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Discovering Variability in Population of T-Lymphocyte Subsets Present in Peripheral Blood of Patients Co-Infected by HIV and HBV Through Laboratory Medicine

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ABSTRACT

An alarming number of 296 million individuals in Egypt have recently been diagnosed as HBV positive; because these two viruses share similar routes of transmission, about 10% of this population is also thought to be HIV positive. It is widely acknowledged that coinfection with HIV and HBV has a significant negative effect on the immune system and accelerates the loss of T-lymphocyte subpopulations, which are essential for immunological defense. This enlightening study, carried out in Cairo, Egypt, explores the complicated dynamics of T-cell subsets and carefully looks at the immunological effects and frequency of coinfection between HIV and HBV. Naturally, of the 35 patients in the cohort under examination, an impressive 8.4% had coinfections with HBV and HIV. Systematic immunophenotyping of several T-cell subsets, such as CD4+, CD8+, CD45RO, CD16+56, and CD45RA, was used in the study, and the results showed clear differences between coinfected and mono-infected peopleUsing trustworthy methods like microfluid inertial separation and flow cytometry has given researchers significant insights into the complex immunological circumstances connected to HIV/HBV coinfection. Interestingly, a significant decrease in CD4+ cells—a crucial marker of HIV progression—was observed in over 50% of the coinfected patients, suggesting an accelerated course of the illness in these individuals. Moreover, the study establishes the foundation for particular treatment strategies and adds to our scientific understanding coinfection dynamics. These therapies, based on the study's detailed findings, can potentially improve patient outcomes despite this difficult coinfection setting. To manage the difficulties of HIV/HBV coinfection, the research emphasizes the importance of ongoing attention and the incorporation of cutting-edge diagnostic techniques, eventually advancing public health activities.

INTRODUCTION

Human immunodeficiency virus HIV mainly targets the immune systems and triggers the autoimmune response leading to Acquired Immunodeficiency Syndrome (AIDS). AIDS is categorized as one of the most destructive epidemics that derogate the lymphocyte subpopulations, eventually leading to various infections and carcinomas. Previous studies have focused on CD4+ T-cell population variations (Yuan *et al.*, 2023). HIV binds to the chemokine receptors CCR5 or CXCR4 and glycoprotein such as gp120 on CD4 T cells (CD4TL), which allow HIV RNA to enter the lymphocyte cells, hijack the machinery and cause apoptosis (Armani-Tourret *et al.*, 2021).

HIV leads to impairment of T-cell subsets by systematically blocking the immune activation and promoting T-cell dysregulation in lymphoid tissues and peripheral blood (Barber-Axthelm *et al.*, 2020). HIV causes functional impairment of HIV-associated CD8+ T-cells, resulting in T-cell exhaustion. T-cell exhaustion is characterized by reduced glycolytic activity, enhanced OXPHOS demands, deregulated mTOR, and reduced cytoplasmic GAPDH (Kirchmair *et al.*, 2023). Ultimately depleting the CD8+ population, due to which the majority of immunotherapies failed to cure it (Rahman *et al.*, 2021).

Not only CD4+ and CD8+ T cells but natural killing cells are equally affected by HIV infection. Natural killer (NK) cells are immune effectors whose activities are intimately associated with the advancement of HIV-1 infection (Cao *et al.*, 2022). Several studies have shown that during HIV-1 infection, there is an overexpression of inhibitory receptors and a downregulation of activating receptors, which leads to NK cell insensitivity and decreased killing capacity (Cao *et al.*, 2022).

It has also been reported that there is a preferential depletion of CD45RA+CD4+ cells in HIV-1-infected patients, with symptoms being more prominent in patients. In HIV-positive CD45RA+, there is an increase in the level of oxidized glutathione and a decreased ratio of reduced to total glutathione as the major characteristics (Kirchmair *et al.*, 2023). The glutathione abnormalities in CD45RA+CD4+ lymphocytes showed significantly low numbers of total CD4+ lymphocytes, ultimately decreasing the proportion of CD45RA+CD4+ lymphocytes in peripheral blood (Kirchmair *et al.*, 2023).

HIV infection leads to the weakening of the immune system, providing opportunities for Coinfection. HIV is well known for its coinfection with HBV. Hepatocellular carcinoma, liver cirrhosis, and chronic hepatitis are frequently caused by hepatitis B virus (HBV) infection (Weldemhret, 2021). Both viral infections, the human immunodeficiency virus (HIV) and HBV, share a mutual mode of transmission, such as unprotected sexual

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activities, unhygienic surroundings, perinatal transmission, and intravenous drug usage (Corcorran & Kim, 2023).

In the case of HBV infection, the T-lymphocyte population decreases due to HBeAg positivity and increased levels of viremia (Kirkoyun Uysal *et al.*, 2023). The immune response to chronic HBV infection plays a multiple complex function; the duration and point of liver damage can be determined by the type of T-cell response to this infection (Yu *et al.*, 2023). One crucial component in developing hepatitis B is the cellular immune response dysfunction mediated by T cells (Dumolard *et al.*, 2023).

According to reports, abnormalities of T lymphocytes and their subsets frequently arise following hepatitis B infection, which is typically characterized by an imbalance in Th1/Th2 lymphocytes, an increase in CD8 + T lymphocytes, and a decrease in CD4 + T cells (Zhang & Ruan, 2023). This type of shift frequently results in an impairment in the organism's immune function, a cell immune insufficiency, difficulty clearing the virus, a protracted illness, and a failure to heal. Hepatitis B surface antigen (HBsAg) expression during chronic infection is frequently attributed to HBV integration into the host genome, which may lead to defective T-cell responses and promote autoimmune responses (Wu *et al.*, 2020)

The Rationale of the Study

There are an estimated two to 296 million people with chronic hepatitis B (CHB) in Egypt, which means nearly 29.6 million people are suspected to be HIV positive as well. It is safe to say that about 10% of HIV-positive individuals also have HBV coinfection (Youssif *et al.*, 2023). According to previous studies, in comparison to chronic HBV mono-infection, HIV/HBV coinfection accelerates the development of chronic HBV into cirrhosis, end-stage liver disease, or hepatocellular carcinoma (Ruta *et al.*, 2023). The HIV/HBV coinfection has become a serious challenge for antiviral therapies. The severity of viral infection in patients with chronic HIV/HBV coinfection is caused by a decline in the number of HBV-specific CD8+ T cells that produce interferon (IFN) as the disease progresses (Lim *et al.*, 2023). This research aims to study variations in lymphocyte subsets such as CD4+, CD8+, CD45RO, CD16+56, and CD45RA in patients co-infected with HIV/HBV.

Significance of Monitoring the T-Lymphocyte Population

Monitoring lymphocyte subpopulations in the context of coinfection with HIV/HBV provides an in-depth understanding of the immune system's dynamic response and the manner in which it affects the progression of the disease (Zhang & Ruan, 2023). Four prominent subpopulations—CD4, CD8, CD45RO, and CD45RA—offer significant data on the immunological response's dynamics. However, prolonged CD8 T-cell activation may trigger immunological exhaustion, emphasizing the delicate balance required for efficient immune system regulation (Zhu *et al.*, 2022).

T-cell populations are represented by CD45RA and CD45RO, which stand for naïve and memory, respectively. The immunological response can be evaluated by examining the ratio of CD45RA to CD45RO (Tang *et al.*, 2020). While an increase in CD45RO+ memory T-cells indicates heightened immunological activation, the depletion of CD45RA+ naïve T-cells impairs the development of new immune responses (Bono *et al.*, 2022). It is necessary to keep these subgroups under continuous monitoring, determining the efficacy of treatment as well as for comprehending the direct effects of HIV/HBV coinfection.

A balanced CD4/CD8 ratio and the restoration of CD4 levels are common indicators of successful ART outcomes (Serrano-Villar *et al.*, 2022). HIV primarily targets CD4 T-cells, which are responsible for managing the immunological response. For the purpose of determining the severity of HIV infection and directing the start of antiretroviral therapy (ART), it is imperative to monitor their levels. A drop in CD4 counts indicates weakened immunity, which makes it a crucial factor in HIV management (Perez-Molina *et al.*, 2023).

However, CD8 T-cells are essential for inhibiting the spread of viruses. During the chronic phase of HIV infection, elevated CD8 counts are frequently seen, suggesting an augmented immunological response. Furthermore, monitoring CD45RO+ memory T-cells influences the long-term health outcomes of coinfected patients by assessing the effectiveness of immune reconstitution. Thus, an extensive comprehension of the host's immune response in the context of coinfection with HIV and HBV is provided by the thorough investigation of lymphocyte subpopulations. In this highly complicated situation, this knowledge aids in developing targeted therapies and targeted therapeutic techniques, eventually improving patient outcomes.

MATERIALS AND METHODS

Sample Collection

The study comprises 35 peripheral blood samples collected from two hospitals in Cairo, Egypt, and samples of infants were collected from two maternity centers. All cryopreserved and fresh blood samples were drawn from patients who tested positive for HIV and HBV. All concerned parties were well informed about the study, and samples were only included in the study after receiving proper consent.

Serological Assessment

All HIV-positive peripheral blood samples were tested for the presence of HBV surface antigen (HBsAg) to identify the presence of HIV/HBV coinfection with the help of commercial enzyme-linked immunosorbent assay (ELISA) kits (Kehua Biotech, Shanghai, China) as per manufacturer instructions (Cai *et al.*, 2023). The same protocol was followed for HBV-positive samples; they were tested to identify the presence of gp120, a glycoprotein marker for the presence of HIV. The samples were then

purified to extract lymphocyte subpopulations. This was done with the help of Microfluidic Inertial Separation. Anticoagulants are added to retrieved cryopreserved whole blood samples to prevent coagulation. The next step is to set up the microfluidic device for the separation of lymphocyte subpopulation. This device has the properties of an inertial spiral design, which can efficiently extract lymphocyte subpopulations. The whole blood sample containing erythrocytes, lymphocytes, and other blood components was injected through the inlet into the microfluidic device. Different-sized cells separate in microfluidic channels due to inertial forces. Lymphocytes fall into a particular size range and travel to a collection chamber from where they are collected.

Lymphocyte Subpopulation Extraction

Peripheral blood mononuclear cells were separated, and preserved blood was collected with the help of density gradient centrifugation using Ficoll-Paque PLUS density gradient media. Once PBMC was isolated, lymphocyte subsets were isolated. CD4+ cells were isolated with the help of an isolation kit for CD4+ (Miltenyi, Bergisch Gladbach, Germany). CD8+ subpopulation was isolated with the help of an immunomagnetic, column-free cell separation (EasySep™) kit. The remaining subsets, including CD45RO, CD16+56, and CD45RA, were extracted by the lymphoprep method.

Immunophenotyping of Lymphocyte Subsets

The immunophenotyping of isolated subsets of lymphocytes was done using the FACS Canto-II flow cytometer. The subsets of lymphocytes, including CD4+, CD8+, CD45RO, CD16+56, and CD45RA, were immune-labeled with the help of monoclonal antibodies. The cells were first washed with a staining buffer and were then incubated with fluorescent-labeled monoclonal antibodies for 15 minutes. Each antibody was labeled with a different fluorescence dye, mainly with fluorescein isothiocyanate (FITC), phycoerythrin (PE), and peridinin chlorophyll protein (PerCP). These antibodies were directed against particular cell surface markers. SK1 antibodies were used for CD8+ t-cells, SK3 for CD4+ cells, NCAM16.2 for CD16+56, L48 for CD45RA, and UCHL1 for CD45RO.The cells were then washed twice again with a staining buffer. The excessive stain was washed off. The stain that remained bound to cells was then fixated with a fixation buffer and was allowed to incubate for 20 minutes. The cells were then permeabilized, which allowed intracellular staining of cell markers and glycoprotein. The cells were stained with fluorescently labeled antibodies and were incubated for 20 minutes. Isotope controls were also included in the antibody panel to differentiate antibody-specific signals from non-specific signals generated in the background. All these monoclonal antibodies and isotope controls were sourced from Immunotech, France. The lymphocyte cell counts were analyzed using Flow Jo V 10 (FlowJOTM software).

Data Analysis

All the statistical analyses were done using SPSS, and the standard deviation was calculated for the population's demographic characteristics. The two-tailed unpaired or paired Student's t-test was used to figure out the statistical differences between the two groups. Multiple regression was calculated to identify a correlation between variables. Odds ratios were also calculated. Hypothesis testing for all statistical analyses was conducted at a significance level of $P<0.01$.

RESULTS AND DISCUSSION

Results

Studied Parameters

Initially, 50 peripheral samples were collected from hospitals and maternity centers. Eight of them were given antiviral vaccination and so were excluded from the study as intake of antiviral can influence the overall impact of HIV, HBV mono-infection, and coinfection of HIV/HBV on lymphocyte subpopulations. Out of the remaining 42 samples, 4 samples were excluded as they gave a cell count of around 800 cells/mm3 and did not align with the chosen criteria for the study. 3 more samples were excluded as the concerned parties did not give their consent to proceed with the study. After applying inclusion and exclusion criteria, the study proceeded with 35 participants.

Immunophenotyping of Lymphocyte Subsets

CD8+ Cell subsets

Three sets of cells were subjected to immunophenotyping

Table 1: Demographic and clinical parameters of patients infected with HIV, HBV, and coinfected with HIV/HBV and their cell count

Studied Parameters	HIV-infected patients (N%)	HBV-infected patients (N%)	HIV patients co-infected with HBV (N%)
No of participants (35)	12 (100.00)	9 (100.00)	14(100.00)
Age factor (years)			
Mean ±SD	21.43±4.08	24.01±3.01	26.07±3.11
0-15 years	1 (8.3)	3 (33.33)	2 (14.28)
16-21 years	4 (33.3)	2 (22.22)	3 (21.42)
22-37 years	5 (41.66)	3 (33.33)	8 (57.14)
38-53 years	1 (8.3)	1 (11.11)	1 (7.14)

>53 years	1 (8.3)	0(0.00)	0 (0.00)
Gender			
Male	3 (25)	4 (44.44)	5 (35.7)
Female	9 (75)	5 (55.55)	9 (64.28)
Mode of acquired infection			
Intravenous drug usage	3 (25)	1 (11.11)	3 (21.42)
sexual practices	3 (25)	3 (33.33)	4 (28.57)
vertical transmissions	4 (30.7)	2 (22.22)	5 (35.71)
unhygenic surroundings	2 (16.66)	3 (33.33)	2 (14.28)
Duration of diagnosis			
0-6 months	3 (25)	2 (22.22)	6 (35.29)
7-12 months	6 (50)	3 (33.33)	4 (28.57)
13-19 months	3 (25)	3 (33.33)	2 (14.28)
20-26 months	0 (0.00)	1(1.11)	2 (14.28)
CD8 cell count:			
>500	2 (16.66)	1 (11.11)	1 (7)
350-500	2 (16.66)	3 (33.33)	2 (14.28)
200-350	6 (50)	3 (33.33)	8 (57.1)
<200	3 (25)	2 (22.22)	2 (14.28)

to determine the variations in CD8 cells. Nearly 50% of mono-infected HIV patients had CD8 cell count in ranges of 250-300 cells/mm3. In the case of acute Hepatitis B, nearly 33.33% of patients showed CD8 cell count lesser than 300 cells/mm3. The same pattern was followed by patients co-infected with HIV/HBV, as nearly 57% of them showed cell count below the range of 350. These results portrayed the inverse relation between cell count and infection progression. Mentioned below is the tabular visualization of data.

Table 1 shows the demographic and clinical parameters undertaken to conduct the study. The median age for HIV-positive patients is 21.43±4.08; for HBV-positive patients, 24.01±3.01, and for HIV/HBV patients is 26.07±3.11. The significance value for CD8 cell count is < 0.01.

Approximately 57.1% of HIV/HBV coinfected patients had a low 300 cells/mm3 population. Out of 14 patients, nearly 2 patients showed more than 350 cells/mm3 populations. This variation may be due to the fact that either the person's immune system is comparatively strong or the viral exposure time is comparatively short. In the case of HIV mono-infection, nearly 25 patients showed less than 300 cells/mm3 CD8+ count, and 22.22% of HVB monoinfected patients showed depleted CD8+ population. It is notable that HIV/HBV coinfection decreases the CD8+ population in an exponential pattern.

Natural Killing Cells (CD16+56) Subsets

CD16+56 cells exhibit natural antiviral properties; however, in case of HIV invasion, the functioning and population of CD16+56 get highly disturbed. This usually results in a low CD16+56 population in peripheral blood. In our study, nearly 50% of HIV patients showed (CD16+56) cell count lower than 300 cells/mm3. A similar trend was followed by HBV patients and coinfected HIV/HBV patients as nearly 44% and 64% of patients showed cell count below 300 cell/mm3, as shown in Table 2:

Table 2 shows the demographic and clinical parameters

Table 2: Demographic and clinical parameters of patients infected with HIV, HBV, and coinfected with HIV/HBV and CD16+56 count

Studied Parameters	**HIV-infected patients (N%)**	**HBV-infected patients (N%)**	**HIV patients co-infected with HBV (N%)**
No of participants (35)	12 (100.00)	9 (100.00)	14(100.00)
Age factor (years)			
Mean ±SD	21.43±4.08	17.01±3.01	16.07±3.11
0-15 years	1 (8.3)	3 (33.33)	2 (14.28)
16-21 years	4 (33.3)	2 (22.22)	3 (21.42)
22-37 years	5 (41.66)	3 (33.33)	8 (57.14)
38-53 years	1 (8.3)	1 (11.11)	1 (7.14)

>53 years	1 (8.3)	0(0.00)	0 (0.00)
Gender			
Male	3 (25)	4 (44.44)	5 (35.7)
Female	9 (75)	5 (55.55)	9 (64.28)
Mode of acquired infection			
Intravenous drug usage	3 (25)	1 (11.11)	3 (21.42)
Sexual practices	3 (25)	3 (33.33)	4 (28.57)
vertical transmissions	4 (30.7)	2 (22.22)	5 (35.71)
unhygienic surroundings	2 (16.66)	3 (33.33)	2 (14.28)
Duration of diagnosis			
0-6 months	3 (25)	2 (22.22)	6 (35.29)
7-12 months	6 (50)	3 (33.33)	4 (28.57)
13-19 months	3 (25)	3 (33.33)	2 (14.28)
20-26 months	0 (0.00)	1(1.11)	2 (14.28)
CD16+56			
>500	1 (8.33)	2 (11.11)	2 (14.28)
350-500	2 (16.66)	2 (22.22)	1 (7)
200-350	6 (50)	4 (44.44)	9 (64)
<200	4 (25)	1 (11.11)	2 (14.28)

undertaken to conduct the study. The median age for HIV-positive patients is 21.43±4.08. For HBV-positive patients, it is 24.01±3.01, and for HIV/HBV patients is 26.07±3.11. The significance value for CD16+56 cell count is < 0.01

CDCD16+56 population has also shown prominent variation in all three sets of samples, i.e., HIV mono-infected patients, HIB mono-infected patients, and HIV/HBV coinfected patients. 64% of HIV/HBV coinfected patients had CDCD16+56 depleted to dangerous levels. The cell count depletion in HIV/HBV was 14% more in mono-infected HIV patients and nearly 20% more in HBV mono-infected patients. Implicating the results attained through this research, the complex interactions between coinfections with HIV and HBV can be better understood, and potential synergistic effects that could worsen cellular depletion are shown. Understanding these disparities is crucial for customized treatment approaches and emphasizes the necessity of attentive observation in coinfected groups to alleviate unfavorable consequences linked to CDCD16+56 depletion.

Naive T-cells (CD45RA subsets)

Correlational analysis of (CD16+56) cell count and coinfection of HIV/HBV showed a negative association between them. The same results were obtained for mono-infection of HIV and HBV. This negative association proved to be significant statistically, giving a p-value of < 0.01. Table 3 below indicates 58.33% HIV, 55.55% HBV, and 64.28% HIV/HBV patients showed cell count below normal ranges, i.e., 300-350 cells/mm3.

Table 3 shows the demographic and clinical parameters

Table 3: Demographic and clinical parameters of patients infected with HIV, HBV, and coinfected with HIV/HBV and CD45RA cell count

Studied Parameters	**HIV-infected patients (N%)**	**HBV-infected patients (N%)**	**HIV co-infected with HBV%)**
No of participants (35)	12 (100.00)	9 (100.00)	14(100.00)
Age factor (years)			
Mean ±SD	21.43±4.08	17.01±3.01	16.07±3.11
0-15 years	1 (8.3)	3 (33.33)	2 (14.28)
16-21 years	4 (33.3)	2 (22.22)	3 (21.42)
22-37 years	5 (41.66)	3 (33.33)	8 (57.14)
38-53 years	1 (8.3)	1 (11.11)	1 (7.14)
>53 years	1 (8.3)	0(0.00)	0 (0.00)
Gender			
Male	3 (25)	4 (44.44)	5 (35.7)

Female	9 (75)	5 (55.55)	9 (64.28)
Mode of acquired infection			
Intravenous drug usage	3 (25)	1 (11.11)	3 (21.42)
sexual practices	3 (25)	3 (33.33)	4 (28.57)
vertical transmissions	4 (30.7)	2 (22.22)	5 (35.71)
unhygienic surroundings	2 (16.66)	3 (33.33)	2 (14.28)
Duration of diagnosis (months)			
0-6 months	3 (25)	2 (22.22)	6 (35.29)
7-12 months	6 (50)	3 (33.33)	4 (28.57)
13-19 months	3 (25)	3 (33.33)	2 (14.28)
20-26 months	0 (0.00)	1(1.11)	2 (14.28)
CD45RA			
>500	1 (8.33)	1 (11.11)	2 (14.28)
350-500	2 (16.66)	2 (22.22)	1 (7)
200-350	7 (58.33)	5 (55.55)	9 (64.28)
<200	3 (21.4)	1 (11.11)	2 (14.28)

undertaken to conduct the study. The median age for HIV-positive patients is 21.43±4.08, and for HBV-positive patients, 24.01±3.01. And for HIV/HBV patients, it is 26.07±3.11. The significance value for CD45RA cell count is < 0.01

Memory T-cells (CD45RO)

In the case of HIV infection, CD45RO shows an interesting trend as it reduces with increased viral titer. The HBV infection and coinfection of HIV/HBV also showed reduced CD45RO population as the infection severs. Table 4 shows that 57.1% of patients with HIV/HBV

Table 4: Demographic and clinical parameters of patients infected with HIV, HBV, and coinfected with HIV/HBV and CD45RA cell count

Studied Parameters	**HIV-infected patients (N%)**	**HBV-infected patients (N%)**	**HIV co-infected with HBV Patients (%)**
No of participants (35)	12 (100.00)	9 (100.00)	14(100.00)
Age factor (years)			
Mean ±SD	21.43±4.08	17.01±3.01	16.07±3.11
0-15 years	1 (8.3)	3 (33.33)	2 (14.28)
16-21 years	4 (33.3)	2 (22.22)	3 (21.42)
22-37 years	5 (41.66)	3 (33.33)	8 (57.14)
38-53 years	1 (8.3)	1 (11.11)	1 (7.14)
>53 years	1 (8.3)	0(0.00)	0 (0.00)
Gender			
Male	3 (25)	4 (44.44)	5 (35.7)
Female	9 (75)	5 (55.55)	9 (64.28)
Mode of acquired infection			
Intravenous drug usage	3 (25)	1 (11.11)	3 (21.42)
sexual practices	3 (25)	3 (33.33)	4 (28.57)
vertical transmissions	4 (30.7)	2 (22.22)	5 (35.71)
unhygienic surroundings	2 (16.66)	3 (33.33)	2 (14.28)
Duration of diagnosis (months)			
0-6 months	3 (25)	2 (22.22)	6 (35.29)
7-12 months	6 (50)	3 (33.33)	4 (28.57)
13-19 months	3 (25)	3 (33.33)	2 (14.28)
20-26 months	0 (0.00)	1(1.11)	2 (14.28)

CD45RO			
>500	3 (25)	1 (11.11)	2 (14.28)
350-500	2 (16.66)	2 (22.22)	1 (7)
200-350	7 (58.33)	5 (55.55)	9 (64.28)
<200	1 (8.33)	1 (11.11)	2 (14.28)

coinfection have cell count reduced to <300 cells/mm3.

Table 4 shows the demographic and clinical parameters undertaken to conduct the study. The median age for HIV-positive patients is 21.43±4.08; for HBV-positive patients, 24.01±3.01, and for HIV/HBV patients is 26.07±3.11. The significance value for CD45RA cell count is < 0.01. The study further explored that the naive T-cells also showed significant variation in mono-infections of HIV and HBV in comparison to coinfection of HIV/HBV infection. CD45RO depleted in 57.1% of patients.

The decrease in CD45RA and CD45RO T-cell subsets in individuals with coinfection can possibly be caused by the cumulative impact of both infections, triggering immunological dysregulation.

The fact that both HIV and HBV infections are chronic may have an impact on memory T-cell exhaustion and the transition of memory T-cells to a naive T-cell phenotype. In addition, these viruses' combined impacts on immune response pathways may have a cascading impact, accelerating the depletion of T cells and changing the delicate equilibrium between memory and naive T-cell populations.

CD4+ Cells

As Table 5 shows, out of 12 HIV patients, 5 patients had a cell count nearly lower than 350 cells/mm3 and showed severe HIV/HBV infection. Out of 9 HBV patients, 5 patients had a cell count below 350 cells/mm3, and in 14 positive HIV/HBV cases, 7 patients showed a cell count below 350 cells/mm3. The statistical analysis has shown a significant negative correlation between the cell count and the severity of infection, giving the P-value of <0.001. As the HIV stage advances from stage B to stage E, the cell count significantly depletes from >500 cells to 200, which

Table 5: Demographic and clinical parameters of patients infected with HIV, HBV, and coinfected with HIV/HBV and CD45RA cell count

Studied Parameters	**HIV-infected patients (N%)**	**HBV-infected patients (N%)**	**HIVco-infected with HBV%)**
No of participants (35)	12 (100.00)	9 (100.00)	14(100.00)
Age factor (years)			
Mean ±SD	21.43±4.08	17.01±3.01	16.07±3.11
0-15 years	1 (8.3)	3 (33.33)	2 (14.28)
16-21 years	4 (33.3)	2 (22.22)	3 (21.42)
22-37 years	5 (41.66)	3 (33.33)	8 (57.14)
38-53 years	1 (8.3)	1 (11.11)	1 (7.14)
>53 years	1 (8.3)	0(0.00)	0 (0.00)
Gender			
Male	3 (25)	4 (44.44)	5 (35.7)
Female	9 (75)	5 (55.55)	9 (64.28)
Mode of acquired infection			
Intravenous drug usage	3 (25)	1 (11.11)	3 (21.42)
sexual practices	3 (25)	3 (33.33)	4 (28.57)
vertical transmissions	4 (30.7)	2 (22.22)	5 (35.71)
unhygienic surroundings	2 (16.66)	3 (33.33)	2 (14.28)
Duration of diagnosis (months)			
0-6 months	3 (25)	2 (22.22)	6 (35.29)
7-12 months	6 (50)	3 (33.33)	4 (28.57)
13-19 months	3 (25)	3 (33.33)	2 (14.28)
20-26 months	0 (0.00)	1(1.11)	2 (14.28)
CD4+			
>500	1 (8.3)	0 (00.00)	1 (7.14)

350-500	3 (25)	2 (22.22)	2 (14.28)
200-350	5 (41.66)	5 (55.55)	7 (50)
<200	4 (30.7)	2 (22.22)	4 (28.57)

shows a strong decline in the CD4+ population.

The study also showed that nearly 50% of HIV/HBV coinfected patients showed CD4+ cell counts in the range of 200-350 cells/mm3, as mentioned in Table 5. Previous studies have shown that HIV/HBV coinfection progression is usually evident from the CD4 cell count

Table 5: Ratios of coinfected patients of HIV/HBV in HIV patients

Characteristics	**Coinfection (HIV/HBV)**		**Univariable Model**			**Multiple Variable mode**		
Studied Parameters			**OR**	**95%Cl**	**P-value**	**AOR**	**95% CI**	**P-value**
	Yes	No			<0.01			
Age factor (years)								
< 37	9	10	1 (Referent)		<0.01	1 (Referent)		
> 37	12	4	3	0.180, 1.95	0.42		0.180, 1.90	<0.01
Gender								
Male	7	8	1 (Referent)		0.36	1 (Referent)		
Female	14	6	2.333333333	8.75,1.030	0.1		8.65,1.025	0.2
Mode of acquired infection								
vertical transmissions	6	9	1 (Referent)		0.11	1 (Referent)		
Intravenous drug usage and other modes	15	5	3	8.75,1.29	0.31	2.13		
Duration of diagnosis (months)								
0-6 months	18	2	1 (Referent)		0.39	1 (Referent)		
> 6 months	4	12	3	8.75,2.12	0.76			
CD4 cell count (cell/mm3)								
> 350	5	10	1 (Referent)		0.0008			
< 350	16	4	4					

ranging below 300 cell count (Tortellini *et al.*, 2023).

The results of the study, as mentioned in Table 6, revealed a striking pattern: nearly half of people with coinfected HIV and HBV had CD4+ cell counts between 200 and 350 cells/mm3. This range is important since previous research has repeatedly shown that the development of HIV/HBV coinfection is shown when CD4 cell counts drop below 300 cells/mm3. The results highlight the likelihood of coinfected individuals and emphasize the importance of tracking CD4 counts to determine the course of the illness. These findings provide important information about the dynamics of coinfection with HIV/HBV, assisting physicians in putting focused therapies into practice and improving patient care approaches for this specific population (de Gea-Grela & Moreno, 2023). In the case of HIV-infected patients, 41.66% of the CD4+ cell count was noted in the range of 200-350 cells/mm3. Nearly 55.55% of positive patients showed CD4+ less than 350 cells/mm3.

Ethical Approval

Ethical approval was obtained from the Institutional Review Board (IRB) of the Health Ministry. Written informed consent was obtained from participants before they were directly involved in the study. All the participants were briefed on the objectives of this study and the possible expected outcomes of participating in the study. They were also assured that there was no pressure, that their participation was entirely voluntary, and that they could quit the interview at any stage.

Discussion

HIV, known to target CD4 T cells, causes a significant decrease in these cells, which makes it more difficult for the immune system to coordinate defenses against infections. The decreased number of T lymphocytes is made worse by the extra burden of HBV. Important for eliminating viruses, CD8 T cells are also impacted, most likely as a result of chronic stimulation and weariness from ongoing infections. Immune deficiency is worsened by T-cell depletion, which leaves the host vulnerable to opportunistic infections and impairs the body's ability to develop potent antiviral defenses. Apoptosis, immunological response dysregulation, or direct viral

impacts could be the cause of the observed reduction.

A total of 35 patients between the ages of 0 and 53 years were involved in the study. The average age of the HIV/HBV coinfected patients was 26.07±3.11. There were higher proportions of females (64.28%) than males (35.72%) generally because of the higher proportion of female participants than males and also due to the fact that women are more prone to STDs (Gruszczyńska & Rzeszutek, 2023). STDS are more likely to be transferred from men to women than women to men. It is estimated that AIDS is 2 times more prevalent in women than men (Nguyen *et al.*, 2020). AIDS transmits more opportunistically in vaginal sex as the vagina allows easy passage of HIV infection within the body. A higher proportion of participants (57.41%) between the ages of 22 and 37 years than any other age group. This study states that the prevalence of HIV/HBV coinfection among patients who have been diagnosed for more than 6 months has been 34.2% with 8.75,2.12% value for 95% confidence interval. A systematic study done in some regions of Cairo has shown the prevalence rate of HBV at 2%, while the HIV prevalence rate is reported to be 0.1% (Azzam *et al.*, 2023). The prevalence rate of HIV/HBV is poorly calculated and is not reported in authentic repositories.

HIV and HBV are most likely to co-infect due to their shared routes of transmission that include Intravenous drug usage, sexual practices, including hetero and homosexual practices, vertical transmission during childbirth, and unhygienic surroundings (Mukasa Kafeero *et al.*, 2006). As per the demographic analysis of this study, most of the patients, nearly accounting for 35.71%, acquired HIV/HBV through vertical transmission, i.e., from mother to child. However, the majority of infected patients lie in the age group of 22-37 years, which may be due to asymptomatic infections. In many cases of HBV and HIV, the patients carry the virus without showing any prominent symptoms. The contribution of unprotected sexual practices and intravenous drug usage rises up to 50% of HIV/HBV coinfected cases (Breen *et al.*, 2024). Non-usage of contraceptives, men-men sex, using syringes multiple times for injecting intravenous fluids or drugs, and improper disposal of body fluids, medical waves pave the way to smooth the prevalence of HIV/HBV coinfection (Breen *et al.*, 2024).

The study also showed that nearly 50% of HIV/HBV coinfected patients showed CD4+ cell counts in the range of 200-350 cells/mm3. Previous studies have shown that HIV/HBV coinfection progression is usually evident from the CD4 cell count ranging below 300 cell count (Tortellini *et al.*, 2023).

One of the main reasons behind depleted T-lymphocytes in patients who have been diagnosed for more than 12 months must be the phenomenon of CD8+ T-cells immune exhaustion. Exhaustion of CD8 T-cells can have a major impact on other immune system lymphocytes. Prolonged exposure to antigens and reduced regulatory capabilities are common characteristics of exhausted CD8 T cells, which might contribute to an immunosuppressive microenvironment for T-lymphocytes (Zhu *et al.*, 2022). The immune exhaustion caused by delayed CD8+ cells may affect how other lymphocytes behave and perform. Exhausted CD8 T cells have the potential to generate cytokines that depress the immune system, like interleukin-10, which can limit the function of other immune cells. Furthermore, worn-out CD8 T cells are less efficient at eradicating aberrant or infected cells due to their decreased cytotoxicity, which permits these cells to endure and possibly influence other lymphocytes (Chaudhary *et al.*, 2022).

The depleting trend for CD45RA and CD45RO also proves the fact that both HIV and HBV infections are chronic and may have an impact on memory T-cell exhaustion and the transition of memory T-cells to a naive T-cell phenotype. In addition, these viruses' combined impacts on immune response pathways may have a cascading impact, accelerating the depletion of T cells and changing the delicate equilibrium between memory and naive T-cell populations (Fromentin & Chomont, 2021).

These results enable us to calculate the variations exhibited by multiple sub-populations of T-cell lymphocytes, allowing us to understand the underlying mechanism that suppresses the immune response in patients, increasing severe symptoms and, in some cases, leading to mortality. It also helps us to design therapies to maintain the T-lymphocyte population to strengthen the immune system (de Gea-Grela & Moreno, 2023).

CONCLUSION

This study showed a prevalence of 8.4% HIV/HBV coinfection among clinically diagnosed HIV-1 positive patients respectively. The study also showed significant differences in the immunologic and virological responses of patients with HIV mono-infections and patients with HIV/HBV coinfections. It was seen that females are more susceptible to HIV/HBV coinfection, and the most susceptible age group was found to be in the range of 22-37 years. The T-cell depletion was seen to be double in HBV and HIV mono infections. The study, therefore, concludes that HIV/HBV coinfections significantly affect the immunologic and virological responses of patients and the T-lymphocyte population.

LIMITATIONS

The sample included in the study may not be able to represent the whole population affected by coinfection of HIV/HBV, and so it contains the potential to be biased. The study was cross-sectional and may limit inferences and social stigmas. Due to the sensitive nature of the study, it requires ethical concern and careful handling of participants.

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