"HEMATOLOGICAL AND MORPHOLOGICAL CHANGES OF BLOOD CELLS IN HIV PATIENTS – AN INSTITUTIONAL STUDY"

By

Dr. KARTHIK KASIREDDY

DISSERTATION SUBMITTED TO THE SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

Under the Guidance of

Dr. MANJULA K M.D

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DEPARTMENT OF PATHOLOGY

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA, KOLAR-563101

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation/thesis entitled "**HEMATOLOGICAL AND MORPHOLOGICAL CHANGES OF BLOOD CELLS IN HIV PATIENTS – AN INSTITUTIONAL STUDY**" is a bonafide and genuine research work carried out by me under the guidance of **DR. MANJULA K** M.D, Associate Professor , Department of Pathology , Sri Devaraj Urs Medical College , Tamaka , Kolar.

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Dr.KARTHIK KASIREDDY

ABBREVATIONS

AIDS – Acquired immunodeficiency Syndrome

ANC – Absolute neutrophil count

ART – Anti retroviral therapy

AZT - Azidothymidine

CA – Capsid p24

CDC – Centre for disease control

CMI – Cell Mediated Immunity

CMV – Cytomegalo virus

CTD – Carboxy terminal domain

DNA – Deoxyribonucleic acid

ENV – Envelope proteins

ER – Endoplasmic reticulum

FDA – Food and drug administration

GP - Glycoprotein

HIV – Human immunodeficiency virus

IL - Interleukin

ITP – Immune thrombocytopenic purpura

MA – Matrix p17

MCV – Mean corpuscular volume

m RNA – messenger Ribonucelic acid

NC – Nucleocapsid p9

PEG – Poly ethylene glycol

PLT - Platelets

RBC – Red blood cell

RDW – Red cell distribution width

RNA – Ribonucleic acid

RT – Reverse transcriptase

SIV – Simian Immunodeficiency virus

TCR – T Cell receptor

TTP – Thrombotic thrombocytopenic purpura

VWF – Von willebrand factor

WBC – White blood cell

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ABSTRACT

ABSTRACT

HEMATOLOGICAL AND MORPHOLOGICAL CHANGES OF BLOOD CELLS IN HIV PATIENTS – AN INSTITUTIONAL STUDY

Background : The Human Immunodeficiency Virus (HIV) infection causes the Acquired Immunodeficiency Syndrome (AIDS). Besides infectious complications, several peripheral blood cell abnormalities have been reported in HIV infection, of which anaemia and neutropenia are reportedly the most common. Very Few studies have been done on the peripheral blood cell abnormalities of HIV infected persons, despite them being common manifestations of HIV infection and AIDS, which may have a considerable impact on the patient's wellbeing, and treatment. Hence this study is to emphasize the need to look for hematological features in HIV Patients to improve quality of life.

Objectives : 1) To study the Changes in Hematological parameters In HIV Patients 2) To study Morphological Changes of Blood cells In HIV Patients.

Methods : The prospective study was conducted from DECEMBER 2014 to AUGUST 2016 at R.L.Jalappa Hospital and Research Center Kolar. 101 confirmed HIV Positive cases were taken with Written informed consent. After taking a brief clinical history 1 to 1.5 ml of venous blood was collected in a sterile EDTA containing tube with universal precautions as per the guidelines of NACO , and it was processed in an automated analyser within 2 hours.

Results : HIV Infection affected the highly reproductive age group of 21-40 years and predominantly affected males in our study. Among the hematological manifestations , anemia (54.5 %) was the commonest. The commonest type of anemia in present study is normocytic normochromic anemia. Among the morphological changes the commonest morphological finding observed in our study was dysplastic neutrophils.

Conclusion : There was significant statistical correlation between declining CD4 counts and Normocytic anemia, leucopenia and Thrombocytopenia. Hence all HIV Patients should be investigated for complete blood count including hematologic and morphological assessment of blood cells to reduce mortality and morbidity .

Key words : HIV , AIDS , Anemia , Normocytic.

INTRODUCTION

INTRODUCTION

Acquired Immunodeficiency syndrome (AIDS) – THE GRAY PLAGUE , is an acquired profound defect in T cell mediated cellular immunity that is caused by a communicable retrovirus and exposes victims to life threatening opportunistic infections and predilection to develop an infective form of Kaposi's sarcoma and certain high grade lymphomas at a relatively young age.[1]

Acquired Immunodeficiency syndrome (AIDS) was first recognized in 1981 and Human immunodeficiency virus (HIV) was identified in 1983. Human Immunodeficiency Virus (HIV) infection is a global pandemic , with cases reported from virtually every country across the globe. The first case of HIV/AIDS in Bangladesh was detected in 1989. Currently , in asia there are about 4.9 million people living with HIV , with an estimated 2.5 million in india alone.[2]

HIV Infection is a multisystem disease , with hematological abnormalities amongst the most common clinicopathological manifestations with a wide range including impaired hematopoeisis , immune mediated cytopenias and coagulopathies , particularly in the later part of the disease.[3,4,5]

The consequences of these hematological problems are two fold ,first , they have major morbidity in themselves , adversely altering the patients quality of life. Second , they hinder the treatment of both the primary viral infection and the secondary infections and neoplastic complications.

The poor hematopoietic tolerance of the therapies often necessitates dose reductions , alteration of drug regimens , or interruption of therapies.

If the hematological complications are better controlled it can result in longer life spans.

The accurate measurements of CD4 Cell counts is essential for assessment of immune system of HIV Infected person as the pathogenesis of Acquired immunodeficiency syndrome is largely attributable to the decrease in the CD4 Lymphocyte counts. In general , hematological abnormalities progress in frequency and severity with the progression of the infection from the asymptomatic HIV carrier state to the later symptomatic stages of the disease.[6]

Granulocytopenias with or without lymphopenia occurs in the asymptomatic HIV carriers , children and adults with AIDS , while anemia and granulocytopenia tend to occur concomitantly with a severity that parallels the course of the HIV Infection.[7,8]

Thrombocytopenia can occur independently of other cytopenias and at all stages of HIV Infection.[8]

Very few studies have been done on the peripheral blood cell abnormalities of HIV Infected persons , despite them being common manifestations of HIV Infection and AIDS which may have a considerable impact on the patients well being and treatment.

Hence this study was done to emphasize the need to look for hematological and morphological features in HIV Patients to improve quality of life. We also tried to evaluate the relationship between various hematological manifestations and CD4 Cell counts.

OBJECTIVE OF THE STUDY

OBJECTIVES

1) To study the Changes in Hematological parameters in HIV Patients
2) To study the Morphological changes of blood cells in HIV Patients

REVIEW OF

LITERATURE

REVIEW OF LITERATURE

The origin of AIDS : By this time we know that the origin of AIDS has its roots in Africa. This is justified by certain simian immunodeficiency viruses - SIVs are closely related to HIV1 and HIV2 ,and almost an exact counterpart in a virus of the sooty-mangabey - a type of African monkey.[9]

The HIV2 connection to the sooty-mangabey – atype of African monkeyalmost justifies for animal to man transfer of HIV. The likely source of HIV 1 is more difficult to justify. The closest simian virus to HIV1 discovered till date exists in certain chimpanzees.[10]

Ofcourse it has not been proved that HIV originated from primates , an SIV was known to infect the humans.[11]

The earliest convincing evidence of HIV infection is from the success after scientists isolating the virus from plasma sample of an adult male who lived - what is now the democratic republic of congo in 1959. Scientists also believe that the ancestor of this strain dates back to the 1940's or 50's and had been introduced to humans a decade or more earlier.[12]

In 1981june and july , the most uncommon opportunistic infection , Pneumocystis carinii pneumonia , and an extremely rare skin tumor of endothelial cell origin , Kaposi s sarcoma , were first diagnosed in Newyork and California in the previously healthy young homosexual and bisexual adult men who were previously not known to be predisposed to these conditions.[13]

As the cases started increasing , it was soon recognized that other neoplastic diseases and life threatening infections were also reported and had an association with an unexplained cell mediated immunity defects.[13]

In early 1982 ,The Centre for Disease Control (CDC) named the group of disease entities as Acquired Immune Deficiency Syndrome (AIDS).[14]

After the original definition of AIDS in september1982 , the CDC subsequently revised this definition to accommodate some other additional syndromes which has been recognized as manifestations in advanced HIV disease.[14]

AIDS is known to be caused by an unknown human retro virus , which was earlier discovered & isolated in 1983 by patients suffering from generalized persistent lymphadenopathy at the "Institut Pasteur" in Paris.[13]

All the related group of viruses which were identifiedwere named as the Human Immunodeficiency Virus (HIV) by the international committee on the taxonomy of viruses in 1986.[15]

CLASSIFICATION :

The CDC Classification of HIV Diseases was initially categorized HIV related symptoms into four groups which was intended mainly for "public health purposes" and not as a staging system , but still it was frequently treated as it was a staging system in AIDS Literature.[15]

The present CDC classification system after the revision in 1993 , combines 3 categories of the CD4 cell counts with 3symptom categories and is nearer to a staging system but still it is not described as such.[14]

CD4 + T Lymphocyte categories

Category 1 :> 500 cells/c.mm (or CD4 % > 28%)

Category 2 : 200 - 499 cells/c.mm (or CD4 % 14% - 28%)

Category 3 :< 200 cells/c.mm (or CD4 % < 4%)

Categories of clinical conditions :

Category A :

HIV infection without any symptoms , generalized persistent lymphadenopathy , acute HIV Infection with coexisting illness or history of acute Infections and Conditions mentioned in category B and C have not occurred.

Category B :

Consists of HIV Infection with symptomatic conditions in an adult that have not been included among the conditions mentioned in clinical category C and should at least meet one of the following criteria : a) the conditions that has been attributed to HIV Infection or due to cell mediated immunity defects , or b) the conditions which are considered by physicians require management and to prevent complications by HIV Infection.

Category C :

Includes the clinical conditions mentioned in the 1993 AIDS surveillance case definition. For classification purposes ,if a patient diagnosed as category C, the person will remain in category C.

Clinical categories/CD4 +	A	B	C
1	A1	B1	C1
2	A2	B2	C2
3	A3	B3	C3
Persons in categories A3 , B3 , C1, C2 and C3 have AIDS under the 1993 surveillance case definition			

HIV Disease is a continuum of gradual progressive damage of the immune system from the time of initial infection to the manifestation of severe immune compromise by oppurtunistic infections , neoplasms , wasting or low CD4 Lymphocyte Count that define AIDS. Almost all infected persons have a CD4 cell counts below the mean than compared to seronegative persons and show a progressive deterioration of these cells with time.[16]

The median incubation period from initial HIV infection until development of AIDS is estimated to be approximately 10 years for young adult persons.[17]

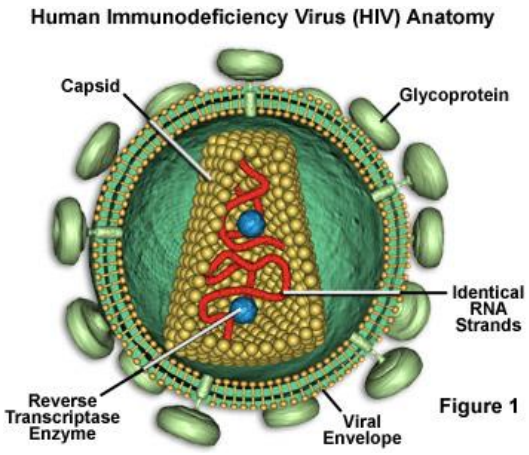

Figure 1

Provirus ,The integrated form of HIV 1 , is approximately 9.8 kilobases in length. The genes of HIV encode atleast 9 proteins and are located in the central region of the proviral DNA.

Structural Proteins :

Gag Proteins :

The gag gene gives rise to p55 which is a 55 kilodalton gag precursor protein ,and it is expressed from the unspliced viral mRNA . After budding , during the process of viral maturation p55 is cleaved by the virally encoded protease into four smaller proteins designated MA (matrix[p17]) , CA (capsid[p24]) , NC (nucleocapsid[p9]) , and p6.

Most MA molecules stabilizes the particle by remaining attached to the inner surface of the virion lipid bilayer. However a small percentage of MA binds integrase and is thereby recruited inside the deeper layers of the virion.[18]Akaryophilic signal on MA is recognized by the cellular nuclear import machinery and these MA molecules subsequently facilitate the nuclear transport of viral genome. This phenomenon allows HIV to infect non dividing cells , an unusual property for a retrovirus.[19]

The conical core of viral particles is formed by p 24 protein .Cyclophilin A interacts with the p24 region of p55 leading to its incorporation into HIV Particles.[20]

For specifically recognizing the so called packaging signal of HIV[21]the NC region of gag is responsiblr. The packaging signal consists of four stem loop structures located near the 5 end of the viral RNA , and is sufficient to mediate the incorporation of a heterologous RNA into HIV 1 Virions.[22] NC also facilitates reverse transcription.[23]

Gag Pol Precursor :

Expression of the viral protease , integrase , RNAse H , and reverse transcriptase are always within the context of a gag-pol fusion protein. The virally encoded protease during viral maturation cleaves the pol polypeptide away from gag and further digests it to separate the protease (p10) , RT (p50) , RNAse H (p15) , and integrase (p31) activities.

HIV 1 Protease :

The HIV 1 Protease is an aspartyl protease that acts as a dimer .During virionmaturation ,protease activity is required for cleavage of the gag and gag-pol polyprotein precursors.[24]

Reverse Transcriptase :

The Pol gene encodes reverse transcriptase. During reverse transcription , the polymerase makes a double stranded DNA copy from single stranded genomic RNA present in the virion.

Integrase :

The integrase protein mediates the insertion of the HIV Proviral DNA into the genomic DNA of an infected cell.

Envelope proteins :

The 160 kDenv (gp160) is expressed from singly spliced m RNA. gp 160 is cleaved by cellular protease to generate gp 41 and gp 120. gp 41 contains the transmembrane domain of env , while gp 120 is located on the surface of the infected cell and of the virion through non covalent interactions with gp 41. On the surface of the cell of the virionEnv exists as a multimer ,most

likely a trimer. Interactions between HIV and the virionreceptor , CD4 , are mediated through specific domains of gp 120.[25]

Regulator proteins :

Tat :

Tat is is essential for HIV 1 replication as it is a transcriptional transactivator. Tat is an RNA binding protein , unlike conventional transcription factors that interact with DNA.[26] Mechanism of tat function remains controversial. In fewstudies , it appears that tat acts principally to promote the elongation phase of HIV 1 transcription[27],While Other studies indicate that tat may be involved in the phosphorylation of the carboxy terminal domain (CTD) of RNA Polymerase II.[28]

Rev :

Rev is a 13 kD sequence specific RNA binding protein .rev acts to induce the transition from the early to the late phase of HIV gene expression.

Accessory proteins :

Nef :

Nef has been shown to have multiple activities , including the down regulation of the cell surface expression of CD4[29], the perturbation of T cell activation[30] , and the stimulation of HIV Infectivity.[31]

Vpr :

The Vpr protein is incorporated into viral particles. Vpr plays a role in the ability of HIV to facilitate nuclear localization of preintefration complex and infect non dividing cells.[32]Vpr can also block cell division.[33]

Vpu :

HIV 2 does not contain Vpu , but instead harbors another gene , vpx. The 16 kDVpu is localized in the internal membranes of the cell and this polypeptide is an integral membrane phosphoprotein.[34] In HIV Infected cells , complexes are formed between the viral receptor , CD 4 and the viral envelope protein in the endoplasmic reticulum causing the trapping of both proteins to within this compartment. Virion assembly is integrated by the formation of intracellular Env CD 4 complexes. Vpu liberates the viral envelope by triggering the degradation of CD 4 molecules complexed with Env[35]. Vpu also increases the release of HIV from the surface of an infected cell.[36]

Vif :

Vif is a 23 kD polypeptide and is essential for the replication of HIV in peripheral blood lymphocytes , macrophages and certain cell lines.

The regulation of HIV gene expression :

The regulation of HIV gene expression is accomplished by a combination of both cellular and viral factors. At both the transcriptional and post transcriptional levels HIV gene expression is regulated. The HIV genes can be divided into the early genes and the late genes. The early genes , Tat , Rev and nef are expressed in a rev independent manner. The m RNAs encoding the late

genes , gag , pol, env , vpr , vpu and vif require rev to be cytoplasmically localised and expressed.

TRANSMISSION :

Sexual Transmission :

The present world wide spread of the AIDS epidemic is primarily associated with sexual transmission of human immunodeficiency virus type 1 (HIV 1) And its future focus to reduce the spread of HIV by sexual transmission with appropriate measures.[37] Sexual transmission among the heterosexuals has been the dominant mode of spread in southern continents like asia and Africa unlike homosexual transmission in USA.[38]

HIV is more commonly transmitted by sexual penile anal intercourse and penile vaginal intercourse and less infrequently by fellatio. Penile Vaginal intercourse can transmit HIV to either the male or female, but risk is always higher to the female partner.[39]

A metanalysis of many studies done on HIV Transmission showed that condom efficacy was 69 % overall.[40]And with the zidovudine therapy there was decreased detection of the HIV 1 in semen.[41]

Injection drug use related HIV Infection :

The HIV Transmission among injection drug users occurs mainly through the contamination of HIV Infected blood by injection paraphernalia , which is again reused by an uninfected persons. Among all the risk factors the more risk is with the sharing of needles , syringes and other injection related equipment. Sharing is still the common practice in injection drug users through out the world.[42]

Transmission of HIV by blood , blood products , tissue transplantation and artificial insemination[43]

The infected persons can transmit the HIV 1 Virus along with other viruses through blood transfusion which was subsequently processed intodifferent blood components (i.e whole blood , fresh frozen plasma , packed red cells , cryoprecipitate and platelets)[44]

Vertical transmission :

The source of all new HIV diagnosed children is mainly by the Perinatal transmission of human immunodeficiency virus.[45]

HIV Testing :

The most common screening method which is also cost effective and accurate followed for HIV test is Testing serum for antibodies to HIV with a standard ELISA (followed by a confirmatory western blot).[46]The other tests which are being marketed and approved by the food and drug administration (FDA) include Rapid serum HIV antibody tests , saliva and urine based antibody tests , and home HIV antibody testing kits .[47] HIV RNA tests are being used in clinical research settings to diagnose primary HIV Infection before detectable antibodies formation.[48]

Virus Entry :

Several studies showed that CD 4 serves as a binding receptor for HIV 1 , with high affinity by binding to gp 120.[49]gp 120 is viral surface envelope protein. The Mechanism of post binding events for HIV 1 and cell membrane fusion are not clearly understood. HIV 1 like most other retroviruses , infects cells in a p H independent manner by direct fusion between viral and cell surface membranes.[50]

Reverse Transcription :

The reverse transcription pathway produces a linear DNA copy of the viral RNA genome[51]. This step occurs within a viral nucleoprotein complex and this step requires the coordinated activities of enzyme reverse transcriptase , an RNA and DNA dependent DNA polymerase , and RNAase , which degrades the RNA component of RNA-DNA hybrid molecules as shown in figure 2. As the viral nucleoprotein complexes are transported rapidly to the host cell nucleus , the majority of viral DNA Synthesis take place within the nuclear compartment.

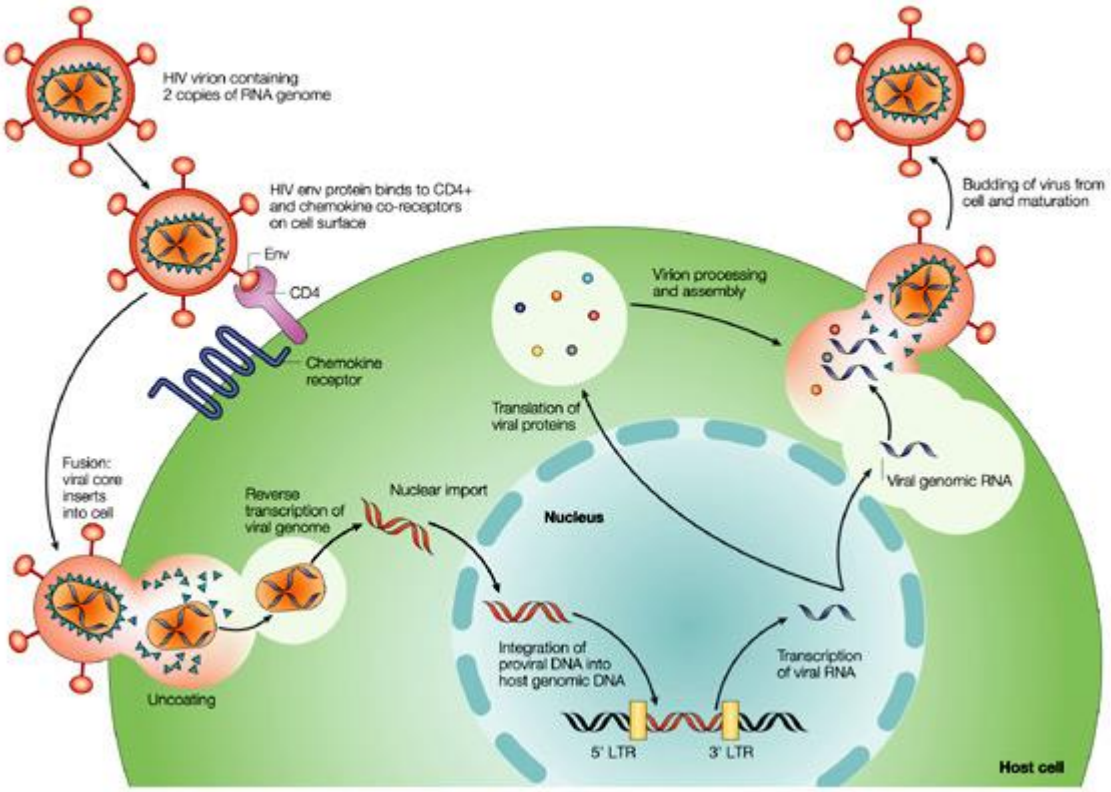

Figure 2 : showing HIV Replication

Integration of the viral DNA into cellular genomic DNA :

The nuclear viral complexes work like machines that integrate viral DNA into host cell chromosomal DNA to form a provirus. This step is essentially dependent on the activity of the viral integrase protein and is required for viral gene expression.[52]

Viral protein expression :

The viral genes expression requires the collaborative activities of the viral regulatory proteins (tat and rev) and host cell transcription machinery (RNA polymerase and transcription factors Sp 1 and NFkB).

Viral assembly :

The attachment of viral gag and gag pol precursor proteins requires N- terminal cotranslational addition of myristic acid to viral MA proteins.[53]ofcourse MA gag protein contains the membrane binding domain and can induce membrane budding , the incorporation of gag and gag-pol precursor proteins into functional viral particles requires the presence of interaction domains of gag and a late acting L domain of the p6 gag protein.[54]

Expression of viral envelope proteins :

Retroviral envelope proteins are synthesized in the endoplasmic reticulum (ER) of HIV infected cells and are transported to the cell surface through the host cell secretory pathway. Within the endoplasmic reticulum , monomers of gp 160 , the precursor HIV envelope protein , associate with BiP , a molecular chaperone , before folding and oligomerisation. Oligomericgp 160 complexes arethen transported from endoplasmic reticulum to golgi apparatus , where it is cleaved by cellular proteinase to produce the surface gp 120 and transmembrane gp 41 subunits

before it gets transport to cell surface.[55] The mature virions that are released from the virus producer cells are then competent to begin the replication cycle again in other target cells.

CLINICAL COURSE OF UNTREATED HIV DISEASE :

Primary infection :

Aborted HIV Infection :

Some individuals may successfully get rid of infection after inoculation and the mechanisms suggested are ,

1) Defective co-receptor needed by Virus to infect cells.

2) Strong Immune response capable of preventing HIV from establishing infection.

3) SDF-1 gene mutations.

Establishing infection[56] :

Once the virus Spread to tissues and cells its hard to eliminate viral reservoirs and the consequences of infection establishment include Extensive damage to lymph node cellular architecture ,Stimulation of an immune response against HIV.

Loss of HIV specific CD4+ and possibly CD 8+ cell clones that may be effective in controlling HIV Infection.

Rapid HIV replication and mutation results in more genetically diverse population of HIV genomes , some of which are more virulent , or more adapted to undergo replication in other micro environments such as coexisting drug therapies and anti HIV cytotoxic lymphocyte clones.

Early impact on T cell clones , T cell diversity , and loss of members of the T cell repertoire :The most important factor determining disease progression is the extent of very early destruction of the sub population of CD 4+ T cell clones which are capable of recognizing HIV antigens. Loss of these clones results in loss of the CD 4+ T cell , which is essential in controlling HIV replication.[56]

The syndrome of primary HIV infection :

Most patients experience presents with acute syndrome within weeks of primary HIV infection and Syndrome persists for several weeks.[57]

Early and middle stages of HIV disease :

HIV antibodies begin to rise resulting in drop of detectable levels of virus , HIV RNA , and viral antigens in peripheral blood. This is called "set point", and is relatively stable for months , perhaps years. Typically there is reduce in the CD 4 count , from normal levels to 200 to 300 cells/cmm which can explain generalized lymphadenopathy syndrome but why lymphadenopathy is prominent only in some patients remains unclear.[58]

Advanced HIV disease :

Untreated patients with advanced HIV disease typically have CD 4 counts less than 200 cells/cmm , increased plasma HIV RNA levels , and clinical features indicative of severe immunocompromised state , qualifying as CDC defined AIDS .

Late stage HIV disease :

In late stages of HIV disease the CD 4+ count drops below 50 cells/cmm ,can be associated with opportunistic infections , non Hodgkins lymphoma , kaposis sarcoma may become extensive and cause disfigurement and clinically significant edema.

Death eventually results from secondary involvement of organs by virus , most commonly the lungs , due to effects of circulating toxins , electrolyte abnormalities , hematopoietic and circulatory failure , and autonomic nervous system damage.

HEMATOLOGIC MANIFESTATIONS OF HIV INFECTION :

Significant hematologic abnormalities are commonly seen in HIV Patients. Impaired hematopoiesis , immune mediated cytopenias , and altered coagulation mechanisms are all described in HIV infected individuals. These abnormalities may occur not only as a result of HIV infection itself but also can be asequelae of HIV related opportunistic infections or malignancies , or as a consequence of the therapies used for HIV infection and the associated conditions.

Anemia :

Anemia is a very common finding in HIV patients , particularly with more advanced Disease. In a study done by Zon li et al (1987) , 8% of asymptomatic HIV seropositive patients , 20 % of those with symptomatic middle stage HIV disease , and 71 % of those with Centre for disease control (CDC) defined AIDS were having anemia at presentation.[59] In a study done by spivac et al (1984) 18% of asymptomatic HIV seropositive patients , 50 % of those with symptomatic middle stage HIV disease , and 75% of those with CDC defined AIDS were having anemia[5]. The multicenter AIDS cohort study found that 3.2 % of HIV patients with mean CD 4 + T

lymphocyte counts above 700 cells/cmm were anemic ,compared to 20.9 % of patients with mean CD4 + T lymphocyte counts below 249 cells/cmm.[3,60]

HIV infection alone , without other complications can produce anemia in some patients. A study of serum immunoreactive erythropoietin in HIV infected patients in various stages of illness showed failure to rise commensurately with increasing anemia suggesting that insufficient amounts of erythropoietin may be one of the cause of anemia.[61]According to some Other studies soluble factors in the serum of HIV patients may play a role in inhibiting hematopoeisis , or , marrow progenitor cells may play a role in producing anemia and other hematologic abnormalities.[62,63]

Drug induced anemia :

Among the drugs ,Zidovudine is most common cause of anemia in HIV patients. In the original phase II clinical trials regarding the role of AZT in advanced HIV patients there was statistically significant reductions in hemoglobin levels (34%) in subjects receiving AZT (1200 mg perday) following 6 weeks of therapy[64] accompanied by a progressive rise in erythrocyte mean corpuscular volume (31%) . AZT Therapy is also associated with Marrow erythroid hyperplasia , aplasia , and megaloblastic maturation. Other studies have demonstrated that anemia is less common in patients with relatively less advanced HIV disease and in those receiving reduced dosages of AZT.[65] More recent studies showed antiretroviral combined therapy have relatively low incidence of severe anemia at low dose of zidovudine.[66]

The treatment for AZT induced anemia is recombinant human erythropoietin. A double blind , placebo-controlled study showed that recombinant human erythropoietin (100 units/kg 3 times

weekly by intravenous bolus) reduced transfusion requirements of AZT treated HIV patients whose serum levels of endogenous erythropoietin were below 500 IU per litre.[67]

Antimicrobial and antineoplastic agents used for prophylaxis also one of the cause of anemia. For example ,Dapsone for treatment or prevention of Pneumocystis Carinii Pneumonia (PCP) may cause hemolytic anemia or generalized myelosuppression[68] , and Anemia is a common finding when myelosuppressive chemotherapy is used to treat HIV related Non-Hodgkins lymphoma.

Anemia caused by bone marrow infections :

Another common cause of anemia in advanced disease is Infection with Mycobacterium avium complex (MAC) causing wide spread[69] ,disseminated infection , usually involving the bone marrow. In such patients , anemia tends to occur out of proportion to other cytopenias. The role of the antimycobacterial therapies currently available for MAC infection is controversial and showed improvement of anemia.

B19 Parvovirus infection - the etiologic agent of the childhood exanthema "fifth disease"(erythema infectiosum) can also cause anemia in HIV patients[70,71]. The anemia of parvovirus infection can be treated with immunoglobulin infusions (400 mg/kg/day over 5 to 10 days).

Tuberculosis ,Cryptococcosis , Histoplasmosis , Pneumocystosis and Non Hodgkins lymphoma can all infiltrate the bone marrow , generally causing pancytopenia.

Other causes of anemia :

In 20 % of HIV Patients with hypergammaglobulinemia ,Antierythrocyte antibodies produce a positive direct antiglobulin test [72] but Hemolytic anemia is rare.

Apart formusual causes of gastrointestinal blood loss , HIV related infections such as cytomegalovirus colitis , Kaposi s sarcoma , non hodgkins lymphoma can produce clinically significant bleeding. So Gastrointestinal bleeding should also be considered.

Thrombocytopenia :

Thrombocytopenia is more commonly seen with HIV infection. In the multicentre AIDS cohort study , of 1500 HIV patients , 6.7 % of patients had platelet counts below 150,000 cells /cmm on at least one semiannual visit , and 2.6 % of all patients had platelet count below 150,000 cells/cmm on two successive semiannual visits.[60] In a study done by Murphy et al (1987) ,thrombocytopenia is seen in 30% of patients with advanced HIV disease and 8% of patients with generalized persistent lymphadenopathy.[73] In a study done by zon Li et al (1987) ,Thrombocytopenia was seen in 15% in asymptomatic HIV patients and 40% in patients with AIDS.[59]

The possible cause of thrombocytopenia in patients with HIV infection include immune mediated destruction , Thrombotic Thrombocytopenia Purpura , impaired hematopoiesis , and toxic effects of medications.

In many other Instances , however , thrombocytopenia is a relatively isolated hematologic abnormality associated increased or normal number of megakaryocytes in the bone marrow and

increased levels of platelet associated immunoglobulin. Most of These patients have the clinical syndrome commonly referred as Immune Thrombocytopenic Purpura (ITP).

HIV Related immune thrombocytopenic purpura :

HIV-ITP manifests earlier before the occurance of any CDC AIDS defining condition.[74]

CD4 + Lymphocyte counts in reported series with HIV-ITP patients have ranged between 300 and 600 cells/cmm. HIV-ITP is therefore commonly included among those conditions associated with thethe middle stage HIV disease. ITP gradually improves as HIV disease progresses.

Several hypothesis have been formulated to possibly explain the pathogenesis of HIV-ITP. One theory says circulating immune complexes are depositednon specifically on platelet membranes , resulting in reticulo endothelial clearance. Studies also showed that these immune complexes contain anti-HIV gp 120 and complementary anti-idiotypic antibody. The hypothesis saying specific antiplatelet antibody binds to the platelet membrane , resulting in platelet destruction , is no longer considerable.

Another theory for impaired platelet dysfunction is that the HIV Virus directly infects megakaryocytes.[75]

Thrombotic thrombocytopenic purpura :

The classical features of TTP is pentad of fever , neurologic dysfunction , renal dysfunction , microangiopathic hemolytic anemia and thrombocytopenia.

Hyaline microvascular thrombi in tissue biopsy helps in diagnosis of TTP. The clinical and pathologic findings are justified byAbnormal interaction between platelets and endothelium. TTP is an early manifestation of HIV infection.

Plasmapheresis is accepted as standard therapy for TTP and other treatment options include plasma infusions , exchange transfusions , antiplatelet drug therapy , corticosteroids , and splenectomy.[76]

Other causes of thrombocytopenia in HIV infection :

Infectious & neoplastic conditions that involve the bone marrow and drugs for treatment of HIV cause generalized myelosuppression in patients with HIV infection can produce thrombocytopenia. HIV infected patients are also susceptible to developing thrombocytopenia for reasons unrelated to their HIV infection , such as alcohol use , splenomegaly , and liver disease or drug effects (heparin , quinidine).

Evaluation of patients with HIV infection and thrombocytopenia :

As in HIV infected patients with anemia , thrombocytopenia patients also should undergo a general evaluation to find out etiology. A bone marrow biopsy should be done to rule out cytotoxic or alcohol or drug related effects. The marrow should be examined for the presence of lymphoma or opportunistic infections such as fungi or mycobacteria that would cause reduced megakarocyte numbers and further causing reduced platelet production.

Other causes of peripheral platelet destruction should also be ruled out such as splenic sequestration resulting from liver disease with portal hypertension , drug induced ITP , lymphoma associated ITP , TTP , or disseminated intravascular coagulation.

Therapy :

Treatment of HIV-ITP should be started up for patients with clinically significant symptoms such as recurrent epistaxis , gingival or subconjunctival bleeding , or gastrointestinal hemorrhage

or petechial hemorrhages. Therapy is also advised for hemophiliac patients with HIV-ITP because of the substantial morbidity and mortality associated with bleeding. AZT and interferon-alpha therapy can increase the platelets while simultaneously providing antiretroviral activity. Therefore , these agents are gaining attraction for the treatment of HIV-ITP. Currently employed antiretroviral combined therapy should be able to ameliorate HIV-ITP through its ability by markedly reducing plasma HIV viremia.

For treatment of ITP patients without HIV Infection , treatment with corticosteroids , cytotoxic agents , Danazol , Intravenous Immunoglobulin Infusions , Plasmapheresis , Interferon alpha , and Splenectomy are all used with varying success rates. Many of these methods have also been used for treatment of HIV-ITP , but were relatively unsatisfactorily.

Granulocytopenia and abnormal granulocyte function :

In HIV Patients Granulocytopenia is commonly seen ,ofcourse low granulocyte counts usually indicate toxicity of HIV infection therapy related or other conditions , studies of untreated patients particularly in patients with more profound immunodeficiency have also shown high incidence of granulocytopenia. For example , in a multicenter AIDS Cohort study it was found that 0.8 % of HIV patients with mean CD4 + T lymphocyte counts of above 700 cells/cmm had abnormally low granulocyte counts whereas granulocytopenia was present in 13.4 % of those with mean CD 4+ T lymphocyte counts less than 249 cells/cmm.[3,60]In a study done by Zon Li et al (1987) he noticed that low granulocyte counts in 13 % of asymptomatic HIV seropositive patients and in 44 % of those with frank CDC-defined AIDS.[59]

In a study done by Murphy MF et al (1987) , the incidence of lymphopenia and neutropenia in patients with AIDS was 75 % and 20 % respectively and in patients with asymptomatic HIV

positive patients the incidence was 15 % and 0% respectively.[73] In a study by Castella A et al (1985) the incidence of granulocytopenia was around 75 %[8].

The pathogenesis of granulocytopenia in HIV patients is multifactorial. An auto immune mechanism involving antigranulocyte antibodies[73] and impaired granulopoiesis has been suggested.[35,36]Granulocytopenia can also be produced by Any infiltrative process involving the bone marrow (infection , malignancy). However drug toxicity is responsible for most of the granulocytopenia seen in patients with HIV Infection in clinical practice.

In another study the investigators found a positive correlation between the level of the absolute granulocyte count and the hospitalization risk for a significant bacterial infection in weeks immediately following the absolute neutrophil count (ANC)[77].

Drug induced Granulocytopenia :

The most common cause of low granulocyte counts in patients with HIV infection is probably AZT Therapy. In one placebo controlled study of advanced HIV Disease patients on therapy Severe granulocytopenia(< 500 cells/cmm) developed in 16 % and 2% of placebo treated patients developed granulocytopenia[37]. There were no reported episodes of bacterial infection or sepsis in the study group despite the relative high frequency of AZT Induced granulocytopenia. In subsequent studies on AZT therapy[38] , the observed risk of bacterial infection was low , justifying the brief duration of AZT induced granulocytopenia; the dosage of AZT was reduced or discontinued when the granulocyte count was in the range of 500 to 1000 cells/cmm.

In a study done by Shaunak and Bartlett severe recurrent (three or more episodes) AZT induced granulocytopenia is seen in 30 patients who are on treatment.[78]

Ganciclovir therapy for symptomatic cytomegalovirus infection is one of the cause of granulocytopeniain advanced HIV disease patients. Jacobson et al found absolute granulocyte counts of below 800 cells/cmm in 10 of 32 patients taking chronic daily maintenance ganciclovir therapy.[77]

Granulocytopenia also reported in patients receiving trimethoprim-sulfamethoxazole ,pentamidine and interferon-alpha treatments.

Impaired bone marrow function due to Antineoplastic chemotherapy is probably the most common cause of low granulocyte counts in patients with absent HIV infection. Post chemotherapy Granulocytopeniaalso , to a greater extent complicates treatment of HIV patients.

In summary , drug induced granulocytopenia is seen often in patients with HIV infection. When the granulocyte count is less than 500 cells/cmm , the risk of infection and sepsis increases significantly. Empiric antibiotic therapy can be started for patients with frank infection, or the granulocyte count is less than 500 cells/cmm.

Defective Granulocyte Function :

Qualitative functions of granulocytes from HIV patients are studied in vitro , and many abnormalities have been observed. Defective chemotaxis , deficient degranulation responses , and ineffective phagocytosis and killing have all been observed.[79]

Lymphopenia :

Increases in both CD 4 and CD 8 cell death and its functional impairement are the sine qua non of HIV infection. IL 2 partially corrects the impaired lymphocyte proliferation and cytotoxicity

seen with HIV infection and can also partially block the enhanced tendancy of lymphocytes obtained from HIV infected patients to undergo programmed cell death.[80]

CD 4+ T Cells :

Progressive loss in numbers of circulating CD 4+ T cells is seen in almost all cases of untreated HIV infection. The number of circulating CD 4+ T cells is widely used as a measure of global immune competence and serves as an indicator of the immediate risk for opportunistic infection[81]. Earlier in the course of infection , many HIV infected persons have used to present with generalized lymphadenopathy characterized by accumulation of lymphocytes within inflammed lymph nodes and upregulation of adhesion molecule expression.

Early in the course of infection , memory CD 4+ T cells are selectively depleted from circulation; as disease advances , CD 4+ T cells of both the native and memory phenotype are lost from circulation.[82] In advanced diseases , all CD 4 cell populations are depleted from circulation and from lymphoid tissue sites.

Functional abnormalities of CD 4+ T cells alsoindicate HIV Progression. Failure of CD 4+ lymphocytes to undergo cell division ,has also been demonstrated following stimulation of T cells from HIV Patients with antigens or mitogens in vitro. A sequential loss of immune responsiveness to recall antigens , followed by alloantigens and then mitogens has been Observed. Decreased expression of IL 2 is readily demonstrable.[83,84] In HIV patients and may be related to the proliferation defects. In contrast , expression of interferon-gamma by these cells is often unimpaired[85] , reflecting that the defective responsiveness is not due to depletion of antigen reactive cells but rather a selective impairement in the ability of these cells to respond after engagement of TCRs.

Using anti-TCR antibody stimulation to characterize proliferation defects in CD 4+ T cells showed proliferation defects in HIV diseases are associated with early G1 phase cell cycle arrest[86] and are more commonly seen in persons who have experienced sustained CD 4 cell losses.[87].CD 4+ T cells facilitates immune response though production of Immunomodulatory cytokines , the loss of these cells and the failure of remaining cells to function properly constitutes a critical impairement in immune capability. Specific CD 4+ T cell responses to HIV antigens appear to be selectively impaired during early HIV infection. However , the exact mechanisms by which HIV causes the loss of CD 4+ T cells are still unknown , and other cell lines – such as CD 8+ T cells , which are presumably resistant to HIV infection – are also gradually decreased over the course of infection.

Any or all of the following mechanisms may contribute to CD 4+ T cell loss , including virus mediated cell killing , immune mediated cell killing , chronic activation leading to the premature death of uninfected cells , generation of auto antibodies and impaired CD 4+ T cell production. Cells of the monocyte/macrophage lineage represent the other major target of HIV infection , but these cells are relatively unaffected by cytopathic effects.

CD 8+ T Cells :

In early HIV Infection , CD 8+ T cell numbers tend to increase , reflecting expansion of memory CD 8+ T cells , particularly HIV reactive cells. CD 8 cell expansions persist until late stages of HIV disease , when all T cell numbers tend to fall.[88] In contrast to memory CD 8 cell expansions , proportions of naïve CD 8 cells tend to fall in early infection , but absolute numbers of these cells do not fall until late stages of HIV disease.[82] For example , in earlier disease CD 8+ T cells that recognize Cytomegalovirus are present in large numbers , but in advanced disease the

cytolytic function of CD 8+ T cells directed against opportunistic pathogens is characteristically impaired.[89] It is not entirely clear whether the CD 8+ cells present in early disease are functionally "normal" , due to maturation phenotype CD 8+ T cells recognizing pathogen derived peptides is variably perturbed.[90]

Whether this is the cause or the consequence (or the interaction of both) of greater exposure to opportunistic pathogen – derived antigens in HIV infected immune-suppressed persons is difficult to sort out.

As is seen with CD 4+ T cells in HIV infection , CD 8+ T cells obtained from HIV infected persons may fail to proliferate in response to TCR activation in vitro.[91]

In this setting , however , it is not clear whether the failure to proliferate is a consequence of failure of CD 4+ T cell help (via provision of IL 2 that is essential for CD 8+ T cell proliferation) , a reflection of an intrinsic failure of CD 8+ T cell function , or a consequence of CD 8+ T cell maturation to a predominantly effector phenotype. As with cellular immune responses , the humoral immune system in HIV infection is characterized by paradoxical hyperactivation and hyporesponsiveness. Hyperactivation is reflected in dramatic polyclonal hyperglobulinemia , only a portion of which is directed against HIV antigens[92] , bone marrow plasmacytosis[93] , heightened expression of activation molecules on circulating B lymphocytes[94,95] , the presence of autoreactive antibodies in plasma[96] , and instances of clinical autoimmune like disease. B cell hyper reactivity may contribute to the increased risk of B cell lymphomas in HIV infected persons , but no casual link has been clearly established.[97]

The etiology of hyperglobulinemia is not well understood. Elevated plasma levels of the endogenous B lymphocyte stimulator are seen in HIV persons and this may contribute to the B

lymphocyte activation of HIV infection and AIDS.[98,99] At the same time , diminished B lymphocyte responsiveness to antigenic stimulation in vitro is characteristic of HIV infected persons[100,101,102] who often fail to develop protective antibody responses after immunization with protein or with polysaccharide vaccines.[103,104,105,106] The characterization of antibody responses to polysaccharides as "T Cell independent" is only partially correct. Although antibodies can be induced to polysaccharides in the absence of linked peptides that induce cognate help by proximate CD 4+ T cells , these responses are not optimal. Moreover , B lymphocyte responses to pure sugars still require some degree of T helper support. Lack of CD 4 help may therefore underlie the poor antibody responses to polysaccharides that are seen in HIV Infection.

Morphological Changes Of Blood Cells In HIVPatients :

Morphologic changes tend to be more pronounced in more immunosuppressed patients and increase in frequency as disease progresses. All lineages can be affected.[32] Using the morphologic criteria established for primary Myelodysplastic syndromes , dysplasia involving at least one lineage was diagnosed in 69 % of patients. Dysplastic changes increase with disease progression.

As noted previously ,cytopenias of all peripheral blood cells have been observed in patients with HIV infection. With the exception of thrombocytopenia , which can occur in asymptomatic individuals with relatively mild immune deficiency , anemia and leucopenia are more frequent and severe in patients with advanced immunodeficiency.[63]

Peripheral red blood cells in anemia patients are typically normochromic and normocytic and exhibit a varying degree of anisocytosis and poikilocytosis. The perturbation in RBC size and shape is reflected in an increased red cell distribution width (RDW). Macrocytosis is rarely seen.

However , in patients receiving therapy with zidovudine or stavudine , macrocytosis is typical , occasionally with MCV values as high as 120 or greater. Rouleaux formation of RBCs may also be seen and likely reflects the presence of concomitant hypergammaglobulinemia. Schistocytes and nucleated RBCs are present in patients with HIV associated TTP.

Peripheral blood neutrophils show left shift and may exhibit morphologic abnormalities , including enlarged size , hyposegmentation , and pelgerhuetanamolies. Atypical plasmacytoid lymphocytes are occasionally seen in asymptomatic individuals but are particularly common in lymphopenic patients with AIDS especiallyduring acute HIV infection. Large , atypical monocytes have also been described with prominent vacuolization and fine nuclear chromatin.[70]

Hemostatic abnormalities :

Thrombosis is seen in 2 % of HIV patients. The risk factors for venous thromboembolic complications are age over 45 years , late stage of HIV infection , the presence of CMV or other AIDS defining opportunistic infections , hospitalization , and indinavir or megestrol acetate therapy.[107]

The coexistence between opportunistic infections and thrombosis may simply indicate immobility due to illness. CMV may promote neutrophil adhesion and platelets to the endothelium , induce release of antiphospholipid antibodies , increase synthesis , and increase secretion and survival of factor VIII and Von Willebrand factor.[108]Like Megestrol , other progestational agents may cause , acquired resistance to activated protein C.[109]

Also HIV patients are at increased risk for thrombosis due to decreased levels of anti thrombin III , free protein S , protein C , or heparin cofactor II; the presence of anticardiolipin antibodies;

coexistence of malignant , inflammatory or autoimmune disorders , or vascular damage due to infection , drug use , placement of intravenous catheters or CMV Infection.[110]

Antithrombin III deficiency can occur with HIV nephropathy due to loss in urine. The nephrotic syndrome seen in HIV nephropathy may also result in compensatory hepatic synthesis of factors V , VIII and X induced by hypoalbuminemia , and increased platelet adhesion and aggregation.[111]

Acquired protein S deficiency can be seen in upto 75 % of HIV infected children and adults especially in patients having CD 4 counts less than 200/microl or AIDS , resulting in thrombotic complications.[112] Acquired free protein S deficiency is due to formation of antibodies against protein S.[113]

The levels of free protein S antigen in HIV patients can be falsely low when assayed by the PEG precipitation technique , so that the prevalence of protein S deficiency in HIV positive patients may actually be lower than it has to be (about 10 %).[114]

The lupus anticoagulant is seen in 0% - 70 % of HIVpatients , depending on the sensitivity of the assay and the type of patient examined. Anticardiolipin antibodies are detected in 46% - 90% of these patients.[115,116,117]

The bone marrow in Human immunodeficiency virus (HIV)Infection:

Morphologic abnormalities are seen in the majority of bone marrow samples from HIV patients , but most are non-specific except in opportunistic infections , in which the bone marrow examination provides valuable diagnostic information. Therefore the bone marrow examination

rarely yields substantial clinical information , except in the diagnosis of concurrent M.avium intracellulare , tuberculosis , or fungal infection or as part of staging for malignancy.[118]

The histopathologic findings in the bone marrow of HIV patients are varied which includes hypercellularmarrow ,Myelodysplastic changes , hypocellular marrow , and fibrosis of bone marrow.[119]

Hyperplasia involving the granulocytic and erythrocytic lineages are reported: the myeloid to erythroid ratio varied from 2:1 to 5:1.[120]As the disease progress , The morphologic changes tend to be more pronounced with increased frequency and severity. All lineages can be involved.[121] Megaloblastic changes , dysplastic changes and ringed sideroblasts are frequent. The cumulative effects of drug toxicities , direct HIV 1 infection of marrow cells , and dysregulated cytokine production may probably explain the morphologic changes that occur especially in late stages of AIDS.

MATERIALS AND METHODS

METHODOLOGY

The present study titled "Hematological and morphological changes of blood cells in HIV Patients – An Institutional Study" was done from DECEMBER 2014 to AUGUST 2016 at R.L.Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

SAMPLE SIZE- Sample size estimated based on expected number of HIV Positive patients in R L JALAPPA HOSPITAL, Sri Devaraj Urs Medical College during the year 2013 was 106

Expected blood disorders among HIV Patients is 81.5 % based on wankah et al.BMC Hematology 2014 ;14:15

$n= 4\ pq/(d)^2$

Prevalence p= 81.5%

Absolute error d= 5%

$n= 4 \times 81.5 \times (100-81.5)/(5)^2$ n = 73

n= 80 at 95% confidence level expecting 10% non-compliance.

All confirmed HIV Positive cases were taken and collected a Total 101 cases to consider for the study.

This is a prospective study conducted in the R L JALAPPA Hospital Kolar.

All the Confirmed HIV positive cases are taken

Written Informed consent is taken from all the cases

After taking a brief clinical history 1 to 1.5 ml of venous blood is collected in a sterile EDTA containing tube with universal precautions as per the guidelines of NACO , and it is processed in an Alere H Automated Analyser within 2 hours.

The following parameters are considered for The study : Complete blood count including Hb % , PCV , Red cell indices , Platelet count , RBC Count , Reticulocyte count , WBC Count and Differential Count , CD4 Counts.

Blood smears are prepared and is routinely stained with Leishman's stain.

A detailed Morphological study of all the blood cell lineages were done on the peripheral smear.

Smears were carefully examined for the organisms.

The inclusion criteria are Confirmed HIV Positive patients symptomatic and asymptomatic.

The exclusion criteria are Patients less than 16 Yrs , Pregnant Patients , Patients who are on ART.

We also tried to exclude the nutritional anemia in most of the cases as far as possible.

STAINING PROCEDURE

Logistics and materials :

1. Leishman stain

2. Buffered distilled water (p H 6.8-7.2)
3. Timer
4. Slide
5. EDTA blood sample

Smear preparation

1. Smear was covered with Leishman's stain
2. It was allowed to stand for 1-2 minutes
3. Without removing the stain , double the amount of buffered distilled water was added
4. Allowed it to stand for 7 minutes
5. Slide was flooded with tap water
6. Back of the slide was washed with soap and water
7. It was air dried in a tilted / upright position

A Well Stained film had the following features :

- The nuclei of leucocytes was purple
- Neutrophilic granules – tan in color
- Eosinophilic granules – red orange in color
- Basophil – dark purple granules
- Platelets – had dark lilac granules
- Cytoplasm of lymphocytes – light blue
- RBCs – pink color.

STATISTICAL

ANALYSIS

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square was used as test of significance. Continuous data was represented as mean and SD. ANOVA (Analysis of Variance) was the test of significance to identify the mean difference between more than two groups. Pearson Correlation was done to find the correlation between two quantitative variables. p value <0.05 was considered as statistically significant.

Correlation coefficient (r)	**Interpretation**
0 - 0.3	**Positive Weak correlation**
0.3-0.6	**Positive Moderate correlation**
0.6-1.0	**Positive Strong correlation**
0 to (-0.3)	**Negative Weak correlation**
(-0.3) to (-0.6)	**Negative Moderate Correlation**
(-0.6) to – (1)	**Negative Strong Correlation**

RESULTS

RESULTS

Table 1: Age distribution of subjects

		Count	%
Age	< 30 years	10	9.9%
	31 to 40 years	38	37.6%
	41 to 50 years	45	44.6%
	> 50 years	8	7.9%
	Total	101	100.0%

Majority of subjects were in the age group 41 to 50 years (44.6%) and 37.6% were in the age group 31 to 40 years.

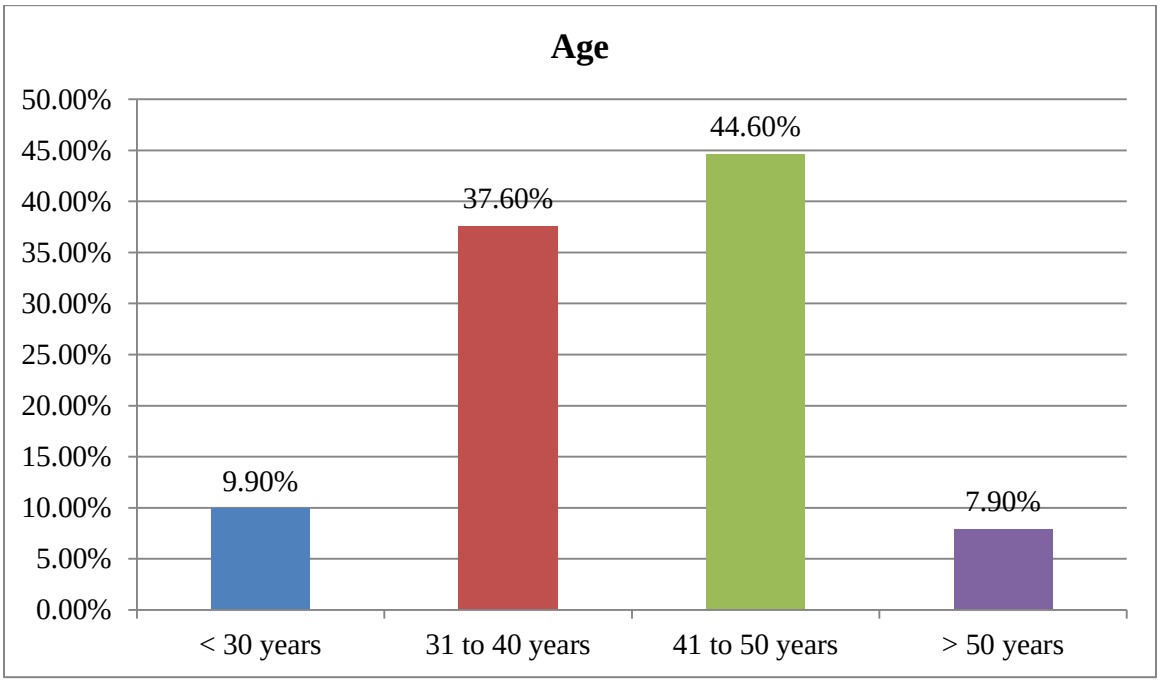

Figure 3: Bar diagram showing Age distribution of subjects

Table 2: Gender distribution of subjects

		Count	%
Gender	Female	28	27.7%
	Male	73	72.3%

72.3% of subjects were males and 27.7% were females.

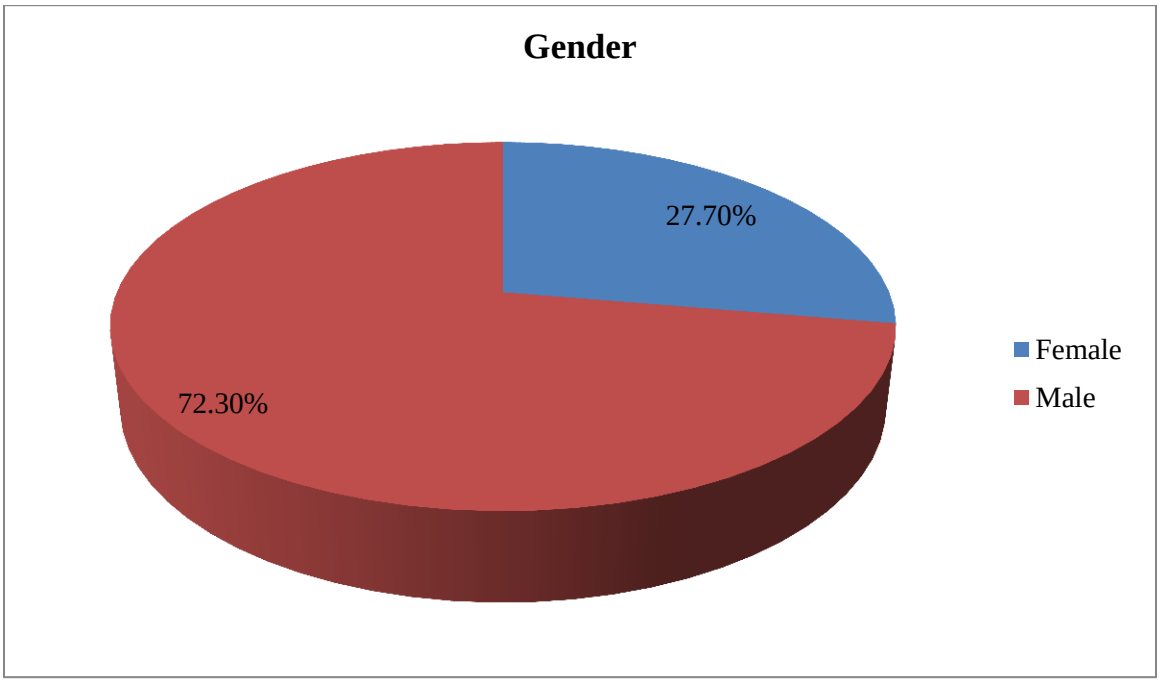

Figure 4: Pie diagram showing Gender distribution of subjects

Table 3: Hematological parameters distribution in subjects

	Mean	SD	Median
RBC (x 1000000/micro L)	4.1	1.0	4.1
WBC (X1000/micro L))	7.2	2.6	7.4
N (%)	49.7	16.4	50.0
L (%)	41.3	16.3	38.0
M (%)	4.9	3.9	4.0
E (%)	2.9	2.9	2.0
B (%)	1.1	.5	1.0
HB (g/dl)	11.9	2.7	12.1
PLT (x 1000/micro L)	236.1	90.4	226.0

Mean and SD values of Hematological parameters are shown in above table.

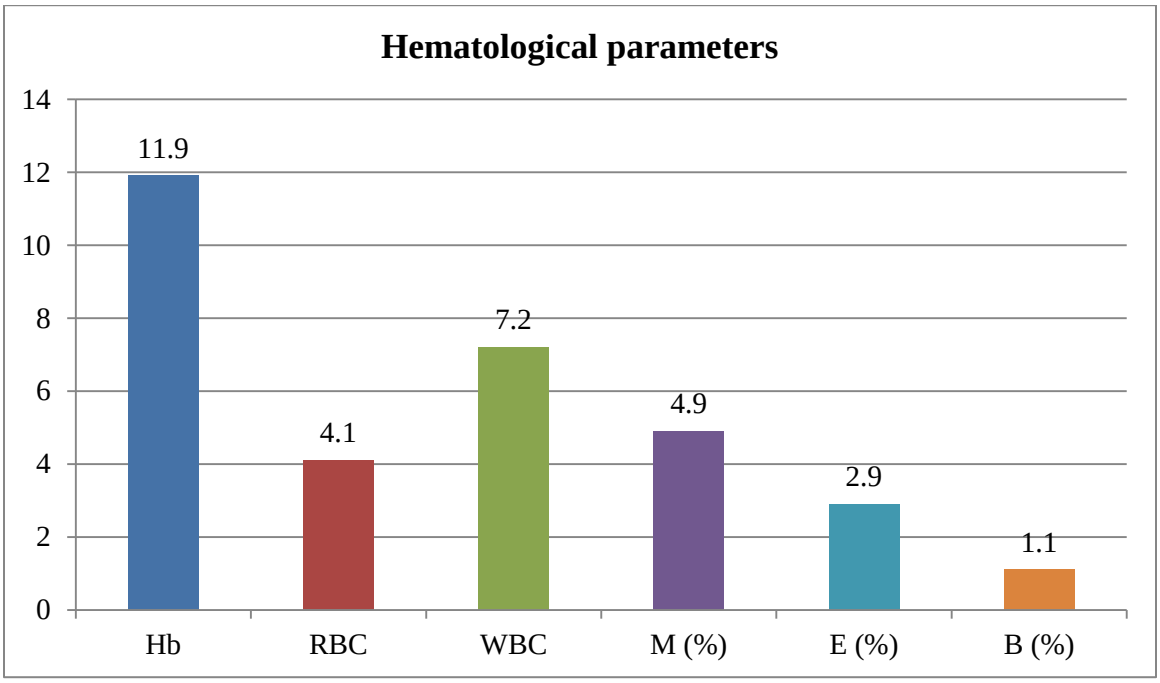

Figure 5: Bar diagram showing various hematological parameters

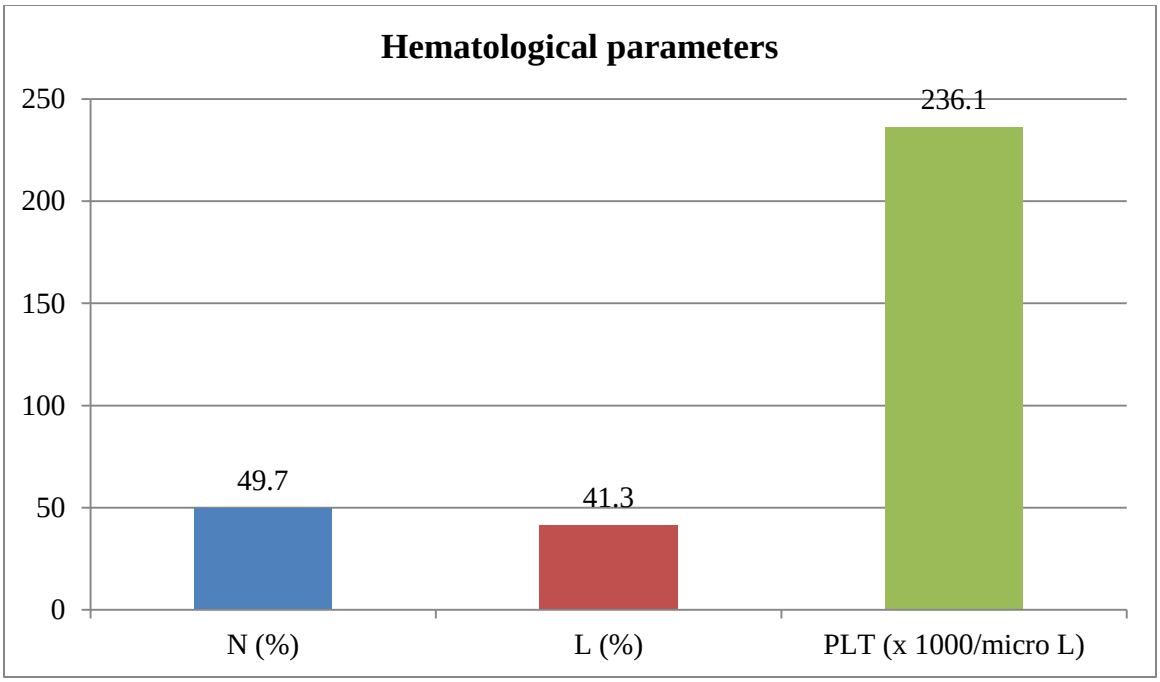

Figure 6: Bar diagram showing Hematological parameters distribution in subjects

Table 4: Peripheral blood smear findings in subjects

		Count	%
Anemia	Absent	46	45.5%
	Present	55	54.5%
Normocytic anemia	Absent	69	68.3%
	Present	32	31.7%
Macrocytic Anemia	Absent	82	81.2%
	Present	19	18.8%
Leucopenia	Absent	74	73.3%
	Present	27	26.7%
Leucocytosis	Absent	96	95.0%
	Present	5	5.0%
Thrombocytopenia	Absent	86	85.1%
	Present	15	14.9%

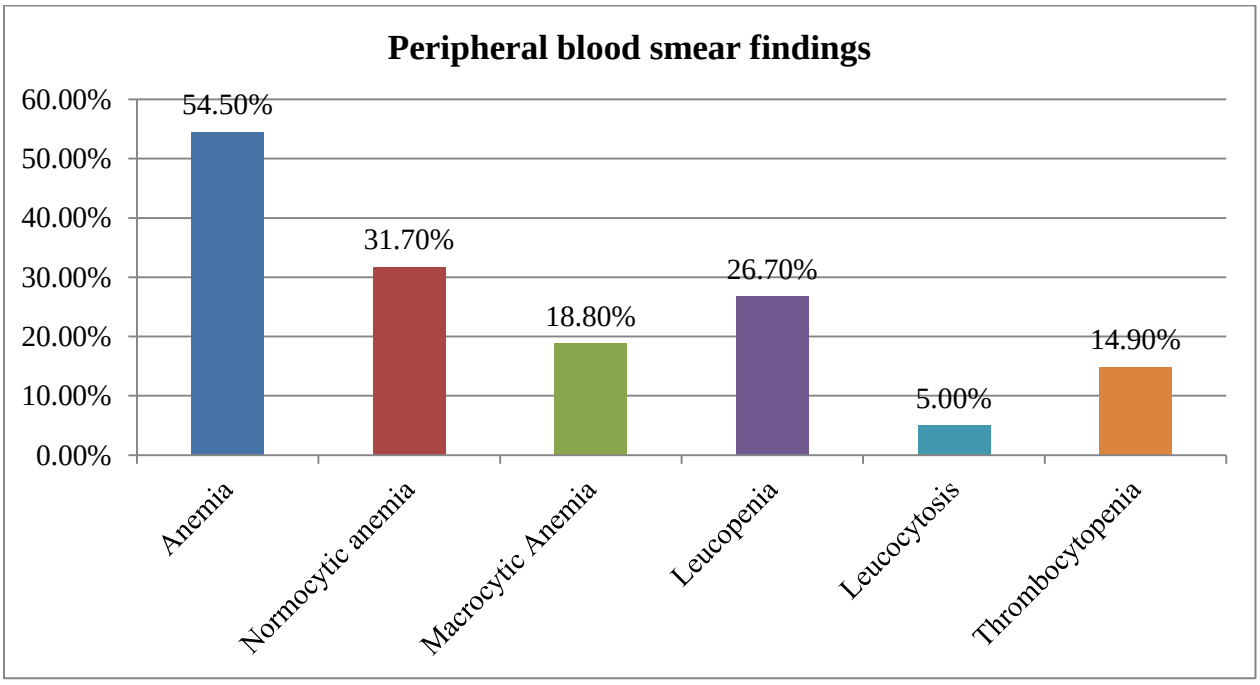

Figure 7: Bar diagram showing Peripheral blood smear findings in subjects

Table 5: Morphological findings in subjects

		Count	%
Macro platelets	Absent	56	55.4%
	Present	45	44.6%
Howell Jolly Bodies (RBC)	Absent	71	70.3%
	Present	30	29.7%
Atypical Lymphocytes	Absent	77	76.2%
	Present	24	23.8%
Monocytic Vacuolations	Absent	81	80.2%
	Present	20	19.8%
Detached Nuclear Fragments	Absent	50	49.5%
	Present	51	50.5%
Dysplastic Neutrophils	Absent	17	16.8%
	Present	84	83.2%
Plasmacytoid Lymphocytes	Absent	72	71.3%
	Present	29	28.7%

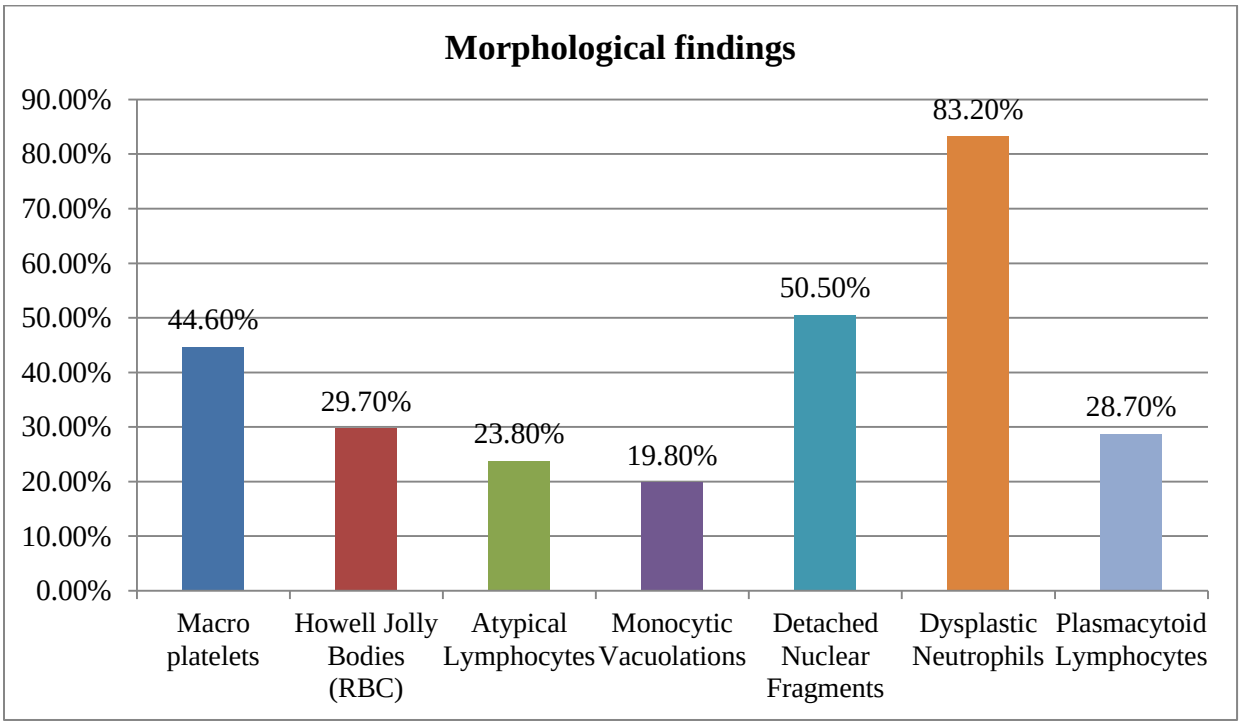

Figure 8: Bar diagram showing Morphological findings in subjects

Table 6: CD4 count in subjects

		Count	%
CD4 count	< 200	10	9.9%
	201 to 500	52	51.5%
	> 500	39	38.6%

Mean CD4 count was 484.7 ± 335.4

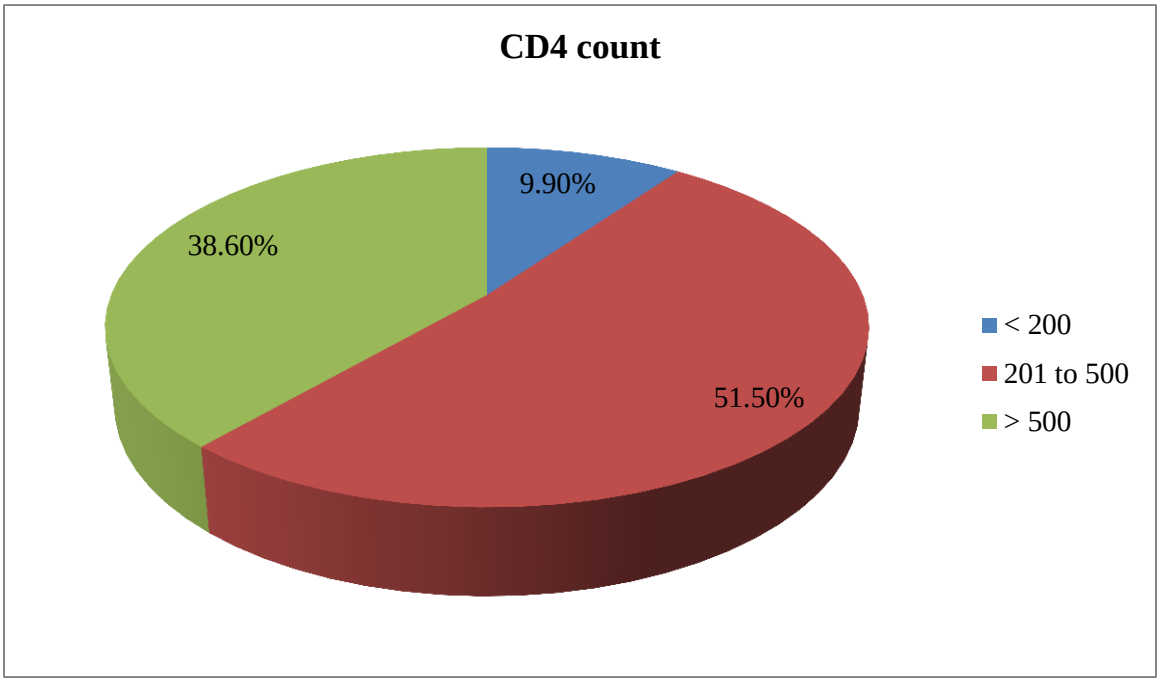

Figure 9: Bar diagram showing CD4 count in subjects

Table 7: Association between Age and CD4 count

	CD4 count						P value
	< 200		201 to 500		> 500		
	Mean	SD	Mean	SD	Mean	SD	
Age	41.1	9.0	41.3	6.8	41.3	8.5	0.998

There was no significant difference in mean age between three groups.

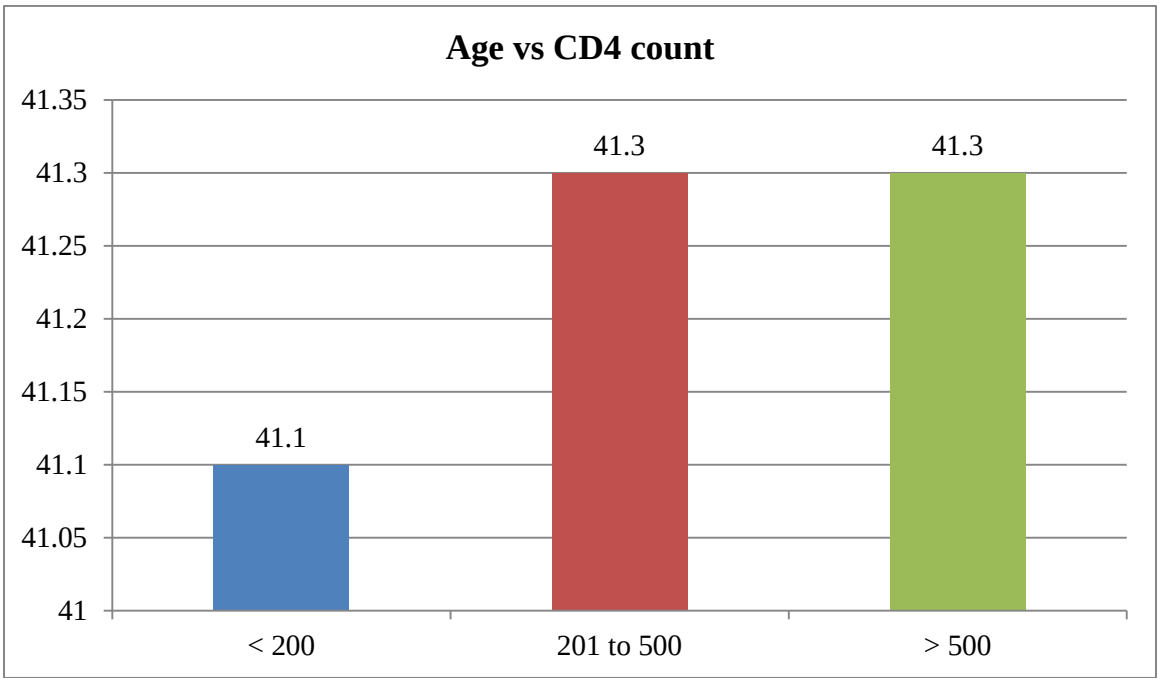

Figure 10: Bar diagram showing Association between Age and CD4 count

Table 8: Association between CD4 count and gender

		CD4countNew					
		< 200		201 to 500		> 500	
		Count	%	Count	%	Count	%
Gender	Female	6	60.0%	12	23.1%	10	25.6%
	Male	4	40.0%	40	76.9%	29	74.4%

$\chi 2 = 5.844$, df =2, p = 0.054

There was no significant association between CD count and Gender.

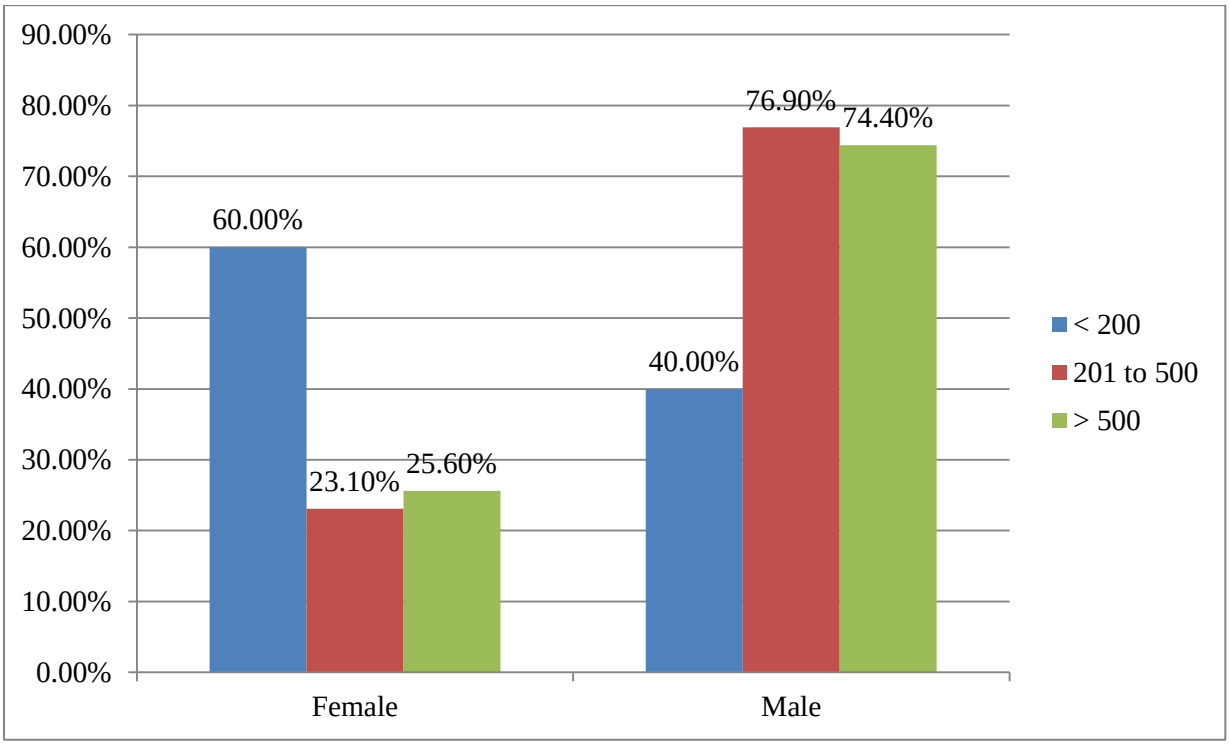

Figure 11: Bar diagram showing Association between CD4 count and gender

Table 9: Association between CD4 count and Hematological parameters

	CD4countNew						P value
	< 200		201 to 500		> 500		
	Mean	SD	Mean	SD	Mean	SD	
RBC (x1000000/microL)	3.8	0.7	4.0	0.9	4.3	1.0	0.189
WBC (X1000/microL))	4.5	1.4	7.3	2.7	7.8	2.4	0.001*
N (%)	63.9	19.0	48.7	16.7	47.5	13.9	0.014*
L (%)	27.3	14.3	41.5	16.5	44.5	15.1	0.011*
M (%)	6.4	4.7	5.2	4.6	4.2	2.1	0.218
E (%)	1.0	0.7	3.4	3.5	2.8	2.1	0.047*
B (%)	1.2	0.6	1.1	0.5	1.0	0.4	0.391
HB (g/dl)	11.3	0.6	11.5	2.9	12.5	2.8	0.199
PLT (x 1000/microL)	149.7	70.5	244.1	108.9	247.5	47.2	0.005*

In the study there was significant difference in mean WBC, Neutrophils, Lymphocytes, Eosinophils and Platelet count.

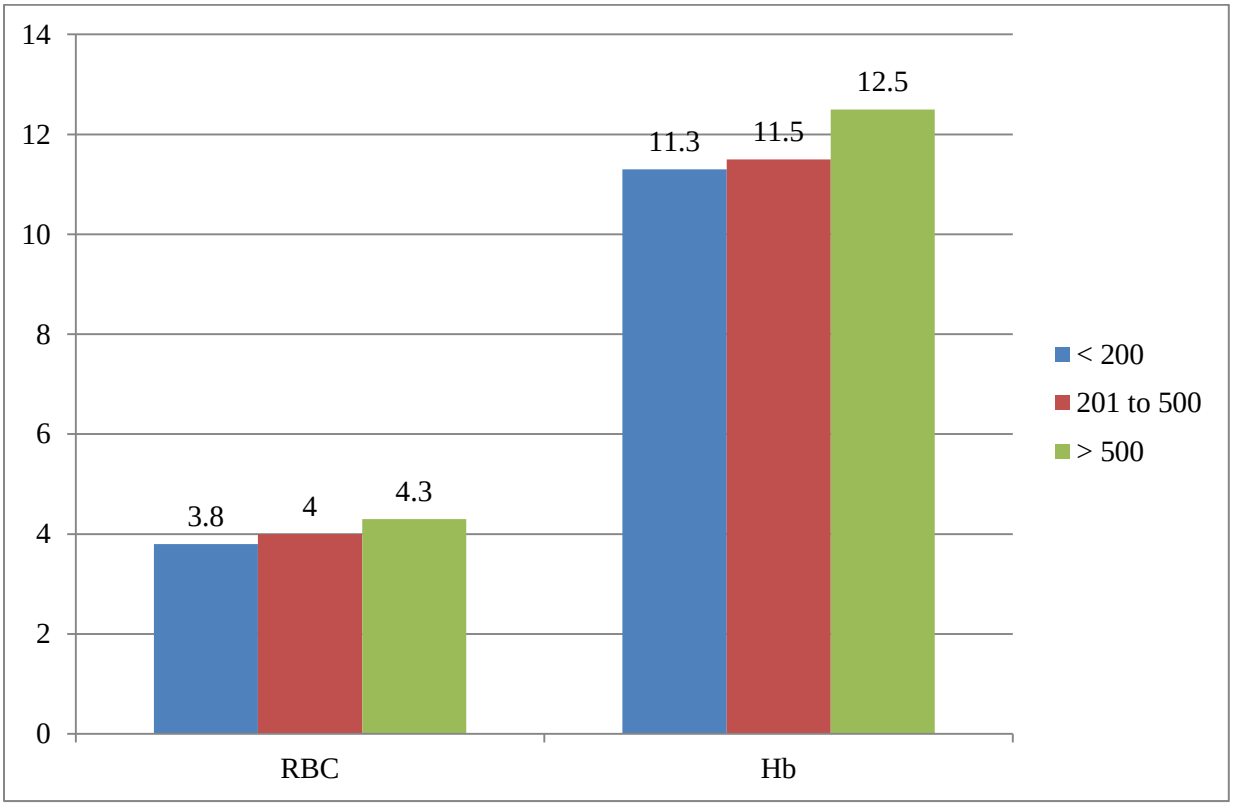

Figure 12: Bar diagram showing Mean RBC and Hb% with respect to CD4 count

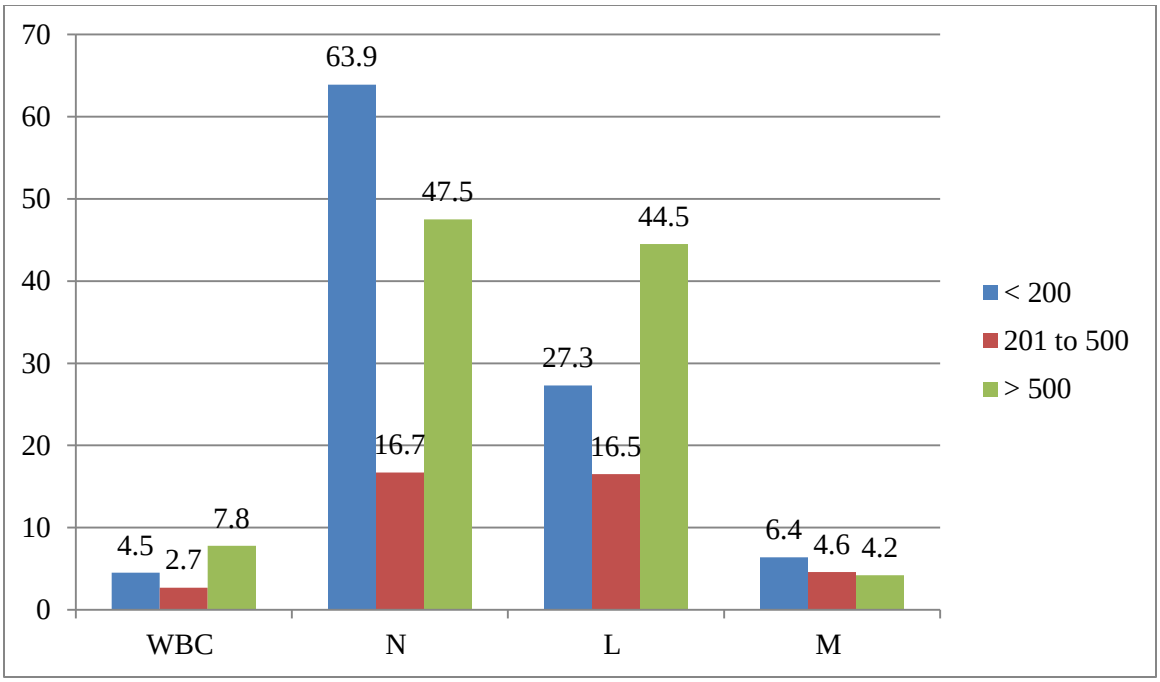

Figure 13: Bar diagram showing Mean WBC and DLC count with respect to CD4 count

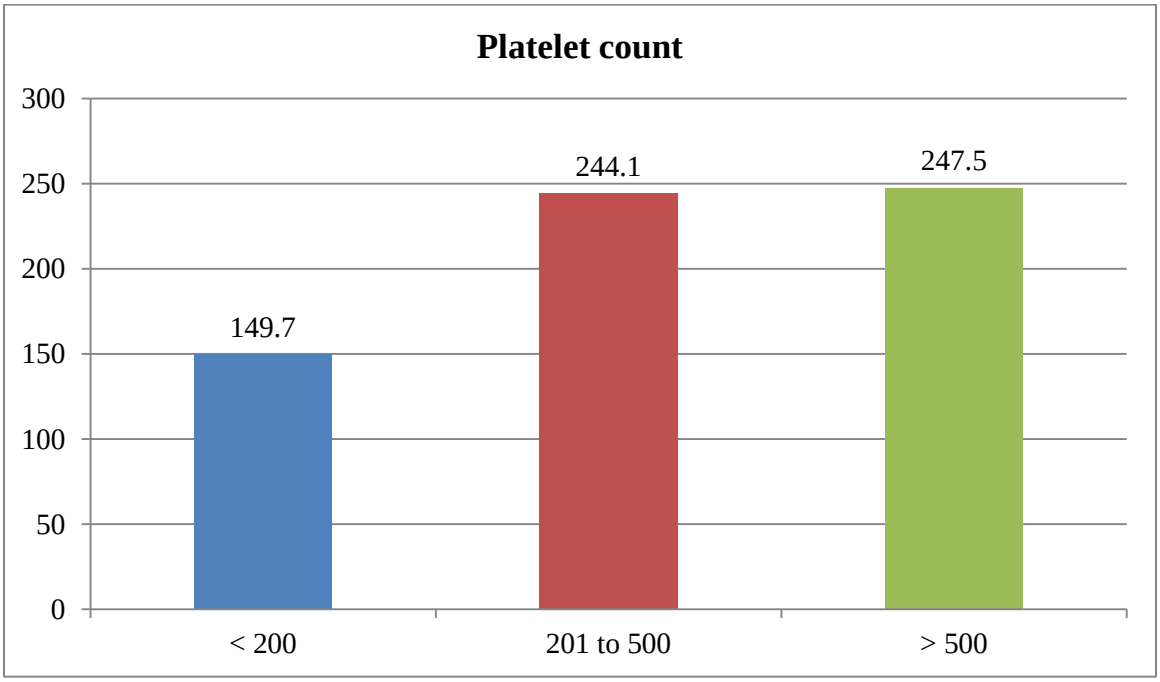

Figure 14: Bar diagram showing Mean platelet count with respect to CD4 count

Table 10: Association between CD4 count and Peripheral blood smear in the study

		CD4 count						P value
		< 200		201 to 500		> 500		
		Count	%	Count	%	Count	%	
Anemia	Absent	0	0.0%	23	44.2%	23	59.0%	0.004*
	Present	10	100.0%	29	55.8%	16	41.0%	
Normocytic Anemia	Absent	2	20.0%	37	71.2%	30	76.9%	0.002*
	Present	8	80.0%	15	28.8%	9	23.1%	
Macrocytic Anemia	Absent	10	100.0%	40	76.9%	32	82.1%	0.228
	Present	0	0.0%	12	23.1%	7	17.9%	
Leucopenia	Absent	4	40.0%	39	75.0%	31	79.5%	0.039*
	Present	6	60.0%	13	25.0%	8	20.5%	
Leucocytosis	Absent	10	100.0%	49	94.2%	37	94.9%	0.742
	Present	0	0.0%	3	5.8%	2	5.1%	
Thrombocytopenia	Absent	5	50.0%	42	80.8%	39	100.0%	<0.001*
	Present	5	50.0%	10	19.2%	0	0.0%	

In the study there was significant association between CD4 count and anemia. I.e. with decrease in CD4 count there was increase in anemia rate.
Similarly there was significant association between CD4 count and Normocytic anemia, leucopenia and Thrombocytopenia.

No significant association was observed between CD4 count and Macrocytic anemia, Leucocytosis.

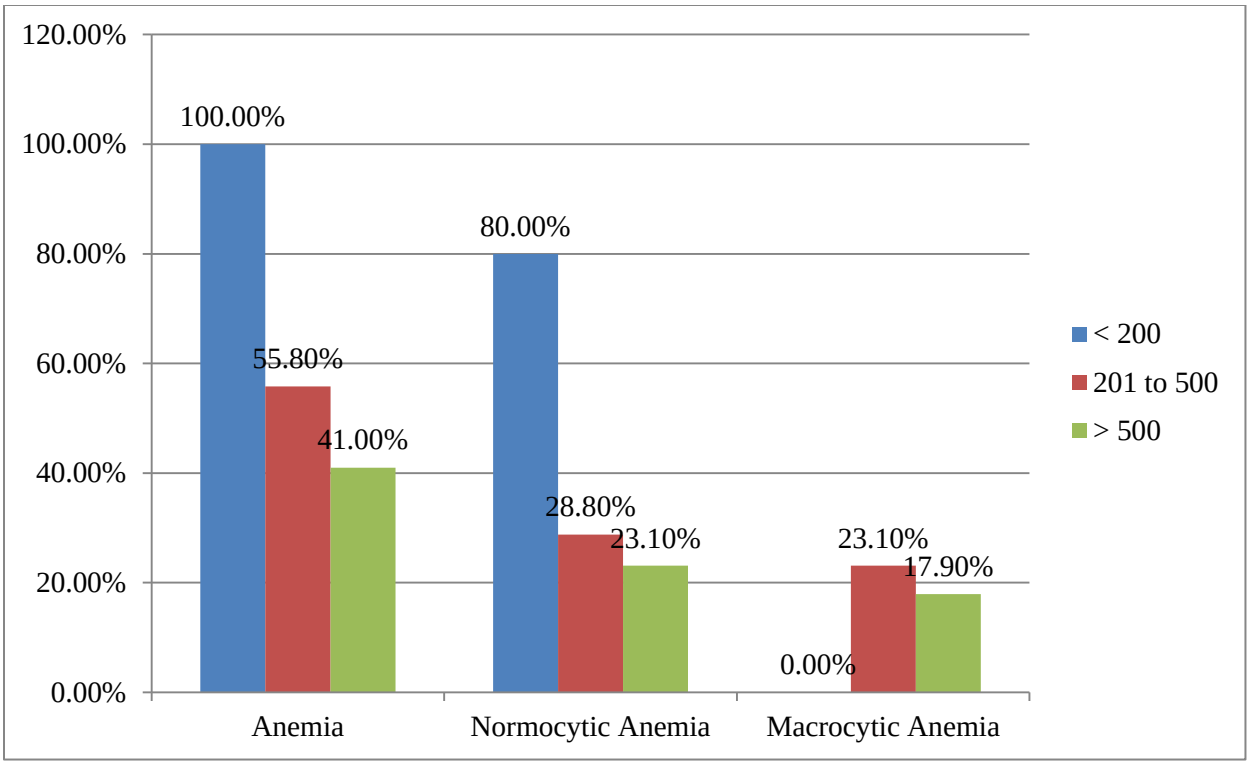

Figure 15: Bar diagram showing Association between CD4 count and Peripheral smear anemia changes in the study

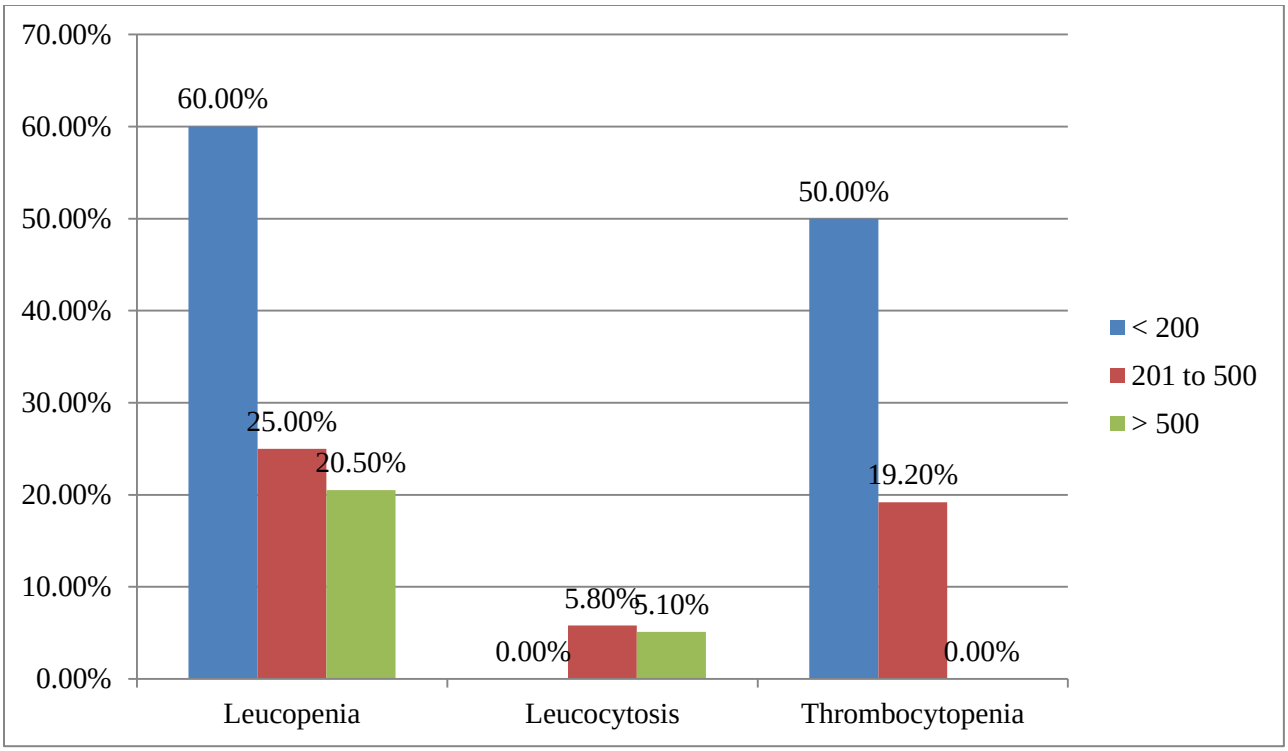

Figure 16: Bar diagram showing Association between CD4 count and Peripheral blood smear

Table 11: Association between CD4 count and Morphological changes in the study

		CD4countNew						P value
		< 200		201 to 500		> 500		
		Count	%	Count	%	Count	%	
Macro platelets	Absent	6	60.0%	28	53.8%	22	56.4%	0.927
	Present	4	40.0%	24	46.2%	17	43.6%	
Howell Jolly Bodies (RBC)	Absent	7	70.0%	38	73.1%	26	66.7%	0.803
	Present	3	30.0%	14	26.9%	13	33.3%	
Atypical Lymphocytes	Absent	4	40.0%	43	82.7%	30	76.9%	0.015*
	Present	6	60.0%	9	17.3%	9	23.1%	
Monocytic Vacuolations	Absent	10	100.0%	41	78.8%	30	76.9%	0.248
	Present	0	0.0%	11	21.2%	9	23.1%	
Detached Nuclear Fragments	Absent	6	60.0%	23	44.2%	21	53.8%	0.519
	Present	4	40.0%	29	55.8%	18	46.2%	
Dysplastic Neutrophils	Absent	1	10.0%	7	13.5%	9	23.1%	0.398
	Present	9	90.0%	45	86.5%	30	76.9%	
Plasmacytoid Lymphocytes	Absent	4	40.0%	39	75.0%	29	74.4%	0.07
	Present	6	60.0%	13	25.0%	10	25.6%	

In the study there was significant association between CD4 count and atypical lymphocytes. I.e. with decrease in CD4 count there was increase in atypical lymphocytes count.

No significant association was observed between other morphological changes and CD4 count.

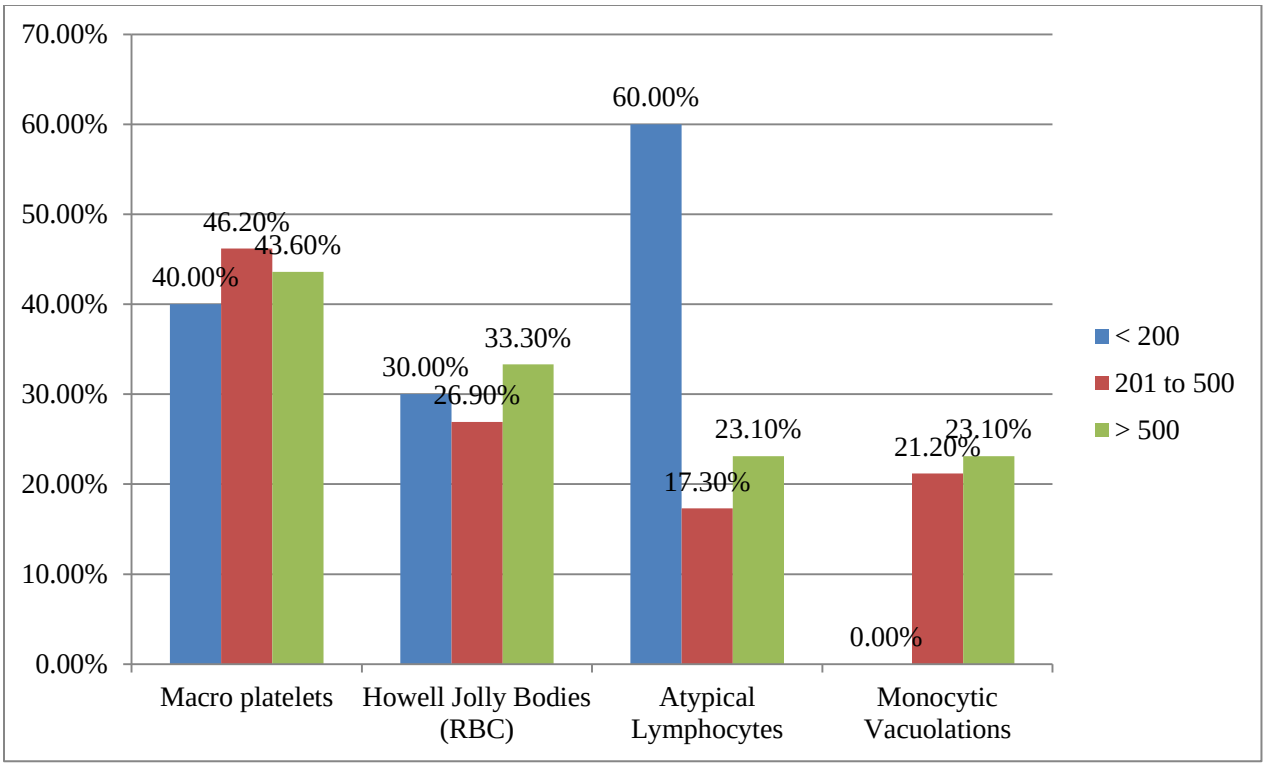

Figure 17: Bar diagram showing Association between CD4 count and Morphological changes in the study

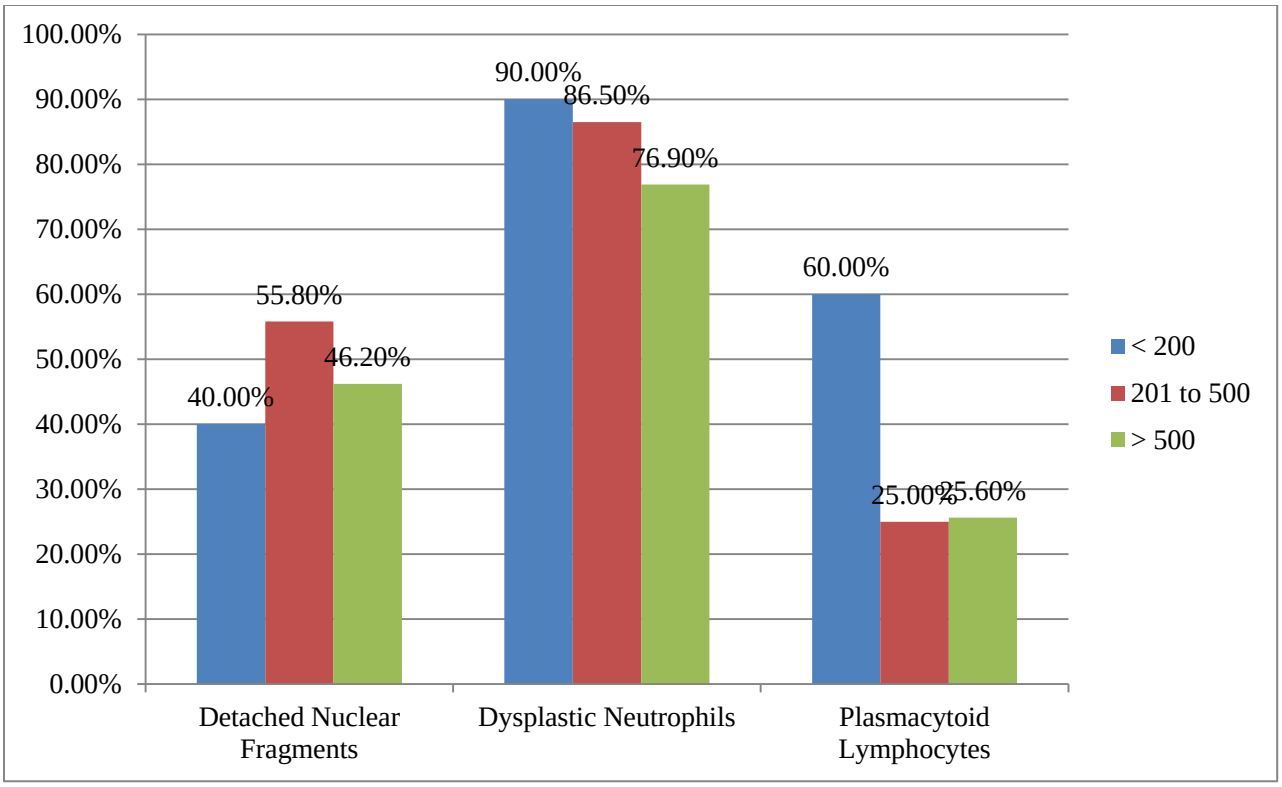

Figure 18: Bar diagram showing Association between CD4 count and Morphological changes in the study

Table 12: Correlation between CD4 Count and Hematological parameters

	N	Pearson Correlation	P value
RBC	101	0.244**	0.014*
WBC	101	0.471**	<0.001*
Neutrophils	101	-0.368**	<0.001*
Lymphocytes	101	0.419**	<0.001*
Monocytes	101	-0.175	0.080
Eosinophils	101	-0.016	0.873
Basophils	101	0.022	0.825
Hb%	101	0.060	0.549
Platelet Count	101	0.270**	0.006*

Significant positive correlation was observed between CD4 count and RBC, WBC, Lymphocytes and Platelet count. i.e. with decrease in CD4 count there was decrease in RBC, WBC, Lymphocytes and platelet count or vice versa.

Significant negative correlation was observed between CD4 count and Neutrophils. i.e with decrease in CD4 count there was increase in Neutrophils count and vice versa.

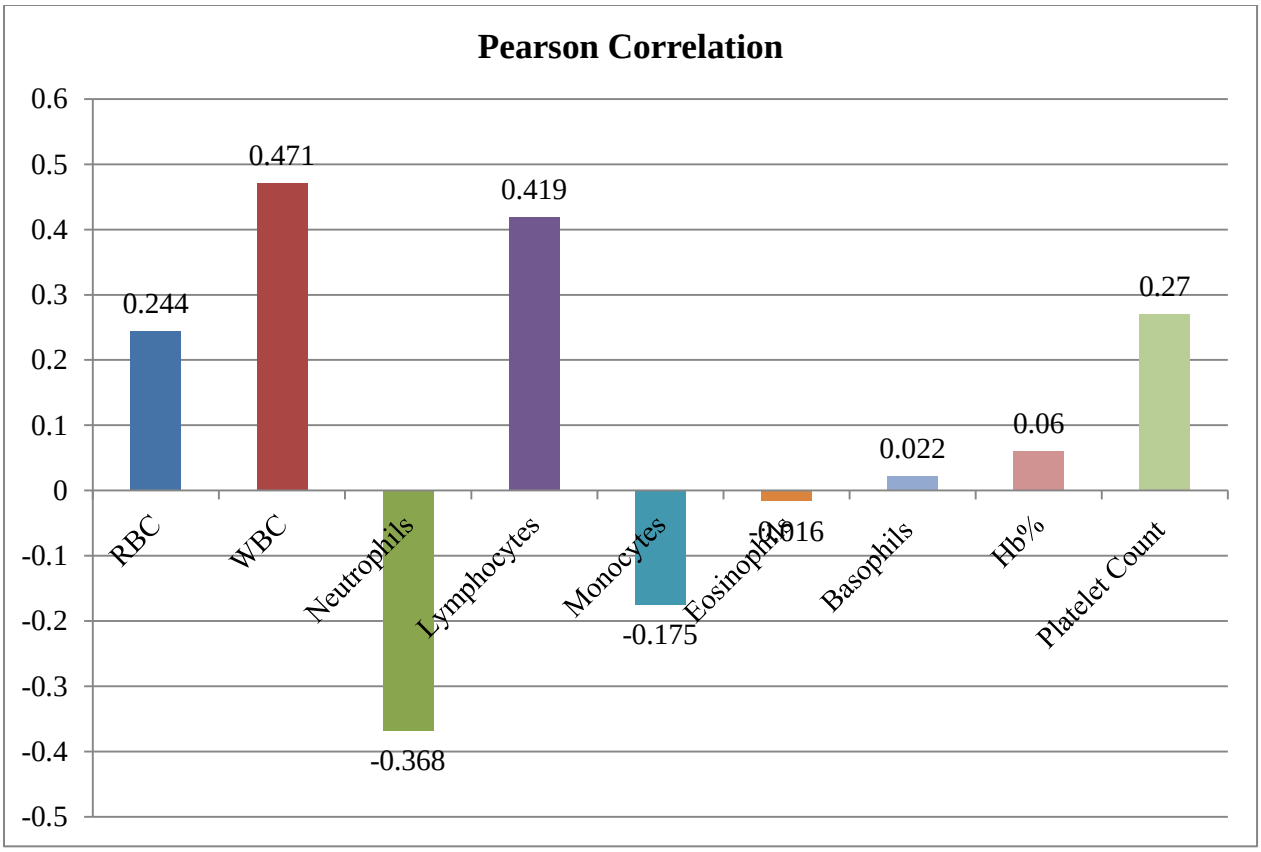

Figure 19: Bar diagram showing Correlation coefficient for hematological parameters

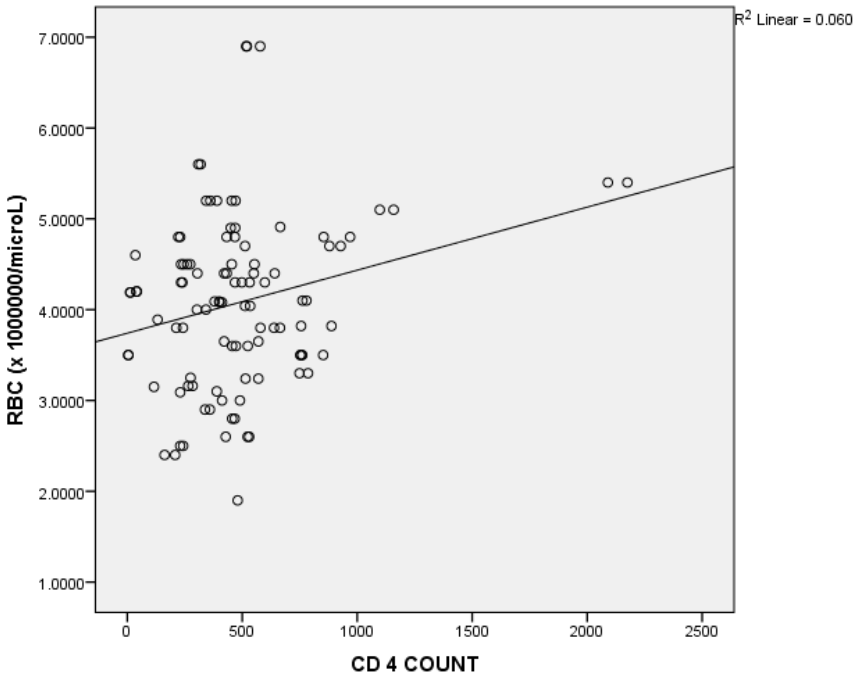

Figure 20: Scatter plot showing significant positive correlation between CD4 count and RBC

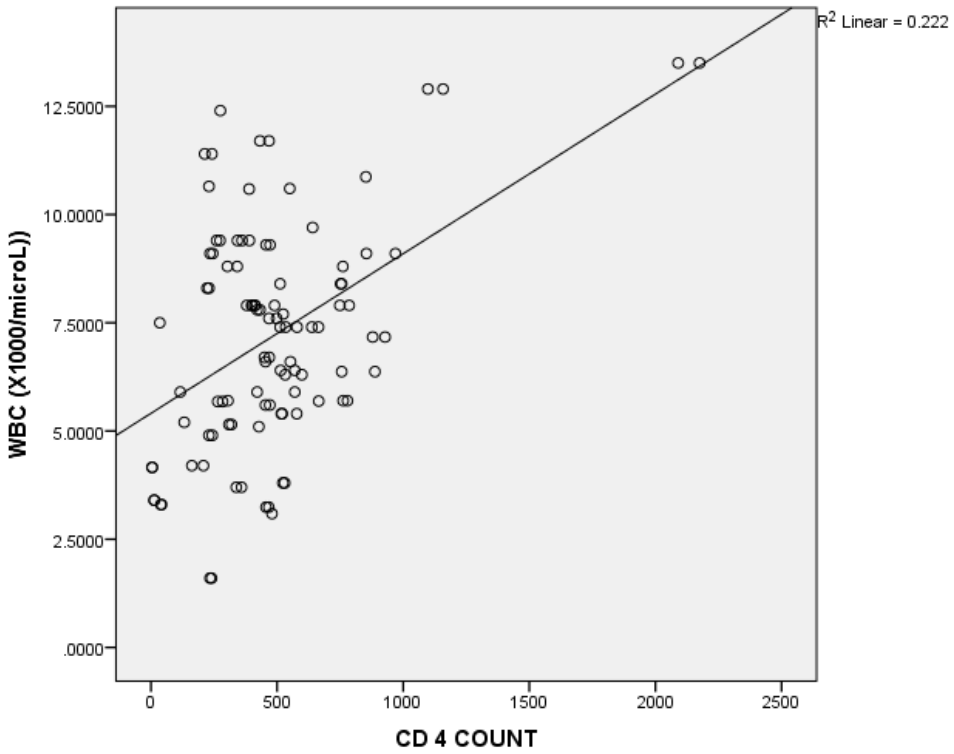

Figure 21: Scatter plot showing significant positive correlation between CD4 count and WBC

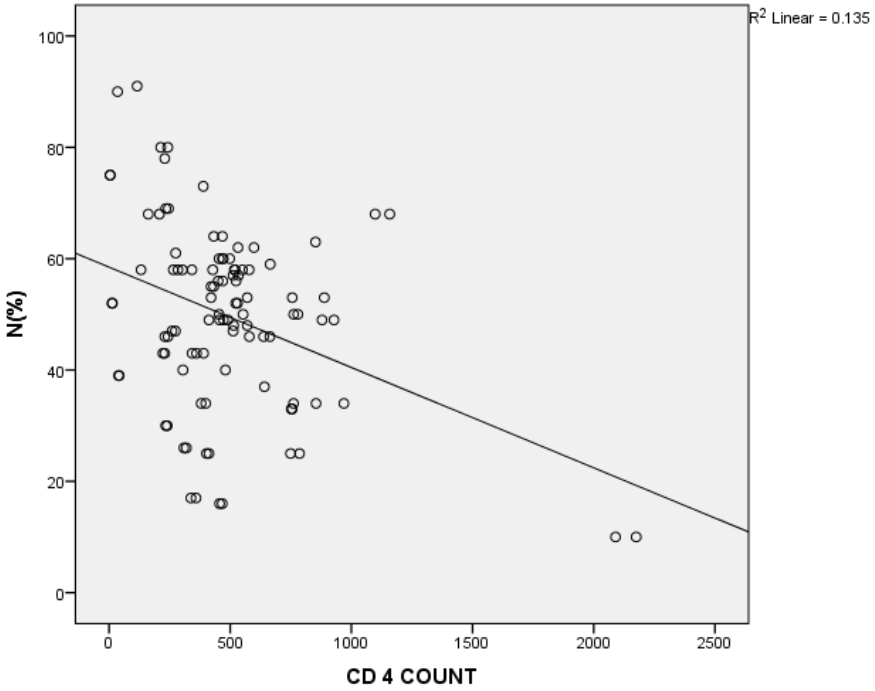

Figure 22: Scatter plot showing significant negative correlation between CD4 count and Neutrophils count

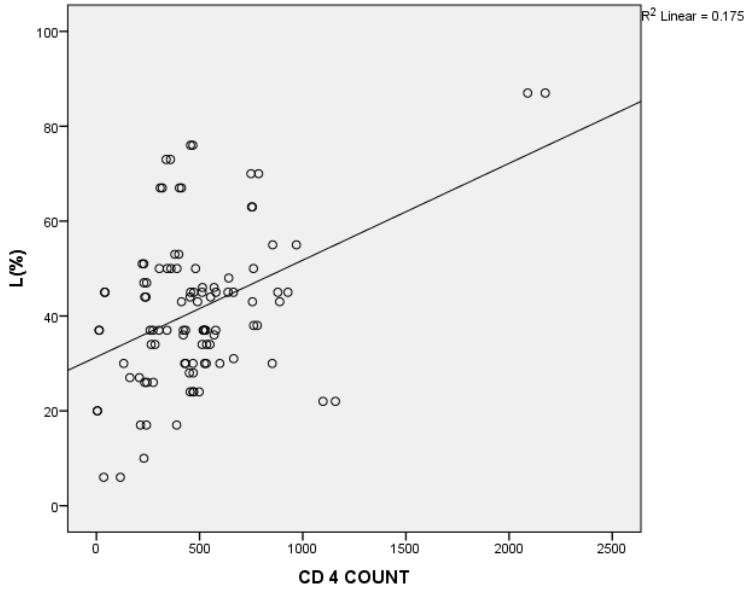

Figure 23: Scatter plot showing significant positive correlation between CD4 count and Lymphocyte count

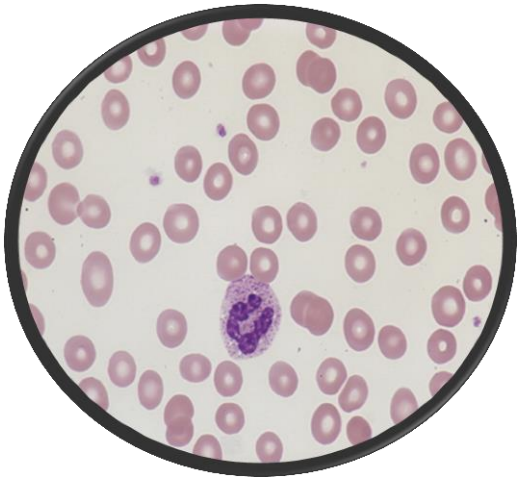

Figure 24 :Peripheral blood smear showing Normocytic Normochromic Anemia.

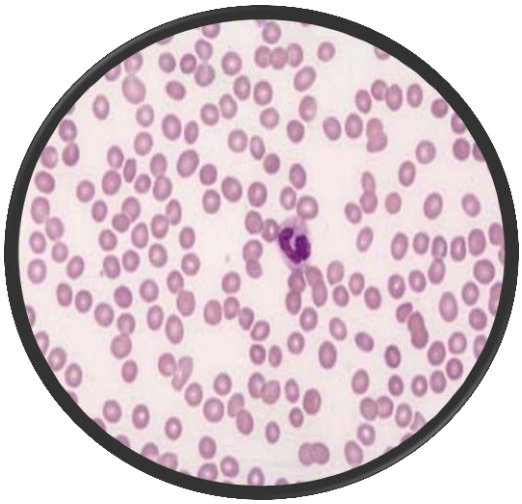

Figure 25 : Peripheral blood smear showing Thrombocytopenia.

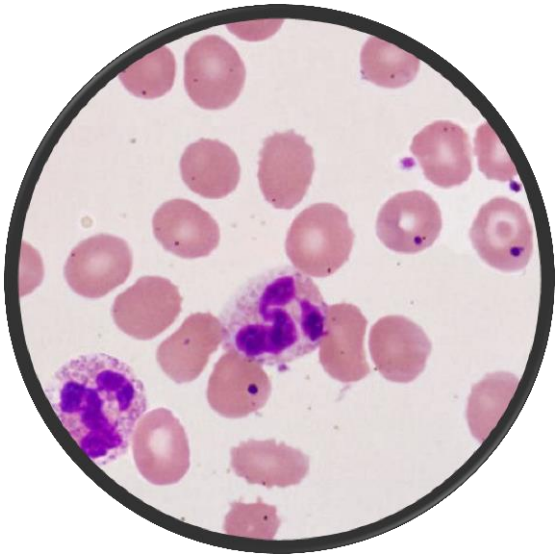

Figure 26 : Peripheral blood smear showing Howell jolly bodies.

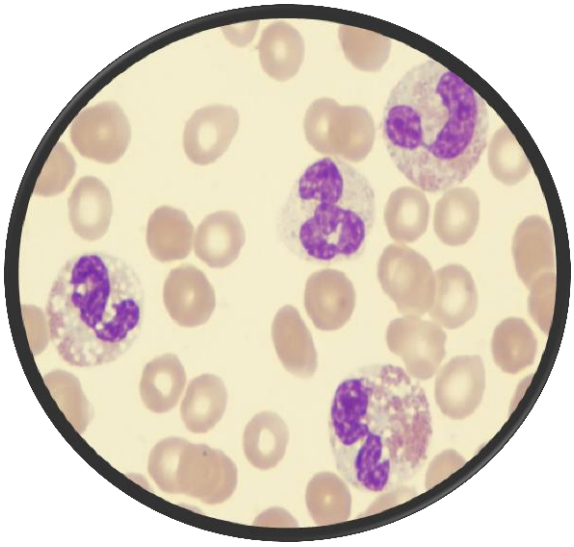

Figure 27 : Peripheral blood smear showing Dysplastic neutrophils.

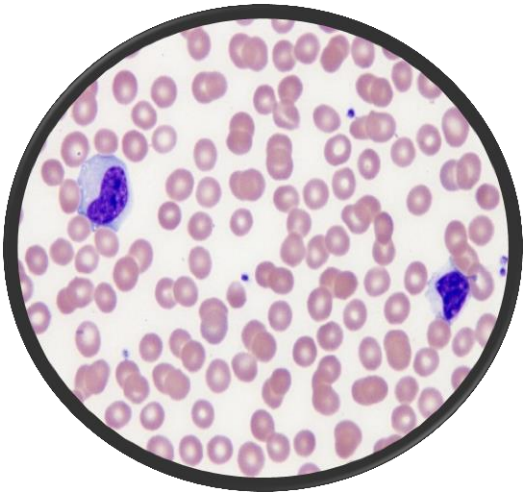

Figure 28 : Peripheral blood smear showing Atypical lymphocytes.

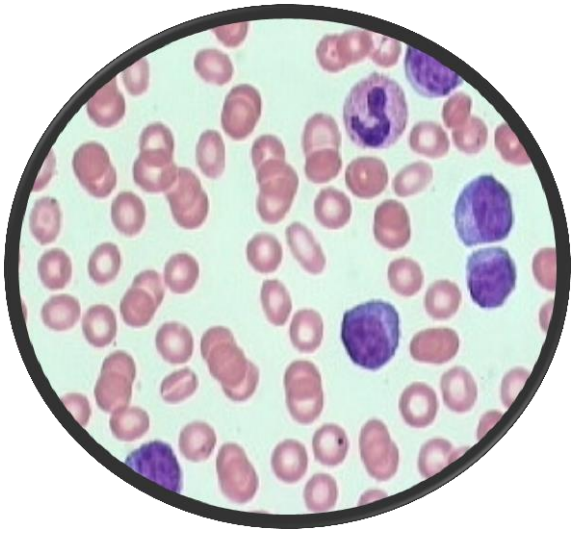

Figure 29 : Peripheral blood smear showing Plasmacytoid lymphocytes.

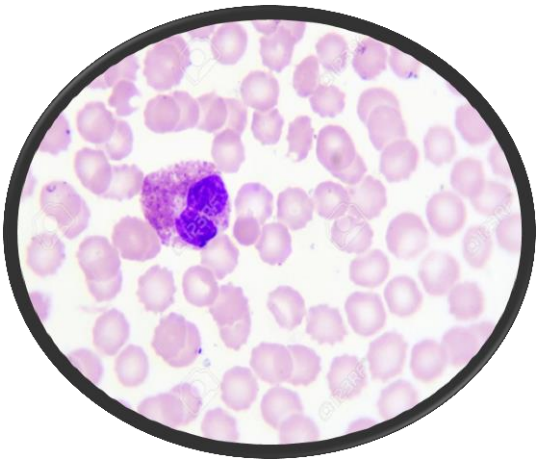

Figure 30 : Peripheral blood smear showingPelgerhuetAnamoly.

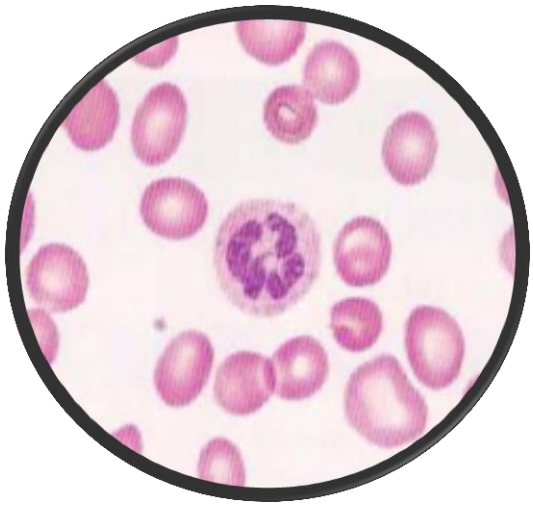

Figure 31 : Peripheral blood smear showing Detached Nuclear fragments.

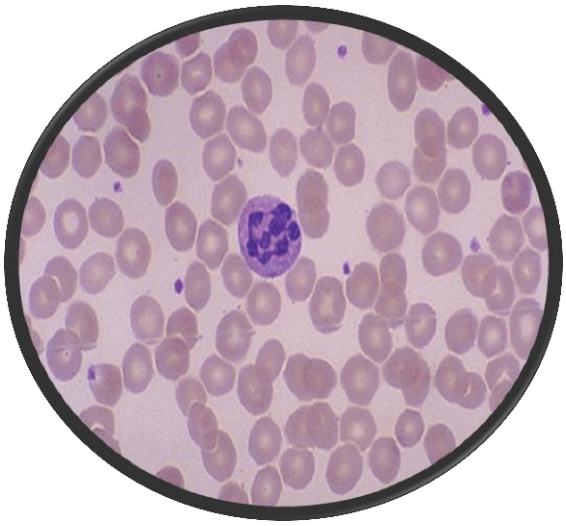

Figure 32 : Peripheral blood smear showing Megaloblastic anemia.

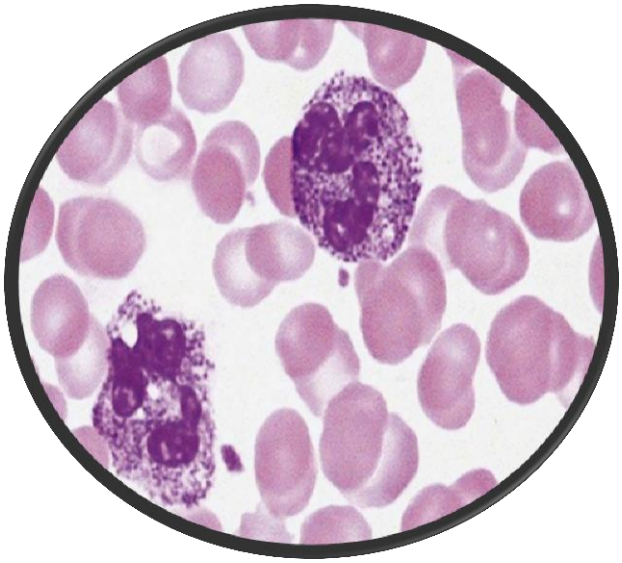

Figure 33 : Peripheral blood smear showing Toxic changes in Neutrophils.

DISCUSSION

DISCUSSION

Disorders of the hematological system are common in HIV Infected patients. The hematological manifestations of HIV are varied and prevalent throughout the course of the disease. Although in the majority of the cases , hematologic abnormalities are detected in middle or advanced stages of HIV Infection , some of these like anemia and thrombocytopenia have been reported to occur in early stages of HIV Infection. [122]

In our study we evaluated 101 HIV Positive patients which were grouped into three groups according to their CD 4 Counts , those having CD 4 (<200/micro l) , CD 4 (201-500 /micro l) and CD 4 (> 500/micro l). we found majority of the patients (51.5%) with CD 4 count between 201-500 /micro l , which was almost similar to the study done by Manisha et al (2002). [119]

AGE DISTRIBUTION

When age distribution was studies , we found 81.2% of the patients were in the sexually active age group of 21-40 years , which is almost similar to the studies done by Manisha et al (2002) [119] and Tripathi et al (2005). [120]

SEX DISTRIBUTION

In present study , it was found that (n=101) male patients (73) outnumbered the female patients (28). High risk behavior and migration opportunities may be attributed to high prevalence of HIV among male patients. These results were similar to the studies done by Manisha et al (2002) [119] and Tripathi et al (2005). [120]

Table 13 : Sex distribution of cases in various studies in relation to present study

Sex	Manisha et al n = 416	Tripathi et al n = 54	Present study n = 101
Males	83.2 %	79.7 %	72.3 %
Females	16.8 %	21.28 %	27.7 %

HEMATOLOGICAL MANIFESTATIONS

In the present study , we defined anemia according to the WHO Criteria as hemoglobin levels of < 13 gm% in males and < 12gm% in females. We found that overall prevalence of anemia was 54.5 % which was little lower as compared to studies done by Aboulafia DM et al (1991) [7] , Zon Li et al (1988) [59] and Spivak JL et al (1984). [5]

Table 14 : Percentage of Anemia in various studies

Study	Aboulafia DM et al (n=54)	Zon Li et al (n=106)	Spivak JL et al (n=124)	Present study (n=101)
Percentage of Anemia	75%	64%	71.5%	54.5 %

The lower incidence of anemia in our study can be explained on the basis of the fact that most of our study population comprised of urban population and nutritional anemia was excluded from these patients.

TOTAL LEUCOCYTE COUNT AND DIFFERENTIAL COUNT

Table 15 : Percentage of Decreased Leucocyte Counts in various studies

Study	Murphy MF et al (n=105)	Zon Li et al (n=106)	Castella A et al (n=55)	Present study
% of leucopenia	75 %	65 %	75 %	26.7 %

In present study 26.7 % of the patients had leucocyte counts less than 4000 cells/micro l while 5 % of the patients had leucocyte counts more than 11000 cells/micro l. This is less as compared to studies done by Murphy MF et al (1987) [72] , Zon Li et al (1988) [59] and Castella A et al (1985) [8] where % of leucopenic patients was 75% , 65% , and 75% patients respectively. Of the 26.7 % of the leucopenic cases in our study , 60 % of the patients CD 4 cell counts are between 201 to 500 cells/micro l and there is a positive correlation between leucopenia and CD 4 Cell counts (p value 0.039). This implies that as the CD 4 levels increased , the total leucocyte count also followed a similar trend and showed a rise in the count.

PLATELET COUNT

Table 16 : Percentage of platelet count (Thrombocytopenia) in various studies

Study	Zon Li et al (n=106)	Murphy MF et al (n=105)	Jost J et al (n=321)	Present study
% of thrombocytopenia	40 %	30 %	9 %	14.9 %

Out of 101 patients , 15 (14.9%) were having platelet counts below 1.5 lakhs/cmm and no thrombocytosis is reported in our study. When compared with the CD 4 cell count there is no single case of thrombocytopenia in patients having CD 4 Cell counts > 500 cells/micro l and 19.2 % of patients had thrombocytopenia with CD 4 Cell counts between 201-500 cells/micro l and 50% of the patients had thrombocytopenia with CD 4 Cell counts below 200 cells/micro l. This is comparitively lower than compared to other studies done by Zon Li et al (1998) [59] , Murphy MF et al (1987) [72] and almost similar to the study done by Jost J et al (1988) [73] .

In the present study we were able to establish a positive correlation between the platelet counts and CD 4 Cell counts (p value = < 0.001) , this implies as there is decrease is CD 4 cell count platelet count followed a similar trend and vice versa.

Table 17 : Comparison of Morphological Patterns of blood picture in present study with other studies

Study	Parinitha et al (n=210)	Tripathi et al (n=74)	Present study
% of Normocytic Normochromic blood picture	48.1 %	17.6 %	45.5 %
% of Normocytic Normochromic Anemia	13.7 %	79.9 %	31.7 %
% of Macrocytic Anemia	7.2 %	4.1 %	18.8 %

In our study Normocytic Normochromic Anemia accounts to majority of patients having anemia (31.7%) which is similar to the study done by Tripathi et al (2005) [120] where normocytic normochromic anemia was most common. We also found significant correlation between normocytic anemia and CD 4 Cell count (p value = 0.002).

Among the morphological changes the predominant finding observed in our study was dysplastic neutrophils (83.2%) which was consistent to the findings seen in a study done by Kulkarni CV , Sachin S.[124]

CONCLUSION

CONCLUSION

In our Present study , out of 101 patients , the commonest hematological manifestation observed was anemia among which normocytic normochromic anemia predominates , followed by leucopenia and thrombocytopenia.

The frequency and severity of these hematological manifestations found increased with decline in CD 4 Cell counts which can have a significant impact on clinical outcomes and quality of life.

There was significant statistical correlation between between CD4 count and Normocytic anemia, leucopenia and Thrombocytopenia.

Among the morphological abnormalities , most common morphological finding observed in our study was dysplastic neutrophils followed by detached nuclear fragments with well defined cytoplasmic border , plasmacytoid lymphocytes , atypical lymphocytes. However significant statistical correlation was seen with atypical lymphocytes when compared with CD 4 Cell counts.

Hence all HIV Patients should be investigated for complete blood count including hematologic and morphological assessment of blood cells and treated accordingly to reduce mortality and morbidity and to improve quality of life.

SUMMARY

SUMMARY

1) Hematological and morphological changes of blood cells are common in HIV patients and has got significant impact on clinical outcomes and quality of life (QOL).

2) The variation in the prevalence of hematological abnormalities in different stages of disease are due to number of factors which includes CD 4 Cell counts , clinical disease status , drug therapy , opportunistic infections and malignancy.

3) HIV Infection affected the highly reproductive age group of 21-40 years and predominantly affected males in our study.

4) Among the hematological manifestations , anemia (54.5 %) was the commonest. The frequency and severity of anemia worsened with declining CD 4 cell counts.

5) The commonest type of anemia in present study is normocytic normochromic anemia followed by macrocytic anemia , which is on par with earlier studies.

6) In the study there was significant association between CD4 count and anemia. I.e. with decrease in CD4 count there was increase in anemia rate.

7) Similarly there was significant association between CD4 count and Normocytic anemia, leucopenia and Thrombocytopenia.

8) Among the morphological changes the commonest morphological finding observed in our study was dysplastic neutrophils but no significant statistical correlation was found.

9) In the study there was significant association between CD4 count and atypical lymphocytes. I.e. with decrease in CD4 count there was increase in atypical lymphocytes count.

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BIBILIOGRAPHY

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ANNEXURE

ANNEXURE I

PATIENT PROFORMA

CASE NO : HOSPITAL NO :

AGE :

SEX :

PRESENTING COMPLAINTS :

PAST HISTORY :

CLINICAL DIAGNOSIS :

HEMATOLOGICAL PROFILE OF THE PATIENT

PARAMETER	NORMAL RANGE	AUTOMATD ANALYSER VALUE	MANUAL	MORPHOLOGY	MISCELLANEOUS
RBC ANY INCLUSIONS					
WBC NEUTROPHILS LYMPHOCYTES MONOCYTES EOSINIOPHILS BASOPHILS					

ATYPICAL CELLS IMMATURE FORM MISCELLANEOUS					
PLATELETS					
HB					
HCT					
MCV					
MCH					
MCHC					
RDW					
ANY ORGANISMS SEEN					

CD4 Count :

ANY OTHER FINDINGS :

IMPRESSION :

ANNEXURE II

LEISHMAN STAINING TECHNIQUE

Logistics and materials :

1. Leishman stain
2. Buffered distilled water (p H 6.8-7.2)
3. Timer
4. Slide
5. EDTA blood sample

Smear preparation

1. Smear was covered with Leishman's stain
2. It was allowed to stand for 1-2 minutes
3. Without removing the stain , double the amount of buffered distilled water was added
4. Allowed it to stand for 7 minutes
5. Slide was flooded with tap water
6. Back of the slide was washed with soap and water
7. It was air dried in a tilted / upright position

A Well Stained film had the following features :

- The nuclei of leucocytes was purple
- Neutrophilic granules – tan in color
- Eosinophilic granules – red orange in color
- Basophil – dark purple granules
- Platelets – had dark lilac granules

ANNEXURE III

KEY TO MASTER CHART

RBC – Red blood cell

WBC – White blood cell

PLT – Platelets

N – Neutrophils

L – Lymphocytes

M – Monocytes

E – Eosinophils

B – Basophils

HB – Hemoglobin

P – Present

A - Absent

CASE	AGE	SEX	RBC (x 1000000/microL)	WBC (X1000/microL))	N(%)	L(%)	M(%)	E(%)	B(%)	HB (g/dl)	PLT (x 1000/microL)	PERIPHERAL BLOOD SMEAR REPORT	Anemia	Normocytic normocytic anemia	MACROCYTIC ANEMIA	Leucopenia	Leucocytosis	THROMBOCYTOPENIA	MORPHOLOGICAL CHANGES	MACROPLATELETS	HOWELL JOLLY BODIES (RBC)	ATYPICAL LYMPHOCYTES	MONOCYTIC VACUOLATIONS	DETACHED NUCLEAR FRAGMENTS	DYSPLASTIC NEUTROPHILS	Plasmacytoid LYMPHOCYTES	CD 4 COUNT	OTHER FINDINGS
S1	40	M	4.4	9.7	37	48	5	6	2	12.4	233	Normocytic normochromic	A	A	A	A	A	A	plasmacytoid lymphocytes , detached nuclear fragments , pseudo pelger huet anamoly , toxic changes	A	A	A	A	P	A	P	641	Nil
S2	70	F	4.7	8.4	47	45	5	1	0.5	9	284	dimorphic	P	A	A	P	A	A	plasmacytoid lymphocytes , detached nuclear fragments , pelger huet anamoly , toxic changes , macroplatelets	P	A	A	A	P	A	P	512	Nil
S3	46	M	1.9	3.09	40	50	10	0.2	1	6	205	macrocytic	P	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	480	Nil
S4	28	F	4.4	5.7	40	50	10	2	1	15.3	214	macrocytic anemia	P	A	P	A	A	A	detached nuclear fragments , atypical lymphocytes , degenerated cells	A	A	A	A	P	A	A	305	Nil
S5	54	M	2.5	4.9	46	47	3	2	1	6.7	363	macrocytic anemia	P	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	230	Nil
S6	45	M	4.8	8.3	43	51	2	3	0.8	12.2	199	Normocytic normochromic	A	A	A	A	A	A	plasmacytoid lymphocytes , detached nuclear fragments , pelger huet anamoly , toxic changes	A	A	A	A	P	A	P	230	Nil
S7	36	F	4.7	7.17	49	45	4	2	1	12.8	226	Normocytic normochromic blood picture	A	A	A	A	A	A	atypical lymphocytes , band forms , toxic changes	A	A	P	A	A	A	A	928	Nil
S8	50	M	3.6	9.3	49	45	4	2	0.8	7.6	523	normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	472	Nil
S9	42	M	4.8	11.7	64	30	1.2	3.2	1.4	14	204	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , pelger huet anamoly	A	A	A	A	P	A	A	468	Nil
S10	43	M	4	8.8	58	37	2	3	0.4	12.6	240	normocytic normochromic anemia	P	P	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	303	Nil
S14	28	M	6.9	5.4	58	37	2	3	1	17.1	178	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	517	Nil
S24	48	M	5.2	9.4	43	50	2	2	1	13.8	201	Normocytic normochromic blood picture	A	A	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	A	A	A	P	P	361	Nil
HH6	42	F	3.8	7.4	46	45	4	2	1	14.8	223	macrocytic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	638	Nil
HH5	52	F	3.5	4.16	75	20	3	1	1	11	149	normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	4	Nil
HH1	45	M	3.65	5.9	53	36	2	8	1	14	242	macrocytic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	570	Nil
HH3	40	F	3.8	11.4	80	17	1	2	1	10.7	280	normocytic normochromic anemia with leucocytosis	P	P	A	A	P	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	242	Nil
HH4	35	M	3.24	6.4	48	46	4	2	1	12.9	294	macrocytic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	570	Nil
H1	38	M	3.5	10.87	63	30	2	4	1	10.2	347	macrocytic anemia	P	A	P	P	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	852	Nil
H2	36	M	4.4	10.6	58	34	2	6	1	14.8	224	Normocytic normochromic blood picture	A	A	A	P	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	550	Nil
H3	35	F	3.25	12.4	61	26	5	7	1	7.9	372	normocytic normochromic anemia with leucocytosis	P	P	A	A	P	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	275	Nil
H4	50	M	3.5	8.8	34	50	6	8	1	10.5	201	macrocytic anemia	P	A	P	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	A	P	761	Nil
H5	38	M	4.8	9.1	34	55	7	2	2	15.9	239	Normocytic normochromic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	969	Nil
H6	42	M	3.82	6.37	53	43	2	2	0.3	14.4	240	macrocytic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	888	Nil
H7	28	F	2.4	4.2	68	27	1.4	3	1	10.9	201	macrocytic anemia with leucopenia	P	A	P	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	208	Nil
H8	42	M	4.5	6.6	50	44	3	2	0.5	12.3	175	Normocytic normochromic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	553	Nil
H9	50	M	4.04	7.4	57	34	2	7	0.8	13.7	271	Normocytic normochromic blood picture	A	A	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	512	Nil
H10	40	M	3.16	5.68	58	34	4	4	0.6	11	149	macrocytic anemia	P	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	284	Nil
H36	38	M	4.5	9.1	69	26	3	2	0.5	13.4	111	Normocytic normochromic blood picture with thrombocytopenia	A	A	A	A	A	P	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	245	Nil
H37	30	F	4.9	6.7	56	28	3	12	2	12.1	218	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	469	Nil
K1	44	M	2.8	3.24	16	76	7	0.3	1.8	6.1	183	macrocytic anemia with leucopenia	P	A	P	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	456	Nil
K2	42	M	3.3	7.9	25	70	3.7	0.5	1	10.6	324	normocytic normochromic anemia	P	P	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	749	Nil
K3	40	F	3.5	8.4	33	63	2	1	0.5	10.6	261	normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	A	P	752	Nil
K4	44	M	2.9	3.7	17	73	9	0	0.3	9.4	216	macrocytic anemia with leucopenia	P	A	P	P	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	A	A	P	A	359	Nil
K5	43	M	4.2	3.3	39	45	13	0.5	2	11.5	125	normocytic normochromic anemia with leucopenia and	P	A	A	P	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	39	Nil
K6	35	M	3	7.9	49	43	6	1.3	0.6	7.4	339	normocytic normochromic anemia	P	P	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	490	Nil
K30	35	F	4.3	1.6	30	44	24	0.5	0.8	8.3	43	normocytic normochromic anemia with leucopenia and thrombocytopenia	P	P	A	P	A	P	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	240	Nil
N1	40	M	5.1	12.9	68	22	7	0.7	1.7	14.8	261	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	1158	Nil
N2	47	M	4.4	7.8	55	37	3	4	1	13.6	182	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	421	Nil

N5	35	F	4.1	5.7	50	38	6	4	1	7.3	226	microcytic hypochromic anemia	P	P	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	779	Nil
N4	42	M	4.3	7.6	60	24	3	11	2	14.7	146	Normocytic normochromic blood picture with thrombocytopenia	A	A	A	A	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	468	Nil
N3	38	M	4.3	6.3	62	30	5	2	1	10	178	Normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	A	A	A	P	P	532	Nil
N27	36	F	5.2	5.6	60	24	3	11	2	15.5	308	Normocytic normochromic blood picture	A	A	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	454	Nil
N46	50	M	5.4	13.5	10	87	2	1	1	9.8	331	microcytic hypochromic anemia with leucocytosis	P	P	A	A	P	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	2175	Nil
N16	36	F	4.19	3.4	52	37	9	0.5	1.7	11.6	248	normocytic normochromic anemia with leucopenia	P	P	A	P	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	14	Nil
N45	36	M	4.08	7.9	25	67	6	0.8	0.9	12.2	487	normocytic normochromic anemia with thrombocytopenia	P	P	A	A	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	A	A	402	Nil
N6	42	M	2.6	3.8	52	37	7	3	0.5	8.3	255	macrocytic anemia with leucopenia	P	A	P	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	530	Nil
R2	38	M	5.6	5.15	26	67	5	1	1	14.4	125	Normocytic normochromic blood picture with thrombocytopenia	A	A	A	P	A	P	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	309	Nil
R6	42	M	4.5	9.4	47	37	6	8	2	15.7	322	Normocytic normochromic blood picture	A	P	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	274	Nil
R3	42	M	4.09	7.9	34	53	10	0.5	2	12	273	Normocytic normochromic anemia	P	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	399	Nil
R10	45	M	3.09	10.65	78	10	8	2	1	10.8	377	macrocytic anemia	P	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	230	Nil
R7	26	F	2.6	5.1	58	30	9	1	1	11.3	198	macrocytic anemia	P	A	P	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	428	Nil
R8	60	M	3.1	10.59	73	17	5	3	0.8	10.2	286	macrocytic anemia	P	A	P	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	389	Nil
R9	36	M	3.6	7.7	56	30	9	3	1	10.6	313	macrocytic anemia	P	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	524	Nil
R4	52	M	4.6	7.5	90	6	3	1	0.2	12	12	normocytic normochromic anemia with thrombocytopenia	P	P	A	A	A	P	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	P	A	A	P	P	35	Nil
R5	29	M	3.15	5.9	91	6	1	1	0.3	10	100	normocytic normochromic anemia with thrombocytopenia	P	P	A	P	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	116	Nil
R13	40	F	3.89	5.2	58	30	9	1	1	11.7	140	normocytic normochromic anemia with thrombocytopenia	P	P	A	A	A	P	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	132	Nil
R22	67	M	4.91	5.69	59	31	9	0.5	0.7	14.9	218	Normocytic normochromic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	A	A	665	Nil
KK1	38	M	4.8	9.1	34	55	7	2	2	15.9	239	Normocytic normochromic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	A	A	P	A	854	Nil
KK2	42	M	3.82	6.37	53	43	2	2	0.3	14.4	240	macrocytic blood picture	A	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	756	Nil
KK3	28	F	2.4	4.2	68	27	1.4	3	1	10.9	201	macrocytic anemia with leucopenia	P	A	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	A	A	162	Nil
KK4	42	M	4.5	6.6	50	44	3	2	0.5	12.3	175	Normocytic normochromic blood picture	A	A	A	P	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	454	Nil
KK5	50	M	4.04	7.4	57	34	2	7	0.8	13.7	271	Normocytic normochromic blood picture	A	A	P	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	P	A	A	P	P	534	Nil
KK6	40	M	3.16	5.68	58	34	4	4	0.6	11	149	macrocytic anemia	P	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	265	Nil
KK7	38	M	4.5	9.1	69	26	3	2	0.5	13.4	111	Normocytic normochromic blood picture with thrombocytopenia	A	A	A	A	A	P	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	234	Nil
LL1	30	F	4.9	6.7	56	28	3	12	2	12.1	218	Normocytic normochromic blood picture	A	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	450	Nil
LL2	54	M	2.5	4.9	46	47	3	2	1	6.7	363	macrocytic anemia	P	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	243	Nil
LL3	45	M	4.8	8.3	43	51	2	3	0.8	12.2	199	Normocytic normochromic blood picture	A	A	A	A	A	A	plasmacytoid lymphocytes , detached nuclear fragments , pelger huet anamoly , toxic changes	A	A	A	A	P	A	P	222	Nil
LL4	36	F	4.7	7.17	49	45	4	2	1	12.8	226	Normocytic normochromic blood picture	A	A	A	P	A	A	atypical lymphocytes , band forms , toxic changes	A	A	P	A	A	A	A	879	Nil
LL5	50	M	3.6	9.3	49	45	4	2	0.8	7.6	523	normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	P	A	A	P	P	456	Nil
LL6	42	M	4.8	11.7	64	30	1.2	3.2	1.4	14	204	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , pelger huet anamoly	A	A	A	A	P	A	A	432	Nil
LL7	43	M	4	8.8	58	37	2	3	0.4	12.6	240	normocytic normochromic anemia	P	P	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	342	Nil
YY1	28	M	6.9	5.4	58	37	2	3	1	17.1	178	Normocytic normochromic blood picture	A	A	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	A	A	578	Nil
YY2	48	M	5.2	9.4	43	50	2	2	1	13.8	201	Normocytic normochromic blood picture	A	A	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	A	A	A	P	P	343	Nil
YY3	42	F	3.8	7.4	46	45	4	2	1	14.8	223	macrocytic blood picture	A	A	P	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	664	Nil
YY4	44	M	2.8	3.24	16	76	7	0.3	1.8	6.1	183	macrocytic anemia with leucopenia	P	A	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	467	Nil
YY5	42	M	3.3	7.9	25	70	3.7	0.5	1	10.6	324	normocytic normochromic anemia	P	P	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	A	A	A	A	P	P	A	786	Nil
YY6	40	F	3.5	8.4	33	63	2	1	0.5	10.6	261	normocytic normochromic anemia	P	P	P	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	P	A	A	A	P	756	Nil
YY7	44	M	2.9	3.7	17	73	9	0	0.3	9.4	216	macrocytic anemia with leucopenia	P	A	A	P	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	338	Nil
YY8	43	M	4.2	3.3	39	45	13	0.5	2	11.5	125	normocytic normochromic anemia with leucopenia and	P	P	A	P	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	42	Nil
FG1	35	M	3	7.9	49	43	6	1.3	0.6	7.4	339	normocytic normochromic anemia	P	P	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	412	Nil

FG2	35	F	4.3	1.6	30	44	24	0.5	0.8	8.3	43	normocytic normochromic anemia with leucopenia and thrombocytopenia	P	P	A	P	A	P	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	P	A	A	P	P	234	Nil
FG3	40	M	5.1	12.9	68	22	7	0.7	1.7	14.8	261	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	1098	Nil
FG4	47	M	4.4	7.8	55	37	3	4	1	13.6	182	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	432	Nil
FG5	35	F	4.1	5.7	50	38	6	4	1	7.3	226	microcytic hypochromic anemia	P	P	A	P	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	762	Nil
FG6	42	M	4.3	7.6	60	24	3	11	2	14.7	146	Normocytic normochromic blood picture with thrombocytopenia	A	A	A	A	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	A	A	498	Nil
FG7	38	M	4.3	6.3	62	30	5	2	1	10	178	Normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	598	Nil
FG8	36	F	5.2	5.6	60	24	3	11	2	15.5	308	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	471	Nil
FG9	50	M	5.4	13.5	10	87	2	1	1	9.8	331	microcytic hypochromic anemia with leucocytosis	P	A	A	A	P	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	2090	Nil
QA1	36	F	4.19	3.4	52	37	9	0.5	1.7	11.6	248	normocytic normochromic anemia with leucopenia	P	P	A	P	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	12	Nil
QA2	36	M	4.08	7.9	25	67	6	0.8	0.9	12.2	487	normocytic normochromic anemia with thrombocytopenia	P	P	P	A	A	P	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	412	Nil
QA3	42	M	2.6	3.8	52	37	7	3	0.5	8.3	255	macrocytic anemia with leucopenia	P	A	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	523	Nil
QA4	38	M	5.6	5.15	26	67	5	1	1	14.4	125	Normocytic normochromic blood picture with thrombocytopenia	A	A	A	A	A	P	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	319	Nil
QA5	42	M	4.5	9.4	47	37	6	8	2	15.7	322	Normocytic normochromic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	A	A	P	A	P	A	260	Nil
QA6	42	M	4.09	7.9	34	53	10	0.5	2	12	273	Normocytic normochromic anemia	P	P	A	P	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	380	Nil
QA7	28	M	6.9	5.4	58	37	2	3	1	17.1	178	Normocytic normochromic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	A	A	A	P	P	A	520	Nil
QA8	48	M	5.2	9.4	43	50	2	2	1	13.8	201	Normocytic normochromic blood picture	A	A	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	390	Nil
QA9	42	F	3.8	7.4	46	45	4	2	1	14.8	223	macrocytic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	A	P	A	579	Nil
QA10	52	F	3.5	4.16	75	20	3	1	1	11	149	normocytic normochromic anemia	P	P	A	A	A	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	A	P	A	A	P	P	5	Nil
QA11	45	M	3.65	5.9	53	36	2	8	1	14	242	macrocytic blood picture	A	A	A	A	A	A	band forms , monocytic vacuolations , dysplastic neutrophils	A	P	A	P	A	P	A	421	Nil
QA12	40	F	3.8	11.4	80	17	1	2	1	10.7	280	normocytic normochromic anemia with leucocytosis	P	P	A	A	P	A	toxic changes , plasmacytoid lymphocytes , dysplastic neutrophils , atypical lymphocytes	A	P	P	A	A	P	P	213	Nil
QA13	35	M	3.24	6.4	48	46	4	2	1	12.9	294	macrocytic blood picture	A	A	A	A	A	A	detached nuclear fragments , dysplastic neutrophils , toxic changes , macroplatelets	P	P	A	A	P	P	A	514	Nil