CD4 cell counts, complete blood picture and lipid profile in HIV infected and AIDS patients in a specific populace from South India

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Received 20 August 2017
Accepted 9 September 2017

The Egyptian Journal of Internal Medicine
2017, 29:151–163

Context
AIDS is caused by a retrovirus known as HIV which breaks down the body's immune system leaving the patient vulnerable to a host of life-threatening opportunistic infections, neurological disorders, or unusual malignancies. According to estimates by WHO and UNAIDS, 35 million people were living with HIV globally at the end of the year 2013. The first AIDS case in India was detected in the year 1986. Seldom studies have been conducted correlating these parameters in the Indian population.
Aim
The present study was carried out to evaluate the CD4 cell counts, complete blood picture, and lipid profile in HIV-infected patients and those with AIDS and correlate these parameters with those obtained in the sero-negative controls.
Materials and methods
This was a cross-sectional, hospital-based study. The included participants were divided into three groups: group A consisted of 500 patients who were without any systemic illness as healthy controls, group B consisted of 500 patients who were diagnosed as having HIV infection, and group C consisted of 500 patients diagnosed as having AIDS depending on their CD4 cell counts. Permission from the Ethical Committee of the Institution as well as Superintendent of Government Hospital was taken to conduct the study. Evaluation of CD4 cell counts in patients with HIV infection and AIDS was done using CyFlow counter, whereas complete blood picture, hemoglobin (Hb), packed cell volume, red blood cell and white blood cells (WBCs) and platelet counts, were analyzed using Sysmex XP 100, a compact, fully automated analyzer. Lipid profile was evaluated using an automated analyzer, Erba EM 360 powered by a diffraction grating photometer.
Results
The results were found to be statistically significant, with the *P*-value being less than 0.001, for the CD4 cell counts, Hb, WBCs, and platelet counts. The levels of total cholesterol and low-density lipoproteins (LDLs) were significantly decreased whereas triglycerides and very LDLs were significantly increased in patients with AIDS when compared with the control group and patients with HIV infection.
Conclusion
CD4 cell counts, Hb, WBCs and platelet counts as well as total cholesterol, LDLs, triglycerides and very LDLs were significantly altered in patients with HIV infection and those with AIDS when compared with the controls.

Keywords:
AIDS, CD4 cell counts, complete blood picture, HIV infection, lipid profile

Egypt J Intern Med 29:151–163

1110-7782

Introduction

AIDS is caused by a retrovirus known as HIV which breaks down the body's immune system leaving the patient vulnerable to a host of life-threatening opportunistic infections, neurological disorders, or unusual malignancies [1]. The two known types of this virus include the HIV-1 and HIV-2, which belong to a family of primate lentiviruses [2]. HIV is a spherical virus enveloped by a lipid bilayer of ~90–120 nm in size. The nucleocapsid has an outer icosahedral shell and an inner cone-shaped core enclosing the ribonucleoproteins. The virus core contains the major capsid protein p24, nucleocapsid protein p7/p9, two copies of genomic RNA, and the three viral enzymes (protease, reverse transcriptase, and integrase). The viral core is surrounded by a matrix protein called p17 which lies underneath the virion envelope. Studding the viral envelope are two viral glycoproteins, gp120 and gp41 [2,3]. According to

estimates by WHO and UNAIDS, 35 million people were living with HIV globally at the end of the year 2013 [4]. Based on HIV Sentinel Surveillance (2008–2009), it is estimated that 23.9 lakh people are infected with HIV in India, of whom 39% are females and 4.4% are children. The first AIDS case in India was detected in the year 1986 [4]. HIV is transmitted by both homosexual and heterosexual contact; by blood and blood products; by infected mothers to infants via either intrapartum, perinatal routes, or breast milk; and by occupational transmission. There is no evidence till date that HIV transmission can occur because of exposure to saliva, tears, sweat, and urine [5]. HIV can infect many tissues; however, there are two major targets of HIV infection: the immune system and the central nervous system. Profound immunosuppression, primarily affecting the cell-mediated immunity, is the hallmark of AIDS. HIV enters the body through mucosal tissues and blood and first infects the T cells as well as dendritic cells and macrophages. The infection becomes established in lymphoid tissue where the virus may remain latent for long periods. Active viral replication is associated with more infection of cells and progression to AIDS. In addition to the lymphoid tissue, the nervous system is a major target of HIV infection. Macrophages and microglia cells in the central nervous system that belong to the monocyte and macrophage lineage are the predominant cell types in the brain that are infected with HIV [3]. The incidence and severity of several common cutaneous diseases are increased in patients with HIV, and this correlates in many instances with the absolute number of CD4 T-helper cell counts. The cutaneous manifestations can occur in all stages of HIV disease, and it is a prognostic indicator for development of AIDS [6]. India carries the third largest number of patients with HIV infection in the world after South Africa and Nigeria [7]. In India, the highest prevalence of HIV/AIDS cases has been observed in Nagaland followed by Mizoram, Manipur, and Andhra Pradesh (0.59%) according to the latest national AIDS statistics (NACO, HIV Sentinel Surveillance 2012–2013) [8]. HIV infection causes depletion of CD4 cells in peripheral blood and lymphoid tissues causing CD8 cell dysfunction. Quantification of CD4 helper lymphocytes is, thus, essential in the staging and monitoring of patients infected with HIV [9]. With reduced CD4 cell counts in HIV infection, granulocytopenia occurs. When the counts of granulocytes decrease less than 500/mm^3, in the presence of an attendant anatomical barrier damage that follows the viral infection, invasion of the bloodstream by microorganisms is facilitated, which results in sepsis and death. The CD4+ T lymphocytes are the primary target of HIV infection because of the affinity of the virus to the CD4+ cell surface marker. Infection with HIV leads to a progressive impairment of cellular functions characterized by a gradual decline of CD4+ T lymphocyte levels in peripheral bloodstream, which results in an increasing susceptibility to a wide variety of opportunistic, viral, bacterial, protozoal, and fungal infections and also, to certain malignancies [10]. Hematological abnormalities are amongst the most common complications of infection with HIV [11]. Chronic thrombocytopenia develops in approximately one-third of individuals infected with the HIV during the course of the acquired immunodeficiency syndrome [12,13]. Different studies have been carried out on hematologic parameters and lipid profile in patients infected with HIV in different parts of the world including Nigeria, Brazil, Thailand, Switzerland, and Ghana. In India, few studies were reported from Uttar Pradesh, Chandigarh, Karnataka, Tamil Nadu, and Manipur. Seldom studies have been conducted correlating these parameters in the Indian population. The present study was carried out to evaluate CD4 cell counts, complete blood picture, and lipid profile in patients with HIV infection and those with AIDS and correlate them with the sero-negative controls. The objectives of the present study included to determine the CD4 cell counts in patients with HIV infection and those with AIDS, to determine complete blood picture in patients with HIV infection and those with AIDS, to determine the lipid profile in patients with HIV infection and those with AIDS, and to correlate the values of the same with the healthy controls as against in patients with HIV infection and those with AIDS.

Study design

This was a cross-sectional, hospital-based study conducted between January 2014 and September 2014 in the outpatient Department of Meghna Institute of Dental Sciences, Nizamabad, and the antiretroviral therapy (ART) Centre of Government Hospital, Nizamabad, and was designed to assess the CD4 cell counts, complete blood picture, and lipid profile in the patients with HIV infection and those with AIDS and to compare the parameters with the healthy controls.

Study population

The study population included all the patients reporting to the outpatient Department of Meghna Institute of Dental Sciences, Nizamabad, and the ART Centre of Government Hospital, Nizamabad, who were interested to participate in the study.

Study sample

The present study consisted of 1500 participants attending the outpatient Department of Meghna Institute of Dental Sciences, Nizamabad, and the ART Centre of Government Hospital, Nizamabad. The said participants were divided into the following three groups:

Group A: consists of 500 patients who were healthy controls without any systemic illness.
Group B: consists of 500 patients who were diagnosed as having HIV infection.
Group C: consists of 500 patients diagnosed as having AIDS depending on their CD4 cell counts.

Obtaining approval from the authorities

The permission from the Ethical Committee of the Institution as well as Superintendent of Government Hospital, Nizamabad was obtained before starting the study. Moreover, an informed consent was obtained from the patients forming the study sample to participate in the study to analyze their CD4 cell counts, complete blood picture, and lipid profile. The patients at the extremes of ages, pregnant women, and those on chemotherapy were excluded from the study because of possible weakened immune status. The patients who did not agree to give consent and were not willing to participate in the study were also excluded.

Materials for examination of the patients (Fig. 1):

(1) Mouth mask.
(2) Sterile gloves.
(3) Mouth mirror.
(4) Explorer.
(5) Kidney tray.
(6) Torch light for artificial illumination.

Methods

All the participants of the groups A and B were explained about the study, and a written, signed informed consent was obtained from each patient. The patients were made to sit in the chair comfortably and a detailed history was taken followed by the clinical examination which was performed following the protocols of the Universal Precautions on each participant in the ART Centre with the help of diagnostic instruments and artificial illumination. All the patients in group C were similarly explained about the study and the same procedure was followed. The findings were recorded in a specialized proforma. All the patients were, then, subjected to phlebotomy procedure.

Phlebotomy procedure

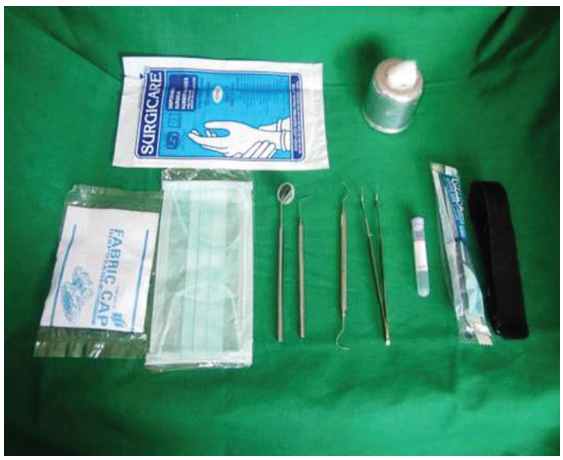
Sample collection

The patient was explained about the procedure. The patient's forearm was rested on the laboratory table comfortably. The antecubital fossa was exposed, and the tourniquet was applied about half an inch to two inches above the antecubital fossa. The area was rendered aseptic with 70% ethyl alcohol, and using a sterile disposable syringe and 23-G needle, a needle puncture was made and maneuvered to enter the antecubital vein and 2 ml of blood was drawn. The tourniquet was then relieved, and the needle was removed. Dry cotton was placed on the site of needle puncture on the forearm, and instructions were given to apply finger pressure for about 5 min and dispose the cotton. The blood was transferred immediately into the tubes containing EDTA (Fig. 2).

Biochemical analysis

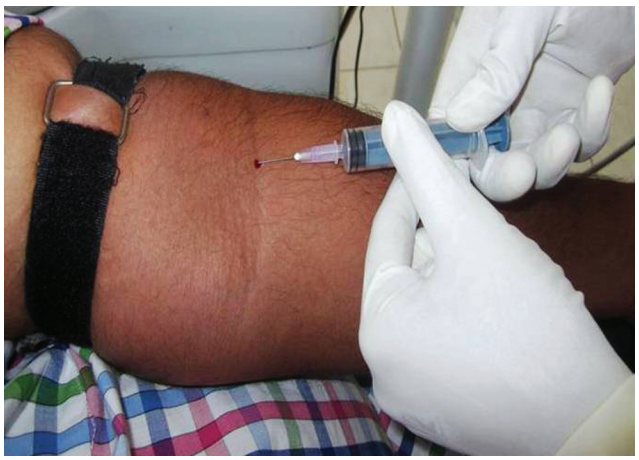
Evaluation of CD4 cell counts in HIV infected and AIDS patients: Evaluation of CD4 cell counts in HIV infected and AIDS patients was done using CyFlow

Figure 1



Armamentarium for sample collection.

Figure 2



Phlebotomy procedure.

Counter by Sysmex Europe GmbH (Fig. 3). 50 µl of EDTA anti-coagulated blood was added to 10 µl of monoclonal antibody. After 15 min of incubation, 1 ml of no lyse dilution buffer was added and the sample tube was attached to the Cyflow counter for automated counting. The results obtained were, then, expressed in the form of a histogram.

Evaluation of complete blood picture, hemoglobin (Hb), packed cell volume (PCV), red cell counts (RBCs), white cell counts (WBCs) and platelet counts, in HIV infected and AIDS patients: Complete blood picture was obtained using Sysmex XP 100, a fully automated, 3 part differential hematology analyzer manufactured in Japan by Sysmex Corporation (Fig. 4). ESR analyzers were manufactured by Diesse Diagnostica, Senesa S.p. A, Italy. 50 µl of blood was taken as the sample. The process was a 2 step procedure. The automated analyzer sampled the blood and quantified, classified and described the cell populations using both electrical and optical techniques. Electrical analysis involved passing a dilute solution of the blood through an aperture across which an electric current was flowing. The passage of cells through the current changed the electrical impedance between the terminals. A lytic reagent was added to the blood solution to selectively lyse the red blood cells (RBCs) leaving only the white blood cells (WBCs) and platelets intact. The solution was, then, passed through a second detector. This allowed the counts of RBCs, WBCs and platelets to be obtained. The platelets were easily separated from the WBCs by the smaller impedance spikes they produced in the detector due to their lower cell volumes. Similarly, optical detection was, also, utilized to gain differential counts of the populations of white blood cells (WBCs). A dilute suspension of cells was, then, passed through a flow cell which passed cells one at a time through a capillary tube past a laser beam. The reflectance, transmission and scattering of the light from each cell were analyzed by software giving a numerical representation of the likely overall distribution of the cell populations.

Evaluation of lipid profile, total cholesterol, triglycerides, high density lipoproteins (HDLs), low density lipoproteins (LDLs) and very low density lipoproteins (VLDLs), in HIV infected and AIDS patients: Lipid profile was evaluated using an automated analyzer, Erba EM 360, powered by a diffraction grating photometer (Fig. 5). Erba is an exclusive partner of Sysmex Corporation, Japan for Hematology analyzers.

Statistical analysis

Comparison of parameters was done using analysis of variance with post-hoc Games–Howell test. A *P*-value of less than 0.05 was considered statistically significant.

Figure 3

Partec Cyflow cell counter for evaluation of CD4 cell counts.

Figure 4

Sysmex XP 100 for evaluation of complete blood picture.

Figure 5

Erba EM 360 for evaluation of lipid profile.

Table 1 Distribution of patients based on age groups

Age group (years)	Control group	Percentage	HIV group	Percentage	AIDS group	Percentage
10–20	40	8	31	6.2	16	3.2
21–30	127	25.4	193	38.6	150	30
31–40	99	19.8	161	32.2	181	36.2
41–50	126	25.2	79	15.8	102	20.4
51–60	73	14.6	21	4.2	38	7.6
61–70	35	7	15	3	13	2.6

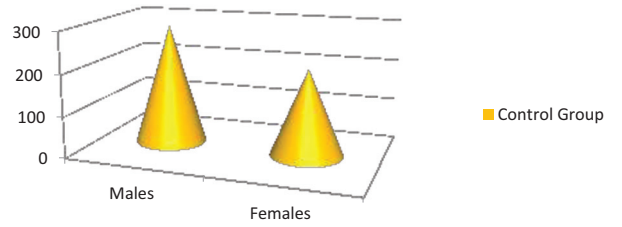
Table 2 Distribution of patients based on sex

Sex	Control group	HIV group	AIDS group
Males	291	235	259
Females	209	265	241

Table 3 Distribution of male and female patients based on age groups

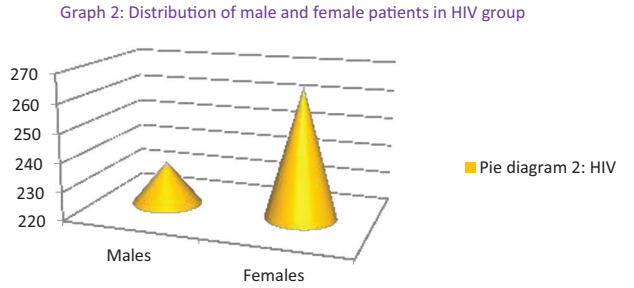
	Control group		HIV group		AIDS group	
Age group (years)	Male	Female	Male	Female	Male	Female
10–20	29	11	11	20	6	10
21–30	79	48	79	114	70	80
31–40	52	47	81	80	90	91
41–50	71	55	41	38	55	47
51–60	38	35	14	7	31	7
61–70	22	13	9	6	7	6

Graph 1



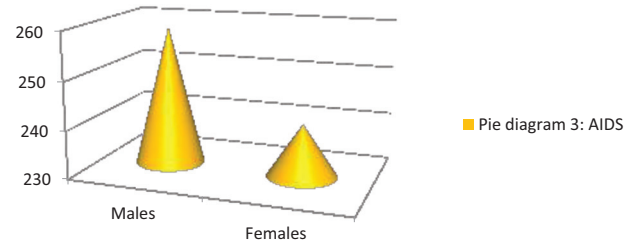
Distribution of male and female patients in the control group.

Graph 2



Distribution of male and female patients in HIV group.

Graph 3



Distribution of male and female patients in AIDS group.

Results

The distribution of patients based on age and sex as well as the distribution of male and female patients based on age is shown in Tables 1–3 and Graphs 1–3.

CD4 cell counts in HIV-infected patients and those with AIDS

The mean CD4 cell counts in the controls was 1125.38, with a SD of 154.73; in the HIV group was 501.35, with a SD of 140.20; and in the AIDS group was 256.41, with a SD of 67.05. The results were found to be statistically significant, with the *P* value being less than 0.001 (Table 4 and Graph 4).

Complete blood picture, Hb, PCV, RBCs, WBCs, and platelet counts in HIV and AIDS groups: a mean Hb value of 13.75, with a SD of 1.76, was observed in the controls; a mean value of 13.38, with a SD of 1.87, was observed in the HIV group; and a mean value of 12.37, with a SD of 1.18, was observed in the AIDS group. The results were found to be statistically significant in this case, with the *P* value being less than 0.001

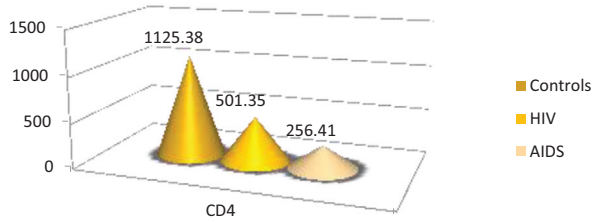
Table 4 Evaluation of CD4 cell counts in the three groups

	Group						*P*-value	Post-hoc test
	Control		HIV		AIDS			
	Mean	SD	Mean	SD	Mean	SD		
CD4 cell counts	1125.38	154.73	501.35	140.20	256.41	67.05	<0.001 (significance)	C>H>A

Table 5 Evaluation of complete blood picture and their mean comparison between the groups

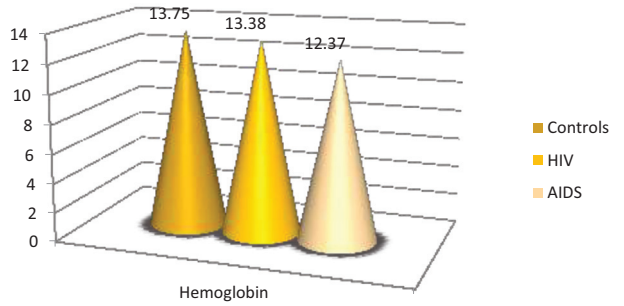
	Group						*P*-value	Post-hoc test
	Control		HIV		AIDS			
	Mean	SD	Mean	SD	Mean	SD		
Hemoglobin	13.75	1.76	13.38	1.87	12.37	1.18	<0.001 (significance)	C>H>A
Packed cell volume	37.88	3.18	38.23	21.24	37.63	5.46	0.614	–
Red cell counts	4.59	0.43	4.57	0.74	4.64	0.73	0.334	–
White cell counts	8134.84	3988.69	9688.40	2813.78	10264.00	5819.57	<0.001 (significance)	H, A>C
Platelet counts	3.37	0.66	3.21	0.64	2.92	1.91	<0.001 (significance)	C>H>A

Graph 4



Mean comparison of CD4 cell counts between the groups.

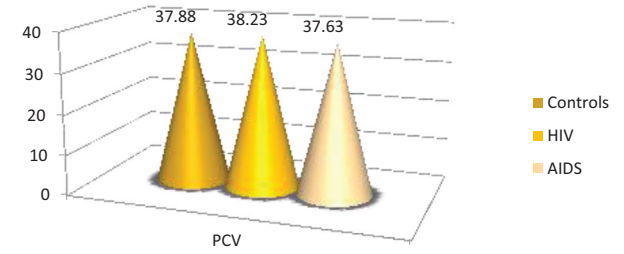
Graph 5



Mean comparison of hemoglobin (Hb) between the groups.

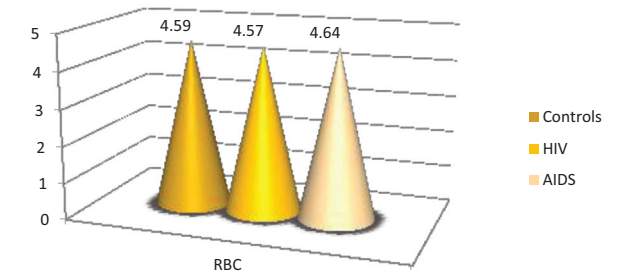
(Table 5 and Graph 5). A mean PCV of 37.88, with a SD of 3.18, was observed in the controls; a mean PCV value of 38.23, with a SD of 21.24, was observed in the HIV group; and a mean PCV value of 37.63, with a SD of 5.46, was observed in the AIDS group. The *P* value was not found to be statistically significant (Table 5 and Graph 6). A mean RBCs of 4.59, with a SD of 0.43, was observed in the controls; a mean value of 4.57, with a SD of 0.74, was observed in the HIV group; and a mean value of 4.64, with a SD of 0.73, was observed in the AIDS group. The *P* value in this case, too, was not found to be statistically significant (Table 5 and Graph 7). A mean WBCs of 8134.84, with a SD of 3988.69, was observed in the controls; a mean value of 9688.40, with a SD of 2813.78, was observed in the HIV group; and a mean value of 10 264.00, with a SD of 5819.57, was observed in the AIDS group. The *P* value in this case was found to be statistically significant, being less than 0.001 (Table 5 and Graph 8). A mean platelet count of 3.37, with a SD of 0.66, was observed in the controls; a mean count of 3.21, with a SD of 0.64, was observed in the HIV group; and a mean count of 2.92, with a SD of 1.91, was observed in the AIDS group. The *P* value in this case too was found to be statistically significant, being less than 0.001 (Table 5 and Graph 9). To summarize,

Graph 6



Mean comparison of packed cell volume (PCV) between the groups.

Graph 7

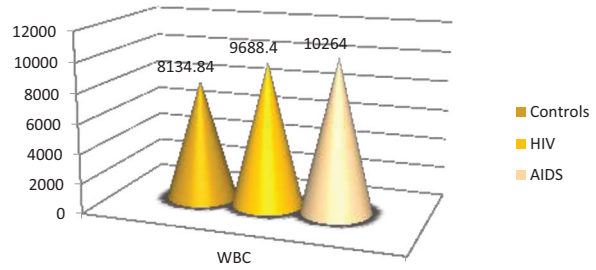


Mean comparison of red cell counts (RBCs) between the groups.

the levels of Hb, WBCs, and platelet counts showed statistically significant results, with the levels of Hb and platelet counts significantly decreased in the AIDS group when compared with the HIV group and the controls, whereas the levels of WBCs were significantly increased in the HIV and AIDS groups as against the controls (Table 5 and Graphs 5, 8 and 9).

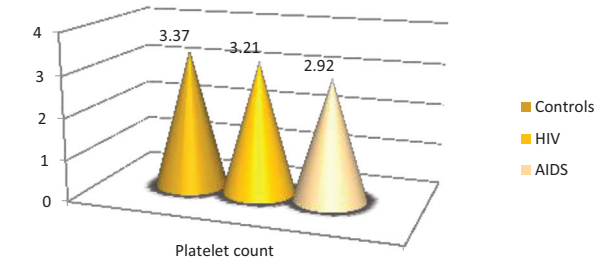
Lipid profile, total cholesterol, TGs, HDLs, LDLs and VLDLs, in HIV-infected and AIDS groups: a mean cholesterol value of 219.49, with a SD of 37.46, was observed in the controls; a mean value of 219.29, with a SD of 43.01, was observed in the HIV group; and a mean value of 200.18, with a SD of 39.36, was observed in the AIDS group. A *P*-value of less than 0.001 was observed between the three groups and was found to be statistically significant (Table 6 and Graph 10). A mean TGs value of 158.23, with a SD of 49.20, was observed in the controls; a mean value of 140.88, with a SD of 67.79, was observed in the HIV group; and a mean value of 167.43, with a SD of 75.40, was observed in the AIDS group. A *P* value of less than 0.001 was observed between the three groups and was found to be statistically significant (Table 6 and Graph 11). A mean HDLs value of 46.57, with a SD of 22.54, was observed in the controls; a mean value of 45.05, with a SD of 17.84, in the HIV group; and a mean value of 45.69, with a SD of 14.70, in the AIDS group. The *P* value in this case was not found to be statistically significant (Table 6 and Graph 12). A mean LDLs value of 144.09 with a SD of 43.44 was observed in the controls; a mean value of 138.47, with a SD of 46.48, in the HIV group; and a mean value of 119.28, with a SD of 27.89, in the AIDS group. A *P* value of less than 0.001 was observed between the three groups and was found to be statistically significant (Table 6 and Graph 13). A mean VLDLs value of 32.55, with a SD of 8.62, was observed in the controls; a mean value of 32.08, with a SD of 10.30, in the HIV group; and a mean value of 37.27, with a SD of 11.09, in the AIDS group. A *P* value of less than 0.001 was observed between the three groups and was found to be statistically significant (Table 6 and Graph 14). To summarize, the levels of total cholesterol and LDLs were significantly decreased whereas TGs and VLDLs were significantly increased in patients with AIDS when compared with the control group and the HIV-infected patients (Table 6 and Graphs 10–14).

Graph 8



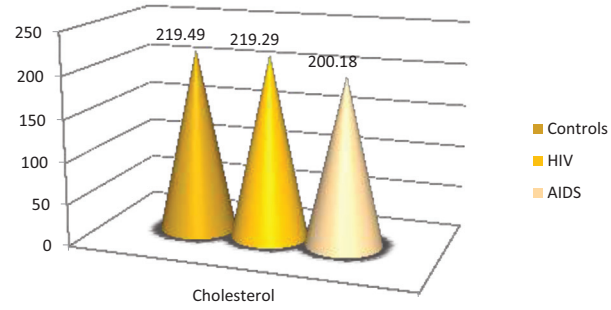
Mean comparison of white cell counts (WBCs) between the groups.

Graph 9



Mean comparison of platelet counts between the groups.

Graph 10



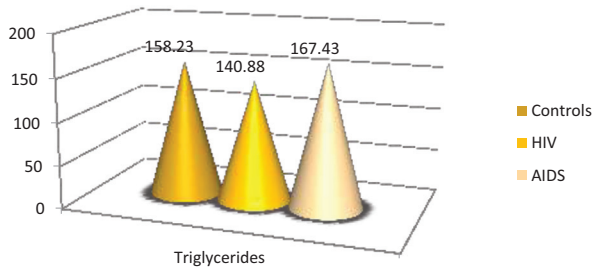
Mean comparison of total cholesterol between the groups.

Table 6 Evaluation of lipid profile and their mean comparison between the groups

	Group						*P*-value	Post-hoc test
	Control		HIV		AIDS			
	Mean	SD	Mean	SD	Mean	SD		
Total cholesterol	219.49	37.46	219.29	43.01	200.18	39.36	<0.001 (significance)	C, H>A
Triglycerides	158.23	49.20	140.88	67.79	167.43	75.40	<0.001 (significance)	C>H; A>H
HDLs	46.57	22.54	45.05	17.84	45.69	14.70	0.497	–
LDLs	144.09	43.44	138.47	46.48	119.28	27.89	<0.001 (significance)	C, H>A
VLDLs	32.55	8.62	32.08	10.30	37.27	11.09	<0.001 (significance)	A>C, H

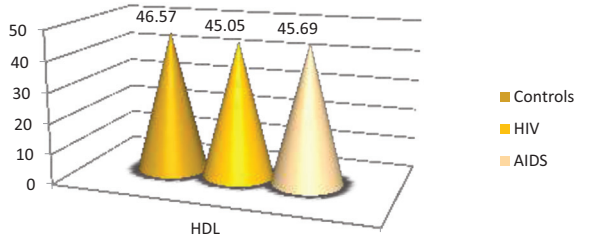
HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Graph 11



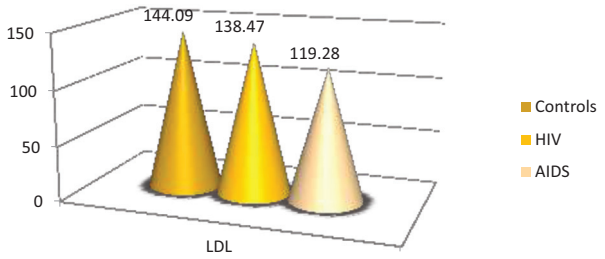
Mean comparison of triglycerides between the groups.

Graph 12



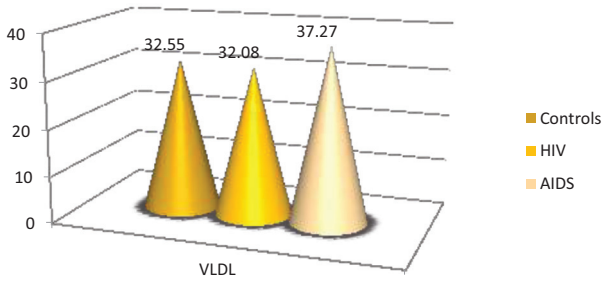
Mean comparison of high-density lipoproteins (HDLs) between the groups.

Graph 13



Mean comparison of low-density lipoproteins (LDLs) between the groups.

Graph 14



Mean comparison of very low-density lipoproteins (VLDLs) between the groups.

Discussion

HIV/AIDS is the most deadly disease which causes devastation to the body by affecting the host's immune system [14]. The pathogenesis of HIV infection is largely attributed to the decrease in the number of T cells (a specific type of lymphocytes) that bear the CD4 cell surface receptors (CD4+). The immune status of a child and/or adult with HIV can be assessed by measuring the absolute number (/mm^3) or percentage of CD4+ cells, and this is considered as the standard way to assess and characterize the severity of HIV-related immunodeficiency. Progressive depletion of CD4+ T cells is associated with progression of HIV disease and an increased likelihood of opportunistic infections and other clinical events associated with HIV including wasting and death. The normal absolute CD4 cell counts in adolescents and adults range from 500 to 1500 cells/mm^3 in blood. In general, the CD4 (%age of CD4+ cells or, absolute count) progressively decreases as the disease advances. In children, individual counts may vary, and assessing the CD4 counts over time is more useful. The CD4 cell counts usually increase in response to an effective combination ART, although this might take many months. The proposed immunological classification outlines four bands of HIV-related immunodeficiency: none, mild, advanced, and severe immunodeficiency. The likelihood of disease progression to AIDS or death without ART increases with increasing immunodeficiency (decreasing CD4 cell counts), opportunistic infections, and other HIV-related adverse conditions, which are related to falling CD4 cell counts, especially, below 200 cells/mm^3 of blood. Response to ART is affected by the immune stage at which it is started, with individuals commencing ART with advanced immunodeficiency (CD4 cell counts >200–350/mm^3) to have better virological outcomes than those who commence with more severe immunodeficiency. Adults starting ART with CD4 cell counts less than 50/mm^3 have a much greater risk of death. On the contrary, adults who commence ART with mild immunodeficiency do not appear to obtain any additional benefits. Pregnancy does affect the CD4 cell counts, although the significance of these changes is not clearly understood, and for practical purposes, the immunological classification remains the same. The present study was carried out to evaluate the CD4 cell counts, complete blood picture, and lipid profile in HIV-infected and AIDS groups and correlate them with those of sero-negative controls.

CD4 cell count is essential for assessment of immune status in HIV-infected individuals as the pathogenesis of AIDS is largely attributed to a decrease in absolute CD4 cell counts [15]. Different methods have been implemented in evaluating the CD4 cell counts by different authors. Chanarat *et al.* [16] used Coulter manual CD4 kit for evaluating the CD4 cell counts. Ghate *et al.* [17] estimated the CD4 cell counts by

using a formula where total leucocyte count was multiplied by lymphocyte %age and divided by 100 and then, multiplied by 100th part of CD4 %age. Pasupathi *et al.* [12] and Srirangaraj and Venkatesha [18] estimated the CD4 cell counts by using fluorescence activated cell sorter (FACS) count system. Sharma *et al.* [9] estimated the CD4 cell counts using flow cytometry (SRL; Ranbaxy). Angelo *et al.* [19] estimated the CD4 cell counts using automated flow cytometer software (multiset). Tiwari *et al.* [20] estimated the CD4 cell counts using flow cytometry absolute cell count system at NPHL. Mbanya *et al.* [21] estimated the CD4 cell counts using conventional flow cytometry using a Becton-Dickinson FACS count. Sen *et al.* [22] estimated the CD4 cell counts using FACS Counter. Edathodu *et al.* [23] estimated CD4 cell counts by standard flow cytometry using FACS Calibur. Pranitha and Kulkarni [15] estimated CD4 cell counts in BD FACS Calibur flow cytometer, an automated multicolor system. In the present study, Partec Cyflow counter was used to estimate the CD4 cell counts as it was relatively small, reputed to be easy to use, and had a high throughput of samples. In the present study, the mean CD4 cell counts in the controls was 1125.38, with a SD of 154.73; in the HIV group was 501.35, with a SD of 140.20; and in the AIDS group was 256.41, with a SD of 67.05. The results were found to be statistically significant, with the *P*-value being less than 0.001. A gradual decrease in the CD4 cell counts was observed in HIV-infected and AIDS groups in the present study when compared with the controls; the mean values were still higher than that observed in the two studies conducted by Pasupathi *et al.* [12,24] who recorded a mean CD4 cell count of 394 in HIV infected patients and 191 in patients with AIDS as well as 375 in HIV-infected patients and 150 in patients with AIDS. However, the results obtained were found in accordance with the results obtained in the studies conducted by Tiwari *et al.* [20], who recorded a mean value of 281 cells/mm^3, and Sharma *et al.* [25], who observed a mean CD4 cell count of 622.4 in HIV-infected patients and 245.39 in patients with AIDS as against 798.81 in the control group. The values obtained in the present study were found to be slightly higher than the values obtained in the study conducted by Sharma *et al.* [9] who divided the patients based on their CD4 cell counts into three groups with group I (10–300), group II (301–600), and group III (>600) and obtained a mean of 163.43 in group I, 325 in group II, and 502.33 in group III. The reason for the higher values obtained in the present study than as compared with most of the studies might be because of the difference in the classification of the patients into HIV infected and AIDS based on the CD4 cell counts. In the present study, HIV-infected patients and patients with AIDS were categorized based on their CD4 cell counts, with 10–350 and 350–500 cells/mm^3 of blood.

The CD4 lymphocytes are the primary target of HIV infection because of the affinity of the virus to the CD4 cell surface receptors (CD4+). Infection with HIV leads to a progressive impairment of cellular functions characterized by a gradual decline in peripheral blood CD4+ lymphocyte levels, which results in an increasing susceptibility to a wide variety of opportunistic viral, bacterial, protozoal, and fungal infections and certain malignancies. Tiwari *et al.* [20] reported that the CD4 cell counts decreased owing to the disruption of the cell membranes of the said cells as HIV buds from the surface or, the intracellular accumulation of hetero-disperse RNAs and un-integrated DNAs takes place with the progression of the disease process. Furthermore, it has also been proposed that an intracellular complexing of CD4 cells with the viral envelope products results in cell killing. Similarly, Tiwari *et al.* [20] proposed untimely induction of a programmed cell death (apoptosis) as an additional mechanism for CD4 cell loss in HIV infection.

Complete blood picture in HIV-infected and AIDS groups: the results of the present study on hematological parameters showed significant reduction in Hb and platelet counts whereas increased WBCs in the HIV-infected and AIDS groups when compared with the controls; however, the results in relation to PCV and RBCs were not found to be statistically significant.

Different methods have been implemented to evaluate the hematologic parameters by various authors. Pasupathi *et al.* [26] estimated the RBC, WBC, and platelet counts and Hb using fully automated hematology analyzer (Pentra XL 80) and observed significant decrease in the RBC and platelet counts and Hb whereas significant increase in the WBC counts in the patients with AIDS compared with the HIV-infected patients and controls. De Santis *et al.* [27] estimated blood cell counts by using an ABX Pentra 120 DX automated hematology analyzer. Sen *et al.* [22] estimated blood cell counts by hematology analyzer and Hb by cyanmethemoglobin method. Tagoe and Asantewaa [28] also estimated the RBC, WBC, and platelet counts and Hb by automated blood analyzer and observed significant decrease in the RBC and platelet counts and Hb whereas significant increase in the WBC counts in the HIV-positive than HIV-negative participants. Pranitha and Kulkarni [15]

estimated the hematology parameters by using an autoanalyzer and observed significant increase in the WBC counts and significant decrease in the platelet counts in patients with AIDS when compared with the HIV-infected patients and controls. In the present study, Sysmex XP 100 automated analyzer was used for the evaluation of complete blood picture, as it has been said to be more reliable, accurate, and less time consuming than other methods. The results of the present study were in accordance with the results of the studies conducted by Pasupathi *et al.* [12] and Dora Mbanya *et al.* [21] who observed decreased levels of RBC counts, platelets, and Hb and increased levels of WBC counts in the HIV-infected patients and those with AIDS. The mean value of Hb was 12.37 in patients with AIDS in the present study, which was in close relation to the Hb level of 11.34 reported in the study conducted by Treacy *et al.* [29] whereas slightly higher than the mean value of 10.20 as reported by Pranitha and Kulkarni [15], a mean value of 10.20 as reported by Tagoe and Evelyn Asantewaa [28], and 10.8 as reported by Kaloutsi *et al.* [30]. The low levels of Hb as well as the RBC counts might be a result of decreased red blood cell production and/or ineffective erythropoiesis seen in the HIV-infected patients and those with AIDS.

Thrombocytopenia observed in the present study was in accordance with the result reported by Erhabor *et al.* [31]. The degree of thrombocytopenia was also found to be directly related to the degree of immunosuppression as was confirmed in the study conducted by Jost *et al.* [32]. Pranitha and Kulkarni [15], Costello [33], and Karcher and Frost [34] also reported the prevalence of thrombocytopenia in their respective studies. According to Pranitha and Kulkarni [15], the mechanism of thrombocytopenia in HIV infection appears to involve increased platelet destruction and ineffective platelet production. Most reports indicate that there is significant platelet sequestration and destruction in the spleen in HIV-associated thrombocytopenia. Platelet destruction is predominant early in the course of the disease process whereas in the later stages, decreased platelet production is assumed to be the major cause of thrombocytopenia observed in the HIV-infected patients and those with AIDS.

Akinbami *et al.* [35] also reported a high prevalence of thrombocytopenia in their study. Possible mechanisms for thrombocytopenia that have been reported are increased platelet destruction either caused by the nonspecific deposition of circulating immune complexes on platelets or by the presence of specific antiplatelet antibodies directed against the platelets as well as direct infection of megakaryocytes by the human immunodeficiency virus with a subsequent decrease in platelet production. The results of the present study were also found to be in accordance with the results of study conducted by Mir *et al.* [36] who reported anemia, thrombocytopenia, and various permutations most HIV-infected patients. According to the results obtained by Walsh *et al.* [37], Karpatkin [38], and Harbol *et al.* [39], chronic thrombocytopenia develops in approximately one-third of the individuals infected with the HIV during the course of AIDS.

The results of the present study, although, were not found to be in accordance with the results found in the study conducted by Tagoe and Asantewaa [28] who observed higher platelet counts in HIV-positive patients compared with the HIV-negative controls. HIV infection is associated with a wide variety of hematological changes because of bone marrow defects and immune cytopenia directly resulting from HIV infection, opportunistic infections or, lymphoma as well as the adverse effects of the drugs used to treat HIV itself or the compounding infections, or lymphoma. Additionally, HIV destruction of CD4+ lymphocytes, which regulate cellular and humoral immunity by interacting with other T lymphocytes, B lymphocytes, macrophages, and natural killer cells, does result in decrease in WBC counts with its associated increased infections in these patients.

Lipid profile in HIV-infected and AIDS group: the present study showed that the lipid profile was altered in HIV-infected and AIDS group. Alteration in the lipid profile occurred even during the early stages of HIV infection and more so, as the disease progressed. Different methods have been implemented in evaluating the lipid profile by various authors. Khiangte *et al.* [1] estimated lipid profile by using enzymatic methods and observed a decrease in HDL and LDL fractions and an increase of VLDL and serum TGs with the progressing disease. Nery *et al.* [40] estimated lipid profile by measuring the total cholesterol, HDL cholesterol, and TGs using an automated enzymatic method. LDL cholesterol was calculated using Friedwald's equation. Pasupathi *et al.* [12] estimated lipid profile by measuring the serum total cholesterol, TGs, HDL, and LDL by a fully automated clinical chemistry analyzer. VLDL cholesterol was calculated by the Friedewald equation. They observed significant decrease of total cholesterol, HDL, and LDL and significant increase in TGs and VLDL in patients with AIDS when

compared with the HIV-infected patients and controls. Anastos *et al.* [41] estimated lipid profile by measuring the serum total cholesterol, TGs, HDL, and LDL using an automated clinical chemistry autoanalyzer. Adewole *et al.* [42] estimated lipid profile by measuring the total cholesterol using ferric perchlorate method whereas HDL was determined after precipitation of the LDL fraction with phospho-tungstate and magnesium, and VLDL cholesterol was calculated from Friedwalds's equation. TGs were measured using the colorimetric enzymatic method. They observed significant increase in the mean LDL and TGs levels whereas a significant decrease in the mean HDL and total cholesterol levels in HIV-infected patients when compared with the controls. In the present study, Erba EM 360 automated analyzer was used for evaluation of the lipid profile as it has been said to be more reliable, accurate, and less time consuming than the conventional methods.

The results of the present study showed that the levels of the total cholesterol and LDL were significantly decreased and the levels of TGs and VLDL were significantly increased in the HIV-infected patients and those with AIDS when compared with the controls, although the results were not found to be statistically significant for HDL in the three groups. Hypertriglyceridemia and a decrease in total cholesterol and HDL cholesterol occurring in advanced phases of HIV infection are considered as markers of a chronic inflammatory process as proposed by Grunfeld [43] and Shor-Posner *et al.* [44]. However, highly active antiretroviral therapy also leads to lipid changes with increases in both TGs and total cholesterol [26]. Infection can increase plasma TGs levels by decreasing the clearance of circulating lipoproteins, a process considered to be the result of reduced lipoprotein lipase or by stimulating hepatic lipid synthesis through increases in either hepatic fatty acid synthesis or re-esterification of fatty acids derived from lipolysis [45]. Other factors that might contribute to dyslipidemia in HIV infection are altered cytokine profile, decreased lipid clearance, and an increased hepatic synthesis of VLDLs. Cytokines such as tumor necrosis factor-α and interleukin-6 appear to promote lipid peroxidation besides endothelial and platelet cell activation and the production of reactive oxygen species [45]. An increase in serum TGs levels is observed in HIV-infected patients as the disease progresses, particularly, in the presence of opportunistic infections possibly owing to an increase in the levels of inflammatory cytokines (tumor necrosis factor-α, interleukins and interferon-α) and steroid hormones. The lower the CD4+ lymphocyte levels in peripheral blood are seen, the higher are the levels of TGs and the lower are the levels of total cholesterol and LDL cholesterol. In contrast, lower levels of LDL cholesterol are found in HIV-infected patients regardless of their CD4+ T lymphocyte counts [45].

Different antiretroviral drugs might be associated with abnormalities in lipid profile. Various studies have shown an association between the use of protease inhibitors (PIs) and dyslipidemia. The prevalence and degree of lipid abnormalities, however, might vary between different drugs within a single class and possibly, with the duration of treatment [46].Young *et al.* [47] carried out a study and concluded that HDL cholesterol levels increase and TGs levels decrease with increasing exposure to non-nuclear reverse transcriptase inhibitors-based therapy whereas TGs levels increase with increasing exposure to PI-based therapy. This might be one of the possible reasons for the patients in the present study to have increased TGs levels as the patients in the present study were on PIs. Different studies on lipid profile carried out in different countries show variations in results. A study by Crook [48] showed that HIV infection is normally associated with hypocholesterolemia, hypertriglyceridemia, and low plasma HDL cholesterol levels. Another study by Pynka *et al.* [49] showed that there was no significant difference in total cholesterol and low-density lipoprotein levels between HIV-infected patients and healthy controls. The results of the present study were in accordance with the results of the study conducted by Iffen *et al.* [50] who concluded from their study an increase in the TGs and VLDL cholesterol in HIV-infected patients compared with the controls. The probable reason given by Iffen *et al.* [50] for the increase in TGs and VLDL cholesterol levels in their study was that increased tumor necrosis factor and other cytokines which occur during the said infection increase lypolysis and insulin resistance. Insulin regulates the uptake of glucose into the skeletal muscle tissue and other cells in the body. As insulin sensitivity decreases in HIV-infected patient with reduction in CD4 cell counts, uptake of glucose into the skeletal muscle tissue and other cells is reduced leading to increased free fatty acids in the circulation and reduced storage of TGs in the adipose tissues. These free fatty acids return to the liver where they are sent back into circulation as triglycerides. Thus, significantly higher TG levels are seen amongst HIV sero-positives compared with the sero-negative controls. VLDLs are composed predominantly of TGs. That is the reason for VLDL to be elevated when the levels of TGs are increased.

According to El-Sadir [51], patients with lower CD4 cell counts of below 200 cells/mm^3 of blood were associated with elevation in VLDL cholesterol and TGs levels ($P<0.05$). This observation was found to be in agreement with the findings from the present study. VLDL cholesterol carries fats around the body, and its elevation can increase the risk of heart disease. Grunfeld [43] also observed decreased total cholesterol levels in both HIV-infected patients and those with AIDS. The results of the present study were, also, found to be in accordance with the results of the study conducted by Pasupathi *et al.* [12] who observed decrease in serum levels of total cholesterol and LDL cholesterol and increase in levels of TGs and very LDL cholesterol in HIV-infected patients and those with AIDS when compared with the controls as against the results obtained in the study conducted by Akpa *et al.* [52] who found increased mean total cholesterol and LDL but decreased TGs and HDL levels in their study and Adewole *et al.* [42] who observed increased total cholesterol, TGs, and HDL levels in HIV-positive patients when compared with HIV-negative patients in their study. The probable reason for lack of association might be related to the close similarity in the CD4 cell counts as most patients were in the CD4 cell count range of 50–220 cells/mm^3 of blood.

Rogowska-Szadkowska and Borzuchowska [53] and Ducobu and Payen [54] determined the levels of plasma TGs, total cholesterol, and HDL cholesterol levels in HIV-infected patients by the level of immunological deficiency according to the CD4 cell counts and concluded that with an increase in the immunological deficiency and clinical development of HIV infection, lipid profile disorders indicated by an increase in TGs levels and decreased concentrations of HDL cholesterol levels intensified. The results of the present study were found to be consistent with the said studies which stated that HIV infection induced an early decrease of cholesterol and a late increase of TGs levels with a reduction of HDL levels. Ducobu and Payen [54] and Crook and Mir [55], however, reported that patients with AIDS had increased levels of LDL cholesterol which contraindicated the results obtained in the present study. Shor-Posner *et al.* [44], also, reported similar findings in which they showed significantly low levels of total cholesterol, HDL, and LDL in HIV-infected patients.

Merits of the present study

The potential points of the present study include that equal number of controls, HIV, and AIDS cases were included following stringent inclusion and exclusion criteria. Automated analyzers were used to evaluate the complete blood picture and lipid profile which were more exact than the traditional methods followed before. Till date, very few studies included these three different parameters in one study. The statistical analysis of the data was done using appropriate statistical tests which included analysis of variance with post-hoc Games–Howell test for comparison of the parameters in between the groups. All the required standardized precautions were taken while phlebotomy procedure was performed. The ethical concern was taken before the start of the study. All the appropriate inclusion and exclusion criteria were followed. A systematic methodology was followed throughout the study starting from sample selection to statistical analysis of the results.

Demerits of the present study

The present study was a cross-sectional, hospital-based study which was designed to assess the CD4 cell counts, complete blood picture and lipid profile in the HIV infected and AIDS patients and to compare the said parameters with the healthy controls. The present study was not a longitudinal study where a patient follow-up could be done and the variations noted.

Conclusion

CD4 cell counts, Hb, WBCs and platelet counts as well as total cholesterol, LDLs, TGs, and VLDLs were significantly altered in HIV-infected patients and those with AIDS when compared with the controls. Further studies are, thus, mandated from across the country with correlation analyses to come to valid conclusions and manage this deadly infectious disease process.

Acknowledgements

The authors thank all the patients who contributed in the study without whom this study would not have been feasible.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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