Blood Superoxide Dismutase (SOD) level has a negative correlation with Dry Eye (DE) degree

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ABSTRACT

Background: The therapy for dry eye (DE) remains controversial. Some studies concluded that the ideal therapy for DE is based etiopathogenesis. Dry eye is caused by a decrease in antioxidant level; one of them is superoxide dismutase (SOD). This study assessed the correlation between SOD level and DE degree as therapy based on the etiopathogenesis of DE.

Method: This study was a cross-sectional study in Sanglah eye clinic, in January until June 2016, with a total of 51 samples with DE. The samples were categorized based on DE degree, namely 0, 1, 2, 3, and 4 respectively. The SOD serum examination was performed on each group. Correlation between SOD level and DE degree was analyzed with Pearson correlation test.

Results: This study obtained data on sex, age, history of diabetes mellitus, history of smoking, and history of ocular surgery from five groups of DE in homogeneity. There was a negative correlation between SOD level and DE degree (r = -0.373; p = 0.007).

Conclusion: SOD level has a negative correlation with DE degree.

Keywords: Superoxide dismutase (SOD), Dry eye (DE) degree


INTRODUCTION

Dry eye (DE) is a dryness condition of the tear film and ocular surface. Dry eye is associated with symptoms such as eye discomfort, visual disturbance, and tear film instability.1 Dry eye therapy based on etiopathogenesis has not been found until now, so the treatment given is limited only to reduce symptoms. Several previous studies have investigated the management of DE therapy, in the hope of reducing morbidity and improving the quality of life for patients. Utilization of artificial tear drops, punctual plugs, and pellet are three of many new therapies that have been conducted, but still, have limitations.

The principle of etiopathogenesis of DE is the presence of inflammation and oxidative stress. Various studies about the role of inflammation and oxidative stress have been conducted in order to understand the etiology and pathophysiology of DE. The research studied direct or indirect examination in inflammation mediators through the formation of enzymes involved in oxidative stress. The results of the study said that one of the therapies are expected to play an important role in the occurrence etiopathogenesis DE is the utilization of antioxidant.4 One of the parameters used to determine antioxidant levels is by using an enzyme protein; one of the important enzymes is superoxide dismutase (SOD).5 Superoxide dismutase (SOD) is an antioxidant enzyme that plays a role in overcoming the oxidative stress that works by changing the free radical superoxide anion (O₂⁻) into other non-dangerous components, namely H₂O₂, which then catalyzed into water (H₂O).4

Therefore, this research was conducted to determine the correlation between SOD level and DE degree, as one of the efforts to provide a therapy that based on the etiopathogenesis of DE. This study is expected to be an additional idea in order to support the development of the use of antioxidants for DE based on etiopathogenesis.

MATERIAL AND METHOD

This was a cross-sectional analytic observational study. In this study, data were collected prospectively, by collecting DE patient’s blood and then examined for the serum SOD levels. This research was conducted at the Eye Clinic Sanglah Hospital, from January to June 2016.

The target population in this study were all patients with DE complaints who went to the Eye Clinic Sanglah Hospital, Denpasar. Affordable population study were all DE patients who came for treatment to Eye Clinic Sanglah Hospital, Denpasar from January until June 2016. The eligible subjects were all patients who meet the inclusion and exclusion criteria. Inclusion criteria were: DE patients who are willing to follow the research and aged 40 years or more. Exclusion criteria were: patients with DE complaint who are on treatment with non-steroid anti-inflammatories, corticosteroids, anti-hypertensive or immunosuppressive drugs.
in the past month, patient taking antioxidant vitamins (vitamins A, C, and E) in the past month, and patient with history of ocular infections such as conjunctivitis, keratitis, and corneal ulcer in the past month.

Fifty-one patients were selected with consecutive sampling technique. The DE degree is the degree of severity of Dry Eye according to The definition and classification of dry eye disease: report of the Definition and Classification subcommittee of the international dry eye workshop (DEWS) 2007, which is divided into five degrees; 0, 1, 2, 3, and 4. The DE degree was assessed on the eye experiencing heavier DE.

Data were entered into the form of research and then recorded in the table. Selection of data, namely editing, coding and tabulation are included in the file navigator program Statistical Package for the Social Sciences (SPSS) version 16. The descriptive statistical analysis to describe the general characteristics and distribution of various variables. Categorical scale data described in terms of frequency and percentage, while for numerical scale data in the form of mean and standard deviation. One-sample Kolmogorov-Smirnov test for normality and Levene's test for homogeneity of data were performed. Pearson correlation test was used to assess the correlation between the DE degree and SOD level. One Way ANOVA test was used to assess the mean difference between SOD level in each DE groups. Generalized linear models were used to analyze the effect of age, gender, history of diabetes mellitus, history of smoking, and a history of ocular surgery, as control variables in the correlation between the level of SOD and DE degree. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

The analysis showed that the data on age, sex, history of diabetes mellitus, history of smoking, and a history of ocular surgery were normally distributed (p > 0.05) and homogeneous (p > 0.05). Characteristics of the subject of this study are shown in Table 1, including sex, age, history of diabetes mellitus, history of smoking, and a history of ocular surgery.

SOD level on each degree of DE can be seen in Table 2. The highest levels of SOD is found in the patients with degree 1 DE, with the mean SOD level of 2026.24 ± 1158.90 unit/mL.

The correlation of SOD level and DE degree was analyzed using Pearson correlation test. As shown in Figure 1, there was a negative correlation between the levels of SOD with DE degree, with the correlation coefficient of -0.373 (p = 0.007).

The influence of independent variables, such as age, sex, history of diabetes mellitus, history of smoking, history of ocular surgery, and the DE degree towards SOD level was analyzed using Generalised linear models. There were two independent variables that affect the levels of SOD significantly, namely: degree 1 DE (1386 ± 263.05) and smoking history (-724.23 ± 283.87); p values were 0.000 and 0.014, respectively (Table 3).

DISCUSSION

There were 33 female patients (64.7%), and 18 males patients (37.3%) participated in this study. Research conducted by the Beaver Dam Offspring Study (BOSS) during 2005-2008 concluded that DE prevalence is higher in women than men, 17.9%, and 10.5% respectively (p < 0.0001). Dry eye incidence in men is relatively in a settled line as the age increased (p = 0.91). However, DE incidence in women tends to increase constantly with increasing age (p = 0.02).7 Research conducted by Moss in 2000 found that the incidence of DE was higher in women when compared with men, 17%, and 11.1% respectively (p <0.001).8 The same result

### Table 1 Characteristics of study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Descriptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender [n(%)]</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33 (64.7%)</td>
</tr>
<tr>
<td>Male</td>
<td>18 (37.3%)</td>
</tr>
<tr>
<td>Age [(years old)(mean±SD)]</td>
<td>59.69 ± 10.22</td>
</tr>
<tr>
<td>Diabetes mellitus [n(%)]</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (21.6%)</td>
</tr>
<tr>
<td>No</td>
<td>40 (78.4%)</td>
</tr>
<tr>
<td>Smoking [n(%)]</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (5.9%)</td>
</tr>
<tr>
<td>No</td>
<td>48 (94.1%)</td>
</tr>
<tr>
<td>Ocular surgery [n(%)]</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (19.6%)</td>
</tr>
<tr>
<td>No</td>
<td>41 (80.4%)</td>
</tr>
</tbody>
</table>

### Table 2 SOD level in each DE degree

<table>
<thead>
<tr>
<th>Degree</th>
<th>n</th>
<th>%</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>5.9</td>
<td>228.54</td>
<td>521.52</td>
<td>420.65±166.44</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>11.8</td>
<td>656.74</td>
<td>3.459.48</td>
<td>2.026.24±1158.90</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>11.8</td>
<td>281.24</td>
<td>1.330.86</td>
<td>577.17±386.77</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>56.9</td>
<td>252.60</td>
<td>1.574.24</td>
<td>491.56±314.61</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>13.7</td>
<td>311.03</td>
<td>1.724.91</td>
<td>577.65±508.36</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100.0</td>
<td>228.54</td>
<td>3.459.48</td>
<td>689.83±693.93</td>
</tr>
</tbody>
</table>

* One Way Anova test
was also obtained by Miljanovic in 2006 where the incidence of DE at age 50 years in the United States is 7.8% or 3.2 million for women and 4.7% or 1.6 million for men. The current study obtained the same result; the incidence of DE is more common in females. The reason behind this is because women over 45 years old tend to be going through menopause and it has been shown that women who receive hormonal therapy during menopause are slightly more prone to DE. After going through menopause, estrogen levels produced by the ovaries will naturally decrease thus estrogen levels in the blood will also decrease.5,8,9 Decreased estrogen levels will result in disruption of the ocular surface, such as decreased tear secretion, meibomian gland, and density of goblet cell in conjunctiva.10

Speaking of age, this current study found the average age of DE patients was 59.69±10.22 years. Research conducted by BOSS in 2005 to 2008, found that the mean age of DE patients was 49 years old, with a range of 21 to 84 year old. The Korean National Health and Nutrition Examination Survey (KNHANES) reported that the mean age of DE patients is 49.9±16.7 year old.11 The incidence of patients diagnosed with DE at age 40 years or more is 10.7% for men and 20.6% for women. In general, the prevalence of DE is known to increase with age.11 The incidence of DE in the age over 50 years in the United States is 7.8%.7 Age is one of the most important risk factors for DE. Dry eye usually occurs at the age of 40 years, and there is a tendency that increasing age is followed by severity of DE. With increasing age and persistent exposure to agent or material from outside, there will be an accumulation of excessive free radicals and disruption to the antioxidant protective mechanisms against the ocular surface.12,13 In addition, along with the increasing age, the secretion of tears from the lachrymal glands tends to decrease, and it will reach its limit in average at 60 year.14 This study found 3 DE patients who smoke (5.9%). Research conducted by Paulsen in 2014 obtained the result that there is no relationship between smoking and DE. Research from BOSS in 2012 obtained a different result, that there is a relationship between smoking and some symptoms of DE.5 Oxidative stress plays an important role in diseases associated with cigarette smoking. Various models showed exposure to cigarette smoking increases leukocytes, platelets, and monocytes adhesion to endothelial cells as well as platelet aggregation, which can trigger an inflammation process.15 Patients with moderate to severe DE presenting a variety of pro-inflammatory cytokines and chemokines, indicating that the ocular surface plays an active role in triggering the inflammation process.16 The current study found 10 (19.6%) samples from DE patients who have a history of ocular surgery. Research conducted by Cho and Kim in 2009 found that DE tests were significantly worse in patients after cataract surgery.17 Another study conducted by Han in 2014 found that there is an increasing of ocular symptoms, worsening of the lid margin, decreasing of meibomian gland secretions, and decreasing of Tear Break-up Time (TBUT) in patients who had cataract surgery. Cataract surgery causes or exacerbates meibomian gland obstruction, while only functional changes in the meibomian glands, such as decreased expression of the meibomian, can be observed without any structural changes as seen on meibography.7 Research conducted by Hovanesian in 2001 obtained an increased incidence in DE within 6 months after Photorefractive Keratectomy (PRK) and laser-assisted in-situ keratomileusis (LASIK) surgery.18

### Table 3  Correlation between SOD levels with independent variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE</th>
<th>t</th>
<th>p</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>First DE degree (reference: fourth degree)</td>
<td>1386</td>
<td>263.05</td>
<td>5.270</td>
<td>0.000</td>
<td>856.04</td>
<td>1916.35</td>
</tr>
<tr>
<td>Smoking (reference: no smoking)</td>
<td>-724.23</td>
<td>283.87</td>
<td>-2.55</td>
<td>0.014</td>
<td>-1296.33</td>
<td>-152.13</td>
</tr>
</tbody>
</table>

* Generalised linear model

### Figure 1  Correlation between SOD levels with DE degree
Those above are caused by various factors affecting the ocular surface after undergoing ocular surgery. Topical anesthetic and eye drops which contain preservatives such as benzalkonium chloride are known to have an effect on the corneal epithelium. Exposure to microscope light also affects DE postoperatively. Various surgical procedures interfere with normal corneal innervations and produce pathological changes in the cornea, which cause discomfort in patients. 17,19

This study found a negative correlation between the levels of SOD and degree of DE with a correlation coefficient of -0.373 (p = 0.007). Research conducted by Holowacz in 2009 obtained the same result, the provision of additional antioxidants in patients with DE can improve the quality and quantity of tears, thereby increasing the lacrimal function and decrease the symptoms. 20 Cejkova research in 2008 is in accordance with the obtained results in current study, which is the expression of antioxidant enzymes like Catalase, SOD, and glutathione peroxidase are lower in conjunctival epithelium of DE patients compared to normal eyes as well as the degree of reduction in expression of the enzyme is inversely related to the severity of the corresponding DE. A study carried out by the Blades in 2001 also concluded that the use of oral antioxidants increases tear stability and health of the conjunctival surface for marginal DE. 21

In this study, it is generally obtained that SOD will decrease in accordance to increasing of the grade of the DE, however, uniquely in DE grade 1 the SOD level were higher and significantly different from grade 0 (Figure 1). It is caused due to the mechanism of compensation that occurs at the beginning of free radical reactions with the body’s natural antioxidants. Free radicals or oxidants can disrupt cell integrity because they can react with various cellular components essential to the survival of cells, both structural components, such as membrane constituent molecules as well as functional components, such as enzymes and DNA. 22

Some research on antioxidants produced by the body in the early phase of the oxidative stress reactions showed that levels of body’s natural antioxidants would be generated excessively in initial phase which aims to bind and neutralize free radicals. Then, if the amount of antioxidant produced already in a certain threshold or saturated while free radicals are still present, the levels of antioxidants will decrease with weight or levels of free radicals that present. 23 It shows that the body’s natural compensatory mechanism in response to the free radical that occurs at the beginning of oxidative stress reactions so that the levels of antioxidants in DE grade 1 is higher than the grade 0.

Compensatory mechanisms can be seen in the case of hypokinesia. Research conducted by Sazontova in 2007 obtained the results that early phase of hypokinesia, between 3 to 72 hours of hypokinesia, will result in increased production of antioxidant enzymes in the liver. However, if the hypokinesia continues then it resulted in decreased activation of antioxidant enzymes against free radicals that are still active. 24

This study found a correlation between the level of SOD and DE grade 1 with a correlation coefficient of 5.270 (p = 0.000). Research conducted by Cejkova in 2008 obtained the same result; a decrease in the expression of SOD which depends on the severity of DE. Particularly at DE grade 1, patients with less dry eye symptoms had a higher level of SOD relatively as much as the normal eye and have the highest correlation with SOD compared with grade 3 and 4. 25

This study found a relationship between the levels of SOD with a history of smoking with a correlation coefficient of -2.55 (p = 0.014). Research conducted by Agnihotra in 2009 obtained the same result, that SOD level in the saliva of smokers is much lower than non-smokers. 26 Free radicals derived from oxygen reaction that occurs in the cell, such as superoxide (O2•-) that is formed by cigarette would be metabolized by SOD into hydrogen peroxide (H2O2) and oxygen (O2) molecules. Hydrogen peroxide is then metabolized by the catalase and glutathione peroxidase enzymes into water molecules (H2O). However, in the case of SOD metabolic disorders, there will be an accumulation of free radicals O2•- that cause damage to the cell’s membrane, essential proteins, and DNA. 27,28 Higher level of free radicals generated by smoking led to increasing SODs that are used to bind or neutralize these free radicals. Thus, the existing reserves of SOD will be reduced.

The limitation of the current study is that the serum samples were taken from patient’s blood sample. In DE patients there is increased osmolarity of the tear, inflammation of the ocular surface, and potential damage to the ocular surface. Oxidative stress overload can cause damage to the epithelial surface and reduce the tears secretion. Thus the sample studied using the tears of patients will make a better result. However, due to limitations in getting samples of tears with the amount of 0.2 ml, then it cannot be done.

REFERENCES
2. Jee D, Park SH, Kim MS, Kim EC. Antioxidant and inflammatory cytokine in tears of patients with dry eye syndrome treated with preservative-free versus preserved eye drops.


