

Markers of HIV-1 Disease Progression and Treatment Response in Highly Active Antiretroviral Therapy (HAART) Era: A Review

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Abstract After the discovery of human immunodeficiency virus 1 (HIV-1) infection more than three decades ago, there has been a significant development in the laboratory diagnosis, treatment and management of patients on highly active antiretroviral therapy (HAART). Initially HIV-1 infection was implicated to cause various cancerous conditions (Kaposi's sarcoma), and infectious diseases (tuberculosis, other bacterial, viral, parasitic and fungal infections). Studies have demonstrated that HIV-1 infection and the disease course is complex and that many HIV infected patients do not progress to acquired immune deficiency syndrome (AIDS) even after 10-15 years (late/non progressors). Introduction of HAART has significantly reduced the morbidity and mortality in HIV-1 infected patients resulting in extended life on par with HIV non infected individuals. Late research has revealed that HIV-1-infected individuals are at greater risks of developing non infectious complications (liver disease, cardiovascular disease (CVD)) that may precipitate with the initiation of HAART. With the increased availability and affordability of HAART, the focus now is on developing effective strategies to monitor HIV -1 disease progression and treatment response.

Keywords: *human immunodeficiency virus (HIV), highly active antiretroviral therapy (HAART), disease progression, treatment response*

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1. Introduction

Human immunodeficiency virus (HIV), since its discovery in 1983 has been a cause of great concern to the medical community [1]. Disease diagnosis and management have been plagued by social and economic limitations. As evidenced from the available literature, Asia and African regions bear the majority of the burden of HIV infected population [2]. From the time when HIV-1 was first discovered, there have been many studies on the pathogenesis and progression of the disease. HIV-1 disease progression is complex and is different in infected population [3]. Though sexual route is the most common means of transmission, needle stick injury, contact with blood and blood products and intrauterine infections also contribute to HIV-1 infection. HIV-1 disease appears to be complex, with infected population being susceptible to various infections (bacterial, fungal, parasitic and viruses), malignancies (Kaposi's sarcoma, Burkitt's lymphoma and many others) and chronic inflammatory conditions related to HIV-1 replication in various systems of the human body. HIV infection results in the development of Acquired ImmunoDeficiency Syndrome (AIDS), due to depletion of CD4+ T cells. AIDS development after HIV-

1 infection has been noted to occur within 5 years after being infected (early progressors), later than 10 years (intermediate progressors) and some infected individuals do not develop AIDS symptoms even after 15 years (late progressors) [4]. CD4+ T cell decline < 200 cells/mm³ indicates AIDS development with corresponding rise in the HIV/RNA viral load. With the introduction of highly active antiretroviral therapy (HAART), the disease progression is slowed and the HIV infected population can live a normal life; however, one cause of concern remains the monitoring of patients on HAART therapy, who are susceptible to toxic drug reactions of HAART [5]. Since the poor and economically weak third world nations cannot afford regular CD4+ T cell counts and HIV-1/RNA viral load testing, cheaper and alternative markers of HIV disease progression are needed [6,7]. HIV-1 disease progression is monitored using various markers including viral markers (plasma HIV RNA load, serum p24 Ag, serum anti p24 antibodies), surrogate markers (antibodies against p17, gp 120, gp 41 and nef gene product) and nonspecific markers including CD4+ T-cell counts, CD8+ T-cell counts and Delayed Type Hypersensitivity test (DTH). Elevated serum β 2 microglobulin, neopterin (D-erythro-1',2',3'-trihydroxypropylprtin), Dehydroepiandrosterone (DHEAS), serum cortisol, CRP, ESR, Tumor Necrosis Factor (TNF), Interferon- γ , Interleukin-2 (IL-2) and IL-4

are also considered as alternate biomarkers [8]. Some studies have also suggested the utility of biochemical parameters including serum albumin, Globulin, Serum Glutamate Oxaloacetate Transaminase (SGOT), Total protein, Total cholesterol, High density Lipoproteins (HDL), Low density Lipoprotein (LDL), Lactate Dehydrogenase (LDH), Creatine Kinase (CK/MB) and Gamma Glutamyl transpeptidase (GGT) as useful markers of HIV-1 disease progression and treatment response [9,10].

2. HIV and Markers of Hematological Abnormalities

HIV-1 infection alone or in combination with HAART therapy, has been shown to influence the hematological parameters including blood hemoglobin (HB), total leukocyte count (TLC), erythrocyte sedimentation rate (ESR) and absolute eosinophilic count (AEC). Studies performed in the past have reported that there was a need to perform tests for hematological abnormalities in HIV infected individuals before initiating HAART therapy [8,11]. This was suggested taking into consideration the adverse effect of HAART. Anemia is well documented in HIV infected population, and initiation of HAART may worsen the condition of the patients or there may be rise in the Hemoglobin numbers following HAART indicating a better prognosis as observed in our study (Figure 1). Performing total leukocyte count (TLC) before initiation of HAART will enable the physician treating HIV-1 infected patients to use it for the prognosis of the

treatment and disease management. Studies have also demonstrated the significance of TLC as an alternate marker to CD4+ T cell counts [12,13,14,15]. Considering the fact that rise in erythrocyte sedimentation rate (ESR) indicates inflammatory condition and that HIV-1 infected patients suffer from various infections and inflammatory conditions, use of ESR in disease management and treatment response has been a subject of debate for a long time [16]. Our study which was carried out in patients attending integrated counseling and testing center (ICTC), Area hospital, Siddipet, Andhrapradesh, India and which included HIV-1 infected population as well as patients on HAART (3-6 months) has demonstrated that levels of ESR may be raised in HIV-1 infected population who are antiretroviral therapy naïve (Figure 2) and that after initiation of HAART a further rise in ESR indicates poor prognosis and may contribute to severe morbidity and mortality. A decrease in ESR may indicate better prognosis and an increase may suggest development of immune reactivation inflammatory syndrome (IRIS), a consequence that results after initiation of HAART in a group of patients resulting in worsening the condition of patients [17,18,19]. In our study we included 56 HIV-1 seropositive patients and evaluated the blood levels of ESR and HB and compared the results with HIV-1 infected population those who were HAART naïve with patients initiated on HAART (3-6 months). The results revealed a significantly positive correlation of the tested parameters before and after antiretroviral therapy as shown in Table 1 indicating their prognostic value in the management of HIV-1 infected patients on HAART.

Table 1. ESR and HB in HIV-1 seropositive HAART naïve and those on HAART

Variables	HAART –ve (Mean±SD)	HAART +ve (Mean±SD)	Correlation co-efficient (r)	Student t-test (p) value
Hemoglobin (HB) (gm%)	10.6605±1.981	10.83613±2.224	0.904*	0.237
Erythrocyte Sedimentation Rate (ESR) (mm Hg)	16.369±8.946	13.0833±7.262	0.726*	0.0089*

*Statistically significant

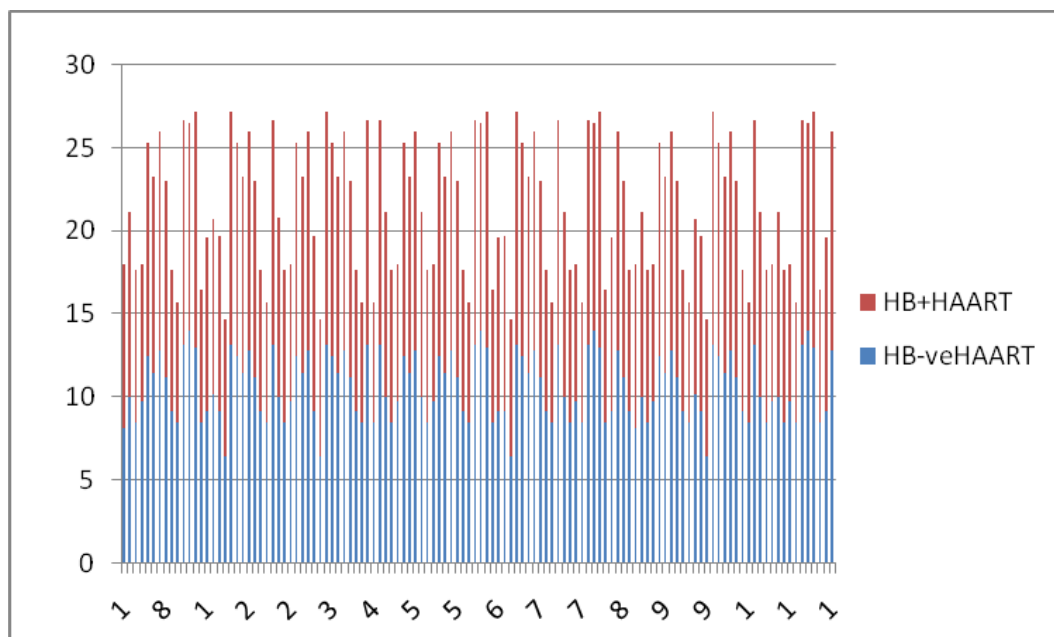


Figure 1. Graph depicting Hemoglobin (HB) levels before and after therapy

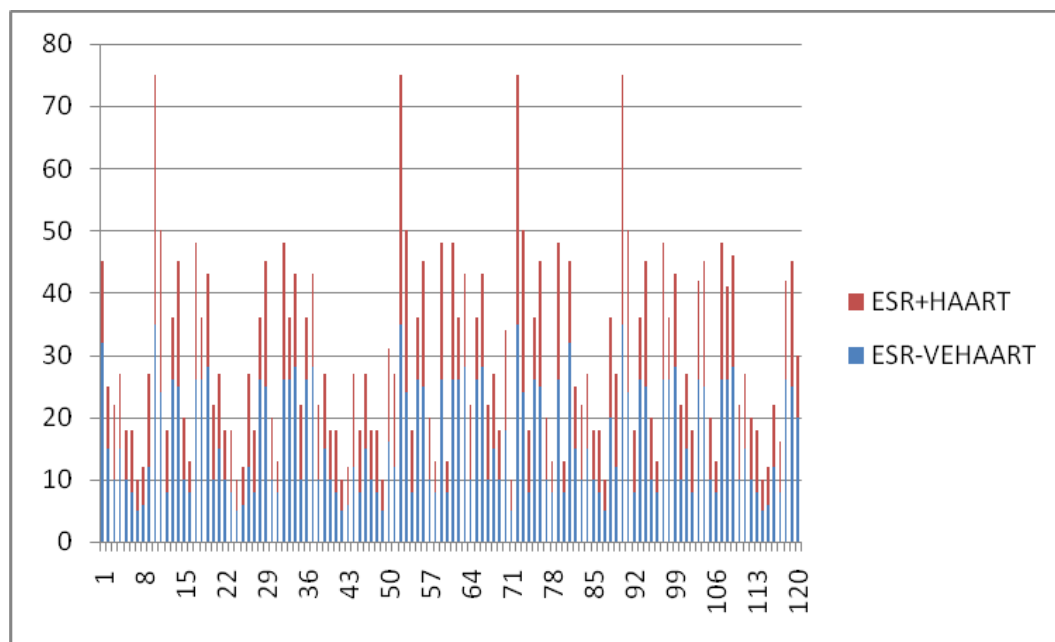


Figure 2. Graph depicting Erythrocyte Sedimentation Rates (ESR) levels before and after therapy

These results emphasize the interest of peripheral blood constituents as a complement to CD4+ T cell counts and HIV/RNA viral load in the HIV-1 disease and antiretroviral therapy management. It should be noted that measurement of CD4+ T cell counts < 200 cells/mm³ in HIV infected population do not always indicate AIDS condition as revealed from previous report documented in women; thus, alternatives to CD4+ T cell counts may help in evaluating the real condition of the HIV-1 infected patient [12,20]. It has been observed that though WHO guidelines recommend using immunological failure criteria to guide switches in antiretroviral therapy, CD4+ T cell count and clinical response alone lack sufficient sensitivity and specificity as surrogates for virological response. Management of patients on HAART requires drug substitutions to prevent serious anemia or drug (i.e., rifampicin) interactions and competent clinical monitoring is warranted [21,22].

3. HIV, HAART and Markers of Liver Injury

Human Immunodeficiency Virus-1 (HIV-1) infection and its role in the initiation of liver destruction can be attributed to apoptosis, mitochondrial dysfunction either by decreasing mitochondrial DNA in various cells or by alteration in mitochondrial membrane by HIV-1 proteins that in turn stimulate inflammatory response [23,24]. HIV-1 infection results in cytopathic effect on cells carrying CD4 receptors including helper T cells, macrophages of various organs, microglial cells, B-lymphocytes, hematopoietic stem cells, rectal mucosal cells and liver sinusoidal epithelial cells [25]. Hepatomegaly was seen as a common feature in both HIV-1 infected asymptomatic patients and AIDS cases. HAART related drug reactions, hepatotoxicity, dyslipidemia and disturbed metabolism should be considered as host factors that may determine and influence HIV-1 disease progression [26]. A recent study that we performed showed that HIV-1 infections result in liver disease and those patients on HAART must

be carefully monitored for possible hepatic destruction by measuring serum GGT, ALT and AST, all non invasive methods which are cost effective and easily performed [27, 28 29 and 30]. Existing literature suggests that in HIV-1 infected and HAART naive patients there was a positive correlation between AST (or ALT) levels and HIV/RNA viral load and that in HIV-1 infected patients without HBV or HCV infection, chronic elevated ALT levels were associated with high HIV-1-RNA levels [27]. The strong and consistent association between higher fibrosis scores at baseline and the risk of liver fibrosis in HIV-1 infected population may be clinically relevant and more proactive interventions may be required in older patients and alcohol abused patients [28]. HIV-1 infected population should also be screened for other hepatotropic viruses including Hepatitis B virus (HBV) and Hepatitis C Virus (HCV) as co-infection because these viruses may increase the liver injury/damage and thereby accelerate disease progression resulting in severe morbidity and mortality [31,32,33].

4. HIV, HAART and Cardiovascular Complications

Among the conditions that contribute to morbidity in HIV-1 infected population, non-HIV-related cardiovascular risk factors and metabolic disorders resulting from chronic inflammatory response and antiretroviral therapy (insulin resistance, lipodystrophy and hypertension) should be considered as significant. Recent studies have emphasized the significance of HIV-1 infection in coronary heart disease (CHD) and showed that the risk of CHD increases after initiation of HAART. Rise of inflammatory markers including C-reactive protein (CRP) and coagulation markers are associated with increased mortality, and possibly Cardio Vascular Disease (CVD), in HIV-1 infection. Antiretroviral therapy in HIV-1 infected population is attributed to initiate pro atherogenic effects and increased risk of Coronary artery disease (CAD). Initiation of HAART has considerably reduced the mortality in HIV infected individuals and the

patients live long enough similarly to the HIV-1 non-infected group and are at increased risk of acquiring CVD/CAD, although a recent study has noted that there was an improved endothelial function initially after starting HAART [34,35,36]. Management of dyslipidemia and other metabolic disorders in people living with HIV-1 requires awareness of the effects of antiretroviral agents on fatty acid and lipid parameters, effect of co-morbidities including age, sex, race, nutrition and related causes, and interactions between lipid-modifying agents and antiretroviral agents.

5. Significance of Alternate Biomarkers in HIV Disease Monitoring and Treatment Response

From the time when HIV epidemic was prevalent and when the number of people being detected for HIV-1 positivity was on the rise, CD4⁺ T cell count and HIV/RNA viral load were used to assess their immunological status and infectivity based on virological load. HIV-infected patients were screened for various opportunistic infections depending on the CD4⁺ T cell counts and clinical examination and prophylactic therapy were initiated where and when necessary [37]. HIV/AIDS, after introduction of HAART, has taken a different course in which people infected with HIV are considerably living longer due to reduced incidence of opportunistic infections and other AIDS-related conditions. HIV-infected individuals are bothered by noninfectious complications that need emergency medical attention and care. Even if HIV-1 disease pathogenesis is complicated, significant research has been done to show that HIV-1 has the ability to disturb the cell metabolism. Oxidative stress and programmed cell death (apoptosis) can result in accumulation of free radicals and in turn be responsible for prolonged inflammatory activity. HAART has worsened the situation by adding adverse drug reactions to already significant HIV-1 disease pathogenesis. From being a life-threatening infection, HIV-1 has now emerged as a chronic infection that can influence most of the human systems. Cardiovascular complications, gastrointestinal infections, hepatic emergencies, pulmonary infections (tuberculosis, cryptococcosis), psychiatric ailments, hematological malignancies/abnormalities, renal and other oncological complications have been on the rise among HIV-infected patients more so after initiation of HAART therapy. Physicians treating HIV-1 seropositive patients should think beyond opportunistic infections and consider other factors including the nutrition, the toxic effects of HAART, the nutrition, age, and other demographic causes. Risk of IRIS after initiation of HAART, which can exacerbate underlying infectious or inflammatory condition, should be considered as a cause of serious concern [38,39,40,41,42].

6. Conclusions and Future Perspectives

From the available literature, it is evident that the HIV-1 disease pathogenesis is complex and that the HIV-1

infection, disease progression, treatment and management of HIV-1 infected population given HAART therapy should be considered based on all factors including the infectious and non-infectious complications. Physicians treating HIV infected patients should consider specific assessment for co-morbidities (physiological, immunological, infectious diseases, lifestyle, alcoholism and smoking, drug use) and other related factors that could influence disease progression. Large scale HIV screening programmes, economically viable strategies and analysis studies of antiretroviral therapy and treatment response, and genotypic resistance testing would be effective in future for making decisions on policies to be initiated for HIV-1 disease management. HIV primary care should include wide range of prevention and treatment programmes including vaccination/prophylactic treatment for Hepatitis A, B, parasitic infestation/infection/fungal infections and others. Currently in India more than 3 lakh HIV seropositive individuals have access to HAART and the number of HIV-1 infected patients put on HAART is on the rise each day through antiretroviral therapy (ART) centers located in peripheral regions [37]. Success in the HIV disease management can only be achieved with improved laboratory diagnosis and effective management strategies of patients on HAART therapy.

7. Statistical Methods

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean \pm SD (Min-Max). Pearson's correlation co-efficient test is performed to investigate the difference between the sample groups. Analysis of variance (ANOVA) has been used to find the significance of study parameters between different groups of patients. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups. Inter group analysis. The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

References

- [1] Barre-Sinoussi F., Chermann J.C., Rey F., Nugeyre M.T., Chamaret S., Gruest J., Dautet C., Axler-Blin C., Vézinet-Brun F., Rouzioux C., Rozenbaum W., Montagnier L., Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS), *Science*, 1983; 220: 868-871.
- [2] UNAIDS, 2006. AIDS epidemic update. Joint United Nations Programme on HIV/AIDS (UNAIDS), Geneva. http://2006_Epiupdate_en.pdf
- [3] K V Ramana. Are we close enough to get rid of AIDS: Insights in to impact of Human Immunodeficiency virus (HIV) infection post Highly Active Antiretroviral therapy (HAART) Era. *International Journal of Molecular Medical Science*, 2013; 3(5): 25-29.
- [4] Ramana K V. HIV Disease Management in the Highly Active Antiretroviral Therapy (HAART) Era. *J Medical Microbiol Diagnosis* 2012; 1:e101.
- [5] Friis-Møller N., Reiss P., Sabin C.A., Weber R., Monforte A.d., El-Sadr W., Thiebaut R., de Wit S., Kirk O., Fontas E., Law M.G., Phillips A., Lundgren J.D., DAD Study Group., Class of antiretroviral drugs and the risk of myocardial infarction, *N. Engl J. Med.*, 2007; 356:1723-1735.

- [6] Miuro G, Nakubulwa S, Watera C, Munderi P, Floyd S, Grosskurth H. Evaluation of affordable screening markers to detect CD4+ T cell counts below 200 cells/ μ l among HIV-1-infected Ugandan adults. *Trop Med Int Health*. 2010; 15(4):396-404.
- [7] Azzoni L, Foulkes AS, Liu Y, Li X, Johnson M, Smith C, et al. Prioritizing CD4 Count Monitoring in Response to ART in Resource Constrained Settings: A Retrospective Application of Prediction-Based Classification. *PLoS Med* 2012; 9(4): e1001207
- [8] Ramana K V, Jagadeeswhwara chary, Sabitha V, S K Mohanty, Ratna Rao. Role of Hematological and Alternate Markers in Human Immunodeficiency Virus Disease Progression. *American Medical Journal* 1 (2): 84-87, 2010.
- [9] Ramana KV, Ratna Rao, Sabitha, Venugopal B, Rafi MD, et al. Biochemical Parameters in Human Immunodeficiency Virus Disease Progression. *J Medical Microbiol Diagnosis* 2012; 1:103.
- [10] Kandi Venkataramana. A Study of Biological Markers in HIV Disease Progression and Management in the Highly Active Antiretroviral Therapy (HAART) Era, *American Journal of Bioscience and Bioengineering*. 2013; 1(2): 24-37.
- [11] Cohen AJ, Steigbigel RT. Eosinophilia in Patients Infected with Human Immunodeficiency Virus. *J Infect Dis*. 1996; 174: 615-18.
- [12] K V Ramana, V Sabitha, Ratna Rao. A Study of Alternate Biomarkers in HIV Disease and Evaluating their Efficacy in Predicting T CD4+ cell counts and Disease Progression in resource poor settings in Highly Active Antiretroviral Therapy (HAART) Era. *Journal of Clinical and Diagnostic Research* [serial online] 2013 July [cited: 2013 Jul 5]; 7:1332-1335.
- [13] Sourav Sen., Akshat Vyas, Sunil Sanghi, K Shanmuganandan, RM Gupta, Ketoki Kapila, et al. Correlation of CD4+ T cell Count with Total Lymphocyte Count, Haemoglobin and Erythrocyte Sedimentation Rate Levels in Human Immunodeficiency Virus Type-1 Disease. *MJAFL*. 2011; 67: 15-20.
- [14] Kapiga SH, Mwakagile D, Spiegelman D, Msamanga GI, Hunter D, Fawzi WW. Predictors of CD4+ lymphocyte count among HIV-seropositive and HIV-seronegative pregnant women in Dar es Salaam, Tanzania. *East Afr Med J*. 2000 Apr; 77(4):206-11.
- [15] de Jong MA, Wisaksana R, Meijerink H, Indrati A, van de Ven AJ, Alisjahbana B et al. Total lymphocyte count is a reliable surrogate marker for CD4 cell counts after the first year of antiretroviral therapy: data from an Indonesian cohort study. *Trop Med Int Health*. 2012; 17(5):581-3.
- [16] Lowe DM. The Erythrocyte sedimentation rate in HIV: a neglected Parameter? *AIDS*. 2010; 24(18):2773-5.
- [17] Paul Collini, Uli Schwab, Stephen Sarfo, Joseph Obeng-Baah, Betty Norman, David Chadwick, David Bibby, and George Bedu-Addo. Sustained Immunological Responses to Highly Active Antiretroviral Therapy at 36 Months in a Ghanaian HIV Cohort. *Clin Infect Dis* 2009; 48:988-91.
- [18] Crum-Cianflone NF. Immune reconstitution inflammatory syndromes: What's new? *AIDS Read* 2006;16:199-206.
- [19] Boulware DR, Meya DB, Bergemann TL, Wiesner DL, Rhein J, Musubire A, et al. Clinical Features and Serum Biomarkers in HIV Immune Reconstitution Inflammatory Syndrome after Cryptococcal Meningitis: A Prospective Cohort Study. *PLoS Med* 2010; 7(12): e1000384.
- [20] Kimmel AD, Weinstein MC, Anglaret X, Goldie SJ, Losina E, Yazdanpanah Y, et al. Laboratory monitoring to guide switching antiretroviral therapy in resource-limited settings: Clinical benefits and cost-effectiveness. *J Acquir Immune Defic Syndr* 2010; 54:258-68.
- [21] Owiredun WK, Quaye L, Amidu N, Addai-Mensah O. Prevalence of anaemia and immunological markers among Ghanaian HAART-naïve HIV-patients and those on HAART. *Afr Health Sci*. 2011;11(1):215.
- [22] Baveewo S, Ssali F, Karamagi C, Kalyango JN, Hahn JA, Ekoru K, et al. Validation of World Health Organisation HIV/AIDS Clinical Staging in Predicting Initiation of Antiretroviral Therapy and Clinical Predictors of Low CD4 Cell Count in Uganda. *PLoS one* 2011; 6(5): e19089
- [23] Casula M, Bosboom-Dobbelaer I, Smolders K, Otto S, Bakker M, et al. Infection with HIV-1 induces a decrease in mt-DNA. *J Infect Dis* 2005; 191: 1468-1471.
- [24] Jacotot E, Ravagnan L, Loeffler M, Ferri KF, Vieira HL, et al. The HIV- 1 viral protein R induces apoptosis via a direct effect on the mitochondrial permeability transition pore. *J Exp Med* 2000; 191: 33-46.
- [25] Stebbing J, Gazzard B, Douek DC Mechanisms of disease - Where does HIV live? *N Engl J Med* 2004; 350: 1872-1880.
- [26] Cooper CL HIV antiretroviral medications and hepatotoxicity. *Curr Opin HIV AIDS* 2007; 2: 466-473.
- [27] Jason T. Blackard, Jeffrey A. Welge, Lynn E. Taylor, Kenneth H. Mayer, Robert S. Klein, David D. Celentano, Denise J. Jamieson, Lytt Gardner, and Kenneth E. Sherman. HIV Mono-infection Is Associated With FIB-4 – A Noninvasive Index of Liver Fibrosis in women. *Clin Infect Dis* 2011; 52 (5): 674-680.
- [28] Monia Mendeni, Emanuele Foca` , Daria Gotti, Nicoletta Ladisa, Gioacchino Angarano, Laura Albini, Filippo Castelnuevo, Giampiero Carosi, Eugenia Quiros-Roldan, and Carlo Torti. Evaluation of Liver Fibrosis: Concordance Analysis between Noninvasive Scores (APRI and FIB-4) Evolution and Predictors in a Cohort of HIV-Infected Patients without Hepatitis C and B Infection. *Clinical Infectious Diseases* 2011; 52(9):1164-117.
- [29] Ramana KV, Ratna Rao, Sabitha Abnormal Levels of γ -Glutamyl Transpeptidase (GGTP), ALT, AST in Human Immunodeficiency Virus-1(HIV-1) Infection. *Biochem Physiol* 2012; 1:101.
- [30] Stonehouse W, Kruger A, Smuts CM, Loots du T, Wentzel-Viljoen E Plasma polyunsaturated fatty acids and liver enzymes in HIV-infected subjects: the Prospective Urban and Rural Epidemiology (PURE) Study. *Am J Clin Nutr* 2010; 91: 729-735.
- [31] K V Ramana et al, Seroprevalence of blood-borne viral infections in post HAART era at a tertiary care hospital in south India: A five year trend analysis (2008-2012) and a comprehensive review. *British Journal of Medicine and Medical Research* 2013; 3 (4) : 1929-1937.
- [32] Mendeni M, Foca` E, Gotti D, Ladisa N, Angarano G Evaluation of Liver Fibrosis: Concordance Analysis between Noninvasive Scores (APRI and FIB-4) Evolution and Predictors in a Cohort of HIV-Infected Patients without Hepatitis C and B Infection. *Clinical Infectious Diseases* 2011; 52: 1164-1173.
- [33] Jain MK . Mortality in Patients Coinfected with Hepatitis B Virus and HIV: Could Antiretroviral Therapy Make a Difference? *Clin Infect Dis* 2009; 48: 1772-1774.
- [34] Dr Aberg Management of Dyslipidemia and Other Cardiovascular Risk Factors in HIV-Infected Patients: Case-based Review. *Top HIV Med*. 2006;14(4):134-139.
- [35] Dr stein. Evaluating and Managing Cardiovascular Disease Risk Factors in HIV-Infected Patients. *Top HIV Med*. 2010; 18(5):164-168.
- [36] Dr Post. Predicting and Preventing Cardiovascular Disease in HIV-Infected Patients. *Top Antivir Med*. 2011;19(5):169-173.
- [37] K. V. Ramana and S. K. Mohanty Opportunistic intestinal parasites and TCD4⁺ cell counts in human immunodeficiency virus seropositive patients. *J Med Microbiol* 2009; 58: 1664-1666.
- [38] Vajpayee M, Mohan T. Current practices in laboratory monitoring of HIV infection. *Indian J Med Res* 2011; 134(6): 801-22
- [39] K V Ramana and Ratna rao. Human Immunodeficiency Virus disease management in Highly Active Anti-Retroviral Therapy era: a comprehensive review *Ann of Trop Med Public Health* 2013; 6(1): 5-9.
- [40] Ramana K V, Rao R, Kandi S, Singh PA, Kumar VP. Elevated activities of serum lactate dehydrogenase in human immunodeficiency virus sero-positive patients in highly active antiretroviral therapy era. *J Dr NTR Univ Health Sci* 2013;2:162-6.
- [41] Valencia ME, Laguna F, Camacho J, Castejón A, Soriano V, Adrados M, et al. Serum activity of the lactate dehydrogenase enzyme in patients with human immunodeficiency virus infection. *An Med Interna* 1994; 11:580-3.
- [42] Venkat A, Piontkowsky DM, Cooney RR, Srivastava AK, Soares GA, Heidelberger CP. Care of the HIV-positive patient in the emergency department in the era of highly active antiretroviral therapy. *Ann Emerg Med* 2008; 52:274-85.