UNIVERSA MEDICINA

January-April ,2014 *Vol.33 - No.1*

Decreased erythrocyte superoxide dismutase in elderly men with early nuclear cataract

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ABSTRACT

BACKGROUND
Imbalance between oxidative processes and antioxidant defenses has been considered to play a role in cataractogenesis, particularly in diabetes patients. Superoxide dismutase (SOD) is an important precursor for oxidative stress in the human lens, and its activity is mainly dependent on the copper and zinc levels in the body. The aim of this study was to compare erythrocyte SOD, erythrocyte zinc and total serum testosterone levels in male patients with early senile nuclear cataract and evaluate the correlations between the parameters in all subjects.

METHODS
A community-based study of cross-sectional design was conducted at Cilandak District Primary Health Center where 52 adult and 17 elderly men with early senile nuclear cataract were chosen as the study subjects. Erythrocyte SOD, erythrocyte zinc, serum testosterone, and fasting blood glucose (FBG) levels were measured in all subjects. Nuclear cataract stage was assessed with the Pentacam® instrument (Oculus, Germany). Independent Student t test and Pearson's correlation were used to analyze the results.

RESULTS
Erythrocyte SOD level was significantly decreased in elderly men compared to adult men (p=0.014). Erythrocyte zinc, serum testosterone and FBG did not differ significantly in adult and elderly males (at p=0.304; p=0.145;and p=0.376, respectively). Erythrocyte SOD activity was significantly associated with erythrocyte zinc level (r=0.486; p=0.048).

CONCLUSIONS
Lower erythrocyte SOD activity was found in elderly males than in adult males with early nuclear cataract. There was a relationship between erythrocyte SOD and erythrocyte zinc level in elderly males with early nuclear cataract.

Keywords: Superoxide dismutase, zinc, testosterone, fasting blood glucose, early nuclear cataract, elderly

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Univ Med 2014;33:65-72

Superoksida dismutase eritrosit menurun pada laki-laki lanjut usia dengan katarak nuklear awal

ABSTRAK

LATAR BELAKANG

Ketidakseimbangan proses oksidasi dan pertahanan dari antioksidan, khususnya pada pasien diabetes melitus diduga mempengaruhi proses terbentuknya katarak. Superoxide dismutase (SOD) merupakan petanda stres oksidasi yang penting pada lensa manusia, dan fungsinya sebagian besar tergantung pada kofaktor cuprum dan seng (zinc). Penelitian ini bertujuan untuk membandingkan kadar SOD, seng di eritrosit dan testosteron total pada pria dengan katarak nuklearis awal dan mengevaluasi korelasi dari parameter tersebut.

METODE

Studi ini merupakan suatu studi berbasis komunitas dengan disain potong lintang yang dilakukan di Puskesmas Kecamatan Cilandak dengan memilih 69 orang pria yang menderita katarak nuklearis awal. Lima puluh orang terdapat dalam kelompok dewasa di bawah 60 tahun dan 17orang dalam kelompok lanjut usia. Kadar SOD, seng eritrosit, testosteron total dan gula darah puasa (GDP) dibandingkan pada kedua kelompok tersebut. Derajat kekeruhan katarak nuklearis diperiksa menggunakan alat Pentacam® (Oculus, Germany). Uji t independent dan korelasi Pearson *digunakan untuk menganalisis data.*

HASIL

Kadar eritrosit SOD menurun secara signifikan pada usia lanjut dibandingkan pada kelompok pria dewasa (p=0,014). Kadar eritrosit seng, testosteron, dan GDP tidak berbeda secara signifikan pada kedua kelompok (p=0,304; p=0,145; p=0,376 masing-masing). Aktivitas SOD eritrosit berhubungan secara signifikan dengan kadar eritrosit zinc (r=0,486; p=0,048)

KESIMPULAN

Aktivitas eritrosit SOD lebih rendah pada kelompok pria usia lanjut dengan katarak nuklearis awal dibandingkan dengan kelompok orang dewasa. Pada lanjut usia dengan katarak nuklearis awal terdapat hubungan antara aktivitas SOD eritrosit dan kadar seng eritrosit.

***Kata kunci** : Superoxide dismutase, zinc, testosteron total, gula darah puasa, katarak nuklearis dini, lanjut usia*

INTRODUCTION

Senile cataract is an age-related disorder, characterized by progressive lens opacification and globally is the leading cause of chronic visual impairment in the elderly. In 2010, the WHO reported that cataract accounts for 51% of all blindness in the world.[1] As the mean age of the global population is rising, cataract will not only pose a major economic burden for the patients but also increase mortality in elderly.[2] Although cataract surgery is a successful way to cure the cataract, it is still unaffordable or inaccessible for many patients in developing countries. Alternative methods to prevent or delay cataract development still remain a challenge for researchers.

Multiple factors involving complex interaction between various physiologic processes contribute to the development of senile cataract.[3,4] Senile cataract starts in the fourth decade as a consequence of aging, with the lens

losing its transparency, its nucleus becoming more rigid, and thicker, and its shape more resistant to change during accommodation, which is the basis of presbyopia. These may be the earliest observable symptoms of age-related cataract.[5,6]

The biochemical cause of senile cataract is not fully known. Numerous studies show that there are other possible risk factors involving oxidative mechanisms associated with age-related cataract, such as gender, nutritional status, smoking, pollution, drugs, ultraviolet light exposure, occupational status, aging and diabetes.[7-9] So far, aging and diabetes remain the most significant contributors to cataract progression.[3,10]

Oxidative stress continues to be the leading mechanism of cataractogenesis.[11,12] Oxidative stress releases free oxygen radicals which are neutralized in the lens by antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxides**,** catalase and by non-enzymatic antioxidants (ascorbate, carotenoids, vitamin E, the glutathione system).[13,14] A study showed that cataractous patients had significantly decreased erythrocyte SOD activity.[15]

During aging, antioxidant system activities continue to decrease. SOD acts as a first order antioxidant enzyme and its function mainly depends on copper and zinc ions. Zinc is one of the important cofactors in humans, and zinc deficiency is related to the sensitivity of oxidative stress. [16-18] As males age, there is also a decline in serum testosterone concentration, an increase in sex hormone binding globulin (SHBG) concentration, and a decrease in free testosterone, but the hormonal decline in aging males is modest compared with estrogen deficiency in menopause.[19]

Researchers have shown that oxidative stress, which is caused by the accumulation of free radicals, plays a role in the pathogenesis of cataracts and that this process can be prevented by antioxidants. We undertook the present study to determine erythrocyte SOD, erythrocyte zinc, and serum testosterone levels in adult and elderly males, and to evaluate any correlation between erythrocyte SOD, erythrocyte zinc, and serum testosterone levels in males with early nuclear cataract.

METHODS

Research design

This was a community-based cross-sectional study which was carried out from May to October 2011 at the Cilandak District Primary Health Center, South Jakarta, Indonesia.

Study subjects

The study subjects were males aged ≥40 years, residing in Cilandak District, Jakarta, Indonesia, and agreeing to participate voluntarily in this study. Sixty nine male subjects who had early nuclear or incipient cataract in both eyes with best-corrected of visual acuity (BCVA) of 1.0 were included in this study. We excluded subjects with mature cataract, glaucoma, diabetic retinopathy, and those unable to undergo complete ophthalmologic and laboratory examinations.

Data collection

All participants were interviewed to collect their demographic data. Based on age, the subjects were categorized into two groups, i.e. adult males (<60 years) and elderly males (≥60 years). The subjects underwent physical examination and routine eye examination, including refractive correction,fundus photography, intraocular pressure examination, and lens imaging for assessment of nuclear cataract using the Pentacam® instrument (Oculus, Germany). This instrument uses a blue light-emitting diode to image the anterior segment of the eye. Nuclear opacity was graded by quantifying the density of the lens opacification in three dimensions with the built-in rotating Scheimpflug camera and analyzing with specific software. The total area of opacity was graded for both eyes. Only early nuclear cataract (grade 1 and 2) was included in this study.

Laboratory measurements

Five-milliliter blood samples were drawn from a peripheral vein in all subjects and collected into 10 ml vacutainer tubes, containing ethylene diamine tetraacetic acid (EDTA), after 10 hours of fasting. Fasting blood glucose (FBG) was assessed by spectrophotometry using the Advia 1800 and Bayer Advia 180074024 glucose reagent (R1: ATP, NAD, sodium azide; R2: Hexokinase, G6PD). Detection limit: 0.6mg/dl, linearity: 0-700 mg/dl. Conversion factor: mg/dl x 0.05555 mmol/L. The glucose in the sample is phosphorylated by hexokinase to yield glucose-6-phosphate (G6P), which is then oxidized by glucose-6-phosphodehydrogenase (G6PD). The oxidative reaction releases nicotinamide adenine dinucleotide (NAD)H, and this is taken as a measure of the glucose concentration in the sample. Erythrocyte SOD activity was determined by a colorimetric enzymatic method, in which xanthine is oxidized to uric acid by xanthine oxidase (XOD), releasing reactive oxygen species (ROS), which is neutralized by SOD. ROS reduces 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) into a red formazan dye. The SOD activity is measured by the degree of inhibition of this reaction, therefore formazan intensity is inversely related to SOD activity. Erythrocyte zinc was measured using flame atomic absorption spectrophotometry. The erythrocyte mass obtained from total blood was centrifuged three times at 4ºC in a centrifuge at 3000 x g for 15 minutes, after washing with 5 ml of 0.9% saline and homogenization. Subsequently the erythrocyte mass was extracted with a micropipette, placed in demineralized Eppendorf tubes, and stored at -20°C until required for zinc analysis. To determine the serum testosterone level we employed an electrochemiluminescent immunoassay (ECLIA) using Roche Elecsys Reagent Kit Cat 11776061 (R1: Anti-testosterone-ad-biotin, R2: Testosterone-Ruthenium) on a 2010/Cobas e601 analyzer. The sample testosterone is attached to anti-testosterone-ad-biotin. This complex is immobilized in the solid phase and bombarded by electrons to induce chemiluminescence, which consitutes a measure of the testosterone concentration in the sample.

Ethical clearance

Written informed consent was obtained from all participants, and the study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Trisakti University, Jakarta.

Statistical analysis

Data were analyzed with SPSS for Windows version 17.00. Descriptive data were expressed as mean ± SD; difference between the levels of SOD, zinc and testosterone in both groups were tested using the independent t-test. Pearson's correlation was used to check the correlation between variables. The statistical level of significance was $p<0.05$.

RESULTS

A total of sixty nine participants were enrolled in this study. Baseline characteristics of the study population are given in Table 1. Mean age, height, weight, and body mass index (BMI) were 53.69 ± 7.77 years (range 42–70 years), 162.25 ± 5.62 cm (range 150–176), 64.11 ± 11.44 kg (range 40–87), and 24.31 ± 3.95 kg/m^2, respectively. Fifty two (75.36%) were adult

Table 1. Distribution of characteristics of subjects with early nuclear cataract (n=69)

Characteristics	n (%)
Age (years)	53.69 ± 7.77
Age group	
Adult (< 60 years)*	52 (75.36)
Elderly (≥ 60 years)*	17 (24.64)
Height (cm)	162.25 ± 5.62
Weight (kg)	64.11 ± 11.44
Body mass index (kg/m^2)	24. 31 ± 3.95
FBG (mg/dl)	111.73 ± 43.78
Testosterone (ng/dl)	511.26 ± 174.78
SOD (U/ml)	196.66 ± 26.88
Zinc (µg/dl)	81.02 ± 12.63

*Values are mean ± SD; SOD = superoxide dismutase

Table 2. Comparison of FBG, testosterone, SOD and zinc levels in adult and elderly males with early nuclear cataract

Parameters	Age group		p value
	Adult males (n=52)	Elderly males (n=17)	
FBG (mg/dl)	114.94 ± 50.13	103.71 ± 22.60	0.376
Serum testosterone (ng/dl)	490.30 ± 169.54	542.62 ± 158.56	0.304
Erythrocyte SOD (U/ml)	200.13 ± 26.31	181.65 ± 26.05	0.014*
Erythrocyte zinc (µg/dl)	60.109 ± 11.08	65.29 ± 16.488	0.145

FBG = fasting blood glucose; SOD = super oxide dismutase; values are mean ± SD; *significant

males and 17 (24.64%) were elderly males. Means of FBG, testosterone, SOD and zinc were 111.73 ± 43.78 mg/dl, 511.26 ± 174.78 ng/dl, 196.66 ± 26.88 U/ml, and 81.02 ± 12.63 µg/dl, respectively.

As shown in Table 2, the erythrocyte SOD level was significantly lower in elderly males (181.65 ± 26.05 U/ml) than in adult males (200.13 ± 26.31 U/ml) with early nuclear cataract (p=0.014). However, the FBG, testosterone and erythrocyte zinc levels were not significantly different between adult males and elderly males.

The results presented in Table 3 show that there was a significant correlation between erythrocyte SOD and erythrocyte zinc levels in elderly males with early nuclear cataract (r=0.486; p=0.048). Relationships between FBG and early nuclear cataract (r=-0.164; p=0.530) and between serum testosterone and early nuclear cataract (r=-0.476; p=0.085) were not significant.

Table 3. Correlation between erythrocyte SOD, FBG, erythrocyte zinc and serum testosterone in elderly subjects with early nuclear cataract

Parameters	Erythrocyte SOD	p value
FBG	- 0.164	0.530
Serum testosterone	- 0.476	0.085
Erythrocyte zinc	0.486	0.048*

SOD = super oxide dismutase; FBG = fasting blood glucose; Values are r (Pearson correlation); *significant

DISCUSSION

Our study showed that erythrocyte SOD was decreased in elderly males with early nuclear cataract as compared to adult males. The human lens grows during life and undergoes dynamic changes in optical, physical and biochemical properties with age. Aging is the most important risk factor for age-related nuclear cataract.[9,11] The aging process is accompanied by extensive oxidative damage to the crystalline lens. Disturbed balance between oxidative processes and antioxidative defenses results in oxidative stress that can damage proteins, lipids, polysaccharides and nucleic acids of the lens.[4,18,20] Some external factors which have been identified as risk factors for the development of nuclear cataract are smoking, excessive ultraviolet-B exposure and diabetes.[5,8,15] We found that the BMI in both groups with cataract was higher than that in the normal population, indicating that obesity is associated with cataract. However, it is not clear whether obesity simply causes cataract or relates to other abnormalities that may contribute to cataract formation. [5,21,22] In this study, we found that mean FBG level was greater than 100 mg/dL. This represents an association between FBG and early nuclear cataract in males. This finding is in contrast to the results of the study by Park et al.[23] who found that the elevated FBG appears to be associated with nuclear cataract only in women and not in males. [23]

Since the FBG level in the present study was inversely correlated with erythrocyte SOD activity, we suggest that hyperglycemia may contribute to the development of early nuclear cataract, the glucose in the blood entering the aqueous humor and the lens, while erythrocyte SOD activity decreases slightly with age.[4,24]

The present study was an evaluation of erythrocyte SOD, erythrocyte zinc and serum testosterone levels with respect to early nuclear cataract in adult and elderly males, based on laboratory examination. Oxidative stress is difficult to monitor by direct examination of the blood, but can be estimated by measuring the antioxidant levels in the blood or erythrocytes. SOD is an essential component of the second antioxidant system that can be evaluated from the red blood cell.[12,18,25] In the present study, the erythrocyte SOD level declined in the elderly male group, showing that erythrocyte SOD correlates with senile cataract. The increased SOD in early nuclear in the adult male group may be a defensive response to the increased oxidation levels in the body in the early stages of nuclear cataract. That the SOD level decreases with the aging process in elderly males may be a reflection of an adaptive protective response in the body system.[12] Chakraborty et al.[16] found a decrease in plasma SOD activity in senile cataract and a significant correlation of SOD activity with serum zinc. In the study of Virgolici et al.,[12] the investigators demonstrated that red blood cell SOD activity was significantly increased in cataract patients,[12] although there was no description of the severity of the senile cataracts included in their study. In contrast, our study compared erythrocyte SOD levels only in male subjects with early nuclear cataract.

In erythrocytes, the level of zinc, an important co-factor of SOD, was found to be significantly higher than the normal reference value of our laboratory. Dawczynski et al.[26] reported that zinc concentration in mature cataracts was significantly higher than in corticonuclear cataracts. Jamil et al.[27] demonstrated that higher zinc levels were found in mature and hypermature cataracts as a consequence of increased protease activity in the lens. Proteases in the human eye may be considered as metallo-enzymes which may lead to cataract formation.[27,28]

One limitation of this study was the small number of subjects with the parameters of interest in the blood. A more complete approach is necessary to evaluate other antioxidant and external factors in cataract formation, involving a larger population with different types of age-related cataracts, and also to assess the protective role of a healthy lifestyle and nutritional status in delaying cataractogenesis in elderly males.

CONCLUSIONS

We found that in elderly males with early nuclear cataract, erythrocyte SOD level was significantly decreased compared to adult males, whereas serum testosterone and erythrocyte zinc levels were not significantly increased. Erythrocyte SOD activity was correlated with erythrocyte zinc level and may be a good predictor of early nuclear cataract in elderly males.

ACKNOWLEDGEMENTS

We would like to express our gratitude to all of the Cilandak Primary Health Center staff and all participants in this research. Thanks are also due to PT. Optik Tunggal and staff for their contribution in providing the ophthalmic equipment and to Prodia Laboratory for the laboratory analyses. Last but not least, we also thank the Dean of the Faculty of Medicine, Trisakti University for the funding of this study.

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