

# The Associations of Plasma Levels of Beta Amyloid, Insulin, Insulin-Degrading Enzyme and Receptor of Advanced Glycosylation End Product with Cognitive Impairment in Type 2 Diabetes Mellitus Patients

Tip 2 Diabetes Mellitus'lu Hastalarda Görülen Bilişsel Bozukluk ile Beta Amiloid'in Plazma Düzeyleri, İnsülin-Parçalayan Enzim ve İleri Glikozilasyon Son Ürünlerinin İlişkisi

Yuliarni Syafrita<sup>1</sup>, Darwin Amir<sup>1</sup>, Eva Decroli<sup>2</sup>

<sup>1</sup>Faculty of Medicine, Andalas University/DR M Djamil Hospital, Jalan Perintis Kemerdekaan No.94 Padang Timur, Padang, West Sumatra, Indonesia, Department of Neurology, Padang, Indonesia

<sup>2</sup>Faculty of Medicine, Andalas University/DR M Djamil Hospital, Jalan Perintis Kemerdekaan Padang Timur, Padang, West Sumatra, Indonesia, Department of Internal Medicine, Padang, Indonesia

## Correspondence:

Yuliarni Syafrita  
Faculty of Medicine, Andalas University/DR M Djamil Hospital, Jalan Perintis Kemerdekaan No.94 Padang Timur, Padang, West Sumatra, Indonesia, Department of Neurology, Padang, Indonesia  
E-mail: ysyafrita@yahoo.com

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## Abstract

**Background:** Diabetes mellitus patients are at a higher risk of developing cognitive impairment compared to the non-diabetes mellitus population. There are various mechanisms underlying the association between diabetes mellitus and impaired cognitive function, including the failure of glucose metabolism, insulin resistance, and increased formation of amyloid beta, insulin-degrading enzyme (IDE) and receptor of advanced glycosylation end product (RAGE).

**Objective:** This study aimed to find the association of the levels of beta amyloid, insulin, IDE, and RAGE with the onset of cognitive impairment in diabetes mellitus patients.

**Methods:** A case control study was conducted on 60 patients classified as 30 subjects with cognitive impairment and 30 subjects without cognitive impairment. Cognitive function was examined using the Montreal Cognitive Assessment Indonesian version (MoCA-Ina) which is a neuropsychological test. The plasma levels of beta amyloid, insulin, IDE and RAGE were measured by Elisa technique. Mean differences in the levels of biomarkers in both groups were analyzed using the Mann-Whitney U test and the association between two variables was analyzed using the chi-square test.

**Results:** It was found that the plasma levels of insulin and beta amyloid 42 were lower in the group with cognitive impairment and there was an association between low plasma levels of insulin and beta amyloid 42 with the occurrence of cognitive impairment ( $p < 0.05$ ).

**Conclusion:** There seems to be a correlation between plasma insulin level, beta amiloid and cognitive impairment in patients with diabetes mellitus..

**Keywords:** Biomarkers, cognitive disorders, diabetes mellitus

## Öz

**Giriş:** Diabetes mellitus'lu hastalarda bilişsel bozukluk görülme olasılığı, bu hastalığı bulunmayanlara göre daha yüksektir. Diabetes mellitus ile bilişsel bozukluk arasındaki ilişkiyi açıklayabilecek insülin direnci, artmış amiloid oluşumu, insülin yıkan enzim (İYE) ve ileri glikozilasyon son ürünleri reseptörü (İGSÜ) gibi bir dizi neden bulunmaktadır.

**Amaç:** Bu çalışmada amaç, diabetes mellituslu hastalarda başlayan bilişsel bozukluk ile beta amiloid, insülin, İYE ve İGSÜ arasındaki ilişkiyi araştırmaktır.

**Yöntemler:** Bu vaka kontrol çalışmasında, bilişsel bozukluğu olan 30 hasta ile olmayan 30 olgu karşılaştırıldı. Bilişsel bozukluk, bir nöropsikolojik test olan Montreal Bilişsel Değerlendirme Testinin Endonezya sürümü (MBDT-End) ile ölçüldü. Beta amiloid, insülin, İYE ve İGSÜ Elisa tekniği ile ölçüldü. Her iki gruptaki biobelirteç düzeyleri Mann-Whitney U testi, iki değişkenin arasındaki ilişki ise Ki-Kare testi ile değerlendirildi.

**Bulgular:** Plazma insülin seviyeleri ile beta amiloid seviyeleri bilişsel bozukluğu olan hastalarda olmayanlara göre daha düşük bulundu. Plazma insülin seviyeleri ve beta amiloid ile bilişsel bozukluk arasında da bağlantı saptandı ( $p < 0.05$ ).

**Sonuç:** Diabetes mellituslu hastalarda plazma insülin seviyeleri ve plazma amiloid düzeyleri ile bilişsel bozukluk arasında bir ilişki izlenmektedir.

**Anahtar Kelimeler:** Biobelirteçler, bilişsel bozukluklar, diabetes mellitus

## Introduction

Several epidemiological studies have shown that diabetes mellitus patients are at the risk of developing cognitive impairment and dementia. It was reported that patients with diabetes mellitus have 1.5 to 2.5 times higher risk of developing dementia than the elderly non-diabetes population.<sup>[1]</sup> It was also reported that high blood sugar levels, especially the two-hour postprandial blood sugar, are positively correlated with the incidence of dementia – both Alzheimer's, and vascular dementia. The mechanism underlying the association between diabetes and dementia remains unclear but tends to be multifactorial, reflecting the complexity of metabolic disorders in diabetes mellitus. The evidence also supports various pathologic changes obtained in the brain of diabetes patients who develop dementia.<sup>[2]</sup>

High blood sugar levels, reduced insulin production by the pancreatic beta cells, and failure of the release of insulin in response to hyperglycemia (insulin resistance) are hallmarks of type 2 diabetes mellitus. If these conditions evolve over time, they are associated with the incidence of cognitive impairment.<sup>[3]</sup> Under normal conditions, insulin and insulin receptors play an important role in cognitive function by modifying the activity of excitatory and inhibitory post-synapse receptors.<sup>[4-6]</sup> It was also found that prolonged hyperinsulinemia leads to reduced expression of insulin receptors on the blood-brain barrier due to lower insulin levels in the cerebrospinal fluid and brain tissue.

Insulin-degrading enzyme (IDE) is an enzyme that plays a major role in degrading insulin and regulating intracellular and extracellular levels of beta amyloid. The presence of hyperinsulinemia, resulting in decreased IDE performance to clear beta amyloid in the plasma since the IDE selectively binds to insulin compared to beta amyloid, subsequently increases plasma A $\beta$  levels. High plasma levels of A $\beta$  increases the secretion of A $\beta$  into the brain by means of the receptor of advanced glycosylation end product (RAGE), and eventually, the brain is affected by excessive A $\beta$ .<sup>[7,8]</sup>

Based on the background mentioned above, this study aims to uncover the associations of plasma levels of beta amyloid, insulin, IDE, and RAGE with impaired cognitive function in patients with type 2 diabetes mellitus.

## Methods

An observational study with a case-control study design has been performed on 60 diabetes mellitus type 2 patients classified as 30 subjects with and 30 subjects without cognitive impairment in the outpatient clinic of Dr. M. Djamil Hospital and Ibnu Sina Islamic Hospital Padang from July until November 2016. Cognitive function tests were performed using the Montreal Cognitive Assessment test version of Indonesia (MoCA-Ina), one of the neuropsychological tests that has been validated for use in Indonesia. Based on the results of the MoCA-Ina test, samples are classified as two groups: a group with cognitive impairment and the those without cognitive impairment. The levels of beta amyloid (40 and 42), insulin, IDE, and RAGE plasma were measured by Elisa method. The measurement of plasma FT4 levels was conducted to rule out hypothyroidism. Differences in the mean level of each variable in both groups were tested by the Mann-Whitney U test, and the association of each variable with the presence of cognitive impairment was tested by the chi-square test. Multivariate logistic regression analysis was also conducted to determine the most dominant variable contributing to the occurrence of cognitive impairment.

## Results

Table 1 shows that the basic characteristics of both subjects were statistically similar ( $p > 0.05$ ).

Table 2 shows significant differences in plasma insulin levels between the two groups ( $p < 0.05$ ). Insulin levels were lower in the group with cognitive impairment. There were no significant differences in beta amyloid, IDE, and RAGE levels between the two groups, but we may observe that mean levels of beta amyloid 42 were lower in the cognitive impairment group than those without.

**Table 1.** Basic characteristics subjects

	Cognitive function		p
	Impaired	Normal	
Age (years)	62.2 $\pm$ 7.9	61.07 $\pm$ 7.5	0.67
Sex (male/female)	12/18	12/18	1.0
Education (years)	12.07 $\pm$ 3.88	11.83 $\pm$ 3.34	0.56
Length of disease (years)	12.1 $\pm$ 6.5	9.83 $\pm$ 7.2	0.60
Compliance (good/bad)	14/16	17/13	0.44

**Table 2.** Differences in mean plasma levels of beta amyloid, insulin, insulin-degrading enzyme (IDE), and receptor advanced glycosylation end product (RAGE) on both groups

Variable	Cognitive function (mean±standard deviation)		p
	Impaired	Normal	
Beta amyloid 40	1737.46 (1171.28–1975.92)	1696.01 (1390.8–2089.0)	0.640
Beta amyloid 42	39.81 (25.19–127.70)	45.44 (28.75–104.81)	0.137
Insulin	90.19 (44.96–302.35)	164.67 (52.21–271.74)	0.002
Insulin-degrading enzyme	0.86 (0.65–3.69)	0.83 (0.42–3.12)	0.193
Receptor advanced glycosylation end product	19.76±12.25	21.26±10.80	0.464

**Association of the Plasma Levels of Beta Amyloid, Insulin, Insulin-Degrading Enzyme (IDE), and Receptor of Advanced Glycosylation End Product (RAGE) with Cognitive Function**

The association of the plasma levels of beta amyloid, insulin, IDE, and RAGE with cognitive function is shown in Table 3. In order to find the association between those variables, they must be classified first of all. Since each variable has no standard normal value, the classification between low or high levels was determined by calculating the cut-off point of each variable using the receiver operating characteristic procedure (ROC), so that each variable can be grouped based on the value of these cut off points. The level was considered high if the level obtained was higher than the cut off point and vice versa.

Table 3 shows significant association of the low plasma Aβ42 and insulin levels with impaired cognitive function ( $p < 0.05$ ).

**Multivariate Analysis**

Multivariate logistic regression analysis was conducted to determine independent variable for the occurrence of cognitive impairment, as shown in Table 4. It was found that the low plasma levels of insulin and Aβ42 were important factors for the occurrence of cognitive impairment in patients with type 2 diabetes mellitus. However, no statistically significant correlation was found between cognitive impairment and insulin or Aβ42.

**Table 3.** The association of the plasma levels of beta amyloid, insulin, insulin-degrading enzyme (IDE), and receptor of advanced glycosylation end product (RAGE) with cognitive function (OR: Odds ratio)

Variable	Cognitive function		p	OR (95% confidence interval)
	Impaired (n)	Normal (n)		
Aβ40				
High (abnormal)	14	10	0.292	1.750 (0.016–4.973)
Low (normal)	16	20		
Aβ42				
Low (abnormal)	20	12	0.038	3.000 (1.046–8.603)
High (normal)	10	18		
Insulin				
Low (abnormal)	17	8	0.018	3.596 (1.216–10.638)
High (normal)	13	22		
IDE				
Low (abnormal)	16	13	0.438	1.495 (0.540–4.136)
High (normal)	14	17		
RAGE				
Low (abnormal)	14	12	0.602	1.313 (0.472–3.653)
High (normal)	16	18		

**Table 4.** Multivariate analysis showing the variables contributing to the occurrence of impaired cognitive function in patients with type 2 diabetes (OR: Odds ratio, CI: Confidence interval)

Variable	Coefficient	p	OR (CI 95%)
A $\beta$ 42	0.823	0.147	2.227 (0.749–6.927)
Insulin	1.058	0.067	2.880 (0.928–8.931)

## Discussion

The baseline characteristics consisting the age, sex, education, patient compliance, and duration of disease between the case and control groups were statistically similar. It was found that the plasma level of insulin is lower in the patients with cognitive impairment ( $p=0.002$ ). They had 3.5 times higher risk of developing cognitive impairment compared to the group with high plasma insulin levels. Low plasma insulin levels indicate the low ability of the pancreas to produce insulin and are associated with severe damage to the pancreas. Burn et al. found that higher plasma insulin levels were associated with the slow decline of cognitive function in patients with early-stage Alzheimer's dementia and high plasma insulin levels are associated with low findings of brain atrophy in patients with early dementia.<sup>[9]</sup>

There was no difference in mean levels of beta amyloid 42 between the two groups but we found that mean level of beta amyloid 42 was lower in the cognitive impairment group than that of those without. After classifying the beta amyloid 42 level categorically (ROC), there was a significant association between low plasma A $\beta$ 42 levels with the occurrence of cognitive impairment in patients with type 2 diabetes ( $p=0.038$ ); the group with low levels of A $\beta$ 42 ( $\leq 42.12$  pg/ml) are at risk of developing cognitive impairment 2.88 times higher than group with higher A $\beta$  levels ( $>42.12$  pg/ml).

Low A $\beta$ 42 in plasma may occur due to reduced clearance A $\beta$ 42 from the brain to the peripheral circulation; or it could be due to increased transportation from the system to the brain and also the increased levels of RAGE (receptors that help the entry of A $\beta$ 42 from the system to the brain, so that the levels of A $\beta$ 42 in brain tissue will increase). Puzzo et al. found that low concentrations of A $\beta$ 42 monomers and oligomers cause elongation of long-term potentiation (LTP) and that high concentration of A $\beta$ 42 (200 nm) causes decreased potentiation in the hippocampus.<sup>[10]</sup>

Various studies have shown that the underlying pathophysiological mechanism of cognitive impairment in patients with dementia is a disorder of the synapse by A $\beta$ . Under normal conditions, low levels of A $\beta$  are required to set up and maintain the plasticity of synapses to improve cognitive function, but the accumulation of A $\beta$  in a higher concentration coupled with the aging process will cause malfunction of normal maintenance of synapses, as seen in patients with dementia.<sup>[10-12]</sup>

No association was found between plasma levels of A $\beta$ 40, IDE, and RAGE with cognitive impairment in patients with type 2 diabetes mellitus.

## Conclusion

This study shows the association of lower plasma levels of insulin and A $\beta$ 42 with impaired cognitive function in patients with type 2 diabetes mellitus.

### Conflict of Interest

There is no conflict interest in this research.

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