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ASSESSMENT OF HIGH-RISK GROUP FOR IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME (IRIS) DEVELOPMENT AMONG PEOPLE LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS/ ACQUIRED IMMUNODEFICIENCY SYNDROME (HIV/AIDS) IN NEPAL

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ABSTRACT

Background: Immune Reconstitution Inflammatory Syndrome (IRIS) a clinical condition which is a side effect of Anti-Retroviral Therapy (ART) due to rapid recovery of Immune system leading to adverse effects. So far, Predicting IRIS have not been difficult but recently some research showed it can be narrowed down to the high-risk group on the basis of cluster differentiation (CD4+ & CD8+) level and of CD4+ /CD8+ ratio. **Methods:** The study was carried out at National Public Health laboratory, the apex laboratory of Government of Nepal; and Sukraraj Tropical Infectious Disease Hospital (STIDH). Includes 1060 HIV infected people and Cluster Differentiation (CD) profile was enumerated by Fluorescent Activated Cell Sorting (FACS) caliber machine. All the data were collected systematically and arranged in a tabular form and statistically analyzed using Graph Pad Prism ver7.0 to check the characteristics of population along with other statistical tests. **Results:** The study was carried out with 1006 HIV infected patients consisting of 60.7% male and 39.2% female. CD4+/CD8+ ratio was taken as major predictor and tested, CD4+/CD8+ ratio value was divided into four group to simplify analysis (>1.5, 0.3-1.5, 0.15-0.3 and <0.15). 75 percentiles of people had CD4+/CD8+ ratio value of below 0.5 indicating poor immune status. Out of 1006 HIV patients included in this study only 44 showed IRIS within six months of starting ART and all of them had CD4+/CD8+ ratio of below 0.15 (considered as high-risk group in this study). Also, chi-square test showed positive association between IRIS cases and CD4+/CD8+ ratio along with sex and Age. All the IRIS confirmed cases lied in the high-risk group and were indicated by CD4+/CD8+ ratio value before starting ART. **Conclusions:** More than 75 percentiles of people living with HIV/AIDS (PLHIV) showed abnormal CD4+/CD8+ratio of below 0.5. The incidence of IRIS among ART initiator PLHIV was identified as High-Risk group using CD4+/CD8+ ratio more accurately in Nepalese people. Hence, CD4+/CD8+ ratio measurement will help narrow down high-risk group of IRIS cases among PLHIV initiating ART. Treatment of IRIS will remain a clinical challenge due to the variety of clinical presentations and the presence of multiple pathogens capable of causing the syndrome. Patients at greatest risk for the development of serious IRIS events, a low CD4+/CD8+ ratio of <0.15, should be screened to exclude an active or subclinical infection with important opportunistic.

KEYWORDS: IRIS, CD4+, CD8+, HIV, Nepal, PLHIV

INTRODUCTION

HIV Immune Reconstitution Inflammation Syndrome (IRIS) is the clinical condition that occurs in response to Highly Active Anti-Retroviral Therapy (HAART), where patients suffer from pathological inflammation reaction mostly due to reactivation of immune system after subsequent HAART treatment.[1] IRIS is mostly characterized by the clinical degeneration of a condition or emergence of new condition after initiation of the HAART.[2] IRIS has been found to be a heterogeneous condition that depends on the associating pathogens, but two distinct patterns of the disease have been described namely, "Paradoxical IRIS" and "Unmasking IRIS".[3] Paradoxical IRIS is a condition in which known opportunistic infection (OI) pronounces as reoccurrence or worsens after HAART albeit treatment is underway and Unmasking IRIS is a condition where new OI prevails itself with distinct inflammation after HAART.[3]

IRIS is mostly associated with other opportunistic infections so it is difficult to describe it based on clinical manifestation due to inconsistency of associated diseases.[3],[4] Depending on the occurrence, it is not easy

to determine from symptoms alone whether it is Paradoxical or Unmasking IRIS but is rather distinguish Unmasking IRIS by atypical exuberant inflammation.[4] The many IRIS associated diseases are TB IRIS, Herpes IRIS, Cryptococcal IRIS, Hepatitis IRIS.[3],[4] etc. Most common OI associated with IRIS are *Mycobacterium*, varicella zoster, Cryptococcal infection, cytomegalo virus infection and *Pneumocystis*.[4] In all of OI that are associated with IRIS however, don't have any distinct pathophysiological distinction from each other, mostly had low baseline CD4+ cell count along with high viral load and abnormal CD4+/CD8+ ratio.[3]–[8]

Significant prediction of IRIS includes, younger age, lower baseline CD4+ cell percentage, lower baseline CD4+ to CD8+ ratio and lower CD4+ count.[9], [10] In a large retrospective analysis, 25% (33/132) of patients suffered from one or more diseases after initiation of HAART expressing good number of cases of IRIS.[10] In HIV patients, CD4+/CD8+ ratio is taken as a tool that measures the immune health system since healthy people without HIV normally have greater CD4+ cells to that of CD8+lying the ratio normally between 1 and 4 (i.e, >1).[11], [12] CD4+/CD8+ratio decreases with age and viral disease while it is opposite in patient with auto immune diseases.[13] The inverted ratio of CD4+/CD8+ occurs as CD4+ count decreases by as much as 30% whereas CD8+ count increases by as much as 40% that occurs during seroconversion period not longer than six month.[13] The major factors that have detrimental role are CD4+ count along with CD4+/CD8+ratio in viral infections, corticosteroid use, seasonal/diurnal variations etc.[13] Though CD4+/CD8+ ratio used to be considered as only self-determining predictor for immune reconstitution inflammatory syndrome (IRIS), now a days the ratio with combination of low baseline CD4+ count and a low CD4+/CD8+ratio are defined as convincing factors as cited by a study, where people with a CD4+/CD8+ratio of less than 0.15 showed more chance of having IRIS than those with ratio greater than 0.30.[13] In this study, we have analyzed CD8+ count, and total CD3+ count in addition to CD4+ count and CD4+/CD8+ ratio. However, CD4+/CD8+ ratio is taken as a major marker and is validated by comparing it between confirmed IRIS cases and also against various variables using statistical tools as a probable predictor and as a marker for the IRIS.

METHODS

A descriptive study was carried out at National Public Health laboratory (NPHL), the apex laboratory of Government of Nepal, and Sukraraj Tropical Infectious Disease Hospital (STIDH), the only Infectious & Tropical Disease Hospital in Nepal and the biggest ART center.

Study population: Patients visited to NPHL and STIDH with HIV infection and having antiretroviral treatment were selected who were attending the centers between January 2013 to November 2013. There were total 1006 subjects enrolled for the study comprising 395 males and 611 females. Further, people ranging in age from 18 months to as old as 72-year-old were included in the study.

HIV diagnosis and CD4+, CD8+ cell count: HIV was diagnosed using National algorithm adopted by NPHL, Government of Nepal's serial test protocol which consisted of three rapid test kits (Abbott Determine™ followed by Uni-Gold™ and in case of discordant result by STAT-PAK®) method.

Whole blood collected from the subjects were traced for CD4, CD8 and CD3 by making panel of monoclonal antibodies conjugated to fluorochromes, fluorescein isothiocyanate (FITC)/CD8, phycoerythrin (PE)/CD8, peridinin chlorophyll protein (PerCP)/CD4. Samples were prepared for the FACS by mixing 20µL of BD Tritest CD4/CD8/CD3 reagent and about 50µL of whole blood using reverse pipetting technique followed by addition of 450µL of 1X BD FACS lysing solution and incubation for 15 minutes in the dark at room temperature. The stained cells thus prepared were acquired in flow cytometry (BD FACS Calibur) and was then analyzed in by cell quest pro software.

Data Collection and analysis: The ethical aspect was taken care by keeping anonymity of the individual and taken approval from Nepal Health Research Council ethical committee. All the information was collected after critical review and approval by the practicing clinician. Collected information included age, sex, and HIV viral load which was collected as a regular checkup during follow-ups. CD4+, CD8+ and CD3+ cell counts were obtained using BD FACSCalibur™ before starting HAART. Also, patients under study were constantly monitored for any signs of IRIS during their subsequent regular visit for 6 months.

The obtained data were first categorically stored in excel followed by the statistical analysis using Graph Pad Prism ver 7.0 to check population characteristics, co-relation analysis and chi-square tests to find the association between various parameters and CD4+/CD+8 ratio to IRIS.

RESULT

Population Under study

The study was carried out with 1006 HIV infected patients consisting of 60.7% male and 39.2% female. The population under study showed normal distribution with co-efficient of skewness -0.5167 in case of its age distribution, and 7.156, 1.668, 14.77 and 2.876 for CD4+, CD8+, CD3 and CD4+/CD8+ratio respectively. The mean for CD4+ count was found to be 393.8 ± 359.3 with SE of 11.33, CD8+ count was 1046 ± 615 with SE of 19.39, CD3 count was 1516 ± 1440 with SE of 45.4 and CD4+/CD8+ ratio was 0.4214 ± 0.3523 with SE of 0.01111 (Table no.1). Taking the baseline value under consideration, about 90 percentiles had CD4+ count

below baseline, 10 percentiles had CD8+ count below baseline as high CD8+ count is bad immune status indicator and 90 percentiles of population had CD4+/CD8+ratio below baseline i.e, 0.8 (Table no.2).

Table no. 1: Non-Parametric t-test of CD4+ count, CD8+ count, Total CD3 count and CD4+/CD8+ ratio performed against baseline value of Nepalese population.

Titles	Observation value	Base line Value*	P-value	Significant (alpha=0.05)
CD4+ count (t=34.62 df=1005)	393.8±359.3	786±248	<0.0001	Yes
CD8+ count (t=15.29 df=1005)	1046±615	567±230	<0.0001	Yes
Total CD3 count (t=26.35 df=1005)	1516±1440	2712±836	<0.0001	Yes
CD4+/CD8+ ratio (t=97.11 df=1005)	0.4214±0.3523	1.52±0.59	<0.0001	Yes

*Base line value was taken in reference to shakya et al 2012.

In order to find out the discrepancy between baseline value found out by Shakya et. al, 2012, Non-Parametric t-test was performed and found to be significant in almost all the parameter that were checked with P-value of <0.0001 (Table no.1).

Table 2: Distribution of different parameter for the population under study.

Distribution	CD4+	CD8+	CD4+/CD8+
Minimum	0.19	92	0.0001902
10% Percentile	21	489	0.04323
25% Percentile	181.8	629	0.1939
Median	329	922	0.3389
75% Percentile	525	1282	0.538
90% Percentile	213.5	1812	0.1366
Maximum	6984	4628	3.202

Immune Reconstitution Inflammatory syndrome

Among 1006 people living with HIV included in this study, 44 of them reported to have shown IRIS after 6 weeks of starting highly active antiretroviral therapy (HAART). Among them, 22 of the patients showed clinical symptoms of Genital Herpes, 10 showed Cryptococcal infection, 4 showed Hepatitis B infection, 4 showed Molluscum contagiosum (Figure 1) and 4 showed Vericella zoster infection (Table no.3). All the reported IRIS cases had CD4+/CD8+ ratio of below 0.15 indicating they had poor immune status before starting of HAART. Patients of all age group were found to have shown IRIS as lowest age showing IRIS was 1.5-year, 27-year age lied in 25 percentiles, 33-year was the median value and 47-year age lied in 75 percentiles indicating almost all age group showed IRIS (Additional data table no.2). Also, most of them were male as 29 were male and only 15 of them were female in this study.

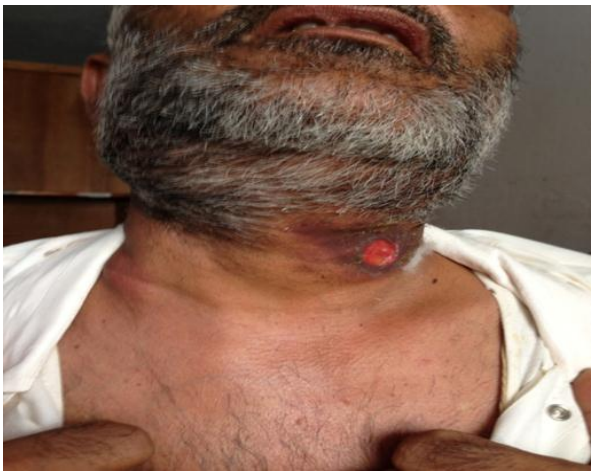

Figure 1: Patient under HAART with Molluscum contagiosum associated IRIS.

Table no. 3: CD4+/CD8+ ratio Before starting HAART and IRIS Confirmed patients under the study with symptoms.

Symptoms	No. of patients	Average Age	Sex	Average CD4+/CD8+ Ratio
Genital Herpes	22	32 yr	14 M/8 F	0.067273
Cryptococcal infection	10	32 yr	5 M/5 F	0.108
Hepatitis B infection	4	21 yr	3 M/1 F	0.1075
Molluscum contagiosum	4	27 yr	3 M/1 F	0.05
Vericella zoster	4	31 yr	4 M/0 F	0.0825

Further, IRIS confirmed cases were compared against those cases that weren't diagnosed with IRIS but was enrolled in the study using t-test. Result of the comparison showed significant difference between CD4+ value and CD4+/CD8+ ratio value with P-value of <0.0001. (Table no.4).

Table no. 4: Comparison of various parameters of IRIS confirmed against Non-IRIS patients.

	IRIS Cases	*Non-IRIS cases*	*Mean Diff.*	*Significant?*	*Adjusted P Value*
CD4+ (95%CI;- 188.1 to 401.1)	99.43±96.2	394.1±359.4	294.6 ± 54.28	Yes	<0.0001
CD8+ (95%CI;- -169.9 to 201.6)	1031±606.7	1047±615	15.85 ± 94.67	No	0.8671
Total CD 3 avg. (95%CI;- -298.9 to 72.46)	1160±598.7	1516±1441	-113.2 ± 94.63	No	0.2318
CD4+/CD8+(95%CI;- 0.2146 to 0.4238)	0.1023±0.125	0.4214±0.3525	0.3192 ± 0.0533	Yes	<0.0001

Co-relation of CD4+/CD8+ ratio to IRIS, Age and Sex in Nepalese population living with HIV

CD4+/CD8+ratio was divided into different groups as below 0.15 as high-risk group, between 0.15 to 0.3 as risk group, 0.3 to 1.5 as low-risk group and above 1.5 was taken as normal i.e. not in risk of having IRIS and co-relation analysis was performed with age, sex and IRIS confirmed and IRIS non-confirmed cases and found to be significant at confidence level of 95% and p-Value of <0.0001 except for age group dependent chi-square test which had significant p-value of 0.0126 (Table no.5).

Table no. 5: Co-relation of CD4+/CD8+ ratio with Sex, age group and IRIS as analyzed by Chi-Square test.

Co-relation	Chi-square, df	P value
Sex vs CD4+/CD8+ Ratio	34.16, 3	<0.0001
Different Age Group vs CD4+/CD8+ Ratio	16.22, 6	0.0126
IRIS confirmed and Non-IRIS vs CD4+/CD8+ Ratio	232.8, 3	<0.0001

Age and Sex in immune status of HIV patients

People of different age group were enrolled in the study, mainly it was divided into three age group of below 15-year-old, 15 to 35-year-old and above 35-year-old. Statistical characteristics of age group specific population is presented in Table no.6. In addition to these, co-relation analysis (one tailed) of Age to CD4+, CD8+, CD3 and CD4+/CD8+ratio showed R square value of 0.09369, 0.04536, 0.01326, 0.0119 with P value of <0.0001, <0.0001, 0.0001, 0.0003 which is significant with confidence level of 95% (Additional data table no.8). Sidak's multiple comparison test performed to compare various parameters (CD4+, CD8+ and CD3 count) between male and female population showed insignificant difference (Additional data Table no.9).

Table no. 6: Statistical parameters of different patients belonging to different age group.

Age Group	total no.	Parameters	CD4+	CD8+	Total CD 3 avg.	CD4+/CD8+
<15	83	Minimum	20	400	354	0.02492
		10% Percentile	176.6	798.4	1120	0.1256
		25% Percentile	335	1200	1698	0.2529
		Median	607	1388	2236	0.3874
		75% Percentile	914	1885	2687	0.6201
		90% Percentile	1243	3000	3460	0.8051
		Maximum	6984	4628	3500	1.549
		P-value	<0.0001	<0.0001	0.5334	<0.0001
15-35	575	Minimum	0.19	108	13	0.0001902
		10% Percentile	99	428.6	666.2	0.1331
		25% Percentile	190	621	991	0.21
		Median	344	891	1326	0.363
		75% Percentile	538	1231	1801	0.5842
		90% Percentile	745.4	1635	2273	0.8553
		Maximum	1525	3000	19925	3.202
		P-value	<0.0001	<0.0001	<0.0001	<0.0001
>35	348	Minimum	14	92	99	0.02235
		10% Percentile	61.7	414.9	574.8	0.09172
		25% Percentile	146.3	616.5	875.5	0.1692
		Median	282	854.5	1266	0.3008
		75% Percentile	430.8	1248	1690	0.4505
		90% Percentile	611.2	1703	2227	0.6188
		Maximum	1477	3000	34494	2.492
		P-value	<0.0001	<0.0001	<0.0001	<0.0001

Additional Data

Table no. 1: Statistical characteristics of all the parameters for the population under study.

Parameters	Minimum	25% Percentile	Median	75% Percentile	Maximum	Mean	Std. Deviation	Std. Error of Mean	Lower 95% CI of mean	Upper 95% CI of mean	Statistics
Age	1.5	27	33	40	72	32.2	10.86	0.3423	31.53	32.87	<0.001
CD4+	0.19	181.8	329	525	6984	393.8	359.3	11.33	371.6	416.1	<0.001
CD8+	92	629	922	1282	4628	1046	615	19.39	1008	1084	<0.001
Total CD 3 avg.	13	965	1337	1849	34494	1516	1440	45.4	1426	1605	<0.001
CD4+/CD8+	0.0001902	0.1939	0.3389	0.538	3.202	0.4214	0.3523	0.01111	0.3996	0.4432	<0.001

Table no. 2: Statistical detail of IRIS confirmed patients

	Number of values	Minimum	25% Percentile	Median	75% Percentile	Maximum	10% Percentile	90% Percentile	Mean	Std. Deviation	Std. Error of Mean	Lower 95% CI of mean	Upper 95% CI of mean	Statistics
Age	44	6	28	33	39.75	44	10.5	41.5	31.05	10.32	1.556	27.91	34.18	0.0127
CD4+	44	16	43.5	74.5	126	570	21	213.5	99.43	96.2	14.5	70.18	128.7	<0.0001
CD8+	44	150	623.3	926	1247	3000	489	1812	1031	606.7	91.46	846.4	1215	<0.0001
Total CD 3 avg.	44	167	717.5	1068	1479	3072	566	2078	1160	598.7	90.26	977.9	1342	0.0087
CD4+/CD8+	44	0.02235	0.05734	0.08175	0.1166	0.8837	0.04323	0.1366	0.1023	0.125	0.01884	0.06427	0.1403	<0.0001

Table no. 3: Pathophysiological parameters of IRIS confirmed patients.

S.No.	Sex	Age	CD4+	CD8+	Total CD 3 avg.	CD4+/CD8+	clinical symptoms
1	F	24	78	1472	1616	0.052989	Genital Herpes
2	F	38	67	997	1092	0.067202	Genital Herpes
3	M	33	98	934	1085	0.104925	Genital Herpes
4	F	8	194	1548	1851	0.125323	Genital Herpes
5	M	39	81	799	892	0.101377	Genital Herpes
6	F	40	76	1070	1152	0.071028	Genital Herpes
7	F	36	227	1921	2224	0.118168	Cryptococcal infection
8	M	31	153	1068	1213	0.143258	Cryptococcal infection
9	M	34	93	682	800	0.136364	Cryptococcal infection
10	F	40	200	3000	2250	0.066667	Cryptococcal infection
11	M	6	20	408	501	0.04902	Molluscum contagiosum
12	M	28	43	580	717	0.074138	Cryptococcal infection
13	M	33	85	633	738	0.134281	Vericella zoster
14	M	38	73	586	678	0.124573	Cryptococcal infection
15	M	41	70	834	895	0.083933	Vericella zoster
16	F	28	272	3000	3072	0.090667	Cryptococcal infection
17	M	20	99	1094	1243	0.090494	Vericella zoster
18	M	11	231	1687	2019	0.136929	Hepatitis B infection
19	F	28	136	1297	1554	0.104857	Hepatitis B infection
20	M	32	89	869	1031	0.102417	Vericella zoster
21	M	28	120	1876	2133	0.063966	Molluscum contagiosum
22	M	35	128	1044	1241	0.122605	Hepatitis B infection
23	M	10	45	620	719	0.072581	Hepatitis B infection
24	F	28	109	1623	1815	0.06716	Cryptococcal infection
25	M	20	21	670	720	0.031343	Genital Herpes
26	M	36	173	1747	2022	0.099027	Cryptococcal infection

27	M	30	70	1173	1272	0.059676	Genital Herpes
28	M	40	34	1073	1179	0.031687	Molluscum contagiosum
29	M	31	99	1106	1208	0.089512	Genital Herpes
30	M	44	46	360	436	0.127778	Genital Herpes
31	F	35	53	937	1050	0.056564	Molluscum contagiosum
32	F	40	51	641	743	0.079563	Genital Herpes
33	F	19	24	640	706	0.0375	Genital Herpes
34	M	7	55	1049	1427	0.052431	Genital Herpes
35	M	37	38	589	631	0.064516	Genital Herpes
36	F	30	50	590	687	0.084746	Cryptococcal infection
37	F	35	570	645	1310	0.883721	Genital Herpes
38	M	44	31	570	665	0.054386	Genital Herpes
39	M	43	45	918	1047	0.04902	Genital Herpes
40	M	41	16	716	761	0.022346	Genital Herpes
41	M	40	28	572	653	0.048951	Genital Herpes
42	M	42	21	299	326	0.070234	Genital Herpes
43	F	33	142	1271	1496	0.111723	Genital Herpes
44	M	30	21	150	167	0.14	Genital Herpes

Table no. 4: Contingency table for Age Groups Vs CD4+/CD8+ ratios

Data analyzed	CD4+/CD8+ Ratio less than 0.15	CD4+/CD8+ Ratio 0.15-0.3	CD4+/CD8+ Ratio 0.3-1.5	CD4+/CD8+ Ratio greater than 1.5	Total
Age (0-15)	10	17	51	2	80
Age (15-35)	60	123	266	12	461
Age (above 35)	95	133	231	6	465
Total	165	273	548	20	1006

Table no. 5: Contingency table for Sex Vs CD4+/CD8+ ratios.

Data analyzed	CD4+/CD8+ Ratio less than 0.15	CD4+/CD8+ Ratio 0.15-0.3	CD4+/CD8+ Ratio 0.3-1.5	CD4+/CD8+ Ratio greater than 1.5	Total
Female	73	157	363	18	611
Male	92	116	185	2	395
Total	165	273	548	20	1006

Table no. 6: Contingency table for IRIS cases Vs CD4+/CD8+ ratios

Data analyzed	CD4+/CD8+ Ratio less than 0.15	CD4+/CD8+ Ratio 0.15-0.3	CD4+/CD8+ Ratio 0.3-1.5	CD4+/CD8+ Ratio greater than 1.5	Total
Iris confirmed	44	0	0	0	44
iris not confirmed	122	273	547	20	962
Total	166	273	547	20	1006

Table no. 7 Co-relation (one-tailed) analysis of Age with CD4+, CD8+, CD3 and CD4+/CD8+ ratio.

	PEARSON R	R SQUARED	P (ONE-TAILED)
AGE VS CD4+	-0.3061 (95% Cl; -0.3611 to -0.249)	0.09369	<0.0001
AGE VS CD8+	-0.213 (95% Cl; -0.2712 to -0.1532)	0.04536	<0.0001
AGE VS CD3+	-0.1151 (95% Cl; -0.1757 to -0.05371)	0.01326	0.0001
AGE VS CD4+/CD8+ RATIO	-0.1091 (95% Cl; -0.1697 to -0.0476)	0.0119	0.0003

Table no. 8: Comparison of various parameters of Male vs Female.

Sidak's multiple comparisons test	Mean Diff.	Adjusted P Value	Significance
CD4+ (95%CI;-205.9 to 52.53)	-76.7	0.451	No
CD8+ (95%CI;-169 to 89.45)	-39.77	0.9038	No
Total CD 3 avg. (95%CI;-202.8 to 55.71)	-73.52	0.4934	No
CD4+/CD8+ (95%CI;-129.4 to 129.1)	-0.135	>0.9999	No

DISCUSSION

The normal population characteristics shows very poor immune status in HIV infected patients with low baseline CD4+ count, exceptionally high CD8+ count and abnormal CD4+/CD8+ ratio before starting ART, all in agreement with increased risk of IRIS in those patients.[2], [14] In contrast to this, recent studies carried out by, Breton *et al* and Martinez *et al.*, the patients with predominantly mycobacterial disease[15], [16] found that IRIS was associated with a higher CD4+ cell percentage and CD4+/CD8+ ratio and a more marked and persistent reduction in viral load.[16] This disparity in findings may be explained by differences in the relative frequency of various IRIS events across studies and in the frequency of CD4+ cell count and viral load monitoring, whereby the reported CD4+ cell counts at event or at 12 weeks after HAART initiation were often based on CD4+ cell counts up to 8 weeks before or after this time point. Resolution of this can only be achieved by undertaking large prospective studies with T cell subset measurement at baseline and monthly for at least 6 months. They found that a higher CD8+ cell percentage (>65%) at baseline and at 12 weeks (as well as a lower CD4+/CD8+ ratio) was associated with 3-fold increase in risk of IRIS, although this was no longer significant after adjustment for CD4+ cell percentage. But, in another study of mycobacterial IRIS, there was no such association with IRIS.[17], [18] This suggests that the pathogenic mechanisms associated with IRIS may differ according to the type of pathogen. In contrast, an enhanced CD8+ cytotoxic T lymphocyte response may be more important in the immunopathogenesis of IRIS to viral infections, such as Herpes Zoster[19], Cytomegalovirus, Hepatitis C, or Human Herpes virus[20], although natural killer cells may also be implicated.[21]

In this study, CD4+/CD8+ ratio was divided into different groups as below 0.15 as high-risk group, between 0.15 to 0.3 as risk group, 0.3 to 1.5 as low risk group and above 1.5 as normal i.e not in risk of having IRIS. Though, CD4+/CD8+ratio cannot be taken as sole marker for IRIS but among all the known marker or risk factor for IRIS so far it has been more reliable marker[1] and this study tests the relationship in Nepalese population to see if high risk group, risk group and low risk group develops IRIS and gets reported or not. Given that, the population under study did had very poor immune status as 90 percentiles had CD4+ count below baseline, only 10 percentiles had CD8+ count below baseline and 90 percentiles had CD4+/CD8+ratio below 1. Among all 1006 HIV infected patients included in this study, about 166 patients were in high risk group, 273 in risk group, 547 in low risk group and 20 were normal/no risk. Out of all 1006 HIV patients enrolled in the study, 44 patients showed IRIS with manifestation of different diseases, about 50% of them showed Genital Herpes associated IRIS, 22% showed cryptococcal associate IRIS, rest showed Hepatitis B, Molluscum contagiosum & Vericella zoster associated IRIS. Mostly, common co-infection associated with IRIS is Mycobacterium and Cryptococcal infections but in this study, none of the cases were found to be associated with Mycobacterium but there was Cryptococcal associated IRIS. Although, most of the IRIS cases were associated with Genital Herpes no distinct pattern could be drawn based on the symptoms and pathophysiological parameters as almost all the cases of IRIS had low baseline CD4+ counts and CD4+/CD8+ ratio well below 0.15. In 44 patients, reported to have confirmed case of IRIS with different symptoms and to see if those patients' CD4+/CD8+ ratio was related to IRIS or not, chi-square test was performed and relationship was found to be significant. Indicating CD4+/CD8+ is a reliable indicator of IRIS, though accuracy might still be poor but it does serves as tool to narrow down the patients who might need medical attention in future and can thus be addressed before IRIS can even develop. Those patients suffering from IRIS was subjected to further analysis by non-parametric two-tailed t-test to see if mean value of parameters differs from the patients not suffering from IRIS, and found to be significantly different indicating the population differ and were predicted by CD4+/CD8+ratio as high-risk group. Immune Reconstitution Inflammatory syndrome (IRIS), a clinical worsening of HIV patients having ART treatment have been very hard to diagnose as it did not yet have any concrete parameters and markers for prediction. CD4+/CD8+ ratio was tested as major risk factor for the IRIS in HIV infected patients after initiation of ART. Though, Florence *et al.* identified several risk factors for the development of IRIS, including younger age at initiation of highly active anti-retroviral therapy (HAART), lower baseline CD4+ cell percentage or CD4+/CD8+ ratio and to a lesser extent, higher baseline CD8+ cell percentage.

Also, standard deviation on all the parameters except for age is very high indicating abnormal distribution supported by the skewness and kurtosis value along with D'Agostino & Pearson normality test. Further, the co-relation analysis showed positive significant relationship

between age and all other parameters which was expected as immune system status and age should be directly co-related which is known and common in HIV infected patients.[9]

More recently, distinct polymorphisms in certain major histocompatibility complex or cytokine genes in association with mycobacterial and herpesvirus-associated IRIS have been described[22]–[25], which indicates a genetic susceptibility to IRIS. Analysis of data in different age group was also performed and found that old age group has comparatively poor immune status and middle age group had comparatively good status and lower age group again showed poor status to that of mid age group but was better than old age group of above 35. But the characteristics of the population under study is not due to age only as all the value was significantly different as shown by the non-parametric t-test to that of the study performed by Shakya *et. al.,* 2007. As the CD4+ count, CD8+ count and CD4+/CD8+ratio is not only due to age, from the data it appears about 25 percentiles were under high risk of having IRIS with low CD4+ count, high CD8+ count and CD4+/CD8+ ratio of below 0.15. whereas 25 to 50 percentiles were in risk group and 50 to 90 percentiles were still in low risk group indicating there is huge chance of people to develop an IRIS as the fraction of population having immunological composition that might later on give rise to IRIS. Further, inter-relationship of immune status was checked by using chi-square test to see if they are significantly related or not and found that CD4+/CD8+ratio was significantly related with sex and Age. The marked relationship between age/Sex and CD4+/CD8+ ratio, meaning age also plays great role in immune status of a patients further increasing the chance of IRIS. However, its effect can be seen in the CD4+/CD8+ ratio thus removing the discrepancy.

CONCLUSIONS

More than 75 percentiles of people living with HIV/AIDS (PLHIV) showed abnormal CD4+/CD8+ratio of less than 0.5. The study provided the baseline data on CD4+, CD8+, and CD4+/CD8+ ratio in HIV infected people of Nepal and will sensitize the clinician about the risk of developing IRIS during ART treatment. Furthermore, no single test is currently available to establish an IRIS diagnosis and CD4+/CD8+ ratio did manage to narrow down the high-risk group and was statistically significant. Hence, CD4+/CD8+ ratio measurement will help to narrow down high-risk group of IRIS cases among PLHIV initiating ART. While exact estimates of incidence are not yet available, IRIS in patients initiating ART has been firmly established as a significant problem in both high and low-income countries. Treatment of IRIS will remain a clinical challenge due to the variety of clinical presentations and the presence of multiple pathogens capable of causing the syndrome. Patients at greatest risk for the development of serious IRIS events, a low CD4+/CD8+ ratio of <0.15, should be screened to exclude an active or subclinical infection with important opportunistic.

DECLARATIONS

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Availability of data and materials

Approval for collection of data and materials was obtained from Sukraraj Tropical Infectious Diseases Hospital (STIDH), Teku, Kathmandu, Nepalwhich is duly acknowledged.

List of Abbreviations

AIDS: Acquired Immunodeficiency Syndrome
ART: Anti retroviral therapy
CD4+: Cluster Differentiation 4
CD8+: Cluster Differentiation 8
CDBT: Central Department of Biotechnology
CMV: cytomegalovirus
FACS: Fluorescent Activated Cell Sorting
HAART: Highly Active Anti Retroviral Therapy
HIV: Human Immunodeficiency Virus
IRD: ImmuneRestoration Disease
IRIS: Immune Reconstitution Inflammatory Syndrome
NAST: National Academy of Science and Technology
NCASC: National center for AIDS and STD control
NPHL: National Public Health laboratory
PLHIV: People Living with HIV/AIDS (PLHIV)
RNA: Ribonucleic Acid
STIDH: Sukraraj Tropical Infectious Disease Hospital
TB-IRIS: Tuberculosis-associated IRIS

Authors' contributions

SKM (Virologist) principal investigatordesignedthe study at each step from inception to manuscript writing. RN (Biotechnologist) performed all the data analysis and manuscript writing to manuscript submission. AP is clinician, who performed all clinical diagnosis and data keeping. KDM (PhD/HOD, Central Department of Biotechnology, Tribhuwan University) supervised in laboratory testing, data analysis, manuscript drafting, editing. Any inquiry and communication should be addressed to him. Email:

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Competing interest

The authors declare that they have no competing interest.

Ethics approval and consent to participate

Informed written consent was taken in local language from all participants and ethical approval obtained from the ethical committee of the Nepal Health Research Council (NHRC).

REFERENCES

1. S. Bonham, D. B. Meya, P. R. Bohjanen, D. R. Boulware, and M. P. H. Dtm, "NIH Public Access," *Microbiology*, 2008; 2(4): 349–361.
2. D. M. Murdoch, W. D. Venter, A. Van Rie, and C. Feldman, "Immune reconstitution inflammatory syndrome (IRIS): review of common infectious manifestations and treatment options," *AIDS Res. Ther*, 2007; 4(1): 9.
3. N. F. Walker, J. Scriven, G. Meintjes, and R. J. Wilkinson, "Immune reconstitution inflammatory syndrome in HIV-infected patients.," *HIV. AIDS. (Auckl)*, 2015; 7: 49–64.
4. S. Bosamiya, "The immune reconstitution inflammatory syndrome," *Indian J. Dermatol*, 2011; 56(5): 476.
5. R. Inflammatory *et al.*, "Increased Incidence of Genital Herpes after HAART Initiation : A Frequent Presentation of Immune," 2006; 20(3): 143–145.
6. L. J. Haddow *et al.*, "Cryptococcal immune reconstitution infl ammatory syndrome in HIV-1-infected individuals : proposed clinical case defi nitions," *Lancet Infect. Dis*, 2010; 10(11): pp. 791–802.
7. S. D. Newsome and A. Nath, "Varicella-zoster virus vasculopathy and central nervous system immune reconstitution inflammatory syndrome with human immunodeficiency virus infection treated with steroids," no. March 2008, pp. 288–291, 2009.
8. S. Leone, E. Nicastri, S. Giglio, P. Narciso, G. Ippolito, and N. Acone, "International Journal of Infectious Diseases Immune reconstitution inflammatory syndrome associated with Mycobacterium tuberculosis infection : a systematic review," *Int. J. Infect. Dis*, 2010; 14(4): e283–e291.
9. S. A. Shelburne *et al.*, "Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy.," *AIDS*, 2005; 19(4): 399–406.
10. M. A. French *et al.*, "Immune restoration disease after the treatment of immunodeficient HIV-infected patients with highly active antiretroviral therapy.," *HIV Med. Oxford*, 2000; 1(2): 107–115.
11. L. S. Zijenah *et al.*, "T lymphocytes among HIV-infected and -uninfected infants: CD4/CD8 ratio as a potential tool in diagnosis of infection in infants under the age of 2 years.," *J. Transl. Med.*, vol. 3, no. 1, p. 6, 2005.
12. R. Seng *et al.*, "Influence of lifelong cumulative HIV viremia on long-term recovery of CD4+ cell count and CD4+/CD8+ ratio among patients on combination antiretroviral therapy.," *AIDS*, 2015; 29(5): 595–607.
13. I. Ratnam, C. Chiu, N.-B. Kandala, and P. J. Easterbrook, "Incidence and risk factors for immune reconstitution inflammatory syndrome in an ethnically diverse HIV type 1-infected cohort.," *Clin. Infect. Dis*, 2006; 42(3): 418–27.
14. A. Pen, "Risk factors for immune reconstitution inflammatory syndrome under combination antiretroviral therapy can be aetiology-specific," 2010; 573–579.
15. S. A. Shelburne *et al.*, "Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy," no. November 2004, 2005.
16. G. Breton *et al.*, "Determinants of Immune Reconstitution Inflammatory Syndrome in HIV Type 1 – Infected Patients with Tuberculosis after Initiation of Antiretroviral Therapy," 2004; 39(2): 1709–1712.
17. D. Salemovic, J. Ranin, and I. Pes, "The prevalence and risk of immune restoration disease in HIV-infected patients treated with highly active antiretroviral therapy," 2005; 140–143.
18. "Immune Reconstitution Syndrome After Highly Active Antiretroviral Therapy in Human Immunodeficiency Virus-Infected PD-1 Expression on HIV-Specific T Cells Is Associated With T-Cell Exhaustion and Upregulation of PD-1 Expression on HIV- Specific CD8 T Cells Leads to Reversible," 2007; 158.
19. Y. Mora, E. M. B, E. Aznar, E. Buira, A. Guelar, and E. Soriano, "High Incidence of Herpes Zoster in Patients with AIDS Soon After Therapy with Protease Inhibitors," 1510–1513.
20. A. Immune, D. Syndromes, H. Retrovirology, A. Carr, and D. A. Cooper, "Authors â€™ Reply : CD8 T - Cell Rèsponse to Antiretroviral Therapy," 2017; 1996–1997.
21. C. International, "soluble factor(s) designated as CD8 cell antiviral factor 120," 2004; 1217–1228.
22. M. Sk, R. Sharma, T. Mahat, and G. Bp, "HIGH RISK OF IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME DEVELOPMENT AMONG PEOPLE LIVING WITH HIV / AIDS IN FAR-WESTERN REGION OF NEPAL," 2013; 4(2).
23. P. Price *et al.*, "Polymorphisms in cytokine genes define subpopulations of HIV-1 patients who experienced immune restoration diseases.," *AIDS*, Oct. 2002; 16(15): 2043–2047.
24. S. F. Stone, P. Price, J. Brochier, and M. A. French, "Plasma Bioavailable Interleukin-6 Is Elevated in Human Immunodeficiency Virus – Infected Patients Who Experience Herpesvirus-Associated Immune Restoration Disease after Start of Highly Active Antiretroviral Therapy," 1073–1077.
25. G. A. D. Hardy, N. Imami, A. Pires, C. T. Burton, and F. M. Gotch, "Reconstitution of CD4 + T cell responses in HIV-1 infected individuals initiating highly active antiretroviral therapy (HAART) is associated with renewed interleukin-2 production and responsiveness," 2003; 98–106.