

Blood and Lens Glutathione and Catalase Activity in Patients with Age-Related Cataract

فعالية glutathione و catalase في الدم والعدسة للمرضى المصابين بساد العين الشيخوخي

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Abstract

Cataract is a complete or partial opacity that develops in the crystalline lens of the eye . There is an accumulation of evidences suggesting that oxidative damage might be one of the primary biochemical events leading to cataract development.

This study was conducted at the Department of Biochemistry ,College of Medicine , Kufa University from December /2006 to July /2007. One hundred and ten cataractous lenses were collected from patients aged 50-85 years (males 52 and females 58) who underwent extracapsular cataract extraction at Al-sadder Teaching Hospital in Najaf. Blood samples were obtained from 60 patients (males 25 and females 35). Lens and blood samples were subjected to chemical analysis to estimate levels of catalase activity and glutathione as a biomarker of oxidative stress in cataractogenesis process. Comparison was made between mature and immature cataract and it was found that the catalase activity and levels of glutathione were significantly decreased ($P<0.01$) in lens and serum of patients with mature cataract and smokers when compared with immature cataract and non smokers respectively.

Keywords : Cataract , Oxidative stress , Catalase , Glutathione

الخلاصة :-

أن ساد العين هو العتمة الكلية أو الجزئية للعدسة البلورية في العين أو لغشائها المحيط . وهناك عوامل خطر عديدة تعتبر من مسببات العتمة منها التقدم في السن والتدخين التي بسبب عمق تأثيرها وشيوعها تم تضمينها في هذه الدراسة. لقد دلت الأبحاث المتواترة على أن الضرر التأكسدي ربما يكون هو الحدث الكيموحياتي الابتدائي الأبرز الذي يقود إلى تفعيل ساد العين وتفاقمه فيما بعد .

تم البحث في مختبرات فرع الكيمياء الحياتية – كلية الطب – جامعة الكوفة للفترة من كانون الثاني 2006 إلى تموز 2007 حيث تم جمع مائه وعشر عدسات من مرضى تتراوح أعمارهم 50-85 عاما (52 ذكور و 58 إناث) بطريقة استخراج الساد خارج المحفظة في مستشفى الصدر التعليمي في النجف وتم سحب عينات الدم من 60 شخصا منهم (25 ذكور و 35 إناث) وأجريت التحليلات الكيماوية الضرورية لتحديد فعالية إنزيم Catalase ومستويات glutathione كونها مؤشرات للإجهاد التأكسدي في عملية تكوين ساد العين.

لقد تمت المقارنة بين ساد العين التام والجزئي وقد تبين إن فعالية إنزيم Catalase ومستويات glutathione قد انخفضت بشكل ملحوظ في العدسات والمصول للمصابين بساد العين التام عند المقارنة مع ساد العين الجزئي كما هي الحال عند مقارنة غير المدخنين بالمدخنين من هؤلاء المرضى .

Introduction :

Cataract is a complete or partial opacity that develops in the crystalline lens of the eye ⁽¹⁾. Age-related cataract is responsible for more than 80% of all cataracts and its incidence increases as longevity increases ⁽²⁾. In addition to altered membrane physiology and protein biochemistry, cataract development is accompanied by changes in lipid biochemistry, decreased enzyme activity, reduced glutathione levels, and diminished ATP production ^(3,4) Oxidative stress, which refers to the cellular damage caused by oxygen radicals, is the major contributor to cataractogenesis ^(5,6,7,8).

To counteract the chronic oxidative stress, the lens has a variety of defense mechanisms including Enzymatic antioxidants like glutathione peroxidase and catalase (CAT) (19) which are decreased in the lens and aqueous humor during aging and in the development of age related cataract^(9,10).

Aim of the study :

This study proceeded to achieve the following points

- 1-To estimate the catalase activity and levels of reduced glutathion in both lens and blood samples in cataract patients and control.
- 2- To assess the mutual relationship between cataract formation or progress and the oxidative stress intensity.

Patients and Methods :

This study was conducted at the Department of Biochemistry ,College of Medicine , Kufa University from December /2006 to July /2007. One hundred and ten (110) cataractous lenses were collected from patients aged 60-85 years (males 52 and females 58) who underwent extracapsular cataract extraction at Al-sadder Teaching Hospital in Najaf. And they were immediately stored at - 20 °C until analyzed.

The cataractous lenses were classified as having mature (n=54) and immature cataract (n=56) on the basis of degree of opacity by examination and diagnosis of senior ophthalmologist. Patients with previous ocular surgery, trauma, iritis, glaucoma and systemic diseases were excluded from the study. Smoking as a risk factor for cataract was enrolled in the study and patients were classified as non-smokers (n=68) and smokers (n=42) with 5 years smoking duration and more. For chemical analysis, the lenses were taken out from the refrigerator, weighed and immediately homogenized in phosphate buffer (pH 7.0) in ratio of 1/10 (W/V).The homogenized lenses were used for the estimation of reduced glutathione.

Blood samples were obtained from 60 patients with cataract (males 25 and females 35). The patients were classified as having mature cataract (n=28) and immature cataract (n=32). They were subdivided into non-smoker (n=34) and smoker (n=26).

Venous blood samples also were taken from control subjects (n=30) aged 60 – 70 years who were 13 females and 17 males. Control subjects had no clinical evidence of any disease state which might affect the antioxidant capacity including cataract and smoking.

The collected blood samples was used to estimate the catalase activity and glutathione concentration in red blood cell .

Statistical Analysis :

In this research the usual statistical methods were used in order to analyze and assess the obtained results.

- 1- Description statistics: Statistical tables , Arithmetic mean (X) , Standard deviation (SD) and Correlation of variation (C.V. %)
- 2- Inferential statistics: These were applied in order to accept or reject the statistical hypothesis. They include student (t-test), in order to find the comparative significance C.S. between the two means for each contrast and determine if it was highly significant H.S (P<0.01), significant S (P<0.05) and nonsignificant N.S(P>0.05).

Results And Discussion :

Catalase activity

Catalase is an enzyme, which is present in most cells and it is a crucial antioxidative enzyme in the normal lens as it is the only enzyme that converts H₂O₂ to H₂O. It is found to act 10⁴ times faster than peroxidase. Accordingly, its inactivation could upset the oxidation- reduction balance in the lens and stimulate lens opacification⁽¹¹⁾.

Catalase enzyme activity was estimated in lens tissue of patients with mature and immature cataract as shown in table (1).The results in this table refer to high significant differences in the means of tissue CAT activity between the mature and immature cataract. There is a decrement in

mature cataract CAT activity with a percent of 49 %in comparison with immature cataract CAT activity. The high significant decrement exists in both smoker and non-smoker subgroups with percents of 54 %and 44 %respectively.

The decrement in CAT activity may be attributed to the enhancement of free radicals production in such circumstances. It is known that the generation of increased free radical flux result in CAT inactivation.

It was thought that cigarette smoke appears to be a major environmental source of cyanide contamination⁽¹²⁾. The inhibition of lens CAT activity by cyanide toxic effect strongly suggests that cyanide imposes oxidative stress on lens tissue.

Erythrocyte levels of CAT activity were also estimated. Figure (1) shows that mean values of erythrocyte catalase activity significantly decrease in cataract patients when compared with control groups. Our results were similar to the results of Ates *et al.*,⁽¹³⁾ who reported that the activity of erythrocyte CAT was lower in cataract patients than in controls.

Table (2) includes the mean values of erythrocyte CAT activity in patients with mature and immature cataract. There is a high significant decrement in mature cataract group levels if compared with immature group. The percent of decrement was 43%. Similar inclination was found in the subgroups of smoker and non-smoker patients with percent of 47 %and 40 %respectively.

The present results are highly consistent with contemplations of Chandrasena *et al.*,⁽¹⁴⁾ who reported that age related cataract (mature and immature) was associated with significantly low levels of erythrocyte CAT activity. Also Bhuyan and Bhuyan⁽¹⁵⁾ found decrease in erythrocyte CAT activity in cataract patients.

Glutathione

The reduced glutathione plays an important role in the maintenance of lens transparency, by preventing the oxidation of SH groups of lens proteins.

Table (3) displays the mean values of lens tissue GSH in patients with mature and immature cataract. The statistical comparison between the levels of lens GSH in the mature and immature cataract refers to a high significant decrement in mature cataract group. Decrement percent is 22 %. In addition, the comparison of the subgroups of smokers and non smokers both show a high significant decrement with percent of 25 %and 20 %respectively. Sulochana *et al.*, reported that free GSH content of clear human lenses was about 18.2µmol/gm protein⁽¹⁶⁾.

Sulochana *et al.*,⁽¹⁶⁾ reported significant low levels of GSH in lenses of smoker patients with cataract when compared to non smokers.

Glutathione levels in erythrocyte were also estimated. Mean values of GSH levels in erythrocyte significantly decrease in cataract patients when compared with control groups as shown in figure (2). Our result was in consistent with Donma O *et al.*, results⁽¹⁷⁾ who reported significant low levels of GSH in erythrocytes of cataractous patients when compared with control group.

As reported in table (4) the mean values of erythrocyte GSH in mature and immature cataract were compared. The results show that there was a high significant decrement in erythrocyte GSH of mature cataract if compared with immature group. The decrement percent was 17%. The decrement of the subgroups smoker and non smoker cataract was obtained to be 18 %and 16 %respectively.

Table(1): Lens catalase activity in patients with mature and immature cataract

	Tissue catalase activity Ku /gm protein								
	Mature Cataract				Immature Cataract				
C.S.	CV%	SD	X	N	CV%	SD	X	N	
H.S	0.26	± 0.13	0.5	32	0.27	± 0.25	0.90	36	Non-Smokers
H.S	0.13	± 0.04	0.3	22	0.20	± 0.13	0.65	20	Smokers
*H.S	0.35	± 0.14	0.4	54	0.31	± 0.24	0.78	56	Total

*P < 0.01

Table(2): Erythrocyte catalase activity in patients with mature and immature cataract

	Erythrocytes catalase activity K/gm Hb								
	Mature Cataract				Immature Cataract				
C.S.	CV%	SD	X	n	CV%	SD	X	n	
H.S	0.09	±13.6	149.1	16	0.13	±33.1	247.8	18	Non-Smokers
H.S	0.27	±26.3	96.1	12	0.09	±16.7	181.8	14	Smokers
*H.S	0.28	±33.7	122.1	28	0.20	± 42.7	214.8	32	Total

*P < 0.01

Table (3): Lens glutathione levels in patients with mature and immature cataract

	Tissue glutathione levels $\mu\text{mol/gm}$ protein								
	Mature Cataract				Immature Cataract				
C.S.	CV%	SD	X	N	CV%	SD	X	n	
H.S	0.13	± 1.2	9.6	32	0.12	± 1.4	12.0	36	Non-Smokers
H.S	0.19	± 0.9	4.8	22	0.13	± 0.8	6.4	20	Smokers
*H.S	0.28	± 2.0	7.2	54	0.25	± 2.3	9.2	56	Total

*P < 0.01

Table (4): Erythrocyte glutathione levels in patients with mature and immature cataract

	Erythrocyte glutathione levels $\mu\text{mol/gm Hb}$								
	Mature Cataract				Immature Cataract				
C.S.	CV%	SD	X	N	CV%	SD	X	n	
H.S	0.18	± 1.3	7.2	16	0.19	± 1.6	8.6	18	Non-Smokers
H.S	0.16	± 1.0	6.4	12	0.15	± 1.2	7.8	14	Smokers
*H.S	0.26	± 1.8	6.8	28	0.23	± 1.9	8.2	32	Total

*P < 0.01

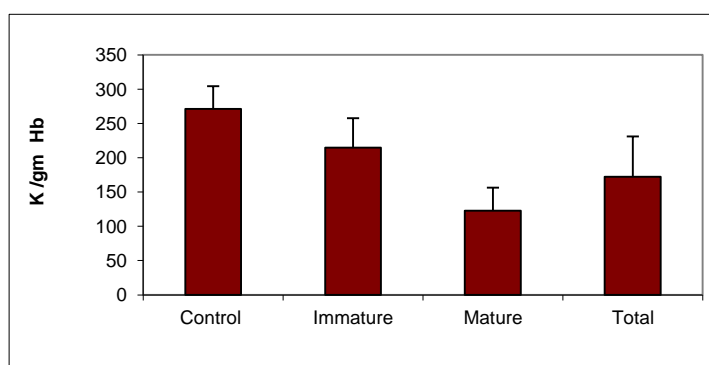


Figure (1): Erythrocytes catalase activity in cataract patients and control.

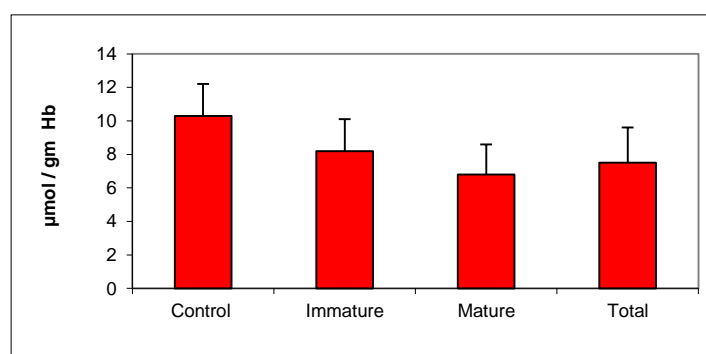


Figure (2): Erythrocyte glutathione levels in cataract patients and control

Conclusion And Recommendations :

- 1-Human lens is widely affected by the high levels of oxidative stress.
- 2- decrement in both catalase enzyme activity and reduced glutathione levels in mature cataract lens when compared with immature reveal that there is a significant association between cataract stage and the severity of oxidative stress.
- 3-Cigarette smoking, especially heavy smokers are at high risk of cataract formation. The deleterious effects of the smoke on the lens may initiate or facilitate the cataract by different ways and mechanisms.
- 4-catalase ,glutathione and other oxidative stress indicators have to be determined regularly to assess the severity of stress status in health and diseases.

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