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COMPARISON OF PROTEIN C LEVELS IN HIV PATIENTS WITH CD4 VALUES <200 CELLS/µL AND CD4 VALUES ≥200 CELLS/µL IN HAJI ADAM MALIK GENERAL HOSPITAL MEDAN

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Abstract

Introduction: Human Immunodeficiency Virus infection is a rapidly growing disease that attacked CD4 as its cell target. The reduction of CD4 value was generally accompanied by abnormality of the coagulation system which one of the components was protein C. Some studies proved that there was a correlation between CD4 cell count and decreased protein activity C, but the data regarding protein C levels with varying CD4 counts were still limited. This study aimed to determine the comparison of protein C levels in HIV patients with CD4 values <200 cells/µl and CD4 values ≥200 cells/µl in Haji Adam Malik General Hospital Medan.

Methods: This study was an analytical study with a cross-sectional design which was conducted from December 2019 until February 2020. Eligible study subjects were at least 18 years old HIV patients who had been confirmed by the 3-methods strategy. Protein C levels were examined by the Coatron A4 machine. Data analysis used independent T-test and Pearson correlation test.

Results: Thirty-eight study subjects were obtained consisting of 81.6% men with an average age of 33.16. ± 10.74 years. The median levels of protein C and CD4 were 79.2% and 192.5 cells µL, respectively. There was a significant difference in protein C levels between both groups (p <0.001).

Conclusion: There was a significant difference in protein C level between HIV/AIDS patients with CD4 <200 sel/µL dan CD4 ≥200 sel/µL (*p* <0.001).

Introduction

Human Immunodeficiency Virus (HIV) infection is a global burden and spreads rapidly, causing a significant increase in morbidity and mortality.[1] Based on World Health Organization (WHO) data, the discovery of HIV cases in the world in 2012 reached 2.3 million cases, of which 1.6 million people died of AIDS (Acquired Immunodeficiency Syndrome).[2]

The high mortality rate due to HIV is caused by HIV attacking CD4 lymphocytes which play an important role in maintaining immune integrity, so that the CD4 value is an important marker in assessing the immunological status of HIV patients.[3] CD4 <200 cells/µL is the best indicator which indicates severe immunosuppression in HIV patients.[4] The reduction of CD4 value was generally accompanied by other organ function disorders such as the coagulation system which one of the components in the anticoagulant system was protein C.[5]

A hypercoagulable state in HIV predisposes to the development of a serious and potentially life-threatening thromboembolic disorder.[6] Several studies have shown that the mean value of protein C activity is lower in HIV patients compared to the control group where there is a correlation between CD4 cell count and decreased protein activity C.[7] However, studies regarding the comparison of protein C levels with varying CD4 cell counts in HIV patients are still limited. Therefore, this study aims to determine the comparison of protein C levels in HIV patients with CD4 values <200 cells/µl and CD4 values ≥200 cells/µl in Haji Adam Malik General Hospital Medan.

Methods

This study was an analytical study with a cross-sectional design to determine the comparison of protein C levels in HIV/AIDS patients with CD4 values <200 cells/µL and CD4 ≥200 cells/µL which was conducted at the

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Department of Clinical Pathology Haji Adam Malik General Hospital, Division of Tropical Medicine and Infections Department of Internal Medicine from December 2019 until the study subjects was fulfilled. Thirty-eight HIV/AIDS patients who were treated in the HIV ward and outpatient treatment at Haji Adam Malik General Hospital were obtained by consecutive sampling.

The inclusion criteria of the study subjects were at least 18 years old who had just been diagnosed with HIV by 3-methods strategy, had CD4 value <200 cells/µL and ≥ 200 cells/µL, and willing to participate in this study. Study subjects were excluded if they consumed antiplatelet drugs such as aspirin, clopidogrel, and cilostazol and had previously consumed protease inhibitor (PI) class antiretroviral drugs such as saquinavir, indinavir, ritonavir, nelfinavir, amprenavir, lopinavir, and atazanavir.

All eligible study subjects were explained and were asked to sign the informed consent. The blood sample was collected from the median cubital vein and placed in a tube containing 3.2% Na-anticoagulant. To separate the plasma, blood was centrifuged at a speed of 3,500 rpm in 15 minutes. Protein C levels were examined through the Coatron A4 machine.

Data analysis used an independent T-test to compare protein C levels between groups with CD4 <200 cells/µL and CD4 ≥200 cells/µL. The correlation between the CD4 and protein C was determined by the Pearson correlation test if the data were normally distributed or the Spearmen correlation if the data were not normally distributed. Analysis was performed at 95% confidence intervals with a significant P value <0.05.

Results

This study was conducted for 3 months from December 2019 to February 2020 with 38 study subjects consisting of 31 men (81.6%) and 7 women (18.4%) with an average age of 33.16. ± 10.74 years (Table 1). The results showed that the median levels of protein C and CD4 were 79.2% and 192.5 cells µL, respectively (Table 2).

Table 1. Characteristic of Study Subjects

Characteristics	N
Gender	
• Men	31 (81,6%)
• Women	7 (18,4%)
Age (Mean ± Standard Deviation)	33,16 ± 10,74 years old

Table 2. Protein C and CD4 Levels in HIV/AIDS Patients.

Variabel	Median (min-max)
Protein C (%)	79,2 (18-161)
CD4 (cells/µL)	192,50 (5-576)

Data analysis using independent T test showed that there was a significant difference in protein C levels between the CD4 group <200 cells / µL and the CD4 group ≥200 cells / µL (p <0.001) with a greater decrease in protein C level was found in the CD4 <200 cells/µL group (Table 3).

Tabel 3. Correlation of CD4 and Protein C in HIV/AIDS Patients

	CD4 <200 cells/µL	CD4 ≥200 cells/µL	*p value*
Protein C (Mean ± Standard Deviation)	58,03 ± 19,68	104,64 ± 26,47	<0,001

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

Discussion

This study involved 38 study subjects who had been diagnosed with HIV/AIDS and met the study inclusion criteria. The results showed that the majority of study subjects consisted of men (81.6%) and only 18.4% women with an average age of 33 years. This is in line with Julianti's and Kunarisasi's studies which also obtained the same proportion of study subjects in terms of gender. Chiapetta et al showed that the highest prevalence of HIV patients was in the range 33-35 years. Kunarisasi also showed that HIV patients who were 33-49 years old had 1.4 times more likely to be infected compared to other age groups.[8]

According to WHO, the immunological status of HIV can be divided into normal with CD4 >500 cells/µL, mild with a CD4 count of 350-499 cells/µL, moderate with a CD4 count of 200-349 cells/µL, and severe with CD4 count <200 cells/µL. The risk of mortality increases along with the rapid decline of CD4 cell count.[9]

The results showed that the median CD4 value was 192.50 cells/µL. These results are in line with the study by Nozza et al which showed that there was a significant decrease in CD4 cell count compared to the control group.[10] CD4 lymphocyte count is an important predictor of HIV disease progression because the surface of CD4 T-helper lymphocytes are the initial targets of HIV infection. CD4 T cells in the host mucosa were partially active for less than one week after exposure. The viral integration enzymes caused the integration of viral dsDNA dan body DNA, which lead to cellular sensors recognizing DNA changes, and causing CD4 cell apoptosis.[11,12]

Based on the results of this study, the mean protein C levels in patients with CD4 values <200 cells/µL were lower than those in the CD4≥200 cells/µL group (58.03 ± 19.68 vs 104.64 ± 26.47; p <0.001). This is in line with a study conducted by Khare, who reported that there was a significant difference in mean protein C levels between HIV and control groups (p <0.001).[7] A study by Erbe et al showed that 12 of 49 HIV patients (25%) had decreased protein C levels that could be induced by opportunistic infections in HIV/AIDS patients.[13]

Protein C is a glycoprotein that is synthesized in the liver and is included in vitamin K-dependent protein because it requires vitamin K for the carboxylation process. Protein C acts as a natural anticoagulant (after activation) by deactivating the procoagulant cofactors Va and VIIIa in the coagulation cascade which is modulated by other factors such as Ca^{2+} ions, phospholipids, and cofactor protein S. Protein C is also an essential blood factor in the blood clotting cascade, so it can help achieve blood hemostasis in several disease conditions such as sepsis, cancer, and HIV. Abnormalities in protein C levels were identified in HIV patients and correlated significantly with immunosuppression which was proved by reduced CD4 cell counts.[6,14] Apart from being a natural anticoagulant, protein C also acts as an anti-inflammatory mediator.[15]

Human Immunodeficiency Virus (HIV) extremely changes the structure of endothelial cells.[16] Infected endothelial cells show selective interference in the storage or excretion of endothelin-1 molecules and von-Willebrand factor. Several molecules also affect tPA (type plasminogen activator) and PAI-1 (plasminogen activator inhibitor). These result in the apoptosis of endothelial cells in small and moderate blood vessels in patients with HIV. The apoptosis of endothelial cells is induced by stimuli released by immunocompetent cells and viral proteins. The HIV activates apoptosis in endothelial cells and changes the function of the single microvascular layer of endothelial cells by increasing the vasopermeability of the protein size.[16,17]

The abnormal increase of inflammatory cytokines such as TNF-α, IL-1, IL-8, monocyte chemotactic peptide-1 caused the damage of endothelial cells. The role of endothelial cells in the control of leukocyte diapedesis is very important in the development of AIDS. During transmigration, the involvement of the integrin system in infected lymphomononuclear and production of inflammatory cytokines by endothelial cells resulted in remarkable viral replication in lymphocytes and monocytes. Furthermore, abnormal leukocyte traffic played a role in the progressive injury of lymphoid tissue and impaired immune response. As a result of the continuation of this process, the activation of protein C was reduced.[16]

Conclusion

There was a significant difference in protein C level between HIV/AIDS patients with CD4 <200 sel/µL dan CD4 ≥200 sel/µL (p <0.001) which lower value was found in CD4 <200 sel/µL group.

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References

[1] Raman RT. Study of basic coagulation parameters among HIV patients in correlation to CD4 counts and ART status. Journal of Clinical and Diagnostic Research. 2016; 10: 1-8.
[2] World Health Organization. Guidelines for HIV/AIDS diagnosis and treatment. 2009. Available from: https://aidsfree.usaid.gov/sites/default/files/hts_policy_vietnam.pdf (Accessed: 10th September 2019).
[3] Pattanapanyasat K. Immune status monitoring of HIV/AIDS patients in resource-limited settings: a review with an emphasis on CD4 + T-lymphocyte determination. Asian Pac J Allergy Immunol. 2012; 30(1): 11-25.
[4] Ladyani F, Kiristianingsih A. Hubungan antara jumlah CD4 pada pasien yang terinfeksi HIV/AIDS dengan infeksi oportunistik di Rumah Sakit Umum Abdul Moeloek Bandar Lampung tahun 2016. JK Unila. 2019; 3(1): 34-41.
[5] Wagener CSC, Esmon BM, Pittet CT, Francois J. Protein C and acute inflammation: a clinical and biological perspective. The American Physiological Society. 2013; 305: 455-466.
[6] Saif, MW, Greenberg, B. HIV and thrombosis: a review. Aids Patient Care. 2001; 15(1): 15–25.
[7] Khare, S, Kushwaha R, Kumar A, Venkatesh V, Reddy HD, Jain M, et al. Prothrombotic state in HIV: a study of protein C, protein S, homocysteine and correlation with CD4 counts. Indian Journal of Medical Microbiology. 2018; 36(2): 201-206.
[8] Kurniasih NK, Manullang E, Wardah, Anam MS, Istiqomah. Situasi HIV/AIDS di Indonesia Tahun 1987-2006. Pusat Data dan Informasi Departemen Kesehatan RI. 2006.
[9] World Health Organization. Clinical Staging of HIV/AIDS and HIV/AIDS Case Definitions for Surveillance. 2005. Available at: https://www.who.int/hiv/pub/guidelines/clinicalstaging.pdf (Accessed: 11th September 2019).
[10] Nozza S, Galli L, Bigoloni A, Nicola G, Pogliaghi M, Cossarini F, et al. Durability and safety of a novel salvage therapy in R5-tropic HIV- infected patients: maraviroc, raltegravir, etravirine. J Acquir Immune Defic Syndr 2011; 56(4): 113–5.
[11] Deuffic-Burban S, Losina E, Wang BX, Gabillard D, Messou E, Divi N, et al. Estimates of opportunistic infection incidence or death within specific CD4 strata in HIV-infected patients in Abidjan, Côte d'Ivoire: impact of alternative methods of CD4 count modelling. European Journal of Epidemiology. 2007; 22(10): 737–744.
[12] Doitsh G, Greene WC. Dissecting how CD4 T cells are lost during HIV infection. Cell Host Microbe. 2016; 19(3): 280-291.
[13] Erbe, M. Rickerts, V, Bauersachs, R, Lindhoff-Last, E. Acquired protein C and protein S deficiency in HIV-infected patients. 2003; 9(4): 325–331.
[14] Lee, JJ, Thiessen, E, Bruley, DF. Protein C production in Oxygen Transport to Tissue XXVI. New York: Springer-Verlag. 2005: 381–387.
[15] Lijfering WM, Sprenger HG, Georg RR, Meulen PAVD. Relationship between progression to AIDS and thrombophilic abnormalities in HIV infection. Clinical Chemistry. 2008; 54(7): 1226-1233.
[16] Bussolino F, Mitola S, Serini G, Barillari G, Ensoli B. Interactions between endothelial cells and HIV-1. The International Journal of Biochemistry & Cell Biology. 2001; 33: 371-390.
[17] Shankar, SS, Dubé MP. Clinical aspects of endothelial dysfunction associated with human immunodeficiency virus infection and antiretroviral agents. Cardiovascular Toxicology. 2004; 4: 261–269.