Original Article

An Observational Study of Immunity Level of Treatment-Naive, HIV-Positive Patients Attending Tertiary Care Centre: A Cross-Sectional Study from North-Western India

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Abstract

Background: Acquired Immunodeficiency Syndrome (AIDS) poses a significant public health challenge in India, driven by the Human Immunodeficiency Virus (HIV). HIV exhibits a selective affinity for CD4+ T lymphocytes, leading to their gradual destruction, resulting in severe immune depression and an elevated risk of opportunistic infections and cancers. The CD4+ T lymphocyte count serves as a valuable tool for initiating and monitoring the response to antiretroviral therapy. This study investigates the immunological status of newly diagnosed, treatment-naive HIV-positive individuals across diverse demographics and transmission categories, providing valuable insights for tailored intervention strategies in this population.

Materials and Methods: This was a cross-sectional study involving 200 newly diagnosed, treatment-naive HIV-positive participants. A structured proforma was used to collect demographic data, education status, occupation, transmission routes, and CD4+ T lymphocyte counts of study participants.

Results: Participants had a mean age of 41.02 years, with the majority being under 50 years old (73%). Most were male (66%), and 48% had primary-level education. Heterosexual contact was the primary transmission route (98.5%), including 18% through commercial partners. The mean CD4+ T lymphocyte count was 289.21 cells/mm^3, indicating significant immunodeficiency. A majority (73%) were late presenters (CD4+ T lymphocyte counts <350 cells/mm^3).

Conclusion: The study highlights the high prevalence of late HIV diagnoses, particularly among less educated and socioeconomically deprived populations. The dominance of provider-initiated testing suggests ongoing stigma surrounding voluntary testing. Urgent interventions are needed, focusing on education, occupation-related vulnerabilities, and awareness campaigns to reduce late diagnoses and enhance public health responses to HIV/AIDS.

Keywords:

HIV, CD4+ T lymphocyte count, Acquired Immunodeficiency Syndrome, treatment-naive HIV-positive people

Introduction

Acquired Immunodeficiency Syndrome (AIDS), a global health issue, is caused by Human Immunodeficiency Virus (HIV), a retrovirus that weakens the immune system. HIV-1 and HIV-2, both primate lentiviruses, are responsible for AIDS, which has caused over 40.4 million deaths since its first detection in 1983 [1,2]. In India, where HIV was first reported in 1986, the National AIDS Control Organization (NACO) established the Integrated Counselling and Testing Centers (ICTCs) in 1997, which facilitate nationwide free HIV counselling and testing services. By 2022, India had 34,600 ICTCs, primarily located in government hospitals, which offer voluntary HIV counselling and testing, provide information on transmission modes, and connect individuals with HIV prevention, care, and treatment services [3]. Although India has a comparatively low HIV prevalence rate of 0.22% in 2022, its large population results in a significant number of individuals living with HIV, which amounts to 23.18 lakh [4].

CD4+ T lymphocytes play a critical role in immune coordination, and their depletion due to HIV infection indicates immune collapse, leaving individuals vulnerable to infection and chronic inflammation. CD4+ T lymphocyte count is a vital marker for immune suppression in HIV, reflecting overall immune function. The normal range for CD4+ T lymphocytes is 500–1500 cells/mm³ [5], and the decline in these cells during HIV infection can occur through various mechanisms, including the virus's cytopathogenic effect on infected cells, CD8+ T lymphocyte cytotoxicity, abnormal distribution of CD4+ T lymphocytes, or hindrance in their renewal [6]. CD4+ T lymphocytes are essential for determining the need for prophylactic treatment of opportunistic infections. Despite control programs, the rising number of HIV/AIDS cases and deaths highlights the importance of monitoring CD4+ T lymphocytes, as opportunistic infections remain a leading cause of mortality [6].

Patients who presented with a CD4+ T lymphocyte count below 350 cells/mm³ at the time of HIV diagnosis, or who presented with an AIDS-defining event regardless of CD4+ T lymphocyte count, were defined as late presenters [7]. They typically show a poorer immunological response to combination antiretroviral therapy (cART) and a higher mortality rate [8]. In December 2020, the Joint United Nations Programme on HIV/AIDS (UNAIDS) introduced ambitious targets within the framework of the 2030 Sustainable Development Goals to eradicate AIDS as a public health threat. This includes ensuring that 95% of all people living with HIV know their HIV status by 2025 [9]. Provider-initiated HIV testing by healthcare providers and voluntary counselling and testing are integral approaches for enhancing testing rates. However, in India, participants' tendency to seek testing is often delayed due to stigma, depression, concerns about confidentiality, fear of ART side effects, inadequate family support, and limited trust in healthcare workers.

These factors, prevalent in the Indian context, contribute to delayed diagnosis, increasing the risk of HIV transmission and raising concerns about AIDS-related deaths [10]. Existing literature underscores the importance of CD4+ T lymphocyte monitoring in managing HIV/AIDS progression and treatment outcomes [11–14]. Early HIV diagnosis is crucial for improved treatment outcomes and reduced transmission risk. A comprehensive understanding of CD4+ T lymphocyte counts and early diagnosis is essential for designing interventions, improving outcomes, and controlling the spread of HIV/AIDS.

There are a limited number of studies examining the immunological status of newly diagnosed HIV-positive individuals prior to treatment initiation and exploring associated factors. Therefore, this study aimed to evaluate the immunity levels in these individuals and identify sociodemographic and behavioural determinants.

Materials and Methods

Study Design, Setting, and Patients: The present study took place at Sawai Man Singh Medical College, Jaipur, a leading institute

in Rajasthan that serves patients not only from Rajasthan but also from neighboring states like Haryana, Madhya Pradesh, and Uttar Pradesh. This is due to the availability of various social security and government insurance schemes, significantly reducing the cost of treatment.

The study was conducted at the Integrated Counselling and Testing Center (ICTC) at SMS Hospital, Jaipur, over a six-month period from July to December 2022. Newly diagnosed, treatment-naive HIV-positive individuals above 18 years of age were included in the study. Individuals who had previously received any form of antiretroviral therapy (ART), had other co-infections that could affect the immune status, such as active tuberculosis or hepatitis B and C, or were pregnant were excluded.

Inclusion Criteria: Newly diagnosed, treatment-naive HIV-positive individuals. Age 18 years and older. Participants who provided informed consent.

Exclusion Criteria: Individuals who had previously received any form of antiretroviral therapy (ART). Participants with co-infections that could affect immune status, such as active tuberculosis or hepatitis B/C. Pregnant women, due to the different immune modulation during pregnancy.

Approval was obtained from the Institutional Ethics Committee and Research Review Board, and prior to participation, consent was obtained from each individual. Participant information was recorded in a pre-structured form that included socio-demographic characteristics, personal details, and risk behavior information.

HIV Testing and Viral Load Determination: The participants' HIV status was confirmed at the ICTC in accordance with the NACO guidelines. To perform the HIV antibody test, 2 ml of the participant's blood was aseptically collected from a peripheral vein and placed in a plain vial. The sample was then centrifuged at 3000 rpm for 15 minutes, and the serum was transferred to another screw-capped vial. The samples were tested using the NACO Strategy III, which required positive results from all three tests, each with a different principle. The three tests used for HIV antibody diagnosis were Combaids – RS Advantage-ST (Arkay Healthcare Private Limited, India), Meriscreen HIV 1-2 WB Test (Meril Logistic Private Limited, India), and Tredro HIV 1-2 Antibody (Meril Logistic Private Limited, India). The interpretation of the results followed the kit manufacturer's instructions.

After confirming HIV positivity, newly diagnosed individuals were enrolled at the ART center for further management and CD4+ T-lymphocyte count estimation. For CD4+ T lymphocyte analysis, 2 ml of blood was collected in K2 EDTA vials from patients older than 18 years who had not initiated ART. CD4+ T lymphocyte count estimation was performed using flow cytometry, which involved the use of fluorochrome-labeled antibodies and a fluorescent nuclear dye. The samples were analyzed using BD FACSCalibur™ (Becton, Dickinson and Company, San Jose, United States of America) as per the manufacturer's instructions, and the MultiSET software automatically identified and calculated CD4+ T lymphocyte counts and percentages. A CD4+ T lymphocyte count below 350 cells/mm^3 was classified as low immunity, while a count below 200 cells/mm^3 was considered extremely low.

Statistical Analysis: The data were recorded on a pro forma, entered into the Microsoft® Excel Workbook, and exported to SPSS software version 26 for statistical analysis. Data were tabulated and analyzed using the Chi-square test for categorical variables and unpaired T-test and one-way ANOVA test. A p-value of <0.05 was considered significant.

Results

The findings of this research shed light on several important aspects of the demographic and clinical characteristics of people

newly diagnosed with HIV at a tertiary care hospital. The study participants comprised 200 individuals with a mean age of 41.02±12.48 years, with a substantial portion falling within the <50 years age group (73%). This reflects the significant impact of HIV infection on the productive age range [Table 1].

Table 1: Demographic Details of Study Population

Age group	**No.**	**%**
≤50	146	73.0
51-60	43	21.5
>60	11	5.5
Gender		
Male	132	66.0
Female	65	32.5
Transgender	3	1.5
Education		
Non-literate	36	18.0
Primary	96	48.0
Secondary	32	16.0
High Secondary	25	12.5
College and above	11	5.5
Occupation		
Non-agriculture labourer	55	27.5
Domestic servant	1	0.5
House wife	30	15.0
Skilled worker	2	1.0
Semi-skilled worker	9	4.5
Self-employed (business)	31	15.5
Service (Govt. or Private)	7	3.5
Student	11	5.5
Hotel staff	19	9.5
Agriculture landholder	32	16.0
Truck driver or helper	3	1.5
Type of visit		
Provider Initiated	145	72.5
Voluntary	55	27.5
Source of transmission		
Not Specified	157	78.5
[a]FSW	36	18.0
[b]MSM	4	2.0
[c]IDU	3	1.5
[a]FSW- Female sex workers, [b] Men who have sex with men, [c]IDU- Intravenous drug users		

In the present study, there was a predominance of males (66%), with a male-to-female ratio of 2.03:1. The study probed the educational and occupational backgrounds of the participants, revealing that more than half had only primary education (66%), and non-agricultural laborers constituted the largest occupational group (27.5%). This provides insights into the socioeconomic status of those affected by HIV [Table 1].

This study focused on explaining the source of HIV infection, where 18% of participants had visited female sexual workers at least once in their lifetime. It was found that the source of transmission is significantly associated with gender, education level, occupation, and type of visit ($p<0.05$) [Table 2].

Table 2: Source of infection in association with various demographic values

	N	Route of transmission								'p' Value*
		Regular Partner		Female sex workers		Homosexuals		IDU		
		No.	%	No.	%	No.	%	No.	%	
Age Group										
≤50	146	110	75.34	29	19.86	4	2.74	3	2.05	0.615
51-60	43	37	86.05	6	13.95	0	0.00	0	0.00	
>60	11	10	90.91	1	9.09	0	0.00	0	0.00	
Gender										
Male	132	98	74.24	30	22.73	1	0.76	3	2.27	<0.001
Female	65	59	90.77	6	9.23	0	0.00	0	0.00	
Transgender	3	0	0.00	0	0.00	3	100.0	0	0.00	
Education										
Illiterate	36	34	94.44	2	5.56	0	0.00	0	0.00	0.008
Primary	96	75	78.13	20	20.83	0	0.00	1	1.04	
Secondary	32	23	71.88	4	12.50	3	9.38	2	6.25	
High Secondary	25	16	64.00	8	32.00	1	4.00	0	0.00	
College and above	11	9	81.82	2	18.18	0	0.00	0	0.00	
Occupation										
Non-agriculture labourer	55	42	76.36	12	21.82	0	0.00	1	1.82	0.013
Domestic servant	1	1	100.0		0.00	0	0.00	0	0.00	
House wife	30	28	93.33	2	6.67	0	0.00	0	0.00	
Skilled worker	2	1	50.00	1	50.00	0	0.00	0	0.00	
Semi-skilled worker	9	8	88.89	1	11.11	0	0.00	0	0.00	
Self-employed (business)	31	24	77.42	4	12.90	3	9.68	0	0.00	
Service (Govt. or Private)	7	5	71.43	2	28.57	0	0.00	0	0.00	
Student	11	6	54.55	2	18.18	1	9.09	2	18.18	
Hotel staff	19	16	84.21	3	15.79	0	0.00	0	0.00	
Agriculture landholder	32	25	78.13	7	21.88	0	0.00	0	0.00	
Truck driver or helper	3	1	33.33	2	66.67	0	0.00	0	0.00	
Type of visit										
Provider-initiated	145	116	80.00	27	18.62	0	0.00	2	1.38	0.016
Voluntary	55	41	74.55	9	16.36	4	7.27	1	1.82	
*Chi-square test, P value <0.05- Significant										

It was seen that the age of study participants [Mean 41.02±12.48 years; Median 42; Range: 19-72] is associated with socio-demographic variables like population seeking testing ($p<0.015$) and education level ($p<0.001$). The study also categorized participants according to the population seeking testing, indicating that 72.5% of them underwent testing at the initiative of healthcare providers. The median age of participants seeking testing was more likely in the younger age group [Voluntary testing—Median Value: 38; Provider-initiated testing—Median Value: 42] [Table 3].

Immunological level was assessed via CD4+ T-lymphocyte counts, which revealed significant immunodeficiency [Mean 289.21±188.29 cells/mm³; Median 229.5 cells/mm³; Range: 22-927 cells/mm³]. In this study, 29.5% of participants had a CD4 count ranging from 200-350 cells/mm³, indicating low-level immunity, while 43.5% had CD4 counts below 200 cells/mm³, classifying them as presenters with very low-level immunity and severe immune compromise at the time of diagnosis.

When analyzing the relationship between immunity levels and socio-demographic and risk behavior variables, it was noted that the prevalence of extremely low immunity was highest (66%) among patients aged over 60 years, followed by those aged 50-60 years and under 50 years (44.45% and 41.78%, respectively). Although no significant association was found with age ($p=0.370$),

Table 3: Comparison of age of study participants in association with socio-demographic variables

	N	Mean	SD	Median	Min.	Max.	'p' Value
Gender[a]							
Male	132	40.89	12.39	42	19	72	**0.094**
Female	65	41.95	12.57	45	19	71	
Transgender	3	26.00	3.46	28	22	28	
Treatment seeking behaviour[b]							
Provider-initiated	145	42.33	12.35	44	19	72	**0.015**
Voluntary	55	37.55	12.25	38	19	62	
Education[a]							
Non-literate	36	48.39	11.43	50	25	72	**<0.001**
Primary	96	42.70	11.50	43.5	20	72	
Secondary	32	34.31	12.78	29	19	56	
High Secondary	25	33.88	10.82	29	20	53	
College and above	11	37.91	9.61	37	23	52	
[a]ANOVA - Analysis of Variance, [b]Unpaired 't' test, P value <0.05- Significant							

Table 4: Immunological status of HIV positive patients as reflected by CD4+ T lymphocyte cells associated with various variables

	<200 cell/mm3		200-350 cell/mm3		350-500 cell/mm3		> 500 cell/mm3 (Normal)		Total	P value*
	N	%	N	%	N	%	N	%	N	
Age (in years)										
<50	61	41.78	43	29.45	21	14.38	21	14.38	146	0.370
50-60	20	44.45	16	35.56	3	6.67	6	13.34	45	
>60	6	66.67	0	0	1	11.11	2	22.22	9	
Gender										
Males	62	46.97	38	28.79	16	12.12	16	12.12	132	0.605
Females	25	38.46	20	30.77	7	10.77	13	20	65	
Transgender	0	0	1	33.34	2	66.67	0	0	3	
Treatment seeking behaviour										
Provider initiated	68	46.89	38	26.21	17	11.72	22	15.17	145	0.389
Voluntary	19	34.55	21	38.18	8	14.54	7	12.73	55	
Education										
Non-literate	17	47.23	10	27.78	2	5.56	7	19.45	36	0.511
Primary	41	42.71	28	29.17	14	14.54	13	13.54	96	
Secondary	14	43.75	11	34.38	6	18.75	1	3.13	32	
High Secondary	9	36	7	28	2	8	7	28	25	
College and above	6	54.55	3	27.27	1	9.09	1	9.09	11	
Route Of Transmission										
Not specified	71	45.23	44	28.03	21	13.38	21	13.38	157	0.332
[a]FSW	15	41.67	13	36.12	2	5.56	6	16.67	36	
[b]MSM	0	0	1	25	2	50	1	25	4	
[C]IDU	1	33.3	1	33.3	0	0	1	33.3	3	
Total	87	43.5	59	29.5	25	12.5	29	14.5	200	
***Chi-square test, P value <0.05- Significant** **[a]FSW- Female sex workers, [b]Men who have sex with men, [C]IDU- Intravenous drug users**										

there was an inverse relationship between age and immunity level. Similarly, 46.97% of male and 38.6% of female participants exhibited extremely low immunity; however, the immunity level was not significantly associated with gender (p=0.605). Provider-

initiated participants showed a higher rate of extremely low immunity (46.89%) compared to self-reporting participants (34.55%). Statistical analysis revealed no significant association between treatment-seeking behavior and immunity level (p=0.389) [Table 4].

Discussion

Human Immunodeficiency Virus (HIV) specifically attacks CD4+ T lymphocytes, thereby impairing the immune system and ultimately leading to the development of AIDS. Recognizing the critical role of CD4+ T lymphocytes in assessing an individual's immune status with HIV, the present study evaluated the immunological status of newly diagnosed, treatment-naive, HIV-positive individuals.

The study's demographic landscape revealed that the mean age of participants was 41.02 years, and the majority of them (73%) were in the <50 years age group. This emphasizes the vulnerability of sexually active populations. Furthermore, individuals aged 60 years and above constituted a smaller proportion, suggesting potential age-related patterns in HIV diagnoses. This aligns with previous studies in India and globally, where sexually active age groups face a higher risk of HIV infection [11,12,13]. Notably, age was not significantly associated with CD4+ T lymphocyte count, which can be attributed to the study design, as cross-sectional studies can only establish associations and cannot determine causation. Further research with a larger sample size may be needed to explore the temporal relationship between age and CD4+ T lymphocyte count over time.

The male-to-female ratio of 2.01:1 in this study contradicted some earlier reports, hinting at potential shifts in the demographic patterns of HIV infection. These shifts may be influenced by factors such as professions requiring migration by male family members, female fear of isolation, and lack of family support if they test positive for HIV [12,13,14,15]. This stresses the importance of employing sex-sensitive approaches in HIV prevention and testing strategies.

This study demonstrated that education played a crucial role, with the majority of participants having only primary education, and 18% being non-literate. This emphasizes the correlation between educational attainment and HIV knowledge, as lower education levels are associated with a limited understanding of HIV transmission and prevention [12,13]. In terms of occupational distribution, 27.5% were non-agricultural labourers, a group prone to migration, unhygienic living conditions, and increased vulnerability to sexually transmitted diseases (STDs), including HIV [16].

The current study shows that the primary mode of HIV transmission is heterosexual, but the specifics of this transmission are not clearly indicated in the majority of participants (96.5%), whether it is from a spouse, non-spousal partner, or client of female commercial sex workers. Female sex workers account for 18% of the cases. This is in accordance with the global trend, in which heterosexual transmission is the most common mode of HIV transmission [12,16]. The findings highlight the need for targeted interventions within high-risk populations who are at an increased risk of acquiring HIV. These interventions should focus on reducing risk behaviours, such as unprotected sex and having multiple sexual partners, and should raise awareness, provide education, and promote safe practices to mitigate the impact of HIV within these populations.

Regarding immunological markers, in the present study, the mean CD4+ T lymphocyte count was 289.21±188.29 cells/mm³. Over half of the participants had CD4 counts below 200 cells/mm³, indicating advanced immunodeficiency at the time of diagnosis. A substantial proportion of the participants (73%) were classified as late presenters (CD4 count <350 cells/mm³). Our findings were consistent with studies conducted across the globe [8]. Late diagnosis poses challenges for disease control and can lead to higher morbidity and mortality rates [8].

The majority of individuals diagnosed with HIV/AIDS typically had CD4 T lymphocyte counts <200 cells/mm³ (43.5%), and their HIV diagnosis is often incidental. It is observed that individuals usually undergo testing only when symptomatic. These individuals undergo testing either as a prerequisite for a diagnostic procedure or a surgical intervention initiated by healthcare providers, resulting in the determination of their HIV status. This showcases the influential role of healthcare providers in encouraging HIV testing. It is apparent that individuals usually undergo testing only when they are symptomatic, thereby limiting the effectiveness of voluntary counselling and testing (VCT). Moreover, the stigma associated with HIV testing continues to act as a barrier, preventing individuals from voluntarily seeking testing.

The present study provides a comprehensive overview of the demographic, educational, and behavioural factors influencing the immunological status of newly diagnosed HIV-positive individuals in India. These findings highlight the need for targeted interventions that specifically address the needs of different population groups and the challenges posed by late diagnosis, inadequate education, and stigma. These findings serve as a vital foundation for the development of tailored strategies to mitigate the impact of HIV/AIDS in the region.

However, it is important to acknowledge several limitations in our study. The relatively small sample size of 200 participants may limit the generalizability of our findings to larger populations. Additionally, the cross-sectional design limits our ability to establish causal relationships, focusing instead on associations at a single point in time. Recruitment of study participants from a single tertiary care center introduces potential selection bias, which may affect the broader representation of HIV-positive populations in India. Furthermore, self-reported data on risk behaviours and educational levels may be subject to recall bias.

Conclusion

In conclusion, this study highlights a concerning prevalence of late HIV diagnosis, particularly among socioeconomically deprived and less educated populations. The majority of participants exhibited compromised immune status at diagnosis, stressing the urgent need for enhanced awareness campaigns and accessible testing facilities. The dominance of provider-initiated testing shows persistent stigma around voluntary testing, emphasizing the pivotal role of initiatives like ICTCs and FICTCs in HIV prevention. Our study identifies critical barriers to voluntary testing, such as stigma, lack of education, and limited awareness about HIV testing facilities. Community-based interventions and tailored educational programs could enhance early diagnosis rates and improve health outcomes. Future research should prioritize longitudinal studies to explore causal relationships between demographic factors and CD4+ T lymphocyte counts over time, and intervention studies targeting stigma reduction and increasing voluntary testing uptake among diverse demographic groups. Tailored interventions addressing educational and occupational vulnerabilities are crucial to mitigating the impact of late diagnoses and advancing public health efforts in combating HIV/AIDS.

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