

CATALASE 1 GENE EXPRESSION (CAT-1) IN HYPERGLICEMIA INDUCED TESTIS, AS AN ANTIOXIDANT THERAPY INDICATORS

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ABSTRACT

Objective: This study aims to analyze the effects of hyperglycemia status on the function of testicular spermatology, especially CAT-1. **Material & Methods:** This study was an experimental pre-clinical study. Twenty-seven rats were divided into 3 groups: normal, 2 weeks, and 4 weeks hyperglycemia. The hyperglycemic state in the Wistar rats was induced by Streptozotocin (STZ). All data were collected and analyzed with SPSS 20.0. At two and four weeks, testicular tissue was extracted and it will be processed using the Total RNA Mini Kit FavorPrep™ then quantitative PCR was performed using the SYBR® Fast qPCR Kit. **Results:** CAT-1 gene expression in the hyperglycemia induction group increased when induced for 2 weeks and again after 4 weeks compared to controls (18.88 ± 4.7 and 21.45 ± 5.52 vs 10.83 ± 3.4). However only induction after 2 weeks was statistically significant ($p = 0.021$). **Conclusion:** CAT-1 (Catalase) gene expression has increased in testicular tissue under conditions of hyperglycemia.

Keywords: CAT-1, hyperglycemia, DM, infertility, gene expression.

ABSTRAK

Tujuan: Penelitian ini bertujuan untuk menganalisa efek status hiperglikemia pada fungsi spermatologi testis, khususnya CAT-1. **Bahan & Cara:** Penelitian ini merupakan penelitian pra-klinis eksperimental. Dua puluh tujuh ekor tikus dibagi menjadi 3 kelompok: normal, 2 minggu, dan 4 minggu hiperglikemia. Keadaan hiperglikemik pada tikus Wistar diinduksi dengan Streptozotocin (STZ). Semua data dikumpulkan dan dianalisis dengan SPSS 20.0. Setelah dua dan empat minggu, jaringan testis diekstraksi dan akan diproses menggunakan Total RNA Mini Kit FavorPrep™ kemudian dilakukan PCR kuantitatif menggunakan SYBR® Fast qPCR Kit. **Hasil:** Ekspresi CAT-1 pada grup induksi hiperglikemia meningkat saat terinduksi selama 2 minggu dan meningkat lagi setelah 4 minggu dibanding kontrol (18.88 ± 4.7 dan 21.45 ± 5.52 vs 10.83 ± 3.4). Namun hanya peningkatan setelah 2 minggu yang signifikan secara statistik ($p = 0.021$). **Simpulan:** Ekspresi gen CAT-1 (Catalase) mengalami peningkatan pada jaringan testis dalam kondisi hiperglikemia.

Kata Kunci: CAT-1, hiperglikemia, DM, infertilitas, ekspresi gen.

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INTRODUCTION

Diabetes mellitus has been widely recognized as a global problem, estimated that around 108 million of the population suffered from diabetes mellitus in 1980 and has since increased four-times in 2014 to 422 million cases. Diabetes is associated with a decrease in life expectancy of 10-30 percent.¹⁻² At the same time, it is known that the infertility rate is also increasing and the subfertile population is relatively increasing in the population of type 2 DM when compared to the normal population.³

Several researches and clinical studies showed a correlation between DM and poor results of sperm conventional parameters, although these results do not across the threshold value. In patients with type 1 DM were also found several abnormalities including, higher DNA fragmentation, mitochondrial DNA deletions, and sperm motility.⁴

Some studies hypothesized that diabetes has the potential to reduce male fertility in pre-testicular, testicular, and post-testicular mechanisms. In pretesticular axis, type 2 DM is closely related to obesity and overweight, this condition causes hyperleptinemia or decreased pulsatile secretion

from GnRH, decreased Leydig cell function, and the mechanism responsible for decreasing serum levels of gonadotropin and testosterone. In addition, the condition of hyperglycemia induces excessive ROS production which results in conditions of subfertility and infertility in men with type 2 DM, high levels of ROS induce conditions of oxidative stress in sperm quality and disrupt spermatogenesis. Catalase (CAT-1) is a mediator that plays a role in maintaining DNA damage due to oxidative reactions. In this study, the gene expression of CAT-1 shows the adaptive response of Sertoli cells to oxidation reactions under hyperglycemia conditions.⁵

OBJECTIVE

This study aimed to analyze the effect of hyperglycemia status on testicular spermatology function, especially CAT-1.

MATERIAL & METHODS

This research is an experimental pre-clinical study located at the UGM Integrated Research Laboratory, Yogyakarta from July 2018 to August 2018. The research subjects were Wistar male rats of 5-8 weeks, weighing 160-200 g were obtained from Lembaga Penelitian dan Pengujian Terpadu (LPPT). The animal procedure protocols were approved by the Animal Care and Welfare Committee of the Faculty of Medicine, University Gadjah Mada (Yogyakarta, Indonesia) and followed the regulatory animal care guidelines of the U.S. National Institutes of Health (No. KE/FK/0628/EC/2019).

Twenty-seven rats were divided into 3 groups: normal, 2 weeks, and 4 weeks hyperglycemia. The hyperglycemic state in the Wistar rats was induced by Streptozotocin (STZ). All data were collected and analyzed with SPSS 20.0. At two and four weeks, testicular tissue was extracted and it will be processed using the Total RNA Mini Kit FavorPrep™ then quantitative PCR was performed using the SYBR® Fast qPCR Kit. The primer sequences used were as follows: Forward: 5'-CCGACCAGGGCATCAAAA-3', Reverse: 5'-GAGGCCATAATCCGGATCTTC-3', with amplicon size of 321 bp. Collected data were presented as mean \pm standard deviation (SD). We analyzed data using the one-way ANOVA with SPSS 15.0 statistical software (IBM Corporation, Armonk, NY, USA).

RESULTS

In this study, the CAT 1 gene expression in the group of mice with no hyperglycemia induction treatment was found to be 10.834 (\pm 3.4). In the hyperglycemia induction for 2 weeks group, CAT1 1 gene expression was found to be 18.88 (\pm 4.7) (p-value 0.021), then further induction increased the gene expression furthermore by 21.45 (\pm 5.52) (p-value 0.34). The increase was found to be different significantly. Exposure to hyperglycemia further increased the gene expression. However, the increase was not statistically significant.

Table 1. CAT1 gene expression trend on diabetic induced wistar mice testis.

	Gene expression of CAT-1 (CI 95%)	p-value
Control	10.834 \pm 3.4	
2 weeks	18.88 \pm 4.7	0.021
4 weeks	21.45 \pm 5.52	0.374

In Figure 1, CAT 1 gene expression had an increasing trend with the duration of hyperglycemia exposure. In weeks 2 and 4, it was observed that the increasing trend in CAT 1 gene expression tend to increase with the increase in the second week being statistically significantly different. However, gene expression value was found to narrow.



Figure 1. CAT-1 gene mRNA expression trend.

DISCUSSION

Hyperglycemia plays an important role in the pathogenesis of microvascular complications of diabetes in type 1 and type 2. This process is caused by increased Reactive Oxygen Species (ROS) such as hydrogen peroxide, superoxide anion, and hydroxyl radicals. In response to high and chronic glucose exposure to cells.⁶ Hyperglycemia, especially in Diabetes Mellitus (DM) patients, is also a factor of infertility in men. Some of the studies explained the negative effects of DM on erectile and ejaculatory function as well as the reduction of semen volume, sperm count, sperm motility, and sperm morphology.⁷ This process is caused by increased ROS in sperm, which can be produced endogenously such as mitochondrial respiration and seminal leukocytes. Beside that, it can be caused by environmental factors. In the sperm, ROS can cause damage to the plasma membrane, DNA integrity, motility, and overall sperm quality. Reducing the production of excessive ROS have important role in the process of spermatogenesis and normal fertilization.⁸

Some enzymatic systems in the body function to protect cells from damage that can be caused by excessive ROS production. These systems include superoxide dismutase (SOD) in the cytosol (CuZnSOD) and mitochondria (MnSOD) which convert superoxide to hydrogen peroxide, along with glutathione peroxidase (GPX) and catalase (CAT) in the cytosol and peroxisome which convert hydrogen peroxide to H₂O.⁹ Catalase (CAT) was demonstrated to be associated with low sperm quality, and one study reported that CAT-262T/T genotype was negatively associated with infertility in idiopathic infertile males.⁸

In this study, we examined the gene expression of CAT-1 in response to hyperglycemic conditions. We compared the control conditions with 2 weeks and 4 weeks of hyperglycemia conditions. We found a trend of increased CAT-1 gene expression in testicular samples at 2 weeks of exposure to Streptozotocin and then again at 4 weeks of exposure to Streptozotocin. An increase at 2 weeks showed a statistically significant difference but not at 4 weeks of hyperglycemia. This result was different from the previous study by Ornoy et al. (2011) where treatment of hyperglycemia, hypoxia, and their combination increased oxidative stress and decreased the expression of both mRNA and protein from the antioxidant enzymes CAT and Mn-SOD.

Although the mechanism is not yet established, it was explained that the conditions of hyperglycemia and hypoxia will cause mutations in genes that cause changes in expression and function of antioxidants.¹⁰ Other studies had also shown decreased CAT gene expression in hyperglycemic conditions.^{6,11}

Catalase activity was decreased under conditions of oxidative stress as a result of the conversion of cysteine to cysteic acid and catalase nitration. This is interesting because in our study we found a trend of increased expression of the antioxidant enzyme CAT in response to hyperglycemia. This difference could be due to differences in genetic mutations that occur so that further research in detecting genotype variants of the CAT enzyme is needed to explain these further.

Previous research showed an increase in CAT gene expression was carried out by Hodgkinson et al. (2003), who examined the effects of hyperglycemia in patients with type 1 diabetes and diabetic nephropathy. This study showed that after exposure to hyperglycemia increased expression was found in CAT, CuZn-SOD, and GPX mRNAs in uncomplicated diabetic patients but decreased enzyme expression of these genes in patients with nephropathic complications. This study explained this difference is likely due to DM patients with nephropathic complications associated with genotype variants that are strongly associated with reduced body's ability to adapt to oxidative stress.^{6,12}

In relation to infertility, CAT is known to be associated with poor sperm quality, and the variant that has been investigated with regard to infertility is the CAT-262T/T genotype.¹³ Previous studies had also shown low testosterone secretion and antioxidant enzyme activity in the testes associated with asthenozoospermia condition.¹⁴

SNP CAT-262C/T has been extensively studied in conjunction with diabetes including type 1, type 2, gestational diabetes, and diabetes complications such as retinopathy, nephropathy, and cardiovascular disease. The CT + TT genotype in the C111T catalase variant has been known to increase blood catalase activity in type 2 DM patients. CAT-62C/T polymorphism is known to contribute to hypertriglyceridemia in DM patients in China. In some studies, there was no relationship between the -262C/T genotype catalase and the risk of type 1 DM. However, in several other studies the C allele was associated with an increased risk of diabetic nephropathy.^{5,15}

This study showed an increase in the gene expression of testicular CAT in hyperglycemic conditions. This result could be an additional base of knowledge on the pathogenesis of hyperglycemia in enhancing oxidative stress reactions on the basis to provide antioxidant therapy in the condition of male infertility. However, some of the shortcomings in this study include the relatively small number of samples, sperm quality not examined from the samples used so that it cannot explain the effect of increased CAT expression on infertility conditions and the absence of internal control (housekeeping gene) in RT-PCR to quantify CAT gene expression.

In the future, further research with larger samples is needed. Genotyping of the CAT gene to determine variants that affect changes in expression, the examination of protein levels and in vitro functional studies to look at antioxidant activity against hyperglycemia is needed to confirm the results of this study and further provide additional basic information for the potential administration of antioxidant therapy in DM patients with infertility.

CONCLUSION

CAT-1 (Catalase) gene expression was increased in testis with hyperglycemia, this can provide the further basis of knowledge in hyperglycemia pathogenesis by increasing oxidative stress reaction. Further research is needed, to utilizing antioxidant therapy for male infertility.

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