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Evaluation of absolute CD4+ and CD4/CD8 ratios before and after antiviral treatment in HIV patients: A single-center experience

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ABSTRACT

Aim: The human immunodeficiency virus (HIV) is a disease with dramatic effects on global health policies. The absolute CD4 and CD4/CD8 ratios are better biomarkers for more accurately describing overall immune dysfunction and disease progression, response to therapy, morbidity, viral suppression, and mortality. Therefore, we aimed to evaluate the absolute CD4+ and CD4/CD8 ratios before and after treatment. In addition, the characteristics of HIV-RNA-positive patients were evaluated.

Method: In this single-center retrospective study, 50,047 anti-HIV tests performed between January 2022 and September 2022 at Samsun University Training and Research Hospital were evaluated. The absolute CD4 and CD4/CD8 values of 94 patients treated in our center were evaluated before and after treatment. In addition, the characteristics of 276 patients who underwent HIV RNA testing during this time were evaluated.

Results: At the specified time, the anti-HIV positivity prevalence was 0.14%. When we compared the absolute CD4 count and CD4/CD8 ratio before and after treatment, we found that both values increased significantly after treatment in the control tests performed in the follow-up of the treatment process. Of the 276 people who underwent HIV RNA testing, 154 (55.80%) were found to be positive. A significant relationship was found between HIV-RNA positivity prevalence and gender. The prevalence of HIV-RNA positive patients was higher in men.

Conclusions: To the best of our knowledge, this is the first study evaluating the HIV RNA positive prevalence, the absolute CD4, and the CD4/CD8 ratio in Samsun. Our results will aid in the management of HIV patients.

Key words: Human immunodeficiency virus (HIV), HIV RNA, absolute CD4, CD4/CD8.

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Introduction

Acquired Immune Deficiency Syndrome (AIDS) is a disease with worldwide implications for global health policies, the social economy, population behavior, and academic research. The human immunodeficiency virus (HIV) causes AIDS. High-, middle-, and low-income countries are not immune to the pandemic, as there are so many different ways HIV can be transmitted sexually, by blood, and from mother to child [1]. Govender et al. stated that more than 0.5% of the world's population is infected [2]. Brooks et al. predicted in 2012 that by 2020, more than 50% of people living with HIV infection will be 50 years of age or older [3]. It has been reported that there are approximately 36.7 million people living with HIV (PLWH), and approximately 2.1

million of these are children younger than 15 years of age. 71% of all patients received antiretroviral therapy (ART), and 62% of new infections were from high-risk populations and their sexual partners [4]. The number of AIDS cases reported to the Ministry of Health from 1985 to 2019 is 24,881 in Turkey [5]. Despite effective antiretroviral therapy (ART), PLWH have a higher incidence of non-AIDS-related morbidity, such as cardiovascular disease and many malignancies, than non-HIV-infected individuals [6]. Patients receiving ART have a permanently lower life expectancy than those in some parts of the world who are not HIV-infected and have a weak immune response [7]. These differences may be due to many factors, such as lifestyle, viral coinfections, and persistent immune dysfunction due to HIV [8]. Chronic immune activation and inflammation leading to immune aging have been observed to be associated with morbidity and mortality, and biomarkers of activation and inflammation remain elevated in patients even years after effective ART [9].

The diagnosis of HIV infection is usually based on antibody tests. Sometimes to show the presence of the virus and sometimes to make a treatment decision, quantitative detection of HIV RNA is recommended with a CD4 count [10]. The absolute CD4 count is important as an indicator of HIV infection management, immune function determination, and opportunistic infection prophylaxis [11]. The HIV viral load and consequent reduction in absolute CD4 T cells have been recognized as biomarkers for HIV immunosuppression and response to therapy. However, with successful ART and viral suppression, absolute CD4 count and HIV viral load may not accurately reflect the risks faced by PLWH because immune dysfunction persists despite normalization of CD4 counts [12]. Therefore, recently, the CD4/CD8 ratio has been used as a good biomarker to more accurately describe general immune dysfunction, disease progression, and response to therapy, viral suppression, morbidity, and mortality [13]. A CD4/CD8 ratio of <1 is associated with biomarkers of activation and inflammation and is a predictor of both AIDS and non-AIDS morbidity and mortality [14].

Therefore, we aimed to evaluate the absolute CD4+ and CD4/CD8 ratios before and after treatment in Samsun. In addition, the characteristics of HIV-RNA-positive patients were evaluated.

Materials and metods

Study population

In this single-center retrospective study, 50,047 anti-HIV tests performed between January 2022 and September 2022 at Samsun University Training and Research Hospital were evaluated. During this period, a total of 276 people were tested for HIV RNA, including those who tested positive for anti-HIV at our center or another center and those who were asked to determine the HIV RNA load. During this period, the treatment and follow-up of 94 patients with total HIV RNA positivity were performed in our hospital. ART treatment regimens were applied according to the diagnosis and treatment guidelines of the Ministry of Health [15]. The absolute CD4 and CD4/CD8 ratios of these patients were evaluated before and after three months of treatment. The principles outlined in the Declaration of Helsinki were followed. The local Ethics Committee of Human Research at the same hospital approved the study (GOKA/2022/7/11).

Flow cytometry analysis

Flow cytometry was used to examine T-cell subsets in a peripheral blood sample. Samples were analyzed first using a CD-45-side scatter

(SSC) plot to map the main hematopoetic cell populations. Further analysis was focused on the CD+3 T-cell population gated on the CD+3-SSC plot. The T-cell differentiation pattern was evaluated on the CD3/CD4 and CD3/CD8 plots. The T-cell-oriented antibody combination applied in the laboratory includes the following antibodies: CD4-FITC/CD8-PE/CD19-ECD/CD3-PC7/CD45-APC. The percentages of CD3+CD4+ and CD4+CD8+ lymphocytes were determined by the lymphocyte distribution gate. 100 ml of Flow-Count fluorostrips (Beckman Coulter) were added to each sample prior to analysis. Absolute CD4 and CD8 T-cell counts were automatically obtained by System II software from the ratio of CD3+CD4+ or CD3+CD8+ lymphocytes to fluorospheres. The CD3+/CD4+/(CD3+/CD8) ratio was used to calculate the CD4/CD8 ratio.

Anti-HIV test

In this study, the ARCHITECT HIV Ag/Ab Combo Test, which is the diagnostic method used for the evaluation of anti-HIV elisa, is a chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of HIV p24 antigen and antibodies to human immunodeficiency virus type 1 and/or type 2 (HIV-1/HIV-2) in human serum or plasma. The ARCHITECT HIV Ag/Ab Combo Assay is a measurement method designed for use in the diagnosis of HIV-1/HIV-2 infection.

HIV-RNA test

For the HIV RNA assay, once the sample was introduced into the automated system, the processes of extraction, amplification, and detection of the nucleic acids took place automatically in a closed system. The COBAS AmpliPrep/TaqMan is based on three principles: i) Sample preparation for extraction of the RNA of HIV type 1. ii) execution of a reverse transcriptase PCR (RT-PCR) to generate complementary deoxyribose nucleic acid (cDNA), and iii) amplification of the cDNA with specific oligonucleotides.

Statistical analysis

The SPSS for Windows 22.0 program was used for the statistical analysis of the study. Continuous variables obtained from the study were given as mean±standard deviation (mean±SD). Categorical variables were expressed as percentage frequencies. Categorical variables were compared with the chi-square test, and continuous variables were compared with dependent and independent sample t tests. p values below 0.05 were considered statistically significant.

Results

In this study, the results of 50,047 anti-HIV tests performed at the specified times were evaluated, and 70 tests were found positive. Accordingly, the prevalence was 0.14%. During this period, the number of patients treated together with patients from different institutions and regions reached 94. Of the individuals with a positive HIV RNA test included in the study, 94 had treatment and follow-up done in our hospital. The patients' average age was 40.3814.15.The majority of patients were male (79.71%). The absolute CD4 ratio decreased in 26 (27.66%) patients, and the CD4/CD8 ratio decreased in 18 (19.15%) patients after treatment. When the absolute CD4 value and CD4/CD8 ratio were compared before and after 3 months of treatment, we found that the absolute CD4 value and CD4/CD8 ratio increased statistically significantly after treatment compared to pre-treatment ($p=0.004$, $p<0.001$, respectively). Information about these patients is given in Table 1.

A Spearman correlation analysis was performed to evaluate the relationship between the absolute CD4 and CD4/CD8 ratios of 94

Table 1. Characteristics of people with HIV RNA positive.

Age (Mean±SD)	40.38±14.15		
Gender Women, n (%) Man, n (%)	 56 (20.29) 220 (79.71)		
	Before treatment (Mean±SD)	**Three months after treatment (Mean±SD)**	***p***
Absolute CD4, cells/micrometer	555.96±375.84	645.94±385.81	**0.004**
CD4/CD8	0.56±0.44	0.78±0.48	**<0.001**

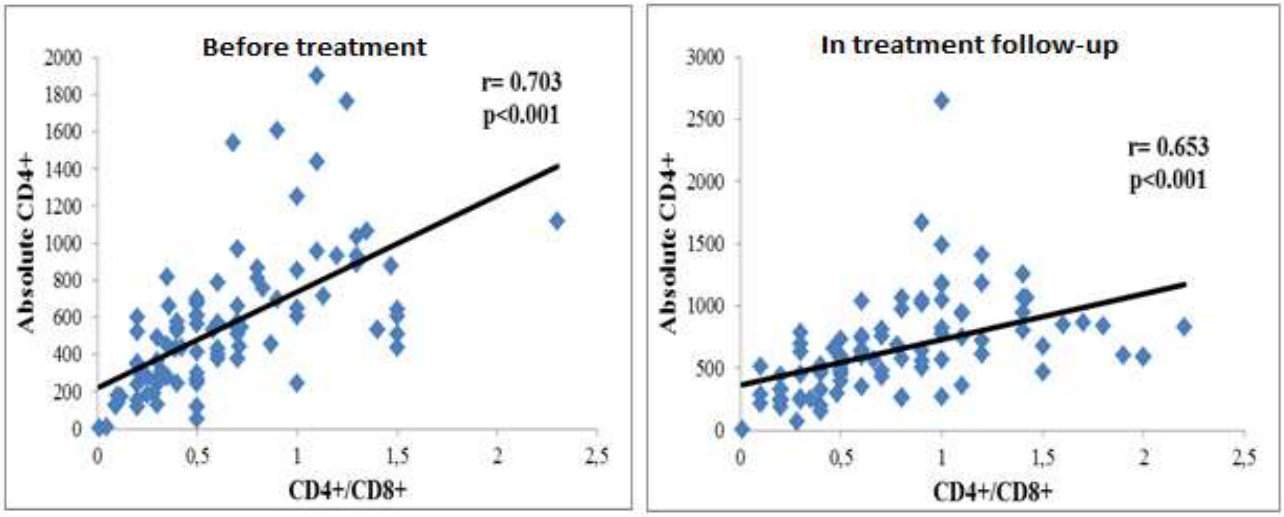

Figure 1. Spearman correlation analysis between absolute CD4 value and CD4/CD8 ratio.

patients followed up in our hospital. A positive correlation was found between absolute CD4 values and CD4/CD8 ratios of 94 patients before treatment (r=0.703) and after treatment (r=0.653) (*p<0.001*).

Table 2. Characteristics of HIV RNA positive patients.

HIV RNA (+) Age, mean±SD	40.69±12.76
HIV RNA test (+) (-)	 154 (55.80%) 122 (44.20%)

Of the 276 people who underwent HIV RNA testing, 154 (55.80%) were found to be positive. The mean age of individuals with positive HIV RNA tests was 40.69±12.76 years.

When we evaluated patients undergoing HIV RNA testing, according to age, there was no significant difference between men and women in terms of age (*p>0.05*). A significant relationship was found between HIV RNA prevalence and gender. Men had a higher prevalence of HIV RNA positivity (*p = 0.005*). Males had a higher mean age than females among those who tested positive for HIV RNA (p =0.05). The youngest of the HIV RNA-positive women was 3 years old, and the oldest was 67 years old. Among men who tested positive for HIV RNA, the youngest was 19 years old, and the oldest was 75. Results are presented in Table 3.

Then, we examined the age distribution of HIV RNA test subjects (Table 4). HIV-RNA

Table 3. Analysis of HIV RNA testing by age and gender.

			p
Age, mean±SD	Women (n=56)	38.55±16.75	
	Man (n=220)	40.84±13.41	>0.05
HIV RNA prevalance	Women (n=56)	22 (40.0%)	
	Man (n=220)	132 (60.0%)	**0.005**
HIV RNA (+), Age, mean±SD	Women (n=22)	35.82±14.59	
	Man (n=132)	41.51±12.30	**0.05**

positivity was highest in the 35-45 age group and lowest in the under 25 age group. Three of those who had HIV RNA tests were younger than 18 years old, and one of them had an HIV-RNA test that was positive.

Table 4. Distribution of people who underwent HIV-RNA analysis by age.

Ages		**n (%)**
≤25 (n=42)	(+)	18 (42.86)
	(-)	24 (57.14)
25-35 (n=66)	(+)	36 (54.55)
	(-)	30 (45.45)
35-45 (n=71)	(+)	45 (63.38)
	(-)	26 (36.62)
45-55 (n=54)	(+)	32 (59.26)
	(-)	22 (40.74)
55-65 (n=29)	(+)	16 (55.17)
	(-)	13 (44.83)
65 < (n=14)	(+)	7 (50.00)
	(-)	7 (50.00)
Total		276 (100)

Discussion

The introduction of ART has brought the life expectancy of HIV-positive individuals closer to that of non-infected individuals and has increased the age of those living with HIV worldwide [16]. The aim of ART is to suppress HIV RNA levels in plasma, increase CD4 cell counts, reduce clinical risks, and prevent the development of drug resistance [17]. According to data from the United States, 81% of infected people taking ART are virally suppressed [18]. Today, the increasing incidence of non-AIDS-related diseases poses a challenge in the management of elderly HIV-positive patients. It has been observed that many organs of HIV-infected individuals are more susceptible to developing inflammatory diseases that directly affect the circulatory, nervous, and metabolic cellular pathways [19]. In a meta-analysis, the worldwide treatment failure rate was 6.08%, but with interregional heterogeneity, it was 7.10% in Africa and 2.55% in Asia [20].

Biomarkers that predict these comorbidities of PLWH have become important for the development and implementation of preventive measures. T cells, which play a fundamental role in the adaptive immune response, enable the activation of naive B cells and plasma cells that secrete neutralizing and opsonizing antibodies [21]. CD4 helper/inducer cells and CD8 cytotoxic/suppressor cells are two T lymphocyte phenotypes characterized by different surface markers and functions. They are found mostly in lymph nodes but are also circulating in the blood [22]. CD4 cells stimulate macrophage activation, recruit phagocytes, primarily neutrophils and macrophages, to the site of infection, and increase the activation of CD8 cytotoxic T cells. The CD4+ count was the most important indicator of mortality risk in HIV positive

patients. Effective ART means that the majority of patients achieve and maintain high CD4+ counts. Naive CD8+ cells, which play an important role in the immune response to viral infections, support their clonal expansion, increase in the number of memory CD8+ cells [23]. CD8+ cell numbers rise following clonal expansion, apoptosis, or senescence as they respond to viruses and CD4+ cell depletion in the gut [24]. The natural course of untreated HIV infection opposes circulating CD4+ and CD8+ lymphocytes. Ratios between 1.5 and 2.5 are generally considered normal, although the normal CD4/CD8 ratio has not been fully defined in healthy hosts. However, gender, age, ethnicity, genetics, exposures, and infections can affect the rate [25].

Before HIV downregulates CD4 cells, a low CD4/CD8 ratio results as circulating CD8 cells typically rise in response to infection [26]. A low or inverted CD4/CD8 ratio is indicative of an immune risk phenotype and is associated with altered immune function, immune aging, and chronic inflammation in HIV-infected and uninfected populations [27, 28]. The incidence of an inverted CD4/CD8 ratio increases with age. Inverse proportions can be seen in 8% of 20-59-year-olds and 16% of 60-94-year-olds [25]. Women are less likely to have an inverse proportion than men across all age groups [25].

Monsalvo et al. noted that an increase in the CD4/CD8 ratio was observed during dual suppressive regimens, and its extent correlated with baseline and prior HIV-related factors [29]. In a study conducted in 3882 HIV-infected individuals treated in Copenhagen between 1995 and 2012, CD8 + cell counts were measured [30]. It was reported that CD8+ cell counts were elevated during HIV infection and did not return to normal despite long-term combined ART. They suggested that significant elevations in CD8+ cell counts after long-term combined ART are associated with increased non-AIDS mortality. Mutoh et al. showed that even after long-term successful combined ART in patients with suppressed viral load, the CD4 count, and CD4/CD8 ratio did not normalize to levels seen in healthy individuals [31]. They stated that the CD4% and CD4/CD8 ratio were not normal in most patients. In a study conducted by Milanés-Guisado et al., annual increases in the absolute CD4, showed weak correlations with CD4% and CD4/CD8 in 1164 patients. However, it was high between CD4% and CD4/CD8 [32].

HIV seroprevalence was 0.11% in Eskişehir Region between 2010 and 2018 [33]. In another study, HIV prevalence was 12% in a study in Istanbul [34]. The prevalence in our study is lower. This difference may be related to the fact that other cities are more crowded. Gezer et al. reported that the 150 patient's baseline CD4/CD8 ratio increased from 0.36 to 0.61 at the 24th week of treatment. At baseline, there were 144 patients with CD4/CD8<1. Those with CD4/CD8≥1 at week 24 after ART were 13.2% [34].

In this study, we aimed to evaluate the absolute CD4 and CD4/CD8 ratios of HIV patients treated in a single center in Samsun before and after treatment. As far as we know, this is the first study conducted in our province. Since our hospital is one of the largest hospitals in the Black Sea region, HIV patients from the surrounding regions come here for treatment. The presence of a decrease in the absolute CD4 and CD4/CD8 ratios after treatment can be interpreted as non-adherence to treatment. However, the absolute CD4 and CD4/CD8 ratios were statistically higher compared to before and after treatment (Table 1). With Spearman's analysis, we found a positive correlation between absolute CD4 and CD4/CD8 ratios before and after treatment. Our results were in agreement with the work of Milanés-Guisado et al. 55.80% of the 276 people who had HIV RNA testing

were positive. HIV-RNA-positive patients had more males (40.0% versus 60.0%) and males had a higher mean age than females. This was consistent with other studies in Turkey [35. 36]. Then we evaluated the people who were positive for HIV RNA tests by dividing them into age groups. The highest incidence was found between 35-45 years of age. The age group with the lowest positivity was under 25.

Conclusions

HIV is the best example of a global health problem. From an epidemiological point of view, two important strategies for HIV/AIDS control are ART and measures for risk reduction.

The introduction of ART for HIV treatment in the early 1990s dramatically changed the course of the infection. The purpose and success of ART regimens are measured by increasing the CD4/CD8 ratio. Our findings aided in determining the efficacy of the treatment methods used in our hospital. We believe that our results will guide clinicians. It would be beneficial to support the results with larger-scale studies.

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