Original Article

Evaluation of Tear Malate Dehydrogenase 2 in Mild Dry Eye Disease

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Abstract

Purpose: To evaluate the effect of tear malate dehydrogenase 2 on monitoring ocular surface injury in mild dry eye (DE) disease.

Methods: A total of 15 DE patients (30 eyes) with mild subjective symptoms but no ocular surface fluorescein staining signs were enrolled in this study (DE group). The control group was 15 healthy age- and sex-matched volunteers (30 eyes). All subjects were asked to fill out a DE symptoms questionnaire and take different tests including tear MDH and MDH2 activities evaluation, tear breakup time (TBUT), Schirmer I, and slit-lamp examination of the ocular surface. We investigated different changes in tear MDH and MDH2 activities in the DE group and control group, discussed the association between tear MDH2 activity and DE symptoms, and the relationship between tear MDH2 activity and diagnostic tests (Schirmer I and TBUT). We also analyzed the changes in tear MDH2 activities after the treatment with artificial tears.

Results: Tear MDH activities in the DE group and control group were 288±102 U/L and 259±112 U/L, respectively, and this difference was not statistically significant (P>0.05). The tear MDH2 activities in DE group were significantly increased compared with control group. Tear MDH2 was significantly and negatively correlated with the Schirmer's value (r=–0.733, P<0.01) and the TBUT value (r=–0.841, P<0.01). MDH2 also had a significant positive correlation with soreness symptoms (r=0.687, P < 0.01). Treatment with artificial tears relieved or eliminated all discomfort symptoms, together with a considerable decrease in MDH2 activities (P <0.01), but no significant changes in the Schirmer and the TBUT tests were observed.

Conclusion: Tear MDH2 activity can indicate ocular surface injury in mild DE patients and may be used to monitor the response to therapy. (***Eye Science 2014; 29:204–208***)

Keywords: dry eye; malate dehydrogenase; mitochondrial isoform; tear

Introduction

Dry eye (DE) is a multifactorial disease of the tear film that results in epithelial cell damage and disruption of the normal homeostasis at the ocular surface[1]. The rising number of video terminal users, rapid pace of life, sleeping insufficiency, and increasing use of corneal lenses has increased the number of patients with DE, and the age distribution has become younger. The prevalence of DE in China is about 21%-30% in recent years[2]. Tear hyperosmolarity causes damage to the ocular surface epithelium[3], and tear osmolarity has been reported to be highly specific and sensitive for identifying DE[4,5]. However, due to the particular instrument requirement, clinical application of tear osmolarity is limited.

Malate dehydrogenase (MDH) catalyzes the reversible oxidation of malate into oxaloacetate, utilizing the NAD^+/NADH cofactor system. MDH2, the mitochondrial isoform of MDH[6], is responsible for intracellular signal transduction, energy metabolism, and exchange of reducing equivalents[7]. Tear MDH is recognized as a membrane damage marker derived from injured corneal and conjunctival epithelium, and particularly from injured corneal epithelium[8,9] so MDH2 activity can indicate the degree of corneal injury in external eye diseases[10]. However, whether a change in tear MDH2 activity occurs in mild DE patients is not clear. The aim of the present study was

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to evaluate the benefits of MDH2 measurement on monitoring ocular surface injury in mild DE patients.

Material and methods

Subjects

A total of 30 participants including 15 mild DE patients (Chinese Classification[2] of DE, 2013) (DE group) and 15 normal controls(control group) in the First Affiliated Hospital of Chinese PLA General Hospital and Chongqing Southwest Hospital from June 2010 to May 2013 were enrolled in this study. All participants gave their informed consent prior to the start of the study. The study was approved by our institution ethics committee.

The inclusion criteria[2] for mild DE patients for this study were as follows: (1) at least one of several mild symptoms (dryness, soreness, grittiness, blurring, redness, ocular fatigue or other), (2)TBUT ≤5sec; Schirmer I test ≤5 mm for 5 minutes without anesthesia, (3) negative corneal and conjunctival fluorescein staining by slit-lamp microscope examination. Exclusion criteria included any previous or present ocular disease other than DE, contact lens wear, and taking topical medications or ocular surface surgery.

Fifteen healthy age- and sex-matched volunteers were enrolled from the persons presenting to the ophthalmology department for routine eye examinations. These subjects had no DE symptoms, were not under any medication, had no history of autoimmune and ophthalmic disorders, and were not contact lens users. These healthy volunteers had TBUT, corneal staining, conjunctival staining, and Schirmer I test results within normal limits.

In all participants, artificial tears (Tears Natural II, Alcon Co.) were administered routinely four times a day for 2 weeks.

Tear collection

A minimum of 5-7 micro liters (μl) of unstimulated tears were collected from the external canthus of the eyes using 10 μl calibrated glass micropipette, in order to avoid additional tear reflex as much as possible. The samples were stored at room temperature until used for analysis.

Diagnostic tests of DE and determination of tear MDH and MDH2 activities

TBUT test

With the patients looking upward, fluorescein strips previously wetted with 0.9% sodium chloride were gently applied to the inferior fornix and then removed. The patients were directed to blink three times, and then look straight forward without blinking. The time lapse between the last blink and appearance of the first break in the tear film was measured, and the mean of three measurements was recorded[11].

Schirmer I test

The Schirmer I test was performed five minutes after the TBUT test. A 5×35 mm filter paper strip was placed at the junction of the middle and lateral thirds of the lower eyelid (without anesthesia). The patients were directed to look forward and to blink normally during the test, and then wetting of the filter paper was recorded after 5 min.

Tear MDH and MDH2 activities

Tear MDH activities were estimated with an autoanalyzer (Hitachi 7600, Japan) according to the method of Wong et al using L-malate as substrate[12]. Reagent 1 contained 30 mmol/L L-malate and 0.1mmol/L sodium pyrophosphate-EDTA buffer (pH 9.2). Reagent 2 contained 4 mmol/L NAD+. The generated velocity of NADH was in direct proportion to the activity of MDH. Tear MDH isoforms were determined by cellulose acetate electrophoresis, using a densitometer (Model CDS 200, USA) to measure fluorescence, based on the revised method of Gopcevic et al[13].

Statistical analysis

Statistical analysis was assessed with SPSS software package, version 13.0 (SPSS Inc., USA). Data were expressed as mean±standard deviation. Significant differences between the groups were determined by using Student's *t*-test. The correlation analysis between tear MDH2 activity and TBUT and Schirmer I test was performed by the Pearson correlation analysis. Spearman's rank correlation was done in analyzing relationships between tear MDH2 activity and DE symptoms. A *P* value less than 0.05 was considered as statistically significant.

Results

Characteristics of the study population

In this study, patients aged from 40 to 63 (medi-

an age 48.2)including 7 males and 8 females. Healthy volunteers aged from 41 to 65 (median age 47.5) including 7 males and 8 females. The most commonly reported symptom of patients in this study was dryness(86.7%),followed by grittiness (73.3%), soreness (63.3%), redness (13.3%), and ocular fatigue (6.7%). Overall, 63.3% of patients reported the occurrence of at least 1 or 2 symptoms, and 36.7% of patients reported the occurrence of at least 3 symptoms (Table 1).

Table 1 Characteristics of the study group

Characteristics	DE group	Control group
Total (eyes)	15(30)	15(30)
Age (years)	48.2±6.8	47.5±6.7
Sex (n(%))		
Male	7(46.67)	7(46.67)
Female	8(53.33)	8(53.33)
TBUT(s)	3.53±0.94	11.09±1.04
Schirmer I (mm)	4.20±0.85	12.73±1.62
Ocular surface	fluorescein staining (-)	fluorescein staining (-)
Symptoms(eye(%))		
<3	19(63.3)	0
≥3	11(36.7)	0

Activities of tear MDH and MDH2

The patients in DE group had slightly higher activities of tear MDH (288±102U/L) when compared to the control group (259±112U/L), but variations were not significant (P>0.05)(Table 2).

The profile of tear MDH isoforms showed the presence of both isoforms (MDH2 and MDH1). In the control group, the dominant MDH isoform was MDH1 with a range of 80.2% to 98.1%; MDH2 activity was low (8.67±6.8%). Our results showed significantly increased tear MDH2 activities in the DE group (20.9±0.84%) compared to the control group (P<0.01)(Figure 1).

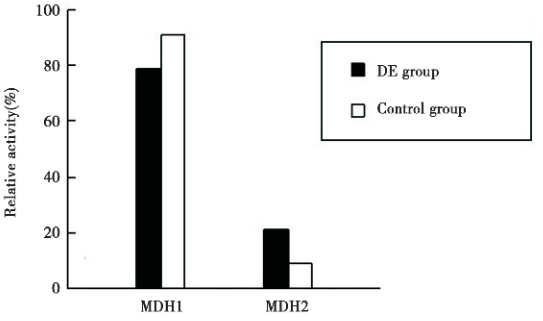

Figure 1 Relative activities of tear MDH isoforms

After the treatment with artificial tears (Tears Natural II, Alcon Co.), all discomfort symptoms in DE group were relieved or disappeared, together with a significant decrease in MDH2 activities (P<0.01). Two weeks after treatment, the tear MDH2 activities were higher than the normal value. In the DE group, the TBUT value had an increased tendency after treatment, although the changes were not significant, but same tendency of the Schirmer's value was not observed (Table 2).

Correlation analysis of tear MDH2 activities with symptoms and diagnostic tests

Correlation analysis was performed between tear MDH2 activities and DE symptoms. The relation between soreness and tear MDH2 activities approached significance (r=0.69, P<0.05). Table 3 shows that tear MDH2 activities showed no significant correlation with other symptoms. Tear MDH2 showed a significantly negative correlation with the Schirmer's value (r=0.733, P<0.01) and the TBUT value(r=-0.841, P<0.01).

Table 2 Changes in tear MDH activities, MDH2 activities, and clinical test parameters

Groups	Eyes	MDH	MDH2(s)			TBUT(s)			Schirmer I (mm)		
			BT	AT	P	BT	AT	P	BT	AT	P
DE group	30	288±102	20.9±0.84	16.7±0.64	<0.01	3.53±0.94	4.03±1.6	>0.05	4.20±0.0.85	4.00±1.65	>0.05
C group	30	259±112	8.67±6.8	8.07±5.7	>0.05	11.09±1.04	11.59±1.24	>0.05	12.73±1.62	12.63±1.5	>0.05
P		>0.05	<0.01	<0.01		<0.01	<0.01		<0.01	<0.01	

Abbr. DE group =dry eye group; C group=control group; BT=before treatment; AT=after treatment

Discussion

Subjective symptoms and objective signs are both important in the diagnosis of dry eye, with the symptom assessment playing a critical role[14]. Dry eye signs and symptoms do not always correlate well, and some controversy currently exists over the ideal roles of signs and symptoms and their actual use in

Table 3 Correlation analysis of tear MDH2 activities and symptoms

Symptoms	Eyes	*r*	*P* values
Dryness	26	0.157	>0.05
Grittiness	22	0.254	>0.05
Soreness	19	0.687	<0.01
Redness	4	0.1195	>0.05
Fatigue	2	0.103	>0.05

clinical practice[15]. In mild DE disease, with mild subjective symptoms but no ocular surface fluorescein staining signs, apart from clinical sign tests, we need a marker for monitoring the ocular surface status and the response to therapy. In this study, we evaluated the potential for tear MDH2 measurements for monitoring ocular surface injury in mild DE patients, and explored the association between tear MDH2 and DE symptoms, tear production, and tear film stability.

The tears of mild DE patients had increased MDH activities compared to normal tears, but the difference was not statistically significant. This result might be related to the relatively high activity and the great fluctuation of the measured value. This great fluctuation in the measured value limits the clinical usefulness of tear MDH as a potential marker of corneal damage.

In mild DE patients, tear MDH2 activities were significantly increased compared to the normal controls. Tear MDH2 also showed a significant correlation with the Schirmer's test and the TBUT, indicating that MDH2 activity correlates with tear production and tear film stability. Two weeks after the treatment with artificial tears, all discomfort symptoms were relieved or had disappeared and MDH2 activities were significantly decreased, but these changes were observed prior to the occurrence of the significant changes in the Schirmer value and the TBUT test. We speculate that tear MDH2 activity could be used as an auxiliary marker for monitoring the response to therapy in mild DE patients. However, two weeks after treatment, MDH2 activities were higher than the normal value, which indicated that ocular surface damage was not completely recovered. The TBUT value had an increased tendency after treatment, but the changes were not significant, which might be related to the therapy time and sample size. We found that the reduction in patient symptoms with treatment was not consistent with improvements in the Schirmer's test, which was also shown in a report by Nichols et al[15]. The reasons for this were probably related to the variability of the Schirmer's test measurement despite of the reasons above. Thus, compared to the Schirmer's and TBUT tests, MDH2 may be a more sensitive indicator for monitoring the response to therapy; this needs to be further investigated.

This study showed that the most commonly reported symptom of patients was dryness, followed by grittiness and soreness. Dryness as the most frequent symptom agrees with the findings of other studies[16,17]. Tear MDH2 activity did not show any significant correlation with dryness and grittiness symptoms, perhaps because of the limited number of patients enrolled in this study. Enríquez-de-Salamanca reported[18] that tear interleukin (IL)-6 and IL-8 had significant correlations with pain sensation scores, and that these cytokines may play a role in pain by direct action upon nerve endings, ion channels, and through other pain mediators. Lam et al[19] proposed that tear IL-6 levels can be related to inflammation-induced hyperalgesia, which may be the cause for irritation symptoms in DE patients. In this study, MDH2 had a significant positive correlation with soreness, which might indicate that MDH2 may induce the response to inflammatory mediators acting on nerve endings.

MDH2 plays an important role in the Krebs cycle for energy metabolism[20,21] and it is sensitive to changes in the metabolic status of its parent tissues. As such, this enzyme is one of the important membrane damage markers. Li reported that MDH2 could play an essential role in protecting corneal cells from ultraviolet B (UVB) irradiation, as MDH2 was overex pressed in UVB-irradiated corneal cells[7]. MDH2 catalyzes malate oxidation in the Krebs cycle and generates NADPH in the malate-aspartate shuttle; thus, the generated NADPH might reduce the accumulation of reactive oxygen species in corneal cells and protect these damaged cells from death. We speculated that the elevation of tear MDH2 activity in mild DE patients correlated with MDH2 overexpress in corneal cells.

In dry eye conditions, conjunctival impression cytology provides information regarding conjunctival cell changes[22], and is thus ideal for studying conjunctival surface epithelium rather than basal epithelium or the basement membrane[23]. However, collecting corneal samples to provide corneal cell information is difficult in clinical practice. Tear MDH2 therefore represents a promising tool for follow-up of the ocular surface, and particularly corneal status.

In conclusion, for mild DE patients, tear MDH2 activities can indicate the extent of surface injury and may be used to monitor the response to therapy. Further research on mRNA expression of MDH2 in DE disease is warranted.

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