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

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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...
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.
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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.

Patients who were included in the study also received antidiabetics therapy, metformin and/or
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 $12.20 \pm 1.84$ mg%, while type 2 diabetic patients with nephropathy have lower GSH, $10.62 \pm 1.84$ mg%. This is also much lower compared to control with $14.21 \pm 2.55$ mg% [27]. In the Egypt study, insulin-dependent diabetes mellitus (with and without vascular complications) had $3.04 \pm 0.38$ mg/g protein (n=40), while non insulin-dependent diabetes mellitus (with and without vascular complications) had $2.35 \pm 0.39$ 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.

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

1) with and without vascular complications

**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.

**Acknowledgment**

We would like to acknowledge the staff in the Endocrinology Polyclinic, Department of Internal Medicine, Mohammad Hoesin Hospital, Palembang, Indonesia, who facilitates the sampling process, especially dr. Yulianto Kusnadi, Sp.PD-KEMD, FINASIM.

**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.
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Declaration of interest
Both authors declare the absent of potential conflict of interest.

Received: 16 March 2021
Accepted: 31 August 2021
Published online: 4 September 2021

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