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Evaluation of serum calcitonin gene related peptide (CGRP) Level in HIV infected patients as an indicator of disease activity

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Abstract

Human immunodeficiency virus (HIV) infection is under global attention due to its rapid spread and high rate of morbidity and mortality. HIV gets an access into the mucosa of genital epithelium through binding to Langerhans cells. While viral load and CD4+ cell count are the main parameters to detect disease activity, new biomarkers are introduced as a potential parameter for monitoring of disease activity in HIV infected patients. Calcitonin Gene Related Peptide (CGRP) is a neuropeptide that is secreted by peripheral neurons at genital epithelia and plays an important role in limitation of HIV transmission and spread to infected CD4+ cells through its effect onto Langerhans cells. This study aimed to evaluate the serum level of CGRP in HIV infected patients and to determine whether CGRP can serve as an indicator of HIV infection activity. The study included 104 HIV patients and 24 normal controls. Patients were divided into four groups. Serum levels of CGRP were measured by ELISA and correlated to viral load and CD4+ cells count for patients in the four groups: primary HIV infection (PHI), chronic HIV infection (CHI) before combinational antiretroviral therapy (cART-naïve), chronic HIV infection after one year of cART-initiation, and chronic HIV infection after two years of cART. Serum levels of CGRP were also measured in sera of controls and compared to patients' groups. Serum levels of CGRP were significantly lower in cART naïve PHI and CHI patients in comparison with normal controls (p<0.05), Also, serum CGRP levels were positively correlated with CD4+ cells count (p<0.01), but negatively correlated with viral load (p>0.05). In conclusion, CGRP could be proposed as an indicator of disease activity in HIV patients.

Keywords: CGRP, PHI, CHI, LCs, cART

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Introduction

Human immune deficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), represents a major global public health issue. In 2013, 35 million people were estimated to be infected with HIV worldwide with 2 million people newly infected in that year.[1]

HIV continues to spread in the Middle East and North Africa (MENA) region[2]. Increasing numbers of HIV-positive persons together with improved access to antiretroviral (ARV) drugs has led to improved survival rates in people living with HIV and AIDS.[2]

During the initial phase of sexual transmission of HIV, the virus crosses the mucosal epithelium that line the vagina, rectum, urethra and eventually spread from these sites to the proximally located lymphoid organs, where it establishes a permanent infection in the host [3].

Genital mucosa like all other mucosal surfaces is equipped to sense threat signals and warn the brain. This protective function is mediated by nociceptors, which are sensory peripheral neurons that innervate essentially all mucosal epithelia and respond to various types of harmful stimuli.[4]

Calcitonin gene related peptide (CGRP) is a 37-amino acid neuropeptide [5], secreted by the sensory peripheral neurons that innervate all mucosal epithelial cells.[6] Langerhans cells (LCs) the resident antigen presenting immune cells which are interspersed in the mucosa of genital tract represents the cellular targets for HIV following sexual exposure and play a crucial role in HIV dissemination.[7]

CGRP plays an essential role in immunomodulation by regulating antigen presenting cells (APCs) and their capacity to express costimulatory molecules and inhibiting antigen presentation for generation of Th1 immunity while enhancing Th2-type immunity. Furthermore, CGRP upregulates the production of IL-10 by LCs.[8] Also, it was found to perform an important role in inhibition of HIV transmission from LCs to infect CD4+ cells as it regulates HIV degradation in LCs.[7]

Three categories of prognostic markers are best documented as having significance in relation to prognosis of HIV infection. These include HIV viral load, CD4+ T-cell levels and plasma levels of soluble markers of immune activation.[9] These markers include CXCL9, CXCL10, sIL-2R, and sCD14 which may be useful as a surrogate marker to monitor immune activation in HIV patients on cART during disease progression.[10] Routine measurement of plasma HIV viral load and CD4+ cell count is central to management of HIV infection in industrialized nations. These biological markers have been demonstrated to predict mortality among HIV infected men and women.[11] Initiation of cART is traditionally based on CD4+cell counts. In conjunction with viral loads, they allow prognostication. Moreover, viral loads provide a measure of infectivity; individuals with low or suppressed viral loads have markedly lower transmission rate.[12] The present study aimed to determine whether CGRP serum levels could be an indicator of HIV disease activity or not.

Subjects and Methods

This was a cross sectional study, included 104 HIV patients who attended Abbassia Fever Hospitals and referred to Central Public Health Laboratories during the period from December 2020 - April 2021. Patients enrolled in the study were clinically diagnosed to have symptoms of acute retroviral syndrome at any phase of disease progression and confirmed to be HIV positive by a polymerase chain reaction (PCR) and western blot assay.[13] They were divided into 4 groups; group (i) involved 32 primary/acute HIV cases of HIV (PHI) and combinational anti-retroviral therapy (cART) naïve (naïve indicate that they never undergone treatment for HIV) and were followed after six months of cART initiation, group (ii) involved 32 chronic HIV cases (CHI) cART naïve, group (iii) involved 24 chronic cases after one year of cART, group (iv) involved 16 chronic cases after two years of cART. Finally, 24 subjects matched for age and sex were included as a normal control group. Patients with history suggestive of chronic infections, cancers, immunological disorders, or receiving immunosuppressive therapy were excluded from the study.

Ethical considerations

The study protocol was reviewed and approved by the Medical Ethics Committee of the Faculty of Medicine, Ain Shams University, Cairo, Egypt (FMASU MD 102/2020). A signed consent form was obtained from each study participant before including in the study.

Sample collection

A blood sample (6 ml) was collected from each patient under complete aseptic conditions. Of these, 2 ml were placed in an EDTA tube for measuring viral load by PCR, 2 ml left to clot then centrifuged and sera collected and stored in aliquots at -20 °C until used in ELISA testing for CGRP serum levels, and the remaining 2 ml placed in EDTA tubes and sent for measuring CD4+ cell count by flowcytometry. In addition, a blood sample (2 ml) was collected from each control subject, centrifuged and sera kept at -20°C until used in ELISA testing for CGRP serum levels.

Quantitation of viral load using a real-time PCR technique

The HIV viral load was measured by commercial real-time (RT)-PCR kits (Catalog No.4513363, Artus HI Virus-1 QS-RGQ Kit, QIAGEN Diagnostics, Hilden, Germany) according to the manufacturer's instructions, using a RT-PCR cycler machine (Rotor-Gene Q for HIV RNA detection and quantitation, QIAGEN Diagnostics, Hilden, Germany).

Measurement of CD4+cell count using flow-cytometry

CD4+ cells count was measured by anti CD4+ monoclonal antibodies CD4+ FITC kits (Catalog No. 340133, BD Biosciences, USA), according to the manufacturer's instructions. The labeled samples were analyzed using a flow cytometer (Becton Dickinson, BD FACSCalibur™, and Company, BD Biosciences, USA) and the BD FAC-Station software.

Determination of CGRP levels using an ELISA technique

The serum CGRP level was measured using commercial ELISA kits (Catalog No. E1061Hu, Human Calcitonin Gene Related Peptide ELISA Kit, Biotech, LTD, China) according to the manufacturer's instructions. Plates were read and interpreted using an ELISA automated system (Lab system, Fenland).

Statistical analysis

Statistical analysis of the results was conducted using SPSS 23.0 statistical software. The mean, standard deviations, and ranges were used to presenting the parametric quantitative data, although the interquartile range (IQR) and median were accustomed to present non-parametric quantitative data. Numbers and percentages were used to represent qualitative variables. Paired t-test was utilized to determine the significance. A *p*-value of < 0.05 was considered statistically significant.

Results

Demographic data

The study included 104 HIV patients (72 males and 32 females), and 24 controls (16 males, and 8 females). The mean age of patients was 40.89 ± 10.70 with a range between 23 and 66 years. While the mean age of the control group was 42.63 ± 11.42 with a range between 27 and 62 years.

The mean value of the viral load of all patients was 4.4 $\log_{10}$ copies/ml. Of the 104 studied HIV patients, 19.2 % had moderate viremia, 42.3% low viremia and 38.5% undetectable level (UDL). The mean value of CD4+ cell count was 400 cell/mm^3, with 39.4% at normal level, 22.1% moderate level, 26.09% low level and 9.6% had count below 200 cell/mm3 (acquired immune deficiency syndrome). The mean level of CGRP was 62.24 pg/ml (with IQR: 34.84-126.64 pg/ml) (Table 1).

Table 1. Median value of viral load, CD4+ cell count and serum CGRP in all study patients

(VL) (copy/ml)	Median (IQR)	4.4 log_{10}
	Range	1.3–5.3 log_{10}
VL (copy/ml)	< 50: UDL	40 (38.5%)
	50 - 100000: Low viremia	44 (42.3%)
	100000 - 1000000: Moderate viremia	20 (19.2%)
	> 1000000: High viremia	0 (0.0%)
CD4+ cells (cell/ ml^3)	Median (IQR)	400 (320 – 590)
	Range	25 – 890
CD4+ cells (cell/ ml^3)	< 200: AIDS	10 (9.6%)
	< 350: Low level	28 (26.9%)
	> 350 - 500 Moderate level	23 (22.11%)
	> 500 Normal level	41 (39.4%)
	NA (not available)	2 (1.9%)
CGRP level (pg/ml)	Median (IQR)	62.24 (34.84 – 126.64)
	Range	5.44 – 267.44

VL: viral load; UDL: undetectable level; AIDS: acquired immune deficiency syndrome; CGRP: calcitonin gene related peptide.

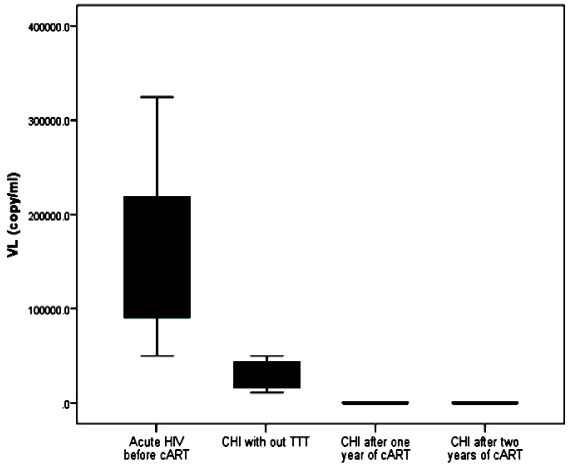
Comparison between the HIV viral load and CD4+ cell counts among the studied groups

For the PHI group, the mean viral load value was 5.04 log_{10} copies/ml, and mean CD4+ cell count value of 380 cell/ml^3. In the cART naive CHI group, the mean viral load value was 4.5 log_{10} copies/ml and mean CD4+ cell count value of 252 cell/ml^3. The viral load and CD4+ cell counts were significantly different among the studied groups (p< 0.0001) except for two groups (CHI after one year of cART and CHI after two years of cART) (Table 2, and Figures 1, 2).

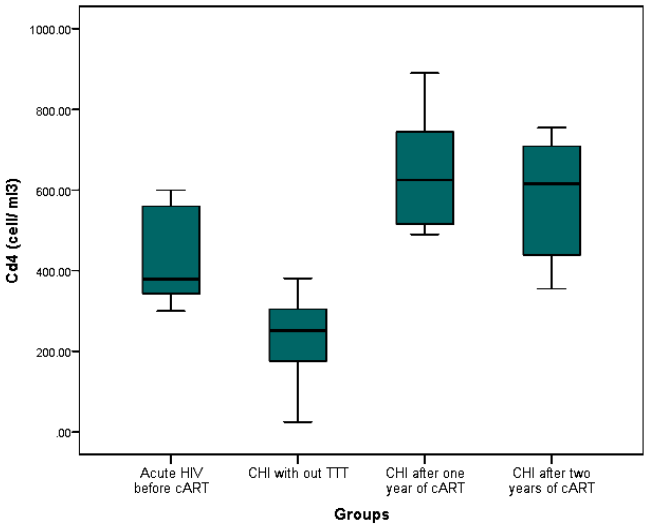
Table 2. Comparison between the viral load and CD4+ cell counts among the studied patients groups.

		PHI	CHI			*p*-value
		before cART	cART naive	After one year of cART	After two year of cART	
		No. = 32	No. = 32	No. = 24	No. = 16	
VL (copy/ml)	Median	5.04 log_{10}	4.5 log_{10}	1.57 log_{10}	1.47 log_{10}	<0.0001
CD4+ cells (cell/ml^3)	Median	380	252	625	615	<0.0001

p-value <0.05: Significant. PHI: primary HIV infection; CHI: chronic HIV infection; cART: combinational antiretroviral therapy.



Figures 1. Measurements of VL count in the studied patients' groups**.**



Figures 2. Measurements of CD4+ Cell count in the studied patients' groups.

Comparison between VL, CD4+ cell count and CGRP levels before and after cART

In PHI group, patients were followed up after six months of cART initiation, the viral load values were significantly declined from 5.04 log_{10} copies/ml to 1.47 log_{10} copies/ml (p<0.0001), and CD4+ cells count significantly increased to normal levels (p<0.0001). CGRP serum levels were markedly increased upon cART initiation (p<0.0001) (Table 3 and Figures 3, 4, 5, 6, 7).

Table 3. Comparison between VL, CD4+ cell count and CGRP concentration among the 32 patients in the acute group (PHI) before and after cART.

PHI		Before	After	*p*-value
VL (copy/ml)	Median	5.04 log_{10}	1.47 log_{10}	<0.0001
CD4+ cells (cell/ ml^3)	Median	380	748	<0.0001
CGRP level (pg/ml)	Median (IQR)	50.84 (31.04 – 77.64)	82.34 (62.34 – 108.84)	<0.0001
	Range	5.44 – 114.84	47.44 – 121.84	

p-value <0.05: Significant; PHI: primary HIV infection; VL: viral load; CGRP: calcitonin gene related peptide.

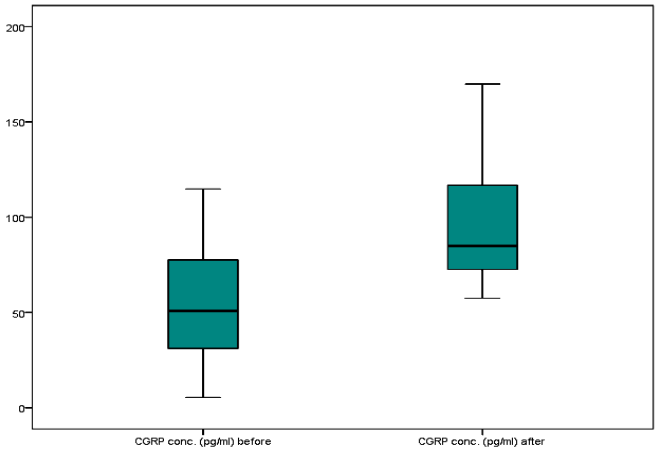


Figure 3. Comparison between CGRP concentration among the PHI group before and after cART.

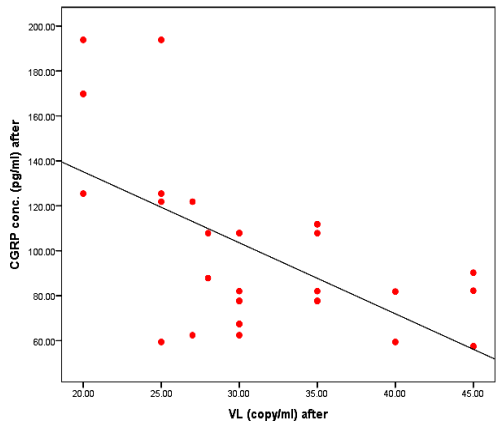


Figure 4. A scatter plot of CGRP levels in PHI group cART naïve against VL (copy/ml).

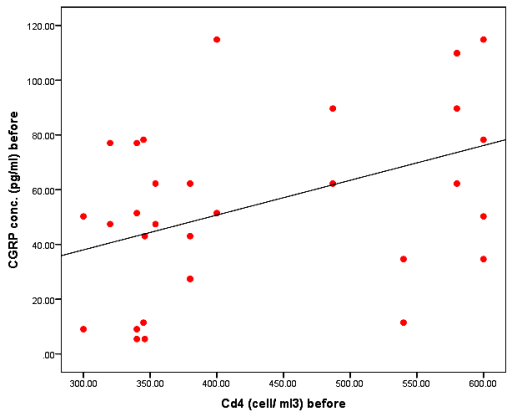


Figure 5. A scatter plot of CGRP levels in PHI group cART naïve against CD4+ cells (cell/mm3).

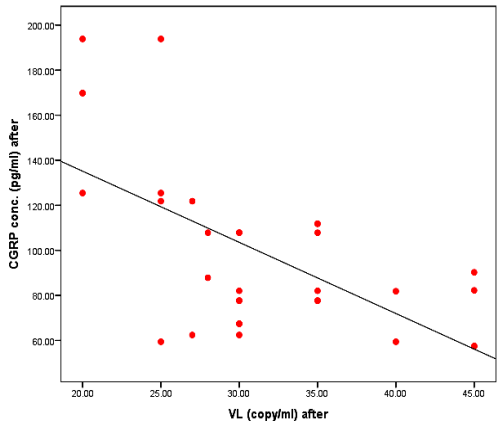


Figure 6. A scatter plot of CGRP concentration against VL (copy/ml**)** in PHI group after cART.

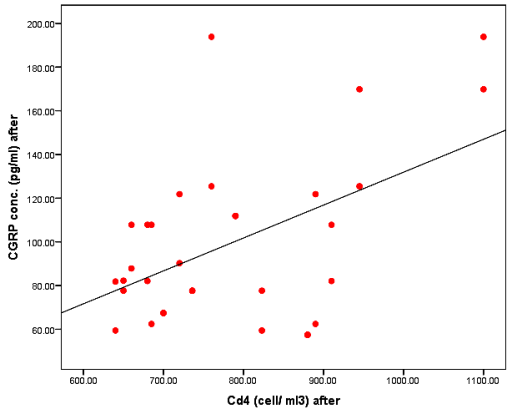


Figure 7. A scatter plot of CGRP concentration against CD4+ cells (cell/mm3), in PHI group after cART.

In CHI group, the viral load decreased from 4.5 to 1.47 log_{10} copies/ml in all patients under cART. However, the CD4+ cell count was near the normal level in contrast with low values in CHI group without cART. The CGRP levels were low in the CHI cART naïve group in comparison to the CHI group after one year and the CHI group after two years of cART. The CGRP highest level was observed in the group after two years of cART. There was a statistically significant difference found between VL, CD4+ cells and CGRP concentrations in CHI group before and after cART. However, there was no difference between CHI groups after one and after two years of cART (Table 4).

Table 4. Comparison between VL, CD4+cell count and CGRP concentration among the CHI groups; cART naïve, after one year and after two years of cART.

		CHI			*p*-value
		cART naïve	After one year of cART	After two years of cART	
		No. = 32	No. = 24	No. = 16	
VL (copy/ml)	Median	4.5 log_{10}	1.57 log_{10}	1.47 log_{10}	<0.0001
CD4+ cells (cell/ml^3)	Median	252	625	615	<0.0001
CGRP conc. (pg/ml)	Median (IQR) Range	40.04 (32.0 – 61.48) 11.44 – 100.64	89.94 (72.04–125.04) 50.24– 167.44	95.84 (70.24 – 143.84) 53.04 – 256.64	<0.0001

p-value <0.05: Significant; CHI: chronic HIV infection; cART: combinational antiretroviral therapy; VL: viral load; CGRP: calcitonin gene related peptide.

Measurements of CGRP values in PHI and CHI groups before and after cART as compared to normal controls

Compared to normal controls, CGRP serum levels were significantly decreased in PHI patients and even further in CHI patients before cART (p <0.0001, Table 5). The CGRP serum levels gradually declined as HIV infection proceeds.

Table 5. Comparison between CGRP levels in the control, PHI, and CHI cART naïve groups.

CGRP conc. (pg/ml)	Control	PHI cART naive	CHI cART naive	*p*-value
	No. = 24	No. = 32	No. = 32	
Median (IQR)	93.04 (78.44–129.84)	50.84 (31.04 – 77.64)	40.04 (32.0 – 61.48)	<0.0001
Range	64.24 – 140.24	5.44 – 114.84	11.44 – 100.64	

p-value <0.05: Significant; PHI: primary HIV infection; CHI: chronic HIV infection; cART: combinational antiretroviral therapy; CGRP: calcitonin gene related peptide.

The mean of CGRP serum levels in the PHI and CHI cART-treated patients was increased upon treatment and reached the normal level as in the control group (p= 0.158) (Table 6, Figure 8).

Table 6. Comparison between CGRP concentration in PHI and CHI groups after cART and the control group.

CGRP conc. (pg/ml)	Control	PHI after 6 months	CHI		*p*-value
			After one year of cART	After two years of cART	
	No. = 24	No. = 32	No. = 24	No. = 16	
Median (IQR)	93.04 (78.44–129.84)	82.34 (62.34-108.84)	89.94 (72.04–125.04)	95.84 (70.24 – 143.84)	NS
Range	64.24 – 140.24	47.44 – 121.84	50.24– 167.44	53.04 – 256.64	

P > 0.05 is not significant (NS). PHI: primary HIV infection; CHI: chronic HIV infection; cART: combinational antiretroviral therapy; CGRP: calcitonin gene related peptide.

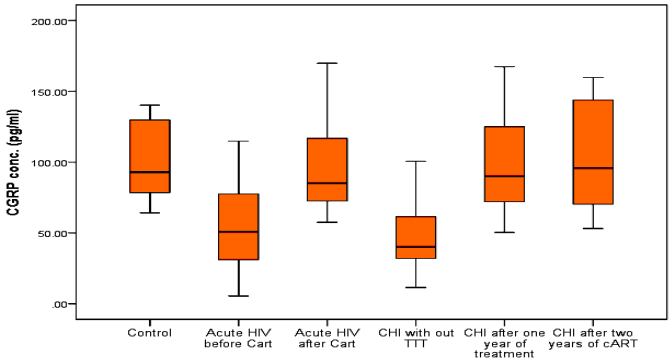


Figure 8. Comparison between CGRP concentration in the studied groups

Correlation between VL level and CD4+ cells in the PHI group

There was a statistically significant inverse relationship between VL level and CD4+ cells in the PHI group after cART**,** Table 7, and Figure 9.

Table 7. Correlation between VL and CD4+ cells in the PHI group.

	CD4+ cells			
	Acute (PHI) cART naive		PHI after cART	
	r	*p*-value	r	*p*-value
VL level	-0.240	NS	-0.468	0.007

P > 0.05 is not significant (NS).

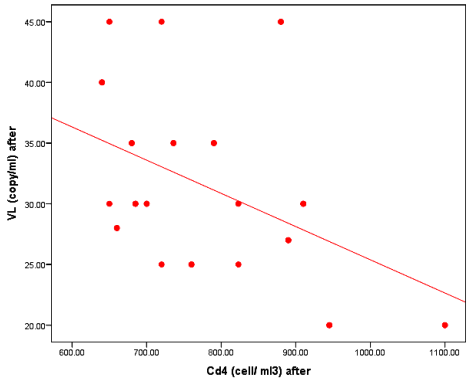


Figure 9. Correlation between VL levels and CD4+ cells in the PHI group.

Correlation between CGRP concentration, VL level and CD4+ cells in the CHI groups

There was a direct relationship between CGRP levels and $CD4^+$ cells in the CHI groups. However, an inverse relationship was observed between CGRP and VL in CHI groups after cART, Table 8.

Table 8. Correlation between CGRP concentration, VL and $CD4^+$ cells in the CHI groups.

	CGRP conc. (pg/ml)					
	CHI cART naïve		CHI after one year of cART		CHI after two years of cART	
	R	*p*-value	r	*p*-value	r	*p*-value
VL	0.099	NS	-0.691	0.000	-0.558	<0.0001
CD4+ cells	0.654	0.000	0.543	0.000	0.762	<0.0001

P > 0.05 is not significant (NS).

Discussion

The present study aimed to determine the role of CGRP as an indicator of disease progression in HIV infected patients by comparing its serum level in patients and normal controls, as well as by correlating its serum levels with measured HIV viral load and CD4+ cell count among the four patients' groups: primary HIV infection (PHI), chronic HIV infection (CHI) before combinational antiretroviral therapy (cART-naïve), chronic HIV infection after one year of cART-initiation and after two years of cART.

In the PHI group, the median of CGRP serum levels was 50.84 pg/mL with IQR (31.04-77.64) and increased significantly after six months of cART to 82.34 pg/mL with IQR (62.34-108.84), this significant increase was consistent with Bomsel et al, 2019[14] who mentioned that serum levels of CGRP were markedly increased in PHI group upon cART from 44.1 pg/mL (28.8-59.4) to 59.8 pg/mL (40.1-79.5).

In the CHI cART naïve group, CGRP serum levels were lower (40.04 pg/ ml) with IQR (32.0 – 61.48) than that in CHI groups after one and two years of cART which showed higher serum CGRP levels (89.94pg/ml) with IQR (72.04–125.04) and (95.84) pg/mL with IQR (70.24 – 143.84) respectively. These results agreed with those of Bomsel et al, 2019[14] who reported that serum levels of CGRP were 28.5 pg/mL with IQR (19.8-37.1) in CHI cART naïve and increased upon cART to 54.3 pg/mL with IQR (23.3- 85.4).

In the current study, CD4+ T-cell count was directly correlated with serum CGRP concentrations in PHI group before and after cART (p =0.015, p =0.003, respectively). On the other hand, viral load was inversely correlated with serum levels of CGRP in the same group before and after cART initiation (p <0.05). These results were in consistent with Bomsel et al., 2019[14] who reported that CGRP serum levels were directly correlated with CD4+ T-cell count and inversely with viral loads.

In our study, the mean serum CGRP concentration in the control group was 93.04 pg/mL, however, Ochoa-Callejero et al., 2021[15] reported that the median level of CGRP in their control volunteers was 220.7 pg/mL. On the other hand, Parlapiano et al., 1999[16] found that the mean plasma CGRP level was 42.80 ±7.12 pg/mL, while Messlinger et al., 2021[17] reported that CGRP plasma levels varied considerably from low <50 pg/mL to very high >500 pg/mL values in apparently healthy subjects. The different levels of serum CGRP in the control groups could be due to the presence of other pathologies, for example sepsis, endocrine tumors, acute psoriasis, or severe pain states other than headaches.[18] This is because nerve regeneration is accompanied by a high expression of CGRP.[19] The wide range of normal CGRP serum levels makes it difficult to detect the change of its level in case of HIV infection or any other associating disorders.[17] Hence, measuring baseline level of serum CGRP in the first visit for HIV patients and its monitoring is mandatory for using CGRP serum level as an indicator of disease activity along with CD+4cell count and viral load.

As the primary source of CGRP is nociceptors, plasma CGRP is generally attributed to spill-over into blood from tissue sites of neuronal release.[4] Decreased levels of

CGRP could be associated with HIV mediated increase in CGRP degrading enzymes [4] and/or factors that inhibit CGRP secretion from nociceptors.[20] In a concurrent manner, as HIV is neurotoxic to peripheral neurons and leads to neuronal apoptosis[21] it could be assumed that uncontrolled HIV infection induces nociceptor loss and subsequently decreased CGRP secretion, which is reflected in plasma.[14]
Also, CGRP serum level was found to be lowered in other viral infections such as COVID-19 and respiratory syncytial virus and its expression was decreased in airway.[22] Also, it was reported that treatment with CGRP has improved severe critical manifestations in these cases.[15] It was shown that CGRP inhibits HIV transmission as it regulates HIV degradation in Langerhans cells,[23] hence, topical application of CGRP to people at risk of HIV transmission might be promising.[3]

In conclusion, we found that CGRP serum levels were markedly decreased in HIV untreated patients in comparison to controls but return to baseline levels upon cART initiation. Therefore, the decreased level of CGRP which directly correlates with CD4+ T- cell count and inversely with viral loads could provide a sign of plasma biomarker for HIV disease activity.

Author Contributions

All authors listed have contributed equally to the work and approved it for publication.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine-Ain Shams University (No. FMASU M D 102 / 2020).

Informed consent

A signed consent form was obtained from each study participant.

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