"IDENTIFICATION OF BACTERIA CAUSING DIARRHEA IN HIV/AIDS PATIENTS & ITS CORRELATION WITH CD4 COUNT"

Submitted By

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in

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Under the guidance of

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LIST OF ABBREVIATIONS

ADCC - Antibody dependent cell cytotoxicity

AIDS - Acquired Immunodeficiency Syndrome

BD Calibur - Beckton Dickinson calibur

C. difficile - *Closridium Difficile*

CD4 lymphocytes - Cluster of differentiation

CMV - Cytomegalovirus

CNS -Central nervous system

DdC - Zalcitabine

Dd I - Didanosine

DNA - Deoxyribonucleic Acid

E.coli - Escherichia coli

EDTA - Ethylene Diamine Tetra Acetic Acid

ELISA - Enzyme linked Immunosorbent Assay

FITC - Fluorescein Isothiocyanate

Gp - Glycoprotein

HAART - Highly Active Antiretroviral Therapy

HEA - Hextoen Enteric Agar

HIV - Human Immunodeficiency Virus

HRP - Horseradish peroxidase

HTLV - Human T- lymphocytotrophic virus

ICMR - Indian Council of Medical Research

Ig - Immunoglobulins

LAV - Lymphadenopathy Associated virus

LTNP's - long term non progressors

MAC	- Mycobacterium Avium Complex
MTCT	- Mother to child transmission
NACO	- National Aids Control Organisation
NK Cells	- Natural Killer cells
p Value	- Probability value
p24	- Capsid protein
PerCP	- Peridinin Chlorophyll Protein
RBC	- Red Blood Cell
RNA	- Ribonucleic Acid
STD	- Sexually Transmitted Disease
WHO	- World health organization
XLD	- Xylose Lysine Desoxycholate

ABSTRACT

BACKGROUND:

The number of HIV-positive patients is increasing in India. Data on the prevalence of diarrhea and the spectrum of bacteria responsible for diarrhea in HIV-positive patients is lacking in our area. The identification of enteric pathogens in patients with HIV/AIDS is important because an increasing array of therapeutic regimens is becoming available to treat many of these infections. Thus an attempt is done to elucidate the associations between causative bacteria of acute and chronic diarrhea and CD4 count.

METHODS:

Stool specimens were obtained between October 2011 and April 2013 from HIV infected adults with diarrhea presenting to Shri B M Patil Medical College, Bijapur. In all patients with diarrhea, stool specimens were examined by microscopy and cultures to identify bacterial pathogens and blood sample was analysed for CD4 count.

RESULTS:

A total of 80 individuals were enrolled in this study. Cases included 46 males and 34 females. Among the cases, maximum subjects were found to be in the age group of 30-40 years in which 23(50%) were males and 14 (41.18%) were females. 56 had acute and 24 had chronic diarrhea. The percentages of bacteria isolated were 5(23.81%) in acute and 16(76.19%) in chronic diarrhea respectively. The most common bacteria isolated was *E.Coli* (17.5%) followed by *Klebsiella* (5%) and *Shigella Sps* (3.75%). Patients with chronic diarrhea had lower CD4 cell counts. The

maximum bacterial isolation was in the patients whose CD4 cell counts were below 200 cells/mm^3.

CONCLUSION:

Bacterial isolation was most strongly associated with low CD4 counts and chronic diarrhea. *E.Coli* was isolated maximum among all the bacteria in the HIV patients. Over two-thirds of diarrheal episodes were undiagnosed, suggesting that unidentified agents or primary HIV enteropathy are important causes of diarrhea in this population. There is a strong negative association between duration of diarrhea and CD4 levels.

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INTRODUCTION

Human immuno deficiency virus / Acquired immuno deficiency syndrome (HIV/AIDS) is a major problem in India. HIV patients are prone to develop numerous variety of opportunistic diseases during their lifetime. Among them diarrhea is a significant cause of morbidity observed in majority of studies. In fact diarrhea is the second leading cause of hospitalisation in developing nations and makes its place in top 10 worldwide. The information on the cause of diarrhea and the possibility of isolation of pathogens has largely come from various cross sectional studies. Expectedly infectious etiologies lead the list in developing nations in contrast to noninfectious etiologies in developed nations. There are many reports regarding frequency of various pathogens causing diarrhea from different parts of India. Some studies also demonstrated regional variability of pathogen, as well as changing trends of etiology in the same population[1].

Diarrheal diseases in AIDS are most often due to infectious pathogens and include various parasitic, bacterial and viral organisms. The degree of immune-suppression, as defined by the CD4+ T-cell count, determines to a large extent when individuals with HIV infection will develop opportunistic infections. The incidence and outcome of many of these complications, however, can be altered by preventive measures, in particular primary and secondary prophylaxis. [2]

Infectious diarrhea is a major cause of morbidity and mortality in patients with HIV infection and AIDS. Most patients with AIDS who develop diarrhea have some degree of malabsorption. Patients with AIDS and diarrhea also have lower numbers of CD4+ cells and a higher incidence of extra-intestinal opportunistic

infections than those without diarrhea, suggesting that patients with AIDS who develop diarrhea, have a higher degree of immunosuppression[3].

Patients with chronic diarrhea have lower CD4 counts than those who have acute diarrhea. Bacteria that are more common in HIV-infected patients include *Salmonella spp., Shigella, Campylobacter jejuni, Escherichia coli (enterotoxigenic, enteroadherent, and enteroaggregative), and Listeria monocytogenes. Clostridium difficile* toxin-associated diarrhea may also be more common among individuals with HIV disease, particularly among hospitalized patients and those who have recently received antibiotic therapy. Other, less common, bacterial causes of enterocolitis include *Aeromonas, Plesiomonas, Yersinia, and Vibrio spp.* Mycobacterial infections of the small bowel are usually associated with late-stage HIV disease and disseminated *M. avium complex*, although enteritis caused by *M.tuberculosis* has been reported[4].

The number of HIV-positive patients is increasing in India. Data on the prevalence of diarrhea and the spectrum of bacteria responsible for diarrhea in HIV-positive patients is lacking in our area. The identification of enteric pathogens in patients with HIV/AIDS is important because an increasing array of therapeutic regimens is becoming available to treat many of these infections. Thus an attempt is done to elucidate the associations between causative bacteria of acute and chronic diarrhea and CD4 count.

OBJECTIVES OF STUDY

1. To identify bacteria causing diarrhea in HIV seropositive patients.
2. To correlate CD4 count of the patients with the causative bacteria.

REVIEW OF LITERATURE

Ever since its recognition in 1981 Human Immunodeficiency Virus infection / Acquired Immuno Deficiency Syndrome (HIV/AIDS) continued to ravage all the continents of the world.[5] Infection with HIV may develop to AIDS at different rates in different individual with a spectrum varying from rapid progression to long term non progression.[6] Once infected the person will be affected for life. AIDS can be called as modern pandemic affecting both industrialized and developing countries.[7]

HISTORICAL REVIEW :

I) HISTORICAL ASPECTS IN THE WORLD :

In 1981 : AIDS was officially recognized for the first time at Centre for Disease Control, Los Angels, USA, in the previously healthy homosexual men dying suddenly with pneumocystic carinii pneumonia and candidiasis and also in a patient with Kaposi's sarcoma[8].

In 1983 : The virus causing AIDS was identified by a team of French scientists led by Dr. Luc Montagnier of Pasteur Institute as "Lymphadenopathy Associated virus" (LAV)[9].

In 1984 : A team of American Scientists led by Dr Robert C. Gallo of National Cancer Institute also identified a virus causing AIDS and named as human T-lymphocytotrophic virus (HTLV-III)[10].

Also Enzyme linked Immunosorbent Assay (ELISA) to detect the presence of antibodies in blood against HIV was developed[11].

The CD4 molecule was identified as the major receptor for HIV[12] during this year.

- HIV was isolated from semen[13] and central nervous system[14]. It became apparent that the virus is lymphocytotrophic and also has neurotropism.

In 1986 : The international Committee on nomenclature of viruses has given the common name for both LAV and HTLV-III as, Human Immunodeficiency virus (HIV)[15].

- The French Scientists, Prof. Montagnier and his co-workers discovered a new type of HIV in West Africa and labelled it as HIV-2[16].

In 1987 : Zidovudine was reported to be useful in managing the patient with HIV infection for the first time[17].

In 1989 : A new antiretroviral drug Didanosine (ddI) was introduced in America.

1n 1992 : First successful use of combination therapy with 2 drugs, i.e, Zidovudine and Zalcitabine (ddC).

In 1994 : Result of the famous protocol of AIDS clinical trail Group-076 (PACTG-076) reported 67% reduction in mother – to –child transmission (MTCT) of HIV-1[18].

In 1996 : Spectacular advances were made in the field of chemokine receptors which are now considered important in the entry of HIV in the CD4 lymphocytes[19].

- International AIDS conference in Vancouver reported on the success of triple drug therapy against dual therapy in the suppression of HIV and named it as Highly Active Antiretroviral Therapy (HAART)[20,21].

In 1997 : HAART is introduced for the first time to the symptomatic AIDS patients.

In 1998 : The International AIDS society, USA panel recommended early therapy with triple drug combination (HAART) in HIV infected patients with HIT EARLY and HIT it HARD[22,23].

15-03-2003 : FDA approved FUZEON (enfuviritide) also known as T-20, for use in combination with other anti HIV medications to treat advanced HIV-1 infections in adults and children aged 6 years and older.[24]

01-12-2003 : World health organization (WHO) and UNAIDS (united nations joint programme on HIV/AIDS) announced a detailed plan to reach the " 3 by 5 target" of providing Anti retroviral treatment (ART) to 3 million people living with HIV/AIDS in the developing countries by the end of 2005[7].

28-09-2005 : FDA approved EMTRIVA (emtricitabine) oral solution 10 mg/ml. The approval of this oral solution formulation allows for dosing recommendation in pediatric patients.[24]

06-08-2007 : FDA approved Selzentry (Maraviroc) 150 mg and 300 mg tablets, the first drug in the new class of anti HIV medications called CCR-5 corecoptor antagonist under priority review.[24]

II) HISTORICAL ASPECTS IN INDIA :

Late 1985 : Surveillance for HIV infection was initiated in India by the Indian Council of Medical Research (ICMR) as a part of AIDS task force.

1986 : Anti HIV antibodies were first detected among the sex workers from Madras in South India.[25] First AIDS case in India was reported from Bombay, in 1986[26].

1987 : National AIDS control programme was launched.

July 1989 : Realizing the high prevalence rates amongst the professional blood donors, HIV screening of all the blood units to be screened for transfusion purpose was made mandatory in 4 metropolitan cities of India.

1991 : Presence of HIV-2 infection in India was reported for the first time from Bombay.[27]

1992 : ICMR established the National AIDS Research Institute in Pune.

- National AIDS control organization was set up by Ministry of Health and family welfare.

2001 : Prevention of HIV transmission from mother to child using single dose Nevirapine.[28]

EPIDEMIOLOGY

GLOBAL SUMMARY OF THE AIDS EPIDEMIC: 2012

GLOBAL HIV /AIDS SUMMARY[29] :

- Global percentage of adult living with HIV has levelled off since 2000.
- In 2011, 34 million people were living with HIV and 1.7 million HIV related death.
- The rate of new HIV infection has fallen in several countries, but globally these favourable trends are at least partially offset by an increase in new infections in other countries.
- Sub Saharan Africa remains the region most heavily affected by HIV, accounting for 69% of all people living with HIV.
- Women account for half the people living with HIV worldwide.
- Young people aged 15-24years account for an estimated 0.8% HIV infection worldwide.

No. of people living with HIV	34 million (31.4 – 35.9 million)
Newly infected with HIV	2.5 million (2.2 to 2.8 million)
AIDS death	1.7 million (1.5-1.9 million)

INDIA – HIV/AIDS SUMMARY 2012 [30] :

- 23.9 lakh people were living with HIV in India in 2008-2009.
- Adult national HIV prevalence was 0.31%.
- India's epidemic is highly varied across states and regions, and diverse trends are evident in different parts of this huge country.
- Reported adult HIV prevalence in six states included in the recent national population-based survey varied from 1.40% in Manipur, 0.90% in Andhra Pradesh, 0.81% in Mizoram,0.78% in Nagaland, 0.63% in Karnataka, 0.55% in Maharashtra.

EPIDEMIOLOGICAL FEATURE :

1. Agent factors :

a. Agent : HIV, which causes AIDS is a lenti virus, one of sub family of retroviruses. Measures 90-120nm in diameter, i.e., 1/10,000 of millimeter^3. They have unique Enzyme- "reverse transcriptase" which is eventually inserted into the host cell chromosome. Hence HIV persist within cell for years.[31]

Fortunately, HIV is very fragile virus, it is susceptible to heat, a temperature of 56°C for 30 minutes (or) boiling for a few seconds kills the virus. Most of the

chemical germicides used in hospitals, laboratories and health care settings kill HIV at much lower concentration.[31]

Thus 0.5% -1% Sodium Hypochlorite, 70%-Ethanol, 2%-Glutaraldehyde, Acetone, Ether, Beta propiolactone (1: 400 dilutions) and Sodium Hydroxide (40 m mol/Litre) inactivates the virus.[31] The virus is relatively resistant to ionizing radiation and ultraviolet light.[7]

b. Reservoir of Infections : These are cases and carriers. Once a person is infected with HIV the virus remains in the body life long.[7]

c. Source of Infection : The virus has been found in high concentrations in blood, semen and CSF. Lower concentration have been detected in breast milk, cervical and vaginal secretions, saliva, tears, urine, faeces and sweat. HIV has also been isolated in brain tissue, lymph nodes, bone marrow cells and skin.[7]

2) Host Factors

a. Age/Sex : AIDS is affecting mainly the young people in sexually active and economically productive age group. Majority AIDS cases (89%) are in the age group 15-44 years. Males account for 78.77% of AIDS cases and Females 21.23%.[31]

b. High Risk Group : Male homosexuals and bisexuals, heterosexual partners (including prostitutes), intravenous drug abusers, transfusion recipients of blood and blood products, hemophiliacs and clients of STD.[7]

HUMAN IMMUNODEFICIENCY VIRUS :

Acquired immunodeficiency syndrome represents the late clinical stage of infection with Human Immunodeficiency Virus (HIV). HIV is a retrovirus belonging to subfamily of lentivirus. It contains a genome comprising of two single stranded RNA molecules. HIV gets incorporated into the chromosomal DNA of host cell by using reverse transcriptase enzyme to produce a double stranded proviral DNA. Majority of proviral particles may go into latency for a variable period of time. Upon activation it reproduces RNA transcripts and proteins which are used to synthesize new virions.[32]

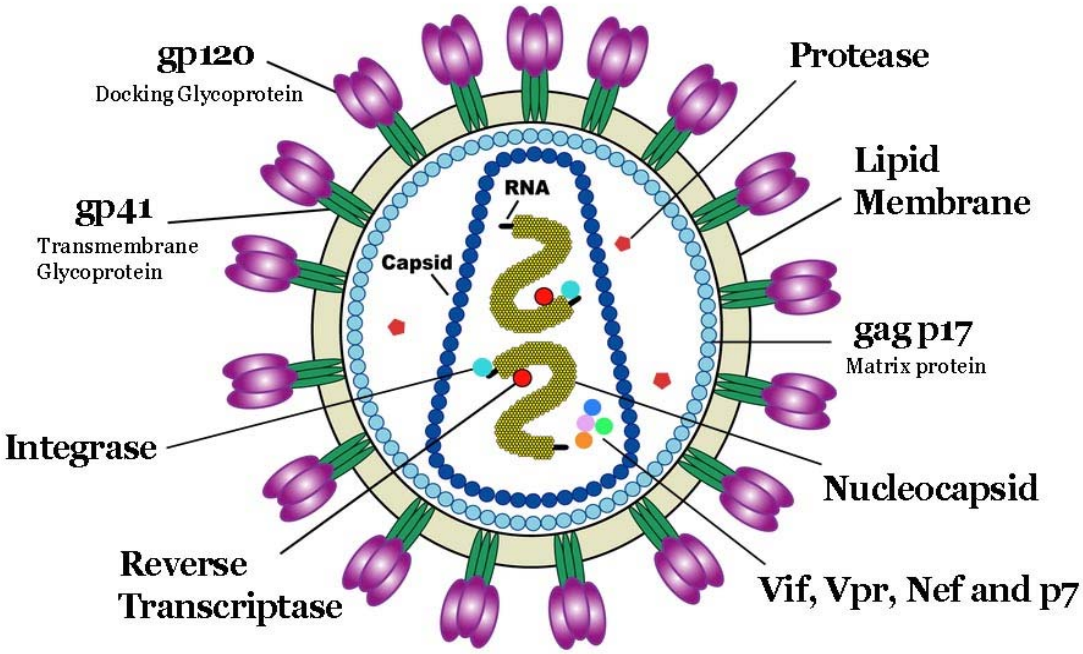


Fig. 1 HUMAN IMMUNODEFICIENCY VIRUS

ORIGIN :

There are 2 types of HIV, HIV1 and HIV2. Both have originated from Simian Immunodeficiency Virus probably from ones found in chimpanzees and in sooty mangabay monkeys.[26] Many theories[33] were proposed to explain maiden entry of HIV into human being. They are

1. Simultaneous injury to monkey and human being.
2. Use of monkey's blood as aphrodisiac,
3. Mutation due to pollution,
4. Development of biological warfare,
5. It might have spread through polio vaccine which was made from monkey kidney cells.

But none of these appears to be correct. Virologist date the first human infection by a lentivirus closely resembling, if not identical to HIV to 1959, most probably with exposure to blood of primates infected with a nearly identical strain of Simian Immunodeficiency Virus.[3]

PATTERN OF SPREAD :

India's epidemic seems to be following the so called type-4 pattern, first described in Thailand. The epidemic shifts from highest risk group (commercial sex workers, drug users) to bridge population (clients of sex workers, STD patients partners of drug users) and then to general population. There is a time delay of 2-3 years between shift from one group to the next. The shift usually occurs where the prevalence in the first groups reaches 5%.[32]

MODES OF HIV TRANSMISSION :

Efficiency of different routes of HIV transmission and their contribution to total number [31]

EXPOSURE ROUTE	PERCENT EFFICIENCY (WORLD OVER)	PERCENTAGE OF TOTAL	
		(WORLD OVER)	(INDIA)
Blood transfusion	90-95	5	7.05
Perinatal	20-40	10	
Sexual intercourse	0.1 to 1	75	74.15(heterosexual) 0.58 (homosexual)
Injecting drugs use	0.5-1.0	10	7.3
Needle stick exposure	<0.5	0.1	0

1. Sexual transmission[32] :

Most common route of spreading of HIV in India.

- Female to male, unprotected vaginal sex -0.03 to 0.1%
- Male to female, unprotected vaginal sex -0.1 to 0.2%
- Male to male, unprotected anal sex -0.5 to 3%

Risk factors for sexual transmission :

- Male to female transmission is 2-4 times more efficient than female to male transmission[27].
- Factors associated with increased risk of HIV transmission[35] :
  - Ulcerative and non ulcerative sexually transmitted disease
  - Lack of circumcision

- In some studies, oral contraceptive pills, alcohol consumption and illicit drug use are associated with unsafe sexual behavior, leading to an increased risk of HIV infection
- Anal intercourse because mucosa is thin, unlike vaginal mucosa, and is prone for injuries.
- Oral sex is a much less efficient mode of transmission of HIV than is receptive anal intercourse.

2. Transmission by blood and blood products[35] :

- The most efficient vehicle of HIV transmission is blood (90-100%)
- Blood and blood products which can transmit HIV virus are :
  - Transfusion of whole blood, Packed red blood cells, leukocytes, Platelets, fresh frozen plasma, concentrates of clotting factors, Transplanted tissue.
- The products which have not been associated with transmission of HIV infection are : Hyperimmune gammaglobulin, Hepatitis B immunoglobulin, Plasma derived hepatitis- B vaccine.

3. Occupational transmission of HIV[35] :

There is a small but definite occupational risk of HIV transmission in health care workers. The risk of transmission of HIV infection following skin puncture from a needle or sharp object is 0.3% and for mucous membrane it is 0.11%. The risk of transmission from an infected health care worker is extremely low.

Factors contributing to the risk for occupational HIV infection[36]:

a. Exposure factors :

- Route of exposure : percutaneous, mucous membrane, cutaneous.
- Inoculum size : Size of the device producing injury, Type of needle (hollow bore or solid), Extent of contamination – visible blood on device, placement of device (in artery/vein), Type of contamination –blood, pleural fluid etc.

b. Source /Donor factors :

Extent of viraemia, Stage of illness, Circulating free virus, Antiretroviral therapy.

4) Maternal - Fetal/Infant Transmission[35] :

- The HIV can be transmitted to the fetus as early as the first and second trimester of pregnancy. Almost 30-50% of neonate acquire HIV infection in infantile period. But 50-70% of transmission occurs late in the gestation or during labour or after delivery.
- The probability of transmission ranges from 15-25% in industrialized countries to 25-35% in developing countries.

Factors which increase the risk of transmission[35] :

Maternal: Advanced maternal HIV disease, P24, CD4+ T cell counts, High plasma HIV-1 RNA levels, Acute HIV-1 infection during pregnancy, Genital STDs and inflammation at the time of delivery, Vitamin A deficiency, Smoking and Drug Abuse during pregnancy.

Labour and delivery factors :

- Chorioamnionitis, Prolonged rupture of membranes (≥4 hours), Premature delivery before 34 weeks gestation, Obstetric procedures - Amniocentesis, Amnioscopy, Episiotomy with severe lacerations, First born twin is more commonly infected, Caesarean section results in decreased transmission to the newborn.

Breast feeding : Account for 5-15% of infants becoming infected after delivery.

Risk factors are : Detectable levels of HIV in breast milk, Presence of mastitis, Low CD4+ T cell counts in mother, Maternal vitamin A deficiency.

5) Transmission by other body fluids[35] :

- Although HIV virus can be identified, if not isolated, from virtually any body fluid, there is no evidence that HIV transmission can occur as a result of exposure to tears, sweat and urine.
- So far there has been no report of HIV transmission through a casual social contact, intact skin, enteric or respiratory route or insect bite.

BIOLOGY OF HIV

THE VIRION[37] :

The HIV-1 virion particle forms a icosahedral sphere with 72 projections consisting of the envelope glycoproteins 120 and 41. Only gp 41 traverses the lipid bilayer. Gp 120 is loosely and noncovalently bound to gp 41.Under the lipid layer, the matrix protein (p17) covers the internal surface of the viral coat. The capsid protein (p24) constitutes the internal core shell, whereas p6 and p7 form part of a

nucleoid structure. The p7 protein and reverse transcriptase (RT) molecule are associated with two copies of the single stranded genomic HIV RNA.

THE GENOME :

HIV has genes that encode the structural and regulatory proteins.

Structural Genes[37,38] :

Gag – encodes proteins that form the core of virion. P17- Matrix protein,

P24-capsid protein, P15-Nucleocapsid precursor processed to P2, P7, P1 and P6,

Pol – Encodes the enzymes, RT – Reverse transcriptase, PR –Protease,

IN -Integrase

ENV - Encodes the envelop glycoproteins gp 120 and gp 41.

Regulatory genes[37,38] :

Tat : Viral transactivator for transcription. More efficient synthesis of full length transcripts.

Rev : Transactivator of structural gene expression.

Vif : Enhances efficiency of viral infection, enhances viral assembly through packaging of nucleoprotein.

Vpu: Down modulation of CD4, efficient maturation and release.

Vpr : Assists the transport of the reintegration complex from the cytoplasm into the nucleus.

Nef protein : Down regulates cell surface expression of CD4+ by inducing endocytosis and lysosomal degradation. Induces and activates resting T lymphocyte leading to productive HIV infection.

REPLICATION CYCLE [31,35,37] :

1. Entry of HIV-I : High affinity binding of gp 120 to CD4 molecule on cell surface. Entry requires binding to one of a gp of co-receptors CCR5 and CXCR4. Viral genomic RNA is uncoated and internalized into target cell.
2. Reverse transcription : The reverse transcription enzyme transcribes DNA copies of the viral RNA.
3. Integration : The pre integration complex (consisting of Integrate, ds DNA, P17, RT) is transported into the nucleus of the cell.

   Viral ds DNA integrated into host DNA. This is called Provirus.
4. Virus gene expression : The provirus is transcribed into RNA copies which are either incorporated into new virus particles as genome or function as mRNA and translated into core, envelope or accessory protein
5. Packaging and assembly : The virus particles are formed, by the assembly of HIV proteins, enzymes and genomic RNA, at the plasma membrane of the cell.
6. Budding : Budding of progeny virus occurs through host cell membrane, where the core acquires its envelope.
7. Maturation : The viral encoded protease cleaves viral polyproteins to yield the mature virion which infects other cells.

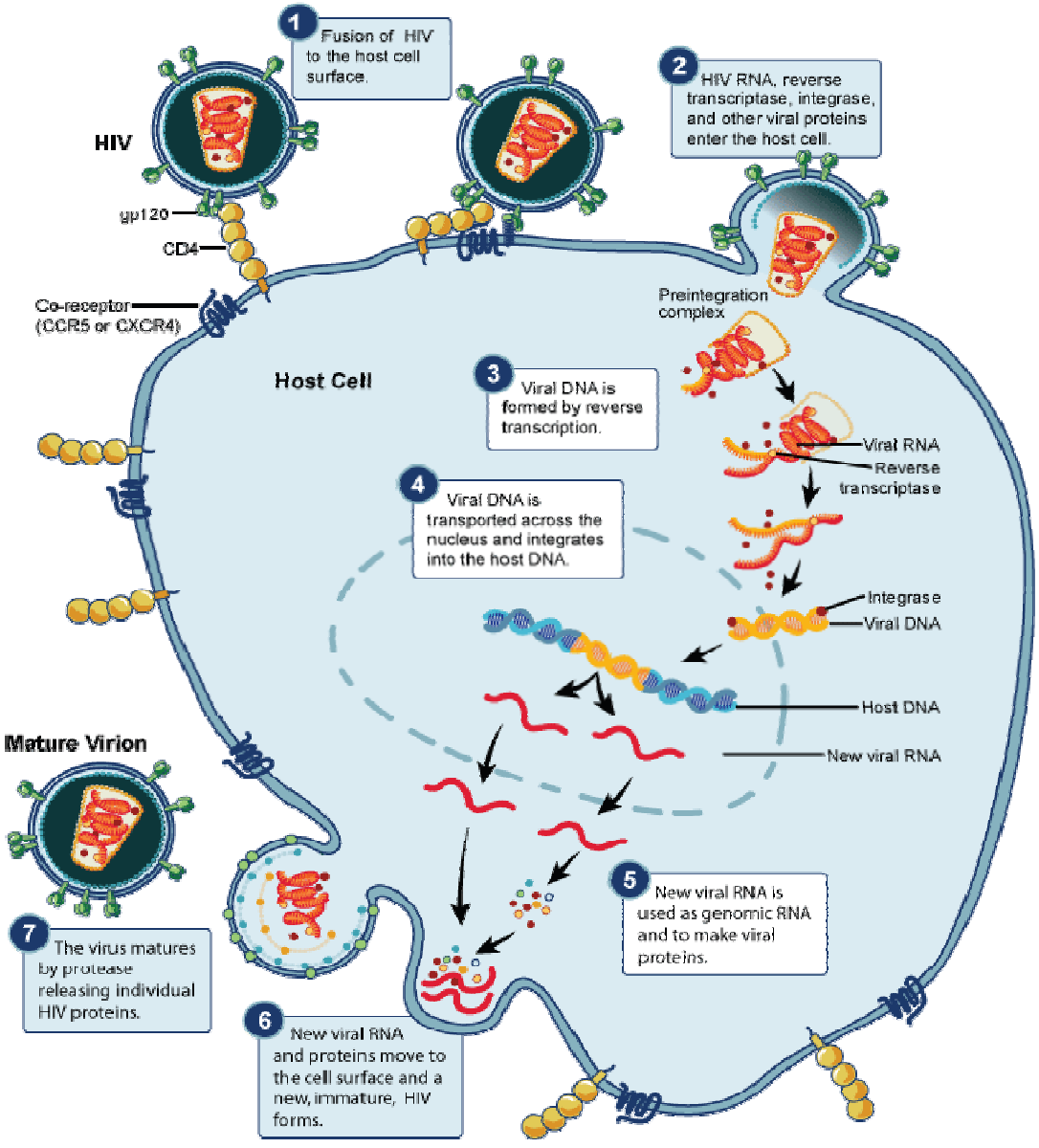


Fig.2 : HIV REPLICATION

TYPES OF HIV[32,35] : There are two types :

1) HIV – 1 : There are 3 groups :

Groups M – Major, Groups O – Outlier, Groups N – New

Subtypes C viruses are the most common type world wide.

2) HIV – 2 : There are six sub types A-F. Sub type A is most prevalent

HIV-2 infection has milder and slower progression of the disease with longer incubation period and is poorly transmitted vertically.

HUMAN CELLS / CELL LINES AND TISSUES SUSCEPTIBLE TO HIV [31] :

HIV practically multiplies in all cells but the extent of replication varies in different cells. The following cell lines are affected.

Haematopoietic system : T lymphocytes, B lymphocytes, macrophages, NK cells, Megakaryocytes, Dendritic cells, Promyelocytes, Stem cells, Thymic epithelial cells, Follicular dendritic cells.

Brain: Capillary endothelial cells, Astrocytes, Macrophages (microglia), Oligodendrocytes. Choroid plexus, Ganglia, Neuroblastoma cells, Glioma cell lines and Neurons.

Skin : Fibroblasts and Langerhans cells.

Others : Myocardium, Renal tubular cells, Synovial membrane, Hepatic sinusoid epithelium, Hepatic carcinoma cells, Kupffer cells, Pulmonary fibroblasts, Foetal adrenal cells, Adrenal carcinoma cells, Retina, Cervix epithelium, Prostate, Testes,

osteosarcoma cells, Rhabdomyosarcoma cells, Foetal chorionic villi, Placental trophoblast cells.

Mechanism of cell death :

1. Increase in cell permeability due to budding of virus. Virus punches holes and kills the cell.
2. Increase in cell permeability due to toxic effects of virus replication.
3. Syncytia formation-involving uninfected cells.
4. Apoptotic cell death of activated T cells.
5. Auto-immune phenomenon involving CD4 molecule.
6. ADCC i.e., antibody dependent cell cytotoxicity.

IMMUNOLOGICAL ABNORMALITIES IN HIV INFECTION [39] :

1. Lymphopenia
2. Selective T-cell deficiency, reduction in number of T4 cells. Inversion of T4 : T8 ratio.
3. Decreased delayed hypersensitivity on skin testing.
4. Hypergama globulinemia-predominantly IgG, IgA and IgM in children.
5. Polyclonal activation of B-cells and increased spontaneous secretion of Immunoglobulins.
6. Decreased invitro lymphocyte proliferative response to mitogens and antigens.
7. Decreased cytotoxic responses by T-cell and Natural Killer cells (NK cells).
8. Decreased antibody response to new antigens.
9. Altered monocyte/macrophage function.
10. Elevated levels of immune complexes in serum.

THE COURSE OF HIV INFECTION [31] :

Three dominant patterns of HIV disease progression have been described. These are based on the kinetics of immunologic and virologic events described above.

- 80%-90% of HIV infected are "typical progressors" with a median survival time of 10 years, approximately.
- 5% to 10% of HIV infected individuals are "rapid progressors" with a median survival time of 3-4 years approximately.
- About 5% of HIV infected individuals do not experience disease progression for an extended period of time and are called "long term non progressors" (LTNPs).

NATURAL HISTORY OF HIV [40] :

The course of the disease from the time of initial infection to the development of full blown AIDS is divided into FIVE STAGES by WORLD HEALTH ORGANISATION :

1. Primary HIV infection.
2. Asymptomatic chronic infection
3. Symptomatic HIV infection (Previously Known as AIDS related complex).
4. AIDS (or) Late stage of HIV diseases.
5. Advanced HIV disease characterized by CD4 cell count below 50/mm.

1.Primary HIV infection/Acute Retroviral Syndrome: (Sero conversion)

It is experienced by 80-90% of HIV infected patients. It takes 2 to 4 weeks for the onset of symptoms. Clinically characterized by fever, pharyngitis, lymphadenopathy, erythematous maculopapular rash, arthralgia, myalgia, nausea, vomiting, diarrhea, mucocutaneous ulcerations involving mouth,

oesophagus and genitals, hepatosplenomegaly, thrush, neurological features include meningoencephalitis, peripheral neuropathy, facial palsy, Guillain-Barre syndrome and psychosis.

Laboratory findings show lymphopenia followed by lymphocytosis with depletion of CD4 cells, Demonstration of HIV RNA, negative HIV serology.

2. Asymptomatic infection with or without Persistent Generalised Lymphadenopathy (CD4 Count>500/mm^3)

Patient is clinically asymptomatic at this state. Incidental finding could be scars from genital ulcer disease or herpes zoster, lymphadenopathy or asymptomatic dermatological manifestations.

Laboratory data shows leucopenia, thrombocytopenia, polyclonal gammopathy and altered serum transaminase levels. The CD4 count continues to decline progressively without Anti Retroviral therapy.

3. Symptomatic HIV infection (CD4 count between 200-499/mm^3)

During the symptomatic HIV infection, the skin and mucous membranes are predominately involved. Wide spread seborrhoeic dermatitis is the most common presentation. Other features include multidermatomal herpes zoster, molloscum contagiosum, Oral Hairy Leukoplakia, Pruritic dermatitis, folliculitis, recurrent vulvovaginal candidiasis and oral candidiasis. Upper and lower respiratory tract infections may also occur.

4. Late Stage of HIV Disease or AIDS (CD4 count 50-200/mm^3)

This stage is characterized by opportunistic infections and malignancies. Other features are persistent and progressive constitutional symptoms, wasting disease and neurological disease.

5. Advanced HIV Disease (CD4 count below 50/mm^3)

It is characterized by AIDS defining opportunistic infections and malignancies. Some of them are more frequently seen like Mycobacterium Avium Complex (MAC) infection, Cytomegalo Virus (CMV) infection, cryptococcal meningitis, and histoplasmosis. CNS involvement is also very predominant like AIDS dementia complex. CNS lymphoma. AIDS wasting syndrome with a weight loss of > 10 % of ideal body weight, in less than 4 weeks is common.

DIAGNOSIS OF AIDS :

Expanded WHO case definition for AIDS surveillance[40] :

For the purpose of AIDS surveillance an adult or adolescent (> 12yrs of age) is considered to have AIDS, if a test for HIV antibody gives a positive result and 1 more of the following condition are present.

1. Unexplained body weight loss or cachexia with diarrhea or both, intermittent or constant for least 1 month not known to be due to a condition unrelated to HIV.
2. Cryptococcal meningitis.
3. Pulmonary or extra pulmonary tuberculosis.
4. Kaposi's sarcoma
5. Neurological impairment i.e. sufficient to prevent independent daily activities, not known to be due to a condition unrelated to HIV infection (eg. Trauma).
6. Oesophageal candidiasis.

7. Clinically diagnosed life threatening or recurrent episodes of pneumonia with or without etiological confirmation.
8. Invasive cervical cancer.

CD4 COUNT :

A number of non HIV specific cellular markers have been used for staging, monitoring progression of HIV infection and assessing response to therapy but the most commonly used cellular marker is the CD4 lymphocyte count.[6]

CD4 is one of the several glycoproteins termed cluster of differentiation antigens, expressed on the surface of the lymphocytes.[2] The normal range of CD4 cell values vary between laboratories but they are around 500 – 1300 /mm^3 for the absolute count and 38 – 65% for the percentage.

CD4 can be measured by Flow cytometry, Microsphere assay and Enzyme immunoassay. CD4 serves as a receptor for HIV, and cells expressing this protein usually decline in number with progressive HIV infection.

The number of cells that express CD4 antigen is therefore a useful guide to pathological effects of HIV on immune system. Its decline is the hallmark of HIV infection and the rate of loss in each person is unique.[6]

Mechanism of CD4 cell depletion and dysfunction in HIV infection[41,43] .

HIV can kill CD4 cells singly or after giant cell and syncitial formation. Single cell killing occurs due to accumulation of unintegrated viral DNA and inhibition of cellular protein synthesis. Syncitium formation is induced by virulent strains of HIV in a multistep mechanism. CD4 cells are destroyed by the viral lytic

process. The host immune response against HIV infected target cells by cytotoxic T lymphocytes or by antibody dependent cell mediated cytolysis could also lyse the infected T-helper cells. The HIV infected cells shed gp 120 molecules into circulation, which are then bound to CD4 + normal T helper cells. These helper cells though uninfected are now coated with gp120 and become perfect targets for immunoreactor T helper cells. This over enthusiastic cell mediated immune response is the most important cause for immune suppression.[41] The non-virologic mechanisms which can damage /destroy CD4 cell include autoimmune mechanisms, anergy, superantigens, apoptosis (programmed cell death) and virus specific immune responses.[4]

The Center for Disease Control (CDC) has proposed the following clinical classification for HIV infection in adults and adolescents. It is based on three ranges of CD4 cell count with three clinical categories.[42]

1993 revised classification for HIV infection and expanded case definition for AIDS in adolescents and adults.[42]

CD_4 cell count	A	B	C
> 500/mm^3 (>29%)	A1	B1	C1
200 to 499/mm^3 (14% to 28%)	A2	B2	C2
<200/mm^3 (<14%)	A3	B3	C3

Category A

- Asymptomatic HIV infection
- Persistent generalized lymphadenopathy
- Acute retroviral syndrome

Category B

- Bacillary angiomatosis
- Oral or recurrent vulvovaginal candidiasis
- Cervical dysplasia
- Constitutional symptoms (fever of 38.5^0C, diarrhea >1 month)
- Oral hairy leukoplakia
- Herpes zoster
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease
- Peripheral neuropathy

Category C (AIDS-defining conditions)

- CD4 count < 200 cells/mm^3
- Persistent oesophageal candidiasis
- Cervical cancer
- Coccidioidomycosis
- Cryptococcosis, Extrapulmonary cryptococcosis
- Cryptosporidiosis
- Cytomegalovirus infection
- Herpes simplex with esophageal, pulmonary, or mucocutaneous involvement of >1 month
- Histoplasmosis
- Isosporiasis

- Kaposi's sarcoma
- Lymphoma
- Mycobacterium Avium complex or M. Kansasii
- Mycobacterium tuberculosis
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent with more than two episodes in 12 months
- Progressive multifocal encephalopathy
- Salmonellosis
- Toxoplasmosis

DIARRHEA OVERVIEW [44,45] :

Diarrhea is defined by the World Health Organization (WHO) as three or more watery or loose bowel movements in a 24 hour period (2009).

Classification of diarrhea

(A) Depending on duration

(a) Acute diarrhea.

Diarrhea can be classified by several methods with duration of the symptom being foremost. Diarrhea lasting less than 2 weeks is considered acute. This phenomenon is most likely caused by an infectious agent, such as bacterial, parasitic or viral invasion, or by a non-infectious agent such as dietary indiscretion or a new medication. Acute diarrhea is typically self limiting and resolves quickly with no lasting sequelae. Infectious agents are one of the factors associated with acute diarrhea. Some of these pathogens can cause an inflammatory response in the gut where the epithelial lining is damaged either by a toxin produced by the organism or by an organism invading the mucosa.

Some organisms that cause an inflammatory response are "*Cytomegalovirus, Herpes simplex virus, Shigella, Salmonella, Chlamydia, Nisseria gonorrheae, Campylobacter jejuni, Clostridium difficile, Escherichia coli O157:H5, Entamoeba histolytica*". Symptoms of acute inflammatory diarrhea include fever (higher than 38.5C), lethargy, and a stool that contains pus, blood, leukocytes and/or mucus. There are organisms that cause acute diarrhea that do not produce an inflammatory response although the person may have a low grade fever, malaise, nausea and vomiting as well as diarrhea. These causative organisms include "*Norwalk virus, Rotavirus, Staphylococcus aureus, Clostridum perfringens, Vibrio cholerae and enterotoxigenic Escherichia coli*" Less commonly, protozoa such *Giardia or Cryptosporidium* may be the causative factor. The elderly are more apt to have a longer illness and have the potential for poorer outcomes than other age groups.

(b) Persistent Diarrhea.

Diarrhea lasting longer than two weeks but resolving within a month is known as persistent diarrhea. This is typically a slower to resolve infection or continuing use of an offending agent .

(c) Chronic diarrhea.

Chronic diarrhea, on the other hand, lasts longer than four weeks. Chronic diarrhea can be the result of disease processes, medication, genetic abnormalities, or a variety of other causes

(B) Depending on pathology

Approximately 10 liters of fluid can move through the small intestine in a 24 hour period from food, fluids, and secretion of various enzymes and fluids necessary for digestion. The small and large intestines have the ability to reabsorb

that and even more fluid when functioning normally. This normal gut physiology relies on a functioning enteric nervous system that coordinates the gut ion transport and motor activity. When any of these pathways are disrupted, diarrhea can result. These disruptions results in osmotic, secretory, motility or mixed diarrhea.

1. Secretory Diarrhea.

Secretory diarrhea occurs when there is an increase in the amount of fluid being drawn into the lumen of the bowel such that the ability of the intestines to reabsorb is overwhelmed. Typically, infectious agents are the cause of secretory diarrhea but any substance (secretagogue) that causes fluid to be pulled into the bowel can be the culprit. Infectious secretagogues include *Vibrio cholerae, E. coli, Camylobacter jejuni, Salmonella, Shigella, and Clostridium difficile*. These pathogens secrete toxins that bind with the structures within the gut, altering, sometimes irreversibly, the amount of fluid secreted into the bowel. As an example, the toxin excreted by the pathogen cholera causes massive secretory diarrhea which, during its acute phase, can be as much as 24 liters in 24 hours. Non-infectious secretagogues include chemicals produced by certain types of cancer, prostaglandins produced in patients with bowel inflammation and substances not well absorbed such as fatty acids and bile acid. Persons with secretory diarrhea will typically have stool volume of more than one liter daily, with neutral pH and have no change in the amount of stool produced with fasting.

2. Osmotic Diarrhea.

Osmotic diarrhea occurs when there is a dysfunction in the ability of the intestine to reabsorb fluid as it flows through the lumen. This may be caused by incomplete breakdown or malabsorption of nutrients in the small intestine allowing a larger and more liquid mass to enter the colon. This fecal matter then creates a negative osmotic gradient causing leakage of more fluid into the gut increasing the stool volume.The causes of this type of osmotic diarrhea are varied but can be broken down into decreased enzymatic availability (lactose intolerance), a genetic abnormality that decreases or eliminates the ability of the body to absorb certain nutrients (celiac sprue), sugars that are poorly absorbed (sorbitol, mannitol or lactose), “laxatives, magnesium containing antacids, amebiasis and antibiotic administration”, as well as malabsorption of certain fats. Other causes have more to do with changes within the bowel that decrease the ability to reabsorb fluid and nutrients as the stool is propelled through the lumen. Malnutrition, especially protein-calorie malnutrition causes “reversible atrophy of the villi and brush border”, the structures within the intestine responsible for absorption. Resection of parts of the bowel, especially the terminal ileum, will mechanically decrease the body’s ability to absorb due to decreased length of intestine available. Inflammation of the bowel due to infection or disease processes (Crohn’s disease) can be another cause of osmotic diarrhea. Typically, osmotic diarrhea responds with decreased stooling when the individual fasts. A person with osmotic diarrhea will have stool volume under one liter per day, the stool will be acidic and more potassium will be lost than sodium.

3. Motility disorders.

During normal functioning of the intestines, solids and fluid are moved through the gut with peristaltic waves of the smooth muscles within the intestines. This movement is slow and may take 3-5 hours for the mass to move from the pyloric valve at the proximal point of the small intestine to the large intestine. It may take as long as 24+ hours for the mass to move from the small intestine to the rectum to be expelled during defecation. When the intestines are not functioning normally, motility can be either increased or decreased and both can lead to diarrhea. Increased motility can be caused by infectious agents, changes within the bowel by inflammatory bowel disease or by irritable bowel syndrome. This increased motility results in faster transport of stool through the bowel so there is less chance for re-absorption of fluid from the large intestine. Counter-intuitively, decreased motility can also lead to diarrhea. Typically, decreased mobility will lead to constipation, which, in its most severe form, can allow a large bolus of stool to form in the lower intestine and cause an impaction. The stool behind this bolus may become liquid again due to the action of bacteria on the stool. This results in liquid stool leaking around the bolus and causing diarrhea. People with altered gut motility and diarrhea will have low volume, liquid stool and cramping. However, disordered motility may be just one factor in a complex mechanism of abnormal gut functioning as seen in infectious diarrhea, and inflammatory bowel disease.

Mixed disorders.

As with most every disease that can befall mankind, there are usually multiple physiological changes within the body that cause diarrhea. Rarely is diarrhea only caused by osmotic or secretory or motility problems. Most diarrheal states have more than one component. As an example, the bacteria *Clostridium difficile* produce toxins that are secretagogues promoting secretory diarrhea. However, the bacteria also produce a pseudomembrane that alters the absorptive ability of the gut, promoting osmotic diarrhea Medications causing diarrhea also fall under the category of mixed disorders.There are a number of drugs that are known to cause diarrhea either as a side effect or as the desired effect of the drug. The mechanism of causing diarrhea can vary from drug to drug. Some offending drug categories include: antibiotics, magnesium and phosphate containing antacids, osteoarthritis medications, cardiac medications, chemotherapeutic medications, Alzheimer's disease medications and oral hyperglycemic drugs.

PATHOPHYSIOLOGY OF BACTERIAL DIARRHEA [3] :

The first effect of HIV infection on the mucosal immune system is to cause depletion of CD4 T cells. This depletion, in turn, leads to altered IgA B-cell development and, ultimately to reduced IgA production in the lamina propria.

Altered IgA production, together with the impaired gastric-acid secretion, reported to occur in HIV infection, leads to colonization of the small intestine with increased numbers of bacteria. These bacteria may contribute to mucosal inflammation through the release of products such as surface proteins that are absorbed into the mucosa and that are capable of recruiting and activating monocytes-macrophages for release of inflammatory cytokines. The chronic

mucosal inflammation promotes villous atrophy, which together with low-grade bacterial overgrowth, leads to malabsorption.

As the disease progresses, CD4+ T-cell depletion becomes more profound, leading to impaired effector T-cell responses such as those of cytotoxic T-cells, which participate in host defense against such viruses as *cytomegalovirus*. HIV infection of the mononuclear phagocytes leads to activation and functional impairment of monocytes-macrophages and, by extension, monocytes passing through the mucosa and possibly resident mucosal macrophages.

With the altered function of cytotoxic T cells and macrophages, the gastrointestinal tract becomes subject to infection with opportunistic pathogens. Infection with HIV leads to a marked reduction of CD4+ T cells in the mucosal T-cell population of the lamina propria. This reduction in CD4+ T cells is accompanied by an increase in the lamina propria CD8+ T cell population, sometimes to a greater extent than that seen in the peripheral blood. Because the increase in CD8+ cells does not correct for the reduction in CD4+ cells, total T- cell numbers are decreased; nevertheless, total mononuclear cells in the lamina propria are increased, possibly because of an increase in the numbers of lamina propria macrophages.

A comprehensive diagnostic evaluation is warranted in patients with persistent and severe diarrhea, because a specific pathogen can be identified in majority of patients (68 – 85%) and because specific therapy can often reduce the volume and frequency of diarrhea.

CLINICAL PRESENTATION [46]

Although bacterial enterocolitis can occur at any stage of immunodeficiency, unusual presentations of these enteric bacteria became apparent early in the AIDS epidemic where *Salmonella sp.* and *Campylobacter sp.* Bacteremia were reported as initial manifestations of AIDS. In general, however, the clinical presentation of these organisms in AIDS is similar to immunocompetent patients except that diarrhea may be chronic. Bacterial enterocolitis usually manifests a s an acute diarrheal illness (less than 2 weeks duration). *Salmonella* gastro enteritis more commonly presents with watery diarrhea, abdominal pain, fever, nausea and vomiting but can manifest as an enteric fever. *Shigella* and *Campylobacter* usually present as a dysentry with the classic "colitis" symptoms mucopurulent bloody diarrhea, tenesmus and fever. Lower abdominal pain and f ever may be prominent, while nausea and vomiting are infrequent. Physical findings include fever, tachycardia and abdominal pain which may be severe. Digital rectal examination may demonstrate frank blood or pus. Initial experience suggested that the clinical presentation of *C. difficile*-colitis was different in the HIV-infected patient, but prospective studies have shown no differences as compar ed to non-immunocompromised patients. *C. difficile* can present fulminantly without diarrhea with clinical signs of peritonitis or even ascites.

The most common manifestations of *MAC* infection are fever, wasting, chronic diar rhea, abdominal pain, night sweats and intestinal malabsorption. Frank colitis or hematochezia, which may be massive, are both rare presentations of gastrointestinal *MAC* infection. Although gastrointestinal tuberculosis may be symptomatic, the clinical picture is often dominated by fever an d wasting and/or pulmonary disease. It is extraordinarily unusual for gastrointestinal TB to present as

chronic diarrheal illness. Most commonly, gastrointestinal TB is identified incidentally at the time of autopsy in patients with disseminated disease.

In a study conducted in United States concluded that *C. difficile* is the most common recognized cause of bacterial diarrhea among persons infected with HIV. The risk for bacterial diarrhea increases with increased severity of HIV disease[47].

HIV-infected persons are at increased risk for infection by several common enteric pathogens. Previous investigations have demonstrated that HIV-infected patients are at 20 times greater risk for infection with *Salmonella* species and 39 times greater risk for infection with *Campylobacter* species than the general population.[48]

A study by the National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia established that HIV-infected persons are more frequently infected with *Salmonella*, *Campylobacter*, *Listeria*, and (possibly) *Shigella* species than are individuals not infected with HIV. In addition, *Salmonella* and (possibly) *Campylobacter* infections are more likely to be severe, recurrent, or persistent and associated with extraintestinal disease when they occur in HIV-infected persons. Infections caused by *Shigella* and *Vibrio* species can also result in more serious disease in HIV infected persons than in those not infected with HIV.[49]

In another study in New York, Bacterial cultures of rectal biopsies frozen at endoscopy yielded *Escherichia coli* in 12 of 18 cases; aggregative adherence was seen in 6 cases. Isolates from 2 cases hybridized with a DNA probe encoding aggregative properties. These results suggest that chronic infection with adherent bacteria may produce a syndrome of AIDS-associated diarrhea and wasting.[50]

In a study in Boston in the year 1997, stools of 68 human immunodeficiency virus (HIV)-infected adults with diarrhea and 60 without diarrhea were examined for enteroaggregative *Escherichia coli*(EAggEc) by HeLa cell adherence assay. *EAggEc* were present in stools of 30 patients with and 18 without diarrhea. [51]

A study conducted in 2009 July concluded that bacteria identified in the stool of HIV seropositive individuals with diarrhea were *Escherischia coli* in 12(24%) cases, *Clostridium difficile* in 5(10%) cases, *Salmonella species* in 2(4%) cases, *Vibrio cholerae* in 2(4%) cases, and *Shigella* species in 1(2%) case.[52]

A study done in 2009 decemeber concluded that *Campylobacter spp*. (24.9%), *Aeromonas spp.* (20.8%), and *Shigella spp.* (8.5%) were the most common bacterial causes of diarrhea among the hospital attendees.[53]

MATERIALS AND METHODS

SOURCE OF DATA:

Type of study : A descriptive cross sectional study design is used.

Study Population : A total of 80 individuals were included in the study after the approval of instituitional ethical committee and with the consent of the subjects.

Cases :80 randomly selected patients seropositive for HIV 1 and/or 2 with diarrhea and all patients presenting with diarrhea and diagnosed HIV1 and/or 2 seropositive on admission for the first time in Shri. B.M. Patil Medical College Hospital and Research Centre, Bijapur as inpatients or outpatients from October 2011 to April 2013

INCLUSION CRITERIA:

Patients admitted to hospital within the study period, irrespective of age and sex with Seropositive for HIV 1 or HIV 2 or both with diarrhea and all patients presenting with diarrhea and diagnosed HIV1 and/or 2 seropositive on admission for the first time. Also patients seropositive for HIV 1 or HIV 2 or both with recurrent diarrhea are also included.

EXCLUSION CRITERIA:

- Patients who are seronegative for HIV 1 or HIV 2 or both with diarrhea.
- HIV positive patients who had received anti-bacterial medications for diarrhea prior to admission.

All the included patients were subjected to the following investigations

- HIV 1 and HIV 2 by ELISA / Tridot / Bidot
- CD4 Count
- Stool Routine
- Stool Culture and Sensitivity
- Other investigations wherever necessary

Method of Test

Sample collection

A freshly passed stool specimens were collected in wide mouthed, leak proof, plastic containers without any preservative. A disposable plastic spoon was kept inside each container for the convenience while collecting the sample. Patients were instructed to drop the spoon along with the sample inside the container and close the lid tight. The containers were labeled properly and transported to the laboratory. The specimens were processed within 1-3 hours of collection.

Sample Processing

Gross examination was done for all the samples to note colour, consistency, presence of blood / pus/mucous, adult worms or segments.

Direct microscopic examination of feces to detect trophozoites, ova, cysts, larvae and oocysts.

BACTERIAL CULTURE TECHNIQUE :

DIFFERENT CULTURE MEDIUMS USED ARE

- Differential, poorly selective media– MacConkey medium
- Moderately selective media– XLD or Hektoen medium
- Highly selective media– Salmonella Shigella medium
- Campylobacter media
- Incubate all media at 35^{o} C for 24 hours

INTERPRETATION OF STOOL CULTURES

- After 24 hours incubation culture plates are evaluated for growth

1. MACCONKEY AGAR

- Evaluate plate for presence of non lactose fermenting colonies (clear colonies)
- Suspect *Salmonella/Shigella* species
- Perform appropriate biochemical screening tests

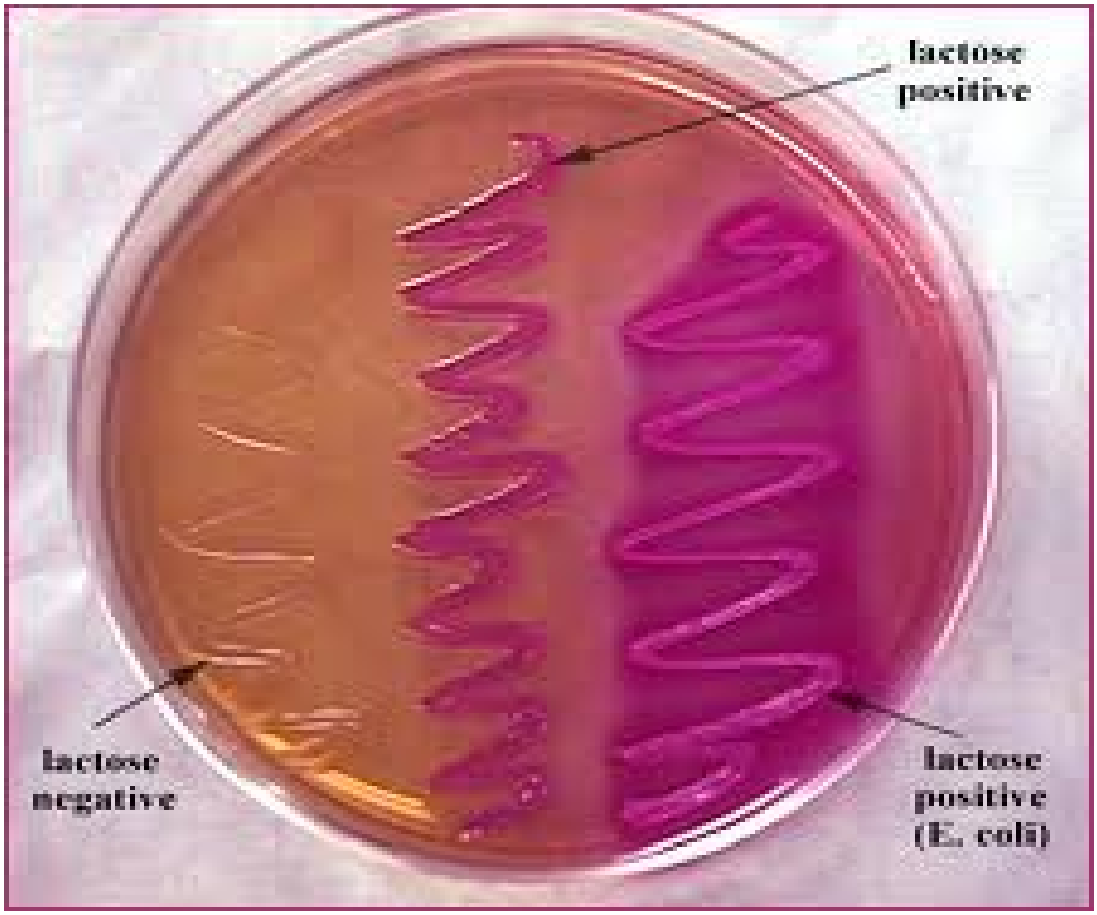


Fig.3 MACCONKEY AGAR

2. HEXTOEN ENTERIC AGAR (HE)

- Non pathogens – bright orange to salmon pink
- *Salmonella* species– Green to blue-green (clear) colonies with black centers
- *Shigella* species– Green to blue-green (clear) colonies

Fig.4 HEXTOEN ENTERIC AGAR

3. XYLOSE-LYSINE-DESOXYCHOLATE (XLD)

- Non pathogens – yellow or yellow with black centers
- *Salmonella* species– Red (colorless) colonies with black centers
- *Shigella* species– Red (colorless) colonies

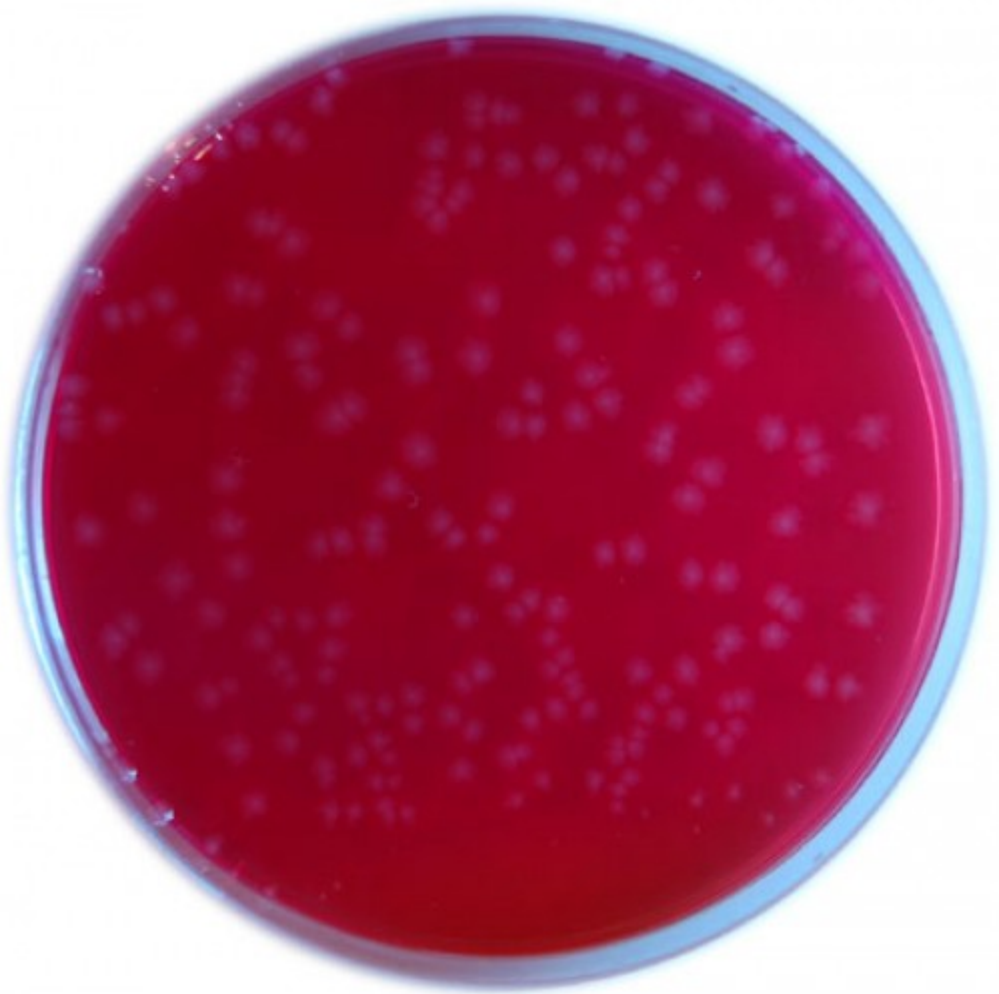


Fig.5 XYLOSE-LYSINE-DESOXYCHOLATE (XLD)

4. CAMPY BLOOD AGAR OR CVA AGAR

- Contain antibiotic mixture to inhibit normal fecal flora
- Incubate at 42°C – this will inhibit normal fecal flora
- Screen growth for both oxidase and catalase production
- Campylobacter will be:
  - Oxidase positive
  - Catalase positive
  - Gram stain suspicious colonies – Curved gram negative rods

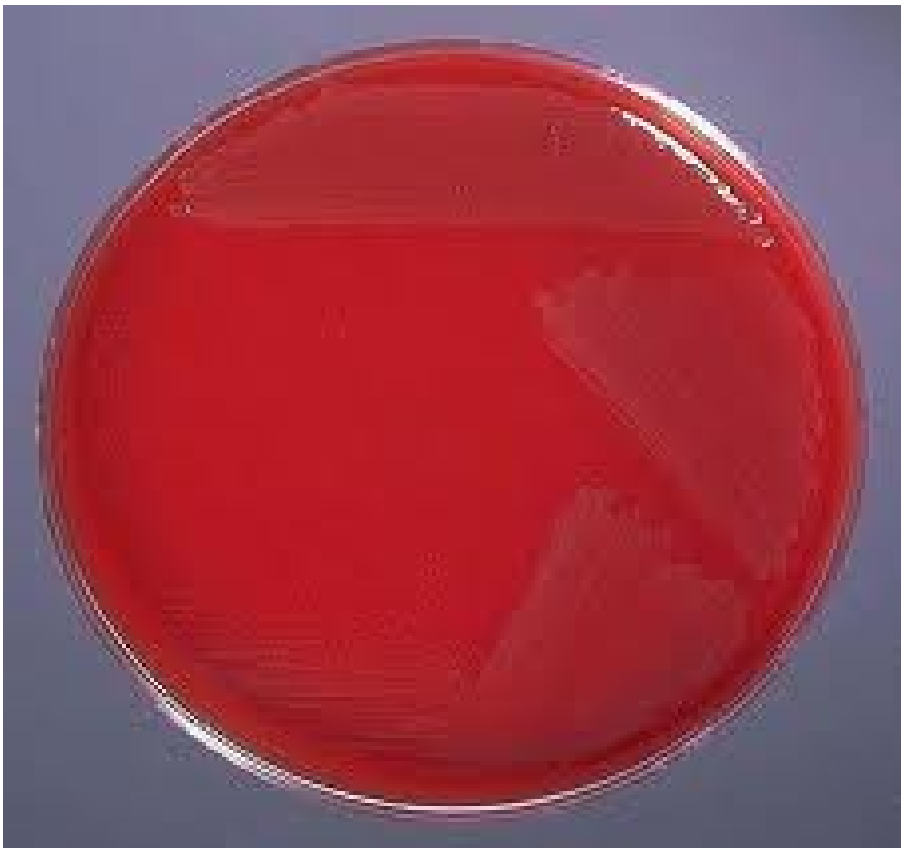


Fig.6 CAMPY BLOOD AGAR

After 48 hours incubation culture plates are evaluate for growth

- If no normal enteric flora is present after 48 hours
  - No growth of normal enteric flora
  - No *Salmonella, Shigella or Campylobacter* species isolated
  - Negative for Shiga-toxin producing *E. coli*

Diagnosis of HIV infection

Sample collection

Serum samples were used for detecting antibodies to HIV virus antigens. After pretest counseling and informed written consent was obtained, with all aseptic precautions 3-5 ml of venous blood was collected and transferred into a properly labeled sterile plastic leak proof specimen container with screw cap. The blood was allowed to clot for 30 min at room temperature. The vial was centrifuged at 3000 rpm for 10 min to separate serum to avoid hemolyis. The serum was transferred to a sterile plastic screw capped leak proof tube.

Appropriate NACO strategy was used to determine HIV status of the individuals. Initially highly sensitive screening test was used. The sample was considered negative if the test gave non-reactive result. In case the test result was reactive the same sample was tested with another test kit (based on 36 different principle of test or having different antigens compared to the first test). If the result was reactive with second test kit also the sample is considered to be positive. In case the sample was positive by first test kit and negative by second test kit, sample was subjected to a tiebreaker third test. If third test is reactive, sample was reported as

indeterminate and follow-up testing was undertaken after 2-4 weeks. In case the tiebreaker third test was negative, sample was reported as negative.

MICROLISA – HIV

Microwell ELISA Test for the detection of antibodies to HIV-1 and HIV-2 in human serum/plasma.

Principle

Microlisa HIV test is an enzyme immunoassay based on indirect ELISA. HIV envelope proteins gp41, C terminus of gp 120 for HIV-1 and gp 36 for HIV-2 representing immunodominant epitopesare coated on to microtiter wells. Specimens and controls are added to the microtiter wells and incubated. Antibodies to HIV-1 and HIV-2 if present in the specimen, will bind to the specific antigens absorbed onto the surface of the wells. The plate is then washed to remove unbound material. Horseradish peroxidase (HRP) conjugated antihuman IgG is added to each well. This conjugate will bind to HIV antigen-antibody complex present. Finally substrate solution containing chromogen and hydrogen peroxide is added to the wells and incubated. A blue colour will develop in proportion to the amount of HIV-1 and/or HIV-2 . 37 antibodies present in the specimen. The colour reaction is stopped by a stop solution. The enzyme substrate reaction is read by EIA reader before absorbance at a wavelength of 450 nm. If the sample does not contain HIV-1 or HIV-2 antibodies then enzyme conjugate will not bind and the solution in the wells will be either colourless or only a faint background colour develops.

Test Procedure

- 100 µl sample diluent was added to well A-1 as blank.100 µl negative control was added to well no. B-1 and C-1 each. 100 µl positive control was added to D-1, E-1 and F-1 wells.

- 100 µl sample diluent was added to each well starting from G-1 followed by addition of 10 µl sample. Cover seal was applied and incubated at 37ºC for 30 min.
- After incubation time was over, wells were washed with working wash solution for 5 times (300 µl, 30 sec soak time).
- 100 µl working of working conjugate solution is added in each well including the blank. Cover seal was applied and incubated at 37ºC for 30 min. Then it was washed 5 times as before.
- 100 µl of working substrate solution is added in each well including A-1. Incubated at room temperature for 30 min in dark.
- 50 µl of stop solution was added to all the wells and absorbance was read at 450 nm within 30 min.

Test Validity

1. Blank must be < 0.100
2. Negative Control mean must be = 0.150
3. Positive Control mean must be = 0.50

Calculation of Results

Cut Off value = (Mean of Negative Control + Mean of Positive Control)/ 6

Interpretation of Results

Test specimens with absorbance value less than the cut off value were considered non-reactive and were considered negative for anti-HIV antibodies. Test specimen with absorbance values greater than or equal to cut off value were considered reactive for anti-HIV antibodies.

Performance Characteristics of Test Kit

Sensitivity 100% Specificity 99.5%

HIV EIA COMB

Rapid visual EIA test for the qualitative and differential detection of antibodies to HIV-1 (including subgroup O and subgroup C) and HIV-2 in human serum/plasma.

Principle

HIV antigens are immobilized circular spot on the polystyrene comb. When incubated with a specimen containing HIV-1 and/or HIV-2 antibodies, these antibodies bind specifically to the immobilized antigens. The comb is washed to remove unbound antibodies. The comb is then placed in microwells 39containing enzyme conjugate (alkaline phosphatase conjugated anti-human IgG). This conjugate will bind to antigen antibody complex present on the comb. Finally the comb is placed in microwells substrate and is incubated. The bound conjugate will react with substrate. The results are directly visualized by the presence of distinct grey-blue dot(s) on the surface of comb.

Setting up of the test

- 250 µl of sample diluent was added to required number of microcuvettes using the dropper provided with the kit. One drop (50 µl) of sample (serum) was added to respective above microcuvettes. The sample was mixed with the diluent by repeatedly aspirating and expelling with dropper. The position and identity of the samples was recorded as they were added.
- Another required number of new microcuvettes in another row were filled with 300 µl of wash buffer.

- Another required number of new microcuvettes in another row were filled with Enzyme conjugate directly from the vial.
- Again another required number of new microcuvettes in another row were filled with 300 µl of wash buffer.

Test Procedure

- Required number of combs were taken and sample numbers marked.
- The comb was placed into the microcuvettes containing samples and incubated for 8 min at room temperature. During incubation comb was withdrawn and inserted in the microcuvettes for 15 sec.
- The comb was removed and tips were blotted on absorbent material.
- The comb was placed into microcuvettes containing wash buffer washed for one minute by carefully mixing the comb up and down in the wash solution. The comb was removed and the tips blotted.
- The comb was placed into microcuvettes containing Enzyme conjugate and incubated for 8 min at room temperature. During incubation comb was withdrawn and inserted in the microcuvettes for 15 sec. The comb was washed in wash buffer as before and blotted.
- Then it was placed into microcuvettes containing chromogenic substrate and incubated for 8 min at room temperature. The comb was removed and the tips blotted. Again the comb was washed as before and the tips blotted.
- The comb was placed into microcuvettes containing Substrate and incubated for 8 min at room temperature. During incubation comb was withdrawn and inserted in the microcuvettes for 15 sec.
- The comb was removed and the tips blotted. Again the comb was washed as before and the tips blotted.

- The comb was washed in distilled water and the tips blotted.
- The comb was placed on a clean surface, reactive side up and allowed to air dry. Results were read only after the comb was completely dry.

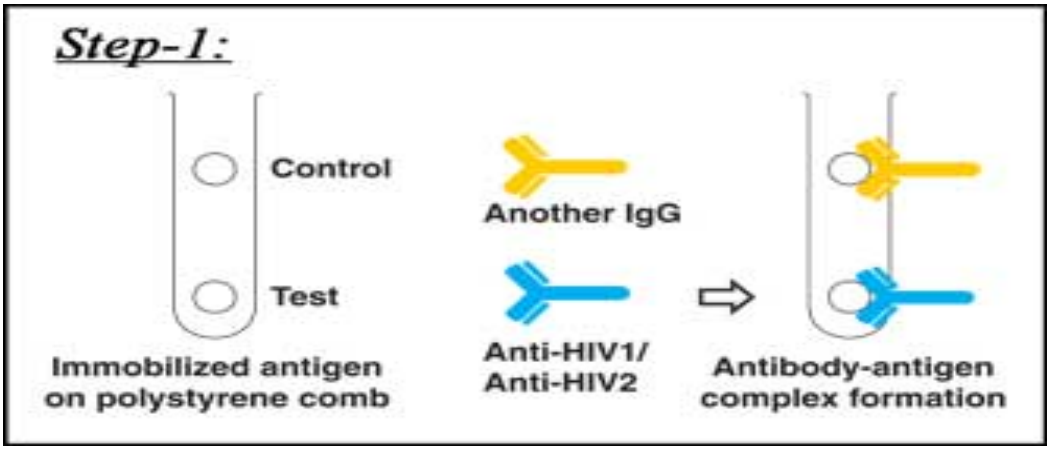
Validity - Appearance of distinct grey-blue dot in the control position indicates the test is valid.

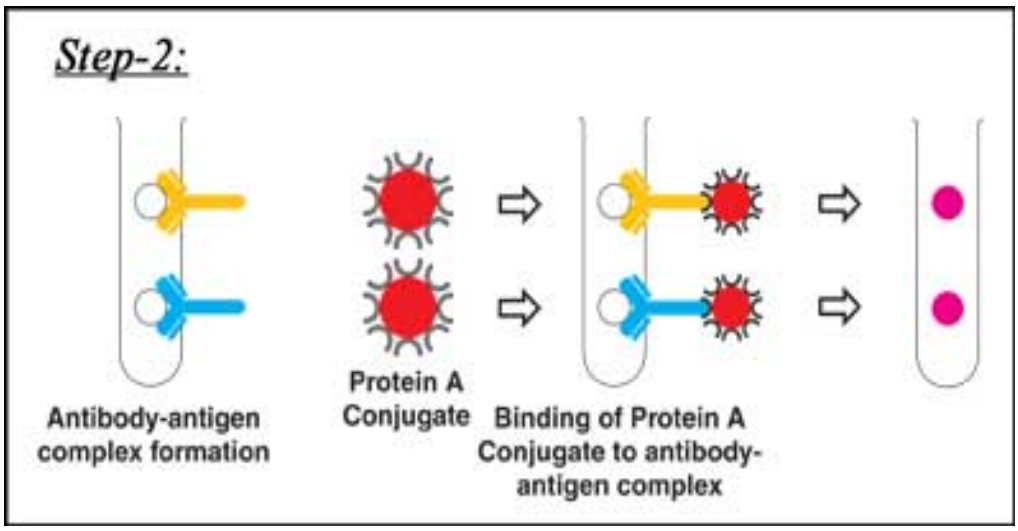
Interpretation of Results

Non-reactive - If only one dot (in the control position) appears, the sample is non-reactive.

Reactive - If two dots, one for the control and the other for HIV1appeared, the sample was considered to be reactive for antibodies to HIV-1. If two dots, one for the control and the other for the HIV-2 appeared, the sample was considered to be reactive for antibodies to HIV-2. If all the three dots, one each for control, HIV-1 and HIV-2 appeared**,** the sample was considered to be reactive for antibodies to both HIV-1 and HIV-2.

Performance Characters: Sensitivity – 100%; Specificity – 99.9%





HIV BI-DOT TEST

Rapid visual test for the qualitative detection of antibodies to HIV-1 and HIV-2 in human serum/plasma.

Principle: HIV antigens (gp41, C terminal of gp120 & gp36 representing the immunodominant regions of HIV-1 and HIV-2 envelope gene structure respectively) are immobilized on a porous immunofiltration membrane and are absorbed into the

underlying absorbent. As the patient sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens. Conjugate binds to the Fc portion of the HIV antibodies to give distinct pinkish purple dot against a white background.

Test Procedure:

- 3 drops of buffer solution are added to the centre of the device.
- The sample dropper provided with the kit is held vertically and 1 drop of patient's sample (serum/plasma) is added. 5 drops of buffer solution are added.
- 2 drops of liquid conjugate are added directly from the conjugate vial.5 drops of buffer solution are added and results are read immediately.

Interpretation of the Results

Non-reactive – If only one dot (the Control Dot) appears, the specimen is non-reactive for antibodies either to HIV-1 or HIV-2.

Reactive – If 2 dots, one for control and the other for test appear, the specimen is reactive for antibodies to HIV-1 and/or HIV-2.

Performance Characteristics: Sensitivity – 100% ; Specificity – 100%

ESTIMATION OF CD4 COUNT

CD4 count was estimated from 5 ml of K 2 EDTA blood collected from each patient and was processed the same day. The CD4 count was done by automated flow cytometry analyzer FACS calibur (Beckton Dickinson).

Principle of Flow Cytometry

Signals are generated by cells or particles in suspension passing through a light (usually LASER) source in the flow cell, in a single file (aligned by Sheath Fluidic System) and are analyzed electronically in a flow cytometer. The parameters measurable include forward scatter (an indicator of cell size), side scatter (an indicator of granularity of the cell) and signals from multiple fluorescent dyes (e.g. FITC, phycoerythrin) tagged to cell surface phenotypic marker-specific antibodies.

BD FACS Calibur is a flow cytometer which is capable of measuring the scatter and the fluorescence parameter. It can detect the scatter parameter namely the forward and the side scatter which gives information about the size and granularity of the cell. The BD FACS Calibur can detect up to 3 fluorescence parameters. It can measure both absolute CD4 + T-lymphocyte count as well as % CD4 count.

Antibody panels - BD TriTEST CD3 fluorescein isothiocyanate (FITC)/CD4 phycoerythrin (PE)/CD45 peridinin chlorophyll protein (PerCP) was used, which is a three-color direct immunofluorescence reagent to identify and determine the percentages and absolute counts of mature human T lymphocytes (CD3) and helper/inducer (CD3+ CD4+) T-lymphocyte subsets in erythrocyte-lysed whole

blood. When used with TruCOUNT Tubes, absolute counts of these populations can be enumerated from a single tube.'

Procedure

- Required numbers of BD Trucount Tubes were taken. 20 µl of Tritest antibody reagent is added to each tube, by placing it on the side wall of the tube, to avoid disturbing the bead at the bottom.
- 50 µl of well mixed whole blood collected in K 2 tube in a similar way and ortexed.
- EDTA is added to each and incubated in dark at room temperature for 15 minutes.
- 450 µl of 1X lysing solution was added and vortexed. This cause lysis of RBCs and fixation of cells on the beads.
- Incubated in dark at room temperature for 15 minutes.
- Reading was obtained on the BD FACS Calibur.

STATISTICAL ANALYSIS

The significance of association of CD4 counts and bacteria isolated was analysed by chi square test. P value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 80 individuals were enrolled in this study, conducted during October 2011 to April 2013 at Shri B.M. Patil Medical College Hospital and Research Centre, Bijapur. It included all patients seropositive for HIV 1 and/or 2 with diarrhea and all patients presenting with diarrhea and diagnosed HIV1 and/or 2 seropositive as cases. Fresh stool samples were collected from all the subjects and were examined for bacteria by stool culture.

Cases included 46 males and 34 females. Among the cases, 37(29.6 %) subjects were found to be in the age group of 30-40 years which constituted major group in which 23(50%) were males and 14(41.18%) were females.

Table no 1: Age and sex distribution of study population.

Age (in yrs)	Female (%)	Male (%)	Total (%)
20-30	13 (38.24)	4 (8.70)	17 (13.6)
30-40	14 (41.18)	23(50.00)	37 (29.6)
40-50	6 (17.64)	12(26.09)	18 (14.4)
50-60	1 (2.94)	6 (13.04)	07 (5.6)
60-70	0 (0)	1 (2.17)	01 (1.25)
Total	34	46	80

Graph no 1: Age and sex distribution of study population.

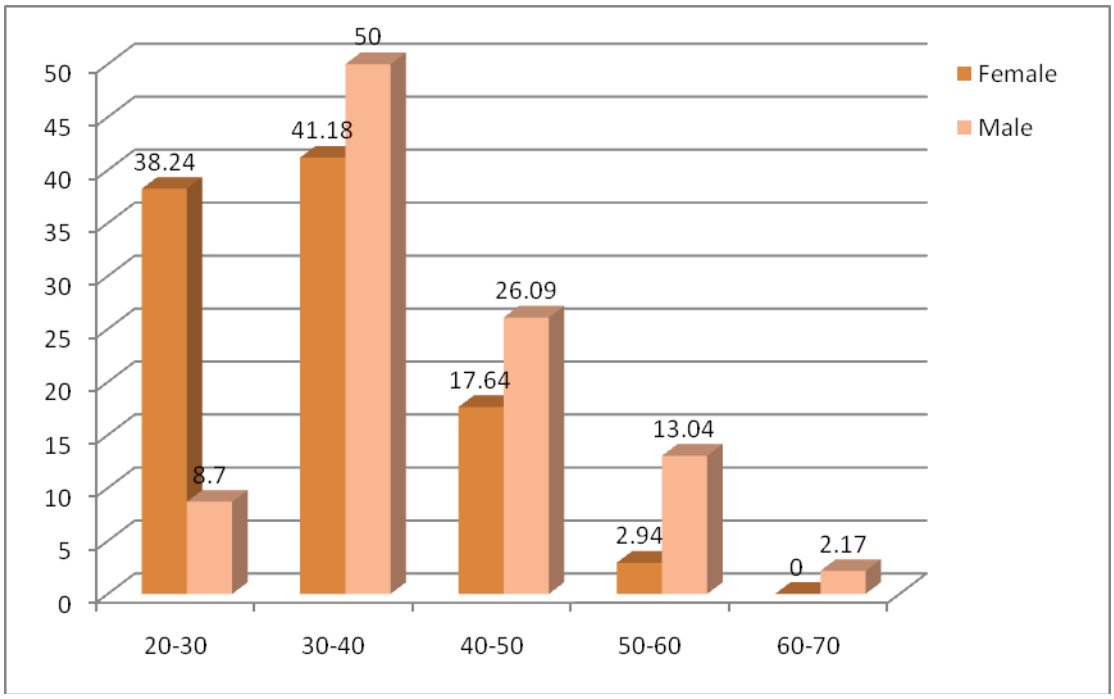
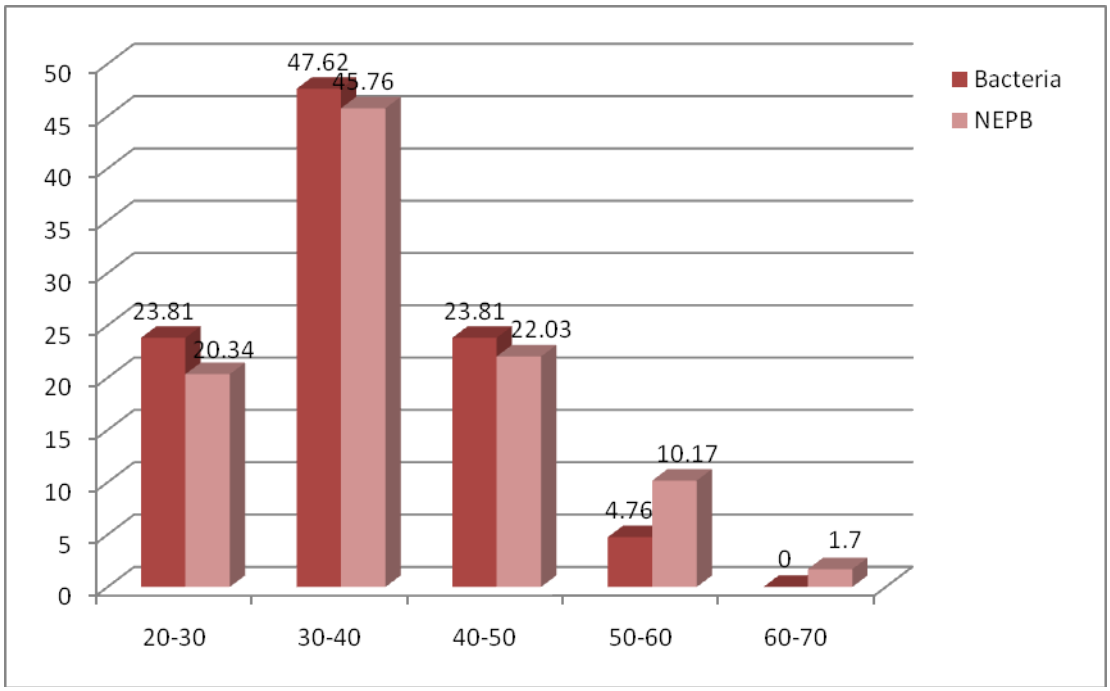


Table no 2: Age distribution of patients according to isolate.

Age (Years)	Isolate				Total
	Bacteria		NEPBI		
	Frequency	Percentage (%)	Frequency	Percentage (%)	
20-30	5	23.81	12	20.34	17
30-40	10	47.62	27	45.76	37
40-50	5	23.81	13	22.03	18
50-60	1	4.76	6	10.17	7
60-70	0	0	1	1.70	1
	21		59		80

Graph no 2: Age distribution of patients according to isolate.

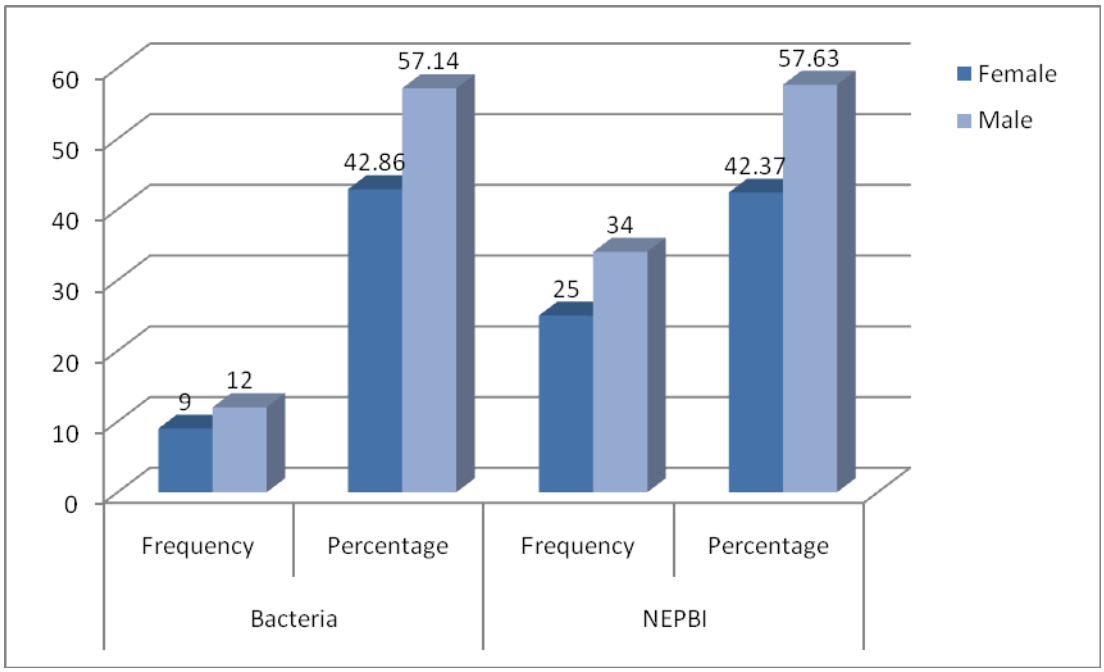


The study groups included individuals of all age groups. Among the cases, 37(29.6%) subjects were found to be in the age group of 30-40 years. About 10(47.62%) bacteria was isolated in the age group of 30-40 years.

Table no 3: Sex distribution of patients according to isolate.

Sex	Isolate					p-value
	Bacteria		NEPBI			
	Frequency	Percentage (%)	Frequency	Percentage (%)	Total	
Female	9	42.86	25	42.37	34	0.532
Male	12	57.14	34	57.63	46	
Total	21		59		80	

Graph no 3: Sex distribution of patients according to isolate.

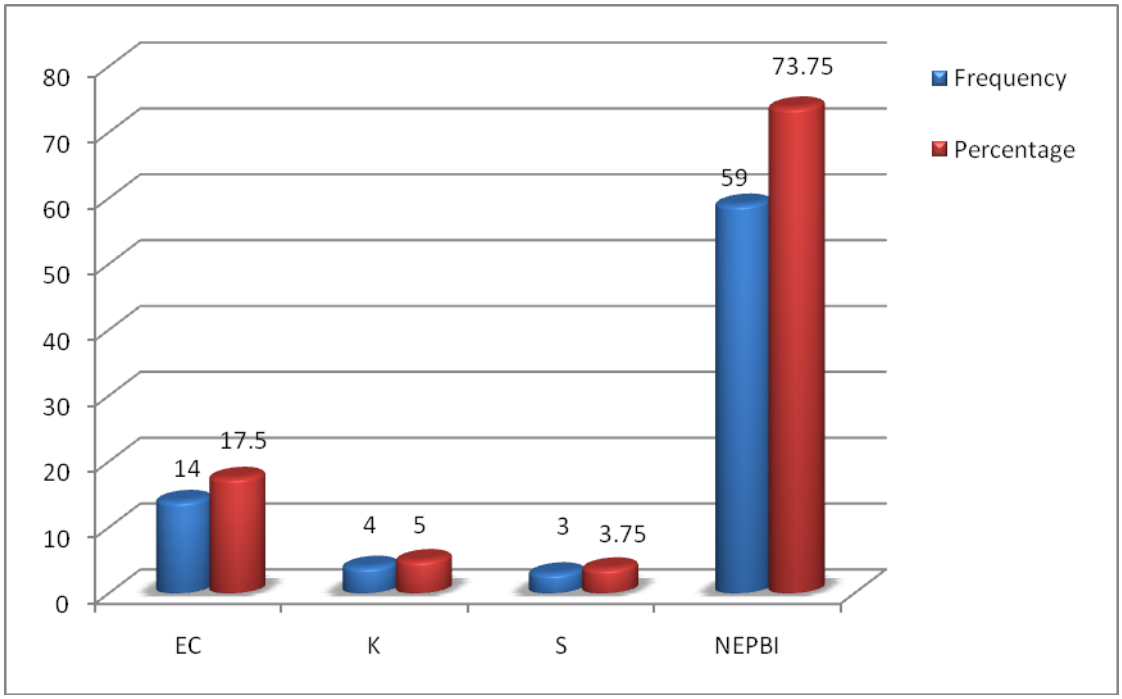


Cases included 46 males and 34 females. Bacteria was isolated among 21 patients out of which 12 (57.4%) were males and 9 (42.86%) were females.

Table no 4: Distribution of patients according to bacteria isolate

Isolate	Frequency	Percentage (%)
E.coli	14	17.5
Klebsiella	04	5.00
Shigella Sps.	03	3.75
NEPBI	59	73.75
TOTAL	80	100

Graph no 4: Distribution of patients according to bacteria isolate

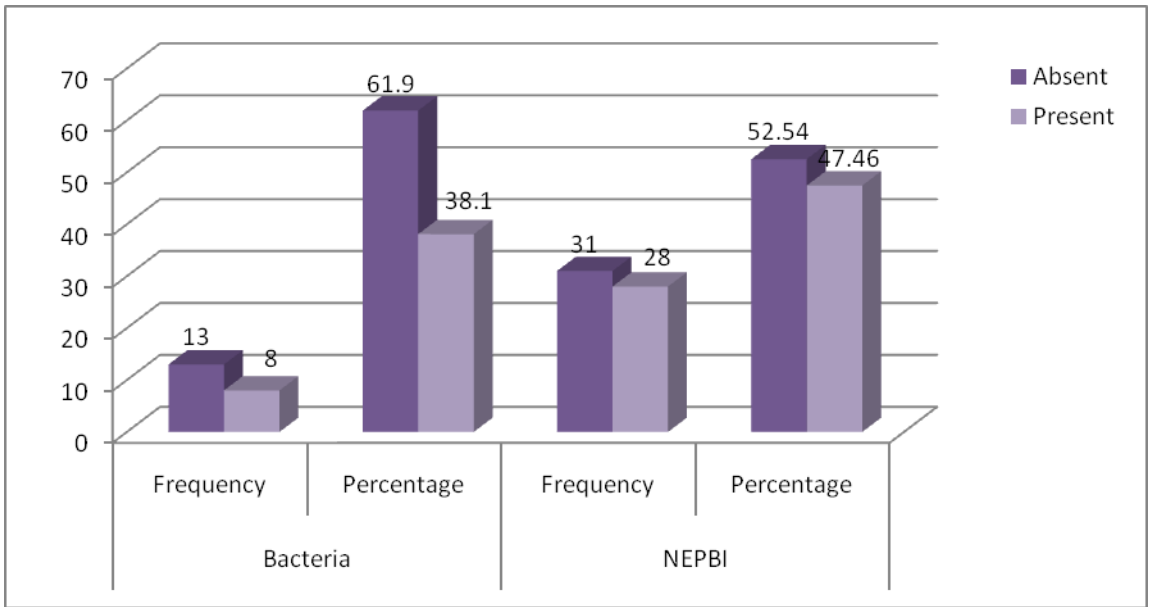


No enteropathogenic bacteria was seen in most of the samples and it constituted about 59(73.75%). *E. Coli* was the most common bacteria isolated which constituted about 14(17.5%). *Klebsiella* was the next most common isolated which constituted about 4(5%) and the last was *Shigella Sps*. which constituted 3(3.75%).

Table no 5: Distribution of patients according to abdominal pain.

Pain Abdomen	Isolate				Total	p-value
	Bacteria		NEPBI			
	Frequency	Percentage (%)	Frequency	Percentage (%)		
Absent	13	61.90	31	52.54	44	0.581
Present	8	38.10	28	47.46	36	
Total	21		59		80	

Graph no 5: Distribution of patients according to abdominal pain

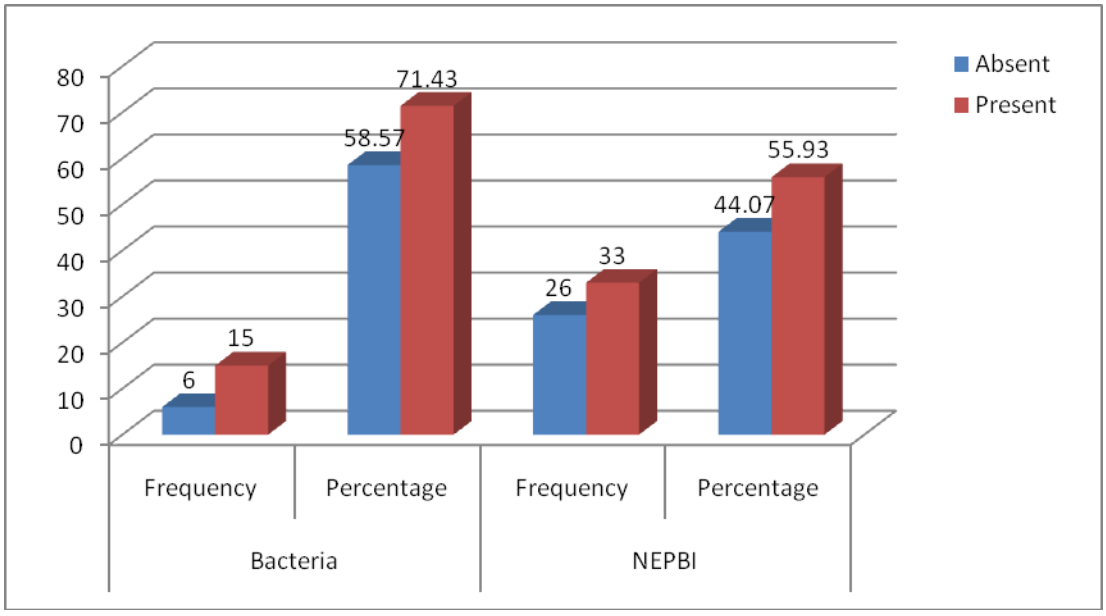


Among 80 patients of diarrhea 36 patients had associated abdominal discomfort in which bacteria was isolated in 8 patients constituting 38.10%. Remaining 44 patients did not had associated abdominal discomfort.

Table no 6: Distribution of bacteria according to fever

Fever	Isolate				Total	p-value
	Bacteria		NEPBI			
	Frequency	Percentage (%)	Frequency	Percentage (%)		
Absent	6	58.57	26	44.07	32	0.162
Present	15	71.43	33	55.93	48	
Total	21		59		80	

Graph no 6: Distribution of bacteria according to fever

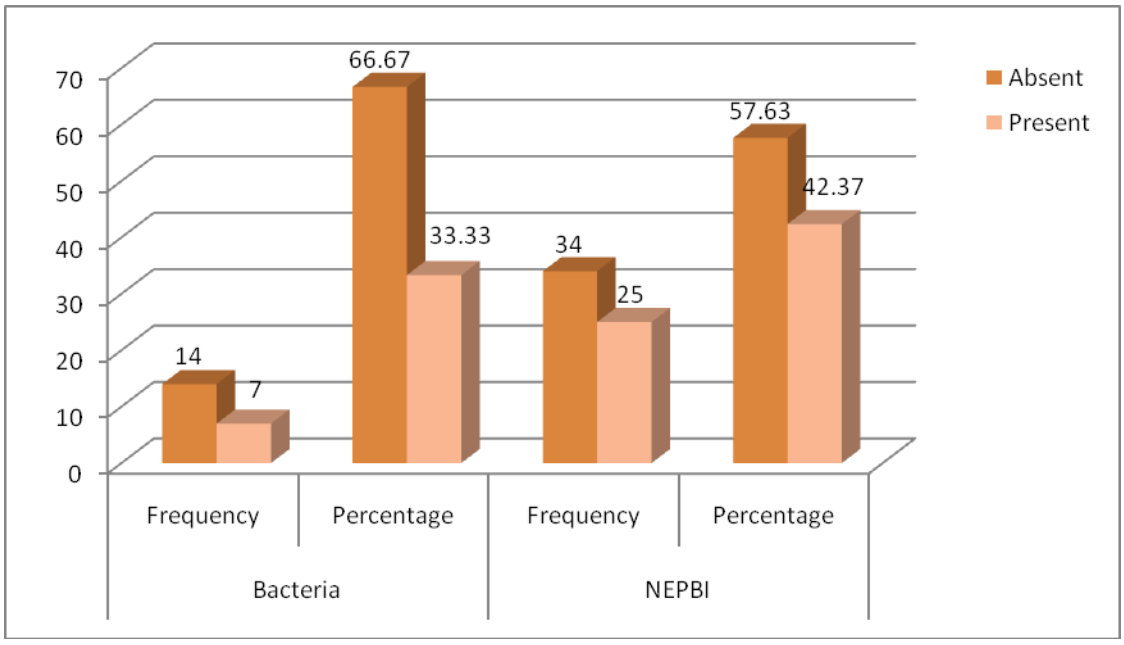


Among 80 patients of diarrhea 48 patients had associated fever in which bacteria was isolated in 15 patients constituting 71.43%. Remaining 32 patients did not had associated fever.

Table no 7: Distribution of bacteria according to vomiting

Vomiting	Isolate				Total	p-value
	Bacteria		NEPBI			
	Frequency	Percentage (%)	Frequency	Percentage (%)		
Absent	14	66.67	34	57.63	48	0.640
Present	7	33.33	25	42.37	32	
Total	21		59		80	

Graph no 7: : Distribution of bacteria according to vomiting

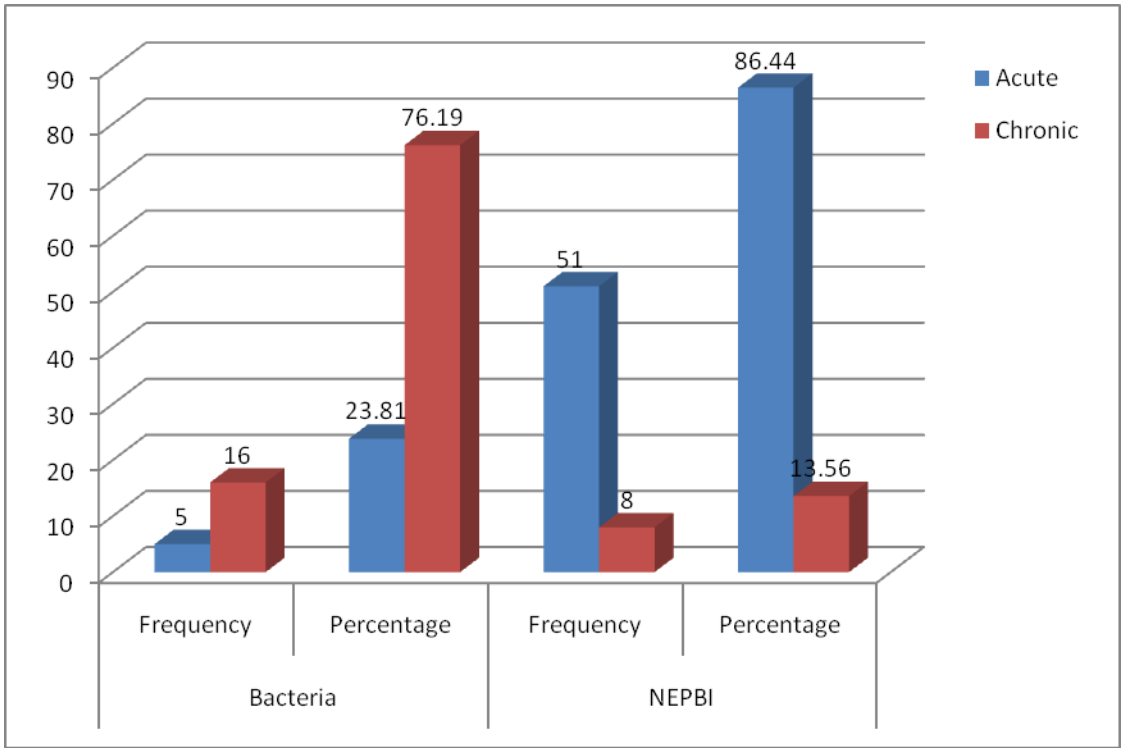


Among 80 patients about 32 patients had associated vomiting in which bacteria was isolated in about 7 patients which constituted about 33.33 %.

Table no 8: Bacteria isolation in relation to diarrhea

Diarrrhea	Isolate				Total	p-value
	Bacteria		NEPBI			
	Frequency	Percentage (%)	Frequency	Percentage (%)		
Acute	05	23.81	51	86.44	56	<0.001
Chronic	16	76.19	08	13.56	24	
Total	21		59		80	

Graph no 8: Bacteria isolation in relation to diarrhea



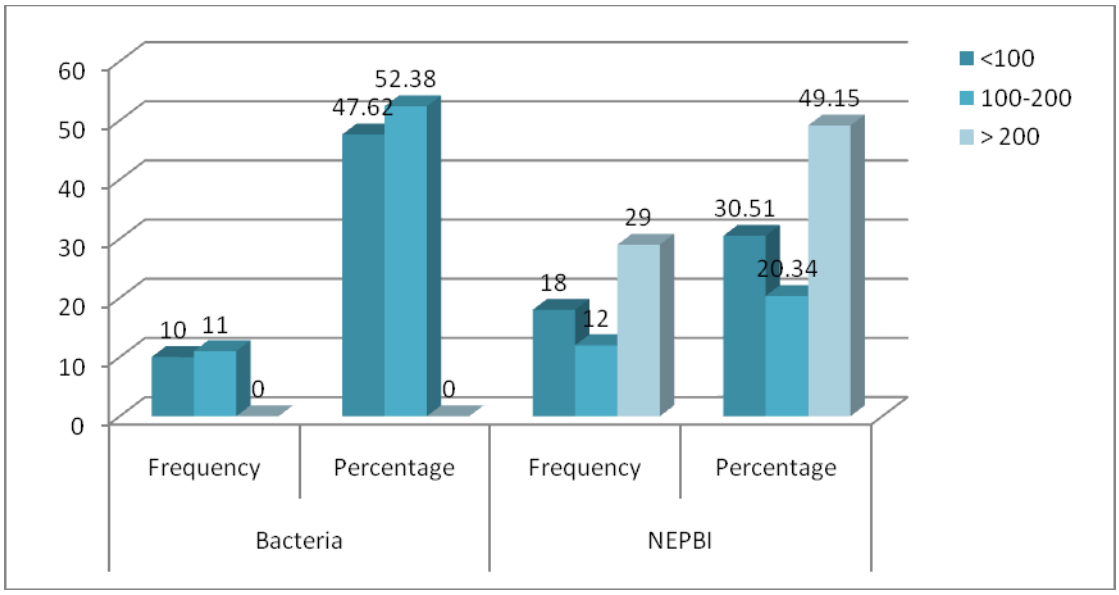
Among 80 patients about 56 patients had acute diarrhea in which bacteria was isolated in 5(23.81%) patients and 24 patients had chronic diarrhea in which bacteria was isolated in 16(76.19%) patients. As p-value < 0.001 indicating that difference is highly significant among acute and chronic diarrhea patients with regard to bacteria.

Bacteria are found more in chronic diarrhea as compared acute diarrhea. P-value was assessed using chi square analysis.

Table no 9: Distribution of bacteria in relation to CD4 count.

CD4count (cells/mm^3)	Isolate				Total	p-value
	Bacteria		NEPBI			
	Frequency	Percentage (%)	Frequency	Percentage (%)		
<100	10	47.62	18	30.51	28	0.0001
100-200	11	52.38	12	20.34	23	
> 200	0	0	29	49.15	29	
Total	21		59		80	

Graph no 9: Distribution of bacteria in relation to CD4 count .



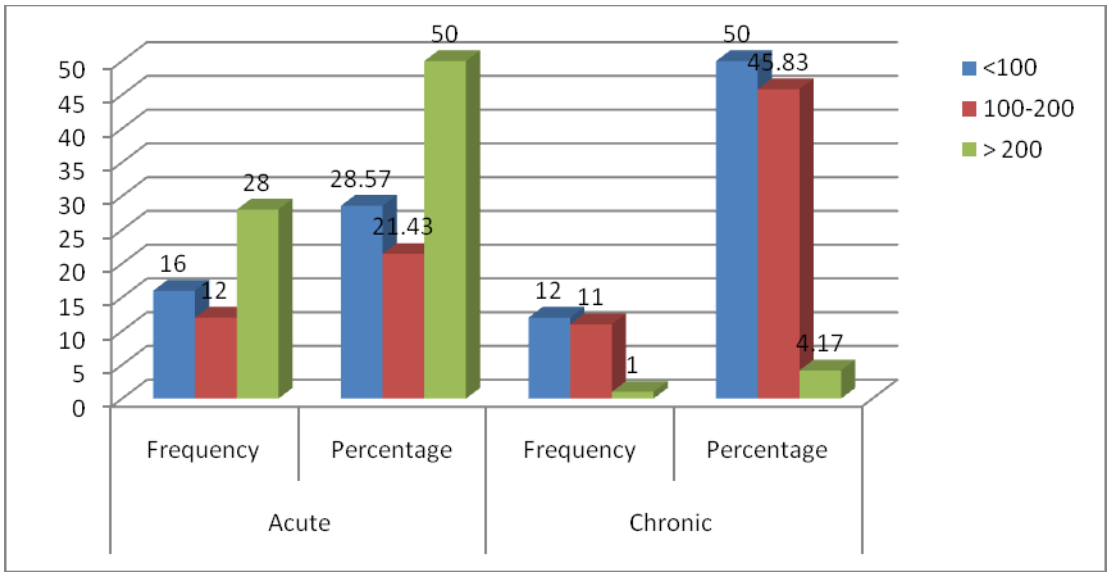
Among 80 patients 28 patients had CD4 count<100, 23 patients with CD4 between 100-200 and 29 patients had CD4 >200 count. Around 11 bacteria was isolated more at a CD4 level 100-200 constituting about 52.38% and next common was at a CD4 <100 constituting about 10(47.62%). As p-value < 0.001 indicating that bacteria and CD4 count is highly associated. Among the diarrhea patients with

presence of bacteria usually have CD4 count less than 200. P-value was assessed using chi square analysis.

Table no 10: Distribution of CD4 count in relation to diarrhea

CD4count (cells/mm^3)	Diarrhea				Total	p-value
	Acute		Chronic			
	Frequency	Percentage (%)	Frequency	Percentage (%)		
<100	16	28.57	12	50.00	28	0.0001
100-200	12	21.43	11	45.83	23	
>200	28	50.00	01	4.17	29	
Total	56		24		80	

Graph no 10: Distribution of CD4 count in relation to diarrhea



Among 56 patients of acute diarrhea about 28(50%) had CD4 >200 and 16(28.57%) had CD4 count <100. Among 24 patients of chronic diarrhea 12(50%) patients had CD4 <100 and 1 (4.17%) patient CD4 >200. As p-value <0.0001 indicate difference is highly significant among acute and chronic diarrhea patients with regard

to CD4 count. CD4 count is usually less than 200 among chronic diarrhea patients which is statistically significant.

DISCUSSION

HIV/AIDS (Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome) is a major problem in India with more than 23.9 lakh recorded cases by the end of 2009 [30]. Diarrhea is a common complication of HIV positive patients and occurs in 90% of AIDS patients in developing countries. Identification of the etiological agent of diarrhea in a patient with AIDS is very important as it can help in the institution of appropriate therapy and the reduction of morbidity and mortality in these patients. This study was therefore conducted to ascertain the scope and frequency of potential enteric bacterial pathogens isolated from stool samples of HIV-positive individuals with diarrhea. The etiology for such diarrhea could be parasitic, bacterial, fungal, enteric viruses, or HIV itself may contribute to diarrhea. In addition to microbes, other factors such as medication, immune deregulation, autonomic dysfunction, and nutritional supplementation play a substantial role in diarrhea of patients with HIV/AIDS [52].

In resource limited countries such as India, enteric infections remain common in the general population and for those people infected with HIV, with geographic differences in the reported prevalence of individual pathogens reflecting differences in pathogen prevalence, standards of hygiene, and diagnostic methods used. There are many reports regarding the frequency of various pathogens causing diarrhea from different parts of India[54] Some studies also demonstrated regional variability of pathogens, as well as changing trends of etiology in the same population [55] . But

reports regarding correlation of the diarrhea with CD4 levels, impact of CD4 levels on isolation of pathogen were not studied in India. So we planned conduct a study to look for the rate of diarrhea, associations between diarrhea and CD4 counts and variation in frequency of identifying a pathogen with stool sample.

1. **Distribution of patients in the study group**

In the present study, there were 80 HIV positive individuals, of which there were 46 males and 34 females. Those having diarrhea for less than 2 weeks were considered to have acute diarrhea and diarrhea lasting more than 2 weeks was considered to be chronic.[56]

Among HIV infected with diarrhea 56 patients had acute form of it and the remaining 24 patients were suffering from chronic diarrhea; acute diarrhea subjects outnumbered chronic diarrhea. Our rate represents a minimum burden of diarrhea, as episodes were only counted if a patient came to the clinic. Diarrhea which was self-limiting or which responded to over-the-counter medication would not have come to our attention.

2. **Distribution of bacteria in the study group**

The overall infection rate in this study was 21(26.25%). In the present study, bacteria were found in higher numbers in males 12(57.14%) than in females 9(42.86%), among HIV positive individuals. Male preponderance in infection rates among HIV infected has been shown in studies by Dwiwedi K *et al* [56]

In this study, higher bacterial infection rate was seen in 30-40 years age group in HIV positive subjects, with 23(50%) males and 14 (41.18%) females being in that age group. In the study by Mukhopadhya *et al* [58] mean

age of infected individuals was found to be in 30-40 years age group. The infection rates observed in the present study were comparable to the studies of Kumar SS *et al* [57] which has reported higher infection rates in HIV positive patients.

3. Spectrum of bacteria identified in the study population

In the present study no enteropathogenic bacteria was seen in most of the samples and it constituted about 59(73.75%). The reason for it might be either patient might be having diarrhea caused by opportunistic parasitic infection which is the most common cause or it may be due to HIV itself which results in enteropathy.

E. Coli was the most common bacteria isolated which constituted about 14(17.5%). *Klebsiella* was the next most common isolated which constituted about 4(5%) and the last was *Shigella Sps*. which constituted 3(3.75%) as compared to the study of *Beena U et al* in which *Escherischia coli* in 24% cases, *Clostridium difficile* in 12.5% cases, *Salmonella species* in 5% cases, *Vibrio cholerae* in 5% cases, and *Shigella species* in 2.5% cases were isolated.[52] Studies conducted in the other parts of world have shown *clostridium difficile, salmonella, campylobacter species* as the predominant bacteria in the HIV-infected[47,48,53]

Figures from various studies demonstrate striking geographic variations in the prevalence of individual pathogens in HIV patients. These variations may relate both to the prevalence of pathogens within the community, and to drugs used prophylactically in patients with HIV infection.

Moreover, the quantitative differences in bacteria infecting HIV patients with diarrhea may be due to differences in genotypic / phenotypic characters.

4. Diarrhea and intestinal bacterial infection

In our study, higher bacterial infection was found in those with chronic diarrhea 16 (76.19%) than those with acute diarrhea 5(23.81%) among HIV infected individuals. Similar observations were made in studies conducted by Christine *et al*., Beena *et al*. and Samie *et al*.[51,52,53].

5. Diarrhea and immune status of the HIV positive patients

Among cases, mean CD4 counts were found to be 196.83 cells/mm^3. Those with chronic diarrhea (111.46 cells/mm^3) had significantly lower CD4 counts compared to acute diarrhea (233.43 cells/mm^3).

There was a strong negative association between the duration of diarrhea and CD4 levels and this was comparable to a study done by Attili *et al* [1]

Mean CD4 counts were observed to be 141 cells/mm^3 diarrhea (265 cells/mm^3 in acute and 123 cells/mm^3in chronic) in the study of Dwiwedi KK *et al*. [15]

The theories for chronic diarrhea at low CD4 is not clear but one of the reasons could be regional immunosuppression as suggested by Schneider et al [59]. They found, loss of CD4 cells in intestinal mucosa of the patients with diarrhea, which were more pronounced than peripheral CD4 levels and their relation is quite variable. The mucosal immunity, an important factor to

prevent diarrhea is therefore variable even in patients with good immunity (i.e. peripheral CD4>200). We did not perform the mucosal CD4 levels thus any comments on the mucosal immune status based on blood CD4 levels would be in appropriate. But we can presume that probably the low mucosal immunity could be a cause of diarrhea in patients with high CD4 levels.

6. Bacterial isolation and immune status of cases

Maximum number of bacteria was seen in those with CD4 counts less than 200 cells/mm^3.The prevalence of bacteria was lesser in CD4 counts of >200 cells/mm^3. Of 80 cases *E.Coli* were identified in 8 patients with CD4 <200 cells/mm^3 and *E.Coli were* seen in 4 patients with CD4 >200 cells/mm^3 . The other two organisms that is *Shigella sps and klebseilla* were isolated at CD4 <200 cells/mm^3.

Studies by Attili *et al*[1] also showed highest prevalence of bacterial infection in patients with a low immune level CD4 < 200 / mm^3

The diagnostic yield of stool analysis is low in patients with higher CD4 cell counts. The probable reasons may be[1]

1. Effective HAART helps eradicating bacterial infection, and associated with the influx of CD4 positive cells into the lamina propria.
2. As the opportunistic infections causing diarrhea in AIDS become less common, other gastrointestinal diseases, which are common in young age group, like inflammatory bowel disease and coeliac disease, irritable bowel syndrome and idiopathic steatorrhoea are presently leading the list of etiological agents.
3. Variety of unknown/unidentified infections or HAART-related toxicities.

CONCLUSION

Individuals with HIV/AIDS, because of their compromised immune status are at a higher risk of infections and especially enteric bacterial infection and produce overwhelming results with grave prognosis.

As bacteria cause prolonged, life-threatening diarrhea in AIDS patients, identification of these bacteria at the earliest will enable the clinician to give effective treatment and save the patient from increasing mortality.

Escherichia coli bacterial diarrhea were found to be more common in HIV. Patients followed by *Shigella sps* and *klebsiella* organisms.

HIV-infected individuals with lower immunity, as indicated by CD4 counts, suffered more with diarrhea, especially in chronic form. Bacteria were common in lower immune status. Highest rate of infection was seen with CD4 counts 100-200 cells/mm^3.

So screening for bacterial pathogens for diarrhea in AIDS patients especially at lower CD4 count should be performed routinely for the management purpose.

SUMMARY

- Stool samples of randomly selected 80 HIV seropositive subjects , in Bijapur, were examined for bacterial pathogens. Stool culture for samples were done. CD4 cell counts were done for HIV infected individuals and were used as indicators of immune status to analyze the results obtained.

- Bacteria were identified in 21(26.25%) cases. Cases with chronic diarrhea had higher infection rates (76.19%) than those with acute diarrhea (23.81%).

- *E. coli* was the commonest bacteria identified in the cases 14(17.5%) followed by *Klebsiella* which constituted about 4(5%) and *Shigella Sps*. which constituted 3(3.75%).

- CD4 cell counts were found to be lower in cases with diarrhea ; and lower in those with chronic diarrhea (less than 200) than those with acute diarrhea (more than 200).

- Maximum number of opportunistic bacteria was seen in cases with CD4 counts between 100-200 cells/mm^3.

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ANNEXURE

CASE PROFORMA

Particulars of patient:

1.Name

2. Age

3. Sex

4. Address

5. Religion

6. Occupation

7. Socio-economic status:

8. Literacy: Literate/ Illiterate:

9. History of present illness:

I.Presenting complaints

- Diarrhea –

Frequency - /day

Duration –

Consistency of stool –

Others – Blood/ Mucus/ Pus/ Frothy

- Other gastrointestinal symptoms – pain abdomen/ nausea/ vomiting/ others
- Weight loss: < 10% , > 10% Duration
- Fever: Duration: < 1 month > 1 month
- Other symptoms

II. Past history

History of tuberculosis - Yes / No

History of anti-tubercular treatment

Antiretroviral therapy

III. Family history

IV. Personal history: Married/ Unmarried

A) History of exposure

B) History of blood transfusion

C) History of IV drug abuse

D) History of occupational exposure

V. Clinical Examination

a) General Physical Examination

b) Systemic examination

CVS:

RS:

PA:

CNS:

VI. Clinical Diagnosis

VII. Investigation

A. HIV antibodies detection

a) HIV EIA Comb

b) ELISA

c) HIV Bidot

d) Tri Dot

B. Stool examination

1) Macroscopic examination
    - Colour
    - Consistency
    - Blood / Mucous
    - Presence of segments
2) Microscopic examination
3) Microbiological Report

C. CD 4 count

REPORT

SIGNATURE

Date:

CONSENT FORM

TITLE OF RESEARCH	**:**	**"IDENTIFICATION OF BACTERIA CAUSING DIARRHEA IN HIV/AIDS PATIENTS & ITS CORRELATION WITH CD4 COUNT"**
GUIDE	**:**	**DR. ANAND . P. AMBALI**
P.G. STUDENT	**:**	**DR. SACHIN**

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to identify bacteria causing diarrhea in hiv/aids patients and to co-relate it with CD4 count

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved and I may experience mild pain during the above-mentioned procedures.

BENEFITS:

I understand that my participation in this study will help in correlate CD4 count to the causative bacteria of diarrhea in HIV/AIDS patients.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations

of hospital. If the data is used for publications the identity of the patient will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw for study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further compensations.

(Signature of Guardian) (Signature of patient)

(If the patient is conscious, well oriented and fully aware)

ETHICAL CLEARANCE CERTIFICATE

Ethical Committee BIJAPUR-586103 OUT WARD 27/2011 Date 21-10-11 B.L.D.E.A's M. PATIL Med.Col College

B.L.D.E. UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 20-10-2011 *at* 10-30 am *to scrutinize the Synopsis/Research projects of postgraduate/undergraduate student/Faculty members of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis/Research project has been accorded Ethical Clearance.*

Title "Identification of bacteria causing Diarrhoea in HIV/AIDS patients & its correlation with CD4 count"

Name of P.G./U.G. student/Faculty member Dr. Sachin
Dept of Medicine

Name of Guide/Co-investigator Dr. A.D. Ambali, Assoc Prof Medicine

DR.M.S.BIRADAR,
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.
Chairman
Ethical Committee
BLDEA'S Shri. B.M. Patil
Medical College
Bijapur-586103

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

KEY TO MASTERCHART

CD4	-	CD4 T lymphocyte count (cells/mm^3)
D	-	Days
EC	-	*Escherichia coli species*
F	-	Female
K	-	*Klebsiella*
M	-	Male
Mo	-	Months
NEPBI	-	No enteropathogenic bacteria isolated
S	-	*Shigella species*
W	-	Weeks
Y	-	Yes

MASTER CHART

Sl. No	Name	Age (in yrs)	Sex	Duration	Other complaints			CD4 count	Isolate
					Pain Abd.	Fever	Vomiting		
1	RENUKA	50	F	3 D	Y	Y	-	163	EC
2	SHARANAPPA	45	M	2 Mo	Y	Y	-	171	NEPBI
3	MANDAKINI	45	F	1 W	-	Y	Y	324	NEPBI
4	SHIVANAND	30	M	1 W	Y	Y	-	38	NEPBI
5	CHANNNAGOUDA	40	M	4 D	-	Y	-	149	NEPBI
6	UMESH	41	M	1 W	Y	Y	-	90	K
7	RAVI	43	M	1 Mo	Y	-	-	98	NEPBI
8	MALLIKARJUN	40	M	1 W	Y	Y	-	256	NEPBI
9	PAVADEPPA	50	M	2 D	-	Y	-	109	NEPBI
10	RENUKA	30	F	1 W	Y	Y	-	17	EC
11	BASAVARAJ	35	M	4 D	Y	-	-	85	NEPBI
12	HANAMANTH	40	M	3 D	Y	-	-	95	NEPBI
13	VITTAL	55	M	3 D	-	Y	Y	95	EC
14	ASHOK	32	M	2 D	Y	Y	Y	33	NEPBI
15	NINGAPPA	35	M	4 D	Y	-	Y	184	NEPBI
16	BASAVANTRAY	60	M	1 W	Y	Y	-	43	NEPBI
17	SAVITA	22	F	3 D	-	Y	Y	16	NEPBI
18	SHIVAPUTRAPPA	42	M	5 D	Y	Y	Y	134	NEPBI
19	LAXMIBAI	27	F	4 D	-	Y	Y	138	NEPBI
20	NAJIMA	38	F	2 D	Y	-	Y	95	NEPBI

Sl. No	Name	Age (in yrs)	Sex	Duration	Other complaints			CD4 count	Isolate
					Pain Abd.	Fever	Vomiting		
21	CHANADRAKANT	35	M	4 D	-	Y	-	65	NEPBI
22	CHIDANAND	39	M	3 D	Y	Y	Y	169	EC
23	KALLAPAA	37	M	4 D	Y	-	-	254	NEPBI
24	SIDAPPA	30	M	3 D	Y	Y	-	320	NEPBI
25	SURESH	42	M	5 D	-	Y	Y	169	NEPBI
26	BASVARAJ	40	M	1 W	-	-	Y	112	NEPBI
27	CHANASANGAYYA	52	M	1 W	Y	Y	-	160	S
28	MALLAPPA	33	M	1 W	-	Y	Y	806	NEPBI
29	SHAILA	28	F	3 D	-	-	Y	544	NEPBI
30	RENUKA	30	F	15 D	Y	Y	-	469	NEPBI
31	ANAND	39	M	3 D	Y	-	-	257	EC
32	SRIDEVI	35	F	5 D	-	Y	Y	482	NEPBI
33	TANGAMMA	26	F	5 D	Y	-	-	371	NEPBI
34	RAMANNA	40	F	1 W	Y	-	Y	212	EC
35	SUNANDA	22	F	15 D	-	Y	Y	178	S
36	JAYASHREE	26	F	3 Mo	-	Y	-	449	NEPBI
37	SATTAVVA	35	F	2 D	-	-	Y	202	EC
38	MAHESH	39	M	3 Mo	-	Y	Y	173	S
39	BASVARAJ	33	M	2 Mo	-	Y	-	16	EC
40	NASEEMA	35	F	2 Mo	Y	Y	-	84	EC
41	ELLAVVA	35	F	2 Mo	Y	-	-	37	EC

Sl. No	Name	Age (in yrs)	Sex	Duration	Other complaints			CD4 count	Isolate
					Pain Abd.	Fever	Vomiting		
42	RENUKA	40	F	20 D	-	Y	-	225	NEPBI
43	ALLABAKSHA	40	M	1 Mo	Y	-	-	192	EC
44	NIJJAVVAA	45	F	1 Mo	-	Y	Y	92	EC
45	MEENAKSHI	38	F	2 Mo	-	Y	-	101	EC
46	LAKSHMI	30	F	1 Mo	-	-	-	122	K
47	PARASHURAM	30	M	1 Mo	Y	Y	Y	155	S
48	BASAVARAJ	40	M	1 Mo	Y	Y	-	112	NEPBI
49	SAYYAVVAA	47	F	1 W	-	Y	-	110	EC
50	JAYDEVI	35	F	1W	-	-	Y	336	NEPBI
51	KASTURI	40	F	2 D	-	-	Y	460	NEPBI
52	BASAVACHALVADI	45	M	1 Mo	-	Y	-	43	K
53	NAGAMMA	38	F	1 W	-	Y	-	210	NEPBI
54	BHIMAPPA	38	M	3 D	-	Y	-	483	NEPBI
55	RAJANNA	38	M	1 Mo	-	-	Y	109	EC
56	ARVIND	55	M	1 Mo	Y	-	-	117	K
57	MALLAPPA	63	M	3 D	-	Y	Y	280	NEPBI
58	RENUKA	26	F	1 Mo	-	Y	-	76	K
59	MANJULA	30	F	1 W	Y	-	-	268	EC
60	SAROJINI	35	F	2 D	-	-	Y	303	NEPBI
61	IMRAN	27	M	1 W	-	-	Y	163	NEPBI
62	MAHANTESH	42	M	1 W	-	Y	-	95	EC

Sl. No	Name	Age (in yrs)	Sex	Duration	Other complaints			CD4 count	Isolate
					Pain Abd.	Fever	Vomiting		
63	MAHADEVI	50	F	2 D	Y	-	-	464	NEPBI
64	UMAMETRI	27	F	5 D	Y	-	-	257	EC
65	SHIVANAND	55	M	1 Mo	-	Y	-	47	NEPBI
66	MEGHA	28	F	1 Mo	-	Y	Y	71	NEPBI
67	MALLAPPA	35	M	2 Mo	-	Y	-	49	EC
68	IJAJ	35	M	1 Mo	-	-	Y	34	EC
69	RENUKA	32	F	2 D	Y	-	-	493	NEPBI
70	VEERUPAKSHA	44	M	2 D	-	Y	Y	134	NEPBI
71	GANAPATI	40	M	2 D	Y	-	-	106	NEPBI
72	SHANTAMMA	45	F	1Mo	-	Y	-	101	NEPBI
73	KALLAPPA	32	M	1 Mo	Y	-	Y	116	EC
74	MALLAPPA	36	M	3 D	Y	-	-	277	NEPBI
75	PANDU	44	M	1 W	-	Y	-	432	NEPBI
76	SADASHIV	45	M	1 W	-	Y	-	182	NEPBI
77	SIDDU	52	M	1 Mo	-	Y	-	110	EC
78	KASTURIBAAI	55	F	4 D	Y	-	-	320	NEPBI
79	MAHESH	38	F	4 D	-	-	Y	270	NEPBI
80	SHANKRAPPA	42	M	5 D	-	-	Y	370	NEPBI