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The comparison of total capacity antioxidant in the serum of people with pterygium and control subjects

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翼状胬肉患者血清总抗氧化能力的临床研究

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摘要

目的:评估翼状胬肉患者血清总抗氧化能力与对照组的比较结果。

方法:病例对照研究。包括 2016 年 Vali-Asr 教学医院眼科患有翼状胬肉的所有患者。对照组来源于 Vali-Asr 教学医院眼科(非翼状胬肉患者),两组年龄、性别和居住地无统计学差异。通过任意抽样选取 66 例受试者[31(47%)例患者,35(53%)例对照组]。用铁还原抗氧化法(FRAP)检测所有患者静脉血液样本。FRAP 是一种 10min 快速检测手段,通过检测样本使铁(Fe^{3+})转化为亚铁(Fe^{2+})的能力以测定样本的抗氧化能力。通过 SPSS 21 软件收集数据,使用 chi-square 和 Mann-Whitney 进行分析($\alpha=0.05$)。

结果:研究组患者平均抗氧化能力为 842.55±161.46 μmol/L,对照组为 856.77±209.41 μmol/L($P=0.8$)。基于性别比较正常个体与翼状胬肉患者平均血清抗氧化能力,结果显示对照组男性平均抗氧化能力为 894.05±176.82 μmol/L,女性为 780.01±118.33 μmol/L,差异具有统计学意义($P=0.008$)。病例组与对照组的同性别间对比结果无显著性差异。

结论:研究结果显示,翼状胬肉患者血清抗氧化能力的总水平低于对照组的平均值,但未观察到显著差异。本研究结果与氧化应激在翼状胬肉发病机制中的影响基本一致。

关键词:总抗氧化能力;翼状胬肉;风险因素

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Abstract

• AIM: To investigate the comparison of total antioxidant capacity in the serum of patients with pterygium and control subjects.

• METHODS: This case-control study was conducted on all persons referred to Ophthalmology Clinic of teaching Hospital of Vali-Asr (peace upon to him) with clinical symptoms of pterygium during the year 2016. The control group was selected among patients referred to the Ophthalmology Clinic of Vali-Asr (peace without pterygium) that the two groups were matched in terms of age, gender and place of residence. Sixty-six persons [31 people (47%) in patient group and 35 people (53%) in the control group] were enrolled by convenience sampling. Venous blood sample was taken from all patients after the sampling using ferric reducing antioxidant power(FRAP); FRAP- as a quick 10min measurement, the antioxidant power measurement of samples according to the conversion of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) was checked. The collected data ware entered to software SPSS 21 and were analyzed by chi-square and Mann-Whitney tests at the level of $\alpha=0.05$.

• RESULTS: The mean of antioxidant capacity in patients was 842.55±161.46 μmol/L and antioxidant capacity in healthy controls was 856.77±209.41 μmol/L ($P=0.8$). In the comparison of mean serum antioxidant capacity in healthy individuals and in the serum of people with pterygium based on gender the results showed that the antioxidant capacity mean in male control subjects has been 894.05±176.82 μmol/L and in females control 780.01±118.33 μmol/L that the observed difference have been reported statistically significant ($P=0.008$) but the other comparison according the gender between cases and control does not show any significant difference.

• CONCLUSION: The results of this study showed that the full level of serum antioxidant capacity in patients has been less than the mean of antioxidant capacity in control subjects, however the observed difference has not been significant. The results of this study were consistent with basic results carried out on the damaging effects of oxidative stress in the pterygium pathogenesis. Recommending diet with minerals and vitamins containing antioxidants may be preventing the onset and progression of pterygium.

• KEYWORDS: total antioxidant capacity; pterygium; risk factors

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INTRODUCTION

Eye is one of the most sensitive organs, therefore the diseases related to it can be very serious and dangerous. Pterygium is a common ocular surface disease in humans, characterized by tissue remodeling, cellular proliferation, neovascularization, and inflammation[1].

Pterygium is a common ocular disease and as a triangular shape mass is thickened in conjunctiva of bulbar and spreads on the cornea. This disease is created due to degeneration of elastoid collagen and the emergence of epithelial fibro-vascular tissue in conjunctiva. Pterygium causes Bowman's layer destruction and inflammatory changes in the cornea. The disease is more prevalent in the certain geographic areas and the maximum age of prevalence is 20-40y[2].

The word pterygium derives its name from "Pteryx" which is the Greek word for wing. Pterygium suggests a small wing[3]. Although the precise pathogenesis of pterygium is unknown the reactive oxygen species (ROS) and chronic inflammation induced by ultraviolet (UV) light, low humidity, and dust are mainly considered to cause pterygium. However aging, the k-ras, mutations in tumor suppressor genes such as P-53 and P-63, the presence of HPV-DNA, expression of new proteins of difensin and phospholipase D, growth factors such as bFGF and VEGF are known risk factors[4-5]. Pterygium symptoms consisted of intermittent congestion accompanied with some degree of photophobia, tearing and foreign body sensation and more over the most important problem of the patient is in terms of beauty and appearance. Another problem of this disease is the condition of astigmatism and visual constriction[6]. The pterygium diagnosis is taken based on clinical appearance of a wedge-shaped lesion on the cornea. Pterygium usually is soft, almost flat, white, irregular borders, thick, pink or red and with fibro-vascular nature[2].

The treatment of this disease varies according to the size and symptoms. Patients with small pterygium can be treated only using artificial tears or other eye drops to relieve symptoms such as redness and irritation. Management of patients with larger lesions which has disrupted eye movement or visual acuity usually includes pterygium surgery. The decision to perform surgery is different in terms of growth rate and degree of astigmatism caused by pterygium. It is recommended surgery for cosmetic reasons be avoided[6]. Despite numerous treatment methods in the treatment of pterygium, the recurrence rate is 30-80% that this high recurrence rate, has led to take advantage of new surgical techniques using adjuvant drugs and radiation therapy among ophthalmologists[7-8].

Since oxidative factors play an important role in creating many eye (optic) diseases, the production of proteins such as Hydroxydeoxyguanosine-8 DAN with therole of the damage to DNA[9]. Therefore in this hot and dusty weather in Birjand there has been no study of this regard, we decided to compare of full capacity of anti-oxidant in the serum of people with pterygium and control subjects.

SUBJECTS AND METHODS

Ethics Statement This study protocol was approved by Deputy of research and technology and the ethics committee of Birjand University of Medical Sciences, Birjand, Iran (approval code: IR. BUMS. REC. 139 Postal Cod; 97179641515.192). At the beginning of the study, oral and written informed consent forms were obtained for both case and control groups. All aspects of the study complied with the Declaration of Helsinki.

The study population included all patients with clinical symptoms of pterygium during the year of 2015 referred to ophthalmology clinic of Vali-Asr Hospital.

Inclusion Criteria Studied group include people referred to the Ophthalmology Clinic of Vali-Asr (peace upon him) with pterygium who have consented to participate in the project, not undergone certain medical therapy and have no certain underlying disease and have relatively equal mean age and sex and distribution of location with studied group and control groups that were among the non-ophthalmic attendance without eye problem was matched and the participants dose not received any salary.

Exclusion Criteria This included consent to participate in the project, underlying chronic disease such as cancer, autoimmune diseases, tuberculosis or immunosuppressive diseases as well as those who were under medical treatment (in the case of corticosteroid drugs more than 2wk). As well as people who didn't respond to positive control in the skin prick test were excluded. In addition people with dermatographism were also excluded due to impossibility of skin testing.

After selecting the cases and control subjects 5 ml of blood were collected from the arm (radial) vein. In the laboratory, the samples were centrifuged for 20min at 3000 rpm and their serum was isolated. It was noted that samples aren't hemolysis or opaque, lipemic or serum containing microbial contamination. The isolated serums were divided into small amounts and until the tests were stored at -20℃. After the sampling using ferric reducing antioxidant power (FRAP); FRAP-as a quick 10min measurement, the antioxidant power measurement of samples according to the conversion of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) was checked. Then the total antioxidant blood in the two groups was compared.

The collected data ware entered to software SPSS 21 and were analyzed by chi-square and Mann-Whitney tests at the level of $\alpha = 0.05$. To examine the groups' consistency for age the

Table 1 The comparison of the frequency distribution of the subjects in the two groups in terms of sex, mean age of group and by location

Mean±SD

Parameters	Case group		Control group		Statistical test
	Frequency	Percentage	Frequency	Percentage	
M	17	54.8	20	57.1	$P=0.8$
F	14	45.2	15	42.9	$\chi^2=0.035$
Age(a)	48.12±14.52		47.00±15.92		$P=0.7$ $z=0.289$
Less than 40	12	38.7	16	45.7	$P=0.7$
More than 40	19	61.3	19	54.3	$\chi^2=0.330$
City	29	93.5	33	94.3	$P=0.7$
Village	2	6.5	2	5.7	$\chi^2=0.330$

Table 2 The comparison of antioxidant capacity mean in two groups of case (patients) and control according to gender and age

Mean±SD, mmol/L

Parameters	Case	Control	*P*	*t*
Overall	842.55±161.46	856.77±209.41	0.8	
Male	894.05±176.82	934.47±237.16	0.56	0.58
Female	780.02±118.33	753.167±100.21	0.51	0.66
<40 y/o	838.56±213.66	836.573±176.25	0.97	0.27
>40 y/o	845.07±124.63	873.782±273.23	0.46	0.46

Mann-Whitney test for gender the chi-square test was used. Due to the lack of normal distribution of antioxidant capacity, non-parametric Mann-Whitney test was used.

RESULTS

This study was conducted on 66 people who were divided into two groups patients ($n=31$, 47%) and control ($n=35$, 53%). Both groups matched regarding the age mean and sex distribution frequency and location. (Table 1).

In the comparison of the sex distribution in the two study groups the results showed that in the patient group and control 54/8 and 57.1% of people were male, respectively ($P=0.8$) (Table 1).

In the investigation of normal age distribution using the Kolmogorov-Smirnov test results showed that this variable is not distributed normally ($P=0.03$), thus to compare the mean age between groups (patients and control) the Mann-Whitney test was used. The mean age of patients was 48.12±14.52y and the mean age of controls was 47.00±15.92y ($P=0.7$) (Table 1).

After dividing patients under 40y of age and over 40 the results showed that in the patient group 61.3% were over 40y and in the control group 54.3% were over 40y ($P=0.6$) (Table 1).

In the comparison of the frequency distribution of location in the studied people the results showed that in the two groups of patients and control most referred people were living in urban centers (93.5% *vs* 94.3%, respectively, $P=0.9$) (Table 1).

According to the non-normal distribution of data the results of Mann-Whitney test showed that the mean antioxidant capacity in patients was 842.55±161.46 mmol/L and antioxidant capacity in healthy controls was 856.77±209.41 mmol/L, that no significant difference was found ($P=0.8$) (Table 2).

In the comparison of antioxidant capacity mean in serum of patients with pterygium based on gender the results showed that the antioxidant capacity mean in males suffering from pterygium was 894.05±176.82 mmol/L and in the female patients was 780.01±118.33 mmol/L that no significant difference was found ($P=0.07$) (Table 2).

The results of this study on comparison of the antioxidant capacity mean in serum of patients with pterygium based on age showed that antioxidant capacity mean in people under 40y was 838.568±213.66 mmol/L versus control 836.573±176.25 mmol/L it also in the people over 40y was 845.07±124.63 mmol/L in comparison to control 873.782±273.23 that no significant difference was found ($P=0.97$, 0.46) (Table 2).

In the comparison of antioxidant capacity mean in serum of patients with pterygium based on gender the results showed that the antioxidant capacity mean in males and female in case and control or gender have not any statistically significant of two groups except of females control group which was 753.16±100.21 mmol/L versus the male control 934.47±237.16 that the observed difference was statistically significant ($P=0.008$).

DISCUSSION

The mean level of antioxidant capacity in case group was higher than control subjects but there was not statistically significant difference ($P=0.8$). The other variable among age and sex the only significant difference that observed was between male and female mean antioxidant capacity in the control groups ($P=0.008$). The Kormanovski[11] explain in their survey that recurrent pterygium have a significant decrease in the level of total antioxidant status and antioxidant enzymes Compared to men. The one significant difference which observed was elevated nitric oxide (NO) level and low total antioxidant status level of women in the primary pterygium group that in spite of higher TAS in male but it was not significant among man and woman of cases and control. Thus our finding could be agreed to above finding to define male have higher antioxidant ratio in comparison to male gender.

Since our efforts were done with the keywords of the antioxidant capacity mean and pterygium in different data

bases including: Elsevier, Google Scholar, Up to date, PubMed and other sources no article similar to our dissertation topic, so the comparison of the results of this thesis paper with similar article is not fully feasible, therefore the results of similar articles on other eye diseases and antioxidants also are brought.

In a reviewstudy by Oduntan *et al*[9-10], it is expressed that the oxidative factors are involved in the pathogenesis of most common serious eye diseases such as cataracts, and primary open angle glaucoma and age-related macular degeneration and the another study indicate development of pterygium.

About the role of ultraviolet radiation, imbalance of oxidant and antioxidant in diseases of the cornea it has been shown that increased levels of Ultraviolet B (UVB) radiation leads to a sharp reduction in the amount of antioxidants of cornea (with high molecular weight, antioxidant enzymes and low molecular weight, mainly ascorbic acid) so that lead to an imbalance of antioxidants and oxidative factors. Reduced antioxidants protect of the cornea causes damage from internal parts of the eye by UVB radiation and reactive oxygen species produced by them[7]. The nitrous oxide and total antioxidant measurement of conjunctival tissue of normal healthy conjunctiva, primary pterygium and recurrent pterygium showed that the primary pterygium group have significantly higher ratio than the value of these parameters for the control or recurrent pterygium groups. The level of total antioxidant in the recurrent pterygium group was lower than that in the control group. This finding was not possible to compare with our study of the different sampling measurement but the higher ratio of total antioxidant in healthy conjunctiva and recurrent pterygium versus primary conjunctiva not only showed controversy in their result but also it was not actually agree with our result that in cases it was lower than control although it was not statistically significant. Therefore this may be due to the different of tissue total antioxidant measurement instead of plasma sampling[11].

In the case of specific role of oxidants and antioxidants in creating pterygium in a study by Chiang and colleagues entitled "the expression of cyclooxygenase 2 in pterygium" in 2007 it was stated that after the recent discovery of an expression of the abnormal gene p53 in the epithelial tissue of pterygium, some researchers thought that pterygium instead of a degenerative disease is a tumor. It has been reported that ultraviolet radiation (UV) is associated with the formation of pterygium; however, the mechanism of following UV radiation will leads to uncontrolled proliferation on pterygium cell is still uncertain. Since the cyclooxygenase 2 (COX2) plays an important role in the incidence of skin cancer related to UV, it is logical that COX2 is present in pterygium. This paper, byimmunohistochemical staining using a monoclonal antibody to COX2 in 90 cases of pterygium and 40 normal conjunctivas, and limbus and 5 were normal which results in pterygium, 75 patients (83.3%) were selected as samples to coloring for COX2. Coloring had been restricted to the cytoplasm of epithelial layers, mainly on the basic epithelial layer without coloring significantly in the fibro-vascular of sub-epithelial layer. All samples in the conjunctiva and limbus of normal (control) group were negative. So they conclude that there has been COX2 role of COX2 in the incidence of skin cancer tumor, also it suggests that COX2 may also play a role in the formation of pterygium. Therefore this study can be used as a basis for future review of causal relationship between COX2 and pterygium as well as COX2 inhibitor effect in preventing initial pterygium or its recurrence[12]. Hence of the more complex phenomenon that could be influenced in generating the pathological stimulation, this conflicting finding needed more study to find the triggering insult.

The decrease of corneal antioxidant protective mechanisms results in oxidative injury of the cornea and causes damage of the inner parts of the eye by UVB rays and by reactive oxygen species generated by them[13].

In the study conducted by Kau and colleagues describe the pterygium is an eye surface disease associated with chronic UV exposure and characterized by factors of proliferative, inflammatory infiltration, fibrosis, angiogenesis and extracellular matrix degradation. In addition, the recent genetic studies on genetic factors and providing a summary of the role of epithelial-mesenchymal transition of endothelial progenitor cells (EPCs) bone marrow cells, and nerve signals that may help in the pathogenesis of pterygium are studied[14].

The treatment options for pterygium also discussed on mechanisms of pterygium growth indeed of other factor such as chondrocyte-derived extracellular matrix that suppresses the growth of pterygium in athymic nude mice[15]. According the Hyesook Lee suggest, the Dichlorofluorescein Diacetate which suppresses pterygium pathogenesis through the inhibition of NF-κB activation and the up regulation of Nrf2 by blocking the p38 MAPK and PKC signaling pathways it seem everyone a factor showed correlation with pterygium as our study and according of above report tile now there is not known a definite risk factor to find preventive or therapeutic approach.

Base on study by Pradhan and collegos they describe 190 people in the age group 50-80y were classified to three morphological types including 73 cases of cortical cataract, 77 cases of sub-capsular cataract and 40 cases of nuclear. The control group included 78 healthy subjects of the same age group were selected in terms of non-cataract. Plasma thiobarbituric acid reactive substance (TBARS) levels were examined in the control group and patients with cataract. The results showed no significant difference in catalase levels of red blood cells and plasma levels of vitamin E between the cataract cases and control group[16]. Therefore the similar pathophysiology of cataract and pterygium permit to compare in the agreement of it to our study.

The study by Orhan[17] and colleagues demonstrated that antioxidant supplementation following selenite exposure may prevent the cataract formation and may enhance antioxidant defense of blood and lens.

In an article by Neelam is stated that proper antioxidant supplements in diet likely helps to retain visual function in patients with age related macular degeneration (AMD) and is

useful in prevention or delay of the progression of early AMD to late AMD. Carotenoids in age-related degenerative maculopathy (CARMA) as a randomized clinical trial and double-blind have been examined that antioxidant supplements versus placebo have been used. Of 433 participants with every feature of ADM at least in one eye or with any level of ADM in one eye with AMD LATE (neovascular AMD or central geographic atrophy) in the opposite eye. The CARMA study aimed to investigate whether lutein and zeaxanthin, combined with antioxidants (vitamins C, E and zinc), has beneficial effects on visual performance and/or prevent disease progression from early to late stages of disease or not? The early result represents improved or maintained visual acuity over 12mo. Secondary outcomes include improvement or maintain of contrast sensitivity, the ability to differentiate shapes, and changes in the severity of AMD using fundus photography[18]. The other study showed that the anti-oxidant such as rosmarinic acid may be having a therapeutic rule in pterygium[19-20]. However the total anti-oxidant only showed significant difference between male and female of control in our study that it may be the protective rule of increased antioxidant ratio of male due to more exposure to outdoor work.

The two RCTs demonstrated that multivitamin/mineral supplements could decrease the risk of nuclear cataracts[21-22]. The Christen[22] study expressed that the oxidative mechanisms have important role in the pathogenesis of eye diseases such as cataracts and age-related macular degeneration which are two important factors of human beings ocular disorders. His findings increase the possibility that vitamins and minerals containing substances with antioxidant could have protection or prevention role in the progression of the diseases. However Christen finally has been contradicted by anticipated results, but he states that likely the cause of this contradiction is lack of having proper diet by subjects and on the other hand lack of appropriate control of confounding factors and limitations of the study, as a result, he recommends to redoing of this study at more volumes and enhanced monitoring[23].

The results of this study showed that the mean total serum antioxidant capacity in patients was less than control subjects. However, the observed difference has not been significant. But among the control group according of gender the difference was significant. So the results of this study could be consistent with carried out basic results on the damaging effects of oxidative stress in the pathogenesis of pterygium.

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