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Original Article

Comparison of Antioxidative Effect of Metformin and Combination of Metformin-Sulfonylurea in Type 2 Diabetes Mellitus Patients

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ABSTRACT

Background: Even though oxidative stress is intensively studied for its role in diabetic nephropathy, the effectiveness of first-line medication on diabetes in preventing oxidative stress and diabetic nephropathy is still unknown. This study aimed to analyze 8-iso-Prostaglandin F2α, UACR, and their correlation on 114 type 2 diabetes mellitus patients who consumed metformin and the combination of metformin-sulfonylurea. **Material and Method:** Urinary 8-iso-Prostaglandin F2α was measured by ELISA and urinary albumin was measured by Bromocresol Green Albumin assay. **Results:** The results showed that HbA1c (p=0.038) and 8-iso-Prostaglandin F2α level higher in the combination group than in metformin group, but not for UACR (p=0.838). However, subgroup analysis on albuminuric patients (UACR>30 mg/g, n=55) showed no different in HbA1c and 8-iso-Prostaglandin F2α level between the medication groups. Linear regression analysis showed HbA1c (β=0.402, p=0.002) and the combination of metformin-sulfonylurea (β=0.364, p=0.004) were the most predictive factors for increased UACR after controlled by age, gender, IMT, systolic blood pressure, 8-iso-Prostaglandin F2α, smoking habit and exercise habit. Conclusion: In conclusion, metformin was more effective in decreasing 8-iso-Prostaglandin F2α level, an oxidative stress marker. The increase of HbA1c and the use of a combination of metformin-sulfonylurea contributed in increased UACR in this study.

Key words: Metformin, Diabetes mellitus, Diabetic nephropathy, Sulfonylurea, UACR, 8-iso-Prostaglandin F2α

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INTRODUCTION

Diabetes mellitus is one of the non-communicable diseases that cause 4% of death in Indonesia.[1] International Diabetes Federation (IDF) predicts diabetic Mellitus patient in the world would increase from 415 million in 2015 to 642 million in 2040.[2] Diabetes mellitus highly needs world attention because it can cause a variety of microvascular complication, such as nephropathy, neuropathy, and retinopathy that can decrease the quality of diabetes mellitus patient's life. Based on data, after 5-20 years suffering from diabetes mellitus type 2, 8-20% of diabetes mellitus type 2 patient will have diabetic nephropathy.[3] Thus early diagnosis and proper treatment are needed to prevent and decrease the progression of diabetic nephropathy. The most common markers that were used to detect the presence of impaired renal function are estimated Glomerular Filtration Rate which could calculate based on serum creatinine (eGFR) value and Urine Albumin to Creatinine Ratio (UACR). However, UACR is still known to be not quite specific and sensitive enough as a prognostic marker for progression of diabetic nephropathy.[4] On the other side, oxidative stress, that could be characterized by increased level of 8-iso-Prostaglandin F2α, is known to occur prior to the manifestation of microalbuminuria and have an important role in the early stage of kidney function decline.[5]

Metformin or combination of metformin-sulfonylurea is the first-line oral antidiabetics for type 2 diabetes mellitus patient, especially at the primary health center. Both medications are reported to have the antioxidative effect. Sulfonylurea has been described as an antioxidant agent through Reactive Oxygen Species (ROS) scavenging effect by reversibly binding to SUR-1 and upregulation of antioxidant enzymes.[6] Metformin also has a beneficial effect against oxidative stress by reducing ROS through inhibiting mitochondrial respiration.[7] However, it is still lacking information regarding the different antioxidative outcomes between metformin with metformin combined with a sulfonylurea.

Moreover, the previous study showed there was no correlation between 8-iso-Prostaglandin F2α and UACR, but the study did not compare the therapeutic treatment of the patients.[8] Therefore, this study aimed to know whether metformin and combination of metformin-sulfonylurea have a different antioxidative effect or not and we wanted to know the correlation between oxidative stress marker with UACR on 114 type 2 diabetes mellitus patient (from ages: 33-75 years) which consume metformin and combination of metformin-sulfonylurea.

MATERIALS AND METHODS

Study Design

The study design is cross-sectional and the processed data in this study are primary data obtained from blood and urine samples analysis and other information from a validated questionnaire.

Location and Time

Sampling was carried out at Pasar Minggu Community Health Center on February to April 2016. Then sample analysis was conducted at Center

of Radioisotopes and Radiopharmaceuticals, BATAN, Serpong on April to June 2016.

Sample and Population

The population study was type 2 diabetes mellitus patients who were under treatment at Pasar Minggu Community Health Center on February 2016 to April 2016, consume metformin or metformin-sulfonylurea for at least 4 months. Inclusion criteria included patients with age ≥ 35 years old and in the fasting state at least 8 hours before sampling. Exclusion criteria patient with hematuria and patient with severe anemia.

Sampling Procedure

Before urine and blood sampling, the patient was asked to not consume anything, except plain water, start from 10 pm until sampling was conducted. For urine sample, patients were asked to collect their first urine in the morning to 30 mL plastic pot. Urine in a plastic pot was kept in a cooler box until the sediment accumulated at the bottom of vacutainer. Urine in the clear layer was then transferred into microtubes. Microtubes were kept in -80°C freezer until analyzed. Blood from the patients was collected by a certified phlebotomist and sent to Prodia Laboratory to determine the HbA1c and serum creatinine level.

Measurement of Urine Creatinine

First, standard creatinine solution was made to 20 mg/dL; 10 mg/dL; 5 mg/dL; 2.5 mg/dL; 1.25 mg/dL; 0.625 mg/dL; dan 0.3125 mg/dL concentration. Then 50 µl blank, standard, and sample solution were diluted with deionized water and then were put into a microplate well. The microplate was then incubated for 30 min at room temperature, and subsequently, the absorbance was measured at λ 490 nm. The analysis was done in duplicate.

Measurement of Urine Albumin

The procedure for urine albumin measurement according to BCG Albumin Kit manual book are first , standard albumin solution was made to 5.0 g/dL; 4.0 g/dL; 3.0 g/dL; 2.0 g/dL; 1.5 g/dL ; 1.0 g/dL; 0.5 g/dL; 0 g/dL. Entire well is given the number and entire solution is done in duplicate. Then 5 µl blank, standard, and sample solution were put into each well. Next, 200 µl reagent, Bromocresol Green (BCG), was put into each well. The plate was shaken to avoid bubbles in the well. After that, the plate was incubated for 5 minutes, then absorbance was measured at λ 620 nm.

Measurement of 8-iso-Prostaglandin F2α

The procedure for 8-iso-Prostaglandin F2α measurement according to competitive Enzyme-Linked Immuno Assay (ELISA) kit manual book (catalog #ADI-900-010, Enzo Life Sciences, Farmingdale, NY, USA). 8-iso-Prostaglandin F2α standard solution was made to 100,000 pg/mL; 25,000 pg/mL; 6,250 pg/mL; 1562.5 pg/mL; 390.6 pg/mL ; 97.7 pg/mL; 24.4 pg/mL; and 6.1 pg/mL. Conjugate solution was made by diluting 50 µl of the supplied conjugate with 450 µl of the assay buffer. Wash buffer was made by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. After addition of 50 µl stop solution, absorbance was measured at λ 405 nm. The analysis was done in duplicate and data is eligible if %CV less than 20%.

RESULTS AND DISCUSSION

Characteristics of the study subject

Basic characteristics of the study subject for the total study populations are presented in Table 1. Participants of this study were patient with type 2 diabetes mellitus at Pasar Minggu Community Health Center. Participants were divided into two groups, the group of patient who consumes metformin (metformin group) and a group of patient who consumes metformin-sulfonylurea (combination group). Total participants were 114 patient, 50 patient of metformin group and 64 patient of combination group (28 samples of metformin-glimepiride and 36 samples of metformin-glibenclamide). Each group was described for the basic characteristics and clinical characteristics to determine whether there are differences in the proportions and the mean in both of groups. The majority participant in both sample groups is female. It is because women have greater risk factors for increasing in Body Mass Index (BMI), which is increasing the risk of diabetes mellitus.[8] The hormonal process through the monthly cycle and after menopause causes the distribution of body fat becomes easy to accumulate and makes the increasing of BMI.[8] The average age of the study subjects in both groups of samples are above 55 years and there are no significant age differences in the two study groups (p=0.429). It may because age affects glucose metabolism caused by changes in the pancreatic beta cells to produce insulin and required the reduction in calorie needs, like at the age of 40-59 years calorie requirement should be reduced to 5%.[8] Age 45 years and above are also associated with increased risk factors such as trends in obesity and decreased physical activity that increases the risk of type 2 diabetes mellitus.

Participants in both groups had different HbA1c level significantly (p=0.038). The average HbA1c at both groups were out of normal level, which was 8.58% in the metformin group and 10.73% in the combination group. These results were not in accordance with established studies since metformin could reduce levels of HbA1c by 1.5-2.0%, while the class of sulfonylureas could reduce HbA1c levels by 0.8 to 2.0%. The result of a high average of HbA1c in this study showed that the effectiveness of treatment metformin and the combination of metformin-sulfonylurea were still inadequate because the long-term glycemic targets for the treatment of diabetes mellitus (HbA1c levels below 7%) was not reached. This could be because non-pharmacologic treatments, such as dietary intake and exercise are not well implemented.

Systolic (p=0.612) and diastolic (p=0.749) blood pressure in both groups was no significant difference and most participants in both groups had normal blood pressure (120/80). The average serum creatinine values in both groups of samples are also in the normal range (0.6-1.50). The difference of serum creatinine (p=0.648) and urine creatinine (p=0.173) in both groups were not significant (p> 0.05). The urinary creatinine and serum creatinine in both groups were still in normal range indicate most of the participants in this study just suffered from diabetes mellitus and have apparently normal renal function.

Even not statistically significant, mean urine albumin in the combination group was higher than that in the metformin group. However, after subgroup analysis, the difference became significant, whereas other parameters were not differ statistically (Table 2). If this situation continued and untreated, it would cause kidney damage characterized by the occurrence of albuminuria.[9] This is in accordance with the previous study that showed therapeutic use of metformin and combination of metformin-sulfonylurea were known to have a poor ability to reduce level of microalbuminuria in patients with type 2 diabetes mellitus.[9,10]

The level of urinary 8-iso-Prostaglandin F2α in metformin group was lower than the number in the combination group, significantly. This result was in line with the study that has been conducted previously, in which the level of urinary 8-iso-Prostaglandin F2α in metformin-sulfonylurea group was lower than sulfonylurea group.[11] It is known that metformin produced significant effect on the malondialdehyde, a lipid peroxidation product, whereas sulfonylurea was not.[12] Thus, metformin could be more effective in reducing the oxidative stress.[12]

Bivariate Correlation

According to Spearman correlation test result (Table 3) , there are no significant correlation between the level of 8-iso-Prostaglandin F2α with UACR in both groups. The results of the other studies also did not reveal a significant correlation (p=0.808 and r=-0.030) between the levels of 8-iso-Prostaglandin F2α and UACR value in patients with type 2 diabetes mellitus with the proportion is normoalbuminuric patients (n=43) and microalbuminuric patients (n=35).[6] Increased levels of 8-iso-Prostaglandin F2α in the whole sample could be a cause in increased glomerular filtration rate, but not for UACR. Glomerular filtration rate is known to have a positive correlation with levels of 8-iso-Prostaglandin F2α.[6,11]

The linear regression analysis results in albuminuric subjects (n=55) indicate HbA1c and the use of a combination of metformin-sulfonylurea were the most predictive factors for increased UACR, after controlled by age, gender, IMT, systolic blood pressure, 8-iso-Prostaglandin F2α, smoking habit and exercise habit (Table 4). It is reported that the use of sulfonylureas compared with metformin for initial treatment of diabetes were associated with an increased hazard of cardiovascular disease events or death, through UACR as one of the markers.[13] Erdmann E [2006] also reported that gliclazide, a sulfonylurea, add-on to metformin increased UACR compared to pioglitazone.[14]

In this study, we also found HbA1c as the most contributor for increased UACR. An uncontrolled glycemic condition characterized by high HbA1c level stimulates a low-level inflammation, oxidative stress and thus dysfunction in kidney glomerular and tubules, such as increased glomerular pressure and flow as well as changes in glomerular permeability.[15] Sustainable oxidative stress cause albuminuria by an accumulation of glycation of proteins known as Advance Glycated End-products (AGEs) that cause oxidative stress.[15,16]

The findings from this study may possibly help in understanding the efficacy of these standard drugs in managing the complications arising from diabetes mellitus which directly proportional to oxidative stress and albuminuria in diabetic patients. In conclusion, the use of metformin was more effective in decreasing 8-iso-Prostaglandin F2α level, an oxidative stress marker, rather than a combination of metformin-sulfonylurea. However, 8-iso-Prostaglandin F2α has no significant effect on the level of UACR. The increase of HbA1c and the use of a combination of metformin-sulfonylurea contributed in increased UACR in this study.

Standard Protocol on Approvals, Registration, & Patient Consents

This study has been registered in Ethics Committee, Faculty of Medicine, Universitas Indonesia–Dr. Cipto Mangunkusumo Hospital (No.44/UN2.F1/ETIK/I/2016). Clinical examinations were undertaken using questionnaire and informed consent was given to subjects before sampling.

Table 1: Characteristics of study subject

Characteristic	Metformin (n=50)	Combination (n=64)	*p*
	Mean±SD /SEM	Mean±SD /SEM	
Gender			
Male (n)	9	10	0.803[a]
Female (n)	41	54	
Age (years)	59.90 ± 7.24	58.81 ± 7.26	0.429[b]
Height (cm)	152.06 ± 6.30	151.51 ± 6.48	0.653[b]
Weight (kg)	61.12 ± 10.66	59.10 ± 8.49	0.265[b]
BMI (kg/m^2)	26.36 ± 3.93	25.77 ± 3.63	0.413[b]
HbA1c (%)	8.58 ± 1.77	10.73 ± 12.71	0.038[c]*
Blood presure			
Sistolic (mmHg)	124.80 ± 17.29	126.56 ± 16.25	0.612[c]
Diastolic (mmHg)	78.00 ± 8.08	78.44 ± 7.39	0.749[c]
Serum creatinine (mg/dL)	0.85 ± 0.41	0.83 ± 0.49	0.648[c]
Urine creatinine (mg/mL)	0.10 ± 0.06	0.09 ± 0.06	0.173[c]
Urine albumin (mg/dL)	24.70 ± 6.21	91.61 ± 34.16	0.710[c]
UACR (mg/g)	326.49 ± 112.19	1893.54 ± 536.96	0.838[c]
Urinary 8-iso-Prostaglandin F2α (pg/mg)	91716.22 ± 17488.32	188167.77 ± 34815.60	0.027[c]*
Smoking habit			
Smoker	48	63	0.581[a]
Non smoker	2	1	
Exercise habit			
Frequent	9	26	0.014[a]*
Not fequent	41	38	

Note : *p* = significance ; SD = Standard Deviation ; [a] = Fisher Exact Test ; [b]= T-Test ; [c] = Mann Whitney Test

Table 2: Characteristics of subgroup (albuminuric patients, n=55)

Characteristic	Metformin (n=27)	Combination (n=28)	*p*
	Mean±SD /SEM	Mean±SD /SEM	
Gender			
Male (n)	4	5	0.766[a]
Female (n)	23	23	
Age (years)	60.07 ± 6.45	59.82 ± 6.57	0.886[b]
Height (cm)	151.67 ± 7.36	152.57 ± 6.88	0.447[c]
Weight (kg)	61.09 ± 11.87	58.66 ± 9.30	0.400[b]
BMI (kg/m^2)	26.44 ± 4.14	25.26 ± 4.16	0.295[b]
HbA1c (%)	8.99 ± 1.8	13.00 ± 19.09	0.316[c]
Blood presure			
Sistolic (mmHg)	122.59 ± 12.59	126.43 ± 15.92	0.414[c]
Diastolic (mmHg)	77.04 ± 6.09	78.21 ± 7.72	0.581[c]
Serum creatinine (mg/dL)	0.77 ± 0.38	0.91 ± 0.62	0.216[c]
UACR (mg/g)	600.42 ± 194.05	4327.16 ± 1071.36	0.001[c]*
Urinary 8-iso-Prostaglandin F2α(pg/mg)	105013.62 ± 25220.88	239393.25 ± 66485.52	0.197[c]
Smoking habit			
Smoker	1	1	0.980[a]
Non smoker	26	27	
Exercise habit			
Frequent	21	17	0.177[a]
Not fequent	6	11	

Note : *p* = significance ; SD = Standard Deviation ; [a] = Fisher Exact Test ; [b]= T-Test ; [c] = Mann Whitney Test

Table 3: Correlation between urinary 8-iso-Prostaglandin F2α with UACR

Subject's Group	Spearman's rho	*p*
Total subjects		
Metformin	0.139	0.342
Combination metformin-sulfonylurea	0.207	0.107
Albuminuric subjects		
Metformin	0.079	0.694
Combination metformin-sulfonylurea	0.317	0.107

Table 4: Linear Regression Analysis for UACR Using Stepwise Method in Albuminuric Subjects

Variables	Beta (standardized coefficients)	*p*
Model 1		
Combination of metformin-sulfonylurea	0.416	0.002*
Model 2		
Combination of metformin-sulfonylurea	0.364	0.004*
HbA1c	0.341	0.006*

*p < 0.05, significant; Model 1, adjusted by age, gender, BMI, systolic blood pressure, 8-iso-PGF2α, smoking habit, exercise habit and HbA1c. Model 2, adjusted by age, gender, BMI, systolic blood pressure, 8-iso-PGF2α, smoking habit, and exercise habit.

CONCLUSION

Metformin was more effective in decreasing 8-iso-Prostaglandin F2a level. The increase of HbA1c and the use of a combination of metformin-sulfonylurea contributed in increased UACR in this study.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATION USED

eGFR: Estimated Glomerular Filtration Rate; **UACR:** Urine Albumin to Creatinine Ratio; **HbA1c:** Glycosylated Hemoglobin, Type A1C; **ROS:** Reactive Oxygen Species; **SUR-1 :** Sulfonylurea Receptor-1; **BCG :** Bromocresol Green.

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