

Biomarkers in Immune Reconstitution Inflammatory Syndrome (IRIS) among People Living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS)

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Abstract

Background: An Immune Reconstitution Inflammatory Syndrome (IRIS) event is a presentation or a paradoxical worsening of a pre-existing infection following initiation of anti-retroviral therapy in the presence of a decreasing viral load and features consistent with an inflammatory process. This study was conducted to find out the significance of different clinical parameters like hemoglobin, albumin, viral load, erythrocyte, body mass index in the people living with HIV/AIDS with IRIS in Nepalese population.

Methods: The study was descriptive with control group. The study included patients who experienced IRIS after initiation of highly active antiretroviral therapy (HAART) with control group as patients who were HIV positive without HAART treatment. This study was carried out on 44 HIV infected individual who initiated HAART and then suffered from IRIS and compared with 56 control HIV infected person without IRIS visiting National Public Health Laboratory (NPHL) for routine HIV viral load testing and CD4 count between April and August, 2014.

Results: The patients were categorized into highly active antiretroviral therapy (HAART) naïve (n=56) and on HAART with immune reconstitution inflammatory syndrome (IRIS) (n=44). Among 56 individuals naïve HAART, viral load <1000 copies/ml was found in 44 individuals, among which 14 were female (34 ± 1.953 years) and 28 were male (39.40 ± 1.290 years) whereas 12 individual had viral RNA >1000 copies/ml among which 02 were female (34.83 ± 2.030 years) and 10 were male (41.34 ± 1.462 years). The comparison of CD4 count between the naïve and patients enrolled for HAART; the risk of having CD4 count <200 cell/mm³ is significantly greater in male than that of female. The BMI ratio of HAART to naïve patients (19.88 ± 0.7290) was lower than that of HAART enrolled patients (21.78 ± 0.3546). The hemoglobin value showed significant (P value < 0.0001) difference among PLHIV having CD4 level less than 200 (9.9 ± 2.156), between 200-500 (11.63 ± 1.946) and more than 500 CD4 level (12.71 ± 1.850). Significant (P < 0.0001) viral load suppression showed among HAART initiated female patients with IRIS when compared with naïve female patients without IRIS. BMI, hemoglobin level, total leukocyte count, albumin level, HDL level, ESR value, CRP level and absolute eosinophil level less than 351 cells/mm³ showed significant (P < 0.05) difference among HAART naïve and on HAART female patients with IRIS. Significant (P < 0.0001) viral load suppression showed among HAART initiated male patients with IRIS when compared with naïve female patients without IRIS. BMI (18-26), hemoglobin level below 8 g/dl, TLC, serum albumin level below 5 g/dl, HDL level below 61 mg/dl, ESR level, CRP value and absolute eosinophil count showed significant (P < 0.05) difference between HAART naïve and on HAART male patients with IRIS. Hemoglobin level, HDL, TLC, ESR, CD8, AEC, viral load, BMI and serum albumin level showed significant (P < 0.0001) difference among HAART initiated patients with IRIS when compared to different level of CD4 T cell count.

Conclusion: Prevalence of anemia was high in HAART naïve patients while leucopenia prevalence was higher in patients on HAART and their prevalence increased as the CD4 count decreased. HIV Patients should be investigated for hematological and immunological changes following with appropriate therapeutic interventions. The study findings reemphasize the importance of nutritional and immunological parameters to assess the stage of the disease, initiate antiretroviral therapy and monitor the response in disease progression.

Keywords: Biomarkers; IRIS; HIV/AIDS

Abbreviations: HIV: Human Immunodeficiency Virus; AIDS: Acquired Immuno Deficiency Syndrome; ART: Anti-retroviral Therapy; CD4: Cluster of Differentiation 4; CD8+: Cluster Differentiation 8; FACS: Fluorescent Activated Cell Sorting; HAART: Highly Active Anti-Retroviral Therapy; HIV: Human Immunodeficiency Virus; IRIS: Immune Reconstitution Inflammatory Syndrome; OIs: Opportunistic Infections; PLHIV: People Living with HIV/AIDS (PLHIV); RNA: Ribonucleic Acid

Introduction

In 2015 there were 2.1 million (1.8 million-2.4 million) new HIV infections worldwide, adding up to a total of 36.7 million (34.0 million-39.8 million) people living with HIV (Global AIDS update, UNAIDS 2016). AIDS is a chronic disease that causes the progressive de-

struction of the immune system, thus leading to recurring opportunistic infections, changes in body composition, adverse nutritional impact,

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Received August 06, 2017; **Accepted** September 12, 2017; **Published** September 19, 2017

Citation: Mishra SK, Khadka S, Dhital S, Mahto RK, Manandhar KD (2017) Biomarkers in Immune Reconstitution Inflammatory Syndrome (IRIS) among People Living with Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS). J AIDS Clin Res 8: 728. doi: [10.4172/2155-6113.1000728](https://doi.org/10.4172/2155-6113.1000728)

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evaluative debilitation and even death [1]. The correlation between the CD4 T cell count and viral load is fluctuating in presence or absence of HAART [2,3]. Incorporating immunological and biochemical markers into routine nutritional assessment provides an often-needed objective dimension [4].

Various authors have reported that the biological markers including nutritional state are a strong predictive factor for survival and functional conditions during the course of HIV infection [5-7]. The WHO classifies a BMI <18.5 as a sign of malnutrition. However, BMI can be insensitive to changes in lean muscle mass (LMM), which is a more accurate marker of recent change in nutritional status. Anemia is a common hematological complication associated with HIV infection and has been implicated as a risk factor for mortality during HIV infection [8]. Approximately 20-80% of the people living with HIV/AIDS suffer from anemia which is directly associated with faster progression of disease [7]. Therefore, prevention of anemia may lead to improved health and survival potential of people living with HIV/AIDS [8]. As HIV/AIDS progresses, the severity and prevalence of anemia also increases [9]. However, leucopenia in patients with HIV/AIDS more common [10]. Since HIV infects CD4+ lymphocytes, it can directly result in lymphopenia [11]. Serum albumin, which is not routinely measured in developing countries and is not available in most of the laboratory at ART center which is the most common laboratory parameter for nutritional assessment with low serum albumin indicating malnutrition [5,6,9,10,12]. Lipid profile abnormalities have been reported in the early stages of HIV infection and become more evident with the disease's progression [13,14]. There is large number of cytokines increase and serum triglyceride, HDL-cholesterol decrease during HIV infection [15]. It is already reported that Interferon-alpha known to be elevated in HIV/AIDS individuals and is positively correlated with plasma triglyceride concentrations [16,17].

An IRIS event was defined as either a first presentation or a paradoxical worsening of a pre-existing infection following initiation of HAART in the presence of a decreasing viral load and features consistent with an inflammatory process. In patients with a previous history of, for example, herpes simplex virus infection, recurrent disease was defined as an IRIS event only if there was documented evidence of a significant increased frequency, severity and/or poor treatment response in the 6 months after initiation of HAART. The objective of this study is to determine anthropometric measurements, immunological markers and nutritional indicators among HIV/AIDS Patients enrolled in HAART and HAART naïve in HIV referral Centre of National public Health Laboratory, Nepal.

Materials and Methods

Study population and definitions

Patients (n=100) visiting National Public health laboratory, Teku, Kathmandu were enrolled in this study. A descriptive study with control group was carried out at National Public Health laboratory (NPHL), the apex laboratory of government of Nepal and Sukraraj Tropical Hospital Infectious Disease Hospital (STIDH) is the only Infectious & Tropical Disease Hospital in Nepal and the biggest ART center. The criteria for the early prediction of people which may suffer IRIS were ratio of CD4/CD8 has been used for prediction of immune reconstitution inflammatory syndrome (IRIS) in future course of treatment. The development of IRIS in HIV-infected patients initiating antiretroviral therapy (ART) results from restored immunity to specific infectious or non-infectious antigens. A paradoxical clinical worsening of a known condition or the appearance of a new condition after initiating

therapy characterizes the syndrome. Some researchers proposed the major criteria like atypical presentation of "opportunistic infections (OI) or tumors" in patients responding to antiretroviral therapy and decrease in plasma HIV RNA level by at least 1 log₁₀ copies/mL and as minor criteria like increased blood CD4+ T-cell count after highly active antiretroviral therapy (HAART), increase in immune response specific to the relevant pathogen and spontaneous resolution of disease without specific antimicrobial therapy or tumor chemotherapy with continuation of antiretroviral therapy.

HIV/AIDS patients were categorized into HAART naïve (n=56, 56%) and with IRIS enrolled (n=44, 44%) patients. Among 56 naïve patients 34 were male and 22 were female whereas in HAART enrolled 44 (44 %), 28 were male and 16 were female. The followings were the inclusion criteria: age between 15 and 60 years and confirmed diagnosis of HIV. Patients who required intensive care during hospitalization were excluded. All patients were initiated on various HAART regimens by strictly following the national guideline. The common first-line regimens used were zidovudine (AZT) plus lamivudine (3TC) plus nevirapine (NVP); followed by stavudine (d4T) plus lamivudine (3TC) plus nevirapine (NVP); zidovudine plus 3TC plus efavirenz (EFV); and d4T plus 3TC plus EFV. The CD4 cell count, viral load, hemoglobin, albumin, high density lipid profile, total leukocyte count, erythrocyte sedimentation rate, presence/absence of C-reactive protein and absolute eosinophil count were performed using standard protocol. The following were the reference values for findings considered normal: HDLc ≥ 40 to <61 mg/dl, ESR ≥ 10 to <21 mm/h, albumin higher than 3.5 g/dl in both sexes; hemoglobin equal to or higher than 12 g/dl and for men; and hemoglobin equal to or higher than 12 g/dl for women. For stratification of anemia, a hemoglobin concentration lower than 8 g/dl was considered severely reduced, a hemoglobin concentration between 8 and 12 g/dl was considered moderately reduced and a hemoglobin concentration equal to or higher than 12 g/dl was considered normal. The CD4 cell count was interpreted based on the recommendations of the Ministry of Health and population, Government of Nepal which uses less than 350 cells/mm³ as the cutoff point (lower than 350 cells/mm³ is one of the criteria used to define AIDS in patients with HIV, together with nutritional status and the presence of opportunistic diseases). Based on the BMI, the patients were classified as either underweight (BMI <18.5 kg/m²) or adequate weight (BMI ≥ 18.5 kg/m²). Data collection, entry and validation, organization and monitoring approaches was used to generate data, double entry, auto correction and validation system was applied during data entry. A backup of every data was maintained. Moreover, results especially for lab experiments such as source data, errors observed and corrective action taken and comments on each activity was maintained. All the study data was entered into a computer database using standard format, checked for errors and verified. The variables were represented by pertinent descriptive statistics: absolute (n) and relative (%) frequency or mean \pm SEM. Student's t-test for independent variables was used to compare means among groups of interest. The level of significance was set to 0.05 ($\alpha=5\%$). The statistical analysis was performed using SPSS 17.0 software.

ELISA

ELISA test for the detection of antibodies to HIV-1 and HIV-2 in human plasma were used. The Bio ELISA HIV-1+2 (rec) (BIOKIT, Barcelona, Spain) is a third generation solid phase enzyme immunoassay in which highly purified recombinant antigens gp41, gp120 and gp36 are used for the combined detection of antibodies to HIV-1, HIV-2 and HIV-1 subtype O. The total number of wells needed for the assay was determined. In addition to test samples, 1 well for the

substrate blank, 3 wells for negative control, 2 wells for HIV-1 positive control and 1 well for HIV-2 positive control was allocated. The 100 µl of sample diluent in each well except blank well, 50 µl of each sample in the designated well, 50 µl of negative control, 50 µl of HIV-1 positive control and 50 µl of HIV-2 positive control was dispensed. The plates along with the adhesive seals were incubated at 37°C for 60 min. 100 French l of detector antibody conjugates with horse reddish peroxidase (HRP) was added and incubated for 30 min at 37°C. After that, 100 µL trimethylbenzidine (TMB) substrate was added and incubated for 15 min at dark. The reaction was stopped by addition of 1 N H₂SO₄ and OD was taken at 450 nm in ELISA plate reader (Bio-Rad). The cutoff value was determined by adding 0.120 to the mean absorbance of the negative control.

RNA extraction and HIV viral load

RNA was isolated from 140 µl of serum sample using Nucleospin viral RNA isolation kit (MACHEREY-NAGEL, Germany) according to the manufacturer's instruction. For the viral RNA amplification and cDNA preparation Artus HI Virus-1 QS-RGQ Kit (QIAGEN, GmbH, Germany) was used and primers were supplied by manufacturer as complete master mix which was used to isolate HIV-1 genome fragment of 93 bp which was later subjected for direct detection in fluorescence channel. Additionally, internal control was used as supplied in kit to identify possible PCR inhibition as detected by fluorescence channel orange in Rotor gene Q. External control was used to determine the amount of viral RNA.

CD4+ T-lymphocytes assay

Proportion of CD4+ T cells (Helper T cells) and CD8+ T cells (Inducer T cells) was calculated using immune-phenotyping by incubating anti-coagulated whole blood with monoclonal antibodies. The antibodies are conjugated to fluorescent tags that emit light of a certain frequency when excited by a laser beam. The specimens were analyzed on a flow cytometer Becton Dickinson (BD) FACS Count system (Becton, Dickinson and Company, California, USA) to determine the proportion of cells of a particular phenotype (that emit light at the right wavelength). The True Count method (Tri TEST 3-color) (Becton, Dickinson and Company, California, USA) uses True Count tubes which contain a lyophilized pellet containing a known quantity of fluorescent beads. A precise quantity of whole blood was added to the tubes, and the lymphocytes are stained with Tri TEST monoclonal antibodies (mAb) as instructed by manufacturer. The absolute count of a full lymphocyte subset profile (CD3+, CD4+ and CD45+) were determined in four tubes with Tri TEST and two tubes with Multi TEST by calculating the ratio of region events for each subset to bead events using the Multi SET (Becton, Dickinson and Company, California, USA).

Results

The patients were categorized into highly active antiretroviral therapy (HAART) naïve (n=56) and on HAART with immune reconstitution inflammatory syndrome (IRIS) (n=44). Among 56 individuals naïve HAART, viral load <1000 copies/ml was found in 44 individuals, among which 14 were female (34 ± 1.953 years) and 28 were male (39.40 ± 1.290 years) whereas 12 individual had viral RNA >1000 copies/ml among which 02 were female (34.83 ± 2.030 years) and 10 were male (41.34 ± 1.462 years). The comparison of CD4 count between the naïve and patients enrolled for HAART; the risk of having CD4 count <200 cell/mm³ is significantly greater in male than that of female. The BMI ratio of HAART to naïve patients (19.88 ± 0.7290) was lower than that

of HAART enrolled patients (21.78 ± 0.3546). The hemoglobin value showed significant (P value<0.0001) difference among PLHIV having CD4 level less than 200 (9.9 ± 2.156), between 200-500 (11.63 ± 1.946) and more than 500 CD4 level (12.71 ± 1.850). The other hematological parameters estimated included were absolute eosinophil count (AEC) (391.8 ± 180.1), total leukocyte count (TLC) (8595.8 ± 2146.9) and erythrocyte sedimentation rate (ESR) (75.625 ± 29). Significant positive correlation was observed between CD4+T-cell counts and AEC, TLC and hemoglobin level. ESR and c reactive protein (CRP) was found to be negatively correlating with CD4+T-cell counts.

Significant (P<0.0001) viral load suppression showed among HAART initiated female patients with IRIS when compared with naïve female patients without IRIS. BMI, hemoglobin level, total leukocyte count, albumin level, HDL level, ESR value, CRP level and absolute eosinophil level less than 351 cells/mm³ showed significant (P<0.05) difference among HAART naïve and on HAART female patients with IRIS as shown in Table 1.

Parameters	Female		Total	P Value
	HIV Patients			
	HAART naïve	On HAART		
Viral Load copies/ml				
<1000	3	18	21	P<0.0001
>1000	16	1	17	P<0.0001
CD4				
<200	1	3	4	P=0.1859
≥ 200-<500	3	23	26	P<0.0001
≥ 500	2	7	9	P=0.0220
BMI				
<18	2	6	8	P=0.0528
≥ 18-<26	4	22	26	P<0.0001
≥ 26	0	3	3	P=0.0253
Hb g/dl				
<8	7	0	7	P=0.0253
≥ 8-<13	1	8	9	P=0.0017
≥ 13	5	19	24	P<0.0001
TLC mm³/ml				
<4000	7	35	42	P<0.0001
4000-11000	0	0	0	
>11000	0	0	0	
Albumin				
<3.5	2	13	15	P=0.0001
≥ 3.5-<5.0	4	15	19	P=0.0004
≥ 5.0	0	5	5	P=0.0253
HDL mg/dl				
<40	7	23	30	P<0.0001
≥ 40-<61	0	9	9	P=0.0253
≥ 61	0	3	3	P=0.0253
ESR mm/1 h				
<10	0	2	2	P=0.0253
≥ 10-<21	1	5	6	P=0.0286
≥ 21	5	29	34	P<0.0001
CRP				
Positive	1	12	13	P<0.0001
Negative	6	19	25	P=0.0003
AEC cells/mm³				
<50	0	4	4	P=0.0253
≥ 50-<351	6	29	35	P<0.0001
≥ 351	1	2	3	P=0.4606

Table 1: Clinical parameters in HAART naïve and HAART used female patients with IRIS.

Significant ($P < 0.0001$) viral load suppression showed among HAART initiated male patients with IRIS when compared with naïve female patients without IRIS. BMI (18-26), hemoglobin level below 8 g/dl, TLC, serum albumin level below 5 g/dl, HDL level below 61 mg/dl, ESR level, CRP value and absolute eosinophil count showed significant ($P < 0.05$) difference between HAART naïve and on HAART male patients with IRIS as shown in Table 2.

Hemoglobin level, HDL, TLC, ESR, CD8, AEC, viral load, BMI and serum albumin level showed significant ($P < 0.0001$) difference among HAART initiated patients with IRIS when compared to different level of CD4 T cell count as shown in Table 3.

Demographics and epidemiological assessment

Among 100 HIV/AIDS patients which were further categorized into HAART naïve ($n=56$, 56%) and with IRIS enrolled ($n=44$, 44%) patients. Among 56 naïve patients 34 were male and 22 were female whereas in

Parameters	Male		Total	P value
	HIV Patients			
	HAART naïve	On HAART		
Viral Load copies/ml				
<1000	8	35	43	$P < 0.0001$
>1000	17	2	19	$P < 0.0001$
CD4				
<200	3	13	16	$P = 0.0006$
≥ 200 to <500	6	23	29	$P < 0.0001$
≥ 500	5	11	16	$P = 0.0344$
BMI				
<18	2	4	6	$P = 0.2595$
≥ 18 to <26	11	45	56	$P < 0.0001$
≥ 26	0	1	1	$P = 0.0253$
Hb g/dl				
<8	10	0	10	$P = 0.0253$
≥ 8 to <13	5	4	9	$P = 0.6801$
≥ 13	3	37	40	$P < 0.0001$
TLC mm³/ml				
<4000	12	44	56	$P < 0.0001$
4000-11000	1	1	2	$P = 1.0000$
>11000	0	0	0	
Albumin				
<3.5	6	19	25	$P = 0.0003$
≥ 3.5 to <5.0	7	21	28	$P = 0.0002$
≥ 5.0	1	4	5	$P = 0.0719$
HDL mg/dl				
<40	17	21	38	$P = 0.3865$
≥ 40 to <61	1	15	16	$P < 0.0001$
≥ 61	0	4	4	$P = 0.0253$
ESR mm/1 h				
<10	2	11	13	$P < 0.0001$
≥ 10 to <21	4	9	13	$P = 0.0574$
≥ 21	7	25	32	$P < 0.0001$
CRP				
Positive	3	14	17	$P < 0.0001$
Negative	10	35	45	$P < 0.0001$
AEC cells/mm³				
<50	2	10	12	$P < 0.0001$
≥ 50 to <351	6	34	40	$P < 0.0001$
≥ 351	5	1	6	$P < 0.0001$

Table 2: Clinical parameters in HAART naïve and HAART used male patients with IRIS.

Parameters	CD4 Count			P value
	<200	≥ 200 to <500	≥ 500	
Hb	9.9 ± 2.156	11.63 ± 1.946	12.71 ± 1.850	<0.0001
HDL	27.25 ± 9.142	40.93 ± 19.08	34.59 ± 15.33	0.003
TLC	57.28 ± 1.383	172.28 ± 1.42	717.1 ± 3.38	<0.0001
ESR	23.11 ± 3.12	34.42 ± 2.12	43.23 ± 4.22	<0.0001
CD8	232 ± 1.23	348 ± 2.12	573 ± 1.11	<0.0001
AEC	221.22 ± 1.21	123.13 ± 3.11	345.21 ± 2.12	<0.0001
Viral Copy No.	4910 ± 1.31	1223 ± 1.11	400 ± 1.12	<0.0001
BMI	15.2 ± 1.12	12.12 ± 1.12	17.13 ± 1.42	<0.0001
Albumin	3.12 ± 1.2	4.121 ± 1.22	5.131 ± 1.12	<0.0001

*values in mean ± SEM

Table 3: Co-relation between CD4 level and clinical parameters among HAART patients with IRIS.

HAART enrolled 44 (44 %), 28 were male and 16 were female. The BMI ratio of HAART naïve patients (19.88 ± 0.7290) was lower than that of HAART enrolled patients (21.78 ± 0.3546). However, HAART naïve patients (35.60 ± 1.746) were younger compared to HAART enrolled patients (38.02 ± 0.8827). Female naïve patients (34.33 ± 3.703) were comparatively younger compared to male naïve patients (36.14 ± 2.008) same as HAART enrolled patients [female (34.45 ± 1.362) and male (40.53 ± 1.020)] (Table 4).

CD4 counts and viral load

The population was divided into 3 groups according to the CD4 counts: CD4 ≥ 500 cell/mm³ (stage 1), CD ≥ 200-<500 cell/mm³ (stage 2), CD<200 cell/mm³ (stage 3). Compared between naïve and HAART enrolled patients the probability of CD4 count, the risk of having CD4 count <200 cell/mm³ is significantly greater in male than that of female in naïve to HAART enrolled patients. Also, the chances of having CD4 counts between ≥ 200 to <500 is greater than 3 times in naïve compared to HAART enrolled males, however its greater than 7 times in naïve versus HAART enrolled female (%). Among individuals naïve HAART, viral load <1000 copies/ml was found in 49 individuals, among which 14 were female (34 ± 1.953 years) and 28 were male (39.40 ± 1.290 years) whereas 51 individual had viral RNA>1000 copies/ml among which 22 were female (34.83 ± 2.030 years) and 29 were male (41.34 ± 1.462 years). The CD4 count and clinical parameters for diagnosis was not significant besides BMI, Hb, TLC, ESR, CD8, AEC and Albumin. The tendency of increment above critical value was observed in above mentioned parameters whenever CD4 count was increasing (Table 3).

Clinical parameters for disease progression

The hemoglobin value showed significant (P value<0.0001) difference among PLHIV having CD4 level less than 200 (9.9 ± 2.156), between 200-500 (11.63 ± 1.946) and more than 500 CD4 level (12.71 ± 1.850). There was no case for macrocytic and normocytic but a single case of microcytic among total patients. High density lipoprotein <40 mg/dl was observed in significant (P value<0.0001) population in male ($n=21$) and female ($n=23$). HAART with IRIS enrolled patients compared to their naïve counterparts. Total leukocyte count was less than 4000 cells/mm³ in 98% of patients among which 56 were male and 42 were female while 2% patients had total leukocyte count of more than 4000 cells/mm³.

Discussion

The prevalence of anemia in patients with AIDS is estimated to be between 63 and 95% [18,19]. Thus, the rate of 35% in the present study is in agreement with findings reported in the literature. There is possibility of multiple cause of anemia in the people living with

Parameters	Male HIV Patients		Female HIV Patients	
	HAART naïve	On HAART	HAART naïve	On HAART
Age				
<25	0	0	0	2
≥ 25 to <50	13	41	6	35
≥ 51	1	3	0	0
Viral Load copies/ml				
<1000	8	35	3	18
>1000	17	2	16	1
CD4				
<200	3	13	1	3
≥ 200 to <500	6	23	3	23
≥ 500	5	11	2	7
BMI				
<18	2	4	2	6
≥ 18 to <26	11	45	4	22
≥ 26	0	1	0	3
Hb g/dl				
<8	10	0	7	0
≥ 8 to <13	5	4	1	8
≥ 13	3	37	5	19
TLC mm³/ml				
<4000	12	44	7	35
4000-11000	1	1	0	0
>11000	0	0	0	0
Albumin				
<3.5	6	19	2	13
≥ 3.5 to <5.0	7	21	4	15
≥ 5.0	1	4	0	5
HDL mg/dl				
<40	17	21	7	23
≥ 40 to <61	1	15	0	9
≥ 61	0	4	0	3
ESR mm/1 h				
<10	2	11	0	2
≥ 10 to <21	4	9	1	5
≥ 21	7	25	5	29
CRP				
+ve	3	14	1	12
-ve	10	35	6	19
AEC cells/mm³				
<50	2	10	0	4
≥ 50 to <351	6	34	6	29
≥ 351	5	1	1	2
ART Regimes				
Nucleoside Reverse Transcriptase Inhibitor	0	39	0	30
Non-Nucleoside Reverse Transcriptase Inhibitor	0	39	0	30
HIV Protease Inhibitors	0	39	0	30
HIV Fusion Inhibitors	0	0	0	0

1. Nucleoside Reverse Transcriptase Inhibitor: Zidovudine (AZT), Didanosine (DDI), Zalcitabine (DDC), Emtricitabine (FTC), Lamivudine (3TC), Tenofovir (TDF), Stavudine (d4T), Abacavir (ABC)

2.Non-Nucleoside Reverse Transcriptase Inhibitor: Nevirapine (NVP), Delaviridine (DLV), Efavirenz (EFV)

3. HIV Protease Inhibitors: Tipranavir (TPV), Amprenavir (APV), Indinavir (IDV), Saquinavir (SQV), Ritonavir (RTV), Atazanavir (ATV), Fosamprenavir (FPV), Nelfinavir (NFV)

Table 4: Clinical parameters in HAART naïve patients and HAART used patients with IRIS.

HIV/AIDS, majority of them have major bone marrow dysfunction and co-infected with other microorganism including virus [20-22]. The other cause of reduction in hemoglobin concentration is the shortage of micronutrients like deficiency of iron, vitamin B12 and B9 required for haematopoiesis [19]. Although, patients with HIV/AIDS are nutritionally compromised and have anemia, it does not seem to depend on the recent intake of iron [23]. This study shows there is an association of low albumin levels among HIV patients [14]. There is positive correlation of serum albumin level of with CD4 count (Table 1). Another, study showed that the acute phase has crucial function for the host's reaction to infection [24]. It has been reported that HIV-infection promotes an enhancement of the fractional and absolute synthesis rates of positive acute-phase proteins, including haptoglobin [25]. Previously reported data on acute-phase proteins in HIV infection are conflicting. Many report on higher plasma concentrations of C reactive protein in a group of HIV/AIDS patients compared to the plasma concentrations of healthy controls [26] which is also shown in this study. Hypertriglyceridemia has been reported as the first lipid alteration in HIV infection and we also found decrement in HDL-cholesterol as reported in the HIV-infection. HDL-cholesterol has been considered as a marker of disease progression however HDL-cholesterol was lower in the patient on ART than naïve patient, indicating a risk of heart disease [27,28]. Hematological parameters are often crucial for disease progression monitoring [11] but due to gold standard HAART and its predefined criteria for CD4 count, these clinical parameters often go undiagnosed. Even in resource limited setting like Nepal, where the CD4 counting infrastructure is limited, these parameters become crucial for diagnosis of disease progression. Despite of the immense utility of these biomarkers, the definitive pattern are not clear. In our study, we find out significant correlation between increments on the relative value defining normal range of BMI, Hb, TLC, ESR, CD8, AEC and Albumin which were on increment along with CD4 counts which was confirmed by another studies as well [10,4,12]. Our work also shed light on the age difference of male and female acquiring infection and provided evidence that in fact female are more prone to HIV infection than male at younger age. The HAART enrolled patients had significantly lower viral copy number in their serum compared to that of naïve but was not consistent with the relapsed number of CD4 count which may provide the case of immunological failure [13]. This study elicits the need of more study related to immunological parameters thus providing precise diagnosis and surveillance of HIV patients.

The correlation between parameters like HDL and viral load was not significant in some HAART enrolled cases, which points out the limitation of the study regarding sample size, maybe better sampling and enlarged cohort may sort out this problem. The work regarding clinical parameters which could be fruitful for developing nation like Nepal, limits crucially regarding routine CD4 and viral load, thus baselines for routine diagnostic taking immunological and physiological parameters are today's need.

Conclusion

Anemia, leucopenia, hypoalbuminemia and lipid disorders were common clinical events among the people living with HIV/AIDS. Prevalence of anemia was high in HAART naïve patients while leucopenia prevalence was higher in patients on HAART with IRIS. Anemia and leucopenia was found to be directly associated with decrease CD4 count.

Prevalence of anemia was high in HAART naïve patients while leucopenia prevalence was higher in patients on HAART and their prevalence increased as the CD4 count decreased. HIV Patients should be

investigated for hematological and immunological changes following with appropriate therapeutic interventions. The study findings reemphasize the importance of nutritional and immunological parameters to assess the stage of the disease, initiate antiretroviral therapy and monitor the response in disease progression. After completion of this study, we suggest the people living with HIV/AIDs should check their CD4 counts regularly and to start HAART when it is appropriate in order to decrease the prevalence of anemia. The CD4+ counts, HIV viral load, nutritional and immunological parameter should be checked in regular basis which help the people to monitor the outcome of disease.

Authors' Contributions

SKM, KDM and RKM were responsible for study design, supervision of work and guidance. SKM, SK and SD were contributed to laboratory work and data analysis. SKM, SK and SD were contributed to writing and manuscript preparation.

All authors read and approved the final manuscript.

Acknowledgement

I would like to acknowledge the all staffs National Public Health Laboratory, HIV Reference Unit, Nepal.

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