The Relationship of Secretory Phospholipase A2 Type IIa Levels with Glomerulus Filtration Rate in Type 2 DM Patients

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Artikel Penelitian

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Abstract: *Phospholipase/sPLA2 type IIA is a mediator that connects the pathogenesis of diabetes and its complications. Diabetic nephropathy is the most frequent microvascular complication, with albumin urine and changes in glomerular filtration rate. This study aimed to discover the relation between sPLA2 type IIA levels in diabetic patients with glomerular filtration rate (GFR). This study used the crossectional method in 62 samples in RSJ. Prof. Dr. Soerojo Magelang. Sampling was done by total sampling, and medical records were used as an instrument. This research was conducted in patients with sPLA2 type IIA levels and had complete laboratory examination data in 2016. The results showed that the level of sPLA2 type IIA was very high in diabetic patients, and GFR was below standard. Pearson correlation analysis showed no significant correlation between type IIA sPLA2 and GFR (p=0.318), with a positive but weak correlation (r=0.129) with the diabetic duration of 8 years. Pearson correlation analysis was conducted in 21 samples in which GFR values were ≤60 (p=0.0712), resulting in a negative direction (r=-0.086) with a diabetes duration of 11 years. It was shown that diabetic patients with increased levels of sPLA2 type IIA and more extended periods of diabetes would affect the decrease in GFR value.*

Keywords: *sPLA2 type IIA enzim, glomerular filtration rate, diabetes*

Introduction

Diabetes mellitus is a chronic disorder of carbohydrate, protein and fat metabolism due to damage or lack of insulin secretion response resulting in hyperglycemia (1). Based on World Health data, Organizational DM is the eighth leading cause of death globally, with the most significant contributor to Southeast Asia and the Western Pacific Region (2). An increase followed the increasing prevalence of DM in the incidence of macrovascular and microvascular complications that would impact the quality of life and the economy of patients and the country (3).

There are several differences in the characteristics of diabetics in Asia and Europe (4). In the Asian region, the onset of DM generally occurs earlier, and in the Asian region, there is also a higher risk of complications in the form of kidney damage than in the European region, which is more dominated by complications in the

heart that lead to stroke (4). The National Health and Nutrition Examination Survey (NHANES) in 1999-2004 showed that the prevalence of microvascular complications was higher than that of macrovascular complications with chronic kidney disease as the most complication, contributing 27.8% (5).

Of DM patients, 20-40% experienced complications in diabetic nephropathy, in which type 2 DM patients dominate. Clinical signs of diabetic nephropathy are increased urine albumin and decreased GFR. Diabetic nephropathy is a process with gradual progression, beginning with an increase in GFR and then a decrease in GFR in later stages (6).

Hyperglycemia in diabetes mellitus can activate four main pathogenic pathways, causing damage to blood vessel walls (7). First, the damage that occurs causes the release of cytokines and growth factors by damaged cells to

increase the activation of phospholipase (PLA2) (8).

Phospholipase (PLA2) is an enzyme that hydrolyzes phospholipids at the 2nd carbon bond into fatty acids and lysophospholipids. In general, this reaction is the first step in arachidonic metabolism, producing bioactive lipid mediators, which are inflammatory mediators, including prostaglandins and leukotrienes (9). That damage to target organs due to PLA2 occurs through the cyclooxygenase (COX) and lipoxygenase (LO) enzymatic pathways (8).

The above conditions can cause scar tissue to form in the glomerulus to decrease kidney function (10). Kidney function is determined by the glomerular filtration rate (GFR) value, whose measurement can be estimated by calculating plasma creatinine levels. The standard GFR value is >90 mL/minute, but if the GFR falls below 90 mL/minute, it indicates a decrease in kidney function and its progression should be monitored (11).

Based on the description, elevated PLA2 levels in DM patients are thought to affect LFG through inflammatory pathways that cause damage to the kidney structure. Until now, PLA2 measurements have been limited to SPLA2 type IIA measurements but can be a parameter that describes PLA2 levels in the body (12). Research on sPLA2 type IIA and LFG and its effect on dm type 2 patients in Indonesia is still tiny, even though this study has the potential to be an essential step in preventing the progressivity of organ damage in DM. Of all hospitals in Indonesia, RSJ Prof. Dr Soerojo Magelang is the only hospital that conducts research on sPLA2 type IIA and RSJ in DM patients in dm-specific internal medicine polyclinics.

Research Method

Research Design

This type of research is a quantitative research using observational analytical methods with *a cross-sectional approach* to finding sPLA$_2$ type IIA levels with LFG at prof. Dr Soerojo Magelang Mental Hospital. Data collection is carried out at once at a time.

Population and Sample

The population in this study was all type 2 DM patients who met the inclusion criteria at RSJ. Prof. Dr Soerojo Magelang, in 2016, as many as 62 people.

The inclusion criteria in this study were all type 2 DM patients in RSJ. In addition, Prof. Dr Soerojo Magelang has sPLA$_2$ type IIA data and complete lab examination data on medical records.

Sampling

The sampling technique uses *a non-probability* technique, namely total sampling. Total sampling is a technique where the number of samples equals the number of populations (13).

Data Collection

The data used in this study is secondary data from the medical records of dm type 2 patients at RSJ. Prof. Dr. Soerojo Magelang.

Research Procedure

The opening of the medical record with the permission of the head of the medical record was carried out, and then data collection was carried out in the form of medical record number, name, age, gender, blood pressure, body mass index, abdominal circumference, blood sugar profile, sPLA2 levels and plasma creatinine levels.

Result and Discuss

Subject Characteristic

The study subjects' characteristics were gender, age, body mass index (BMI), abdominal circumference and blood pressure.

Based on **Table 1**, it can be seen that the majority of subjects in this study are female, with the most age ranging from 53-64 years, with a median BMI value of 25.9 kg / m2 accompanied by an average abdominal circumference of 90.79 cm. Furthermore, of the 62 subjects of the study, the median value of systole pressure was 135 mmHg with a minimum value of 90 mmHg and a maximum value of 190 mmHg, while for diastole, it had a median value of 90 mmHg with a minimum value of 60 mmHg and a maximum value of 90 mmHg.

Table 1. Characteristics of the study subjects by sex, age, body mass index (BMI), abdominal circumference and blood pressure

Characteristics of Respondents	N	%
Gender		
Man	24	38.7
Woman	38	61.3
Age (Years)		
<52	17	27.4
53-64	26	41.9
>65	19	30.6
	Average (s.b)	**IK95%**
Abdominal circumference (cm)	90.79 (11.75)	87.81-93.77
	Median (Minimum-Maximum)	
BMI (Kg/m²)	25.9 (19.0-45.2)	
Blood Pressure (mmHg)		
Systolic	135.0 (90-190)	
Diastolic	90 (60-90)	

Source: Secondary data, 2016

Table 2. Distribution of subjects by blood sugar (GDS), fasting blood sugar (GDP) and HbA1c

Characteristics of Respondents	Average (s.b)	IK95%
GDS (mg/dL)	225.45 (87.55)	203.22-247.69
	Median (Minimum-Maximum)	
GDP (mg/dL)	132.5 (62.0-419.0)	
HbA1c (%)	8.4 (4.7-14.7)	

Source: Secondary data, 2016

Table 2 shows that the average current blood sugar level of the 62 samples was 225.45 mg / dL, while the blood sugar level had a median value of 132.5 with a minimum value of 62 mg / dL and a maximum value of 419 mg / dL. Blood sugar control can be seen through HbA1c values in people with diabetes. In this study, the median value of 62 samples was 8.4%, with a minimum value of 4.7% and a maximum value of 14.7%.

From the results of Kolmogorov-Smirnov's statistical analysis on the sPLA data variable, a $p<0.001$ value was obtained so that it could be known that the distribution of t data was not standard with the median level of sPLA2 type IIA in 62 study subjects was 3650.2 ng / dL with a minimum value of 1300 ng / dL and a maximum value of 14570.9 ng / dL.

From the results of Kolmogorov-Smirnov's statistical analysis on the glomerular filtration rate (LFG) data variable, a p-value = 0.2 was obtained, which is more than 0.05 so that it can be known that the distribution of standard distributed LFG variable data with the average glomerular filtration rate value of 62 study subjects was 70.60 ml/min / 1.73 m².

Discussion

Based on the results of the analysis of 62 samples of type 2 diabetes patients, the majority of the samples were women, with a percentage of 61.3%, this is following who is data that in 2012 globally, the mortality rate due to diabetes in women was higher than in men, and this mortality rate is predicted to continue to increase in the following years.

Table 3. Distribution of subjects by SPLA Grade$_2$

Characteristics of Respondents	Median (Minimum-Maximum)
SPLA$_2$ (ng/dL) levels	3650.2 (1300.0-14570.9)

Source: Secondary data, 2016

Table 4. Distribution of subjects by LFG Values

Characteristics of Respondents	Average (s.b)	IK95%
LFG (ml/min/1.73^{m2})	70.60 (24.48)	64.38-76.81

Source: Secondary data, 2016

Furthermore, in middle-income countries such as Indonesia, deaths due to high blood sugar levels are dominated at the age of more than 50 years, in contrast to high-income countries, dominated by the age group of 60-69 years (14). This study is dominated by the age of 53-64 years, which is 41.9%.

From the results of data analysis, the median BMI value was 25.9 kg / m2 and an average abdominal circumference of 90.79 cm for the Southeast Asian region BMI with a figure of >25 kg / m2 was included in the obesity classification. In contrast, abdominal circumference>90 cm in both men and women showed the presence of central obesity (14). In general, the presence of obesity and central obesity are two interrelated risk factors in the occurrence of type 2 diabetes (15) (14).

Based on 2014 data, it is estimated that more than one in three people over the age of 18 is obese. WHO states that the prevalence of obesity is higher in the Americas than in the Southeast Asian region. One of the causes of the high rate of obesity is the lack of physical activity. From all areas covered by WHO, without differentiating state income, it shows that women are less active than men, and this lack of physical activity is also a risk factor for obesity and diabetes (14).

Of the 62 subjects of the study, the median value of systole pressure was 135 mmHg with a minimum value of 90 mmHg and a maximum value of 190 mmHg, while for diastole, it had a median value of 90 mmHg with a minimum value of 60 mmHg and a maximum value of 90 mmHg. Based on the JNC VIII blood pressure classification, the systole blood pressure value of

135 mmHg and diastole of 90 mmHg are included in the pre-hypertension classification. Diabetes and obesity are risk factors that can be controlled in the occurrence of hypertension. Diabetes and obesity interconnect things in the pathological path of diabetes (16). Besides, hypertension is a significant risk factor in diabetic nephropathy because it can accelerate the progressivity of microvascular damage in people with diabetes (16). According to the Centers for Disease Control and Prevention 2007, about 75% of people with diabetes also have hypertension.

The statistical analysis results show that the average blood sugar level (GDS) is 225.45 mg / dL, the median fasting blood sugar level (GDP) is 132.5 mg / dL, and the HbA1c value is 8.4%. Therefore, the results showed a high blood sugar condition in the sample because, according to the American Diabetes Association (ADA), in normal conditions, GDS was <140mg/dL, GDP was <100mg/dL and HbA1c <5.7% (17).

From the results of Kolmogorov-Smirnov's statistical analysis on the sPLA2 data variable, a value of p<0.001 was obtained so that it can be known that the distribution of sPLA level variable data is not normally distributed. From the results of the statistical analysis above, it is known that the median value of sPLA2 type IIA levels in 62 study subjects was 3650.2 ng / dL with a minimum value of 1300 ng / dL and a maximum value of 14570.9 ng / dL. The sPLA value was said to be expected when it ranged from 130-242 ng/dl, so in this study, all the subjects had very high sPLA levels compared to normal values(18). This study follows other studies that state that hyperglycemic conditions in DM patients can

activate various pathways that cause oxidative stress states. This condition will then cause the release of cytokines and growth factors so that they can also activate the enzyme phospholipase (8)(19). The increase in phospholipase enzyme levels is expected to accelerate the progressivity of damage to an organ with the resulting lipid inflammatory mediators (9)(20).

From Kolmogorov-Smirnov's statistical analysis of the glomerular filtration rate (LFG) data variable, a p-value = 0.2 was obtained, which is more than 0.05 so that the distribution of standard distributed LFG variable data can be known. The data analysis results show that the average glomerular filtration rate of 62 study subjects was 70.60 ml/min / 1.73 m2. According to Barret et al., in 2016, there was a difference in LFG values in men and women. Women had a value 10% lower than men. Besides, a person's body surface area also influences the LFG value. Of the 24 study subjects with male sex, an average LFG value of 65.42 ml/min / 1.73 m2 was obtained, while 38 samples with female sex had an average LFG value of 73.87 ml/min / 1.73 m2. The existence of differences in theory and circumstances in the sample could be due to the presence of pathological factors in subjects, such as obesity, hypertension and hyperglycemic states (18)(15).

Bivariate Analysis Results

Relationship of sPLA$_2$ levels with LFG

The relationship between sPLA2 levels and LFG at RSJ Prof. Dr Soerojo Magelang can be seen in **Table 5**.

Based on the results of univariate analysis, sPLA levels have an abnormal distribution, and after the normality of data using log10, the data distribution can be expected. At the same time, dependent variables in the form of LFG values show a normal data distribution. Because both variables have a normal distribution, a bivariate analysis of the Pearson Test can be performed. From the results of the Pearson Test, a p-value = 0.318 was obtained, which showed that the correlation between sPLA$_2$ levels and LFG values was not meaningful. Pearson's correlation value of 0.129 indicates a positive correlation direction with a fragile correlation strength.

Based on **Table 6** of the analysis results in the LFG group ≥60 ml/min / 1.73m2, a p-value = 0.542 was obtained, which showed the correlation between sPLA$_2$ levels and LFG values ≥ 60 ml/min / 1.73m^2 was not meaningful. Pearson's correlation value of 0.098 indicates a positive correlation direction with a fragile correlation strength. In the LFG group <60 ml/min/1.73m^2, a p = 0.712 value was obtained, which indicated that the correlation was not meaningful, with a Pearson correlation value of - 0.086, indicating a negative correlation direction with a fragile correlation strength.

Table 5. Relationship of Knowledge to Compliance with The Use of Personal Protective Equipment

	LFG
sPLA$_2$ levels	r = 0.129 p = 0.318 n = 62

Source: Secondary data, 2016

Table 6. Relationship between sPLA content and LFG value with LFG value limit of 60 ml/min/1.73 m2

	LFG (≥60)	LFG (<60)
sPLA$_2$ levels	r = 0.098 p = 0.542 n = 41	r = - 0.086 p = 0.712 n = 21

Source: Secondary data, 2016

Discussion

The results of data analysis obtained a value of p = 0.318, which shows that the correlation between $sPLA_2$ levels and LFG values is not meaningful. On the other hand, Pearson's correlation value of 0.129 indicates a positive correlation direction with a fragile correlation strength. Here is a linear graph between the $sPLA_2$ levels and the LFG values in 62 samples.

Figure 1 shows that the higher the sPLA2 level, followed by an increase in the LFG value, the relationship with the positive direction between the two variables is evident. $SPLA_2$ levels may increase in inflammatory states. People with diabetes are mild inflammatory conditions of a chronic nature. The increase in $sPLA_2$ levels is induced by a hyperglycemic state that can cause oxidative stress conditions that will produce cytokines and growth factors that increase the amplification and activation of the $sPLA_2$ enzyme.

In theory, diabetic nephropathy proceeds slowly and is divided into five stages, and the examination carried out to monitor renal function in this disease is the value of LFG (Verdiansah, 2016). In the early stages of diabetic disease, nephropathy LFG values can be monitored commonly or increased, called hyperfiltration, while in stages 3 to 5, LFG values will decrease until they can be affected by terminal renal failure gradually [18]. The 62 samples had an average LFG value of 70.60 ml /min / 1.73 m^2, indicating their kidney function had decreased. When this LFG value is correlated with a high sPLA2 level, a high value indicates that an increase will follow the higher sPLA2 level in LFG. It is estimated that this high level of sPLA can cause a thickening of the basal membrane in the glomerulus, the accumulation of the extracellular matrix and affect the permeability of the kidney microvascular so that this mechanism can likely cause hyperfiltration or an increase in LFG in the kidney organs in people with diabetes. Manifestations of damage to the kidney organs can be seen after five years of diagnosis of DM [18][20], this is in line with this study which has a median value of 62 samples having suffered from DM for eight years, so this corresponds to the course of the disease in diabetic nephropathy.

This study used all samples without classifying samples based on LFG values based on physiological theory so that the limit $sPLA_2$ levels would affect LFG values more. Subsequent bivariate analysis was carried out by data processing divided into two groups considering physiological LFG value classification and the minimum number of samples for data analysis.

From the analysis results in the LFG group ≥60 ml/min / 1.73m2 with a total of 41 subjects, a p-value = 0.542 was obtained, which showed the correlation between $sPLA_2$ levels and LFG values ≥ 60 ml/min / 1.73m^2 was not meaningful. Pearson's correlation value of 0.098 indicates a positive correlation direction with a fragile correlation strength.

The above analysis showed the same results as the results of the previous analysis, which did not distinguish subjects based on LFG values, and it was increasingly seen that the high levels of $sPLA_2$ were also followed by an increase from LFG, which was more pronounced than the previous bivariate test. Furthermore, the duration of diabetes has a median value of 8 years, which shows that at this stage, the high levels of $sPLA_2$ have not caused a decrease in the value of LFG.

The analysis of the ≥60 groups showed the same results as the previous analysis, which did not distinguish the subjects based on LFG values. However, from **Figure 2**, it can be seen that an increase also follows the high level of sPLA2 in LFG, which is more pronounced than the previous bivariate test. Furthermore, the duration of diabetes has a median value of 8 years, which shows that at this stage, the high levels of $sPLA_2$ have not caused a decrease in the value of LFG.

In the analysis of the LFG group <60 ml/min / 1.73m^2, different correlation direction results were obtained, namely negative, meaning that the higher the $sPLA_2$ level followed by a decrease in the LFG value, this following the stage of diabetic nephropathy stage 3-5. At that stage, kidney function will be observed to decrease and continue to deteriorate even though it occurs slowly. Furthermore, an analysis of the diabetes duration variables in this group showed that the average sample had been suffering from diabetes
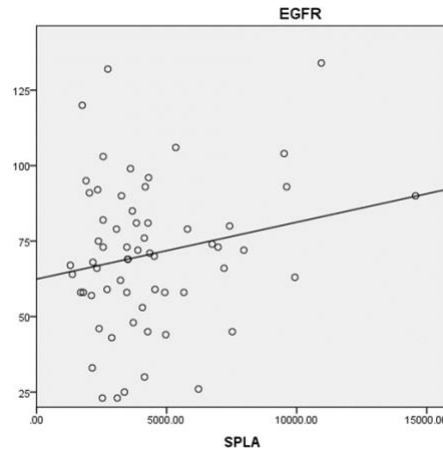
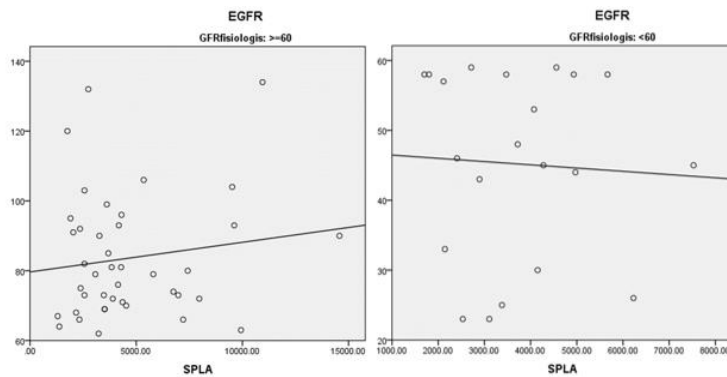**Figure 1** Linear graph of the relationship of sPLA2 levels with LFG

Figure 2 Linear graph of the relationship of sPLA2 levels with LFG with a limit of 60 ml/min/1.73m2

for 11 years. Those abnormalities in the kidney organs' progress decrease so that patients with a duration of DM 11 years and have high $sPLA_2$ levels will show a decrease in LFG values, and it can be concluded that at this stage, kidney function has decreased and can be predicted that the longer the DM and the higher the $sPLA_2$ levels will cause the LFG value to decrease (18).

Conclusion

Based on the study results and discussion, it can be concluded that data obtained from 62 study subjects showed no significant relationship between $sPLA_2$ levels and LFG. However, it can be seen that different correlation directions in the data analysis group with LFG values <60 have a negative correlation direction.

References

1. Perkumpulan Endokrinologi Indonesia 2015, *Management and prevention of type 2 diabetes mellitus in Indonesia 2015,* First Edition, Executive Board of the Indonesian Endocrinology Association, Jakarta
2. World Health Organization 2016, *Global Report on Diabetes,* Geneva, WHO, accessed June 2018, https://apps.who.int/iris/ bitstream/handle/10665/204874/WHO_N MH_NVI_16.3_eng.pdf;jsessionid=7066016C B18F12A3E9D5A2D4A5AB48B?sequence=1

3. Indonesia, Departemen Kesehatan RI, 2014, *data and information centre of the Indonesian Ministry of Health,* Ministry of Health of the Republic of Indonesia, Jakarta.

4. Ma, RCW & Chan, JCN 2013, 'Type 2 diabetes in East Asians : similarities and differences with populations in Europe and the United States, *Annals of The New York Academy of Sciences*, Vol.1281, hal.64-91

5. Deshpande, AD, Harris-Hayes, M & Schootman, M 2008, 'Diabetes-Related Complications', *Physical Therapy Journal*, Vol.88, hal.1254-1264

6. Gheith, O, Farouk, N, Nampoory, N, Halim, MA & Al-otaibi, T 2015, 'Diabetic Kidney disease: World Wide Difference of Prevalence and Risk Factor', *Journal of Nephropharmacology*, Vol.5, hal.49-56

7. Kakouros, N, Rade, JJ., Kourliouros, A & Resar, JR 2011, 'Platelet Function in Patients with Diabetes Mellitus : From a Theoretical to a Practical Perspective', *International Journal of Endocrinology,* Vol.2011, hal.1-14

8. Natarajan, R, Nadler, J. L & Cohen, R. A 2015, 'Lipid Inflammatory Mediators in Diabetic Vascular Disease', *Journal of the American Heart Association,* Vol.24, hal.1342-1349, accessed March 2018, https://www.ahajournals.org/doi/full/10.1161/01.ATV.0000133606.69732.4c?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

9. Sato, H, Taketomi, Y, dan Murakami, M 2016, 'Metabolic regulation by secreted phospholipase A2', *Biomed Central,* Vol.36, hal.1-7,

10. Beckman, J, Creager, MA & Libby, P 2014, 'Diabetes and Atherosclerosis: Epidemiology, Pathophysiology, and Management', *JAMA*, Vol.287, hal.2570-2581

11. Verdiansah 2016, 'Pemeriksaan Fungsi Ginjal', *Cermin Dunia Kesehatan Journal,* Vol.43, hal.148-154

12. Rosenson, R. S dan Hurt-camejo, E 2012, 'Phospholipase A2 enzymes and the risk of atherosclerosis, *European Heart Journal,* Vol.33, page. 2899–2909

13. Sugiyono 2013, *Qualitative and R&D Quantitative Research Methods,* Alfabeta, Bandung

14. World Health Organization 2000, *The Asia-Pacific Perspective: Redefining obesity and its treatment,* Geneva, WHO

15. Maric, C 2011, 'Obesity, Metabolic Syndrome and Diabetic Nephropathy', *Contrib Nephrol. Basel*, Karger, vol 170, hal.28–35, d

16. Long, AN, DO & Dagogo-Jack, S 2013, 'The Comorbidities of Diabetes and Hypertension: Mechanisms and Approach to Target Organ Protection', *NIH-PA Author Manuscript*, Vol.13, hal.244-251

17. American Diabetes Association 2018, 'Introduction: Standards of Medical Care in Diabetes 2018', *Diabetes Care*, Vol.41 (Supplement 1), page. 1-2

18. Levey, AS, Astor, BC, Stevens, LA & Coresh, J 2010, 'Chronic kidney disease, diabetes, and hypertension, *Kidney International Journal*, Vol.78, hal.19-22

19. Sun, GY & Jensen, MB 2011, 'Phospolipases A2 and Inflammatory Responses in the Central Nervous System', *Neuromolecular Medicine,* Vol.12, hal.133-148,

20. Murakami, M, Sato, H, Miki, Y, Yamamoto, K, & Taketomi, Y 2015, 'A new era of secreted phospholipase A2', *Biomed Central,* Vol.36, hal.1-7