The correlation between total antioxidant status with malondialdehyde and caspase 3 epididymal fluid type 2 diabetic rat and non diabetic

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Abstract: The aim of this study is to determine the correlation between total antioxidant status (TAC) with malondialdehyde (MDA) and caspase 3. This study was divided into non-diabetic (n = 8) and type 2 diabetic rats (n = 8) induced by streptozotocin 65 mg / kg body weight. Epididymal fluid was examined after 28 days. Antioxidant status was examined by the trolox equivalent method, MDA with the thiobarbituric acid (TBA) reaction and caspase 3 expression with immunohistochemistry. Our results show that the mean of TAC in non-diabetic was significantly higher (P <0.000) than diabetic rats, MDA (P <0.000) and caspase 3 expression (P <0.001) in non-diabetic rats were significantly lower than diabetic rats. TAC was negative correlation with MDA (r = 0.881, P = 0.004) and caspase 3 (r = 0.898, P = 0.002) in the non-diabetic group. In the diabetic group, TAC was a negative correlation with MDA (r = 0.856, P = 0.007) and caspase 3 (r = 0.886, P = 0.003). In conclusion, total antioxidants have a negative correlation with MDA and caspase 3 expression.

1. Introduction
Diabetes mellitus (DM) refers to a group of general metabolic disorders characterized by hyperglycemia caused by decreased insulin secretion factors, decreased glucose utilization and increased glucose production [1]. Type 2 diabetes (T2DM) is the body's inability to respond properly to the action of insulin. This type of diabetes is more common and accounts for around 90% of all diabetes cases worldwide [2]. Data from the Basic Health Research (RISKESDA) shows that the prevalence of DM in Indonesia has increases from 2007 by 5.7% to 6.8% in 2013 [3].

Hyperglycemia in diabetes increases the production of free radicals which can induce oxidative stress. Oxidative damage can be evaluated from lipid peroxidation reaction products, DNA oxidation and protein oxidation [4]. Lipid peroxidation is the process of free radicals attacking lipids that contain carbon-carbon double bonds, especially polyunsaturated fatty acids [5]. Malondialdehyde is one that can be relied upon as a marker of oxidative stress to measure the lipid peroxide index [6].

Increased levels of plasma MDA, serum, and many tissues in diabetics may be involved in diabetes complications. Increased lipid peroxidation is also an indication of decreased enzymatic and non-enzymatic antioxidant defense mechanisms [7]. Non-enzymatic antioxidants include vitamins A, C, and E, bioflavonoids, minerals Cu, Zn, Mn, Se, albumin and vitamin B. Enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase [8]. The antioxidant power of the epididymal fluid can be assessed by measuring various kinds of antioxidants or total antioxidant capacity (TAC) [9].
Oxidative stress can cause cellular dysfunction through the formation of AGEs (advanced glycosylated end products), activating poly ADP-ribose polymerase (PARP) and other stress-activated pathways causing apoptosis [10]. Caspase is a family of proteases; this proteolytic enzyme has an important role in apoptosis induced by specific proteins in the cytoplasm and nucleus [11]. Caspase-3 is a major factor in the initiation of apoptosis from both receptor and mitochondrial pathways [12].

2. Methods

2.1. Animals

Male Wistar rats aged more than 8-10 weeks with a body weight of 170-200 grams. There were two groups, non-diabetic rats (n = 8) and diabetic rats with streptozotocin (STZ) induction 65 mg / kg body weight. Rats were kept in individual cages with standard feed and drinking ad libitum for 28 days. Epididymal fluid was obtained from the caudal epididymis for measurement of TAC, MDA and caspase 3 expression. This study was approved by the health research ethics commission (KEPK) FK UNDIP / RSUP Dr. Kariadi Semarang No. 75 / EC / H / FK-RSDK / X / 2017

2.2. Laboratory Analysis

TAC was measured using a kit from Biovision (K274-100) using the CUPRAC method [13] with the principle of reducing Cu2+ to Cu. Absorbance was measured at 570 nm using a spectrophotometer, the final result displayed as mmol/l. MDA in epididymal fluid is reacted with Thiobarbituric Acid (TBA) (Biovision, Catalog # K739-100). Seminal plasma protein is precipitated by the addition of trichloroacetic acid (TCA). Thiobarbituric acid (TAB) reacts with malondialdehyde (MDA) to form the reactive product of thyobarbituric acid. Absorbance was measured at 534 nm and results were expressed in nmol/ml [14]. Caspase 3 expression is measured by immunohistochemistry (Biocare Medical, Catalog number: CP 229 A, B, C). The method used refers to [15]. All slides were examined using a fluorescent microscope (Mic Convocal CARL ZEISS NGO 800). The percentage of caspase-3 is calculated from 100 sperm. The expression of active caspase 3 is shown in brown, while the purple color in sperm has no expression.

2.3. Statistical analysis

Data are presented as mean ± SD. Comparisons between the two groups were carried out using independent sample t-tests. Pearson's correlation coefficient test is used to determine correlations between various factors. Statistically significant with a P value <0.05.

3. Results and Discussion

The measurement results of the variables are shown in Table 1. The TAC level of non-diabetic mice (1.16 ± 0.12 mmol / l) was significantly higher than that of diabetic rats (0.48 ± 0.25 mmol/l). MDA levels (1.86 ± 0.20 nmol / ml) and caspase 3 expression (9.63 ± 4.50%) in the non-diabetic group were significantly lower than those in the diabetes group (9.66 ± 0.40 nmol / ml and 26.63 ± 9.84%).
Table 1. Mean ± SD from TAC, MDA Levels dan Caspase 3 expression in Non Diabetic and Diabetic Rats

| Parameter     | Non-diabetic | Diabetic   | P   |
|---------------|--------------|------------|-----|
| TAC (mmol/l)  | 1.16 ± 0.12  | 0.48 ± 0.25| 0.000|
| MDA (nmol/ml) | 1.86 ± 0.20  | 9.66 ± 0.40 | 0.000|
| Caspase 3 (%) | 9.63 ± 4.50  | 26.63 ± 9.84| 0.001|

Negative correlations occurred between TAC and MDA (Figure 1.a) and caspase 3 (1.b) expression in a group of non-diabetic rats. There was also a negative correlation between TAC and MDA (Figure 2.a) and caspase 3 (2.b) in the diabetic group.

![Figure 1](image)

Figure 1. Correlation between TAC and MDA level $r = 0.881$, $P = 0.004$ (a) and caspase 3 expression $r = 0.898$, $P = 0.002$ (b) in a group of non-diabetic rats
Figure 2. Correlation between TAC with MDA level $r = 0.856$, $P = 0.007$ (a) and caspase 3 expression $r = 0.886$, $P = 0.003$ (b) in diabetic rats

The results showed that MDA as a marker of oxidative stress in the group of diabetic rats was significantly higher than non-diabetic. Same with previous researchers who stated that in DM conditions MDA levels increase due to oxidation of free lipid radicals [4]. Hyperglycemia due to STZ induction will increase free radicals in the epididymis which results in membrane lipid peroxidation. Increased levels of MDA correlate with hyperglycemia due to glucose auto-oxidation which can produce free radicals [16].

This study shows that there is a negative correlation between total antioxidants and MDA in both diabetic and non-diabetic rats. The increased MDA level in diabetic rats is the opposite of the total decrease in antioxidant in response to oxidative stress events. Oxidative stress due to exposure to hyperglycemia causes an imbalance between the oxidant system and antioxidants in patients with type 2 diabetes [17].

The total antioxidant of epididymal fluid has an important contribution as potentially protecting sperm from oxidative attack during storage [18]. Sperm has a lot of polyunsaturated fatty acids in the plasma membrane so it belongs to one cell that is very
susceptible to oxidative stress [9]. Antioxidant protection system both enzymes and non-enzymatic role interactively and synergistically neutralize free radicals by contributing or blocking the electron uptake reaction [19].

Excessive reactive oxygen radicals activate this process of apoptosis through increased caspase-3 activity and suppress Bcl-2 expression [20]. Oxidative stress due to hyperglycemia will trigger apoptosis through both the intrinsic and extrinsic pathways [21]. Mitochondria as the main target will be activated by oxidative stress so that it disrupts membrane permeability [22]. Cytochrome C released from the membrane activates the caspase-3 executor protein [23].

4. Conclusions
The total antioxidant status of the diabetic rats group was significantly lower than the non-diabetic group. Total antioxidants were negatively correlated with MDA and caspase-3 expression.

5. Reference
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