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Association of protein disulfide isomerase family A, member 4, and inflammation in people living with HIV

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ABSTRACT

Objectives: : Protein disulfide isomerase (PDI) family members are specific endoplasmic reticulum proteins associated with inflammation, obesity, and cancer. In HIV infection, the role of PDI family A, member 4 (PDIA4), is unclear. This study aimed to clarify the association between plasma PDIA4 levels and inflammation in people living with HIV (PLWH).

Methods: : In this study, 287 PLWH and 74 healthy participants were enrolled. The plasma PDIA4 values, demographic data, laboratory data, and other inflammatory markers were recorded. The association between PDIA4 level and inflammatory extent was analyzed using logistic regression and Spearman rank-order correlations. Other results were analyzed using Student's *t*-test or chi-square test.

Results: : In PLWH, the PDIA4 levels were positively associated with the inflammatory markers, interleukin 6 ($r = 0.209$, $p = 0.001$), and tumor necrosis factor-α ($r = 0.162$, $p = 0.01$) levels, but not with high-sensitivity C-reactive protein levels. Moreover, the plasma PDIA4 level of PLWH decreased after anti-viral treatment ($p = 0.0001$).

Conclusion: : Plasma PDIA4 levels are closely associated with inflammation in PLWH and have a positive correlation with the viral load during anti-viral therapy.

Introduction

HIV infection can cause CD4 T-cell depletion and an immunodeficiency state. HIV causes immune activation that is associated with massive CD4 T-cell depletion in the gut-associated lymphoid tissue (Guadalupe et al., 2003). Both innate and adaptive immune responses are involved in HIV infection. Furthermore, cytokines secreted by the T cells changed during infection, and dysregulation of the cytokine profile contributes to the pathogenesis of the disease by impairing cell-mediated immunity (Kedzierska and Crowe, 2001; Ramana, 2014).

Combination antiretroviral therapy (cART) includes the combination of different types of highly effective antiretroviral agents, including nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-NRTIs (nNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), and entry inhibitors. This effective treatment suppresses viral replication and increases the CD4 T-cell counts in most cases, but it only partly reduces T-cell activation, cell death, and soluble inflammatory markers (Lederman et al., 2011; Massanella et al., 2010). The residual chronic inflammation and persistent immune activation may increase morbidity and mortality in people living with HIV (PLWH) receiving cART (Wada et al., 2015). A previous study demonstrated that interleukin 6 (IL-6) and high-sensitivity C-reactive protein (hs-CRP) are linked to a higher risk of subsequent mortality (Neaton et al., 2010). Furthermore, a sub-study of AIDS Clinical Trials Group shows that tumor necrosis factor-α (TNF-α) levels are significantly associated with increased risk of AIDS and non–AIDS-related events (McComsey et al., 2014).

The endoplasmic reticulum (ER) plays a crucial role in protein folding, assembly, and secretion. Disruption of ER homeostasis may lead to the accumulation of misfolded or unfolded proteins in the ER lumen. ER stress markers such as glucose-regulated protein GRP

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78, calreticulin, calnexin, and protein disulfide isomerases (PDIs) are associated with lipid metabolism, insulin resistance, cardiovascular disease, type 2 diabetes mellitus, and obesity (Hotamisligil et al., 2010; Lee et al., 2014). PDIs, oxidoreductases of the thioredoxin superfamily, are expressed mainly in the ER of eukaryotic cells (Appenzeller-Herzog et al., 2008). They participate in the redox-dependent chaperones process, catalyzing the formation and rearrangement of disulfide bonds, which is an important physiological function of the ER (Grek et al., 2014; Okumura et al., 2015). PDIs are also expressed on the cell surface of hepatocytes, lymphocytes, and platelets. Protein disulfide isomerase family A, member 4 (PDIA4), a member of the PDI family, has been found to be located in the cytoplasm and nucleus (Su et al., 2022a; Turano et al., 2002).

PDIs have been implicated as potential therapeutic targets for influenza A and B viruses (Kim et al., 2018). Additionally, PDIA4 is involved in genome uncoating during human astrovirus cell entry (Aguilar-Hernández et al., 2020). The reductase activity of surface-associated PDI is important in the HIV fusogenic process (Markovic et al., 2004). Several studies have indicated that ER stress markers are elevated in the inflammatory state (Galligan et al., 2012; Spee et al., 1999). HIV induces a chronic inflammatory condition; however, there is limited knowledge about the clinical significance of ER stress-induced PDIA4 in PLWH. Hence, this study aimed to assess whether plasma PDIA4 levels are associated with inflammation in PLWH receiving treatment.

Patients and methods

Study population and setting

PLWH are provided with free-of-charge inpatient and outpatient medical services at designated hospitals in Taiwan. These services include management of opportunistic infections, cART prescription, and laboratory measurement of HIV RNA viral load, CD4 T lymphocyte count, viral hepatitis, blood cell count, renal and liver function, glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol, and uric acid levels. Plasma HIV RNA load, CD4 lymphocyte count, and liver and renal function tests are conducted every 3 months in the 1st year of cART initiation. These laboratory monitoring schedules can be adjusted to every 6 months thereafter if the HIV RNA load remains <50 copies/ml. This study enrolled 361 adults who followed up at the Tri-Service General Hospital from July 2018 to June 2019. This study was approved by the Institutional Review Board of Tri-Service General Hospital.

Study design

In this cross-sectional study, we reviewed the medical records, physical examinations, clinical symptoms, and laboratory results of PLWH. HIV treatment-naïve patients aged ≥20 years and eligible for cART were assessed for inclusion in this study. We excluded PLWH who had active opportunistic infections or could not attend regular follow-up visits post cART treatment. Blood samples of PLWH (n = 260) who had received at least three effective cART regimens, such as two NRTIs plus nNRTI, PI, or INSTI for ≥6 months, were obtained once in the analysis. In addition, we enrolled additional PLWH (n = 27,) and their blood samples were collected at three different points: before treatment (baseline), 6-month, and 12-month post cART treatment. Participants in the control group (n = 74) were randomly selected from the health examination department, and their clinical and laboratory data were collected. Traditional inflammatory markers, such as TNF-α, IL-6, and hs-CRP, were also measured using the sandwich enzyme-linked immunosorbent assay method.

Measurement of PDIA4 level

The PDIA4 protein levels were measured using a sandwich enzyme-linked immunosorbent assay kit (USCN Life Science Inc., USA). Briefly, each 100μl plasma sample was directly added to the micro-test plate coated with an antibody specific to PDIA4, followed by incubation at 37°C for 2 hours. Avidin conjugated with horseradish peroxidase was then added to each microplate well and allowed to incubate, followed by aspiration, the addition of 100μl of Detection Reagent A, and incubation for 1 hour at 37°C. After washing thrice, 100μl of Detection Reagent B was added, followed by incubation for 30 minutes at 37°C. After washing five times, 90μl of 3,3′,5,5′-tetramethylbenzidine substrate solution was added, followed by incubation for 15-25 minutes at 37°C. Subsequently, only the wells containing PDIA4, biotin-conjugated antibodies, and enzyme-conjugated avidin changed color. The enzyme-substrate reaction was terminated by the addition of 50μl of sulfuric acid solution, and the color change was measured spectrophotometrically at a wavelength of 450 ±10 nm. The concentration of PDIA4 in the samples was determined by comparing the optical density of the samples to the standard curve.

Statistical analysis

Descriptive continuous variables are presented as means and SDs, and categorical variables as numbers and percentages. Statistical comparisons between the two groups were performed using Student's *t*-test (unpaired *t*-test) or chi-square test, according to the type of data. Biochemical variables associated with PDIA4 levels were analyzed using Spearman rank-order correlations and partial correlation analysis after adjusting for age. Multivariate logistic regression analysis was used to study the independent factors of plasma PDIA4 levels and other covariates. All statistical analyses were performed using GraphPad Prism 7.0 software (version 18.0; SPSS Inc., Chicago, Illinois).

Results

Demographics and biochemical values of healthy participants and PLWH receiving cART

From July 2018 to June 2019, 484 PLWH met the inclusion criteria, and the recruitment strategy is shown in Figure 1. The demographics and biochemical values of the healthy participants and PLWH are presented in Table 1. The PLWH were younger than the healthy participants (38.3 ± 12.2 vs 46 ± 12.8 years; $p < 0.001$) and had significantly lower total cholesterol levels (168 ± 36.9 vs 193 ± 34.1 mg/dl; $p < 0.001$). However, the PLWH showed higher triglyceride (147 ± 20.1 vs 102 ± 47.6 mg/dl; $p < 0.001$), creatinine (0.92 ± 0.18 vs 0.78 ± 0.18 mg/dl; $p < 0.001$), and PDIA4 (24.7 ± 18.8 vs 15 ± 28.1 ng/ml; $p = 0.001$) levels than those of the healthy participants.

Demographics and laboratory values stratified by different treatment regimens

The demographic and biochemical variables of the PLWH stratified according to different treatment regimens are shown in Table 2. Age, body mass index, hepatitis B surface antigen positivity, anti-hepatitis C virus positivity, highly active antiretroviral duration, $\log_{10}$ HIV viral load, CD4 count, CD8 count, CD4/CD8 ratio, white blood cells, and the platelet, fasting glucose, total cholesterol, LDL, high-density lipoprotein cholesterol, triglyceride, creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin, TNF-α, IL-6, and hs-CRP levels were

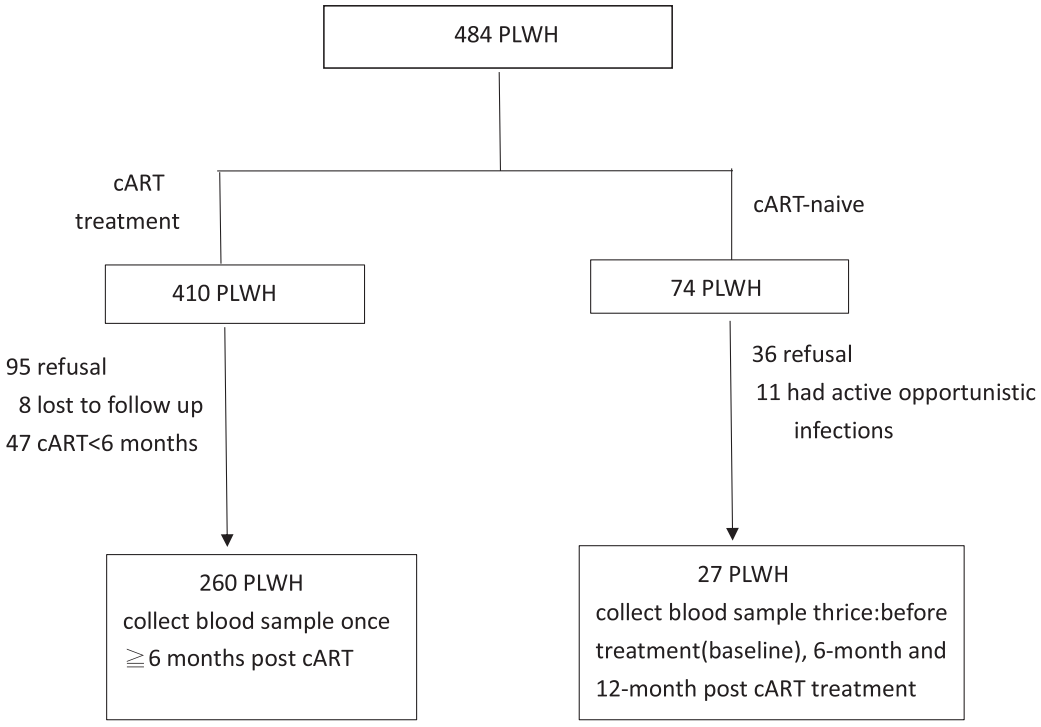

Figure 1. Study flow.
Abbreviations: cART, combination antiretroviral therapy; PLWH, people living with HIV.

Table 1
Demographic and biochemical variables among healthy participants and PLWH receiving combination antiretroviral therapy.

	Healthy participants(n = 74)	PLWH receiving cART (n = 260)	*P*-value
Age, years	46 ± 12.8	38.3 ± 12.2	<0.001*
Body mass index, kg/m^2	23.6 ± 3.4	23.4 ± 3.5	0.679
Fasting glucose, mg/dl	85.97 ± 0.9	97.1 ± 21.2	<0.001*
Total cholesterol, mg/dl	193 ± 34.1	168 ± 36.9	<0.001*
Triglyceride, mg/dl	102 ± 47.6	147 ± 20.1	<0.001*
Creatinine, mg/dl	0.78 ± 0.18	0.92 ± 0.18	<0.001*
Alanine aminotransferase, U/l	21.5 ± 15.6	41.3 ± 21.5	0.428
Protein disulfide isomerase family A, member 4, ng/ml	15 ± 28.1	24.7 ± 18.8	0.001*

Note: The demographics and biochemical values of healthy participants and PLWH are presented. *p < 0.05.
Abbreviations: cART, combination antiretroviral therapy; PLWH, people living with HIV.

similar among the three treatment groups of PLWH. PLWH receiving nNRTI had higher hemoglobin levels than those of the other two treatment groups (15.1 ± 1.2 vs 14.7 ± 1.5 vs 14.7 ± 1.5 g/dl; p = 0.026). PLWH of the INSTI group had higher blood urea nitrogen (15 ± 5 vs 14.5 ± 4.45 vs 13.4 ± 3.36 mg/dl; p = 0.027), and PDIA4 (28.1 ± 24.9 vs 24.9 ± 16.5 vs 19.3 ± 10.7 ng/ml; p = 0.024) levels than those of the other two treatment groups.

Demographic and biochemical variables of cART-naïve, 6-month, and 12-month post cART patients

The demographic and biochemical variables of cART-naïve, 6-month, and 12-month post cART patients are shown in Table 3. PLWH receiving cART 6-month and 12-month dramatically reduce HIV viral load (0.67 ± 1.40 vs 4.83 ± 1.29 $\log_{10}$ copies/ml; p < 0.0001; 0.34 ± 0.66 vs 4.83 ± 1.29 $\log_{10}$ copies/ml; p < 0.0001) and elevation CD4 count (511.6 ± 226.5 vs 287.5 ± 174.3 cells/mm^3; p = 0.0002; 569.0 ± 228.0 vs 287.5 ± 174.3 cells/mm^3; p < 0.0001) compared to naïve status. However, HIV viral load and CD4 count were similar between PLWH receiving cART 6-month and 12-month group. PLWH cART-naïve patients have higher PDIA4 levels than those receiving cART 6-month and 12-month group (77.4 ± 60.1 vs 57.6 ± 39.5 ng/ml; p = 0.02; 77.4 ± 60.1 vs 42.0 ± 30.0 ng/ml; p = 0.0001).

Positive correlation between inflammatory markers and PDIA4 levels in PLWH

To emphasize the association between inflammatory markers and PDIA4 plasma levels, an age-adjusted Spearman partial correlation was used (Table 4). The TNF-α, IL-6, and hs-CRP levels and white blood cell count correlated positively with the PDIA4 levels. Additionally, TNF-α and IL-6 showed significant differences (p < 0.05). In contrast, the platelet count and CD4 and CD8 T lymphocyte counts were inversely correlated with PDIA4 levels.

Factors associated with high PDIA4 levels in PLWH

Table 5 shows an analysis of the potential factors that may influence the PDIA4 levels in PLWH using univariate and multivariate logistic regression analyses. The HIV viral load and TNF-α and IL-6 levels showed a significant positive correlation with elevated PDIA4 levels (p < 0.05), but age, lipid profile, CD4

Table 2
Demographic and biochemical variables of PLWH stratified by treatment regimen.

	nNRTI (n = 123)[a]	PI (n = 57)[b]	INSTI (n = 80)[c]	p-value
Age, years	38.5 ± 12	36.9 ± 11.2	39 ± 12.3	0.591
Body mass index, kg/m^2	23.5 ± 3.84	23.5 ± 3.4	23.8 ± 3.1	0.78
Hepatitis B virus surface antigen positivity, n (%)	15(12.2)	9(15.8)	6(7.5)	0.308
Anti-hepatitis C virus positivity, n (%)	6(4.9)	4(7)	7(8.8)	0.564
Highly active antiretroviral therapy duration, months	30.6 ± 29.2	27.0 ± 28.1	39.4 ± 36.1	0.14
HIV viral load, $\log_{10}$ copies/ml	0.42 ± 0.92	0.24 ± 0.91	0.51 ± 1.05	0.283
CD4, cells/mm^3	546 ± 242	536 ± 259	571 ± 274	0.689
CD8, cells/mm^3	886 ± 398	913 ± 392	902 ± 426	0.905
CD4/CD8 ratio	0.71 ± 0.39	0.68 ± 0.44	0.74 ± 0.44	0.688
White blodd cell, x 10^3 cells/mm^3	5.77 ± 1.61	6.43 ± 1.91	5.95 ± 1.72	0.056
Hemoglobin, g/dl	15.1 ± 1.2	14.7 ± 1.4	14.7 ± 1.5	0.026
Platelet, cells/mm^3	220 ± 53.4	239.7 ± 62.2	228 ± 57.8	0.103
Fasting glucose, mg/dl	94.3 ± 11.3	96.8 ± 15.2	101.5 ± 32.6	0.061
Total cholesterol, mg/dl	179 ± 38.4	168 ± 36.1	164 ± 35.1	0.485
Low-density lipoprotein cholesterol, mg/dl	101 ± 27.5	103 ± 25.9	98.8 ± 27.7	0.66
High-density lipoprotein cholesterol, mg/dl	44.4 ± 10.9	43.8 ± 9.62	40.9 ± 7.93	0.681
Triglyceride, mg/dl	155 ± 128	131 ± 100	148 ± 121	0.471
Blood urea ntrogne, mg/dl	13.4 ± 3.36	14.5 ± 4.45	15 ± 5	0.027
Creatinine, mg/dl	0.92 ± 0.17	0.91 ± 0.17	0.94 ± 0.21	0.632
Uric acid, mg/dl	5.87 ± 1.25	6.13 ± 1.6	6.03 ± 1.37	0.467
Aspirate aminotransferase, U/l	38.2 ± 171	24.8 ± 14.7	22.2 ± 8.81	0.595
Alanine aminotransferase, U/l	26.5 ± 21	31.2 ± 60.8	71 ± 382	0.33
Total bilirubin, mg/dl	0.81 ± 0.69	0.82 ± 0.71	0.83 ± 0.8	0.988
Albumin, mg/dl	4.52 ± 0.36	4.49 ± 0.47	4.53 ± 0.37	0.828
Tumor necrosis factor-α, ng/ml	0.9 ± 1.18	1.06 ± 1.32	1.1 ± 1.33	0.512
Interleukin -6, pg/ml	8.48 ± 25.2	7.29 ± 14.8	8.81 ± 19.7	0.915
High-sensitivity C-reactive protein, mg/l	4.67 ± 8.84	3.66 ± 6.44	4.99 ± 8.77	0.637
Protein disulfide isomerase family A, member 4, ng/ml	24.9 ± 16.5	19.3 ± 10.7	28.1 ± 24.9	0.024

Note: We stratified PLWH into three groups according to different treatment regimens. Continuous data are presented as means±SD, and categorical data as numbers (%).
Abbreviations: CD, clusters of differentiation; INSTI, integrase inhibitor; nNRTI, non-nucleoside reverse transcriptase inhibitor; PLWH, people living with HIV; PI, protease inhibitor.
[a] Among PLWH received nNRTI group, 68 patients received efavirenz; 44 patients received rilpivirine; and 11 patients received nevirapine.
[b] Among PLWH received PI group, 20 patients received atazanavir; 29 patients received lopinavir/ritonavir; and eight patients received darunavir/ritonavir.
[c] Among PLWH received INSTI group, 25 patients received raltegravir, and 55 patients received dolutegravir.

T lymphocyte count, and other biochemical markers did not show a significant correlation. Moreover, TNF-α showed a significant association with high PDIA4 levels in the multivariate analysis.

Patients with high PDIA4 levels presenting high IL-6 and TNF-α levels

In Figure 2, the PLWH were stratified by the PDIA4 levels (<20, 20-50, and >50 ng/ml) (Figure 2a). The IL-6 levels were significantly higher in the group with PDIA4 levels >50 ng/ml than in the other groups (Figure 2b). Furthermore, the TNF-α levels were higher in the group with PDIA4 levels >20 ng/ml than in the other groups (Figure 2c). There was no difference in the inflammatory marker, hs-CRP, among these groups (Figure 2d).

Positive correlation between PDIA4 levels, and HIV viral load

To study the trend of PDIA4 level change in correlation with viral activity, 27 patients were followed up for their PDIA4 levels and viral load during the highly active antiretroviral therapy (HAART) course. We recorded the PDIA4 levels and HIV viral load before and during the treatment course (at 6 months and 12 months). PLWH responded well to HAART and showed a significantly reduced viral load after 6 months of treatment (Figure 3a). Notably, the PDIA4 levels also showed a decreasing trend during the HAART course (Figure 3b). The change in the PDIA4 levels during the treatment course showed a significantly positive correlation with the viral logic response ($r = 0.242$, $p = 0.029$) (Figure 3c).

Discussion

HIV infection is characterized by dysregulated production of pro-inflammatory cytokines and leads to persistent chronic inflammation. Our results showed that the plasma PDIA4 levels in PLWH were significantly higher than those in the healthy controls. The PDIA4 levels reduced significantly after cART and correlated with a decreasing trend in the HIV viral load. Moreover, PDIA4 levels positively correlated with the TNF-α levels.

PDIA4, comprising 645 amino acids, is upregulated in tumor cell lines and human cancer tissue (Li et al., 2021; Wang et al., 2019). Furthermore, the PDIA4 level is elevated in metabolic syndrome, and it is involved in the pathogenesis of diabetes. (Chien et al., 2017; Kau et al., 2021). Pickup et al. reported the role of inflammation and activated innate immunity in the pathogenesis of type 2 diabetes (Pickup, 2004). Persistent systemic inflammation remains a characteristic feature despite continuous cART in PLWH (Hunt et al., 2016; Legarth et al., 2016). We have demonstrated that the plasma PDIA4 levels in PLWH were significantly higher than those in the healthy controls, implying a higher inflammatory state exists in the former than in the latter, and a similar trend is observed in diabetes patients.

The current HARRT regimen comprises different regimens and may have various effects on the patient's inflammatory status (Hattab et al., 2014; Massanella et al., 2014). Our results revealed that the PDIA4 level was the highest in PLWH receiving INSTIs, followed by those receiving nNRTIs and those receiving PIs. The possible explanation is that INSTI-treated individuals have a higher chance of weight gain (Sax et al., 2020). A recent study showed

Table 3
Demographic and biochemical variables of cART-naïve, 6-month and 12-month post cART patients.

	cART-naïve (n = 27)	Six-month post cART (n = 27)	Twelve-month post cART (n = 27)	p-value[a]	p-value[b]	p-value[c]
Body weight, kg	62.8±11.8	63.7±12.2	65.7±13.5	0.77	0.39	0.57
Body mass index, kg/m^2	21.2±3.23	21.5±3.28	22.2±3.71	0.72	0.29	0.48
HIV viral load, log_{10} copies/ml	4.83±1.29	0.67±1.40	0.34±0.66	<0.0001	<0.0001	0.28
CD4 count, cells/mm^3	287.5±174.3	511.6±226.5	569.0±228.0	0.0002	<0.0001	0.36
CD8 count, cells/mm^3	1041.5±703.1	975.6±451.4	892.1±428.1	0.69	0.36	0.49
CD4/CD8 ratio	0.35±0.23	0.60±0.38	0.73±0.34	0.005	<0.0001	0.22
White blood cell, cells/mm^3	5.69±1.94	6.03±1.77	6.54±2.14	0.50	0.13	0.34
Hemoglobin, mg/dl	13.7±2.11	14.6±2.08	14.8±1.36	0.11	0.02	0.65
Platelet, x 10^3 cells/mm^3	222.6±80.3	246.8±77.4	275.4±68.7	0.27	0.01	0.16
Fasting glucose, mg/dl	90.9±18.8	92.2±18.6	96.0±15.7	0.79	0.28	0.42
Total cholesterol, mg/dl	153.2±32.6	161.8±31.4	155.9±27.6	0.33	0.74	0.47
Low-density lipoprotein cholesterol, mg/dl	97.1±23.6	103.6±24.2	102.6±22.7	0.33	0.39	0.88
Triglyceride, mg/dl	127.0±80.4	124.4±53.7	116.1±59.4	0.89	0.58	0.59
Blood urea nitrogen, mg/dl	11.4±2.89	12.2±2.68	13.0±4.13	0.29	0.10	0.39
Creatinine, mg/dl	0.74±0.14	0.87±0.13	0.90±0.14	0.001	0.0002	0.49
Uric acid, mg/dl	5.98±1.42	6.62±2.33	6.25±1.42	0.23	0.49	0.48
Aspirate aminotransferase, U/l	36.7±33.8	27.2±25.6	35.0±55.4	0.25	0.89	0.51
Alanine aminotransferase, U/l	34.3±32.4	30.3±42.4	44.6±101.3	0.70	0.62	0.50
Total bilirubin, mg/dl	0.83±0.56	0.76±0.27	0.65±0.24	0.58	0.13	0.10
Protein disulfide isomerase family A, member 4, ng/ml	77.4±60.1	57.6±39.5	42.0±30.0	0.02	0.0001	0.11

Note: We compare biochemical variables between cART-naïve, 6-month and 12-month post cART. Continuous data are presented mean ± SD.
Abbreviations: cART, combination antiretroviral therapy; DC, clusters of differentiation.
[a] cART naïve vs 6-month post cART.
[b] cART naïve vs 12-month post cART.
[c] Six-month post cART vs 12-month post cART.

Table 4
Age-adjusted Spearman partial correlation coefficients between Protein disulfide isomerase A4 and inflammatory markers.

	Correlation coefficients (n = 260)	
	r	p-value
Tumor necrosis factor-α, ng/ml	0.162	0.01
Interleukin-6, pg/ml	0.209	0.001
High-sensitivity C-reactive protein, mg/l	0.025	0.694
White blood cell count, cells/mm^3	0.01	0.873
Platelet count, x 10^3 cells/mm^3	-0.106	0.089
Clusters of differentiation 4 T lymphocyte count, cells/mm^3	-0.056	0.374
Clusters of differentiation 8 T lymphocyte count, cells/mm^3	-0.029	0.64

Note: Potential inflammatory markers and protein disulfide isomerase family A, member 4 levels in people living with HIV were analyzed using Spearman partial correlation analysis

that the expression of PDIA4 in the adipose tissue was upregulated in obese participants when compared with lean participants (Su et al., 2022b). Therefore, PLWH receiving INSTI regimen present higher PDIA4 levels than those treated with the other regimens.

HIV infection is accompanied by a robust inflammatory cytokine response, and several studies have linked surrogate markers to the clinical outcomes (Hunt et al., 2012; Neuhaus et al., 2010). Traditional soluble inflammatory markers, such as TNF-α and IL-6, can be biomarkers predicting the state of inflammation and treatment outcomes in PLWH. Ledwaba et al. found that elevated pre-ART levels of IL-6 are strongly associated with early mortality after commencing ART (Ledwaba et al., 2012). Baker et al. also found that higher IL-6 levels during entry in The Strategic Timing of AntiRetroviral Treatment trial were associated with an increased risk of AIDS events (Baker et al., 2017). McComsey et al. showed that higher TNF-α levels are associated with increased risk of AIDS-related and non-AIDS-related clinical events after cART initiation (McComsey et al., 2014). In our study, age-adjusted Spearman par-

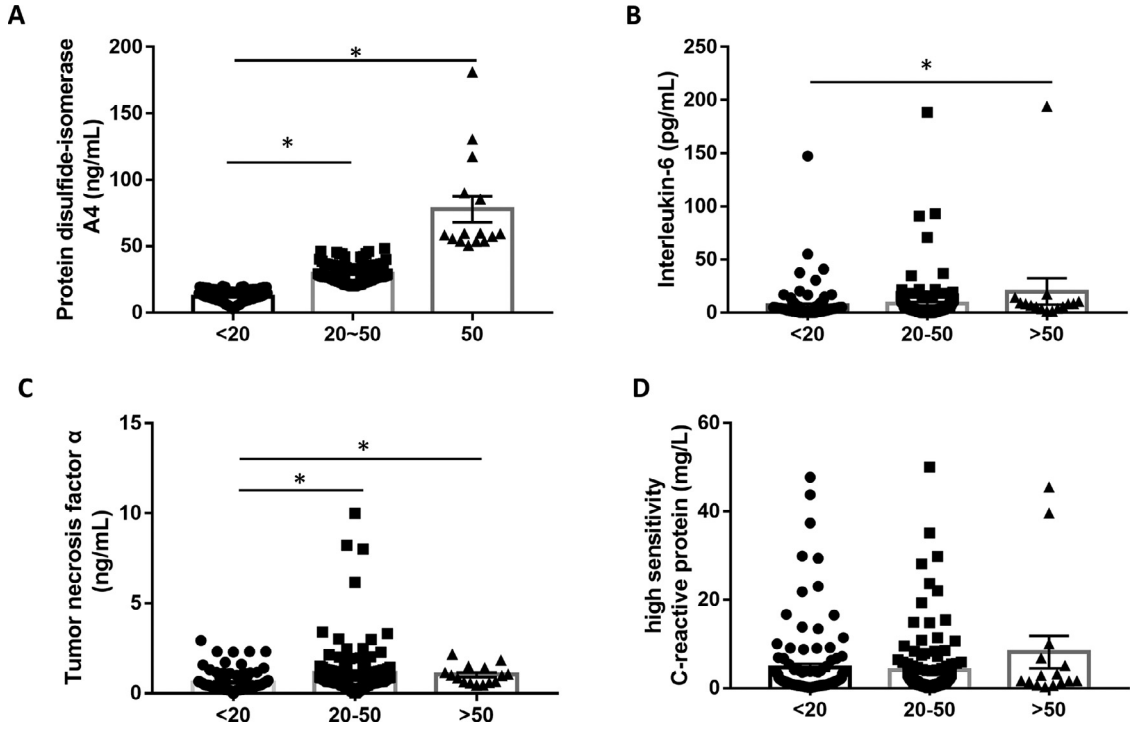

Figure 2. People living with HIV with high protein disulfide isomerase family A, member 4 levels presenting high interleukin-6 and tumor necrosis factor-α levels.
People living with HIV were stratified by protein disulfide isomerase family A, member 4 levels (<20, 20-50, and >50 ng/ml) (a), and their interleukin-6 (b), tumor necrosis factor-α (c) and high-sensitivity C-reactive protein (d) levels were analyzed; *p < 0.05.

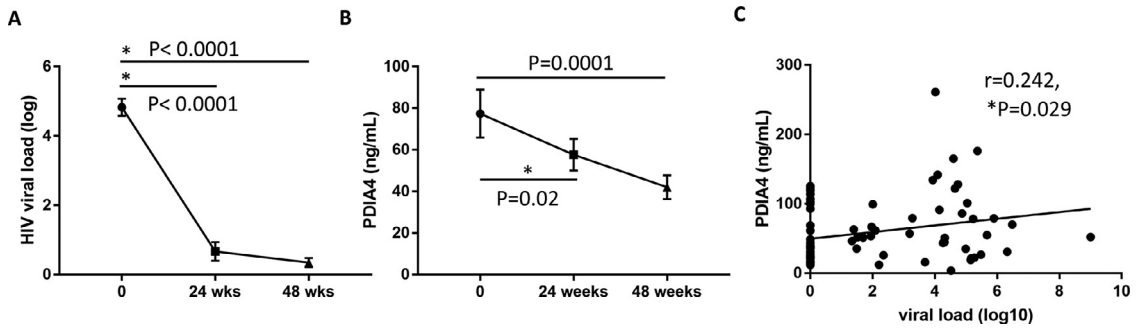

Figure 3. Association between the PDIA4 levels and viral load HIV.
In total, 27 people living with HIV were followed up for PDIA4 levels and viral load during the highly active antiretroviral therapy course. We recorded the PDIA4 levels and HIV viral load before treatment (a) and during the treatment course at 24 weeks and 48 weeks (b). The correlation between the change in PDIA4 expression and the viral logic response during the treatment course was analyzed (c); *p < 0.05.
Abbreviation: PDIA4, protein disulfide isomerase family A, member 4.

tial correlation coefficients revealed that PDIA4 positively correlated with TNF-α and IL-6 (Table 4). Additionally, logistic regression analysis of the association between biochemical variables and PDIA4 levels showed that PDIA4 levels positively correlated with TNF-α ($p < 0.001$) and IL-6 ($p = 0.021$) (univariate analysis). Moreover, TNF-α showed a significant correlation with PDIA4 in the multivariable logistic analysis (Table 5). Our findings that PDIA4 levels positively correlated with the TNF-α and IL-6 levels imply that PDIA4 could be a potential surrogate marker of inflammation in PLWH.

The cART effectively suppresses HIV viral replication after initiation. Several pro-inflammatory cytokines are elevated in PLWH and markedly decrease after treatment (Hamlyn et al., 2015; Roff et al., 2014). Our study showed a significant viral load decline at 6 months and 12 months during the treatment course (Table 3 and Figure 3a). Baker et al. found a reduction in the IL-6 levels after cART initiation (Baker et al., 2011). We also demonstrated that PDIA4 levels markedly decreased at 6 months and 12 months during the treatment course (Figure 3b). Osuji et al. found that pro-inflammatory cytokines, TNF-α, and IL-6 significantly decreased in PLWH at 24 weeks and 48 weeks of cART treatment (Osuji et al., 2018). Furthermore, they found a significant positive correlation between viral load and the IL-6 and TNF-α levels. Our study demonstrated a significant positive correlation between viral load and PDIA4 levels, implying that PDIA4 could be a good surrogate inflammatory marker in PLWH.

Our study has several limitations. First, as this was a cross-sectional study, it was difficult to assess the causality relationship of PDIA4 in PLWH. Second, only 27 PLWH cART-naïve patients were recruited in the observation cohort, which is a relatively small sample size; therefore, future studies with a larger population size are warranted. Third, biological PDIA4 pathways are complex and interconnected; hence, we were unable to interpret the observational data in a comprehensive manner.

Our study demonstrates a significant association between PDIA4 levels and inflammation in PLWH. Moreover, PDIA4 lev-

Table 5
Logistic analysis to identify factors associated with high protein disulfide isomerase family A, member 4 values in people living with HIV.

Variables	Univariate analysis		Multivariate analysis	
	Odds ratio (95% confidence interval)	p-value	Odds ratio (95% confidence interval)	p-value
Age (ref: <30)				
31-40	0.73 (0.37-1.43)	0.356		
41-50	0.77 (0.37-1.64)	0.503		
>50	0.50 (0.24-1.07)	0.073		
Hepatitis B virus surface antigen positivity	0.46 (0.21-0.99)	0.069		
Anti-hepatitis C virus positivity	2.35 (0.87-6.33)	0.091		
DM	1.15 (0.35-3.86)	0.817		
Biochemical data				
Glucose ante cibum (ref: <100 mg/dl)	0.95 (0.52-1.72)	0.858		
Total cholesterol (ref: <200 mg/dl)	1.05 (0.54-2.03)	0.449		
Triglyceride (ref: <150 mg/dl)	1.06 (0.61-1.85)	0.827		
Low-density lipoprotein (ref: <100 mg/dl)	1.24 (0.56-2.73)	0.591		
Aspirate aminotransferase (ref: <40 U/l)	0.37 (0.13-1.11)	0.076		
Alanine aminotransferase (ref: <40 U/l)	0.54 (0.27-1.07)	0.078		
Blood urea nitrogen (ref: <25 mg/dl)	2.06 (0.22-18.7)	0.521		
Creatinine (ref: <1 mg/dl)	1.34 (0.68-2.65)	0.398		
Total bilirubin (ref: <1.2 mg/dl)	1.62 (0.79-3.30)	0.188		
White blood cell count (ref: $<4 \times 10^3$ cells/mm^3)	3.29 (0.94-11.5)	0.062		
Platelet (ref: $<150 \times 10^3$ cells/mm^3)	1.74 (0.56-5.33)	0.336		
HIV viral load, log 10 copies/ml	2.31(1.06-5.05)	0.036		
CD4 count (ref: >500)				
<200	2.15 (0.69-6.72)	0.190		
201-500	0.85 (0.49-1.46)	0.551		
CD4/CD8 ratio (ref: <0.8)	1.35 (0.79-2.30)	0.275		
Tumor necrosis factor-α	4.22 (2.27-7.86)	<0.001	4.32 (2.29-8.17)	<0.001
Interleukin-6	2.22 (1.13-4.37)	0.021		
High-sensitivity C-reactive protein	0.96 (0.50-1.92)	0.959		

Note: Multivariable analysis used forward logistic regression and adjusted for other variables. Univariate and multivariate analysis of factors associated with high protein disulfide isomerase family A, member 4 levels were conducted.
Abbreviation: DC, clusters of differentiation; DM, diabetes mellitus.

els are associated with HIV viral load and TNF-α and IL-6 levels. The plasma PDIA4 level may be considered a potential surrogate marker of inflammation in PLWH.

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Ethical approval

This study was approved by the Institutional Review Board of Tri-Service General Hospital (No. 2-105-05-027).

Author contributions

All authors made a significant contribution to the work reported in terms of conception, study design, execution, acquisition of data, analysis and interpretation, or areas all of these; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Declaration of competing interests

The authors have no competing interests to declare.

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