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Effect of glucose on reduced glutathione level in Malay uncomplicated type 2 diabetes patients

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ABSTRACT

Background: Increasing blood sugar level may increase free radical compounds in type 2 diabetes. Free radical compounds can cause oxidative stress, thereby decreasing endogenous antioxidants such as reduced glutathione (GSH).

Objective: This study aimed to determine whether random blood glucose levels affect GSH in type 2 diabetes patients within the Malay race.

Methods: This study was observational with case-control, involving 25 patients with uncomplicated type 2 diabetes (receiving metformin and/or glimipiride) and 25 healthy controls. Random blood glucose levels were determined by using ACCU-CHECK® Kit. Blood GSH levels were determined by using Sigma GSH Assay Kit.

Results: Results showed that type 2 diabetes patients have a significantly lower random blood glucose level compared with those of age-matched normal subjects ($p<0.0001$). Type 2 diabetic patients had significantly lower levels of GSH ($p=0.01$) than those of age-matched normal subjects. We found a moderate negative correlation ($r=-0.437$ and $p=0.02$) between the level of random blood glucose and the level of GSH.

Conclusion: The depletion of GSH during hyperglycemia may neutralize the free radicals indirectly generated by the abundant of glucose.

Keywords: free radicals, random blood glucose level, reduced glutathione, type 2 diabetes

Introduction

Type 2 diabetes is a condition characterized by hyperglycemia due to insulin resistance, with a 1.4-1.6% prevalence in Indonesia. Such condition is usually triggered by multiple factors, such as age, race, family history, diet, lifestyle, and several other metabolic diseases [1–3]. Hyperglycemia could lead to chronic complications such as nephropathy and retinopathy. Such complications may be caused by the increasing free radicals that will accelerate microangiopathic and macroangiopathic complications due to damaged lipids, DNA, and protein [3–8]. This condition can be detected by the increase of oxidative stress markers such as malondialdehyde (MDA) and carbonyl compounds. It can also be measured by the decrease of endogenous antioxidants such as glutathione (GSH), as it is depleted to counteract the free radicals [1,4]. Research in Egypt showed a significant rise in SOD, MDA, and GSH levels in patients with type 2 diabetes compared to healthy people. Other studies also show an increase in free radicals in type 2 diabetes patients [9,10]. The expression of enzyme related to glutathione metabolism based of racial profile have been explored, but none has discussed ethnic population in Indonesia [11,12].

Unfortunately, previous research that measures GSH either did not distinguish diabetic type 2 patients based on the absence of complications [13–15], or they use a limited number of uncomplicated diabetic patients (<10 subjects) [16]. Within Malay race patients, there is also an absence of data

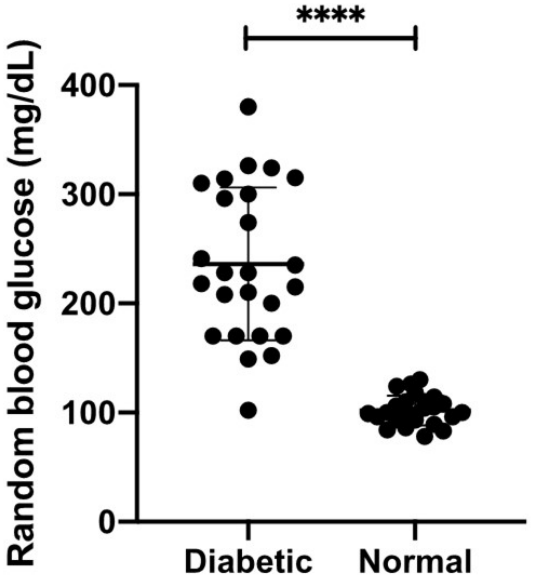

Figure 1. Random blood glucose level of diabetic patients and normal subjects. n = 25. **** p<0.0001

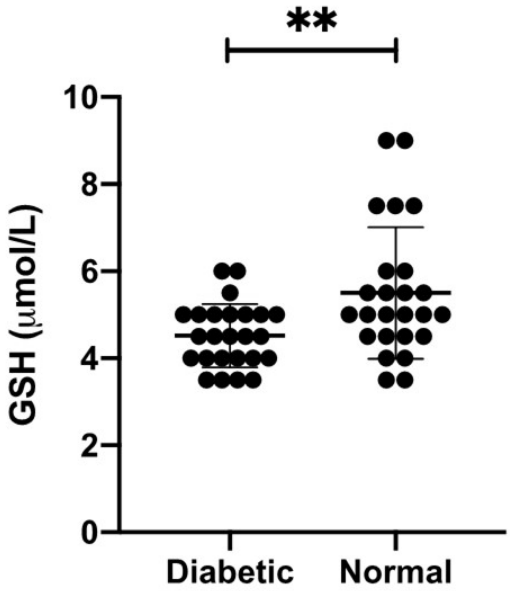

Figure 2. GSH level of diabetic patients and normal subjects. n = 25. ** p=0.01

regarding the relationship between random glucose levels versus GSH in uncomplicated type 2 diabetes patients. This study aimed to evaluate GSH in the early stage of diabetic type 2 pathogenesis and the effect of race. This research measured the correlation between glucose level and GSH, to evaluate the impact of hyperglycemia on GSH as an endogenous antioxidant.

Methods

Subjects

This study was an observational analytic study conducted from July to November 2015 with a case-control approach. The research subjects were 25 type 2 diabetes patients treated at the Internal Medicine Polyclinic in Mohammad Hoesin Hospital, Palembang, Indonesia. The inclusion criteria were Malay race and agree to participate by signing informed consent. Exclusion criteria were suffering from other endocrine diseases, diabetes complications (including, but not limited to, complicated diabetic foot, retinopathy, nephropathy), carcinoma, and infectious disease. For the controlled group, we recruited 25 healthy people with matching ages and sex. The study was conducted with ethical permission from The Bioethical and Humanities Unit of Mohammad Hoesin Hospital Palembang and Faculty of Medicine, Universitas Sriwijaya number 157/kepkrsmhfkunsri/2014.

Patients included in this study were type 2 diabetes mellitus patients without complications, which were verified by asking the patient's history and checking their medical records. The patient has received antidiabetics therapy from the doctor in the form of metformin and/or glimepiride in accordance with the guidelines for treating diabetes mellitus from The *Indonesian Society* of *Endocrinology [17]*.

Measurement of glucose and GSH levels

Two mL of blood samples were taken from the median cubital vein in the left arm. Blood glucose was measured by using ACCU-CHECK ® Active Kit. The plasma was obtained through centrifugation. GSH plasma was measured by using Sigma GSH Assay, according to the manufacturer's instruction.

Statistical analysis

All data were presented as mean ± SEM. GraphPad Prism 8 (California, US) was used to generate the figure. Data were then analyzed using GraphPad for unpaired t-test, and SPSS 16 for the Pearson correlation test.

Table 1. Characteristics of type 2 diabetes patients

Characteristics	Patients	Control
Gender		
Male	5	7
Female	20	18
Average age	51.9 years	48.8 years
Average duration of diabetes	5.8 years	-

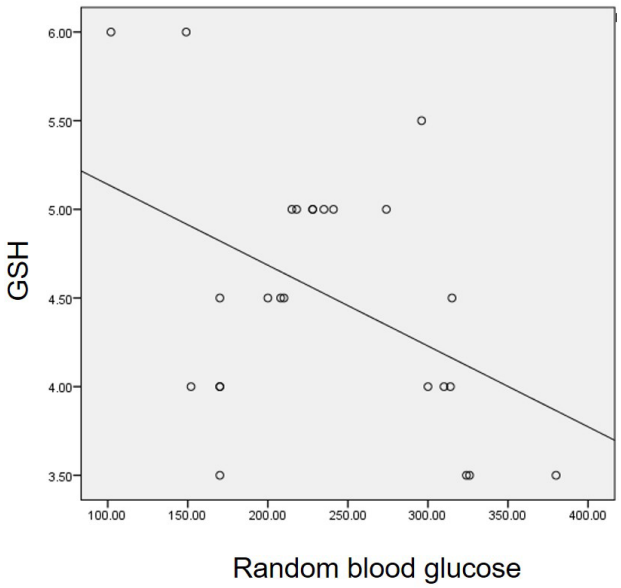

Figure 3. Linear association between random blood glucose and GSH level of diabetic patients. (r = -0.437, p=0.02, n =25)

Results

Characteristics of subjects

Table 1 shows characteristic of subjects including of gender, age, and duration of experienced with diabetes. Type 2 diabetes patients enrolled in this study were dominantly female (20 women, 5 men) with an average age of 52 years old. They experienced diabetes for an average of 5.8 years. Results showed that the diabetic patients have a higher average random blood glucose levels (236.2 ± 69.9 mg/dL) than those of the control (101.9 ± 13.6 mg/dL) (Figure 1).

GSH levels

The average GSH level of type 2 diabetes patients were 4.52 ± 0.72 µmol/L, which is significantly lower than the control group with 5.50 µmol/L ± 1.51 µmol/L (p=0.01) (Figure 2). The Pearson correlation test showed that a negative correlation between blood sugar levels and GSH levels (r = -0.437, p=0.02) (Figure 3).

Discussion

The lower GSH levels result (compared to the control) in our research indicates increasing free radicals and higher oxidative stress. It concurs with a previous smaller study in which nine diabetes type 2 patients with microvascular complications had significantly lower GSH than the remaining seven patients who had no complications [16]. The aldehyde group of glucose is a highly reactive compound. The high blood glucose concentration in diabetic type 2 patient will cause non-enzymatic protein glycation through the binding of monosaccharides to an amino group in the protein [5]. Such reactions will alter protein structure and produce advanced glycosylation end compounds, which have a significant role in long-term diabetic complications.

The abundant glucose could also trigger auto-oxidation to produce superoxide radicals and hydroxyl radicals. Free radicals are neutralized with various antioxidants, which includes GSH. This mechanism is evident by the negative correlation between blood sugar levels and GSH levels in our study (Table 2). Other studies also show the decrease of antioxidant enzyme activity, such as superoxide dismutase, catalase, and glutathione peroxidase [9,10,17,18]. These results underline the importance of maintaining adequate antioxidant levels, which may prevent subsequent complications.

Patients who were included in the study also received antidiabetics therapy, metformin and/or

Table 2. Comparison of GSH value

Study	Patient group	Sample size	GSH value
Our study	Normal	25	5.50 ± 1.51 µmol/L
	Uncomplicated type-2 diabetes	25	4.52 ± 0.72 µmol/L
India study [27]	Normal	30	14.21 ± 2.55 mg%
	type-2 diabetes **without** nephropathy	30	12.20 ± 1.84 mg%
	type 2 diabetes **with** nephropathy	30	10.62 ± 1.84 mg%
Egypt study [28]	Normal	20	4.19 ± 0.58 mg/g protein
	Insulin-dependent diabetes*	40	3.04 ± 0.38 mg/g protein
	Non insulin-dependent diabetes*	55	2.35 ± 0.39 mg/g protein

*) with and without vascular complications

glimepiride. Studies have shown that metformin does not affect glutathione metabolism [19]. There is no human study on the effect of glimepiride. However, an animal study also shows that glimepiride has no effect on glutathione [20]. It shows that the effect of the medication can be ignored. Related to the use of the ACCU-CHECK ® kit, it has been shown to have comparable performance with standard laboratory assay [21,22] and relatively superior to other blood glucose self-monitoring test [23] while also being simplistic. This self-monitoring device were also used were frequently used in various research to measure blood glucose in the diabetic patients [24–26].

In terms of racial profile, this study's reach a similar conclusion to research in other populations, such as in India and Egypt (Table 2). In the Indian study, type-2 diabetic patients without nephropathy has 12.20 ± 1.84 mg%, while type 2 diabetic patients with nephropathy have lower GSH, 10.62 ± 1.84 mg%. This is also much lower compared to control with 14.21 ± 2.55 mg% [27]. In the Egypt study, insulin-dependent diabetes mellitus (with and without vascular complications) had 3.04 ± 0.38 mg/g protein (n=40), while non insulin-dependent diabetes mellitus (with and without vascular complications) had 2.35 ± 0.39 mg/g protein (n=55) [28]. Our findings along both studies indicate a GSH-related antioxidant protecting mechanism in uncomplicated diabetes type 2 patients, irrespective of race.

Conclusion

In Malay type 2 diabetes patients, the blood GSH levels decreased significantly compared to controls, showing that the increased blood sugar levels may generate free radicals and deplete antioxidant pool, including GSH. Further research should examine endogenous antioxidant expression at the cellular level. A control group of uncomplicated diabetes type 2 patients who do not receive metformin and/or glimepiride should also be included to observe how metformin and glimepiride affects the antioxidant mechanism.

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Author contribution

Conceptualization, S.; Methodology, S. and R.R.; Formal Analysis, S.; Investigation, S.; Resources, S.; Writing – Original Draft, R.R; Writing – Review & Editing, S. and R.R.; Visualization, S.; Funding Acquisition, S.; Resources, S. and R.R.; Project Administration, S..

Declaration of interest

Both authors declare the absent of potential conflict of interest.

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