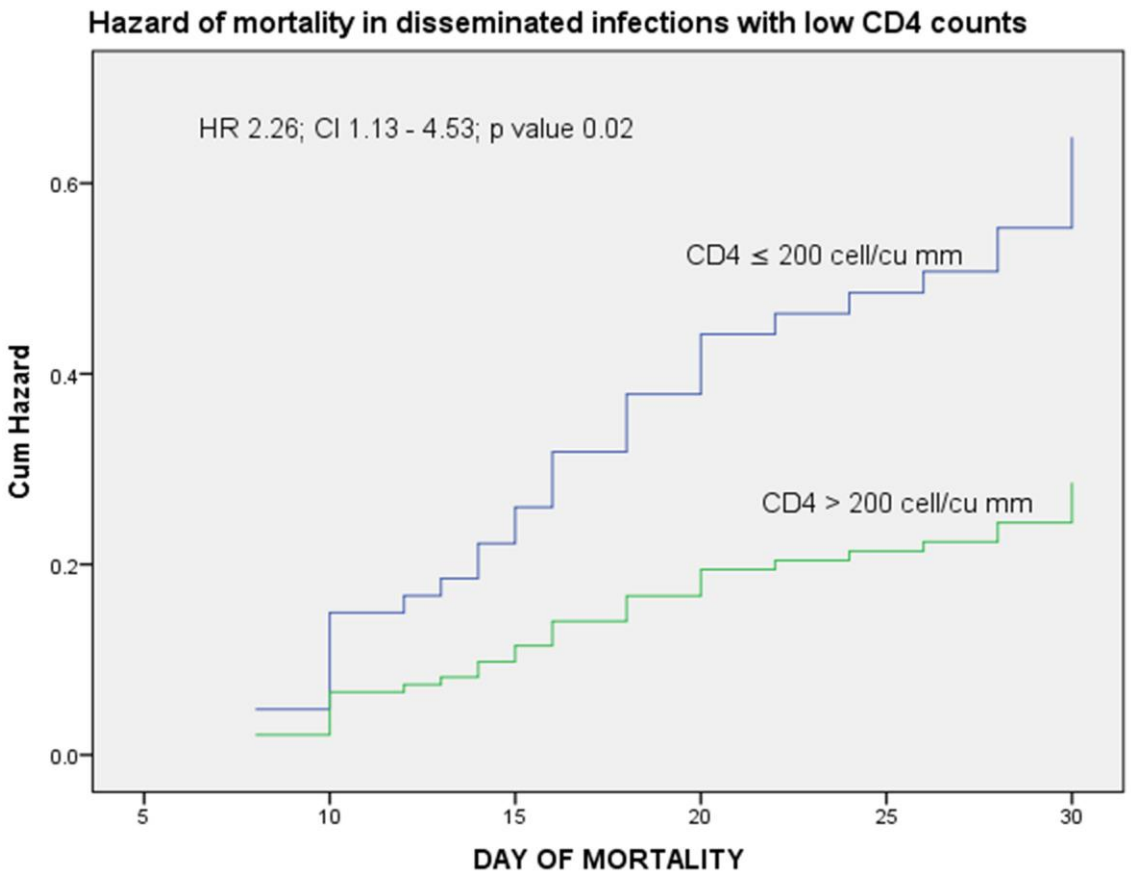

Figure 14: Hazards of mortality in disseminated infections in relation to the low CD4 counts by Kaplan-Meier Curve

Cumulative survival was higher among patients with CD4 count > 200 cells/cumm at Diagnosis as compared to patients with CD4 count ≤ 200 cells/cumm as represented by Kaplan Meyer curve in Fig 15. Also, the survival probability is decreasing within the first 30 days of diagnosis and is almost static later.

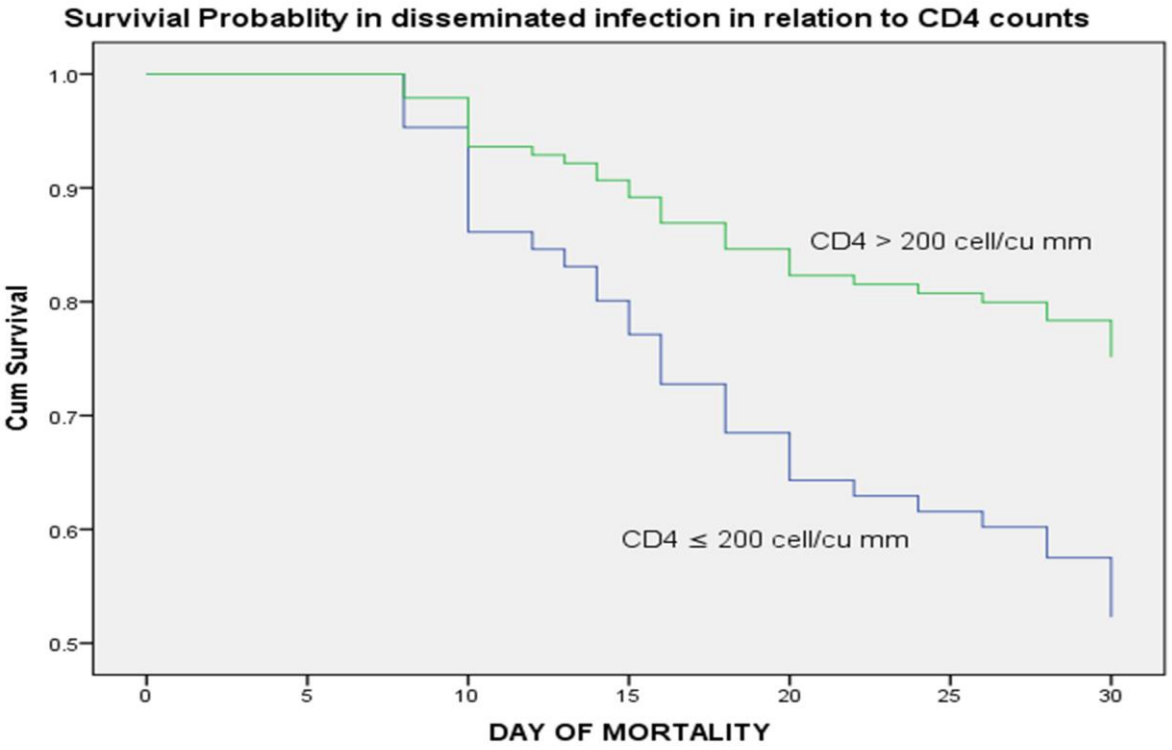

Figure 15: Kaplan-Meier Survival Probability Curve in disseminated infection in relation to CD4 count

DISCUSSION

DISCUSSION

Idiopathic CD4 lymphocytopenia is a very rare disease. ICL is a heterogeneous condition diagnosed typically in middle age, usually after an opportunistic infection, although it can also be an incidental laboratory finding[28].

In this study, a total of 110 patients of disseminated infections were enrolled after written informed consent. The mean age of total population was 42.7 ± 19.2 years (range:18-90). This was comparable to that seen in the study by Smith et al., in which the mean age was 43 years, Ahmad et al., (40.7 years), Regent et al., (44.2 years), and Yarmohammadi et al., (45 years)[11,28,39,40]. The mean age in various subgroups in our study was comparable to the other studies of ICL where mean age in Disseminated TB was 37.39 ±17.12 years[40,41]; Bacterial Sepsis was 46.21 ±21.05 years, Fungal infections were 46.11±15.98 year[25].

Males (64%) were affected predominantly. Many studies have similar male predominance with a proportion of 62% in the study by Smith et al., 64% in the study by Ahmad et al., and 64% in Vijaykumar et al (10,27,42). Most of the study population belonged to the Urban population (69%). Only a few familial cases have been reported in the literature but no such association was studied among our population[43].

The clinical spectrum of ICL varies from asymptomatic stage to florid opportunistic infections. Our study was restricted to the patients with disseminated infections, so the clinical presentation of ICL in our study was an opportunistic infection, even without any identifiable underlying immunosuppressed status. This presentation was similar to that of other studies by Smith et al., Ahmad et al., and Yarmohammadi et al., where infection led to the presentation of underlying ICL[10,27,39]. ICL patients are susceptible to various opportunistic infections, including AIDS-defining illnesses. Among the disseminated infections included in the study population, the majority of patients had Bacterial Sepsis (47%) followed by Disseminated TB (33%), Fungal (8%), Viral (7%), and Nocardia (5%).

There were other reported clinical presentations of ICL like Autoimmune phenomenon reported about 33% in studies of Ahmad et al., 23% in Zonis et al., 35% in Regent et al., 33% in Yarmohammadi et al., and 18% in Vijaykumar et al.,[27,28,38,39,42]. Sjogren's disease was the most common reported autoimmune diseases in ICL patients. Kertava et al. retrospectively studied 115 patients with primary Sjogren's syndrome. Six patients met the criteria of ICL. One patient developed lymphoma in 3 years. They suggested that ICL patients should be

screened for primary Sjogren's syndrome[44]. None of our patients showed association with autoimmune disease.

Malignancies were reported in 41% cases in Yarmohammadi et al., 16% in Vijaykumar et al., 13% in Zonis et al., 18% in Ahmad et al.,13% in Regent et al., of the ICL patients. Lymphoma, in general, was the most common reported malignancy in ICL patients[27,28,38,39,42]. However, the population with malignancies and autoimmune disorders was excluded from our study.

In a few studies, lymphocytopenia had been largely overlooked for several months or years before eventually ICL was diagnosed from an opportunistic infection. Data obtained from blood banks confirm the rare existence of a small population of otherwise healthy persons with low CD4 T-cell counts (0.25%-0.5% of blood donors), (45) although it is not clear if this represented a transient or persistent low count. Investigation toward alternative diagnoses at disease presentation should always include lymphoproliferative diseases or lymphomas and other forms of immunodeficiency, such as common variable immunodeficiency.

Pathogenesis:

The pathogenesis for ICL has not yet been defined clearly. Decreased CD4 cell production and differentiation, and increased destruction with tissue sequestration (such as spleen and lymph nodes) are most probably involved in the pathogenesis of ICL[7]

The two important factors related to CD4+ lymphocyte functions were increased activation of CD4, which may result from stimulation by an unidentified pathogen, resulting in a persistent decrease in the number of CD4+ lymphocytes and this was the main mechanism of ICL in our study population. Lee et al. found increased levels of serum lipopolysaccharide (LPS) and markers of CD4+ lymphocyte activation in patients with ICL[46].

Second, apoptosis of CD4+ lymphocyte which may be associated with enhanced expressionof Fas and Fas ligand. Roger et al. demonstrated that a patient with ICL and disseminated Mycobacterium xenopi infection had overexpression of Fas/CD95c and spontaneous and Fas-induced apoptosis[47].

Various other immune defects have been described in some ICL patients. Low CD8 T-cell counts at diagnosis represent a subset of ICL with a worse prognosis and increased risk for a serious opportunistic infection or death. Patients with CD8 counts <180 cells/mm3 in a study

of 39 patients were found to have a higher risk of serious opportunistic infections and death[48]. The complete absence of specific CD8+ cells (CD8+ 28+) has been reported in a small number of patients with ICL[46]. Genetic factors might be involved in the pathogenesis of ICL as well. Zonios et al. found higher proportions of HLA DR+ CD4 cells in ICL patientscompared to controls, which suggests that there could be a genetic predisposition to ICL[28].In contrast to HIV infection, there is no decrease in CD8 cell counts and the number of B cells is within the normal limits in most patients with ICL. The CD4 cell levels in ICL patients are stable (without significant decline during follow-up) over a long period in contrast to HIV-infected patients in whom without treatment CD4 decline is mandatory[42].

In summary, diminished precursors (reduced clonogenic potential), accelerated apoptosis (Fas/Fas ligand), reduced chemotaxis (reduced CXCR expression), poor response to TCR stimulation, impairment of activation (low expression of Lck, MAGT1 defect), defective cytokine production (TNF-α and IFN-γ), elevated IL-17, dysregulation of IL-7 and its downstream targets, others mutations involving RAG1, UNC119, ITK, STK4, and CD45, cytotoxic antibodies to T cells, and sequestration all can contribute to reduced CD4 T cell counts[42].

Clinical Presentation and Findings:

In our study, the most common clinical presentation of disseminated infections was Fever (73%) followed by Dyspnoea (42%), Cough (35%). Others Symptoms include weight loss, altered sensorium, abdominal symptoms, and Urological symptoms. The most common clinical finding in our study was Pallor (53%) followed by Pedal edema (21%) and Lymphadenopathy (10%). Other findings include Icterus, Clubbing, skin changes.

On reviewing the comorbidities in our study, 22% had a history of previous infections and the most common comorbidity was Diabetes Mellitus (21%) followed by Hypertension (15%), and CKD (8%). 34% have other comorbidities like cardiovascular disease (Coronary artery disease, Valvular heart disease), CVA, Bronchial Asthma, Bronchiectasis, and Coeliac disease. This was comparable to other studies[27,28].

In this study around 50% had positive Ultrasound findings in which the whole abdomen mostly depicted lymphadenopathy and organ enlargement, mostly in the Disseminated TB subgroup. Common Chest X ray findings were consolidation, fibrosis, tree in bud appearance, and ground-glass opacities being more common and almost equally found in the

Disseminated TB and Bacterial Sepsis group. More than 80% had Positive CT or MRI findings, among which Brain imaging findings were prominent and mostly found in Disseminated TB and Bacterial Sepsis group. The common ECG findings in this study population were LVH, ST-T wave changes, arrhythmias, and ischemic changes and mostly inBacterial Sepsis group.

Among the study population, 19% of patients had positive Blood culture findings in which Escherichia coli was the most common organism encountered followed by Klebsiella. 6% had positive Urine cultures in which Escherichia Coli which was MDR was the most prominent. Other cultures like CSF, Pus, BAL fluid, and synovial fluids were positive in 1% population in only the Disseminated TB group with MRSA being the most common organism. Most Blood and Urine culture findings were positive in Bacterial Sepsis followed by the Disseminated TB group. No cultures were positive in the Nocardia population.

Immunological characteristics:

In the present study, at the time of diagnosis, mean CD4 counts were 690.54 ± 703.08 cells/cumm with a median of 412.38 cells/cumm. Minimum count was found to be 16 cells/cumm and the maximum was 3428 cells/cumm which were both distributed among the Bacterial Sepsis group. This was similar to a study done among the ICU population, Arthur et al., with a mean of 494 ± 282 cells/cumm and with the least count of 50 cells/cumm[49]. Mean counts among the Disseminated TB group was 472.83 ± 529.93 with a median of 264, which were comparable to the study population of Aska et al., and Sten Skogmar et al,[40,41].

The mean CD4 counts of the study population at Follow up visit were 601.94±626.70 cells/cumm with a median of 442.19 cells/cumm. Minimum count was found to be 63 cells/cumm and the maximum was 3011 cells/cumm which were both distributed among the Bacterial Sepsis group. Mean among the Disseminated TB group was 435.49±248.54 with a median of 347.

Among our study population, 27% were found to have a CD4 lymphocyte count of fewer than 200 cells/cumm, and 41% were found to have a CD4 lymphocyte count of fewer than 300 cells/cumm at Diagnosis. This was comparable to Arthur et al., in which 51% population had a CD4 count of fewer than 500 cells/cumm and this was proven to be a significantly associated risk factor for mortality[49]. Among these both categories in the present study

highest percentage of the population belongs to the Disseminated TB group followed by Bacterial Sepsis, Viral, Nocardia, and least in the Fungal group.

Prevalence of ICL:

In our study, ICL was observed in 9% of patients with disseminated infection. Mean CD4 count among our ICL population was found to be 179 ± 85 cells/cumm. This was similar to the studies of Smith et al., and Vijayakumar et al.,[10,42].

The spectrum of opportunistic infections in ICL seems to overlap with that found in HIV-positive patients with similar CD4 T-cell counts. The majority belong to the Disseminated TB group which was the most prevalent infection among our ICL population (50%), followed by Viral infections (30%), Nocardia (10%), and Sepsis (10%). The fungal infection group has no ICL population. This proves that ICL is most predominant among the disease groups where T cell-mediated immunity plays an important role in pathogenesis. This is comparable to Zonis et al., Regent et al where Cryptococcal infections were the most prevalent infections in ICL patients (26.6%), followed by mycobacterial infections (17%), candidal infections (16.2%), and VZV infections (13.1%) (28,38). In the search of literature regarding the infections in ICL, we found the that disseminated Cryptococcal (50–56) and mycobacterial infections[57–63]were the most common.

In our study, among the Bacterial Sepsis, infection encountered were of Urosepsis followed by CNS, Pulmonary, Bloodstream infections, and abscess. Cryptococcus was most common among Fungal infections followed by a few cases of Histoplasma and Mucormycosis. CMV, EBV was predominant viral infections followed by Herpes and COVID 19 related disease. The majority of the ICL population belong to the age group of 18-30 (50%) with 80 % belonging to Urban backgrounds and most of them were females (60%).

Outcome

In the study population, 30% had mortality at 3 months with the highest being among the Sepsis group (49%) followed by Disseminated TB (30%), Fungal (9%), Viral (6%), and Nocardia (6%). Survival population was least among the Nocardia (4%). Mortality data was similar to Arthur et al.,(30.5%)[49].

Of the mortality population, 47% had CD4 ≤ 200 cells/cumm and this association was statistically significant with a P-value of 0.03 while 40% had CD4 count less than 300 cells/cumm.

On univariate and multivariate analysis, higher age, high respiratory rate, Pedal edema, raised Hs CRP, deranged blood urea, and serum creatinine at follow-up, abnormal Chest X ray, abnormal ECG findings, and CD4 count ≤ 200 cells/cumm at the time of diagnosis of disseminated infections were found to have independent predictor of mortality at 3 months. Comparison of Demographic data, clinical presentation, immunological findings, and outcome of our study with the major studies of ICL in literature was done in Table 22.

Table 22: Demographic and Clinical Features – A Summary of Case Reports, Case Series and Cohorts of ICL

Parameter	Zonis et al	Smith et al	Ahmad et al	Vijayakumar et al	Yarmoham-madi et al	Regent et al	OUR STUDY
Source of study	Prospective single centric	Review of AIDS reporting system	Literature search	Literature search from only case reports	Retrospective single centric	Retrospective Multicentric	Prospective; Observational
Population	39	47	259	164	24	40	110
Age in years (mean)	29 (20-90)	43 (17-78)	40.7 (SD: 19.2)	45.4 (0.5-85)	45 (7-76)	44.2 (19-70)	42.7 (18-90)
Sex distribution (Women %)	22 (56%)	18 (38%)	91 (36%)	55 (36%)	14 (58%)	24 (60%)	40 (36%)
Infections	15 (42%)	43 (91%)	226 (87.6%)	114 (70%)	18 (75%)	25 (63%)	We studied ICL in infections group (100%)
Autoimmune	23%	Not reported	33%	18%	33%	35%	Not studied
Malignancy	13%		18%	16%	41%	13%	
Mortality	7 (18%)	2 (4%)	24 (9%)	25 (15%)	0	8 (20%)	33 (30%)
CD4 counts in cells/cumm (mean)	139	144	143	140	119	127	178

Treatment:

We did not compare the effect of treatment in our study however, infections were treated as per the standard protocol of our institution. It was found from the literature that treatment of ICL revolves around the treatment of presenting illness, appropriate prophylaxis and screening, and the treatment of ICL itself.

Prophylaxis and Screening

Prophylaxis should be considered for a subset of ICL patients with the worst prognosis, such as those with low CD8 counts or patients presenting with an "AIDS-defining condition". We treated our patients with the standard prophylaxis as given in Table 23. However, it remains unclear which opportunistic infection should be targeted by such prophylaxis or what CD4 count might prompt it, although the HIV approach might be appropriate[25].

Table 23: Prophylaxis for Opportunistic Infections

CD4 counts	Organisms	Treatment
< 200 cells/cumm	Pneumocystis jirovecci	TMP-SMX (DS tablet) daily
< 100 cells/cumm	Toxoplasma gondii Histoplasma capsulatum	TMP-SMX (DS tablet) and Itraconazole 200 mg daily
< 50 cells/cumm	Mycobacterium avium intracellulare	Azithromycin 1200 mg/week

Abbreviations: CD4: Cluster of differentiation; TMP-SMX: Trimethoprim/sulfamethoxazole

In the prospective study by Zonios et al., one-fifth of their patients resolved their lymphocytopenia within 3 years of diagnosis. Therefore, they suggested that it is reasonable to consider following ICL patients more closely during the first 3 years because of the risk of serious infections and the possibility of normalization of CD4 T-cell counts, allowing discontinuation of any prophylaxis if initially given[28].

Specific therapy of ICL

As the underlying pathophysiology is poorly understood and the condition is rare, therapy of ICL is mostly experimental. Studies showed improvement with Interleukin 2 (IL-2), IL-7 infusion. Other therapies under development were Interferon Gamma (INFγ), Stem Cell Transplant, Intravenous immunoglobulin. The efficacy of vaccination in ICL patients is

unknown. Live vaccines are contraindicated and should be avoided in ICL patients. All other types of vaccines (dead, recombinant) may be given but their efficacy and protective effects are not known and are unpredictable[42].Prospects in the studies regarding specific therapy of ICL are required.

CONCLUSION

CONCLUSION

ICL is a heterogeneous yet distinctive condition that is quite different clinically and immunologically from infection with HIV and is likely multifactorial in etiology. It commonly presents as opportunistic infections, autoimmune diseases, and/or neoplasia. A high index of suspicion is necessary for diagnosis, mainly in the disseminated infections involving T cell immunity. We found that a significant number of disseminated cases had ICL and an initial CD4 T lymphocyte count of less than 200 cells/cumm represent a poor prognosis. This study emphasized the routine incorporation of CD4 counts measurement in patients with disseminated infections, especially in which T cell immunity is essential, which could further reveal the mystery of ICL. Currently, management revolves around the treatment of the presenting symptoms and close follow-up. More research into this obscure disease will provide us with better insights into its pathology and open new avenues for therapy.

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ANNEXURES

IEC CERTIFICATE

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2062

Date: 01/01/2020

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/958**

Project title: **"The Study of Prevalence of Idiopathic CD4 T cell lymphocytopenia in HIV negative patients with disseminated infections"**

Nature of Project:	**Research Project**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr.Tejaswee Banavathu**
Guide:	**Dr. M.K. Garg**
Co-Guide:	**Dr.Deepak Kumar, Dr.Satyendra Khichar & Dr.Abhishek Purohit**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure I

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

Annexure I

Institutional Ethics Committee
All India Institution of Medical Sciences, Jodhpur

Meeting of Institutional Ethics committee held on **23-12-2019 at 10:00** AM at Committee Room, Admin Block AIIMS Jodhpur.

Following members were participated in the meeting:-

S/No.	Name of Member	Qualification	Role/Designation in Ethics Committee
1.	Dr. F.S.K Barar	MBBS, MD (Pharmacology)	**Chairman**
2.	Justice N.N Mathur	LLB	Legal Expert
3.	Dr. Varsha Sharma	M.A (Sociology)	Social Scientist
4.	Mr. B.S.Yadav	B.Sc., M.Sc. (Physics), B.Ed.	Lay Person
5.	Dr. K.R.Haldiya	MD (General Medicine)	Clinician
6.	Dr. Arvind Mathur	MBBS, MS (General Medicine)	Clinician
7.	Dr. Surajit Ghatak	MBBS, MS (Anatomy)	Basic Medical Scientist
8.	Dr. Vijaya Lakshmi Nag	MBBS, MD (Microbiology)	Basic Medical Scientist
9.	Dr. Sneha Ambwani	MBBS, MD (Pharmacology)	Basic Medical Scientist
10.	Dr. Kuldeep Singh	MBBS, MD (Paediatric), DM (General Medicine)	Clinician
11.	Dr. Abhinav Dixit	MBBS, MD (Physiology), DNB (Physiology)	Basic Medical Scientist
12.	Dr. Pradeep Kumar Bhatia	MBBS, MD (Anaesthesiology)	Clinician
13.	Dr. Tanuj Kanchan	MBBS, MD (Forensic Medicine)	Basic Medical Scientist
14.	Dr. Pankaj Bhardwaj	MBBS, MD (CM&FM)	Clinician
15.	Dr. Praveen Sharma	M.Sc., Ph.D. (Biochemistry)	Member Secretary

Dr. Praveen Sharma
Member Secretary
Institutional Ethics Committee
AIIMS,Jodhpur

APPENDIX-1

All India Institute of Medical Sciences

Jodhpur, Rajasthan

Informed Consent Form

Title of Thesis/Dissertation: **The Study of Prevalence of Idiopathic CD4 T cell Lymphocytopenia in HIV Negative Patients with Disseminated Infections**

Name of PG Student : Dr. TEJASWEE BANAVATHU, Contact No. - 9704234919

Patient/Volunteer Identification No.: _____

I,_____S/o or D/o_____

R/o,_____give my full, free, voluntary consent to be a part of the study **"The Study of Prevalence of Idiopathic CD4 T cell Lymphocytopenia in HIV Negative Patients with Disseminated Infections"**, theprocedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from_____(Company Name) or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place:_____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date: _____ _____

Place:_____ Signature of PG Student

APPENDIX-2

अिखलभारतीयआयुिव]ा

नसं5थान जोधपुर, राज5थान

सूिचत सहमित पा

थीिसस का शीषक: िनि®य सं मण के साथ एचआईवी नकारा क रोिगयोंम अ yातहेतुक सीडी 4 टी सेल

िल%फोसाइटोपेिनया के)सार का अ*यन

पीजी छाIT का नाम: डॉ। तेजसवी बानवथु, संपक नंबर - 9704234919

रोगी / 4यं सेवी पहचान सं%ा.: _____

म,_____पुT /पुTी_____

िनवासी_____िन5िलिखत अ*यन का

िह4ा बनने के िलए मेरी पूण, िन: शु9, 4ैि;क सहमित देता 5ँ

“िनि य सं मण के साथ एचआईवी नकारा&क रोिगयोंम अ]ातहेतुक सीडी 4 टी सेल

िल+फोसाइटोपेिनया के -सार का अ4यन”, िजसकी)ि या और)कृ ित मेरी पूरी संतुिJ के िलए मेरी अपनी भाषा म मुझे समझायी गयी है। म पुिJ करता 5ं िक मेरे पास)D पूछने का अवसर था।

म समझता 5ं िक मेरी भागीदारी 4ैि;क है और िकसी भी कारण के िबना, िकसी भी समय अ*यन से बाहर िनकलने के मेरे अिधकार से अवगत 5ं

.म समझता 5ं िक मेरे और मेरे िकसी भी मेिडकल रकॉड के बारे म एका की गई जानकारी एG जोधपुर से या िनयामक)ाधिकरणोंसे िजHेदार D4 Kारा देखी जा सकती है । म इन D4योंके

िलए अपने रकॉड तक प5ंचने की अनुमित देता 5ं.

िदनांक: _____ _____

Mथान : _____ ह™ाIOर/बाएं अंगूठे कीछाप

यह)माणित करने के िलए िक उपय4 सहमित मेरी उपMथित म

_____

)ा™ की गई है। तारीख :_

Mथान: _____ ह™ाOर पीजी छाIT

APPENDIX-3

PATIENT INFORMATION SHEET

Name of the patient: **Patient ID.:**

THE STUDY OF PREVALENCE OF IDIOPATHIC CD4 T CELL LYMPHOCYTOPENIA IN HIV NEGATIVE PATIENTS WITH DISSEMINATED INFECTIONS

1. **Aim of the study**: To evaluate role of CD4 counts in prediction of treatment response and mortality in disseminated infections in HIV Negative patients.

2. **Study site:** In-patient services of Department of Internal Medicine, All India Institute of Medical Sciences, Jodhpur, and Rajasthan.
3. **Study procedure:** Prospective observation study by detailed clinical history and go for physical examination, laboratory investigations including viral markers HIV, HbsAg, HCV in patients with disseminated infections. We will go for first CD4 and CD8 counts on the diagnosis and follow up after 2 months and correlate with trends.
4. **Confidentiality:** All the data collected from each study participant will be kept highly confidential.
5. **Risk:** Enrollment in above study poses no substantial risk to any of the study participant and if any point of time participant wants to withdraw himself/ herself, he/ she can do so voluntarily at any point of time during the study.

For further information / questions, the following personnel can be contacted:

Dr, Tejaswee Banavathu,

Junior Resident, Department of Internal Medicine, All India Institute of Medical Sciences, Jodhpur, Rajasthan. Ph: 9704234919

APPENDIX-4

रोगी सूचना पा

रोगी का नाम: रोगी आईडी :

‘िनि य सं मण के साथ एचआईवी नकारा&क रोिगयों म अ]ातहेतुक सीडी 4 टी सेल

िल+फोसाइटोपेिनया के -सार का अ4यन’

1. आप **एचआईवी नकारा&क रोिगयों म -सारित सं मण म उपचार -ितिया और मृ6ु दर**

की भिव®वाणी म सीडी 4 की भूिमका का मू ांकन। संबंध का अ*यन म भाग ले रहे ह।

2. अ*यन Mथल: मेिडिसन िचिकTा िवभाग, अिखल भारतीय आयुिवyान संMथान, जोधपुर, राजMथान के आउट पेशट, आईपीडी और आपातकालीन सेवाएं।

िधक जानकारी /)Dों के िलए, िन5िलिखत किमयों से संपक िकया जा सकता है:

डॉ, तेज4ी बनवथु, जूिनयर रेिजडट, आंतरिक िचिकTा िवभाग, अिखल भारतीय आयुिव]ान संथान, जोधपुर, राज5थान। Ph: 9704234919

APPENDIX-5

SOCIO-DEMOGRAPHIC AND CLINICAL PROFORMA

Patient ID:

Name of patient **Age** **Gender**

Address

Contact number

CHIEF COMPLAINTS

BRIEF HOPI:

PAST HISTORY:

TREATMENT HISTORY:

FAMILY HISTORY:

PERSONAL HISTORY:

VITALS:

TEMP:

PULSE:

RR:

BP:

GENERAL PHYSICAL EXAMINATION:

SYSTEMIC EXAMINATION:

INVESTIGATIONS:

CBC		
KFT		
LFT		
RBS		
URINE ANALYSIS		
CHEST XRAY		
CULTURE AND SENSITIVITY REPORTS		
VIRAL MARKERS		
CD4 COUNTS	**DIAGNOSIS**	**TWO-MONTH FOLLOW UP**

CD8 COUNTS	**DIAGNOSIS**	**TWO-MONTH FOLLOW UP**

FINAL DIAGNOSIS:

TREATMENT GIVEN:

TOTAL DURATION OF ILLNESS:

DURATION OF HOSPITAL STAY:

ANY COMPLICATIONS DURING HOSPITAL STAY: