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EFFECT OF HAART ON HEMATOLOGICAL PROFILE AND OXIDATIVE STRESS IN HIV NAIVE PATIENTS.

Chapter 1

INTRODUCTION

1.1 INTRODUCTION AND BACKGROUND

HIV a worldwide pandemic, revealing cases from all nations. In accordance with UNAIDS (United Nations Program on HIV/AIDS) 2017, around 36.9 million individuals were living with HIV/AIDS around the world. An expected 940,000 deaths of individuals because of AIDS-related ailments worldwide in 2017, contrasted with 1.4 million in 2010 and 1.9 million in 2004 [1]. According to recent statistics at the end of 2019, worldwide there are 38 million people living with HIV, with 68% adults and 53% children and 25.4 million are on ART. In India (2017), 2.1 million people were living with HIV.

From 2000 to 2019 new HIV infection fell by 39% and HIV related death fell by 51%. This was due to joint efforts by national HIV program supported by civil society and international development partners which worked together to bring improved medical drug treatment with new antiretroviral drugs, HAART (Highly active antiretroviral treatment) regimen and also active preventive measures.

HIV infection is regularly connected with a hematopoietic framework [2]. Disorders of the hematological framework incorporate, anaemia, leucopenia, thrombocytopenia which may be the result of HIV infection or medication therapy

impacts (adverse effects)[3]. HIV infection attacks and assaults significant human resistant cells, for example, T helper cells, macrophages and dendritic cells(with CD4 receptors), that cause immunodeficiency. Continued immune system failure (low CD4 count) leads to immunodeficiency syndrome (AIDS), making patient susceptible to opportunistic infections and malignancy.

Presently, there is proof that HIV infection (affecting various signalling pathways and viral proteins including Gp120, Tat, Nef and Vpr, and reverse transcriptase) causes oxidative stress imbalance leading to mitochondrial dysfunction [4]. Oxidative stress and disturbed mitochondrial function is important cellular and molecular mechanism responsible for HIV pathologies and also increases viral replication. Reactive oxygen species (ROS) likewise play role in autophagy and apoptotic pathway, altogether influencing cell survival and increases cell mortality [5]. HIV infection worsens the oxidative stress which keeps on expanding with the utilization of ART [6]. However, individuals with HIV show a decline in total antioxidant capacity and reduction in the substance of glutathione (GSH) in the blood.

We can reduce the mortality and morbidity of the disease by proper monitoring. Monitoring of HIV disease progression most successfully done by prognostic markers like CD4+ T cell count and viral load [7,8]. Lipid peroxidation has been recognized as one of the biomarkers for diagnosing oxidative imbalance in HIV infected individual. Study done by Friis-Moller et al [9] have indicated that HIV-positive patient's shows oxidative imbalance.

Without treatment, the future of a HIV-positive individual is assessed to be 9-11 years. In India, NACO(National AIDS Control Organisation) and MSACS(Maharashtra State AIDS Control Society) has incorporated a system of testing and

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treatment in HIV/AIDS. Improved and new antiretroviral drugs have been found to prolong life of HIV patients by efficiently suppressing HIV viral load. The principle antiretroviral medication (HAART) is currently being suggested as a standard treatment for HIV disease. HAART incorporates a nucleoside and non-nucleoside analogues which have potential to inhibit HIV reverse transcriptase and protease. Combination of three or four medications that make numerous obstructions to HIV replication and holds the number down and lessen chances of DNA mutations [10]. This helps to improve life quality of individuals living with HIV/AIDS [11]. Depending on utilization of antiretroviral combinations may have a positive or negative effect on hematological parameters. HIV treatment shows adverse effects like nausea, vomiting, diarrhoea, anaemia, neutropenia and so forth [12]. There are reports that antiretroviral drugs likewise show an increase in oxidative stress.

There are studies describing hematological parameters of HIV infected individuals and there correlation with CD4 count after receiving HAART. Also few studies are on HIV and oxidative stress and associated pathology. However there are very few studies where correlation between both hematological profile, CD4 level and oxidative stress levels are studied at same point. Hence the aim of the present study is to assess and compare hematological profile and oxidative stress levels with CD4 count in HIV infected adults before and after initiation of HAART and to see whether we can find any oxidative marker which may help us in prognosis and in line of treatment of the disease.

Study hypothesis: HIV infection sets in motion variety of cellular derangements, causing altered hematological profile and accelerates ROS (Reactive Oxidative Species) which in the absence of antioxidants, accelerate apoptosis and immune cell

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death, leading to progression of HIV replication and Acquired Immunodeficiency Syndrome (AIDS).

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1.2 AIMS AND OBJECTIVES OF THE STUDY

1] To study hematological profile and CD4 count before and after 3 month of HAART.

2] To study correlation of hematological profile and CD4 count before and after 3 month of HAART.

3] To study oxidative parameters in HIV patients before and after 3 month of HAART.

4] To study correlation of oxidative parameters and CD4 count in HIV patients before and after 3 month of HAART.

5] To find any oxidative marker which may help us in prognosis and in line of treatment of the HIV disease.

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Chapter 2

REVIEW OF LITERATURE

2.1 History and epidemiology of the Human Immunodeficiency Virus (HIV)/AIDS.

The human immunodeficiency virus (HIV) was obscure until the mid-1980. First clinical case observed was in 1981 in the United States, since that time has tainted a large number of people in an overall pandemic. HIV infection resulted in destruction of the immune system further landing into AIDS, where HIV infected people are in danger of death because of life threatening infections and neoplastic outcomes of the inescapable complications of AIDS[13]. HIV is thought to have started in non-human primates in sub-Saharan Africa and was moved to people (Zoonosis) late in the nineteenth century, [14] most likely through introduction to primate blood. The earliest retrospectively described case of AIDS is accepted to have been in Norway, in 1966.

AIDS was first clinically observed between late 1980 and early 1981 in U.S. Drug users (injections) and gay men with no cause known for impaired immunity showed manifestations of Pneumocystis carinii pneumonia (PCP) and uncommon skin malignant growth called Kaposi's sarcoma (KS)[15]. Alerting to this U.S. Centre for Disease Control and Prevention (CDC), framed task force to monitor the outbreak. Subsequent to bizarre manifestations introducing in patients, the CDC team named the condition Acquired Immune Deficiency Syndrome (AIDS)[16] in July 1982 meet. Around the world in 2016, there were around 37 million individuals living with HIV, around 1 million AIDS-related deaths and 1.8 million new HIV infections. 70 percent of those living with HIV, newly infected or dying from HIV were from sub-Saharan

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Africa and notably in eastern and southern Africa [1]. There are an expanding number of nations who are accomplishing epidemic control, India is one of them. India is the third most burdened nation of HIV disease in the world [17].

According to data released in 2018, India in 2017 estimated as, 2.1 million individuals living with HIV, 0.2% adult HIV prevalence (age15-49), 88,000 new HIV cases, 69,000 AIDS related deaths and 56% on ART(antiretroviral treatment). In 2017, adults HIV prevalence is estimated at 0.25% (0.18-0.34) among males and at 0.19% (0.14-0.25) among females. At national level adult prevalence has continued steady fall from peak of 0.38% in 2001-03 through 0.34% in 2007, 0.28% in 2012 and 0.26% in 2015 to 0.22% in 2017.

2.2 Classification and types of human immunodeficiency virus (HIV).

HIV is a unique type of retrovirus containing RNA. There are three groups of retroviruses, Oncoviruses (Causing malignant growths), lentivirus (slow infection of which HIV is one) and foamy viruses or spumaviruses (less known). Retroviral animal infections, for example SIV(Simian immunodeficiency virus) infects nonhuman primates, FIV(feline immunodeficiency virus) influences cats and Visna virus infects sheep's. HIV is an organism from the genus *Lentivirus*, part of the family *Retroviridae*.

Types of HIV.

There are two primary types of HIV, HIV-1 and HIV-2. HIV-1 was found by Luc Montagnier and his associates at institute Pasteur in Paris in1983 and HIV-2 was first recognized among patients in Cameroon in 1985. Around the world, the prevalent infection is HIV-1, which records for around 95% of all infections. HIV-2 is estimated

to be more than 55% genetically different from HIV-1[18]. Both HIV-1 and HIV-2 have many groups within them and further branch out into subtypes, or strains.

Comparison of HIV species

Species	Virulence	Infectivity	Prevalence	Inferred origin
HIV-1	High	High	Global	Common chimpanzee
HIV-2	Lower	Low	West Africa	Sooty mangabey

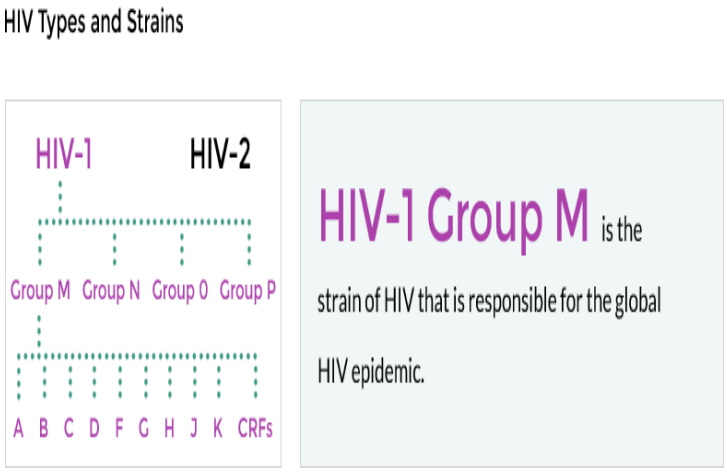

Figure 2.1 HIV types and strains

HIV-1- Is most common type and has four groups M,N,O,P.

Group M (Major) is responsible for HIV pandemic. Almost 90% of all HIV-1 cases originate from this group. This M group subdivided in nine strains, A,B,C,D,F,G,H,J and K. B strain is the most well-known in U.S. Around the world, the most widely recognized HIV strain is C. Furthermore, these subtypes can combine

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genetic material to form hybrid virus called as a 'circulating recombinant form' (CRFs). Around 89 of these are known to exist [19]. The other three groups N, O and P are very uncommon. Group O, found up to 5% of diseases in a few west and central African countries, and Group N and P have been seldom distinguished in Cameroon. All groups can be analysed by HIV-1 antibody tests.

HIV-2- is seen in smaller number of individuals from generally West Africa, more similar to SIV, is less virulent and is less progressive. The subtype of HIV-2 have been designed from A to F. There is up to 25% distinction in genetic homology among these subtypes.

Transmission of both HIV-1 and HIV-2 is similar, specifically sexual contact, blood-borne exposure (blood transfusion, sharing needles), and perinatal transmission, Person can be co-infected with HIV-1 and HIV-2[20]. Both types can be serologically detected independently.

2.3 Structure of HIV.

HIV includes from a class known as Retroviruses. These viruses store their genetic data as ribonucleic acid (RNA), not at all like most viruses which store their genetic data as deoxyribonucleic acid (DNA). Before viral replication can occur, the RNA must be changed over to DNA by reverse transcription, consequently the Latin expression Retro, signifying 'turning back' [21]. Viruses are absolutely reliant on living cells to get by as they use the host cell's own replication measures, so as to recreate themselves. At the point when the viruses leaves the host cell it takes the bilayer lipid membrane from the host cell.

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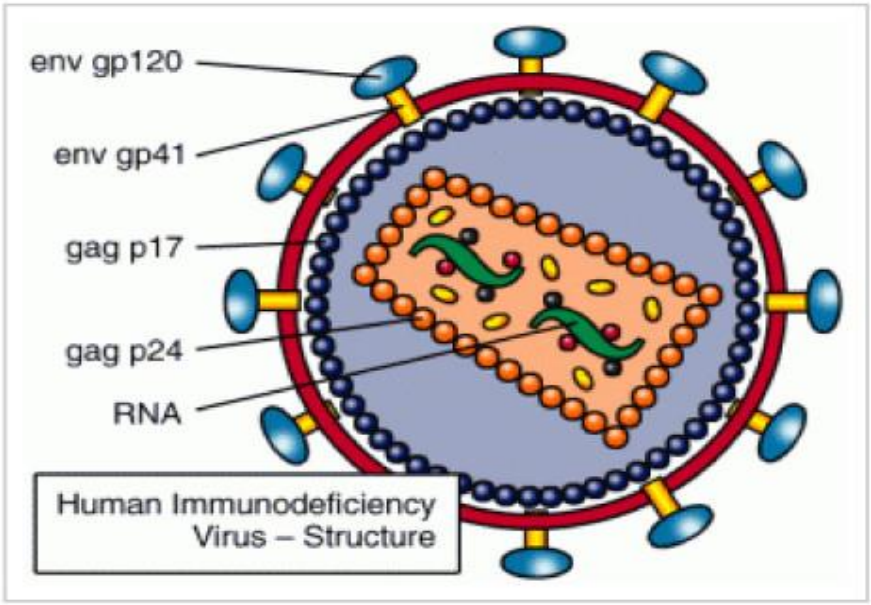

Figure 2.2: A diagrammatic representation of HIV [22]

The human immunodeficiency viruses are roughly 100-120 nm in measurement. It has a lipid envelope, wherein are inserted the trimeric transmembrane glycoprotein gp41 to which the surface glycoprotein gp120 is attached. These two viral proteins are liable for connection to the host cell and are encoded by the *env* gene of the viral RNA genome. Underneath the envelope, is the matrix protein p17, the core proteins p24 and p6 and the nucleocapsid protein p7 (bound to the RNA), all encoded by the viral *gag* gene. Inside the viral core, lies 2 duplicates of the ~10 kilobase (kb) positive-sense viral RNA genome (diploid RNA genome), along with the protease, integrase and reverse transcriptase enzymes. These three proteins are encoded by the viral *pol* gene [23].

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2.4 Retroviral Genome of HIV

a) Structural gene of HIV-1: These genes encodes for products which take part in development of useful functional structures of virus. HIV is made out of three genes, which code for the internal core proteins (*gag* gene), the viral enzymes (*pol* gene) and envelop proteins (*env* gene).

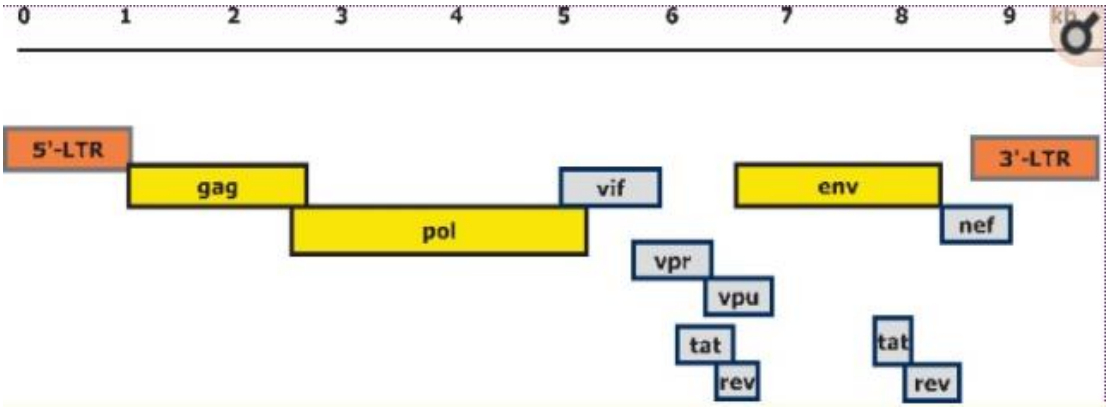

Figure 2.3 Structural of genome of HIV-1[24]

The *gag* gene- codes for core and shell of virus. It is liable for formation of the dense cylindrical shaped core protein (p24) and a few internal proteins (p7, p15, p17 and p55). The gag protein can coordinate the development of viral like particles when all other genes (pol and env) are absent. It is just when the gag gene is non-functional that the retroviruses (HIV) lose their ability to bud out of the host cell [25]. The p24 antigen (significant core antigen) can be detected in serum during beginning phases of infection till appearance of antibodies.

The *pol* gene- Encodes for polymerase reverse transcriptase and other viral enzymes like protease and integrase. It is precursor protein, which is cleaved in to parts like p64 which has reverse transcriptase and RNAse activity, p51with just reverse transcriptase action, p10 is a protease and p32 is an integrase. The polymerase is comprised of two

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subunits, alpha and beta. The alpha subunit has three capacities: (1) to make DNA from RNA; (2) to digest the RNA from RNA: DNA hybrid and (3) to make DNA from DNA. The beta subunit is non-enzymatic [26].

The *env* gene- codes for two significant envelope glycoprotein gp120, (situated on external spikes of HIV) and gp41,(the transmembrane protein that joins gp120 to the surface of HIV) that become inserted all through the host membrane, which eventually turns into the envelope that surrounds the virus as it 'buds' out.

b) Regulatory genes of HIV-1.

i) Positive Regulatory Genes-There are four positive regulatory genes that produce regulatory proteins which influences expression of other genes and positively regulate formation of viral particles and bring assembly of viral components.

1*) tat* gene- (trans activator of transcription)- encodes the trans-activator of transcription protein. Tat that emphatically increases the transcription of incorporated proviral DNA to messenger RNA (mRNA) that is further utilized by the cell to produce proteins. *tat* is made out of two isolated pieces that must be spliced together to become functional[27].

2) *rev* gene- (regulator of expression of viral proteins) encodes a protein named Rev that is necessary to regulate HIV protein production by bringing the transition from first to the second phase of HIV proviral DNA transcription and translation. *rev* is also made of two isolated pieces that must be joined together to become functional[27].

3) *vif* gene- (virus infectivity factor) impacts the infectivity of the viral particles and to bring release of infectious virus from the cell[27].

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4) *vpr* gene Stimulate promotor region of the virus, which further helps in transcription.

5) *vpu*-(viral protein u)(in HIV-1) and *vpx* (in HIV-2), enhance development, maturation and release of virus from the cells . *Vpu* additionally encourage production of the HIV envelope Env [27]. Detection of type specific sequences *vpu* and *vpx* may help in differentiating between infections by HIV-I or HIV-2.

ii) Negative Regulatory Genes.

***nef* gene** (negative factor), produces proteins which act on a segment of the long terminal repeat (LTR) called NRE (negative regulatory element) which communicates down regulating viral replication by inhibiting production of structural protein.

2.5 Replication of HIV

Retroviruses can't reproduce outside of living host cells and don't contain deoxyribonucleic acid (DNA). The pathogenesis of HIV infection is a function of the viral life cycle, cellular environment of host cell, and amount of viruses in the infected person. Subsequent to entering the body, the viral molecule get attached with CD4 receptor molecule which is fused to a susceptible cell membrane, undergoes endocytosis which helps to enter the cell. The likelihood of infection is a function of both the quantity of infective HIV virions in the body fluid which contacts the host and the number of cells present at the site having appropriate CD4 receptors [28]. Replication can be summed up in six stages 1) binding and entry 2) uncoating 3) reverse transcription 4) provirus integration 5) synthesis of viral protein 6) assembly and its release.

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1) Binding and entry of HIV.

HIV initially infects the cells that have CD4 cell-surface receptor particle, utilizing receptor to get entry. Numerous cell types share common epitopes with this protein, however CD4 lymphocytes play crucial job. Cells with CD4 receptors susceptible to infection includes cells of the mononuclear phagocyte system, mainly blood monocytes and tissue macrophages, T lymphocytes, B lymphocytes, natural killer (NK), dendritic cells (Langerhans cells of epithelia and follicular dendritic cells in lymph nodes), hematopoietic stem cells, endothelial cells, gastrointestinal epithelial cells, and microglial cells in cerebrum.

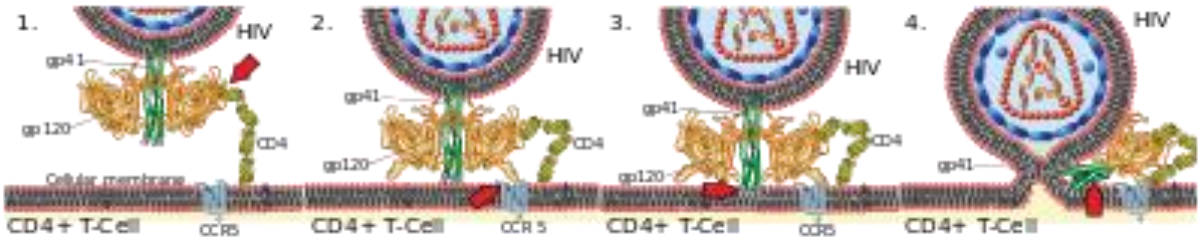

Figure 2.4: Fusion of HIV with host cell membrane (Natural Immunology 2002)

Viral entry into the cell starts through interaction of the trimeric envelope complex (gp160 spike)(gp 120 and gp41) and both CD4 and a chemokine co-receptor (for the most part either CCR5(on macrophage) or CXCR4(on T cells).The gp160 spike contains binding sites for both CD4 and chemokine receptors. First step in fusion, includes the high affinity attachment of the gp120(CD4 binding domains) to CD4. Once gp120 is bound with the CD4 protein, the envelope complex goes through an auxiliary change, exposing the chemokine receptor binding domains of gp120 and permitting them to interface with the target chemokine receptor. This considers a more steady two dimensional connection, which permits the N-terminal fusion peptide gp41 to enter the

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cell membrane causing the breakdown of the extracellular portion of gp41 into a hairpin shape. This loop like structure brings the virus and host cell membranes near one another, permitting fusion of the membranes and resulting passage of the viral capsid into host cell. The differential articulation of chemokine receptors on cell targets has been demonstrated to be a significant determinant of the HIV-1 tropism [29]. The term viral tropism alludes to the cell types a virus infects.

A few people are resistant to specific strains of HIV, For instance, individuals with the CCR5 mutation are impervious to be infected by the R5 virus, as the mutation leaves HIV unfit to bind to this co-receptor, reducing its capacity to infect target cells.

2) Uncoating of HIV.

Following fusion of membrane, the virus uncoats into the cytoplasm of the target cell. Cell enzymes eliminates the capsid, delivering the RNA and 3 enzymes.

3) Reverse transcription of the HIV genome into double strand DNA.

Soon after the viral capsid enters the cell, a compound called reverse transcriptase liberates the positive-sense single strand RNA genome from the viral proteins and duplicates it into a complementary DNA (cDNA) molecule. The reverse transcription process is very error prone and result into mutation which may be responsible for drug resistance or permits virus to enter the body's immune system. The reverse transcriptase additionally has ribonuclease activity that further degrade viral RNA during the formation of cDNA. Together, the cDNA and its complement, forms double strand viral DNA that is then moved into the cell nucleus [30].

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4) Provirus integration.

The incorporation of the viral DNA into the host cell's genome is completed by another viral enzyme called integrase.

5) Virus protein synthesis.

Proviral incorporated DNA transcribe to form viral mRNA. Messenger RNA coding for long fragments moves into the cytoplasm, where structural proteins of new virions are synthesized. The cleavage of the precursor molecule (large protein particle) by the HIV-1 protease is vital for formation of infectious viral particles. The pol and gag gene coded protein forms nucleus of the developing HIV particle, the gene items coded by the env forms the glycoprotein spikes [30].

6) Assembly and release.

The arrangement of new viral particles is a stepwise cycle: two viral RNA strands comes together along with replication enzymes, while centre core proteins collect over them forming the virus capsid. This juvenile molecule relocates towards the cell surface. The huge precursor protein are then cleaved by the HIV-1 protease, bringing about new infectious viral particles, which bud through the host cell membrane (Figure 2.5). During budding the viral lipid membrane get incorporated with many host cell proteins and become enhanced with phospholipids and cholesterol. Uniquely in contrast to T-lymphocytes, where budding happens at the cell surface and virions are delivered into the extracellular space, the budding process in monocytes and macrophages brings about the accumulation of virions in intracellular vacuoles which are then delivered. The formed virion is yet immature as the gag polyproteins still should be divided into the matrix, capsid and nucleocapsid proteins. This cleavage is

interceded by the viral protease and can be restrained by antiretroviral drugs of the protease inhibitor class. The different structural components get assembled to form mature virion capable to infect another cell [30].

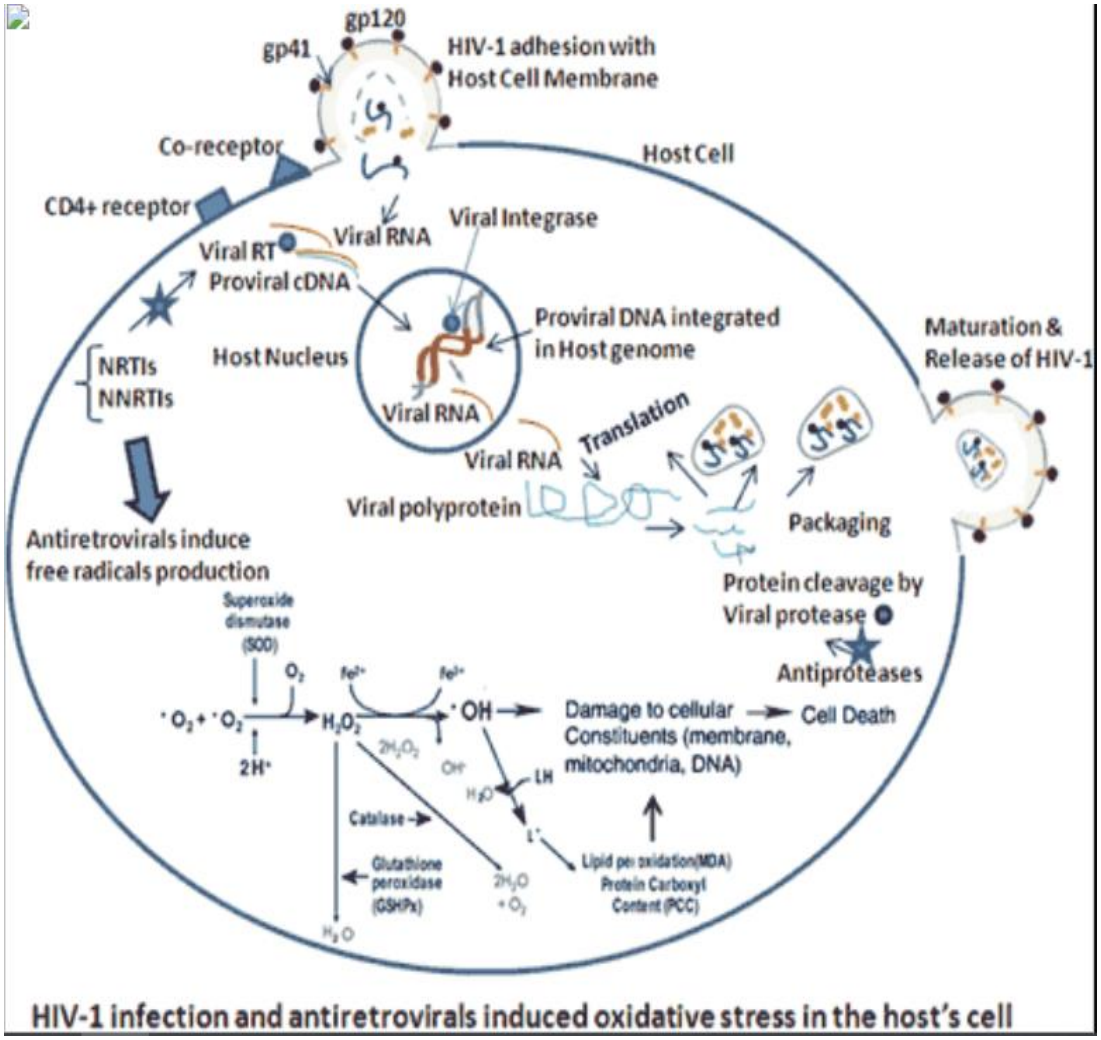

Figure 2.5: Replication cycle of HIV [31].

2.6 Mode of transmission of HIV.

HIV is an infectious disease and can be communicated from individual to individual. It is most ordinarily sent by engaging in sexual relations without a condom or by sharing needles contaminated with the virus. HIV is found in all the body fluids

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including salivation, blood, semen, pre-seminal fluid, vaginal discharges, nervous tissue, spinal fluid, anal secretions and breast milk. Only blood, semen, and breast milk mostly have been seen to transmit disease to other people. HIV is spread by three primary courses, sexual contact (most common), critical introduction to contaminated body fluids or tissues, and from mother to child during pregnancy, parturation, or breastfeeding (vertical transmission)[32]. It is likewise possible to be co-infected by more than one strain of HIV, a condition known as HIV superinfection [33].

Sexual transmission.

The most incessant method of transmission of HIV is through sexual contact with an infected person [29]. Globally, the most widely recognized method of HIV transmission is by means of sexual contacts between individuals of opposite sex, but pattern of transmission differs among the countries. Danger of transmission increases in presence of genital ulcers. Genital ulcers seem to expand the danger around fivefold [34]. Other infections sexually transmitted, for example, gonorrhoea, chlamydia, trichomoniasis, and bacterial vaginosis, are related to some degree of risk of transmission[35].

Body fluid transmission.

The second most incessant method of HIV transmission is by means of blood and blood products [32]. Blood-borne transmission can be through needle sharing during intravenous drug use, needle stick injury, contaminated blood transfusion, unsterilized medical injections, Individuals giving or getting tattoos, piercings, and scarification are hypothetically in danger and risk of infection.

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Mother-to-child transmission.

This is the third most way of HIV transmission around the world. HIV infection can likewise be gained as an inherent perinatal infection or in infancy. Mothers with HIV can pass the infection to their children transplacentally, at the hour of parturation through the birth canal or through breast milk. Without breast-feeding, intrauterine transmission represents 25 to 40% of infection, while 60 to 75% during labour and delivery [36].

Spread within the body.

The process of infection of a cell by a virion, called “cell-free spread", in which viral particles bud from an infected T cell, enter the blood or extracellular fluid and afterward infect another T cell. More recently explained process is called “cell-to-cell spread” in which, HIV can show direct transmission from one cell to the next cell.

2.7. Immunological response and clinical phases of HIV.

Immune Responses:

The general impact of HIV infection and its interaction with the body's natural response mechanism brings damage to the immune system reaction, annihilating the methods by which the human body normally protects itself against various infections. Following passage into the host, infection is spread by means of the blood and circulatory system to various tissues in the body. From this snapshot of infection, the virus replicate at very fast rate. As the infection recreates and spreads all through the body, the immune system recognizes the virus and mounts a prompt neutralizer reaction forming antibodies. This typically happens within two to four weeks of exposure and referred as seroconversion (since antibodies to HIV can be detected in the blood)[37].

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Initially cell resistance that is T cells go into fight against attacking microbes. T helper cells additionally been known as the "general" of the immune system, since they call up troops of B cells, cytotoxic T cells, and other helper cells to fight against microorganisms. Macrophages alert helper T cells to the microorganisms. These phagocytic macrophages engulf microscopic organisms and destroy foreign antigen.

During the course of the disease, infection is spread to the lymphoid tissues. The lymph nodes are situated all through the body and contain a mesh like structure of follicular dendritic cells (FDCs) in their germinal centers, which trap microbes and viruses. The lymph nodes are additionally the site of a concentration of immune system cells, including T – lymphocytes. As the microbes are caught by the FDC network, these cells of immune system attack and destroy them. During the earlier phases of infection, the FDC network is intact and can trap extra-cell virions, which in blend with immune system can result in very low level of virus in different tissues just as in blood and plasma. As viruses are trapped in more concentration, they infect the T lymphocytes and different other cells in the lymph nodes. Progressively, more and more cells in the lymph nodes get accumulated. In the end, the FDC network breaks down completely. This decimation of lymph node architecture has been seen in lymph node biopsies [38].

At last, with this total destruction of the lymph nodes, microscopic organisms spill into the blood system and spread around the body. At this stage the virus can infect and wreck CD4 T lymphocytes at a quicker rate than the body can produce new immune cells (including CD4 T - lymphocytes). At this leaves the body become unfit to mount an effective immune response against these microorganisms. This entire event can take average of, roughly 12 years.

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Clinical Phases of HIV

1) Acute phase of HIV infection- Occurs after two to four week after exposure to the disease. Influenza like side effects, fever, muscle pain, vomiting, diarrhoea and rash are seen. During this intense stage there is dynamic viral replication and marked HIV viremia (50.000 copies/ml), decline CD4 count, p24 antigen test is normally positive. HIV antibody tests are often negative. The viremia is more prominent in symptomatic primary HIV infected people [39].

2) Latent stage- At this stage immune system become very active to fight against viruses. During this stage viral burden is reduced. CD4 lymphocyte remains moderately reduced (> 500 cells/mm3).Test for HIV antibodies will stay positive during this time however p24 antigen tests are generally negative. Patient will be asymptomatic and may show generalized lymphadenopathy. This latent period may extend from7 to 10 years.

3) Clinical AIDs stage of HIV infection- In this stage the immune system is depleted because of extreme destruction of T cells. There is enhancement of caspase-1 which stimulates pro-inflammatory IL B1 production and develop inflammatory environment in the cell which kills normal T cells called pyroptosis, which bring chain response and destroys exceptionally huge number of T cells and crushes immune system. Viral burden keep on expanding, CD4 cells keep on decreasing (<200 cells/mm3).Patient gets opportunistic infections and life threatening conditions. Individuals with low level (<45000 copies/ml) of HIV have advanced to AIDS at 10 years following seroconversion. For people in the top quartile (>36,270 copies/ml) advancement of AIDS is 3.5 years [40]. There are 4 clinical stages of HIV. Clinical stage I: Asymptomatic with generalised lymphadenopathy. Clinical stage II: Moderate

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unexplained weight reduction with intermittent respiratory tract infection. Clinical stage III: Unexplained serious weight reduction with unexplained severe diarrhoea, fever, pneumonia. Clinical stage IV: Recurrent respiratory infection, pneumonia, herpes simplex disease, TB, Kaposi's sarcoma, HIV encephalopathy and so on.

2.8 HIV immunology and pathogenesis of HIV.

2.8.1 Immunohemapoietic cells infected by HIV.

Disease is transmitted when infection enters the blood or tissue of an individual and interacts with a suitable host cell. The virus infects cells bearing the CD4 antigen on the surface (called antigen presenting cells) or co-receptors either CCR5(C-C Chemokine receptor type 5) for macrophage and CXCR4(C-X-C Chemokine receptor type 4) for T cells. Principally these are CD4+ helper T lymphocytes and others like monocytes, B lymphocytes, macrophages,(alveolar macrophages in lungs and Langerhans in dermis) glial and microglia cells in CNS[41]. Many of these infected cells are killed due to cytopathic effect of virus, immune response of host or apoptosis. Some of them forms a steady pool of long lived memory T cells. These memory T cell has proviral DNA which don't transcribe viral gene, but if they get re-infected they will gets activated.

CD4+ T helper cells-CD4+T lymphocyte are generally idea target for HIV as they express CD4 receptors and both CCR5 and CXCR4 co-receptors. The hall mark of HIV/AIDs is depletion of CD4 T cells, which might be consequence of direct cytopathic impact, or killed by activated CD8+ cytotoxic cells][42]. During acute stage generally CD4 cells in GIT get depleted and shows GI symptoms. Furthermore, in chronic stage CD4 are depleted by immune response.

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Monocytes and Macrophages-Monocytes and Macrophages are infected by HIV in the later phases of disease as shows low expression of CD4 antigen. Even however not many of them are infected, monocyte activation initiate ROS production which adds to pathogenesis of HIV disease [43]. Macrophages are the main insusceptible cells encountered during sexually transmitted HIV. This is the most commonly studied immune cell because of its part in viral dissemination all through the host [44]. Non-multiplying mature macrophages which are impervious to cytopathic impacts of HIV are not easily killed by virus and can support HIV production for long period, which act as viral store for viral transmission to different lymphocytes. Activated macrophages are liable for production of pro-inflammatory cytokines, further responsible for inflammation and oxidative stress. In this way macrophages has initial role in HIV disease and furthermore liable for neurological and innate immune system dysfunction in chronic stages.

Dendritic cells-These are antigen presenting cells (APCs) which recognize the antigens and present them to T cells. The dendritic cells have surface lectin, an adhesive molecule called dendritic cell-specific intracellular adhesion molecule/particle, 3-grabbing non-integrin (DC-SIGN) which traps the HIV on their surface from site of disease and move to lymph nodes and transfer virus to CD4 T cells [45]. Fusion of dendritic cell membrane doesn't happen so less possibilities for dendritic cell getting infected.

Cells of the nervous tissue-Though the cells like glial cells oligodendrocytes, astrocytes and brain macrophages express low degree of CD4 antigen, may get infected and cause disease central nervous system. During infection, the viral protein Tat binds neural cells by CD91 antigen.

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2.8.2 HIV mediated depletion of CD4+ T cells.

There are numerous theory's, which clarifies the system of CD4 depletion. Important among these are 1) Direct cytolytic impact of virus 2) Chronic immune activation and apoptosis.

1) Direct cytolytic impact by virus- As virion increases in CD4 cells, they cause budding from the cell and penetrating the membrane which bring devastation of CD4 cells. Additionally there are syncytia development that help in infection spread to uninfected cells. Different theory which clarifies this is, Nef and Tat gene which get shaded off from viral particles may bind to uninfected cells through CD4 antigen, consequently may show up as infected cell and get eliminated by host immune system [46]. In beginning phases there is constant and progressive decrease in CD4 T cells.

2) Chronic immune activation and apoptosis-Due to chronic immune activation, triggers monocytes and macrophages which delivers pro-inflammatory substances like cytokines and chemokines(IL,TNF alpha), which brings inflammation and inflammation induced oxidative stress, further liable for apoptosis and CD4 exhaustion which further advances the disease. Different theory of pyroptosis i.e highly inflammatory form of programmed cell death, in which there is release of cytoplasmic substance, including inflammatory cytokines from dying cells, which further triggers pyroptosis in other T cells demonstrating vicious cycle of CD4+ T cells destruction[47].

2.8.3 Inflammation, immune activation and disease progression.

After initiation of infection by gaining viral entry in CD4 cells through DC, the infected cells produce pro-inflammatory cytokines and chemokines which bring enrolment of inflammatory cells (neutrophil, monocytes, macrophages, natural killer

cells) at the site of infection to wipe out the infection. Supportive of provocative cytokines are created by these cells known as cytokine storm, which is key element of acute inflammatory response [48]. The cytotoxic T lymphocytes (CTL) are formed, that kills infected cells and reduces replication of virus.

Due to persistent systemic inflammation there is disease progression which increases morbidity and mortality. Also due to persistent immune activation, the activated macrophages/monocytes, discharges cytokines like, IL-1, IL-6, IL-12, TNFα and chemokines like IL-8, MIP-1.MCP-1 and interferon [43]. The cytokines TNF bring underlying inflammation, cell activation, proliferation, apoptosis and necrosis. Hence TNF liable for both intracellular inflammatory and signalling pathway of apoptosis through TNF receptor. TNF additionally trap neutrophils to inflammatory site and brings neutrophil respiratory bust, causing creation of superoxide anion and ROS. There is additionally actuation of nuclear factor of kappa (NF-kB), responsible for pro-inflammatory cytokines and ORS[49]. Cyclooxygenase-2 is produced due to incitement of IL-1 and TNF which bring about creation of PGE2 or prostacyclin from endothelium, causing vasodilatation causing pain during inflammation. As the result of chronic inflammation, accelerates depletion of endogenous antioxidant agents like glutathione which results increase in level of H_2O_2, further contributing death of CD4+T cells.

2.9 Natural course of HIV infection.

It takes 8 to 10 years to develop full blown out AIDS from the time of infection. In some instances it might take just 2 years which relies on immune status and nutrition of the person.

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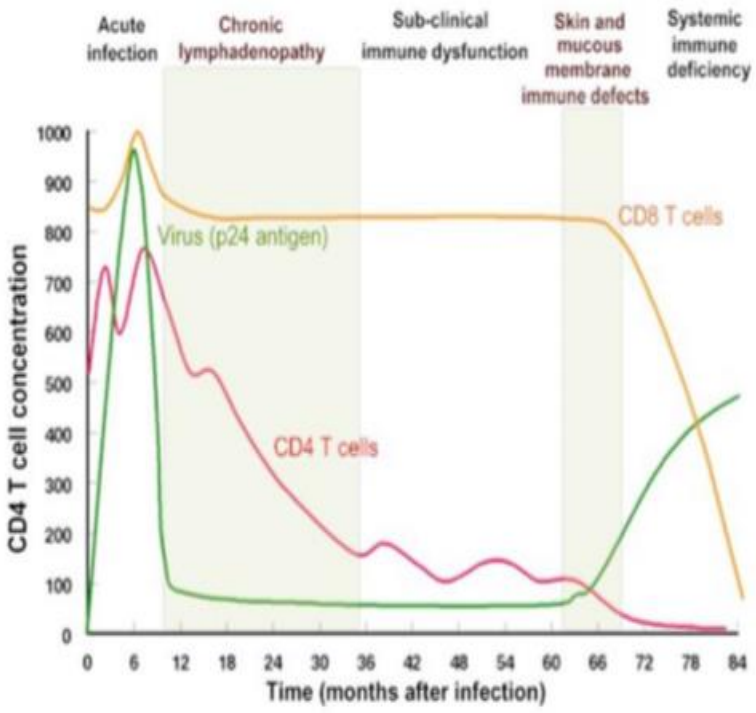

Figure 2.6: Natural course of CD4 Count and viral load in various phases of HIV infection [50]

At first, infection produce mild disease and might be asymptomatic. Acute phase of infection goes on for 6-12 weeks. There is excess destruction of CD4 T-cells of MALT because of actuation of immunity at site of infection. Later T-lymphocytes and cytotoxic B builds their protection and viral infection cells are decimated, however small amount survives as resting memory cells which act as reservoir and start replicating if get stimulated. This resting memory cell is critical in drug therapy [51]. Also actuation of humoral immune system produces humoral antibodies (anti-HIV antibody). During this period around 10 billion viral particles are created which are cleared by immune system. It has been seen, because of immune activation, there is upregulation of CCR5, consequently along with CD4 receptor, thus there is increase entry of viruses into the cells and expands replication and infection [52].

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Indeed, even strong immune response eliminates viral particles in circulatory system but they keep on replicating in other cells and infect more and more cells landing patient in latent phase which may last for 1 year to more than 15 year. CD4 cells which are actuated and susceptible for apoptosis shows their continuous depletion. As CD4 exhausts and when falls under 200 cell/mm^3 it can't control virus and now virus titre rises and immune response falls. Further due to excess stimulation of immune system, releases pro-inflammatory substances which brings inflammation and oxidative stress leading to apoptosis, initiating further exhaustion of CD4 T-cells, which is responsible for opportunistic infection and HIV associated cancer characterizing AIDS [53].

2.10 HIV screening.

HIV confirmation is done by utilizing three rapid tests of three distinct antigens or principles. The individual is viewed as HIV-negative, if the first test is non-receptive and as HIV-positive, when every test of the three tests show reactive outcomes. For indeterminate outcomes (one positive and other two negative), testing ought to be repeated on a second sample taken following 14-28 days or ought to be screened by ELISA or western blot method (antibody reaction to viral proteins and glycoproteins)[54].

A positive outcome for the most part requires presence of bands correlating to p24, p31 gp41 and gp120 or gp160. Other test for precise checking is polymerase chain reaction (PCR). This can identify the presence of viral genetic material. Nucleic acid sequence based amplification (NASBA) method includes isolation by lysis and binding to silicon dioxide particles, followed by an isothermal amplification methodology dependent on an enzyme-linked gel assay or electro chemiluminescence [55]. Thus

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from these advancements have made it conceivable to quantitatively measure plasma viral levels in patients, from which disease status and danger of progression of disease can be found. Different examinations like CD4 count, blood profile, serum creatinine and liver function tests done which offers manual or guide to start the ART combination (cART).

2.11 Current ART regimens.

As per WHO and UNAIDS,(2018) 37.9 million individuals living with HIV and 23.3 million individuals with HIV were getting to antiretroviral treatment universally, an expansion of 1.6 million since 2017 and up from 8 million in 2010. In India (2017), 2.1 million individuals are living with HIV and 56% adults are on ART. The Indian HIV program has advanced extended and executed various new changes over years. WHO in 2010 suggested the initiation of ARV treatment for adults at CD4 count of ≤350 cells/mm3. In 2013 modified WHO guidelines on utilization of ARVs for preventing HIV infection, suggests to start treatment for all individuals with diagnosed HIV disease with a CD4 of ≤500 cells/mm3. As indicated by current NACO guidelines 2017 all people determined to have HIV disease, should be put on ART regardless of CD4 count. ART controls the multiplication of HIV and improves CD4 cell count, consequently delaying the asymptomatic period of disease, slows progression of the condition and furthermore helps in diminishing the danger of transmission of disease. Standard ART combination includes at least three or more antiretroviral drugs, termed as “Highly active antiretroviral therapy” (HAART)” or (cART)[56].

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2.11.1 FDA-approved HIV drug classes.

1. Reverse transcriptase inhibitors.

Reverse transcriptase inhibitors are group of medications, which can bind and repress the reverse transcriptase enzyme to block the multiplication of HIV. There are two kinds of inhibitors: nucleoside reverse transcriptase inhibitors (NRTIs) like Zidovudine, Stavudine Lamivudine, Abacavir, Didanosine, Tenofovir, Combivir and Non-nucleoside reverse transcriptase inhibitors (NNRTIs) like Nevirapin, Efavirenz. Etravirdine.and Delavirdine [56].

2. Protease inhibitor (PIs)

Protease inhibitors effectively hinder the function of protease enzyme in acute and chronically HIV infected CD4 cells. PIs repress the proteolytic cleavage of the gag/pol polyproteins in HIV infected cells. Due to inhibition of HIV protease enzyme which prevent assembly of particles of viruses, which result in the liberation of immature and non-infectious viral particles. Example-Ritonavir,Indinavir, Darunavir, Saquinavir Lopinavir, Nelfinavir and Amprenavir [57].

3. Fusion Inhibitors.

Fusion inhibitors combines to the envelope glycoprotein gp41 and forestall viral fusion to the CD4 T cells, further prevent entry into CD4 cell [58].

Example-Enfuvirtide

4. Chemokine Receptor 5 (CCR5) Antagonist.

CCR5 antagonists specifically and reversibly block entry into the CD4 T cells by hindering interaction between CD4 cells and the gp^{120} subunit of viral envelop

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glycoprotein. Without empty CCR5 receptors, HIV fails to be picked up and fails to gain entry and infect the target cell [59]. Example- Maraviroc

5. Integrase Strand Transfer Inhibitors.

Integrase strand transfer inhibitors forestall the combination of viral DNA into the host genome of CD4 cells and prevents HIV from replicating [60].

Example-Raltegravir, Dolutegravir and Elvitegravir.

Regimen composition

Fixed-dose combination(FDC) of ARVs are favoured as they are easy to be prescribed and easy for patients to be taken, thus improving adherence of treatment which help in reducing development of drug resistance, mutation and treatment failure. It has been proved FDCs are more tolerated, acceptable and improved compliance. ART choice depend on co-morbidities (hepatitis and psychiatric disorder). First line ART contains a NRTI as backbone, ideally tenofovir (TDF), in addition to Lamivudine (3TC) and one NNRTI, preferably Efavirenz(EVF). Example, Tenofovir 300mg+ lamivudine 300mg+ Efavirenz 600mg as FDC single pill once per day. Other combination favoured is blend of zidovudine(AZT), Lamivudine (3TC) and Nivirapine (NVP). If individual is anaemic, zidovudine combination isn't used as it has bone marrow suppression which will further accelerate anaemia. In this case we can use tenofovir or stavudine instead of zidovudine [61]. Protease inhibitors are reserved for II line drugs in HIV.

Because of accessibility of new drugs, formulations and dosage recommendations, ART is updated continuously. Though adverse effects are been reported with many ARTs, the newer regimen use has decreased these adverse effects. However various factors, as gender, concomitant use of other medications,

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comorbidities, drug-drug interactions and genetic factors, impact the advancement of adverse effects.

2.11.2 ART associated ADRS.

Nucleoside/Nucleotide reverse transcriptase inhibitors (NRTIs)[62 63].

- NRTIS cause mitochondrial toxicity, which may present as neuropathy and lactic acidosis. Few NRTIS may likewise cause bone marrow depression, anaemia and lipodystrophy.
- Tenofovir with good tolerance, however may cause kidney injury or diminished bone mineral thickness. Cessation of tenofovir formulas will require clinical monitoring and monitoring of hepatic functions as suspension of drug may cause an acute exacerbation of HBV.
- Abacavir is associated with a CD8 mediated hypersensitivity reaction in individuals having HLA-B*5701 mutation.
- Didanosine is infrequently utilized because of the danger of pancreatitis and hepatomegaly.

Non-nucleoside reverse transcriptase inhibitors (NNRTIS) [64 65].

- NNRTIS may cause rashes that resolve within month, however may advance to stevens-johnsons syndrome. Hepatitis and fulminant hepatitis advancing to liver failure which may occur within six weeks of HAART. QT prolongation can be seen mostly with rilpivirine. NNRTIS additionally cause interaction with hepatic cytochrome p450, so should be checked intently for dose modifications or interaction with concurrent medications.

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- Efavirenz is known to cause mental and CNS impacts including, sleep disturbance, dizziness, headache, delusions, psychosis and increases suicidal tendency.

Protease inhibitors PIs [66 67].

- PIs class incorporate hepatotoxicity, insulin resistance, hyperglycaemia, lipodystrophy, hyperlipidaemia and prolongation of PR interval.
- Saquinavir and Indinavir are not used much due to reduced efficiency, unwanted adverse effects as nephrolithiasis and drug resistance.

Integrase Strand Transfer Inhibitors (INSTIS) [68].

- INSTIS are very well tolerated. Due to its neutral effects on cholesterol and triglyceride levels they are favourably used as third agent in HAART regimen. Few patients may show sleep disturbance, dizziness, depression. There are reports of myopathy in patients taking dolutegravir and raltegravir, so creatinine phosphokinase (CK) checking is required.

Fusion inhibitors (FIs)

- Enfuvirtide is very much tolerated but few patients may encounter injection site reactions.

Chemokine receptor antagonists (CCR5 antagonists) [69 70].

- Maraviroc is likewise very much endured, however a few patients may show dizziness or allergic reactions like hypersensitive responses and hepatic failure. In the event that patients are taking simultaneous CYP3A4 inhibitors or inducers, drug-drug interactions should be watched for.

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2.12 HIV and hematological profile.

Hematological abnormalities are regular entanglements of human immunodeficiency virus infection which increases as disease progresses. In both antiretroviral-treated and untreated people various kinds of hematological abnormalities are seen,[71] like impaired hematopoiesis, cytopenia and coagulopathies especially in the later stage of the disease[72, 73]. Cytopenia might be brought about by increased destruction or decreased formation of red blood cells (anaemia), platelets (thrombocytopenia), white blood cells (leucopenia or neutropenia).

Pathogenic component of cytopenia-Hematopoietic stem cells (HSCs) are self-renewing cells that are present in the bone marrow from which different types of blood cell lineages are formed and differentiate into mature blood cells, white blood cells, megakaryocytes, and lymphoid cells[74,75]. The microenvironment of bone marrow is altered by HIV infection which is responsible for peripheral blood cytopenia. These hematologic irregularities brought about by altered stem cell differentiation and reach beyond the loss of CD4 cells and could be because of abnormal expression of specific cellular genes as cytokines interleukin 6 (IL-6) and granulocyte colony-stimulating factor which play role in regulation of hematopoiesis[76]. It is also mentioned, HIV mediated hematopoietic suppression is mediated by HIV-1 encoded envelope HIV-1 glycoprotein gp120, the accessory extracellular protein negative factor (Nef) and cellular proteins as tumor necrosis factor alpha. HIV also directly infect the microvascular endothelial cells of bone marrow and give a persistent source of the virus. The viral proteins released is thought to have toxic effect on blood stem cells [77].

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Comorbid opportunistic infections, physiological stress, neoplastic disorders, immune factors and the cytotoxic impacts of antiretroviral and antimicrobial treatment further confound the hematopoietic suppression. The relationship of HIV-infection with hematological irregularities is dependent on viral replication, as the severity of these abnormalities increases with progression of the disease. Hematopoiesis might be re-established or revised by HAART, but prolonged use of these drugs may bring persistent hematopoietic suppression [78 79].

Anaemia- Over duration of disease, roughly 70 to 80% of patients suffer from anaemia[80]. Recurrence and seriousness of anaemia relate with HIV factors for example CD4 count and viral burden. In few cases on contrary, anaemia in these patients is essentially connected with an expanded danger of death and is independent of CD4 level and viral load [81,82]. Normocytic normochromic iron deficiency anaemia being the dominating type followed by microcytic anaemia [83]. Myelosuppression for the most time, macrocytic anaemia (MCV>100fl) is the dose limiting toxicity of zidovudine which can be utilized as a target sign that patient has been compliant with this medication [84].

Causes of HIV-associated anaemia

Decreased RBC production-There may be reduction in red cell production due to infiltration of bone marrow by infection, neoplasms, mylosuppressive medication (including AZT) there is diminished production of endogenous erythropoietin or decreased response to erythropoietin[85, 86]

Increased destruction of RBCs- Destruction of RBC may occur in the spleen or circulatory framework by RBC autoantibodies, disseminated intravascular coagulation,

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thrombotic thrombocytopenic purpura, glucose-6-phosphate hydrogenase inadequacy and medications [87,88,89].

Ineffective RBC production- There may be ineffective RBC production, principally because of nutritional insufficiencies of iron, folic acid and nutrient B12, might be because of malabsorption in the ileum, gastric infection or other gastric mucosa pathology. Adverse impacts of ART for example anaemia, neutropenia and thrombocytopenia have been seen in HIV positive people before and after introduction of HAART and continues even with use of these potent drugs [90].

Consequences of anaemia- Results of anaemia, there is decline of survival, increase disease progression and reduced quality of life. Some cohort studies have shown, independent risk factor for survival in HIV individuals is anaemia. It has been observed that there is association of anaemia with increased death risk for all CD4 ranges but death risk is increased by 60% in anaemic individuals with CD4 level < 200 cells/mm^3[54]. Anaemia also shows independent association with progression of HIV disease. In few cohort studies, disease progression was 2.2% with mild anaemia, whereas in severe anaemia it was 7.1%. Anaemia is additionally connected with decline in quality of life. Recovery from anaemia shows independent association with improved survival.

Neutropenia-neutropenia announced in around 10% of patients with early asymptomatic HIV and 50% with advanced HIV related immunodeficiency [91,92]. HIV tainted cells produce soluble inhibitory substances which reduce production of neutrophils in vitro proposing that autoimmunity has an influence in development of neutropenia in HIV disease. Diminished serum level of granulocyte colony stimulating

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factor (G-CSF) have been portrayed in HIV patients with afebrile neutropenia (<1000/microliter), proposing a relative deficiency of this growth factor may add to persistent neutropenia [93]. Other reasons for neutropenia in HIV incorporates, presence of opportunistic infections, malignancies and myelosuppression and medication induced neutropenia. When absolute neutrophil count (ANC) falls below 1000/microliter, increases risk of bacterial infection

Thrombocytopenia- Immune thrombocytopenic purpura (ITP) occurs in 30% of HIV patients and is most regular reason for thrombocytopenia in HIV. The mechanism of thrombocytopenia in HIV seems to include, increased platelet destruction and insufficient platelet production. Most reports show that there is platelet sequestration and obliteration in the spleen in HIV related thrombocytopenia [94]. Antibodies coordinated against platelet glycoprotein IIIa, like those found in classic ITP [95]. Megakaryocytes express CD4 and CXCR4 are susceptible to infection by HIV. Studies of megakaryocytes from HIV infected patients have shown viral RNA and proteins, proposing that these cells are infected in vivo [96]. Different other causes includes, marrow infiltration by opportunistic infection, lymphoma, and myelosuppressive impacts of medication treatment.

2.13 Oxidants, Free radicals and Reactive oxygen species (ROS)

Historical Background of Free Radicals

Free radicals in the biological materials was discovered less than six decades ago [97]. Denham Harman theorized that oxygen radicals might be produced as results of enzymic reactions in vivo. In 1956 he portrayed free radicals as," Pandoras box" of evils that may account for cellular harm, mutagenesis, malignancy and degenerative

process of aging[98].In second era, McCord and Fridovich 1969 found the enzyme superoxide dismutase (SOD) and found that free radicals are significant and important in biology. Numerous scientists at that point were inspired to investigate oxidative harm dispensed by radicals upon DNA, proteins, lipids, and other components of the cell. A third era, started with the primary reports by Mittal and Murad in 1977 portraying advantageous biological effects of free radicals and gave intriguing proof, that the superoxide anion through its derivative, the hydroxyl radical stimulates activation of guanylate cyclase and development of the second messenger cGMP[99].

Now there are many proofs demonstrating that living organisms have not just adapted to an unfriendly coexistence with free radicals but also in addition have the advantageous use of free radicals. Significant physiological functions that include free radicals or their derivatives include the regulation of vascular tone, sensing of oxygen tension and guiding of functions that are controlled by concentration of oxygen, improvement of signal transduction from various membrane receptors including the antigen receptor of lymphocytes and oxidative stress responses that guarantee maintenance of redox homeostasis [100]. The term "oxidative stress" was coined just 30 years ago [101]. Oxidative stress is a circumstance when steady-state ROS concentration is briefly or chronically enhanced [102].

Free radical production and Reactive Oxygen Species (ROS)

Oxidants are generally defined as exogenous or biological substances which are capable of oxidizing the target molecules, either straightforwardly through electron abstraction or by creation of intermediate chemical substances which are highly reactive. Free radicals are chemical substances which contain at least one unpaired

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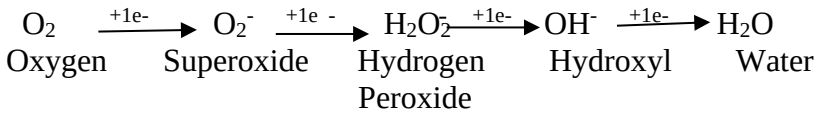
electron in their nuclear structure, which is liable for its reactivity [103] and are fit for independent existence for brief time periods. ROS includes all reactive oxygen sources, both radical and non-radical species engaged in initiations and transmission of radical chain responses. Aggression of ROS brings about oxidative destruction of essential molecules including DNA, proteins, and lipids [104, 105].

Endogenous sources of oxidant radicals

1.Along electron transport chain (ETC)- During oxidative phosphorylation in mitochondria during ATP synthesis, because of standard breathing of aerobic respiration the mitochondria absorbs O2, decreasing it by sequential steps to produce H_2O (needs 4 electrons) and forms O_2^- ,H_2O_2 ,and OH^- radicals, which are unavoidable by-products of this process[106 107].



2. In peroxisomes-These organelles create H_2O_2 as by-product, further processed by catalase and are responsible for degradation of fatty acids and other substances. Few peroxide which do not degrade under some conditions, may enter other part or compartment of cell and cause oxidative DNA damage [108].

3. During infection and inflammation-phagocytic cells for example neutrophils, macrophages destroy infected cells and/or viral cells which bring oxidative burst and responsible for production of nitric oxides (NO), O_2^-, H_2O_2 ,and OCl^-[108].

4. During hypoxia-When oxygen is limited to cells the mitochondria pumps ROS[109].

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Exogenous sources of oxygen radicals

1. Ionizing radiations- UV beams and ionizing radiation changes over H_2O into OH^- (hydroxyl) radicals which are very harmful. It activates the development of mitochondrial reactive oxygen species, with the aid of upregulation of the role of a mitochondrial electron chain transport [110]

2. Tobacco smoke and pollution- Nitrogen oxide causes macromolecules oxidation in tobacco smoke (about 1000ppm) and deplete antioxidant concentrations [111].

3. Iron and copper salts- The formation of oxidizing radicals are facilitated by abundance of iron and copper salts from peroxides (H_2O_2 converted to OH^- radicals [112].

4. Drugs/Xenobiotic-Drugs like acetaminophen in harmful dose produces excess oxidant radicals [113] through cytochrome p450 in liver.

5 Natural toxic chemicals from plants-Cytochrome p450 in animals involve one of the defensive mechanism against natural toxic substances from plants. Such catalysts are induced to avoid toxic responses but produces oxidants as by-products [108].

Types of ROS

The most well-known ROS includes, superoxide anion (O_2^-), hydrogen peroxide (H_2O2), hydroxyl radical (OH^-), hypochlorous acid (HOCl), peroxyl radicals, nitric oxide(NO) and peroxynitrite ($ONOO^-$).

Superoxide anion (O_2^-)- The superoxide (substantially less reactive) is an anionic radical formed by reducing molecular oxygen by accepting a single electron. They are created during oxidative phosphorylation by means of ETC, during electron transport

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in mitochondria commonly with the escape of 1–2% of electrons that are caught by molecular oxygen [114]. The chemical needed to change over oxygen to superoxide is NADPH oxidases (NOX/DUOX). NADPH enzyme contains 7 isomers DUOX1,DUOX2 AND NOX1-NOX5. Activation of NOX family protein will produce ROS [115,116]. Cytochromes p450 that catabolize endogenous compounds and xenobiotics, additionally generates superoxide anions. In response to bacterial infection, the activated phagocytes do have metabolic pathway to generate superoxide radicals. Superoxide anion may likewise induce metabolism of arachidonic acid to produce more superoxide and release the $Fe2^{+}$ from the stores of ferritin [117]. Due to negative charge the superoxide anion is less active, yet its protonation produces a higher oxidizing perhydroxyl radical (HO_2^{-})

Hydrogen peroxide (H_2O_2)

Superoxide dismutase(SOD) changes superoxide into hydrogen peroxide and molecular oxygen, but superoxide normally can also create hydrogen peroxide as well as singlet oxygen by redox reaction[118]. Hydrogen peroxides capacity to react is extremely weak, but with an improved oxidation power it is changed over into hydroxy-radical. H_2O_2 in presence of myeloperoxidase(neutrophil-derived enzyme) can be changed over to hypochlorous acid having reactive potential. H_2O_2 has a novel capacity to transduce biological tissues that changes it into a classical signalling molecule [119].

Hydroxyl radical (OH^{-})

The extreme powerful oxidant hydroxyl radical (OH), is produced via Fenton reaction, formed within in the presence of Fe_2^{+} or Cu (catalyst) by the reaction of O_2^{-} with H_2O_2 (Fenton reaction) [120 121] or Haber-Weiss cycle, which involves the

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reduction by superoxide anions of ferric ions($Fe3^{+}$) into ferric ions, accompanied by Fenton reaction. Hydroxyl radical, attributable to its low half-life and has the capacity to oxidize the nearby vicinity to almost every molecule, including DNA, phospholipids and proteins. Hydroxyl radical is the most volatile ROS[122 123]. The oxidation of these molecules adds to the accumulation of a normal lipid peroxidation and other oxidized nucleic bases, malondialdehyde (MDA) and 4-hydroxynonenale(HNE) (lipid peroxidation products) and cause protein harms, manifest by the protein carbonyl content[124].

Peroxyl radicals

During peroxidation of lipids, peroxyl radicals are dominatingly produced. Despite the fact that lipid peroxidation has proved valuable in certain cellular processes, membrane peroxidation i.e. hydroxyl-radical can react with polyunsaturated fatty acid (eliminates one hydrogen) not only to change the structure and functionality of that fatty acid, but in addition produce various fatty acid radicals, which react with other lipids, proteins or core acids, hence spreading an electron transmission cascade and oxidizing those substances. Cell damage brought about by cell membrane lipid peroxidation can affect membrane fluidity, increase penetrability and modify the electrical potential which can add to cell lysis [125]. These free radicals likewise denature proteins both structural and enzymatic [126]. Toxic oxygen metabolites can likewise target nucleic acids directly causing straightforward hydroxylation, cross-linkage and/or breaking of DNA strands which can bring mutation, apoptosis and cell death [127].

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Lipid peroxidation steps-The oxidation of lipids by ROS comprises of three steps

1 Initiation step

The hydroxyl radical extracts a hydrogen atom from methylene carbon of a polysaturated fatty acid which forms carbon-centered lipid radical. The lipid radical can interact with molecular oxygen to form peroxyl radical

2) Propagation step

By removing H-atom from methylene carbon, peroxyl radical is transformed into lipid hydro peroxide to make a new lipid radical, thereby causing a propagating sequence of the peroxidation. Moreover hydroperoxides might be decayed to hydrocarbons, epoxides, alcohols, and aldehydes, Malondialdehyde and the hydroxynonenal, of these ingredients are capable of forming cross-linking between these molecules to inactivate phospholipids, proteins and DNA [128].

3) Termination step

There is interaction between radicals themselves or radical and antioxidants, which produce non-radical or inert radicals, further prevents the chain reaction. The chain is ended as lipid radicals association with E vitamin, that forms tocopherol radical and lipid alcohol[128].

Singlet oxygen ($^1O_2$)

Singlet oxygen is profoundly responsive species with high energy. There is no free radical so it doesn't carry any valence electron. Singlet oxygen is additionally engaged with photochemical reaction such as transferring energy due to type II photosensitivity, thermal decomposition of endoperoxides and dioxetans[129]. During

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the reaction when hypochlorous acid combines with the hydrogen peroxide to form water and form singlet oxygen. Singlet oxygen is a highly reactive ROS which induces carcinogenic, genotoxic and mutagenic effects by acting on DNA[130].

Nitric Oxide (NO.), Peroxynitrite (ONOO) (Reactive nitrogen species)

Radical nitric oxide (NO•) in the biological structure, which is produced by nitric oxide synthase (NOS), from L-arginine oxidation to citrulline [131]. The radical superoxides and nitric oxide contributing to peroxynitrite are among the most critical reactions in physiological conditions.

$$NO + O_2^- \longrightarrow OONO^- \text{(peroxynitrite)}$$

This reaction is important in redox control and aids to preserve the equilibrium of ROS and superoxide radicals. The protonated form of peroxynitrite (ONOOH) is a strong oxiding agent that may result in sulfhydryl (SH) depletion and oxidation of a number of molecules which causes damage similar to OH [132].

2.14 Antioxidants

Halliwell and gutteridge (1989), have identified antioxidants to be substances that are capable of competing with other oxidizable substrates at genuinely low concentration and further reducing and altogether inhibiting their oxidation. Their creation and evacuation (redox state) is steady. Cells guard against the harmfulness of abundance ROS/RNS in different ways, enzyme and non –enzyme antioxidants (to preserve oxido/redox equilibria).

a) Enzymatic antioxidants (endogenous)

Superoxide dismutase(SOD), catalase (CAT) glutathione peroxidase (GPx) glutathione reductase (GRx)

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b) Non-enzymatic antioxidants

i) Metabolic antioxidants- lipoid acid, glutathione L-arginine, uric acid, bilirubin.

ii) Nutrient antioxidants(exogenous) vitamin C (ascorbic acid), vitamin E, carotenoids, trace metal components(Se, Cu, Zn, Mn)

Mechanism of action of antioxidants

1. Preventive antioxidants- They work by joining and sequestering oxidation promoters and metals like iron and copper that include unpaired electrons. E.g. heptoglobin (binds haemoglobin), caeruloplasmin (bind copper), transferrin and lactoferin (bind ferric particles), catalyzes the oxidation of ferrous particles to ferric and accelerates iron binding to transferrin [133].

2. Enzyme antioxidant-The endogenous catalysts bring interaction with ROS species and convert them to less unsafe substances e.g. glutathione (GSH), catalase(CAT) and superoxide dismutase (SOD), SOD changes the radical, super oxide to hydrogen peroxide which isn't a real free radical, yet a forerunner of very reactive hydroxyl radical. The principle intracellular enzymes are GSH, SODS and catalase [133].

3. Chain breaking antioxidants (Scavenging)-These antioxidants work by oxidizing in the chain reaction of free radicals and result in forming low-energy substances, that can't continue to spread the chain reaction. In cellular conditions, β-carotene, coenzyme Q (CoQ), lipid soluble scavengers [134] and water soluble scavenger like ascorbic acid, uric acid and bilirubin, capacities as chain breakers.

Important antioxidants

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a) Enzyme antioxidants

1. Superoxide dismutase (SOD)- superoxide dismutase destroys the free radical superoxide by changing over it into peroxide that is additionally destroyed by glutathione peroxidase (GHPX) or catalase. The superoxide radical transform into the less-responsive H_2O_2 by SOD[135]. There are three types of SOD, cytosolic-copper-zinc superoxide dismutase(Cu-zn-SOD) (SOD-I), mitochondria-Mn-SOD (SOD-II) and extracellular-SOD (ECSOD).Largely SOD catalyses the dismutation of O_2^- by progressive oxidation and reduction of the change metal ion at the active site in a ping-pong-type response with high response rates[136].

2. Catalase (CAT)- It is a tetrameric heme enzyme having 4 comparative tetrahedral subunits. Catalase interact with H_2O_2 to frame nontoxic substances, water and oxygen. Catalase is amazingly organized that H_2O_2 at any concentration is difficult to get saturated [137].

3. Glutathione framework- It is a tripeptide types of L-gamma-glutamyl-L-cysteinyl glycine. Glutathione mechanism (glutathione reductase, glutathione transferase and glutathione peroxidase) is a primary protection against H_2O_2 and different other peroxides. Glutathione peroxidase eliminates H_2O_2 by utilizing it to oxidize reduced glutathione (GSH) into oxidized glutathione(GSSG). GPx likewise reduces lipid or nonlipid hydroperoxides during glutathione-oxidation. [138 139].

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b) Non enzyme antioxidants

1. **Nutrient antioxidant**

Vitamin E- is a lipid soluble vitamin act as antioxidant. Help in chain breaking reaction. It is an eight stereoisomer chiral compound: α, β, γ, δ tocotrienol and α, β, γ, δ tocopherol. α-tocopherol is most bioactive. As it is fat soluble, α-tocopherol shields cell membrane by protecting them from lipid peroxidation and free radicals injury. Vitamin C improves LDL susceptibility to oxidation [140]. Low utilization of vitamin E, C and beta carotene is identified and related to reduced immune response, with rise in malignancy hazards. Vitamin E containing dietary substances includes, consumable oils, sprout butter, oats, grain, nuts berries, milk, poultry and beef. Cooking or storing food for long period will destroy d-α-tocopherol. The daily recommended dietary allowances of vitamin E in adult is 15mg. High doses and taking vitamin E for longer period should be avoided.

Vitamin C- It is a water-soluble nutrient additionally called ascorbic acid (AscH).Vitamin C has scavenging action against superoxide, peroxide, hypochlorite, hydroxyl radical, peroxyradicals, and O_2. A stable ascorbic radical is formed by giving hydrogen atom to a free radical. Ascorbic acid can likewise shield lipid layers from peroxidation by enhancing the action of lipid soluble tocopherol antioxidant. Citric fruits, green vegetables, tomatoes are vitamin C containing natural sources. Ascorbic acid is subjected to be destroyed during cooking [141].

B-Carotene and Vitamin A-The fats solvent carotenoids are called as provitamins, as they get changed over into active vitamin A (retinol). B-carotene is a potent antioxidant and is the best quencher of singlet oxygen[142]. The turn of events and action of T-

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cells, B-lymphocytes and natural killer cells get modulated by vitamin A and so act as immune stimulant. Beta-carotene has been found in numerous natural products like nuts, fats, and vegetables (carrots green seeds, beans, spinach). Carotenoid lycopene (tomato) has antioxidant and anti-proliferative impact.

Flavonoids- polyphenolic substances, which are found in many plants are flavonoids. Around 4000 flavonoids are known as flavanones, flavanols, flavones, proanthocyanidins, catechins, anthocyanins, and isoflavones. Effective and more efficient antioxidant property is found in flavones and catechins (green tea) [143].It has been shown that, many diseases like cancer, degenerative diseases, asthma, stroke, heart disease, ageing, Alzheimer's, inflammation infection are delayed or prevented by use of flavonoids. Soybean, broccoli, grapes(red wine) berries, cocoa, ginkgo biloba, green tea, curcuma, apple, onion, and so forth are the sources of flavonoids.

2. Metals- Zinc a metallic divalent cation bound to proteins in cell and cell lipid layers. Zinc present in many zinc metalloenzymes in biological framework. Zinc keeps up the integrity of biological membrane by balancing out thiol group and phospholipids and ensures against oxidative injury. Zinc finger which is a transcription factor and interact with DNA, which helps in controlling action of gene[144]. Selenium comprises a functioning site of numerous antioxidant enzyme, (including GPx), so has cell immunomodulatory security impacts and anti-cancerous effect at low consumption [145]. This mineral is available in vegetable items (garlic, cereals, seeds, and soybean) water, meat, liver, yeast.

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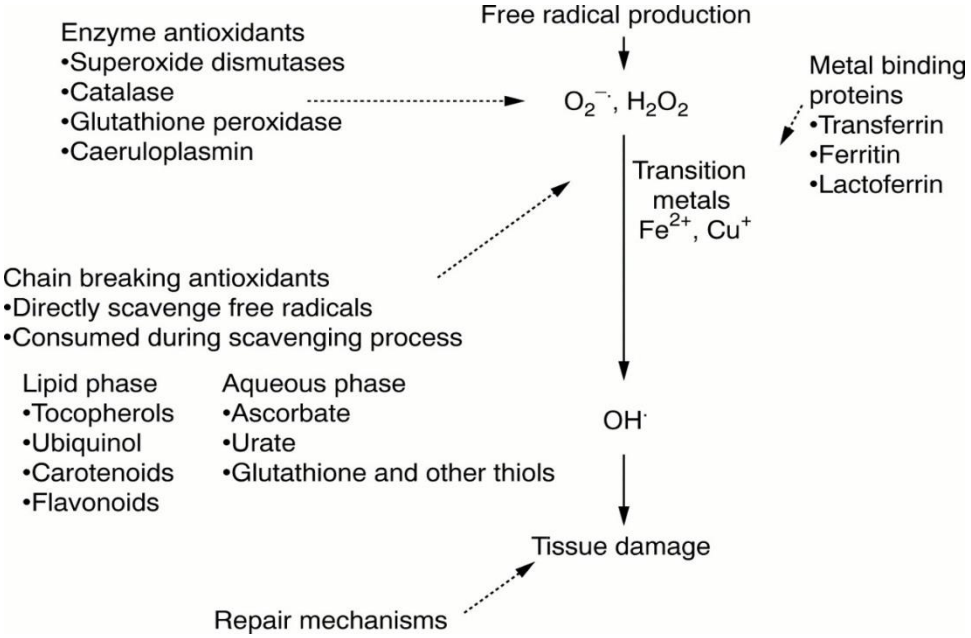
3. Transitional metal binding proteins– Caeruloplasmin for copper and metal binding protein ferritin for iron, function as the key part of the antioxidant protection by the sequestration of iron and coppers to forestall the making of hydroxyl radical.



2.15 Oxidative stress.

It is a state where there is disturbance between production of free radicals and limit of cell to defend against them [146]. It results due to exorbitant making of ROS with diminished antioxidant defence system. Constitutively ROS and receptive nitrogen species (RNS) are produced as ordinary physiological and metabolic pathway, for instance aerobic respiration. ROS are oxygen-containing responsive chemical species and RNS are nitrogen containing species for example, nitric oxide (NO), nitric dioxide (NO_2) and peroxynitrite. ROS system isolated into two groups, first, free radicals having one unpaired electron in their outer nuclear orbitals for example, superoxide, hydroxyl radicals and other groups and second, Non-radicals which don't have unpaired electrons, yet they are chemically responsive which gets changed over to free radical species, for example, hydrogen peroxide (H_2O_2) and peroxynitrate (ONOO)[147].

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Mitochondria a critical and significant source of ROS, creating electron spillage in the cells from the mitochondrial respiratory chain. The electrons spilled out reacts with molecular oxygen to frame superoxide and other ROS.

ROS have a basic role in biological functions, as regulators of many sign transduction pathways, cell development, signalling and differentiation, and regulation of some enzymes (e.g. ribonucleotide reductase)[147]. They are occupied with bringing inflammation due to abundance cytokine production. They moreover help destruction of microorganisms and foreign particles, particularly in oxygen dependent microbial decimation of neutrophil respiratory burst. ROS as especially reactive substance, liable to make oxidative damage in biomolecules like lipids, proteins, DNA and altering their functions and liable for various pathological conditions and diseases. Excess increase in ROS in the cells may cause oxidative harm, which may result to decrease in immune function, inflammation and even cell death[148].

2.15.1 Antioxidant defence system, oxidative stress and HIV.

As referred to as activated immunity (due to HIV), the activated phagocytes which are liable to increase formation of ROS (both locally and systemic). This expanded ROS carry oxidative alterations and damage to proteins, carbohydrates, lipids and nucleic acids. To fight this, cells have progressed and evolved complex antioxidant system [149]. Antioxidants are the substances that at low concentration hinder or forestall oxidation of previously mentioned biomolecules. This antioxidant system have, primary preventive antioxidants, which limits formation of oxygen centred radicals. And secondary scavenging or chain breaking antioxidants, which bring trapping of intermediate ROS. The third line of defence is further repair system for damaged lipids, nucleic acids and proteins.[150,151].

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First fundamental, essential safeguard action includes antioxidant enzymes like CAT, GPx, GR and SOD. Catalase(CAT) present in peroximes of the greater part of the tissues. It is a ubiquitous catalyst, which catalyse the two stage change of H_2O_2 into water and oxygen. It is amazingly capable as saturation doesn't happen at any concentration of H_2O_2. Catalase has a peroxidatic action in which low molecular weight alcohols can serve as electron donors. Critical degrees of catalase is found in human erythrocytes, kidneys, liver, where it brings excessive deterioration of H_2O_2, thus essential to protect cells against extraordinary oxidative pressure. There is an observation, a vital decrease in erythrocytes catalase has been found in AIDS patients, when compared with HIV illness and sound subjects [152].

Glutathione peroxidase (GPx) (selenium containing peroxidase) and glutathione reductase (GR) catalyses different hydroperoxides at the expense of glutathione in the cytosol and mitochondria ($2\ GSH + H_2O_2 = GSSG + 2H_2O$) and thus shield the cell from oxidative damage. Glutathione reductase (GR) converts oxidized glutathione (GSSG) to the reduced glutathione (GSH). Along these lines if there is low GR activity, it lead to raised GSSG levels, which will bring about increased oxidative pressure and instigates viral replication[153]. There are studies that demonstrates that there is decrease in erythrocyte of GPx action in HIV individual than healthy individuals.[154]

SOD a metalloenzyme which is a principal component of the cell antioxidant system. SOD catalyse the dismutation of superoxide anion to molecular oxygen and H_2O_2. Both cellular and extracellular SOD are essential for prevention of oxidative stress related harm. It is available in high amount in liver, brain, heart, kidneys and erythrocytes. There are different types of SODs as explained before, from those mitochondrial Mn-SOD removes the mitochondrial superoxide radicals, where

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respiratory chain acts as main source of oxygen radicals. Copper and zinc SOD responsible for catalysing the dismutation of superoxide radicals to produce H_2O_2 and oxygen. Extracellular SOD (EC-SOD) present in interstitial spaces and extracellular fluid liable for SOD activity of lymph, plasma, and synovial fluid. It has been shown that there is decreased SOD action in HIV patients, may be an eventual outcome of inhibition of Mn-SOD by HIV tat protein and shows negative association between Mn-SOD level and SOD activity[155 156].

Other system of defence incorporates chain-breaking cell antioxidants. During this chain breaking reaction, stable by-product result by getting electrons from a radical or by giving an electron to a radical. Exogenous antioxidant are lipid and water soluble. Lipid soluble antioxidants like vitamin E and carotenoids, restrains lipid peroxidation by scavenging free radicals in membrane layer and lipoprotein particles by trapping peroxyl radicals [157]. The water soluble antioxidants like vitamin C, uric acid, phenolic substances and bilirubin scavenges free radicals from blood plasma. Vitamin C moreover involved in recycling of free radicals produced by oxidation of vitamin E. Other different endogenous non–enzymatic antioxidants are metals and metal binding proteins such as, transferrin, manganese and copper. Metal binding proteins (ferritin, transferrin and lactoferrin) help in capturing copper and iron, hence they are unavailable for hydroxyl radicals. Vitamin E supplement help in diminishing oxidative stress and decreasing the risk of AIDS progression [158].

Catalase and potentially GSH are liable to bring repair in harmed DNA. There is exhaustion of these enzymes in AIDS and so result in failure to bring repair in damaged DNA, may prompt malignancies. Any lymphoid-borne malignancies are common than in other cell type under similar circumstances, [159] as lymphocytes have

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less active antioxidant capacity than macrophages, thus lymphocyte are more sensitive for DNA harm than macrophages. For HIV-affected people, chronic antigenic exposure is important as it relates to level of intracellular antioxidant involvement in DNA damage repair. Another possible factor in the headway of malignancies is a direct result of DNA damage with mutagenic effects.

2.15.2. Glutathione redox status and HIV

One of the principal endogenous scavenger is glutathione, having key part in cellular redox balance and apoptosis. As seen before glutathione (GSH) is procured from tripeptide (glycine, amino glutamine, and cysteine). Glutathione present in two structures, reduced glutathione (GSH) and oxidized glutathione (GSSG). Reduced form is commonly present in the body having strong antioxidant activity. Glutathione reductase (GR) oxidises GSH to GSSG and later recycled back to GSH by NADPH in the GPx catalysed reduction of peroxides to water and alcohol[160].

$$H_2O_2 + 2GSH \xrightarrow{GPx} GSSG + 2H_2O$$

$$GSSH + NADPH + H^{+} \xrightarrow{GR} 2GSH + NADP^{+}$$

Glutathione is a major scavenger of free radical which hinders transcription of HIV-1 by inhibiting activation of NF-kB. NF-kB is available as inactive form as bound to Ikb in the cytoplasm. Factors like TNF-α and ROS can cause its activation and its release from Ikb to the nucleus where it binds with DNA. Intracellular GSH is furthermore known to control T-lymphocyte function. In human the ratio of GSH is about 90% to 10% GSSG. Changed ratio or decrease in GSH shows an increase in GSSG, which brings about expanded lipid peroxidation and therefore increases cell oxidative stress. It has been shown in HIV disease there is fundamental decline in GSH, which could be a direct result of its increased use and low synthesis, secondary to

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reduced accessibility of the substrates [161,162]. There is glutathione redox unevenness in HIV disease, which increases ROS synthesis and brings oxidative damage, inflammation, viral replication, deficiencies in vitamins, further obligated for apoptosis and progression of disease. There is likewise association seen in between glutathione and CD4 level. Decrease in glutathione also shows decrease in CD4 count [163].

2.15.3 Oxidative stress, immune activation and HIV.

Chronic immune activation is seen during HIV infection. The viral tat protein progresses and which accelerates synthesis of ROS through mitochondrial generation of superoxide anion. This ROS bring activation of NFkB, a nuclear transcription factor, which upgrades HIV transcription and further bring more generation of ROS and subsequently reduces antioxidant (GSH) [164]. Thus shows vicious cycle of actuation oxidative stress and replication of virus through factor kappa B (NF-Kb). In cytosol NF-kB is complexed to other administrative particle Ikb, so it stays as inactive form. Incitement of this inactive complex by inflammatory factors, increased oxidative stress, cytokines, and mitogens brings phosphorylation and degradation of Ikb protein from complex and releases NF-kB heterodimer. NF-kB by then move to the core nucleus and trigger transcription of required gene of virus which results in viral replication. Few cytokines also have influence on viral replication e.g.tumor necrosis factor Alpha(TNF-α)[165].

The immune system cells are profoundly sensitive to oxidative stress, as their plasma membranes contain significant levels of polyunsaturated acyl lipids, which is liable to undergo peroxidation[166,167]. Peroxidation of the polyunsaturated acyl chain in the cell membranes bring disturbance of intracellular signalling and the general cell

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function because of loss of membrane integrity and hamper membrane fluidity. ROS may alter protein structures making them liable for fragmentation, proteolysis and eventually disabling their activity. Due to expanded ROS which brings oxidative damage of DNA causing mutation and deletion in gene, liable for some illnesses, including loss of function of mitochondrion, cell senescence and aging [168,169].

In HIV infection, it targets fundamentally the CD4+ T-cell pool and less significantly macrophages and dendritic cells, which are vital immune cells. In chronic period of HIV disease, the principle target cells for virus are CCR5, $CD4^+$ activated T-lymphocytes(memory cells)[170,171]. 80% of these cells are available in the lymphoid tissues, for example, lymph nodes in MALT(mucosa associated lymphoid tissue)mainly in GIT associated lymphoid tissue (GALT)[172]. As these mucosal CD4 cells comprise principally of memory CD4+ T-cells expressing the HIV co-receptor CCR5 are therefore perfect target for the HIV virus[171]. The "leaky gut" phenomenon happens in early HIV infection, and there translocation of microbial substances, as LPS, flagellin and CpG DNA (toll like receptor ligands) over the epithelial surface which result in loss of mucosal integrity [173]. This microbial substances liable for enactment of natural immunity and initiate immune cells, for example, neutrophils, macrophages/monocytes and dendritic cells, straightforwardly to create ROS and pro-inflammatory cytokines for example TNF-α, IL-6 and IL-1β, bringing about expanded oxidative stress, exhaustion of antioxidant agent which increases apoptosis, bring consumption of CD4 T cells, resulting danger of fast progression of diseases[174]. The apoptosis or programed cell death, is a characteristic physiological cycle, however it is increased over the span of oxidative stress. CD4 + T cell culture with HIV is related to the the cytopathic activity of the virus, which is demonstrated by cells ballooning and

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syncytia formation that adds to cell death of both infected and non-infected cells. CD4 + T cell death can directly be mediated by HIV replication or indirectly, as non-infected cells being primed for apoptosis. The commencement of apoptosis in both infected and non-infected cells is intervened by altering expression of the viral envelope gp120/gp41 complex. Chronically HIV infected cells can serve as effector cells to bring apoptosis in uninfected target CD4 + T cells. TNF-α is likewise a solid apoptosis inducer [175].

Presently incessant fact that chronic activation of CD8 and CD4+ T-cells characterizes HIV-1 infection and their continues activation brings depletion of CD4+ T-cells and progress to AIDS. The markers of immune activation are presently viewed as more important predictor of disease progression to AIDS than the CD4 count and viral load [176,177]. Oxidative disturbance in the beginning stage of HIV disease, which is characterized by low GSH and SOD and high lipid peroxidation products (MDA) has been reported. The immune system framework's capacity is influenced by its glutathione redox status and it’s potential. Subsequently, oxidative stress and diminished glutathione levels in lymphocytes, cause changes in cell function, abnormal cytokine production and a dysfunctional proliferation response [178]. Different projects reports, glutathione-replenishing agents restore in vitro proliferative response in HIV-infected cells and in cells where chemical depletion of glutathione was done in the cell [179, 180].

2.15.4 Oxidative stress, inflammation and HIV

Due to interminable increase in ROS, activate monocytes and macrophages which further potentiating them to create pro-inflammatory cytokines, which are liable for producing inflammatory process in HIV. This aggravated inflammation is again

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responsible to produce excessive ROS, which is connected to pathophysiology of chronic diseases such as chronic inflammatory diseases and atherosclerosis. Inflammation straightforwardly initiate oxidative stress and may cause endothelial damage in HIV-infected people, leading to thrombotic complications [181]. In spite of the fact that inflammation is a necessary protective reaction to tissue injury by a microbe or any pathogen, but if not appropriately controlled it may be fatal [182]. The reaction of inflammation is partitioned into two stages; an acute stage which is important for innate immunity and a chronic stage, part of adaptive immunity. Acute inflammation is the quick reaction of the body to injury. The injured tissue cells discharges chemical signals that activate the endothelium of the nearby capillaries. The activated endothelial cells releases selectin or cell adhesion molecules, which arbitrarily attract and attach neutrophils to the endothelial cells, slowing them down. The neutrophils encounter the mediators of inflammation that activate adhesion receptors (integrins) on the cells. Now the neutrophil integrins attach firmly to endothelial adhesion molecules. Neutrophils go through configurational change in shape and squeeze through the endothelial wall into interstitial tissue. It move to the site of injury, and assault the pathogen and kills them. The activated neutrophils and macrophages shed arachidonic acid (AA) from the membrane. Arachidonic acid, going through a progression of eicosanoids changes and synthesize prostaglandins which is liable for pain, vasodilation, erythema and fever that are associated with inflammatory reaction. Depending upon the seriousness of insult caused to tissue, other leukocytes for example lymphocytes, monocytes, and macrophages may follow the neutrophils [183].

In chronic phase of inflammation, Macrophages and neutrophils has oxygen-dependent enzyme (NADPH oxidase system) which helps in killing pathogen by its

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capacity of produce noxious ROS. The expression of NADPH oxidase, NOX2 (gp91 phox) is responsible for ROS production during inflammatory process, which is induced by NF-kB. This happens during respiratory burst, there is increased oxygen use and ATP creation and further leads to oxidative stress, tissue injury and inflammation [183]. Inflammatory cytokines, for example, TNF-α, IL-1, IL-6, are known to enhance HIV transcription, replication and fast exhaustion of CD4+ T-cells by means of apoptosis, which further liable for progression of HIV to AIDS[184,185]. It has been proved that, TNF-α may cause inflammatory harm in the hosts immune responses independent of CD4 depletion. In HIV disease there is association among inflammation and higher mortality risk, even in patients with high CD4 count [186,187].Inflammatory biomarkers C-receptive proteins (CRP) and fibrinogen were strong and independent predictors of mortality in HIV patients [188]. Thus the chronic infection characteristic of HIV infection, leads to a continuous production of ROS and chronic state of inflammation, which promotes chronic oxidative stress.

2.16 Biomarkers in oxidative stress.

A] Estimation of antioxidants- There are various techniques for assessment of antioxidant level in blood.

1) Enzymatic antioxidants - superoxide dismutase (SOD), catalase, glutathione peroxidase.

2) Non-enzymatic antioxidants- Vitamins, glutathione, uric acid.

Evaluating of above levels, independently is tedious and furthermore require more expository method and instruments, so total antioxidant capacity (TAC) is estimated which is internationally acknowledged. Despite the fact that now a days

numerous commercial detection kits accessible for assessment of SOD, glutathione, glutathione reductase and so forth. There are various strategies for TAC assessment like TEAC (Trolox equivalent antioxidant capacity) bases on capacity of inhibiting the oxidation of chemical substance, which is the direct method [189]. Other indirect techniques like, FRAP(Ferric reducing antioxidant power) and CUPRAC(cupric reducing antioxidant capacity) depend on electron transfer responses where antioxidant agents are utilized as reductants in redox-linked colorimetric method[190,191].

TEAC assay for assessment of TAC.

Principle is that when ABTS (2,2'- azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) is mixed with a proper chemical compound, an ABTS radical (ABTS•+) is formed. The ABTS•+ shows maximum absorption at 650, 734 and 820 nm. This mixture with radicals show blue-green colour. When sample is added antioxidants in the sample reduces ABTS•+, suppressing this colour production to a degree that is proportional to their concentration. In human plasma, TEAC assesses mainly, albumin and uric acid and also ascorbic acid, α-tocopherol, and bilirubin [192].

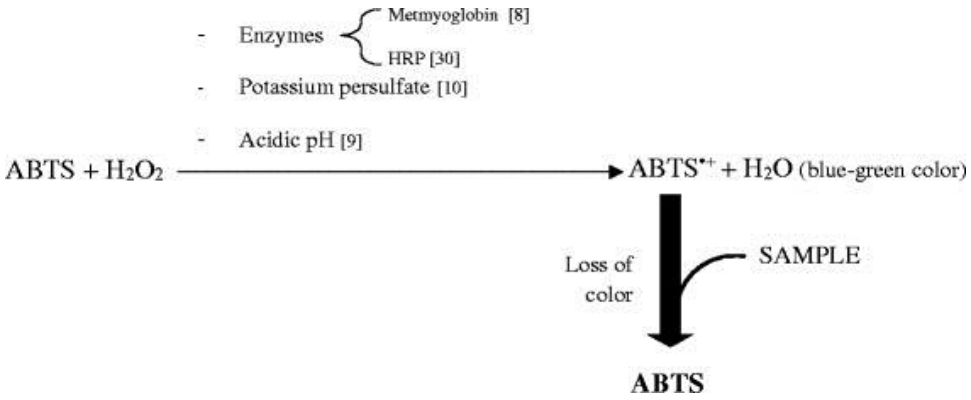

Figure 2.7: The principle of the TEAC method [193].

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FRAP technique for assessment of TAC

It is based on the reduction of ferric-tripyridyltriazine (Fe3+-TPTZ) complex to ferrous tripyridyltriazine (Fe2+-TPTZ) by the antioxidants of a sample at low pH. The final product (Fe2+-TPTZ) has blue colour with maximum absorption at 593 nm and the change in absorbance is related to the antioxidant capacity of the plasma. FRAP assesses estimate mostly uric acid, additionally it also quantifies ascorbic acid, bilirubin, and α-tocopherol[190].

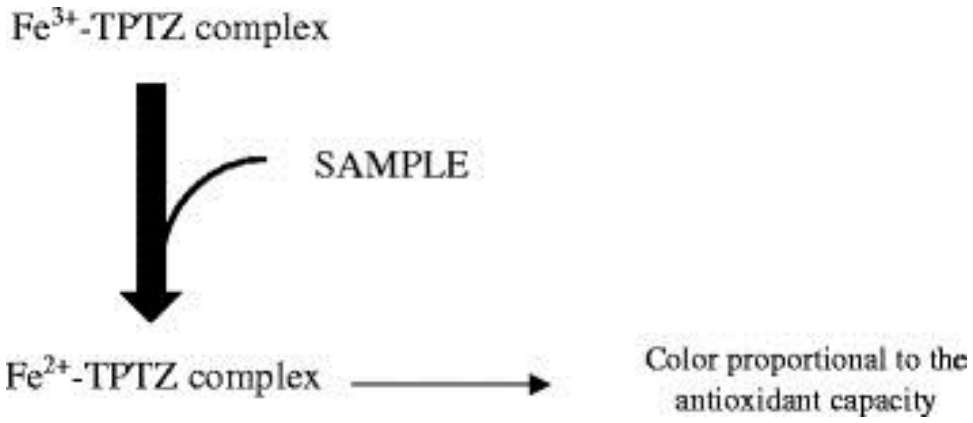

Figure 2.8: The principle of the FRAP method [193]

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CUPRAC assay for assessment of TAC

This measures the capacity of antioxidants of a sample to reduce the Cu^{2+} to Cu^{1+} in the presence of a chelating agent. These chelators produce stable complexes with Cu^{1+}, which is coloured that have a maximum absorption at 450–490 nm [191]. CUPRAC mainly estimates the thiol-group antioxidants and other such as, α-tocopherol, β-carotene, ascorbic acid, uric acid, bilirubin, and albumin.

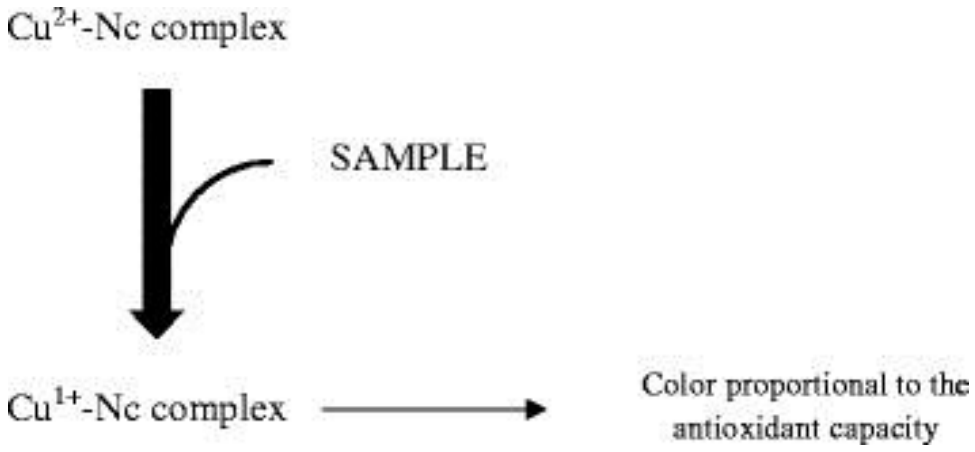

Figure- 2.9: The principle of the CUPRAC method [193]

B] Estimation of oxidant levels- Estimation of oxidative stress is commonly done by measuring degree of lipid peroxidation, which estimates MDA(Malondialdehyde), which is by-product of lipid peroxidation reaction and estimation of Nitric oxide(NO), as reactive nitrogen species.

Estimation of MDA(TBARS assay)

The reaction of MDA with thiobarbituric acid (TBA) produces thiobarbituric acid reactive compound (TBARS), which gives the name to the test. Specifically, the

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MDA–TBA adduct formed by the reaction of MDA and TBA under high temperature and acidic conditions in the samples, is measurable by colorimetry or fluorimetry [194].

Estimation of Nitric Oxide (Griess test)

Total levels of nitrite or nitrous acid is estimated by Griess test. The nitric oxide (NO^-) derived compounds in the serum combined with alpha-naphthylamine to produce pink azo dye, whose absorbance was measured at a wavelength of 540 nm. Total nitric oxide metabolites is total measure of nitrite and nitrate levels which is considered a direct marker of in vivo NO production[195].

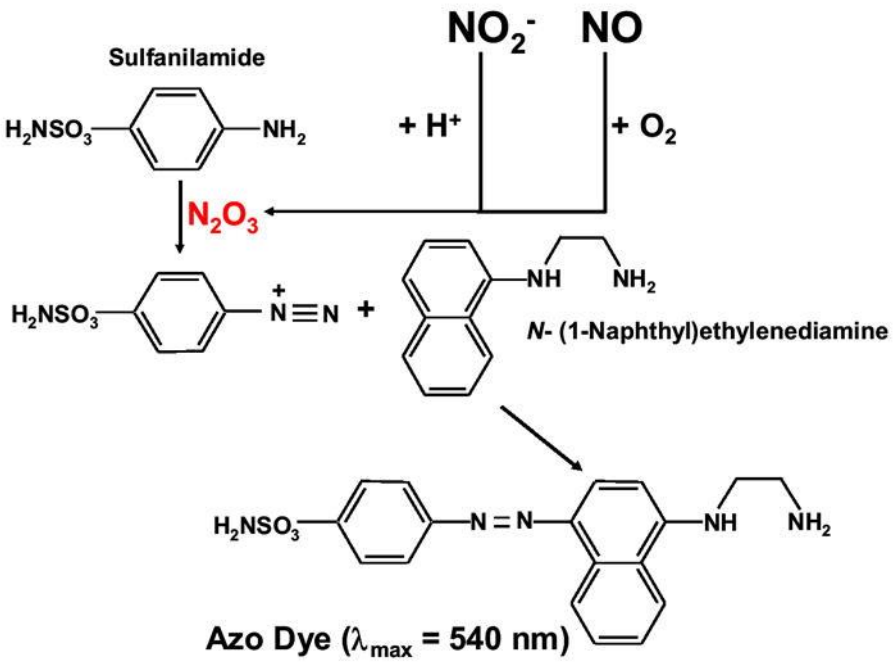

Figure 2.10: Griess reaction for estimation of NO [196]

2.17 Oxidative stress and antiretroviral treatment in HIV patients

According to 15th World Health Organisation (WHO) guidelines, recommend combination antiretroviral therapy (HAART), which is based on scientific evidence from the Strategies for Management of Antiretroviral Therapy (SMART) trial

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[197] and the HIV Prevention Trials Network (HPTN) O52 study[198]. It is well established that a high number of patients who delay HAART until the $CD4^+$ count drops below 200 cells/mm^3 do not achieve a normal $CD4^+$ count, even after a long therapy. It is proved that earlier the ART better the immune system and less inflammation is present, with subsequently less mortality and morbidity[199]. There are fixed dose combination of antiretroviral drugs used. India has adopted, single pill of tenofovir disoproxil fumarate + 3TC (lamivudine) + EFV (efavirenz), as per 2013 WHO guidelines [200].

The induction of oxidative stress during antiretroviral therapy (ART) is a finding in HIV-1 redox biology. Several studies indicate that nucleoside and non-nucleoside inhibitors and inhibitors of the viral protease cause significant development of ROS in different cell types. In addition to the persistent redox imbalance of HIV-1 infection, several studies reported an increase in oxidant stress, due to a rise in oxidants and a decrease in serum antioxidant rates [201]. The study carried out in 84 patients infected with HIV in 6 months of ART shown the rise in serum peroxidization, total hydroperoxide, MDA, and glutathione levels decreased significantly in comparison with their rates before therapy and for healthier controls [148]. This may be due to rise in GSH utilization or limited reduction of its oxidized form intracellularly. Experiments performed on cell lines exposed to ART and in laboratory animals showed that increased oxidized metabolite production is caused by mitochondrial interference. Mitochondrial dysfunction with ART results from altered mitochondrial DNA replication and inhibited oxidative phosphorylation[202].

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There are five drug classes of antiretroviral drugs making number of possible HAART combinations. Selecting these combinations mostly dependent upon knowledge of antiretroviral toxicities. There are studies which shows that potential side effects is a primary reason for patients discontinuing ART among HIV patients. Extra attention for drug toxicity is needed in elderly HIV patients due to concurrent medication use and so more chances of harmful drug-drug interaction, as well as renal and hepatic dysfunction due to age may affect drug activity [203].

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Chapter 3

MATERIALS AND METHODS

3.1 Study population and design

This study was conducted in D Y Patil medical college, after getting permission from institutional ethical committee (IEC). During the study, 100 HIV positive individuals were incorporated by consecutive and convenience sampling after taking consent. Data collected from private HIV clinic kolhapur and Civil hospital Gadhinglaj. During the study confidentiality was maintained. Participants were reassured that they will be given standard of care and will not be burdened for extra investigations. Participants included in the study were HIV positive, of age 18-60 years, of either sex, clinically stable, not started HAART and who were ready to take part in the study. Participants excluded from the study includes, chronic diseases like diabetes, TB, liver and kidney disease, additionally patients on chemotherapy, steroid treatment or taking any antioxidant vitamins.

Hematological profile and CD4 count were collected from routine investigations done during visit in HIV OPD for 100 participants, before beginning treatment and following after 3 months of starting HAART. Oxidative parameters like Total antioxidant capacity (TAC), nitric oxide (NO) and vitamin E were done in first 50 participants from these above 100 participants, before beginning treatment and following after 3 months of starting HAART.

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3.2 Collection of data

The sample (blood) were collected from the vein (antecubital) in the lower arm in both (ethylenediaminetetraacetic acid (EDTA) and plain bulb. EDTA blood samples were utilized for assessment of complete blood count (CBC) which incorporates hemoglobin concentration (Hb), white blood cell count (WBC), differtial count, blood indices (MCV, MCH, MCHC), platelets by automated analyser and CD4 count by flow cytometry, while blood in plane bulb was permitted to clump and was centrifuged for 5 minutes at 3000rmp, the clear serum was isolated and kept in deepfreeze until analysis. Serum sample was utilized for investigation of oxidative parameters (TAC, NO and vitamin E).

3.3 Hematological Assay

Various hematological parameters including, Hemoglobin concentration(Hb), white blood cell count(WBC) with differtial count, blood indices like MCV(mean cell volume), MCH(mean cell hemoglobin), MCHC(mean cell hemoglobin concentration) and platelets were determined by automated hematological (CBC) analyzer (sys mex-KX-21,Transasia model) by electrical impedance technique.

Principle of automated analyzer-

Automated CBC analyzer is computerized highly specialized equipment. It determines size and volume of the cell, differentiates WBC cells into neutrophils, lymphocytes and mixed cells. It aspirates the required quantity of blood, quantifies, classifies and describes cell population using electrical impedance technique. In this technique blood cells pass through aperture, as each cell passes through aperture, a change in electrical resistance occur generating a voltage pulse. Number of pulses

during a cycle corresponds to number of cell counted whereas amplitude of each pulse directly proportional to the cell volume [204].

CD4 Count. T lymphocytes of CD4 type are estimated by Flow Cytometry

Principle of Flow Cytometry- Flow cytometry involves enumeration of cells in a liquid flow. For HIV detection, the blood cells are generally tagged using fluorescent labeled antibodies specific for CD4 enumeration and suspended in a liquid stream within a flow cell. The cells flow in a narrow stream toward the laser source and the beam hits only one single cell at a time. The incident light on the cell is then scattered forward as well as sideways. This scattered light is captured by the optics in the analyzer and directed to the detectors arranged. This detectors are connected to the computer, where the cells are analyzed on the basis of their shape, size as well as internal complexity [205]. Flow cytometry is the recognized gold standard for CD4 testing and is used to stage HIV/AIDs, guide treatment decision for HIV infected persons and evaluate effectiveness of therapy. In HIV positive patients initial CD4 count is estimated to see immunological status and used to determine whether prophylaxis for opportunistic infection is needed (If CD4<200 start co-trimoxazole treatment) and CD4 estimation done after 3 month of initiation of treatment to evaluate response of ART, disease progression and survival.

3.4 Markers of Oxidative stress

1] Total antioxidant capacity estimation (TAC) (CUPRAC assay)

TAC was measured by using an assay kit (Bioassay Systems QuantiChrom^{TM} USA) (DTAC-100) following the manufacturers protocol, which shows colorimetric

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determination of total antioxidant capacity. Copper reduction can be used as a sensitive indicator of potential pro-oxidant activity of antioxidants

Principle-TAC estimation-CUPRAC assay by cupric reducing antioxidant capacity, based on determination of ability of a sample to reduce a metal complex Cu^{2+} to Cu^{1+} in presence of chelating agent (bathocuproine-BC) which forms colored stable complexes with Cu^{1+} , have maximum absorbance at 450-490 nm which is measured[165].

2] Nitric oxide(NO) estimation (Griess method)

In our study we have used BioAssay Systems QuantiChrom^{TM} Nitric Oxide Assay Kit, following the manufacturer's protocol which is designed to accurately measure NO production following reduction of nitrate to nitrite using improved Griess method.

Principle - NO estimation by Colorimetry - Nitric oxide (NO) is a reactive nitrogen radical estimated by colorimetric method. Since NO is oxidized to nitrite and nitrate, it is common practice to quantitate total NO2/NO3 as a measure for NO level. First Nitrate is converted to Nitrite by nitrate reductase (NR) enzyme. Now this nitrite is treated with diazotizing reagent e.g sulfanilamide (SA), in acidic medium to form transient diazonium salt .This intermediate is then allowed to react with a coupling reagent, N-naphthyl-ethylenediamine (NED) to form stable Azo compound .The intense purple colour of product allows nitrite assay with high sensitivity and can be used to measure nitrite concentration at low as 0.5µm level. The absorbance of this adduct at 550nm is linearly proportional to the nitrite concentration in the sample [167].

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3] Vitamin E estimation (Colorimetry)

In our study we have used Vitamin E Assay kit(Catalog No-MBS2540415) following the manufacturers protocol which snows colorimetric determination of vitamin E.

Principle--Fe^{3+} can be deoxidized to Fe^{2+} by vitamin E. Fe^{2+} Can react with phenanthroline and form pink compound under certain condition. After colorimetric assay, Vit E content can be figured out according to standard curve .

3.5 Normal values of blood and oxidative parameters[206]

Parameter assessed	Male	Female
Hemoglobin	13.5 -17.5g/dl	12.0-16.0g/dl
Total leucocyte count(WBC)	4.0-11.0×10^3/ mm^3	
Differential count	Neutrophils- 40-75%	
	Lymphocytes- 20-50%	
	Monocytes- 2-10%	
Blood indices	MCV- 73-99 fl	
	MCH - 24-35pg	
	MCHC- 32-36g/dl	
Platelet count	1.5-3.0 lakh/mm^3	
CD4 Count	500-1200/mm^3	
TAC(Total Antioxidant Capacity)	0.8-1.3mmol/L	
NO(Nitric Oxide)	11.5-75µmol/L	
Vitamin-E	5.5-17µg/ml	

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3.6 Statistical analysis.

Data collected, feed to Microsoft excel and analyzed. The finding are described as the mean ±standard deviation. All measurable investigations were performed with the program statistical package for the social science (SPSS for windows version 20). To test significant difference between hematological investigations and oxidative parameters, before and after HAART, Paired t test was used. To test significant difference between CD4 count (CD4<200,CD4 200-499, CD4≥500) with hematological parameters, ANOVA test was applied. Spearman's rank correlation coefficient was applied to discover correlation between studied parameters. A "p" value estimation of <0.05 was acknowledged as statistically significant.

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Chapter 4

OBSERVATIONS AND RESULTS

4.1 Demographic of study population

Table 4.1: Age and sex distribution

Age in years	n(100)	Male	Female
18-30	26	15	11
31-40	32	12	20
41-50	25	10	15
51-60	17	7	10
Total		**44**	**56**

In our study among 100 subjects, 56 are female and 44 are male. Patients are grouped in four groups according to age between 18-30(26%), 31-40(32%), 41-50(25%), 51-60(17%). Most of the patients female are in age group of 31-40(35.71%) and males are in age group of 18-30(34.09%).

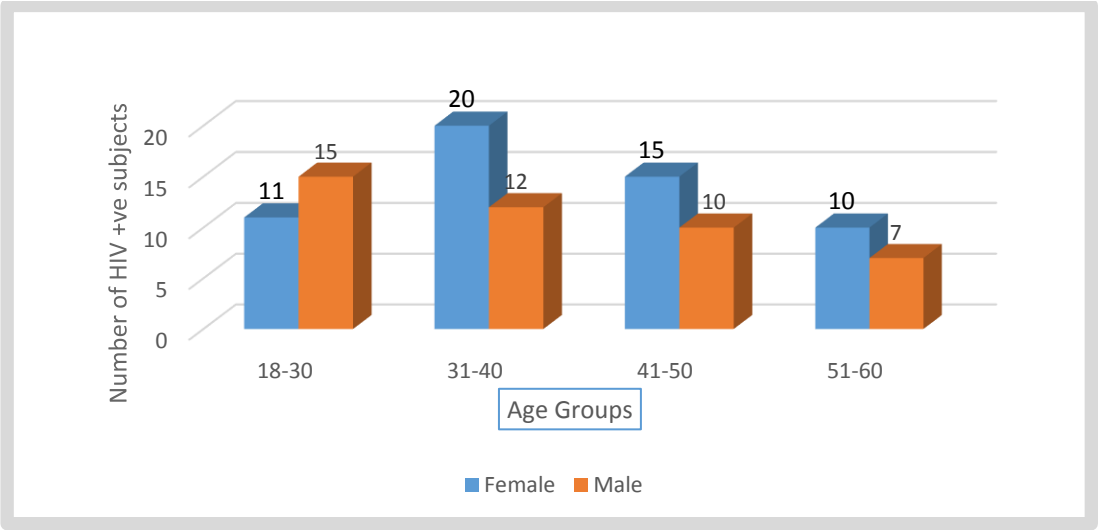

Figure 4.1: Distribution of HIV subjects according to age and sex

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4.2 Hematological investigations and Hematological abnormalities

Certain hematological abnormalities were determined in the subject. In our study anemia defined as hemoglobin less than 11 gm/dl(female) and less than 13 gm/dl (male). Mild anaemia as hemoglobin value of 10-11 gm/dl(female) and 12-13gm/dl(male). Moderate anaemia as hemoglobin value of 8-9gm/dl(female) and 10-11gm/dl(male) and severe anaemia as hemoglobin value less than 8gm/dl(female) and less than10gm/dl(male). Leucopenia as total WBC count less than 4000 cells/µl. Neutropenia as absolute neutrophils/granulocyte count less than 1000 cells/µl and lymphocytopenia considered at lymphocyte count of less than 800 cells/µl. Thrombocytopenia considered as platelet count less than 150x 10^3 /µl. Macrocytosis as (MCV) mean cell volume more than 100 fl.

Table 4.2: Hematological characteristics of HIV subjects before and after HAART

Parameters	**Before starting HAART (0 month) n=100** mean ± SD	**After starting HAART (3 month) n=100** mean ± SD
Hemoglobin(g/dl)-	9.8 ±0.69	12.51 ±1.19***
WBC (10^3 /mm^3)-	5.5 ±0.35	4.1 ±0.13*
Neutrophil(10^3 /mm^3))	2.41±0.17	2.14 ±0.18*
Lymphocyte(10^3/mm^3)	1.64±0.04	1.54±0.14*
MCV(fl) -	82.05±3.25	103.33±2.25***
MCH(pg)	32.03 ±2.18	34.25 ± 2.53*
MCHC(Gms/dl)	29 ± 1.55	32.02±1.17 *
Platelets(10^3/mm^3)	215.25± 4.88	220.02±11.11*
CD4(mm^3)	340.27± 98.91	382.72± 113.62***

Data presented as group mean ± SD, Significantly different: *****p<0.01,******p<0.001, *******p<0.0001

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Table 4.2 represents hemoglobin level, WBC count, neutrophil count, lymphocyte count, MCV, MCH, MCHC, Platelets and CD4 count estimated before and after HAART.

4.2.1 Hemoglobin- The mean hemoglobin at baseline (before HAART) is 9.8 ±0.69 while after HAART is 12.51 ±1.19 ($p<0.0001$). Thus highly significant increase in hemoglobin level was seen after HAART.

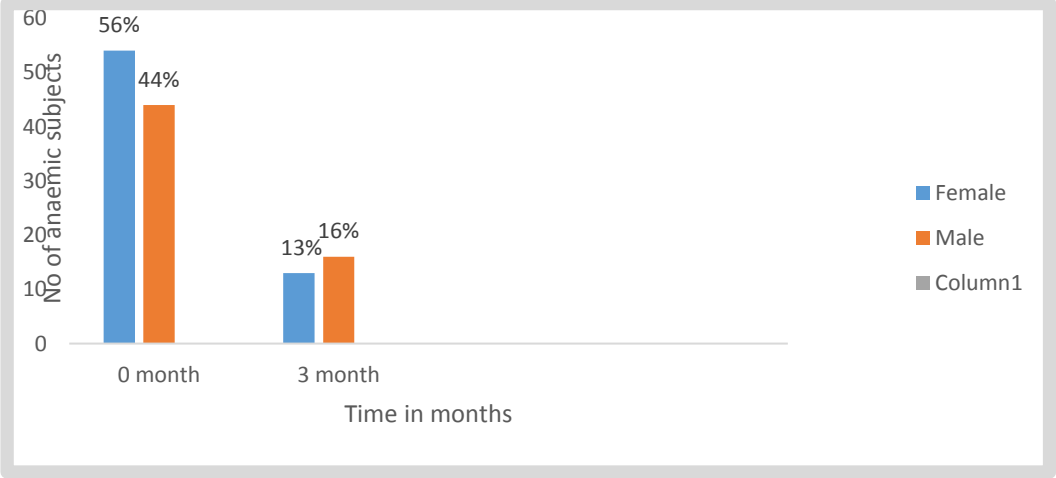

Figure 4.2: Improvement in anemic patients after HAART

Figure 4.2, shows all patients were anaemic and after HAART, 71% showed improvement in hemoglobin and could overcome anaemia,, only 29% remained anaemic.

4.2.2 White blood cell (WBC) and differtial count- Mean WBC before and after HAART is 5.5 ±0.35 and 4.1 ±0.13 respectively. There is significant difference between two ($p<0.01$), showing significant reduction in WBC count after HAART . Also there is significant reduction in neutrophil and lymphocytes after HAART ($p<0.01$).

4.2.3 Blood indices-MCV, MCH and MCHC-Mean MCV before and after HAART is 82.05±3.25 and 103.33±2.25 respectively. There is highly significant difference between two ($p<0,0001$), showing highly significant increase in MCV (macrocytosis)

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after HAART. Also there is significant increase in MCH and MCHC levels after HAART with $p<0.01$ respectively.

4.2.4 Platelets- Analysis of platelet count, data revealed significant increase in platelet count after HAART ($p<0.01$) .

4.2.5 CD4 count - The mean CD4 at baseline (before HAART) is 340.27±98.91 while after HAART is 382.72± 113.62 ($p<0.0001$). Thus highly significant increase in CD4 count is seen after HAART.

4.3 CD4 Count distribution in HIV positive subjects

Table 4.3: CD4 count distribution in HIV subjects

CD4 Count(mm^3)	Before HAART (No of subjects)	After HAART (No of subjects)
<200	19	20
200-499	81	50
≥500	0	30

Table 4.3, among 100 HIV subjects, before treatment 19 subjects showed CD4 count below $200mm^3$ and 81 subjects with CD4 count between 200-$499mm^3$. After treatment, 20 subjects with CD4 <200, 50 subject with CD4 count in range of 200-499 and 30 subjects showed CD4 count above 500.

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4.4 Comparison of CD4 and hematological profile in HIV positive subjects

Table 4.4: Hematological profile of HIV subjects stratified by CD4 count after HAART

Parameters	**CD_4 Count stratification**		
	<200 (n=20)	**200-499(n=50)**	**≥500 (n=30)**
Hb (g/dl)	11.25±0.92	12.28±0.91	13.74±0.41***
WBC (10^3 /mm^3)	4.14±0.12	4.12±0.15	4.15±0.09
Neutrophil(10^3/mm^3))	2.15±0.16	2.12±0.17	2.14±0.10
Lymphocyte(10^3/mm^3)	1.59±0.16	1.54±0.14	1.50±0.11
MCV(fl)	103.28±2.14	103.58±2.14	102.93±2.49
MCH(pg)	34.14±2.46	33.88±1.91	34.93±2.70
MCHC(Gmg/dl)	32.20 ± 1.28	31.97 ± 1.12	31.94± 1.14
Platelets(10^3/mm^3)	214.40± 22.64	222.48±4.93	219±3.44 *

Data presented as group mean ± SD, Significantly different:*p<0.05, **p<0.01, ***p<0.0001

Table 4.4 shows, Hemoglobin level, WBC count, neutrophil count, lymphocyte count, MCV, MCH, MCHC, and Platelets are compared with CD4 count level(CD4<200,CD4 200-499 and CD4≥500) after HAART.

4.4.1 Hemoglobin compared with CD4 count after HAART –The mean Hb level at CD4 count<200, CD4 200-499 and CD4≥500 is 11.25±0.92, 12.28±0.91 and 13.74±0.41 respectively (p < 0,0001). Thus highly significant difference is seen in subjects when CD4 compared with Hemoglobin. Thus higher level of hemoglobin is seen in subjects having higher CD4 count.

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4.4.2 White blood cell count and differtial count compared with CD4 count after HAART - The mean WBC level at CD4 count<200, CD4 200-499 and CD4>500 is 4.14±0.12, 4.12±0.15and 4.15±0.09 respectively(p>0.05). Thus no significant difference seen with CD4 count and WBC after HAART. There is also no significant difference seen with CD4 count and neutrophils (p>0.05) and CD4 count and lymphocytes (p>0.05) after HAART

4.4.3 Blood indices-MCV, MCH and MCHC compared with CD4 count- There is no significant difference seen with CD4 levels and blood indices, MCV,MCH,MCHC, each showing P>0.05.

4.4.4 Platelets compared with CD4 count-The mean platelet count at CD4 count<200, CD4 200-499 and CD4≥500 is 214.40±22.64, 222.48±4.931 and 219±3.44 respectively(p <0.05). Thus significant difference is seen in subjects when CD4 compared with platelet. Thus higher level of platelet is seen in subjects having higher CD4 count after HAART.

4.5 Oxidative parameters before and after HAART

Table 4.5: Oxidative parameters of HIV subjects before and after HAART

Parameters	**Before HAART (0 month) n=50 mean ± SD**	**After HAART (3 month) n=50 mean ± SD**
Nitric oxide µmol/L	26.40±3.65	35.16±5.14***
Total antioxidant level (TAC)mmol/L	0.70±0.08	0.86±0.13***
Vit-E µg/ml	3.43±0.92	4.8±0.87**

Data presented as group mean ± SD, Significantly different: *p<0.01,**p<0.001,***p<0.0001

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4.5.1 Nitric oxide -The mean nitric oxide at baseline (before HAART) is 26.40 ±3.65 and after HAART is 35.16 ±5.14 ($p<0.0001$). Thus highly significant increase in nitric oxide level is seen HAART.

4.5.2 Total antioxidant (TAC) –The mean TAC at baseline (before HAART) is 0.70 ±0.08 and after HAART is 0.86 ±0.13 ($p<0.0001$). Thus highly significant increase in TAC level is seen after HAART.

4.5.3 Vitamin E –The mean vitamin E at baseline (before HAART) is 3.43 ±0.92 and after HAART is 4.8 ±0.87 ($p<0.001$). Thus shows significant increase in vitamin E levels after HAART .

4.6 Comparison of CD4 and Oxidative parameters

Table 4.6: Oxidative parameters of HIV subjects, stratified by CD4 count after HAART

Parameters	**CD4 Count stratification** (n=50)	
	≤350 (n=23)	>350 (n=27)
Nitric oxide level µmol/L	31.60±3.64	38.20±4.23***
Total-antioxidant level (TAC)mmol/L	0.76±0.09	0.95±0.07***
Vit-E level µg/ml	4.47±0.83	5.08±0.82*

Data presented as group mean ± SD, Significantly different:*p<0.05, **p<0.001, ***p<0.0001

Table 4.6 showing comparison of oxidative parameters, Nitric oxide, TAC and vitamin E with CD4 levels(CD4 ≤350 and CD4>350).

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4.6.1 Nitric oxide compared with CD4 count –The mean nitric oxide level at CD4 ≤350 and CD4>350 is 31.60±3.64 and 38.20±4.23 respectively ($p<0.0001$).Thus highly significant difference is seen in subjects when CD4 compared with nitric oxide. Thus significantly higher level of nitric oxide is seen in subjects having higher CD4 count.

4.6.2 TAC compared with CD4 count –The mean TAC level at CD4 ≤350 and CD4>350 is, 0.76±0.09and 0.95±0.07 respectively ($p < 0,0001$).There is highly significant difference is seen in subjects when CD4 compared with TAC. Thus significantly higher level of TAC is seen in subjects having higher CD4 count.

4-6.3 Vitamin-E compared with CD4 count-The mean vitamin E level at CD4 ≤350 and CD4>350 is 4.47±O.83 and 5.08±0.82 respectively ($p<0.05$). Thus significant difference is seen in subjects when CD4 compared with vitamin E. Thus higher level of vitamin E is seen in subjects having higher CD4 count.

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4.7 Correlation of CD4 count with hematological parameters and oxidative parameters before and after HAART

Table 4.7 Correlation of CD4 with hematological and oxidative parameters

BEFORE HAART				AFTER HAART			
		r value	p value			r value	p value
CD4	**Hb**	-0.28	0.0039*	**CD4**	**Hb**	0.78	p<0.0001***
CD4	**WBC**	-0.017	0.86	**CD4**	**WBC**	0.04	0.69
CD4	**Neutrophils**	-0.003	0.97	**CD4**	**Neutrophils**	0.058	0.56
CD4	**Lymphocytes**	0	>0.99	**CD4**	**Lymphocytes**	-0.32	0.0008**
CD4	**MCV**	-0.003	0.97	**CD4**	**MCV**	-0.04	0.67
CD4	**MCH**	0.08	0.41	**CD4**	**MCH**	-0.01	0.85
CD4	**MCHC**	0.05	0.55	**CD4**	**MCHC**	-0.03	0.76
CD4	**Platelet**	0.045	0.65	**CD4**	**Platelet**	-0.02	0.83
CD4	**NO**	-0.03	0.81	**CD4**	**NO**	0.75	p<0.0001***
CD4	**TAC**	0.04	0.75	**CD4**	**TAC**	0.82	p<0.0001***
CD4	**VIT E**	0.12	0.36	**CD4**	**VIT E**	0.34	P<0.01*

r- Correlation coefficient between +1& -1. Significantly different:

*p<0.01,**p<0.001,***p<0.0001

4.7.1Correlation between CD4 count and Hemoglobin before and after HAART

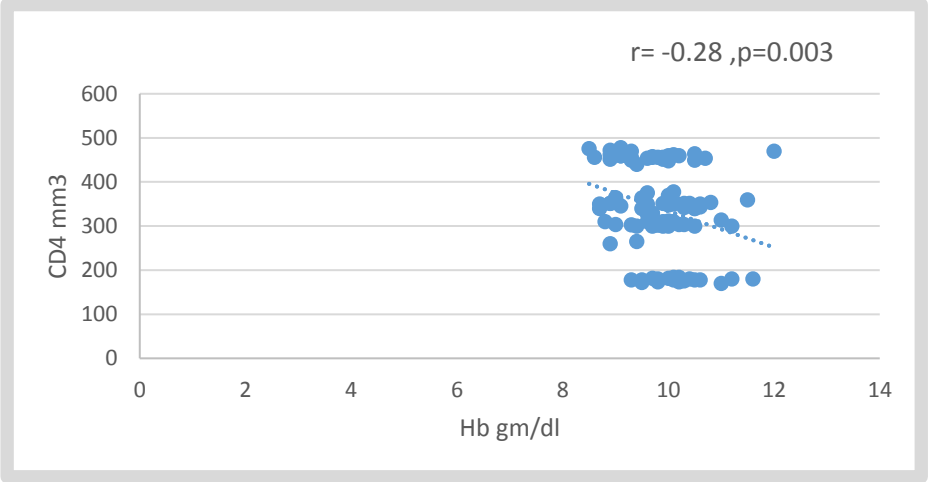

Figure-4.3: Correlation plot of CD4 count with Hemoglobin before HAART
As shown, significant negative correlation(r= -0.28) (p<0.01)

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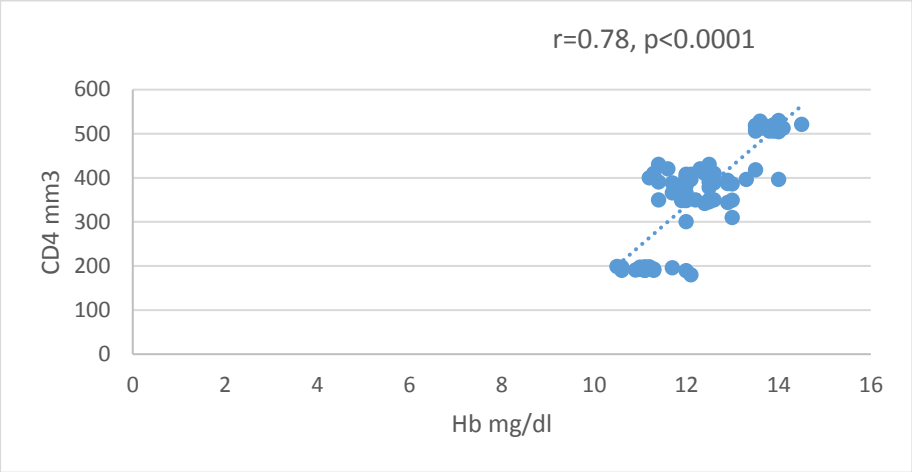

Figure-4.4: Correlation plot of CD4 count with Hemoglobin after HAART
As shown, highly significant positive correlation(r=0.78) (p<0.0001)

Figure 4.3-There is significant negative correlation between CD4 count and hemoglobin before treatment (r= -0.28, p<0.01)(fig 4.3), but there is highly significant and positive correlation (r=0.78 ,p<0.0001) between the CD4 count and their respective Hb levels after treatment (fig 4.4).Thus as the blood CD4 count of subject increases there is highly significant increase in their respective blood hemoglobin levels. There is no correlation seen between CD4 count and lymphocyte, but show significant negative correlation (r= -0.32,p=0.0008) after HAART.

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4.8 Correlation of CD4 count with oxidative parameters before and after HAART

4.8.1 Correlation between CD4 count and Nitric oxide before and after HAART

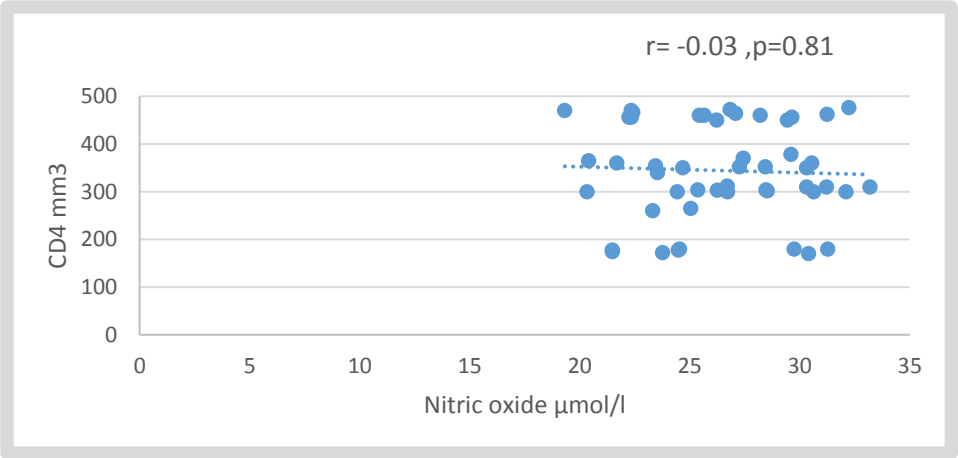

Figure 4.5: Correlation plot of CD4 count with Nitric oxide before HAART
As shown, No correlation(r=-0.03))(p=0.81)

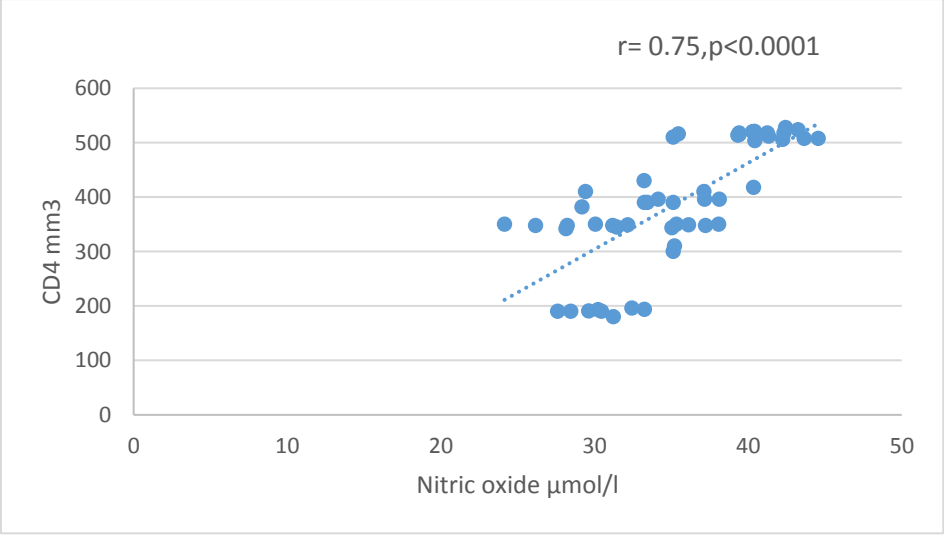

Figure 4.6: Correlation plot CD4 count with Nitric oxide after HAART
As shown, highly significant positive correlation(r=0.75)(p<0.0001)

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Figure 4.5, There is no correlation of CD4 with NO(r= -0.03,p=0.81) before treatment but show highly significant and positive correlation of CD4 and NO(r= 0.75,p<0.0001) after treatment (fig 4.6). Thus as the blood CD4 count of subject increases there is highly significant increase in their respective blood nitric oxide levels.

4.8.2 Correlation between CD4 count and TAC before and after HAART

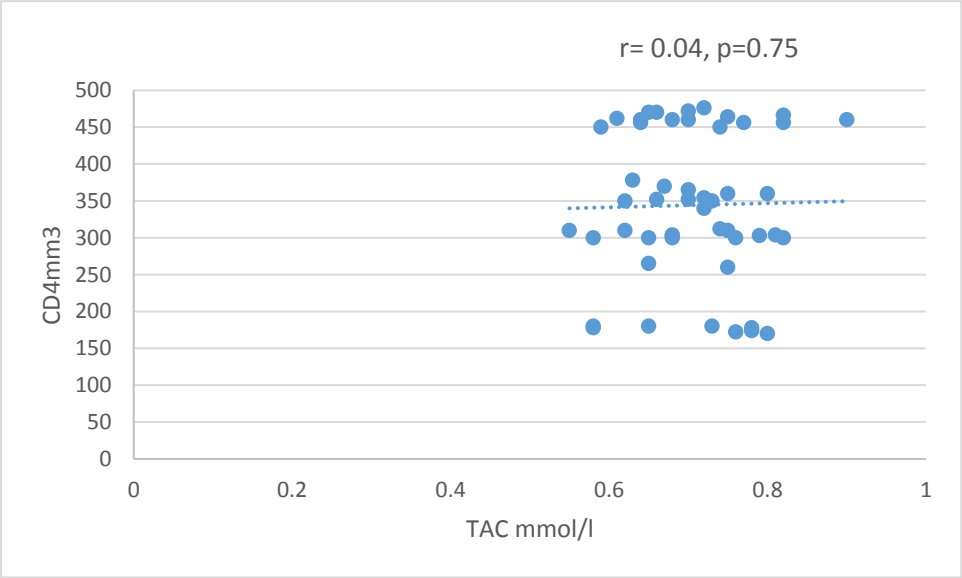

Figure 4.7: Correlation plot between CD4 count with TAC before HAART
As shown, No correlation(r=0.04)) (p=0.75)

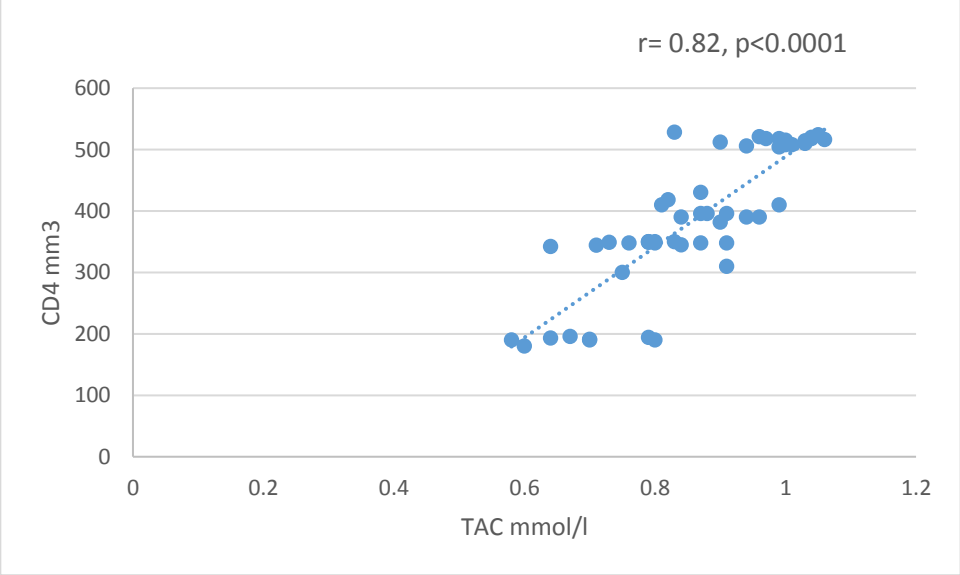

Figure 4.8: Correlation plot of CD4 count with TAC after HAART
As shown, highly significant positive correlation (r=0.82) (p<0.0001)

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Figure 4.7-There is no correlation of CD4 with TAC(r= 0.04,p=0.75) before treatment but show highly significant and positive correlation of CD4 and TAC(r= 0.82,p<0.0001) after treatment(fig 4.8). Thus as the blood CD4 count of subject increases there is highly significant increase in their respective blood TAC levels.

4.8.3 Correlation between CD4 count and Vitamin E before and after HAART

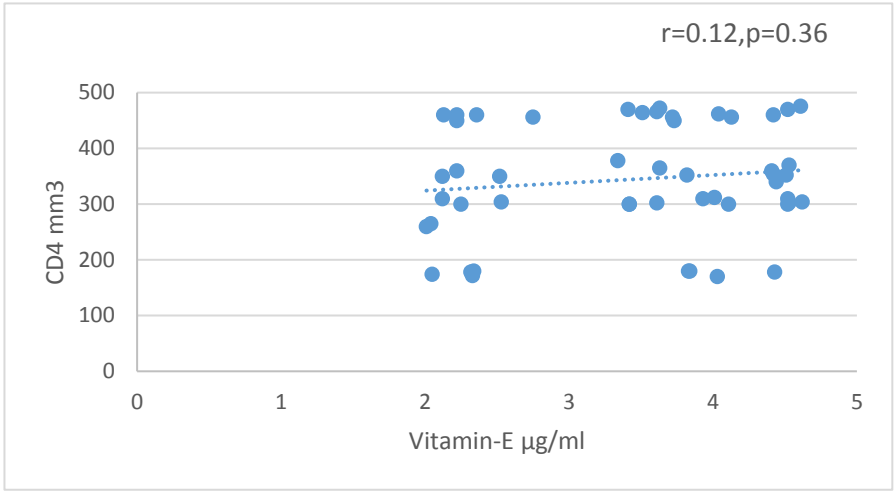

Figure 4.9: Correlation plot of CD4 count with Vitamin E before HAART
As shown, No correlation (r=0.12)) (p=0.36)

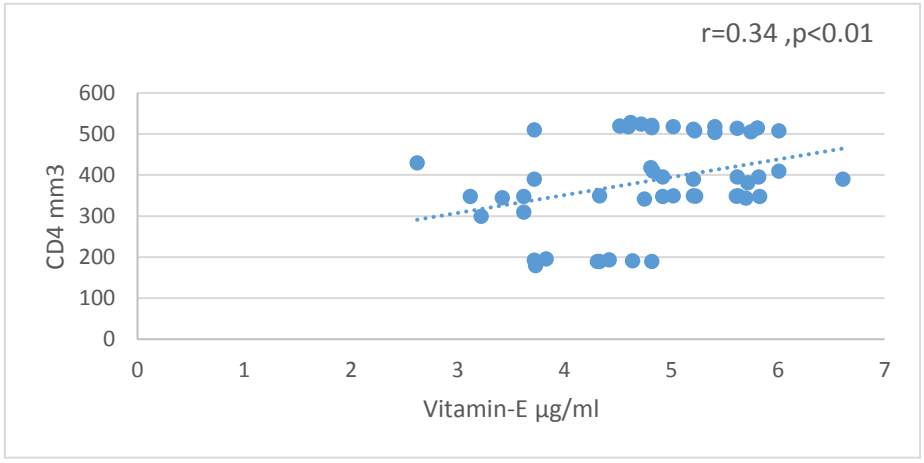

Figure 4.10: Correlation plot of CD4 count with Vitamin E after HAART
As shown, significant positive correlation (r=0.34) (p<0.01)

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Figure 4.9, There is no correlation of CD4 count with vitamin E(r= 0.12,p=0.36) before treatment but shows significant and positive correlation of CD4 and vitamin E(r= 0.34,p<0.01)(fig 4.10) after treatment. Thus as the blood CD4 count of subject increases there is significant increase in their respective blood vitamin E levels.

4.9 HAART and ADRs

From total 100 subjects on HAART, 68% subjects showed 164 ADRs.

Table 4.8: Distribution of ADRs in HIV subjects on HAART

ADR	Number of ADRs(164)
Gastritis	42(25.61%)
Itching	36(21.96%)
Nausea, vomiting	32(19.51%)
Rashes	28 (17.07%)
Diarrhea	26(15.85%)

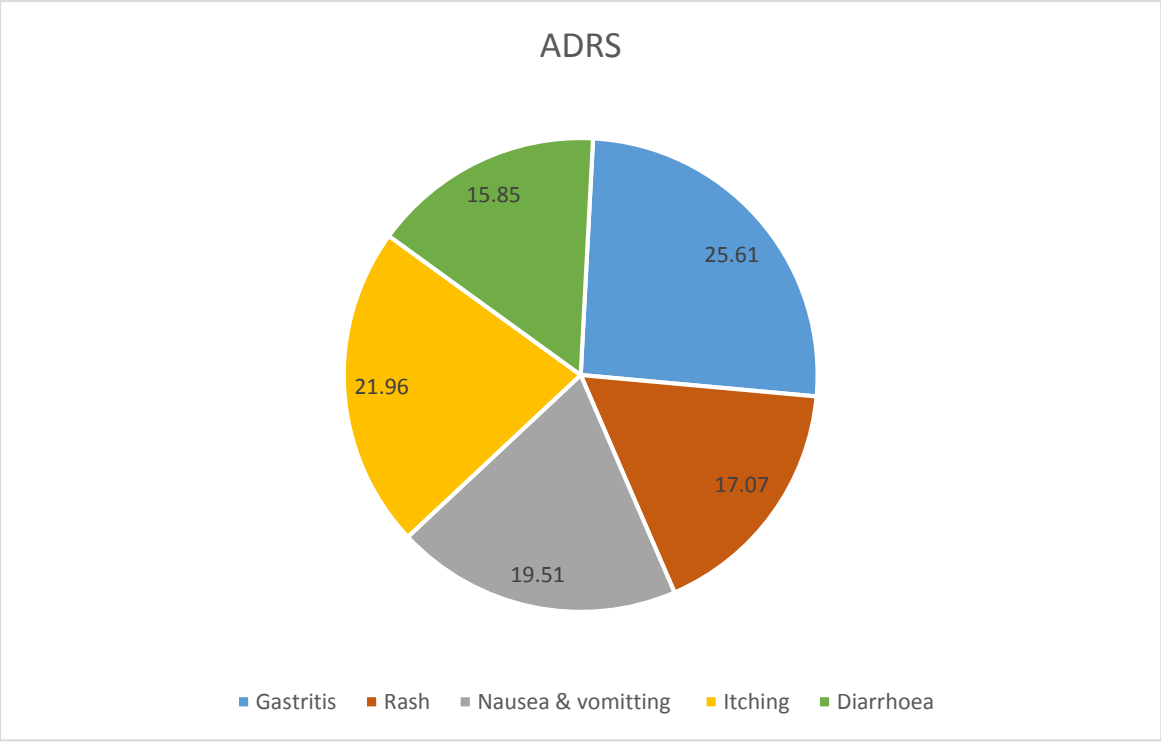

Figure 4.11: Distribution of ADRS in HIV subjects on HAART

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Maximum ADRS were seen in males (n=38)(55.88%) than in females(n=30)(44.12%) subjects. Among 68 subjects,40 subjects showed 2 types of ADR each and 28 showed 3 types of ADR each. Among total 164 ADRs, gastritis seen is 25.61%, rash is 17.07%, nausea vomiting is 19.51%, itching is 21.96% and diarrhea is 15.85%.

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Chapter 5

DISCUSSION

The different phases of HIV disease are firmly associated with quantifiable laboratory findings. HIV disease markers are by definition, quantifiable attributes that associate with advancement of clinical AIDS. There are numerous HIV markers found, however not many has demonstrated promising outcomes. HIV Markers ought to satisfy following criteria:

(i) To analyse and diagnose the patients at highest risk of disease progression.

(ii) Estimating the span and staging of HIV disease.

(iii) Early Prediction of opportunistic infections in AIDs and

(iv) Proper judgment of drug efficacy (Antiretroviral treatment).

These markers must be effectively quantifiable, clinically accessible, dependable, and ought to be affordable.

In AIDS, trademark include is a specific depletion of CD4+ T helper cells. The most significant lab finding considered is the level of CD4+ T-cell depletion, when suggestions are made regarding therapy with antiretroviral drugs or antibiotics to be given to prevent opportunistic infection. Mostly therapeutic protocols enrol patients on the basis of CD4+ T-cell count, as well as the presence of viral load.

There are numerous research going on to discover markers for HIV. Lipid peroxidation is found to be one of the biomarkers to evaluate oxidative stress status in human diseases, including HIV. Study done by Friis-Moller et al have shown that HIV-infected patients have oxidative imbalance. Similarly in our study we are attempting to

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discover parameters like total antioxidant level, nitric oxide, vitamin E, if can be utilised as markers for HIV patients. Likewise we are associating or correlating hematological parameters and CD4 count which can be used for clinical evaluation.

5.1 Age and sex distribution in HIV subjects

In the data obtained, shows a prevalence of female among 100 patients that is female established 56% in present study. Same type of results are reported by Manisha SP et.al [207]. Age distribution showing, 35.71% of female patients in the age group 31 to 40 years, (which is sexually active age group) and 34.09% of male patients in age group 18-30 years. Similar observations are referenced by Mitra & Hora, showing more female in this 31 to 40 age group [208] .

5.2 Association of hematological parameters, HIV infection and initiation of HAART.

Anaemia is the most common hematological anomaly identified in our study (mean Hb 9.8g/dl). In our study anaemia characterized as, Hb <11g/dl in females, while in male it was characterized as Hb<13g/dl. We found all patients included were iron deficient(anemic), might be expected due to low socioeconomic condition. Occurrence of anaemia in HIV disease may be due to many reason like enhanced inflammatory cytokine (TNF,IL-1,INF gamma), which diminishes erythropoietin concentration and diminishes red cell formation, deficient dietary admission, frequent opportunistic infections, hemolytic anaemia(i.e malignancies), and bone marrow suppression. Accordingly anaemia prevalence seen in other studies like, 65.5% by Dikshitet.al [71] and 61% by Kasthuri et.al [209]. This variation in anaemia might be because of difference in socio-demographic characteristics of study population. There are

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numerous studies done in developing countries which demonstrate anaemia as strong independent indicators of morbidity and mortality and advancement to AIDS [210,211].

Drug treatment like HAART prevents anaemia and may promptly bring improvement in health and increases survival rate of HIV patient [212]. In our present study prevalence of anaemia was altogether diminished to 71% subsequently after starting antiretroviral therapy (ART). Anaemia also known to occur as an adverse effect or complication of antiretroviral treatment. Myelosuppression predominantly macrocytic anaemia is the dose limiting toxicity of zidovudine. Conversely, Kiragga et.al demonstrated that patients with AZT-containing HAART likewise indicated improvement in hemoglobin level and recommended, baseline anaemia is not the rules to avoid AZT in HAART[213]. In recent study, mean hemoglobin level before treatment is 9.8g/dl and following after HAART, mean hemoglobin altogether increased to 12.51g/dl($p<0.0001$). This finding are comparative with other studies,[214,215] demonstrating that use of ART brings down the prevalence of anaemia. Estimation of hemoglobin has an important role in management of HIV in West Africa,[216] which shows its strong relationship with AIDS disease, as laboratory markers like CD4 lymphocyte and viral load which are not accessible in many developing nations. Regular estimations of hemoglobin would help to find which patients are at greatest risk of disease progression and permitting these patients to be identified for closer monitoring or therapeutic intervention.

Neutropenia in HIV patients might be aftereffect of immunosuppression, decreased formation of granulocyte colony-stimulating growth factor (G-CSF), autoimmunity or drug induced myelosuppression. There are many studies showing resolution of neutropenia ,thrombocytopenia and anaemia after 6 month of starting

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HAART along with improvement in CD4 count level[217,218]. In contrast to above studies, in our study we found significant decrease in total WBC, neutrophils and lymphocyte count ($p<0.01$) after 3 month of treatment. This may be because of advanced staging of HIV and low level of CD4 count before starting of HAART. We found in our study significant increase in platelet levels ($p<0.01$) after HAART, which goes with study of Waidearnanuel et al and Deressa et al . In contrast to our study O'Bryan et al demonstrated persistent thrombocytopenia after HAART with higher viral load [219].

Most of the studies show low MCV level, which reflects microcytic hypochromic anaemia in HIV infected individual (iron and nutritional deficiency) [220]. In present study there is significant increase in MCV level (macrocytosis) ($p<0,0001$) after HAART. Most basic reason for macrocytosis is anti-retroviral treatment (NRTIS) induced vitamin B12 and folate deficiency. Comparative similar results were seen in different studies conducted by Enawgawet et al and Owiredu et al, reporting macrocytic normochromic anaemia after ART than before starting ART [221,222]. And this increase in MCV in patients taking nucleoside reverse transcriptase inhibitors is taken as surrogate marker for adherence to ART [223]. In our study MCH and MCHC also show significant increase ($p<0.01$) in HIV individual after HAART. Similar findings were seen with study by Woldeamahuel et al [224].

5.3 Association of CD4, HIV disease and initiation of HAART.

CD4 count is analysed for evaluating the improvement and progress of the management of HIV patient on ART. Antiretroviral treatment has an important role in preventing depletion of CD4+ cell. There are studies showing, ART inhibits viral

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replication and quick increase in CD4 T cells [225,226]. In our project, mean baseline CD4 level is 340.27± 98.91mm3 and 19% subjects were with CD4 <200mm^3. Other studies in India demonstrated 89.2% cases with baseline CD4-T cell count < 200 cell/mm^3[227]. This variation might be because of choice of patients during study, who may be in advance stage during selection. There are studies showing, quantitative assessment of CD4 T cells and high association with progress of disease [228]. In our project mean CD4 T cell count before and after HAART is 340.27±98.91 and 382.72±113.62 respectively. There is highly significant increase in CD4 T cell count ($p<0.0001$) after HAART. Similar results of highly significant increase in CD4 T cell count after early initiation of ART has been demonstrated by Bajpai etal and Smith etal.[229,230]

In our recent study, highly significant and positive correlation is seen when CD4 levels when compared with haemoglobin after HAART.($r= 0.78$ & $p<0.0001$). This outcomes are comparative with different other studies that have indicated an association among low CD4 counts with anaemia [231,232].

5.4 Association of oxidative stress, HIV disease and initiation of HAART.

As seen earlier in HIV patient there is initiation of macrophages, T cells and dendritic cells bringing increased formation of cytokines, which are further responsible for bringing chronic inflammatory condition which promotes generation of reactive oxygen species (ROS) and nitrogen species(RNS). ROS and RNS respond with biological molecules like lipid, protein, carbohydrate and nucleic acid which alter cell function and further result in oxidative and nitrosative stress [233].

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Oxidative stress actuates viral replication through NF-κ B which induces the expression of gene and bring viral replication in Tcells. There is also DNA damage in infected cells results in depletion of CD4 cell (immunosuppression) [234] and depletion of antioxidant defence system resulting in susceptibility of cell to apoptosis, this cycle gets autocatalytic and encourages progression of disease. There are numerous studies showing HIV disease and its relationship with an imbalance in redox system and high oxidative stress profile described by 1)depletion of defensive framework (GSH, superoxide dismutase (SOD), catalase, vitamin C and E, Selenium, Zinc. 2)Activation of signalling molecule (cytokines,chemokines) 3) Increased formation of ROS and RNS.

Increased ROS production and consequent buffering activity by antioxidant enzymes associated with HIV disease and HAART may be responsible for depressed catalase action. There are studies showing significant higher level of erythrocyte antioxidant enzyme like SOD and catalase in HIV and AIDs patients than compared to control [127]. Contrast to this, a few studies reports, diminished activity of the antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione oxidase (GPx) in HIV individuals [235,236]. In study of Quaye O et al , SOD action was altogether diminished in ART-naive patients than those on ART control group and minor components, trace elements demonstrated a negative correlation with SOD action and a positive significant correlation with CD4+ count[237]. Study by Stephen et al watched diminished glutathione in HIV-infected subjects with CD4+ T-cell count < 200 cells/mm^3[238]. MDA a marker of lipid peroxidation is studied widely. In Rosario et al study, MDA serum level were altogether higher in HIV patients with respect to healthy individuals [239].

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In our study we have assessed NO, TAC &Vitamin E.

5.4.1 Nitric oxide (NO) as oxidative stress marker.

There are numerous studies who have utilized, GSH, SOD, catalase, vitamins (A,C,E) and minerals (selenium zinc) to appraise oxidative stress. There are not many studies done to detect NO levels (which is an indirect technique) to recognize stress and also there is controversy in the role of nitric oxide, whether the nitric oxide level are increased or decreased in HIV infected patients. Thus we chose to detect nitric oxide level before and after ART and to study its relationship with CD4 count.

Nitric oxide has been involved in numerous biological functions like support of vascular homeostasis, neurotransmission and cascade course to modulate normal function of the cell [152]. In case of chronic inflammation in HIV, nitric oxide rapidly interact and is consumed in a response with superoxide anion (free radical) yielding peroxynitrite ($ONOO^-$) and other profoundly reactive NO species, which are liable for cell injury, suggesting reduced amount of NO level in HIV infected individual. Contrast to this, decreased NO creation has likewise been accounted in people on indinavir-based antiretroviral treatment, suggesting HAART itself may bring NO reduction in these patients. This process is intervened by gp120 of HIV-1, which could diminish expression of NO synthase in some tissues [240]. In our project we discovered low mean NO level before treatment (26.40±3.65) and after treatment the mean NO level is 35.16±5.14, altogether highly significantly increased ($p<0.0001$) and positive correlation was seen with CD4 T cell count and NO level after treatment ($r=0.75$, $p< 0.0001$), suggesting there is increased immunity and reduced oxidative stress which result in less NO consumption in formation of peroxynitrite ions. Comparable finding

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as our study by Auguesta et al, higher NO level in HIV patient on HAART than HAART naïve patients [240]. Contrast to our discoveries, in study by Reneta et al, higher NO level in HIV untreated group than HIV treated one and association of increased NO level with lower CD4 cells with higher NOX level has been reported [241]. This result revealed that NOX level were increased in HIV infection and that derangement of immune system function was associated with increased NO level.

5.4.2 TAC (Total antioxidant capacity) as anti- oxidative marker.

There are numerous interaction that happen in vivo among various antioxidant substances, so there is no single antioxidant complex compound which completely reflects the defensive efficiency. Total antioxidant capacity (TAC) considers the aggregate impact of all antioxidants agents present in blood and body liquids [242]. Thus TAC (by copper reducing antioxidant power assay) is viewed as a sensitive indicator pro-oxidant activity of antioxidants.

In our project mean TAC is low (0.70±0.08) in HIV naive patient. Comparable outcomes are found in study by Teto et al, [228] which shows low TAC in HIV naïve individuals than the control and TAC diminishes with diminishing CD4 count. This might be because of the virus which utilizes antioxidant substances during its replication [243]. In our project we found elevated level of mean TAC (0.86±0.13) after HAART. There is highly significant increase in TAC level after HAART ($p<0.0001$) .This outcomes were comparable in different other studies, by Nsonwu et al and Awodele et al [244 245]. Contrast to our results, a few studies have reported lower level of oxidative markers in naive patients as compared to those on HAART [246].This may be because of perhaps due to cART's influence or host genetics. It has been

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proposed that HIV is a quicker generator of free radicals than drugs generating free radicals. When TAC was compared with CD4 count at CD4 <350 & CD4>350, it revealed, significant difference ($p<0.0001$).Thus increased level of TAC found in subjects with higher CD4 count. Also in our project, highly significant positive correlation observed in CD4 T cell count and TAC level ($r=0.82$, $p<0.0001$) after HAART Thus as the blood CD4 Tcell count of subject increases there is increase in their respective blood TAB levels. TAC levels are directly proportional to CD4 count. Thus antioxidant therapy can be useful to monitor and optimize the management of HIV infected patients. Similar results are shown by Vamseedhar & Suresh [247].

5.4.3 Vitamin E (Alpha-tocopherol) as antioxidant marker.

Vitamin E is an antioxidant which is fat soluble. It has been demonstrated that vitamin E has significant role in immune system and shows immunostimulant property. It has been proved that vitamin E supplement improves the immune response [248]. Also in spite of the fact that vitamin E is antioxidant, there are numerous clinical studies to see medical benefits of micronutrients including vitamin E in HIV individuals. Excess oxidant level may bring exhaustion of vitamin E level and may bring viral replication and progression of disease. It has been demonstrated supplementation of vitamin E diminishes progression of HIV, [249] as vitamin E decreases pro-inflammatory cytokine levels (interleukins and TNFα) through restraint of lipoxygenase path and expands prostaglandin E2 (PGE2) which reduces creation of IL-2. PGE2 additionally repress activation of natural killer (NK) cells. There are few reports showing vitamin E elevates expression of CCR5 co-receptor in HIV patients [250].

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This study meant to find the impact of HAART on vitamin and its connection with CD4 count. In our project baseline vitamin E is 3.43±0.92 and after HAART was 4.8±0.87, There is highly significant increase in vitamin E level after HAART ($p<0.001$), comparable outcomes according to our outcome indicating low level of baseline and progressive increase of vitamin E after HAART [240 251 252]. In our project, there is significant and positive correlation found between CD4 count and vitamin E($r=0.34$, $p=0.01$) after HAART. Hence as CD4 increases, there is also increase in vitamin E level. Comparable relationship seen with study of paucht et al, plasma alpha tocopherol diminishes with time during HIV infection and CD4 count also decreases [253]. It has been proved that, vitamin E supplementation has likewise been found to reduce NF-κB levels in HIV-1 affected lymphocyte cell culture and lowers oxidant production in lymphocytes, in this manner diminishing viral replication and hindering cell death [254].

5.5 Antiretroviral drugs (HAART) and ADRs.

As we are aware that there are many ART combinations approved for treatment of HIV, among these favored regimen is TLE(tenofovir (TDF), lamivudine(3TC), and efavirenz (EFV) as FDC). Other combination favored is zidovudine (AZT), lamivudine (3TC) and nivirapine (NVP).Recently new recommended regime is TLD (tenofovir (TDF), lamivudine(3TC), and dolutegravir as FDC). In our study 76% were on TLE (TDF+3TC+EFV), and 14% on Abacavir+3TC+EFV combination, 10% on AZT+3TC+EFV. There are numerous studies, who have tried to find different ADRs with various combinations. In our project different ADRs were noted. Among 100 enrolled subjects, 68 subjects indicated ADRs. Prevalence of ADR seen is more in

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males, 55.88% (n=38) than in females, 44.12% (n=30). Contras to our result, Rajesh et al in his study discovered more prevalence of ADR in female than in male. This difference in results of ADRs of male and female might be because of differences in in them, like body mass index, fat content, genetic, hormone and enzyme impact, which may acquire alteration in antiviral drug metabolism and responsible for ADRs. We have seen in our project, more frequency of ADRs to cART (68%) than appeared in the study by Rajesh et al (43.85%) [255] and Ghate et al (35.32%) [256]. This differences in the result might be due to related conditions like, opportunistic infections and other comorbid conditions for which given treatment may bring about increased ADRs. There are studies done to study ADR pattern with different cART regimen used. Maximum ADR seen in zidovudine, nevirapine and stavudine based cART[255 257]. In the study by Issac et al, indicating relationship among ADRs and virological failure with lipodystrophy, skin rash and anaemia as most common ADRs [258]. In our project, most common ADRs seen are gastritis (25.61%) and itching (21.96%).

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Chapter 6

CONCLUSIONS

In our project most affected hematological profile is anaemia. Our results have demonstrated that commencement of HAART improves hemoglobin levels and overcomes anaemia in 71% of subjects. Other hematological parameters like WBC, neutrophil and lymphocytes show noteworthy decline level after HAART. 29% of subjects didn't arrive at normal hemoglobin levels while on HAART. This implies extra intervention should be done for the subjects in whom anaemia persists even after HAART, as these individuals are in danger and risk of progression of disease and death. In this manner screening for clinically important hematological parameters preceding to initiation of HAART and also during HAART must be prioritized. Also other factors should be considered as gender based risk, nutritional status and other contributing components responsible for immune suppression. Highly significant correlation is seen with hemoglobin and CD4 count, as Hb increases there is also increase in CD4 count. Along these lines screening of hematological parameters before and during treatment is very important so as to intervene where the abnormalities would be potentially reversed. Randomized controlled trials on big population taking HAART is necessary to affirm the causal pathway between HIV burden, hematological variations and clinical outcome.

. We propose in addition to other things that, numerous HIV affected patients experience the ill effects from oxidative stress coming about because of an increase in plasma ROS, RNS and decrease in antioxidant concentration. Intracellular ROS is associated with the enactment or activation of NF-kB, a trans-activator of HIV-LTR.

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Additionally, these ROS are probably going to work in synergy with other hematological and biochemical indices like hemoglobin, CD4 count, to reflect a wide range impact at cell level which may lead to programed cell death and disease progression.

It is obvious from this study that, during the progression of HIV disease, there is corresponding increase in cell oxidative stress, portrayed by decrease in copper reducing ability of Plasma (CUPRAC), reflecting an overall diminishing in antioxidant capacity in HIV individual. CUPRAC measures predominantly detects the thiol-group antioxidant and furthermore others as, α-tocopherol, β-carotene, ascorbic acid uric, bilirubin, and albumin.[167]. TAC level was increased after HAART, reflecting reduction in oxidative stress after HAART. There is additionally an overall decline in Vitamin E, a scavenging antioxidant measure, this accounts for derangement of antioxidant system in HIV infected subject. Likewise, in our project we discovered low degrees of NO in HIV infected subjects, which reflect increase in RNS (reactive nitrogen species), as NO rapidly interact and is utilized in a reaction with superoxide anion (free radical) yielding peroxynitrite (ONOO-). Level of NO is enhanced after HAART, which reflects decline in oxidative stress. Along these lines NO can be utilized as one of the biomarker in oxidative stress in HIV patients, however must be additionally further investigated or explored with larger population size. Likewise after HAART, CD4 count compared with level of TAC, NO, vitamin E, shows positive correlation among them which indicates potentiation of immunity, reduction in oxidative stress and increased antioxidant level in HIV infected individual. We subsequently infer that HIV infection advances to AIDS, is not a single event but combination of different factors with increased ROS, a main factor. With this can

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conclude, Antiretroviral drugs(HAART), which at cell level will reduce the impact of ROS and increase in antioxidant level and thus estimation of these parameters during treatment lead to the proper management of people living with HIV/AIDS. The information acquired from the hematological, biochemical tests and oxidative parameters(TAC,NO) could be utilized as pre-intervention data for management of HIV/AID. Despite the fact that in our project it was of value in demonstrating the significant difference in different markers in HIV patients, before and after HAART, it will be imperative to follow up these markers in longitudinal cohort studies on larger population to decide their actual surrogate values, which will help to decide strategies of treatment intervention.

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