Dynamic Thiol Disulphide Homeostasis in Patients with Surfer's Eye: A Case-Control Study

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Abstract

Purpose

The role of oxidative stress in the pathogenesis of pterygium is still unclear. However, abnormal thiol disulfide homeostasis levels are involved in the pathogenesis of various systemic or ocular diseases. We aim to analyze dynamic thiol disulphide homeostasis in patients suffering from conjunctival pterygium using a contemporary technique.

Methods

Thirty-eight subjects suffering from pterygium and 35 age-gender matched healthy volunteers were recruited for study. For each case, total thiol, disulfide and native thiol levels in blood were obtained. Additionally, ratio of disulfide over total thiol, native thiol over total thiol and disulfide over native thiol were computed.

Results

The level of median native thiol was lower in pterygium group (318.2 μmol /L vs 333.4 μmol /L) and median disulfide was slightly higher in pterygium group (24.3 μmol /L vs 22.8 μmol /L) compared to control group. Both disulfide over total thiol and disulfide over native thiol ratios were higher in pterygium group, ratio of native thiol over total thiol was found to be higher in control group. Nevertheless, none of those differences were statistically significant at 95% confidence level. Notably, correlation test pointed to a negative correlation both between pterygium grade and native thiol and between total thiol and pterygium grade in pterygium group (*P = 0.03 and 0.02* respectively).

Conclusion

A negative correlation hinting that slightly weakened dynamic thiol disulphide homeostasis in subjects with pterygium, a local ocular disease. Further studies with larger sample sizes may shed light on this potential relationship and justify systemic antioxidant therapies in these cases.

Introduction

Conjunctival pterygium (CP) (aka Surfer’s eye) is a degenerative condition characterized by a slightly elevated, triangular shaped conjunctival overgrowth extended to cornea [1]. Many theories investigating its pathogenesis showed that CP is a condition stemmed by many triggers among which ultraviolet radiation (UV) the most important one. UV irradiation relays its toxic impacts either directly by UV phototoxicity or indirectly by formation of primary molecules causing oxidative damage (aka reactive oxygen species or ROS). Imbalance between growth factors and cytokines, changes in tear film, viral

infections, genetic mutations, immunologic disturbances and chronic inflammation are also believed to be the important factors [2].

Pterygium is usually observed to be asymptomatic in the beginning, however, if it grows over the pupillary axis or induces astigmatism causing chronic ocular irritation, cosmetic imparity or visual disturbance, surgical intervention becomes inevitable. The major problem with pterygium surgery is the repeated surgeries due to high recurrence rates [3].

Thiol is a biological derivative embodying sulfhydryl (-SH) which plays a critical role by impeding oxidative strain inside the cells. Oxidation takes place in thiol groups by some oxidant compounds in the environment resulting in disulfide bonds. Thiol disulfide homeostasis (TDH) is maintained by reducing disulfide bonds back to thiol groups. While dynamic TDH plays an important role in vital cellular systems such as apoptosis, detoxification, cell growth, antioxidant defense, regulation of enzyme activities, transcription factors and cellular signaling mechanism; degradation of TDH is thought to trigger many diseases through oxidative stress and tissue inflammation [4–7]. It has been shown in the literature that abnormal levels of TDH are active in the pathogenesis of various ocular conditions including pseudoexfoliation syndrome, central serous chorioretinopathy, glaucoma, cataract, keratoconus and macular degeneration [8–13].

On the other hand, despite the extensive studies, the etiology of CP has not been fully elucidated. Furthermore, the role of TDH in the etiology of CP has not been investigated before, in which context, we designed this study aimed at analyzing TDH in subjects with CP.

Materials And Methods

This is a comparative and observational study conducted fully compliant with the Declaration of Helsinki and HIPAA rules, and commended by the Duzce University ethical committee (IRB: 2019/152). Informed consent was obtained from each participator before enrolment to the study. This study was carried out with a collective corporate discipline as certain processes were performed at ophthalmology department of Duzce University and remaining parts at biochemistry department of Ankara Yıldırım Beyazıt University.

Subjects

Sample size was determined as 28 subjects per group in order to reach 95% statistical significance and 80% power level, by which the false null hypothesis will be rejected in 80% of tests.

Thirty-eight eyes from 38 subjects with conjunctival pterygium (pterygium group, PG) and thirty-five eyes from 35 gender-age matched healthy volunteers (control group, CG) were included in the study. Subjects in PG were healthy other than CP whereas there were no ocular or systemic diseases in CG.

Exclusion criteria consisted of a history of systemic and ocular conditions other than CP; smoking; history of preexisting ocular surgery or intraocular injection or systemic/ophthalmic drug usage (especially antioxidant or anti-inflammatory therapies); pregnancy (for woman volunteers); alcohol usage; being younger than 18 or older than 65 years old.

Upon obtaining written consent, standard eye examination including intraocular pressure (IOP) measurement with air-puff tonometry (NT-2000, Nidek Co., Ltd., Aichi, Japan), slit-lamp examination, best corrected visual acuity (BCVA) with Snellen eye chart and fundoscopy were performed for each participant. Pterygium was also graded in the study group as described before [14]. Subjects were not subsidized.

Laboratory method

Venous blood samples were taken from the patients in 3 ml tubes containing EDTA in the morning between 08:00 and 10:00 A.M. following an 8-hour fasting. A 10 minute centrifugation at 1500 rpm was applied to separate plasma and the obtained plasma was stored at − 80°C until biochemical tests. Blood samples were collected in Duzce University Eye Clinic and transported to biochemistry laboratory of Ankara Yıldırım Beyazıt University School of Medicine sustaining cold chain and then analyzed in a single session. The novel method by Erel and Neselioglu is employed in analyzing plasma thiol disulfide homeostasis [15]. To summarize, measurement of native thiol (–SH) and total thiol (–SH + –S–S–) concentrations is followed by subtraction of native thiol content from total thiol content. The obtained difference is then divided by 2 yielding the amount of dynamic disulphide bonds (–S–S–). Additionally, disulfide/native thiol [(–S–S–) × 100/(–SH)], disulfide/total thiol [(–S–S–) × 100/(–SH + –S–S–)], and native thiol/total thiol [–SH × 100/(–SH + –S–S–)] ratios were computed using these parameters.

Statistical analysis

All statistical analyses were carried out using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Normality of data was tested via Shapiro–Wilk procedure where the difference between means of two groups was tested by independent samples t-test and Mann Whitney U-test. Normally distributed continuous data were expressed as mean ± standard deviation whereas remaining continuous data were expressed as median (IQR = interquartile range). Categorical variables were tested by Chi-squared test and presented as the number or percentage of cases. The correlation between numerical variables was evaluated using Spearman's coefficient. Statistical significance level was set at $p < 0.05$ (corresponding to 95% confidence level).

Results

The mean age was 50.17 ± 13.41 and 51.34 ± 9.72 years in pterygium and control groups respectively. Demographic data were presented in Table 1. The median native thiol was found lower (318.2 µmol/L vs 333.4 µmol/L) and the median disulfide was found slightly higher (24.3 µmol/L vs 22.8 µmol/L) in PG compared to CG. Yet the differences were not statistically significant (P = 0.428 and 0.316 respectively). While disulfide/native thiol and disulfide/total thiol ratio were shifted in favor of PG, native thiol/total

thiol ratio was deviated towards CG. However, these differences did not reach statistical significance level as well (Table 2). As a remarkable result, correlation test suggested a statistically significant negative relation between both native thiol and grade of pterygium and total thiol level and grade of pterygium in PG (Table 3).

Table 1
Demographics and grade of pterygium

Variables		**Pterygium group (*N* = 38)**	**Control group (*N* = 35)**	***P***
Age (years)		50.17 ± 13.41	51.34 ± 9.72	0.791[a]
Gender (n, %)	Female	18 (47.4%)	22 (62.9%)	0.184[b]
	Male	20 (52.6%)	13 (37.1%)	
cIOP		12.94 ± 3.15	13.65 ± 3.36	0.356[a]
Pterygium grade		1 (2)	-	-

[a]: Independent samples t-test; [b]: Chi-squared test; n = number of patients, cIOP = corrected intraocular pressure

Parameters are expressed as mean ± standard deviation and median (IQR = inter-quartile range)

Table 2
Thiol disulphide homeostasis by groups

Variables	Pterygium group (*N* = 38)	Control group (*N* = 35)	*P*[a]
Native thiol (µmol/L)	318.2 (104.8)	333.4 (73)	0.428
Total thiol (µmol/L)	368.7 (91.8)	376.5 (74.8)	0.724
Disulfide (µmol/L)	24.3 (7.4)	22.8 (13.5)	0.316
Disulfide/Native thiol (%)	7.4 (3.7)	6.5 (2.9)	0.226
Disulfide/Total thiol (%)	6.4 (2.9)	5.8 (2.4)	0.226
Native thiol/Total thiol (%)	87.0 (5.8)	88.3 (4.7)	0.226

[a]Mann Whitney U test; n = number of patients, P = P-value

Parameters are expressed as median (IQR = inter-quartile range)

Table 3
Correlation between thiol disulphide homeostasis and grade of pterygium in pterygium group

Variables	Pterygium grade	
	r	*P*[b]
Native thiol	-0.394	*0.031*
Total thiol	-0.437	*0.016*
Disulfide	-0.039	0.839
Disulfide/Native thiol	0.157	0.407
Disulfide/Total thiol	0.157	0.407
Native thiol/Total thiol	-0.157	0.407

[a]: Spearman's correlation test. r = correlation coefficient, P = P-value

Discussion

Pterygium is a benign yet incorrigible degeneration associated with elevated lesions of the conjunctiva and contiguous cornea. Despite highly effective surgical interventions, it may still affect life quality of patients due to the local depth of invasion and recurrence. Therefore, the search for the exact etiopathogenesis that could pave the way to find the best treatment option for CP with minimal risk of recurrence and/or complications, remains to be an interest of modern ophthalmology [1, 3]. On the other hand, TDH is a new topic for many researchers to investigate the underlying etiology of several irrelevant systemic or ocular conditions.

Pterygium is characterized by inflammatory infiltrates, fibrosis, proliferation, angiogenesis and extracellular matrix breakdown and its pathogenesis is mainly associated with long-term exposure to ultraviolet radiation [1]. Moreover, apoptotic and oncogenic proteins, DNA methylation, lymphangiogenesis, viral infection, extracellular matrix modulators, inflammatory mediators, loss of heterozygosity, microsatellite instability, alterations in cholesterol metabolism and epithelial-mesenchymal cell transition have been identified as other causes [2].

Several studies attempted to discover the underlying molecular mechanisms affecting the course of pterygium as this may help in exploring new therapeutic targets. In this context, the effects of oxidative stress on CP have been well investigated to date [16].

ROS has an important role in the pathological process of pterygium formation, progression and recurrence through suppression of local/systemic immune responses, mutations in certain genes, pyroptosis (a highly inflammatory form of programmed cell death) and glucose-6-phosphate-dehydrogenase (G6PD) deficiency with the help of oxidative damage [17–19].

Oxidative stress and free radicals are closely related to pathophysiological processes in many ophthalmological diseases including but not limited to glaucoma, keratoconus, age-related macular degeneration, and cataractogenesis [20]. It is also claimed to take part in pathogenesis of ocular surface inflammation [21]. Photooxidative products of this network can be revealed immunohistochemically at systemic and/or tissue level. Free radicals are oxygen-containing molecules which can cause large chain chemical reactions in human body as they react so easily with other molecules thanks to the uneven number of electrons they contain. These reactions are called oxidation. Free radicals may arise either naturally in our body through physical activity or inflammation or by environmental elements such as exposure to free radicals by factors such as UV light, ozone, chemicals (certain pesticides/cleaners, metals, solvents, etc.), smoking, radiation, air pollution and infectious organisms. Oxidation is an ordinary and necessary procedure of the body’s intricate mechanism of protecting its health. On the contrary, oxidative stress is a result of the imbalance between antioxidant and free radical activities. Antioxidants can transfer an electron to a free radical while keeping their stability which in turn causes stabilization and reduced reactivity of the free radical getting the electron. Free radicals can be of help in case they function properly. Excess free radicals may cause imbalance against antioxidants and in turn parts of the body including DNA, proteins and fatty tissues. This damage may cause various diseases over time as the aforementioned body parts constitutes a big part of our body [22].

In fact, the molecular pathogenesis of pterygium and the role of oxidative stress in this process have been investigated by several studies before. The diminished activities of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase (CAT); higher levels of malondialdehyde and nitric oxide (NO); increased 8-hydroxydeoxyguanosine (8-OHdG); altered p53 protein, and augmented inflammatory elements were found in pterygium tissue [20, 23]. However, they were mainly based on pterygium tissue analysis and it is not clear if pterygium pathogenesis is caused by oxidative stress. Furthermore, to the best of our knowledge, dynamic TDH in CP patients was not studied before.

There are only three articles in the literature analyzing oxidative stress in primary pterygium patients systemically. In their study with 61 subjects suffering from pterygium, Kurtul BE et al. [24] showed that distribution width of red cells was significantly larger in patients with pterygium than control group. Based on the results, they concluded that oxidative stress and inflammation cytokines could play a major role in the pathogenesis of pterygium. In another study, Rasool M. Et al. [25] pointed to a higher level of oxidative stress and inflammatory clinical parameters such as nitric oxide, malondialdehyde, tumor necrosis factor-α and matrix metalloproteinases-9 and a lower level of non-enzymatic small molecules and antioxidant enzymes in cancer patients following systemic chemotherapy. The authors of this study argued that these parameters could also take part in the pathogenesis of ocular complications related to systemic chemotherapy such as retinal degeneration, glaucoma, cataract, blepharitis, pterygium, macular degeneration and retinitis pigmentosa. In the third study evaluating serum total oxidant status (TOS) and total antioxidant status (TAS), Kilic-Toprak et al. [26] claimed that suitable environment for pterygium progress might be created by surged systemic oxidative state and offsetting antioxidant feedback.

Various tests namely TOS, TAS, oxidative stress index, lipid hydroperoxide, paraoxonase, arylesterase and thiols can be employed in measuring the level of antioxidant and oxidant enzymes as along with molecules to determine state of oxidative stress in organisms. Thiols consist of 52.9 % of the total serum antioxidant capacity containing sulfhydryl (-SH) groups and are responsible for modulating glutathione related antioxidant enzymes. Given the presence of ROS in the environment, –SH groups form disulphide bonds by oxidizing, and which implies a prospective protein oxidation mediated by radicals. The disulphide bonds can be reduced back to thiol groups thereby maintaining TDH. Weakened thiol disulphide homeostatic state and shifted balance towards disulphide or thiol side are the two main abnormalities found in TDH tests.

Although lower molecular weight thiol compounds such as cysteine disulfide (CySS), Cys (cysteine), cysteinylglycine (CysGly), reduced glutathione (GSH), oxidized glutathione (GSSG), homocysteine (HCysSS) and glutamylcysteine (GluCys) could usually be measured in the past, only a small portion of the total thiol in the organism is made up by lower molecular weight thiols. The remaining large fraction, on the contrary, is fundamentally consisted of albumin and thiols in other proteins. When analyzing oxidative stress, only the level of thiol could be measured as level of disulphide could not be gauged since 1979. Nonetheless, the new technique developed by Erel and Neselioglu [15] allows simultaneous measurement of both the disulfide and thiol levels resulting in a thorough assessment. In addition to that, the method is superior to other sophisticated methods such as bioluminescent systems, fluorescence

capillary electrophoresis and high-performance liquid chromatography (HPLC), in terms of simplicity, ease of use, practicality, sensitivity, cost-efficiency, speed, reliability and the possibility of remeasurement [27].

Among above mentioned studies, the research conducted by Kilic-Toprak et al. [26] is similar to our study. However, our work is more comprehensive compared to it because thiols are the most important reductant compounds in organisms and TDH enables dynamic regulation, performs redox signaling, and sits in a key position as a spot of oxidative stress, it is a useful marker as a clinical measure of oxidative stress in blood serum than TAS and TOS. Moreover, although our sample size was similar to theirs, contradicting with their results, we could not reach statistical significance potentially because our patients' pterygium grades (grade 1–2) and hence cumulative oxidative load, were lower than their study (grade 2–3). Nevertheless, we found a slight yet insignificant deviation in thiol disulfide balance towards oxidation and a statistically significant negative correlation between both native thiol grade of pterygium and total thiol levels and grade of pterygium in PG. Erel and Neşelioğlu noted that, under oxidative stress conditions, increase in reduced thiol concentration is accompanied by a concurrent decrease in native (non-reduced) thiol concentrations and raise in disulfide amounts. Additionally, they argued that level of plasma disulphide were higher in subjects with degenerative disorders whereas lower in others with proliferative conditions. Moreover, a shift in the balance towards either disulphide or thiol side is at least a sign of weakened TDH even if it is not statistically significant [28]. In the light of this information, though not significant, our results cast doubt on the possibility of a predisposing systemic oxidative background for pterygium formation suggested by above-mentioned studies.

The sole limitation in our study is that it was carried out in a single center with a relatively small group of patients. Yet, it is an invaluable study as it is the first one in its kind to investigate the potential relationship between TDH and CP.

In conclusion, our study is remarkable since it is the first study investigating TDH in patients with conjunctival pterygium. A negative correlation hinting that slightly weakened dynamic TDH in CP patients was showed in this study. Although statistical significance could not be achieved in all the evaluated parameters, we showed that thiol oxidation slightly increased in CP patients implying that this topic must be further examined. Should this relationship be supported by studies with larger samples, systemic antioxidant therapies can be a strong treatment candidate for CP patients.

Declarations

Acknowledgements

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Ethics approval and consent to participate

The Institutional Review Board of the Duzce University, Duzce, Turkey approved this study (IRB: 2019/152). All procedures adhered to the Declaration of Helsinki and HIPAA rules were conducted in accordance with the approved protocol. Written informed consent was obtained from each patient before his/her participation.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the present study are not publicly available (obtained from Duzce University Hospital, Duzce repository), but are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Conflicts of interest/Competing interests

The authors declare no competing interests or conflict of interest.

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Authors' contributions

HTA, OE: study design, article writing, critical revision. OE: performing laboratory tests. HTA: data acquisition, interpretation. OE: supervision. All the authors have read and approved the final manuscript.

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