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The effects of HIV on immune cells and correlation with cardiovascular diseases

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ABSTRACT

Although the roll-out of anti-retroviral therapy has slowed down the spread of HIV-AIDS, studies have shown that the treatment can still increase the risk of developing cardio-metabolic issues. In this study, we hypothesized that the effects of HIV on immune cells could contribute to the development of cardiovascular diseases. The goal of our study was to analyze the changes in the T and monocyte cell subsets of the immune system caused by HIV. We also examined the relationship between these changes and the viral load. The study was conducted in Egypt, where 80 participants were recruited. They were divided into groups based on their CD4 count, as well as those who were HIV-nave and those who were treated with cART. The control group had a CD4 count of 500 cells/L, while the group that was HIV-positive had a count of 200 cells/L. The data collected during the study were used to analyze the effects of the HIV treatment on the monocyte and T cells subsets. They were then analyzed using flow cytometry. In addition to these, the researchers also used tissue factor and CD38 to identify the changes in the monocyte subpopulation.The results of the study revealed that the levels of coagulation and inflammation markers significantly increased in the CD4+ and CD8+ T cell populations. The co-expression of these markers also increased.

Keywords: *Immunology HIV , CD4,cardiovascular diseases, lipid*

INTRODUCTION

The challenges faced by healthcare systems globally due to AIDS are partly due to its social impact and health consequences. The main cause of this illness is the HIV virus, which attacks the body's immune system and renders it ineffective[1]. Because of this, individuals with AIDS are at risk of acquiring other infections. HIV can destroy CD4 T cells, which are the part of the immune system that fights against various types of pathogens and cancer cells[2,3].

The natural course of an HIV infection involves three phases. In the acute stage, the virus can cause a rapid decrease in the number of CD4 T cells. In the asymptomatic phase, the count of these cells can decline gradually. A dysfunctional immune system can lead to the development of other infections during the AIDS stage. The duration of the illness's various phases can vary depending on the infected individual[4].

Despite the immense efforts being made to find a cure or a vaccine for HIV, there is currently no effective treatment or prevention of this illness[5]. The development of HIV-mediated cardiovascular disease (CVD) has been shown in various studies[6–8]. Individuals infected with HIV are known to have a higher risk of experiencing a heart attack than those without the virus. Studies also suggest that the roll-out of effective treatment for HIV has led to an increase in the lifespans of people with this condition[9,10].Due to the increasing prevalence of these conditions, the number of deaths caused by sudden cardiac death and pulmonary hypertension has also increased. In 2022, a study revealed that over 86% of the deaths of individuals with HIV were caused by SCD[11]. Although the effectiveness of anti-viral therapy has led to the development of new HIV-infected individuals with longer lifespans, the risk of developing other co-morbidities such as heart disease is still high. This has led to the emergence of CVD as a major cause of death and morbidity in this group. Through studies, it has been revealed that the development of CVD in people with HIV is a complex process that can involve various factors. Some of these include the drug toxicity and the persistence of immune activation[12–14].

The HIV is a member of the lentivirus family. Like other retroviruses, it has a single-strand RNA genome. During its life cycle, this virus makes use of reverse transcription to integrate its DNA into the host genome.Before the virus can replicate, it needs to enter host cells. This happens by targeting CD4 T cells as this cell surface is the viral receptor[15]. The term inflammation refers to the natural process that occurs when a soluble factor or injury triggers the interaction between immune cells and the body's tissues. When a pathogen is identified, inflammatory cells start producing chemokines and pro-inflammatory cytokines. Inflammation usually appears within a couple of weeks following an infection. Once the pathogens are cleared, the immune system's activation levels can return to normal[16].

When acute inflammation is triggered, various types of plasma proteins and immune cells are activated at the site of the infection. These actions are carried out by a group of PRRs that are expressed on phagocytes, natural killer cells, and DCs[17,18]. When the development of inflammation is triggered, certain types of immune cells are stimulated early. These include NK and macrophages, which are known to produce various types of pro-inflammatory cytokines and chemokine receptors[19].The acquired immunity response can then follow. Antigen-specific T-cells can amplify the inflammatory response by producing more cytokines. CD4 T helper cells are responsible for the overall immune response. On the other hand, CD8 T cells are mainly used to kill pathogens and malignant cells. Inflammatory mediators are involved in the movement of immune cells and tissues to the site of the infection. They can also cause blood vessels to dilate and release plasma. In addition, certain types of immune cells can kill the pathogens[20].

When damaged tissue is repaired, these cells can then eliminate the trigger. Through a regulatory process, mediators can switch the mechanisms that contribute to the development of inflammation. These mediators have properties that can stimulate the clearance of mucous membranes and mucosal antimicrobial resistance. This process recruits monocytes and macrophages instead of neutrophils. It plays a role in the anti-inflammatory activity of IL-10, which is involved in the tissue repair process. Continued inflammation if the resolution phase isn't achieved can lead to chronic inflammation[21,22].

The HIV virus can also affect the development of inflammation by causing various types of immune cells to produce a variety of inflammatory cytokines. It is believed that the presence of viral components and HIV can trigger immune activation. A study revealed that when compared with controls, the lower level of HIV virus in the blood significantly increased the inflammation[23].The presence of HIV infection could increase the risk of developing cardiovascular disease (CVD) in treatment-nave individuals. This condition could be triggered by the persistence of inflammation and the development of lipid disorders and coagulation markers. The SMART study also showed that stopping treatment with antiretroviral therapy can

increase the risk of developing cardiovascular disease (CVD). In support, another study revealed that elevated levels of immune activation were linked to coronary atherosclerosis[24,25].

The SMART study also showed that stopping treatment with antiretroviral therapy can increase the risk of developing cardiovascular disease (CVD). In support, another study revealed that elevated levels of immune activation were linked to coronary atherosclerosis[26,27]. It has been known that prolonged low-level HIV infection can also lead to chronic inflammation and immune activation in people with HIV. Douek and Bernley noted that the co-infecting of microbes and the translocation of HIV into the gastrointestinal tract can fuel the development of inflammation and immune system activation. Individuals infected with HIV who are on treatment for their condition could also develop atherosclerosis[28].

Early infection and exposure to the virus can trigger the development of adaptive immunity. When a person is infected with HIV, the virus' particles are taken up by the DCs, which are specialized cells that recognize various types of antigens. These are then broken down into protein fragments[29]. Histocompatibility complex II (MHC-II) and class I MHC provide information to CD4 and CD8 T cells. These two types of cells recognize the antigens presented by these two complexes. When activated, the TCRs of these two types of cells recognize the pathogen[30,31].After activation, the T cells can divide and proliferate to create a clonally expanded population that can recognize different types of antigens. These T cells then secrete various proteins, which can be defined by the specific function of the subsets. During an HIV infection, persistent activation of T cells can lead to the exhaustion of these cells, which can result in the development of a reduced proliferative capacity. This can also affect the production of other cytokines and co-stimulatory substances.[32,33]

As the number of CD8 and CD8+CD28 T cells increases, this can lead to the development of various chronic conditions such as cardiovascular disease and cancer. This is because the diminished ability of these cells to regenerate can result in the establishment of a senile immune system. In addition, the depletion of the CD4 count can result in the emergence of premature aging markers. Lower CD4 counts can be an indication of an increased risk for developing atherosclerosis. In a study, it was revealed that individuals with low CD4 counts had a higher likelihood of experiencing carotid plaques. In addition, it was also found that both loss of function and depletion of CD4 are linked to the development of atherosclerosis[34].Studies have shown that the immune system plays a significant role in the development of HIV pathogenesis. It is believed that the activation of the immune system is the main factor that contributes to the development of this condition. The development of AIDS was not seen in the natural host species, such as the African Green Monkey. On the other hand, pathogenic species, such as the Rhesus macaque, exhibited high immune activation[35].

Treating HIV-infected individuals with retroviral therapy can improve their quality of life and ward off various complications associated with the condition. However, despite its effectiveness, this treatment can still fail to fully restore the immunological recovery. Studies have shown that the presence of chronic HIV infection can also increase the risk of developing cardiovascular disease (CVD). Although previous studies focused on the effects of viral infection on the immune system, they did not examine the relationship between the changes in monocytes, T cells, and their CVD risk markers[10].

This study, which will be conducted in South Africa, is the first of its kind to be carried out in this country. The goal of the study is to analyze the effects of HIV-1 infection on the functioning of various immune cells. It is hypothesized that these changes could contribute to the development of CVD and atherosclerosis.

METHODOLOGY

study sampling

the study amples were collected form several Hosbitals in Alexandria, Egypy during the period of july 2021 to decamber 2021 . All participants were informed of the various procedures

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involved in the study, and all of them signed the consent form. Following the completion of the study, blood samples were obtained from the participants.Participants were selected according to the inclusion criteria, which included individuals aged 18 to 55 years old who were HIV-positive or HIV-negative. Subjects who were infected with tuberculosis or HIV were excluded.The objective of the study was to determine the relationship between the various socio-demographic and health factors of the participants and their HIV-related CVD risk.

The study provided details about the participants' demographics and characteristics. Some of these included their age, and duration of the disease. It also collected information about their cART history, viral load, blood pressure, and diabetes.

A research nurse was then able to collect blood samples from the subjects. They were sent to a nearby laboratory for further analysis. For instance, for the evaluation of CRP, CD4, and lipid profiles, blood samples were analyzed by the NHLS laboratory in 30 minutes.A total of 1 ml of blood was collected during this process. It was then placed in a polypropylene tube, which contained a BD FACS LYSING solution. This was used for the later analysis of two flow cytometric screens. The blood samples were then centrifuged for about 10 minutes. They were then stored at a temperature of 80 degrees Celsius until they were thawed for various analyses. Some of these analyses included the assessment of viral load and lipoprotein subclass.

Cی4 count

BD's TruCOUNT tube was then used to perform CD4 counts. The MultiTEST CD3 FITC, CD8 PE, CD45 PerCP, and CD4 APC reagent was then added to the tube. The mixture was then gently stirred.The samples were then placed in a glass tube, which was then lysed with a BD FACS LYsing solution. After 15 minutes, the samples were allowed to remain in the room temperature. They were then analyzed using a flow cytometer, which was made by BD Biosciences. . It was performed within 30 minutes after the samples were collected.

Viral load (hiv-1 quantitative assay)

The goal of this study was to evaluate the level of viral load (VL) in the blood samples of the subjects. After a period of time, a small amount of frozen plasma was then placed into a micro centrifuge tube. It was then tested using the NucliSense EasyQ HIV-1 v1.2 test. This quantitative test was performed using a combination of molecular beacons and a sequence-based technology. It can detect up to 106 copies/ml of HIV-1. It was carried out at a South African university's laboratory.

Flow-mediated dilatation

FMD can be performed on patients with endothelial dysfunction, an early stage of atherosclerosis progression. It is based on how the endothelium can release nitric oxide to respond to a stress stimulus. The diameter of the brachial artery can also be measured using ultrasound.

The brachial artery diameter is initially evaluated after 5 minutes of ischemia, which can be done by placing a pressure cuff around the forearm. After that, the blood pressure is lowered and the subsequent vasodilation is computed. This method can be used to measure the function of the vascular system.The percent FMD is a measure of the change in the diameter of the brachial artery's flow-media. It is used to determine the function of the vascular system and the NO-mediated vasodilatation.

RESULTS

Patient demographics

The objective of this study was to determine the effects of first and second line HIV treatment on the participants' health. The group was composed of 67 HIV-positive individuals and 15 HIV-negative individuals. The participants ranged in age from 18 to 55 years old, and the majority of them were females. According to the WHO's classification system, about 65% of people living with HIV can be classified into one of four stages: stage 1, 2, 3, or 4. Data collected from patient records revealed that 51% of them have a family history of cardiovascular disease (CVD), while 59% were smokers.

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TABLE 1: participants demographical analysis

Variable	(HIV-) Control (mean ± SD)	(HIV+) Naïve (mean ± SD)	HIVtreated1st line (mean ± SD)	HIVtreated2nd line (mean ± SD)	p-value
Numberofdonor	15	24	21	20	
Age(years)	45±10	35 ±8	36±7	38±11	0.02
Median	50	36	37	36	
SBP(mmHg)	126±12	118±14	125±17	121±19	N/S
Median	120	120	130	115	
DBP (mmHg)	78±6	73±7	78±6	71±10	0.02
Median	80	75	80	70	
HR(minutes)	77±8	76 ±7	75±7	74±7	N/S
Median	80	80	80	75	
CD4nadir(cells/µL)	N/A	553±280	203±163	135±150	<0.01
median		510	195	86	
CD4count(cells/µL)	N/A	548±333	378±295	331±270	0.01
Median		503	332	278	
HIV/DX (month)	N/A	49 ±37	66 ±40	104±45	<0.01
Median		36	63		
TimecART(month)	N/A	N/A	49 ±31	78 ±45	0.02
Smoking(number cigarettes per week	0.6±0.3	0.6 ±0.3	0.4 ±0.3	0.3±0.3	<0.01

Testing the CD4 COUNT in the Samples

Participants were randomly assigned to receive either first or second line HIV treatment. They were shown their CD4 count, ranging from 800 to 3,600 cells/L. The mean count for the HIV-infected group was 548 333, while the HIV-treated group had a mean count of 354 280. When subdividing them into groups based on their CD4 count, the mean count for the HIV-positive groups was 753 219, 353 73, and 127, 76 cells/L. The results of the analysis revealed that the CD4 counts were negatively correlated with various immunological markers.

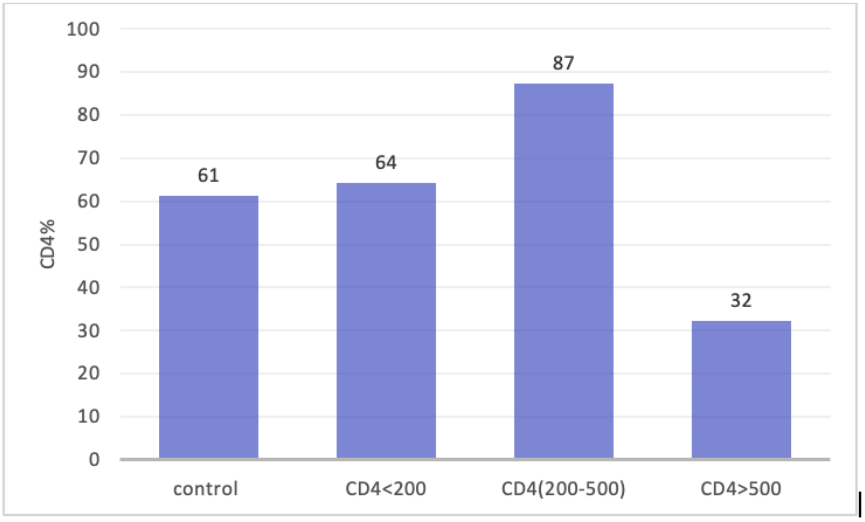

FIGURE 1: CD4 count in HIV positive patients

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TABLE 2: correlation between CD4 count and immunological markers

CD4 count versus;	R-value	p-value
CD8+CD38+ (%)	-0.74	0.005
CD8+CD142+ (%)	-0.34	0.01
CD8+142+38+ (%)	-0.24	0.001
CD4+FOXP3+ (%)	-0.76	0.0001
CD25++FOXP3+SATB-1+ (%)	-0.02	0.4
CD25++GARP+ (%)	-0.33	0.0001
VL (C/mL)	-0.44	0.001

Testing Viral Load in the samples

The mean number of copies/mL of the HIV-positive individuals was 53,532. When subdividing them into three groups, the mean number of copies/mL for the group with the highest CD4 count was 547, while the group with the lowest count was 31. The Spearman correlation analysis revealed that the relationship between the number of copies/mL of the HIV-positive individuals and the CD4 count was negative.

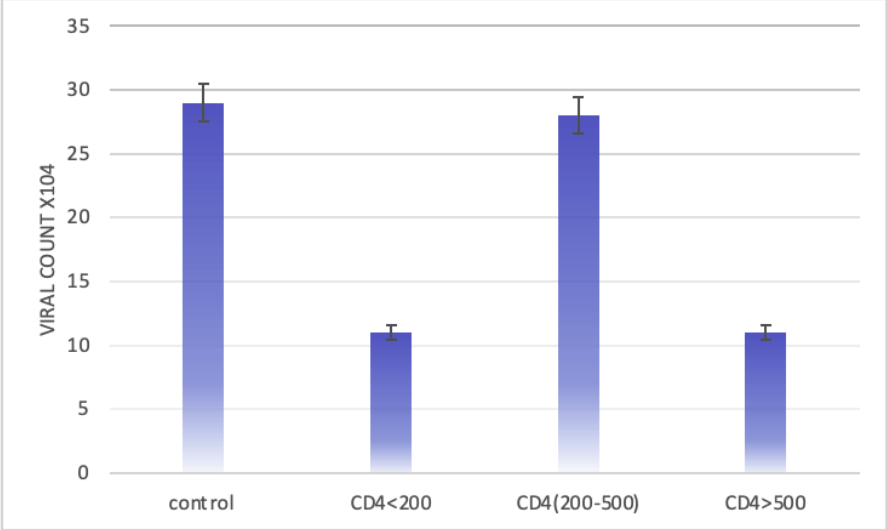

FIGURE 2: viral count in in HIV positive patients

Immune Activation

During the course of an HIV infection, cytotoxic T cells (CD8 T cells) are activated and expand to express certain markers that can help identify the presence of the virus. The analysis of the total number of T cells revealed that the control group had a lower number of total lymphocytes than the other groups. The number of T cells in the groups with high CD4 counts was higher than in those with low counts.

The growth and activation of cytotoxic T cells during an HIV infection are known to help identify the presence of the virus. The analysis of the total number of T cells revealed that the control group had a lower number of total lymphocytes than the other groups. The number of T cells in the groups with high CD4 counts was higher than in those with low counts.

figure 3 summarises the Immune Activation Statusof CD8+CD38+ when Comparisoed with varying CD4 groups. Where both CD4<200 and CD4>500 groups were equal with a percentage 35%. both CD4 (200-500) and control group are over 90%.

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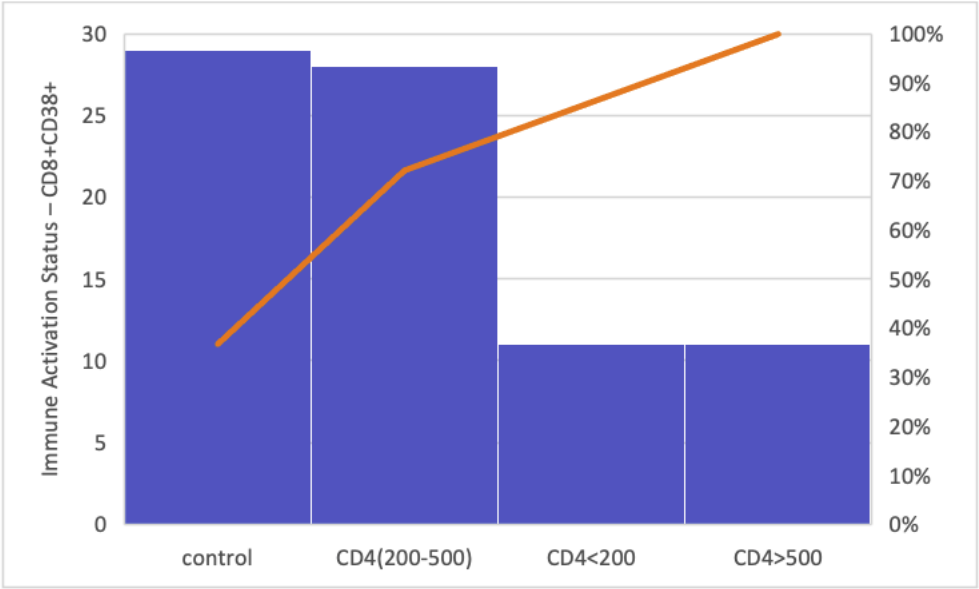

FIGURE 3: Immune Activation Status – CD8+CD38+

The level of immune activation exhibited on CD3+CD8 was significantly higher between the groups that were HIV-positive and those that were non-infected. For instance, the control group's CD4 count was 33 to 11% higher than that of the HIV-positive individuals. On the other hand, the nave groups' CD4 count was 59 to 12% lower. Further analyses revealed that the increase in CD4 count with lower counts was progressive

Correlation between Viral Load and Immune Activation and Coagulation Markers

The correlations between the various factors were also analyzed for the CD8 T cells with different VL versus CD8+CD142 or CD8+CD38+ variants. There was a positive correlation between the two instances. Correlations were then examined between the various markers of coagulation and immune activation. It was concluded that the increase in immune activation and the level of coagulation were positively correlated.

TABLE 3: The correlation between immune activation and coagulation markers and other markers

	CD8++142+		CD8 +38+	
	R-value	P-value	R-value	P-value
CD4 count (cell/µL)	-0.29	0.02	-0.34	0.005
viral load (C/mL)	0.26	0.04	0.27	0.04
GARP (%)	0.49	0.001	0.43	0.001
SATB-1 (%)	0.31	0.008	0.39	0.001
CRP (mg/L)	0.25	0.03	N/S	N/S

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Correlation between Viral Load and lipid content

The usual method was used to determine the plasma levels of various lipid components, such as total cholesterol, HDL, LDL, and triglycerides. The results of the study revealed that the total cholesterol levels of study participants decreased significantly when they were infected with HIV.

The objective of this study was to evaluate the effects of TC on the plasma levels of HIV-positive individuals. In two groups, control groups had lower TC levels than those who were treated with HIV. In the third group, the control group had higher TC levels than those who were treated with HIV. The results indicated that the levels of TG for HIV-positive individuals decreased compared to those in the control group. Further analyses revealed that the groups with different CD4 counts showed significantly different results.

The results indicated that the control group had lower TG than the nave or the HIV-treated groups. On the other hand, the groups with varying CD4 counts had significantly different results. No significant differences were found in the levels of HDL and LDL in the analyses that were carried out.

The study analyzed the effects of HIV infections on HDL levels in the subjects. It divided the group into two: one that was a control group and one that was an HIV-infected group. The study also analyzed the effects of HIV infection on the LDL levels of the subjects. It divided the group into two: a control group and an HIV-infected one. The LDL levels of the HIV-infected individuals with different CD4 profiles were compared with those of the control group.

More detailed analyses performed on the data revealed that the LDL levels and total cholesterol of the study participants decreased. The researchers then performed a correlation analysis between the various immunological markers and the traditional lipid biomarkers. However, no significant results were obtained.

TABLE 4: Correlation between lipid content and immune markers.

HDL	**r- value**	**p-value**
CD14+CD16++	-0.2	0.44
CD14+CD16+	0.16	0.17
CD14+CD16-	-0.18	0.25
CD8+CD142+	-0.12	0.29
LDL	r-value	p-value
CD14+CD16++	-0.16	0.23
CD14+CD16+	0.21	0.33
CD14+CD16-	-0.19	0.23
CD8+CD142+	-0.1	0.36

DISCUSSION

The study revealed that a decrease in the CD4 count and higher levels of immunological activation were observed during infection. In the group with a low CD4 count, the immune system responded significantly. Previous studies have shown that the correlation between the level of immunological activation and the CD4 count is strong. In addition, the immune response is significantly different between the groups with high CD4 counts and those with low counts[20,36]. Even though viral suppression through combination therapy (cART) can reduce the level of immunological activation, it does not eliminate the high levels of immunological activation that were observed in the individuals with HIV. This suggests that the persistence of this response is related to the depletion of CD4 cells[21,22,37].

In 2021, a study conducted by researchers revealed that in HIV-infected individuals, the increased levels of T cell activation were linked to higher levels of inflammation and

coagulation[28,38,39]. This condition, known as RIDS, is more common in people who have a low CD4 count. The findings support the idea that increased immunological activation in HIV-positive individuals is linked to the development of immune dysregulation. This condition is also known to trigger the activation of the coagulation pathways.

In support, the researchers noted that RIDS was a syndrome that was characterized by an increase in T-cell activation and high levels of coagulation and inflammation. It was most common in people who did not increase their circulating CD4 cells.[31] The study also supported the idea that the increased levels of immunological activation in people with HIV are linked to the development and maintenance of immune dysregulation, which can trigger the activation of various coagulation pathways[37,40–42].

CONCLUSION

The study, which analyzed the data from South Africa's Worcester region, showed that in people with HIV, the higher levels of immunological activity were observed in T-cells that had lower CD4 counts. In addition, there was evidence of an increase in the coagulation levels in these cells. The findings support the link between the development of immune disorders and the activation of coagulation pathways. The findings support the idea that the increasing levels of immunological activation in people with HIV are linked to the development and maintenance of immune dysregulation, which can trigger the activation of various coagulation pathways. This condition could therefore lead to the development of atherosclerosis and cardiovascular disease. Treating HIV using combination therapy can lower the risk of developing CVD and decrease the incidence of inflammation and coagulation in people with this condition. However, it is important to note that another group of patients, who have a poor immune recovery, are at high risk of developing inflammation and coagulation.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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