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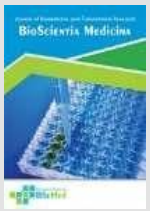

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The Relationship between Dickkopf-Related Protein-1 (DKK-1) Plasma Levels and Impaired Cognitive Function in HIV Patients

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A B S T R A C T

Background: Dickkopf-related protein – 1 (DKK-1) is an antagonist protein to the Wingless (Wnt)-β catenin signal. Dysregulation of Wnt-β catenin signaling by DKK-1 causes disruption of neuronal synapses, which can result in impaired cognitive function. Impaired cognitive function in people with HIV or HIV-associated neurocognitive disorder (HAND) is a spectrum of cognitive disorders related to HIV neuroinvasion and neuroinflammation, which significantly results in impaired cognitive function and daily activity. This study aimed to assess the relationship between plasma DKK-1 levels and impaired cognitive function in HIV patients. **Methods:** This study used a cross-sectional design, consisting of 84 HIV sufferers who went to the voluntary counseling and testing (VCT) polyclinic at Dr. M. Djamil General Hospital, Padang, Indonesia, in the period December 2022 - March 2023, who met the inclusion and exclusion criteria. Cognitive function was assessed using the Montreal Cognitive Assessment Indonesian version (MoCa-Ina), and daily activity function using the instrumental activity of daily living (IADL) examination. Plasma DKK-1 levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. **Results:** The mean plasma DKK-1 level of HIV sufferers was 358.4 (+ 157.6) pg/mL. ANI type disorder was found in 78.6%, MND in 21.4%, and no HAD type. There was a significant relationship between plasma DKK-1 levels and impaired cognitive function in HIV patients (OR = 2.82, CI95% = 1.128-7.043, p = 0.025), but there was no significant difference in plasma DKK-1 levels between types of cognitive impairment ANI and MND (p = 0.858). **Conclusion:** Plasma DKK-1 levels are significantly associated with impaired cognitive function in HIV patients.

1. Introduction

Dickkopf-related protein-1 (DKK-1) is a protein antagonist to the Wingless signal (Wnt)-β catenin. Wnt-β catenin signaling has an important role in cell communication, cell cycle, cell defense, new cell growth, cell differentiation, and organogenesis.[1] Dysregulation of Wnt-β catenin signal by DKK-1 causes disturbance of neuronal synapses. This becomes one of the pathogenesis theories of cognitive dysfunction. Impaired cognitive function is a neurological complication that is often found in people with HIV.[2] Impaired cognitive function in people with HIV or HIV-associated neurocognitive disorder (HAND) is a spectrum of cognitive disorders related to HIV neuroinvasion and neuroinflammation, which causes attention, memory, language, problem-solving, and decision-making disorders. Resulting in significantly impaired function of daily activities. HAND can develop from mild types, namely asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND) to HIV-Associated Dementia (HAD) severe.[3]

Several biomarkers have been studied related to the incidence of cognitive dysfunction in HIV sufferers, such as markers of cell stress (ceramide,

sphingomyelin), oxidative stress (carbonyl protein, hemoxigenase), energy metabolism (Krebs cycle substrate), and glutamate regulation (glutamine). This biomarker requires sampling cerebrospinal fluid via lumbar puncture.[4] This is less practical to do in clinical practice than a venous puncture. Other biomarkers are Dickkopf-related protein – 1 (DKK-1), reported to have a positive predictive value and high specificity in assessing the risk of cognitive dysfunction events in HIV sufferers.[5]

Several studies have reported a relationship between DKK-1 levels and the incidence of cognitive impairment. Ross reported that DKK-1 levels at baseline were significantly associated with decreased global cognitive function in older adults after 18 months of age follow-up.[6] This dysregulation of Wnt-β catenin signaling has been associated with the incidence of neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease.[6-8] Furthermore, Orellana also studied in vitro cultures of human neurons and astrocytes, reporting an increase in DKK-1 secretion in an HIV model. Infected astrocytes, leading to disruption of neuronal synapses. This is evident in the decrease in the number and length of neuronal processes, which ultimately have an impact on cognitive impairment.[9] Meanwhile, research conducted by Yu et al. of HIV patients reported that levels of Dickkopf-related protein 1 (DKK-1) did not differ significantly between the groups of HIV-positive and HIV-negative patients, but DKK-1 levels were found to be significantly increased in HIV-positive patients with impaired cognitive function, so it was identified as a biomarker specific for the incidence of cognitive dysfunction in HIV patients. This study aimed to determine the relationship between plasma DKK-1 levels and impaired cognitive function in HIV patients at Dr. M. Djamil General Hospital, Padang, Indonesia.

2. Methods

This study is a cross-sectional study. The study consisted of HIV sufferers who were treated at the VCT Polyclinic at Dr. M. Djamil General Hospital, Padang, Indonesia, in the period December 2022-March 2023, who met the inclusion and exclusion criteria. As for criteria inclusions, among others, the patient has been diagnosed as living with HIV based on what is written in the medical record, is over 18 years of age, and is willing to participate and sign informed consent. In contrast, the exclusion criteria were patients with a history of opportunistic infections of the central nervous system, cerebrovascular disease, head trauma, intracranial tumors, depression, or other mental disorders. psychiatric other; conditions that affect DKK-1 levels, such as acute infectious diseases, history of tumors, hematological disorders, and musculoskeletal diseases; or uncooperative patient. The sample size was set at 84 people, which were divided into 2 groups, namely the group with cognitive impairment and the group without cognitive impairment, each consisting of 42 people.

Each subject will undergo a Moca-Ina and IADL examination, then proceed with taking 3 cc of venous blood, which will then be carried out centrifuge to obtain a plasma sample. Examination of plasma DKK-1 levels using the ELISA technique was carried out at the laboratory of the diagnostic and research center for infectious diseases, Faculty of Medicine (FK) Universitas Andalas (Unand). This research was approved by the medical and health research ethics committee at the Faculty of Medicine, Universitas Andalas, Padang, Indonesia. Data analysis was carried out using SPSS software version 25. Univariate analysis was performed to present the data frequency distribution for each test variable. Bivariate analysis was carried out to present the relationship between the test variables, with a p-value <0.05.

3. Results

Table 1 shows that the patient group is aged more than 50 years and experiences cognitive disorders (83.3%), with the largest percentage being women at 61.1%. In addition, patients with an educational level $\leq$ 12 years also experienced more cognitive impairment, namely 71.4%. Meanwhile, the

characteristics of the stage of the disease have an almost equal incidence of cognitive impairment between each group. Overall, the mean Moca-Ina score was 26.0 (11-30), the mean IADL score was 8 (6-8), and the mean plasma DKK-1 level of HIV sufferers was 358.4 (± 157.6) pg/mL. From the results of statistical analysis, a significant relationship was found between age and impaired cognitive function (OR=6.25; 95% CI=1.277-30.580; p-value = 0.013). In addition, a significant relationship was also found between education level and impaired cognitive function (OR=6.25; 95% CI=0.612-5.140; p-value = 0.000). Meanwhile, for the characteristics of sex and disease stage, no significant relationship was found with impaired cognitive function (p>0.05).

Table 1. The relationship between the basic characteristics of the sample and impaired cognitive function.

Characteristics	Impaired cognitive (n=42)	No impaired cognitive (n=42)	OR (95% CI)	p-value
Age			-	
≥ 50 years, n(%)	10 (83,3%)	2 (16,7%)	6,25	0,013*
< 50 years, n(%)	32 (44,4%)	40 (55,6%)	(1,277-30,580)	
Gender				
Female, n(%)	11 (61,1%)	7 (38,9%)	1,774	0,287*
Male, n(%)	31 (47%)	35 (53%)	(0,612-5,140)	
Level of education				
≤ 12 years, n(%)	30 (71,4%)	12 (28,6%)	6,25	0,000*
> 12 years, n(%)	12 (28,6%)	30 (71,4%)	(2,425-16,108)	
Disease stage				
Stage 3, n(%)	14 (53,8%)	12 (46,2%)	-	
Stage 2, n(%)	17 (58,6%)	12 (41,4%)		0,259*
Stage 1, n(%)	11 (37,9%)	18 (62,1%)		

* Chi-square test.

Furthermore, for plasma DKK-1 levels, because they still do not have standardized normal values, the researchers made calculations of cut-off point (COP) plasma DKK-1 levels associated with impaired cognitive function in HIV patients. From the analysis results, it was found that the plasma DKK-1 COP level was 313.5 pg/mL. Furthermore, the sample was divided into two groups based on the COP value so that it became a group of patients with DKK-1 values > 313.5.6 pg/ml, which totaled 52 people, and patients with DKK-1 values≤ 313.5 pg/ml, which totals 32 people. The results of the analysis using the test who squares showed that there is a significant relationship between plasma DKK-1 levels and cognitive dysfunction in HIV patients (OR=2.82; 95% CI=1.128-7.043; p = 0.025).

Table 2. The relationship between plasma DKK-1 levels and impaired cognitive function in HIV patients.

	Cognitive function			
Plasma DKK-1 levels	Impaired cognitive n=42	No impaired cognitive n=42	OR (95% CI)	p-value
> 313.5 pg/mL (n=52)	31 (59,6%)	21 (40,4%)	2,82	0,025*
≤ 313.5 pg/mL (n=32)	11 (34,4%)	21 (65,6%)	(1,128-7,043)	

* Chi-square test.

Impaired cognitive function in HIV sufferers based on criteria Frascati divided into three types, namely asymptomatic neurocognitive impairment (ANI), mild neurocognitive disorder (MND), and HIV- associated dementia (HAD). In this study, out of 42 patients with impaired cognitive function, 33 patients (78.6%) had ANI-type disorders, 9 patients (21.4%) had MND, but no patients with HAD. From the statistical analysis,

there was no significant difference significantly on plasma DKK-1 levels of patients with cognitive impairment ANI type (386.8± 151.9) pg/mL with the MND type (376.4± 159.5) pg/mL, with a value of p = 0.858.

Table 3. Differences in DKK-1 levels between ANI and MND type impaired cognitive.

	Types of impaired cognitive		
Variable	**ANI (n=33)**	**MND (n=9)**	**p-value**
Plasma DKK-1 levels (pg/mL), mean (±SD)	386,8 (±151,9)	376,4 (±159,5)	0,858*

* Unpaired T test, SD standard deviation.

4. Discussion

Kaul's research states that age plays an important role in causing cognitive impairment related to neuroinflammation and increased permeability of the blood-brain barrier with increasing age.[10] Cherner's study found a significant relationship between age and levels of HIV RNA in cerebrospinal fluid (CSS), where older individuals with HIV RNA (+) in CSS had a 2-fold risk of experiencing cognitive impairment compared to HIV RNA (-) in CSS. In contrast, young individuals found, no significant results. This shows that the age factor can affect the effect of HIV on the brain, causing cognitive impairment.[11]

In this study, it was found that the incidence of cognitive dysfunction in women (61.1%) was higher than that of male (47%), where it was found that women had a tendency to have a cognitive disorder of 1.774 compared to male. These results are consistent with Widyastuti's research on HIV sufferers, which showed that the incidence of cognitive impairment in women was higher than in men.[12] This is possibly due to a lower level of education in women, cerebrovascular comorbidities which are more common in women, genetic factors, and hormonal factors.[13,14] However, the results of statistical analysis in this study showed no significant relationship between gender and impaired cognitive function in HIV sufferers. The differences found in the current study indicate that there are other factors that predominate as a cause of impaired cognitive function in people with HIV other than gender.

Furthermore, this study found a significant relationship between educational level and impaired cognitive function, where patients with a low level of education (under or equal to 12 years) have 6.25 times the risk of experiencing impaired cognitive function compared to HIV patients with a high level of education (over 12 years) (95% CI = 2.425-16.108). These results are consistent with research conducted by Aung, where a high level of education increases and optimize neuronal circuit.[15]

As for the stage of the disease, no significant relationship was found between the stage of the disease and impaired cognitive function (p = 0.259). The prevalence of impaired cognitive function increases up to four times higher in HIV clinical grade C (45.8%) compared to clinical grade A (9.4%). HIV with clinical degree C is known to be an independent predictor of cognitive function disorders.[15] Several possible causes include increased adherence to taking ART medication or the need for a more sensitive neurocognitive screening tool to detect cognitive impairment, especially for the early stages of the disease. Research by Dore suggests that the use of ART drugs has significantly reduced the incidence of cognitive dysfunction in people with HIV.[16]

Because there is no standardized normal value for plasma DKK-1 levels, the researchers looked for the COP value for plasma DKK-1 levels of HIV sufferers who experienced cognitive impairment and obtained a COP value of 313.5 pg/mL. These results are inconsistent with Yu's study, which found higher plasma DKK-1 COP levels, namely 735 pg/mL.[5] This

difference is probably due to different population backgrounds and demographics. The statistical results of this study show that there is a significant relationship between plasma DKK-1 levels and cognitive impairment, where patients with plasma DKK-1 levels of more than 313.5 pg/mL have a 2.82 times risk of experiencing cognitive impairment compared to patients with DKK-1 plasma levels ≤ 313.5 pg/mL. HIV infection of astrocytes can lead to increased dilatation connexin 43 hemichannels (Cx43 HCs), which results in increased DKK-1 secretion.[9] DKK-1 is a Wnt-β catenin antagonist which plays a role in cellular communication, cell cycle, cell survival, cell renewal, regulation of synaptogenesis, plasticity, and organogenesis.[17] Wnt signaling pathway blockade mechanism-β catenin begins with the binding of Wnt to Wnt Frizzled (FZD) receptors as well as receptors of low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6). DKK1 does not bind directly to Wnt or FZD but forms a complex with LRP6 on the cell surface. As an antagonistic ligand with a high affinity for LRP6, DKK1 inhibits interactions between Wnt, FZD, and LRP6, resulting in β degradation-catenin by the proteasome resulting in inactivation of the β transcription complex-catenin/T-cell specific factor (TCF). The end result of the process is the downregulation of several genes downstream regulated by the TCF. Downstream gene in the Wnt pathway plays a role in cellular communication, cell cycle, cell survival, cell renewal, differentiation, regulation of synaptogenesis, plasticity, and organogenesis.[17] In addition, the Wnt/β signaling pathway catenin also functions as a restriction factor for HIV in astrocytes, monocytes, and T cells. Thus inhibition of the Wnt/β pathway catenin by DKK-1 can enhance viral replication.[18] Then DKK-1 also affects the ability to scavenge glutamate by astrocytes, which can inhibit transcription regulation excitatory amino acid transporter 2 (EAAT-2) and glutamine synthetase. This will cause interference to reuptake glutamate resulting in excess extracellular glutamate, which is an excitatory neurotransmitter. This condition causes excitotoxicity which can lead to synaptodendritic destruction, which in turn results in impaired cognitive function.[5]

The results of this study showed that there were 33 patients (78.6%) with cognitive dysfunction of the ANI type, 9 patients with MND (21.4%), and no patients with HAD. In addition, there was no significant difference in plasma DKK-1 levels between ANI and MND patients (p=0.858). These results are consistent with Antinori's study, which stated that the incidence of ANI was higher than that of MND.[3] As for the incidence of HAD, another cohort study found the incidence of HAD to be 2-7% in people with HIV.[19] This is probably due to increased mortality among HAD patient population (Mukherjee, 2018). HAD is also associated with HIV viremia (e.g., low CD4+ nadir or high viral load plasma).[3] A low CD4 count represents a severe deficiency and is associated with a viral load high in plasma and cerebrospinal fluid (CSF). This is related to the occurrence of severe neuroinflammation and neuronal injury.[20] Research by Sukarini stated that HIV sufferers with CD4 levels ≤ 200 cells/µL have a 9x risk of experiencing cognitive impairment.[21] In addition, the use of anti-retroviral regimens (ART) with scores of central nervous system penetration effectiveness (CPE) is associated with better cognitive function in HIV patients.[22] There are several limitations in this study, including not assessing other factors that may be associated with impaired cognitive function, such as CD4 levels and CPE scores. It is also suggested for future research to use several combinations of neurocognitive tests for the accuracy of assessing cognitive impairment in HIV sufferers.

5. Conclusion

There is a significant relationship between plasma DKK-1 levels and impaired cognitive function in HIV patients.

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