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Evaluation of oxidative stress levels using glutathione peroxidase (GPx) expression on hyperglycemia-induced rats testis

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ABSTRACT

Diabetes mellitus (DM) is a global health problem with an estimated 422 million cases worldwide. Previous studies reported a correlation between hyperglycemia and oxidative stress-related male infertility in DM. Glutathione peroxidase (GPx) can cause DNA damage due to oxidative reactions. Therefore, it could be used as potential indicator of antioxidant therapy. The study aimed to evaluate the expression level of GPx on the hyperglycemia-induced rats. This was an experimental case-control study using 27 Wistar rats divided into three groups i.e. hyperglycemia induction for four weeks group, eight weeks group, and a control group with no intervention. Following after induction, total RNA from the rats' testis was extracted, and GPx expression was analyzed using qPCR. Data were analyzed using SPSS, and a p <0.05 was considered significant. The study showed a significantly higher GPx mRNA expression level after hyperglycemia induction in both 4 and 8 weeks groups (16.93 ± 3.32 and 17.62 ± 3.42) compared to control group (9.94 ± 2.91) (p< 0.05). However, no significantly different between the 4 weeks group and 8 weeks group was observed (p >0.05). In conclusion, hyperglycemia increases GPx mRNA expression in rats. It may change the testicular environment's oxidative processes and impairs male reproductive function in the Sertoli cells with no exception.

ABSTRAK

Diabetes melitus (DM) merupakan masalah global dengan estimasi 422 juta kasus diseluruh dunia. Penelitian terdahulu menunjukkan adanya korelasi antara hiperglikemia dan infertilitas pada DM. Glutation peroksidase (GPx) dapat menyebabkan kerusakkan DNA akibat reaksi oksidatif. Oleh karena itu, GPx dapat digunakan sebagai indikator potensial terapi antioksidan. Penelitian ini bertujuan untuk mengkaji tingkat GPx pada tikus yang diinduksi hiperglikemia. Penelitian ini merupakan kasus kontrol eksperimental pada sembilan tikus Wistar yang dibagi menjadi tiga kelompok, yaitu kelompok yang diinduksi hiperglikemia selama 4 minggu, 8 minggu dan kelompok kontrol yang tidak diinduksi. Setelah induksi, total RNA dari testis tikus diekstraksi dan ekspresi GPx dianalisis menggunakan qPCR. Data dianalisis menggunakan SPSS dan nilai p <0,05 dianggap signifikan. Hasil penelitian menunjukkan peningkatan tingkat ekspresi GPx mRNA bermakna setelah induksi hiperglikemia selama 4 dan 8 minggu (16,93 ± 3,32 dan 17,62 ± 3,42) dibandingkan dengan kontrol (9,94 ± 2,91) (p<0,05). Namun demikian, tidak terdapat perbedaan bermakna antara kelompok 4 minggu dan 8 minggu (p>0,05). Dapat disimpulkan, hiperglikemia meningkatkan ekspresi mRNA GPx pada tikus. Hiperglikemia ini kemungkinan dapat mengubah proses system oksidatif dalam lingkungan testis, tanpa terkecuali seel Sertoli.

Keywords:
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expression
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antioxidant

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INTRODUCTION

Diabetes mellitus (DM) has been widely recognized as a global health problem due to high its prevalence. It was estimated that around 108 million of the population suffered from DM in 1980 and has increased more than 4 times in 2019 to 463 million cases.[1,2] Diabetes mellitus is associated with the decrease of life expectancy by 10-30 percent.[1,3] Moreover, DM is also associated with the increase of infertility rate. The subfertile population in type 2 DM (T2DM) population is relatively higher than in normal population.[3] Several studies reported that there is a correlation between DM and poor sperm conventional parameter. Patients with type 1 DM (T1DM), several sperm abnormalities such as higher DNA fragmentation, mitochondrial DNA deletion, and low sperm motility were reported.[3-8] The T2DM potentially reduce male fertility in the pretesticular, testicular, and post-testicular. On the pretesticular axis, T2DM is closely associated to obesity and overweight. These condition cause hyperleptinemia or decrease pulsatile secretion of GnRH, decrease Leydig cell function and decrease gonadotropins and testosterone serum levels.[5-7]

Hyperglycemia induce excessive production of reactive oxygen species (ROS) resulting in subfertile and infertile conditions in men with T2DM. High levels of ROS induce oxidative stress conditions in sperm quality and interfere with spermatogenesis. Glutathione peroxidase (GPx) is a mediator that plays a role in DNA damage due to oxidative reactions.[4,5] The GPx exists in the cytosol as a homotetramer, and each subunit contains selenium atoms incorporated into catalytically active selenocysteine residues.[6] The amino acid is spatially exposed to the protein with a flat lipophilic impression, allowing it to be oxidized by the hydroperoxide process.[6] The GPx expression increase in diabetic rats' studies.[4,5] In addition, GPx is highly expressed testicular tissue and could be used as Sertoli' functional parameter.[9,10] However, studies concerning the effect of hyperglycemia on GPx expression specifically in testicular rats is limited.

MATERIALS AND METHODS

Study design and samples

This was experimental case-control study conducted in the Integrated Research and Testing Laboratory, Universitas Gadjah Mada and Anatomical Pathology Laboratory, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada from May-June 2019. Twenty-seven male Wistar rats with 250-300 g body weight and 12-16 weeks old were used in this study. The rats were divided into three groups with nine rats in each group. Group 1 was induced for hyperglycemia using streptozotocin for four weeks; group 2 was induced for eight weeks using the same dose of streptomycin and group 3 as control group with no intervention. All rats were maintained according to the Australian Animal Research Review Panel (ARRP) guideline.

Ethical considerations

The study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nurshing, Universitas Gadjah Mada/Dr. Sardjito General Hospital, Yogyakarta (No. KE/FK/0628/EC/2019).

Streptozotocin induction

One week after adaptation and fasting for one night, the rats were induced by streptozotocin (STZ) at a dose of 40 mg/kgBW. Three days after induction, the blood glucose level was measured using a One Touch Basic blood

glucose monitoring system to confirm STZ injection-induced hyperglycemia. The rats with a blood glucose level ≥ 280 mg/dL would be considered hyperglycemia and included in the study. If the rats died, the cause of death was documented whether it was hypo- or hyperglycemia. The mortality ratio caused by STZ induction is around 10-15% of the study population.

GPx expression analysis

A unilateral orchidectomy on the included rats was conducted. Fifteen mg of tissue was extracted for total RNA using Total RNA Mini Kit FavorPrep™ (Favorgen, Taiwan). Quantitative PCR was performed using the KAPA SYBR® FAST qPCR Kit (Sigma Aldrich, USA) with the following primer *GPx*: forward primer-CGCCAAGAACGAAGAGATTC and reverse primer-CAACATCGTTGCGACACAC using the DT lite qPCR System. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as internal control: forward primer-CATGTTCGTCATGGGTGTGAACCA and reverse primer-AGTGATGGCATGGACTGTGGTCAT.

Statistical analysis

Data were analyzed using SPSS version 15.0. The data were first tested for homogeneity using Levene's test and normality using Shapiro-Wilk test. If the data was normally distributed, the parametric test was conducted using two-way Anova and if found statistically significant was continued with the Duncan test. If the data was not normally distributed, a non-parametric test was conducted using Kruskall-Wallis test and if found significant, continued with Mann-Whitney U test. A p value < 0.05 was considered significant.

RESULTS

Three groups of rats based on hyperglycemia duration were analyzed the GPx mRNA expression levels using the qPCR method. Diabetic rats after successful induction showed specifically diabetic's symptoms such as polyuria and polydipsia. A significantly higher GPx expression level after hyperglycemia induction in both 4 and 8 weeks groups (16.93 ± 3.32 and 17.62 ± 3.42) compared to control group (9.94 ± 2.91) was observed ($p < 0.05$). However, no significantly different between the 4 weeks group and 8 weeks group was observed ($p > 0.05$). It was indicated prolonged hyperglycemia conditions did not significantly increase the GPx expression (FIGURE 1).

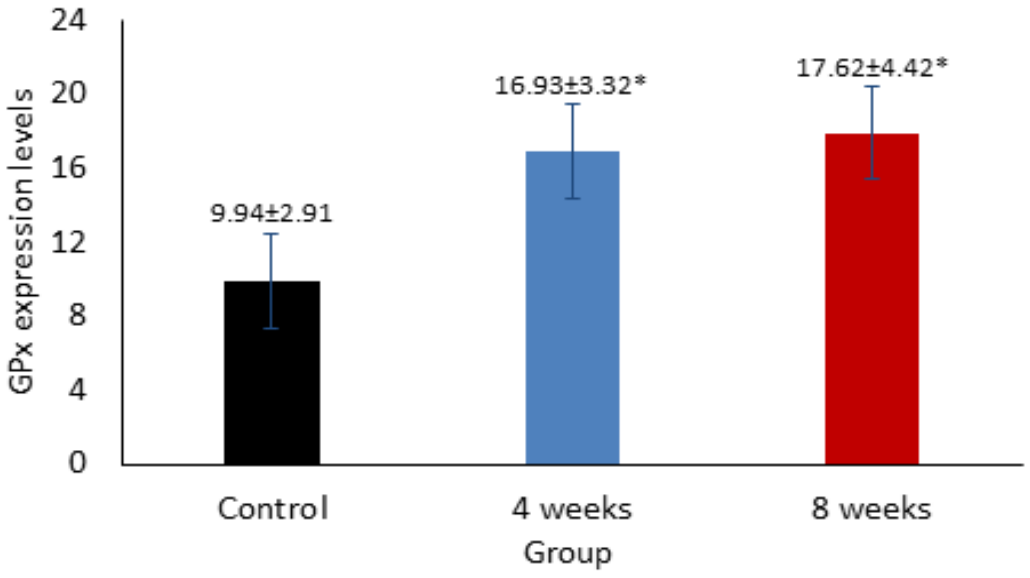

FIGURE 1. GPx expression among groups. Mann-Whitney U test; *p<0.05

DISCUSSION

Although it is suggested that hyperglycemia may affect sperm conventional parameter, however the association between key indicator of oxidative stress and hyperglycemia is still controversial.[4–8] In this study, testicular GPx expression as the indicator for increased oxidative stress in diabetic-rendered rats was investigated. All of the hyperglycemia-induced groups had an increase of GPx expression compared to control rats.

Glucose metabolism in the Sertoli cells of the testis involves a comprehensive system. In the end, glucose will be converted into pyruvate and then lactate, which has an essentially active role in the spermatogenesis process. This Sertoli metabolism is also known to be the center of the spermatogenesis process.[11] The lactate will be excreted as an essential metabolite in the formation and duplication of germ cells, a mechanism that plays a role in supplying high amounts of lipids in cell wall synthesis. This process is modulated by lactate dehydrogenase A (LDHA) expression.[12] The LDHA at high levels is pro-oxidative that has similar properties to ROS.

Reactive oxygen species may directly destroy sperm DNA by attacking purine and pyrimidine bases. It can also initiate apoptosis in sperm, causing the activation of caspase enzymes to degrade sperm DNA.[13] However, the body is equipped with a set of defense systems to ward off free radicals or oxidants to limit the damage caused by free radicals. These antioxidant defense systems include the superoxide dismutase (SOD) found in mitochondria and cytosol, GPx, glutathione reductase (GR), and catalase.[14] These defense systems work in several ways, including direct interaction with free radicals, oxidants, or single oxygen, preventing the formation of reactive oxygen compounds, or changing reactive compounds to become less reactive.[14,15] But under certain circumstances, the production of free radicals or reactive oxygen compounds can exceed the body's defense system, a condition known as oxidative stress.[13,16] Under oxidative stress conditions, free radicals will cause lipid peroxidation of cell membranes and damage the cell membrane organization. The cell membrane is vital for receptor and enzyme function, so the peroxidation of cell membrane lipids by free radicals can result in a total loss of cellular function.[13]

In men, oxidative stress is thought to be one of the factors that can cause decreased testosterone function. The increase in nitric oxide (NO), which is often associated with an increase in lipid peroxidase in various types of stress, causes a decrease in testosterone secretion.[15] In oxidative stress conditions, the average balance between the production of free radicals or reactive oxygen compounds with the body's natural antioxidant ability to eliminate them is disrupted, affecting the standard chain of oxidation-reduction ultimately causes oxidative tissue damage. This tissue damage also depends on several factors, including molecular targets, the level of stress that occurs, the mechanisms involved, and the system's timing and nature being attacked.[17]

The apoptosis process is controlled by various levels of cell signals, which can come from extrinsic or intrinsic triggers. Included in extrinsic signaling which includes hormones, growth factors, NO, and cytokines. All these signals must be able to penetrate the plasma membrane or be transduced to cause a response. The intrinsic apoptosis signal is a cell-initiated response to stress and can eventually result in cell death. Glucocorticoid binding to nuclear receptors, heat, radiation, nutritional deficiencies, viral infections, and hypoxia are conditions that can cause the release of intrinsic apoptotic signals through cell

damages oxidative stress conditions, the average balance between the production of free radicals or reactive oxygen compounds with the body's natural antioxidant ability to eliminate them is disrupted, affecting the standard chain of oxidation-reduction ultimately causes oxidative tissue damage. This tissue damage also depends on several factors, including molecular targets, the level of stress that occurs, the mechanisms involved, and the system's timing and nature being attacked.[13,16]

In these parts of the spermatozoa, main oxidative defense enzymes, SOD, GPxs, GR are very important. The GPxs and GR have a unique role in semen. The results of a study by Alvarez and colleagues showed increased membrane damage after the addition of mercapto succinate inhibitors. The complete glutathione system includes a complex set of biochemical reactions. In brief, GPx catalyzes the reduction of organic and inorganic hydroperoxides, with glutathione as the reduction equivalent, through the action of GPx. GR acts to restore glutathione to its reduced form. GPxs are enzymes with various families and differences in their properties. Intracellular GPx-1 is almost ubiquitous. In sperm and the genital tract, GPX-1 has a direct association with sperm motility.[15,18] GPx is highly expressed in several previous studies involving testicular tissue and could be used as Sertoli' functional parameter.[9,10,19]

Increased expression of GPx in this study may indicate that the Sertoli cells have an internal compensation system, and this increase may indicate that antioxidants have a therapeutic opportunity to prevent DNA damage due to exposure to hyperglycemic conditions. In this study, GPx expression was observed to increase significantly in the first four weeks. Then the increase slowed down. This study can be evidence that the provision of synergistic antioxidant therapy with GPx can provide a therapeutic and preventive effect on sperm DNA damage in the spermatogenesis process.

CONCLUSION

In conclusion, there is an increase in the expression of GPx on hyperglycemia-induce rats' testis indicating that hyperglycemia can change the oxidative system processes in the Sertoli cells. If this oxidative process continues, it can result in defragmentation of DNA, causing cell death, and the spermatogenesis process fails. The GPx could be used as an indicator for antioxidant therapy on the fertility due to hyperglycemia.

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