Original Article

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Tear Lactate Levels as a Surrogate Marker of Oxidative Stress in Myopia: A Case-control Study

Biochemistry Section

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ABSTRACT

Introduction: Myopia is most common refractive error leading to visual dysfunctions worldwide. Oxygen deficit due to environmental stress leads to increase in anaerobic glycolysis which results in lactate production. Increase in Reactive Oxygen Species (ROS) and inability of defense system to eliminate them is also responsible for alteration of regulatory pathways. The patient with elevated lactate levels are at significant risk of morbidity and hence, require a prompt treatment.

Aim: To estimate and study the lactate levels in tear in various degree of myopia.

Materials and Methods: This case-control study was conducted in tertiary care centre, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India. Twenty five patients each with low, medium, high myopia, age and sex-matched healthy controls (i.e, group 1-4). Diagnosis was established with help of detailed history, clinical and ocular examination. Lactate levels were estimated in tear samples by enzymatic method on autoanalyser within 30 minutes of collection. Independent Student's t-test was used to determine the significance between myopia and controls.

Results: Out of total 100 subjects, 68% were males and 32% were females. The mean±SD age was 24.56±6.35, 24.12±5.45, and 23.24±5.17, 21.69±3.95 in the group 1, group 2, group 3 and group 4, respectively, in the control group, group with low, medium and high myopia, respectively, which was statistically not significant. The results of the present study showed that lactate levels in tears were significantly higher (p-value=0.013) in myopia patients as compared to controls (1.34±0.46 vs 1.06±0.50). Additionally, the lactate levels increased with the progression of degree of myopia.

Conclusion: Increase in the level of lactate in tear was directly proportional to the degree of myopia. Tear lactate levels can be used as a non invasive method to study oxidative stress in individuals. Further studies with more oxidative markers in tears can also be compared with blood values in larger groups of patients with long-term follow-up to validate the finding.

Keywords: Aqueous humour, Glycolysis, Visual impairment

INTRODUCTION

Myopia is the most common type of the refractive error and a major cause of medium to high visual impairment worldwide especially in early adulthood. In myopia, the image is formed in front of the retina, leading to blurred distant vision. Other symptoms may include discomfort after near work, sensitivity to light, floating black spots and sometimes, flash of light. High myopia increases the risk of retinal breaks, retinal detachment and glaucoma [1,2].

Corneal function are affected adversely depending upon the severity of hypoxia in case of any environmental stress that restricts supply of oxygen. As hypoxia starts, the dependence on anaerobic glycolysis in epithelium increases, which is approximately 18 times less effective production of energy than aerobic metabolism. The end product of anaerobic glycolysis i.e., lactate is mostly shifted posteriorly from the corneal epithelium to aqueous humour. The patient with elevated lactate levels are at significant risk of morbidity and hence, require a prompt treatment [3].

Whenever, there is an imbalance between the production of Reactive Oxygen Species (ROS) and the ability of the biological defense mechanism to eliminate it, oxidative stress occurs. It's role is well-described in many ocular conditions including age-related macular degeneration, uveitis, cataract, retinopathy of prematurity, inflammation of cornea and keratitis [4-8].

Elevated levels of lactate and pyruvate along with the decreased activity of the antioxidant system in the tear were found in a study, which concluded that lactate is an important biomarker to monitor due to its reflectiveness of the current status of the underlying oxidative stress [9]. Lactate measured in tears suggests an alternative and non invasive method as compared to blood sample which is invasive and painful [10]. The tear lactate levels are not influenced by the haematocrit, which may be a confounding factor while assessment of serum lactate levels [11].

Recent awareness of association of damage due to oxidative stress in ocular surface diseases has incited the researchers to establish the mechanisms behind it. It faces challenges such as extremes of temperature, wind, ultraviolet radiations, pollutants, irritants, and smoke. These factors can affect the severity of ROS and can cause potential damage [12].

It is important to know the oxidants levels in tears as ocular surface is the most exposed mucosal surface of body to the environment. Hence, the current study evaluates the level of tear lactate in patients with myopia, and their comparison with healthy controls. The present study also proposes that tear lactate levels may be used as a point of care non invasive surrogate marker of oxidative stress in individuals.

MATERIALS AND METHODS

This case-control study was conducted in the Department of Biochemistry in association with Department of Ophthalmology, at a tertiary care centre, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India from January 2018 till April 2019. Prior approval of the study protocol was obtained from the Institute Ethics Committee (approval no. IEC/17/487 dated: 18/12/2017). Diagnosis was established with help of detailed history, clinical and ocular examination.

Sample size: A convenient sample size of 100 subjects, based on number of myopic patients in routine Outpatient Department (OPD) of hospital was taken and 25 patients each were enrolled in four groups as in classification given below.

Myopia, which is measured in diopters by the strength or optical power of a corrective lens that focuses distant images on the retina, has also been classified by degree or severity:

- Group 1: Low myopia-3.00D or less.
- Group 2: Medium myopia- 3.00 and -6.00D.
- Group 3: High myopia- -6.00D or more [13], and
- Group 4: Age and sex matched healthy controls.

Inclusion criteria: Patients between 20-40 years of age and diagnosed clinically as myopia were included.

Exclusion criteria: Patient having glaucoma, any systemic disease like diabetes, hypertension, thyroid disorders, any surgery or trauma to eye within one month of presentation, any major illness like liver disease, cancer, in Intensive Care Unit (ICU), any form of local or systemic steroids and or sex hormones, any antioxidant supplementation were excluded.

Study Procedure

Tear collection [14]: After explaining the procedure and making the person comfortable an informed consent was taken then the tear sample was collected from one eye of each subject.

- One drop of 0.5% proparacaine (a topical anaesthetic agent) was instilled on the cornea and conjunctiva and the patient was made to wait for five minutes.
- Tear collection was accomplished by using graduated 100 µL glass capillary rods which was procured from Scientific Drummond company Pennsylvania, USA.
- The tip of the glass capillary rod was placed in contact intermittently with the tear fluid at the lower cul-de-sac for a minimum time to obtain fluid without irritating the subject's eye (30 to 60 seconds).
- The collected sample was placed gently into a 1.5 mL Eppendorf tube.
- The sample was immediately transferred to the laboratory in an insulated container on cold packs for analysis of various parameters.
- The sample was stored at -700°C, if it could not be analysed immediately.

Sample processing: Tear sample was analysed for lactate levels within 30 minutes of collection and estimation of lactate was done by enzymatic method on autoanalyser [15]. Composition of reagent and its concentration is given in [Table/Fig-1].

Contents	Concentration of the test
R1a. Buffer	
Pipes buffer	100 mM/L, pH 7.20
TOOS	2.1 mM/L
Sodium azide	1 g/L
R1b. Enzyme reagent	
4-aminophenazone	0.4 mM/L
Peroxidase	≥1000 U/L
Lactate oxidase	≥600 U/L
Ascorbate oxidase	≥10000 U/L
Calibration standard	4.38 mM/L

[Table/Fig-1]: Reagent composition and its concentration.

STATISTICAL ANALYSIS

Data were expressed as mean (±SD). The data was compiled and analysed by IBM Statistical Package for Social Sciences (SPSS) version 20.0 using appropriate statistical methods. For comparison of mean, Independent Student's t-test was used to determine the significance between myopia and controls. Control population was compared with each subgroup, 25 controls with 25 patients each with myopia of low, medium and high degree respectively. A p-value <0.05 was considered statistically significant. Charts and graph were prepared using IBM SPSS statistics version 20.0 and Microsoft office 2007.

RESULTS

In the present study, 68% of the subjects were males and 32% were females in the control and myopia group [Table/Fig-2].

Subjects	Controls	Myopic patients
Total number	25	75
Males	17 (68%)	51 (68%)
Females	8 (32%)	24 (32%)

[Table/Fig-2]: Distribution of patients and control with respect to sex.

The mean (±SD) age was 21.69±3.95, 24.56±6.35, 24.12±5.45, and 23.24±5.17 in the control group, group with low, medium and high myopia respectively. Age was comparable in all the four groups [Table/Fig-3].

Characteristics	Control n=25	Degree of myopia			p-value
		Low n=25	Medium n=25	High n=25	
Gender					
Male	17 (68%)	19 (76%)	18 (72%)	14 (56%)	0.61
Female	8 (32%)	6 (24%)	7 (28%)	11 (44%)	0.45
Age (in years)					
Mean±SD	21.68±3.95	24.56±6.35	24.12±5.45	23.24±5.17	0.83
Range	20-37	20-37	20-38	20-36	
Median	20.40	20.86	21.50	20.58	

[Table/Fig-3]: Gender and age distribution in cases and controls (N=100).

The mean lactate level in tear was high in myopia patients as compared to controls. The increase was statistically significant (p-value <0.05). This is summarised in [Table/Fig-4].

Lactate (mmol/L)	Control	Myopia	Total	Student's t-test (p-value)
Mean±SD	1.06±0.50	1.34±0.46	1.27±0.48	0.013
Range	0.23–2.30	0.61±2.66	0.23-2.66	
Median	0.96	1.31	1.26	

[Table/Fig-4]: Mean lactate levels in tears of various degrees of myopia and controls. p-value <0.05 was considered statistically significant.

[Table/Fig-5] shows the mean level of lactate in tears were increased in the various degrees of myopia patients as compared to the controls. Mean level (mmol/L) was 1.06±0.50 in the controls, in low cases 1.14±0.37, in medium cases 1.34±0.39 and 1.54±0.52 in the high cases group. The increase in the level of lactate in tears was directly proportional to the degree of myopia. The increase was statistically significant when compared to control group (p-value <0.05) in medium and high degree of myopia.

Lactate (mmol/L)	Control	Degree of myopia		
		Low	Medium	High
Mean±SD	1.06±0.50	1.14±0.37	1.34±0.39	1.54±0.52
Range	0.23-2.30	0.63-1.92	0.71-2.04	0.61-2.66
Median	0.96	1.07	1.33	1.44
Student's t-test p-value		0.542	0.035	0.001

[Table/Fig-5]: Mean lactate levels in tears of various degrees of myopia and controls. p-value <0.05 was considered statistically significant

[Table/Fig-6] shows there was statistically significant difference in the lactate values in low versus high degree of myopia (p-value <0.05).

Lactate (mmol/L)	Degree of myopia intragroup comparison	Mean difference	Student's unpaired t-test (p-value)
Low	Medium	0.196	0.131
	High	0.399	0.003
High	Medium	0.203	0.118

[Table/Fig-6]: Intragroup comparison of lactate levels in tears of various degrees of myopia.
The increase was statistically significant in the cases with high myopia (p=0.003); p-value <0.05 was considered statistically significant

DISCUSSION

The study was conducted to estimate lactate levels in tears of patients of various degree of myopia and to establish its importance as a surrogate marker of oxidative stress in myopia. It was found that lactate levels in tears were high in myopia patients as compared to controls. The levels of lactate in tears were increased in the various degrees of myopia patients as compared to the controls which was directly proportional to the degree of myopia. On intragroup comparison, there was statistically significant difference in the lactate values in low versus high degree of myopia (p-value <0.05).

Fullard RJ and Carney LG first suggested the use of lactate as a prognostic tool [16]. Van Haeringen NJ studied composition of tear fluid and found levels of lactate ranging from 1-5 mM/L in tears which were elevated than the normal blood (0.5-0.8 mM/L), whereas levels of pyruvate were from 0.05-0.35 mM/L, about the same as is normal for blood (0.1-0.2 mM/L). Mechanical irritation with filter paper strips was done and no significant changes were noticed in these markers [11]. The present study results were in consensus with the present study, all the values of lactate were between 1-5 mM/L.

Alam F et al., also described need of point of care testing of lactate as a rapid, accurate and non invasive way for measuring oxidative stress. Various techniques like optical and electrochemical were used to detect lactate and to improve the selectivity and sensitivity, Lactate Oxidase (LOx) and Lactate Dehydrogenase (LDH) were used. Further small size, low cost, portability were other important points he reported in his study. The reference range of lactate concentration for a healthy person in blood, interstitial fluid, saliva, tear fluid, breath and sweat was given as 0.5-2 mM, 1.9-2.2 mM, 0.1-2.5 mM, 1-5 mM, 13.5-22 μM and 10-25 mM, respectively [17]. Current study results were in consensus with the present study, all the values of lactate were between 1-5 mM/L.

Shkrebets GV conducted a study for same age group (15 to 27 years) in three sets (G1,G2 and G3), such that G1 having patients of rapidly progressive high-grade myopia, G2 of slowly progressive high-grade myopia and G3 of healthy individuals (controls). The G1 and G2 patients had elevated levels of lactate and pyruvate along with the decreased activity of the antioxidant system in the tear. They concluded that lactate is an important biomarker to monitor due to its reflectiveness of the current status of the underlying oxidative stress [9]. Present study is also in consensus of above finding, additionally we grouped myopia into low, medium and high degree and found levels of lactates were increased in progression to the degree of myopia.

Revenga-Parra M et al., developed taurine and lactate biomarkers in saliva and tears for the diagnosis of different pathologies using electrochemical liquid chromatography detectors. For detection purpose, SWCNT (Single Walled Carbon Nano-Tube) based SPE (Screen Printed Electrode) was used. The electropolymerised Schiff base metallate film were used to modify the working electrode [18].

Lin CE et al., found that during intensive exercise when there is oxygen deficiency, lactate can be used as biomarker for clinical diagnostics and fitness monitoring. Lactate measured in tears suggests a non invasive and possible sample than blood sample which is painful. If sampling is done properly and measured with novel sensor for tear, which has lactate oxidase impregnated on the Schirmer's test strips, the results were found to be in the critical relevant range [10]. The present study also suggests lactate in tears as surrogate marker in various degree of myopia.

Scherer described that raised lactate levels have association with morbidity and mortality of disease [19]. The present study also found increase in the level of lactate in tears was directly proportional to the degree of myopia. Findings of similar studies have been summarised in [Table/Fig-7] [9-11,17,18].

Authors and year of study	Place of study	Sample size	Findings	Conclusion
Van Haeringen NJ [11] 1981	Amsterdam, Netherlands	Not mentioned	They found lactate levels of 1-5 mM/L in tears are elevated than the normal blood levels of 0.5-0.8 mM/L in comparision to pyruvate that were about the same as is normal for blood.	Lactate in tear can be used to evaluate status of antioxidant activity.
Revenga-Parra M et al., [18] 2017	Madrid, Spain	Not mentioned	Developed taurine and lactate biomarkers in tears and saliva.	Lactate as biomarkers in tears and saliva for the diagnosis of different pathologies.
Alam F et al., [17] 2018	Miami, United States	Not mentioned	Described need of point of care testing of lactate in a rapid, accurate and non invasive way. Various techniques like optical and electrochemical were used to detect lactate.	Defined reference range of lactate concentration for a healthy person in various biological fluids and secretions.
Shkrebets GV [9] 2010	Russia	Three sets of patients such that G1: Rapidly progressive high-grade myopia G2: Slowly progressive high-grade myopia G3: Healthy individuals (controls).	Studied the relation of myopia and glaucoma in terms of hypoxia. Rise in hypoxia index by more than 1.0 and a decrease in the lacrimal fluid content of superoxide dismutase by more than 40%, may be a predictor of glaucoma in persons with rapidly progressive high grade myopia.	Similarly, lactate in tears can also be checked to see oxidative stress.
Lin CE et al., [10] 2018	Tempe, USA	Not mentioned	Lactate measured in tears suggest a non invasive and possible sample than blood sample which is painful.	Found that in oxygen deficit state, lactate can be used as biomarker for clinical diagnostics and fitness monitoring.
Present study 2022	Rohtak, Haryana, India	25 patients each were enroled in four groups: Group 1: Low myopia, Group 2: Medium myopia Group 3: High myopia, and Group 4: Age and sex matched healthy controls	Evaluated lactate in tears of various degree of myopic patients and compared with normal controls of same age group.	Found elevated levels of lactate with increasing degrees of myopia. Poor prognostic signs with progression of disease.

[Table/Fig-7]: Various studies describing role of lactate in myopia [9-11,17,18].

Limitation(s)

Other parameters of oxidative stress like glutathione, uric acid, superoxide dismutate, if have been included in study would have provided more insight but could not be done due to finanacial limits.

CONCLUSION(S)

Lactate in tears was increased in the various degree of myopia patients as compared to the controls. The increase in the level of lactate in tears were directly proportional to the degree of myopia. Lactate values were increasing in different grade of myopia indicating the increasing hypoxic condition of the eye. Thus, lactate can be used as biomarker for clinical diagnostics and fitness monitoring. Beside this, there is significant role of oxidative stress in development of myopia and other degenerative disorders. Tear can be used as a non invasive specimen to study various oxidative and other markers for this purpose. Further studies with more oxidative markers in tears can also be compared with blood values in larger groups of patients with long-term follow-up to validate the finding.

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