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Original Article

The influence of long-term corneal contact lens wearing on the stability and quality of tear film in Qinghai

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Abstract: This study was designed to explore the influence of long-term corneal contact lens (CL) wearing on the stability and quality of tear film in Qinghai. Thirty subjects with CL over five years and thirty myopia subjects without CL as control were recruited in our study. Basic tear secretion test, tear film break-up time (TBUT), and corneal fluorescein staining were determined. For further evaluating the quality of tear film of two groups, the mucin levels including mucin 1 (MUC1) and mucin 5AC (MUC5AC) were measured by enzyme-linked immunosorbent assay (ELISA), meanwhile MUC1 and MUC5AC mRNA expression were processed by real-time polymerase chain reaction (RT-PCR) after samples were collected. Compared with control group, basic tear secretion and TBUT decreased, obvious corneal fluorescein staining presented in CL group. The mean protein quantity of MUC1 and MUC5AC in CL group (1.1612±0.2119 ng/ml, 1.6731±0.2457 ng/ml respectively) was less than that of control group (1.2369±0.1825 ng/ml and 1.7892±0.2461 ng/ml respectively), there were significant differences between the two groups (t=2.098, P=0.038; t=2.586, P=0.011 respectively). Both the mRNA expression of MUC1 and MUC5AC in the CL group (1.3944±0.1212 and 1.622±0.1988 respectively) decreased in comparison with control group (1.4630±0.1028 and 1.7056±0.1638 respectively), significant differences was observed between the two groups (t=3.343, P=0.001; t=2.514, P=0.013 respectively). These results suggest that long-term CL wearing in Qinghai might pose an impact on the stability and quality of tear film.

Keywords: Contact lens, tear film, mucin 1, mucin 5AC

Introduction

China becomes one of the countries with high prevalence of myopia, and the prevalence rate has been increasing year by year and reaches 50-78.4% among young ages [1, 2]. Contact lens (CL), which is the most common way for the correction of myopia, has been accepted by many people over the last two decades in China due to its thinness, less weight and convenience, not only corrects the refractive error, but avoids the trouble of being brought by wearing glasses and helps people achieve the cosmetic goals.

However, the disorders that caused by this application such as dry eye couldn't be underestimated. CL contributes to the chronic and progressive sub-clinical conjunctival impairment, and dry eye is one of the most common complaints. The pre-ocular tear film has three major components including lipids, aqueous phase and mucus. Abnormalities in presence and character of any of these components may affect the homeostasis of the ocular surface and induce dry eye syndrome, which is characterized by the feeling of dryness and foreign body, light phobia, and vision blur. Meanwhile the increase of the tear evaporation frequency makes the mucin protein unable cover the ocular surface evenly and therefore affects the stability of water and lipid layer adhering to the ocular surface.

Currently, CL is also widely used in Qinghai for refractive correction. Qinghai, located at the plateau area, in the north-west area of China, has an average 3000 meters altitude and prominent climate characteristics with high evaporation and low humidity, where the ozone layer is weak and the ultraviolet (UV) radiation is accordingly strong, these environment fac-

tors might contribute to the occurrence of dry eye. In term of this view point, it is necessary to evaluate the extent of the clinical changes for subjects with CL in this area.

Materials and methods

This study was performed in accordance to the tenets of the Declaration of Helsinki and was approved by the hospital ethics committee. Written informed consent was obtained from each subject after an explanation of the purpose and the procedures of the study.

Subject selection

The study was consisted of 30 CL daily wearers (eyes, n=60) over five years and 30 myopia subjects (eyes, n=60) without CL as control. Inclusion criteria included that subjects aged between 18 and 45 years with myopia, myopic spherical refractive error ranged from -2.00 D to -9.00 D. Subjects with a history of ocular or systemic disease, or ocular surgery were excluded from this study.

A survey of general medical and ophthalmologic history, slit-lamp biomicroscopy, tear film break-up time (TBUT) test, corneal fluorescein staining procedure, and Schirmer I test without anesthesia were performed.

TBUT determination

A fluorescein sodium ophthalmic strip (Tianjin Jingming New Technological Development Co, China) was put into the lower conjunctival sac without the anesthetic and the strip was removed until it was wet by tear. The subjects were advised to blink several times to ensure that the corneal surface became evenly coated with fluorescein. Tear breakup time was measured for a mean value after 3 successive measurements as the number of seconds between the last complete blink and the first visible random dry spot, as visualized at the slit lamp equipped with a cobalt blue filter. The slit lamp and filter were standardized across the studies.

Schirmer I test

The test (measured without topical anesthetic) was performed by placing a standard Schirmer test strip (Tianjin Jingming New Technological Development Co. China) at the junction of the medium and lateral third of the lower lid for 5 minutes and measuring the length of the wet portion.

Corneal fluorescein staining

After the TBUT observation, the corneal fluorescein staining was continuously detected with the cobalt blue filter. The corneal fluorescein staining pattern was graded from 0 to 3, which were scored individually as 0 (no staining), 1 (mild staining with a few scattered dots of stains), 2 (moderate staining between 1 and 3), and 3 (severe staining with confluent stains or corneal filaments).

Enzyme-linked immunosorbent assay (ELISA)

According to the method published previously [3], tears were collected from unanesthetized eyes by micropipette after instillation of 60 uL sterile water in the cul-de-sac, followed by movement of the eyes to mix the tear fluid content. Individual tear samples were centrifuged for 30 minutes at 14,000 rpm at 4°C. Expression of the mucin 1 (MUC1) and mucin 5AC (MUC5AC) was quantified utilizing ELISA assays (ELISAs; USCN-life, China). All samples were run according to manufacturer's guidelines, absorbance was measured at 450 nm using microplate reader (Thermo Fisher Scientific, America).

Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis

Samples were collected via scraping cytology of the superior and temporal conjunctiva from each eye using sterile swabs after the conjunctiva was anesthetized with 0.5% proparacaine hydrochloride (Alcon, Belgium). All samples were immediately placed in RNA-Be-Locker A solution (Sangon Biotech, China) for storage until processing.

Total RNA was extracted using an RNeasy kit (Sangon Biotech, China). RT-PCR was performed with a Green-2-Go qPCR mastermix kit (Sangon Biotech, China) according to the manufacturer's protocol. The primer pairs (MUC5AC: Forward: 5'-TCCACCATATACCGCCACAGA-3'; Reverse: 5'-TGGACCGACAGTCACTGTCAAC-3'; MUC1: Forward: 5'-GTGCCCCCTAGCAGTACCG-3'; Reverse: 5'-GACGTGCCCCTACAAGTTGG-3'; GA-

Table 1. The evaluation of Schirmer I test and TBUT in two groups ($\bar{X}$±s, n=60)

Group	Schirmer I test (mm)	TBUT (s)
CL	9.18±2.3179	8.76±2.5202
Control	10.15±2.0488	10.1667±1.9844
t	2.420	3.381
P	0.017	0.001

Note: tear film break-up time (TBUT).

Table 2. Corneal vital staining in two groups ($\bar{X}$±s, n=60)

Group	Corneal fluorescein staining		
	0	1	2
CL	24 (40.00%)	24 (40.00%)	12 (20.00%)
Control	48 (80.00%)	8 (13.33%)	4 (6.67%)
χ^2		20.000	
P		<0.001	

Note: The intensity of corneal fluorescein staining pattern was graded from 0 to 3 (0, no staining; 1, mild staining with a few scattered dots of stains; 2, moderate staining between 1 and 3; 3, severe staining with confluent stains or corneal filaments).

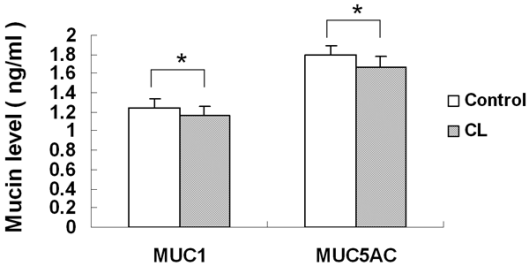

Figure 1. Mucin protein level in different groups. The comparison of mucin protein level including MUC1 and MUC5AC between CL wearing group and control group disclosed that the mucin level in CL group decreased and significant change was detected in the production of MUC1 and MUC5AC between the two groups. Statistically different data are indicated by asterisks, *represent P<0.05 compared with controls.

PDH: Forward: 5'-TCATGGGTGTGAACCATGAGAA-3', Reverse: 5'-GGCATGGACTGTGGTCATGAG-3') were used in RT-PCR. Relative gene expression was normalized by the median expression of GAPDH as a housekeeping gene.

Statistical analysis

Differences for TBUT value, *Schirmer I* test, *mucin protein level and mucin mRNA expression* between two groups were determined using independent samples *t*-test. The χ^2 test was employed to assess the proportional values in gender, age and ocular surface vital staining score between the two cohorts. For all statistical analyses, the level of significance was set at a probability of 0.05. Statistical analyses were conducted with SPSS 18.0 statistical analysis software (SPSS Inc., Chicago, IL), with data presented as mean ± SD.

Results

The two groups were matched in age (*t*=0.327, *P*=0.745) and gender (x^2=0.693, *P*=0.405).

Evaluate the stability of ocular surface

The average TBUT value for eyes in CL group (8.76±2.5202 s) was significantly shorter than control eyes (10.1667±1.9844 s), there was a statistically significant difference; the Schirmer I test result (9.18±2.3179 mm) of CL group decreased compared with control subjects (10.15±2.0488 mm), and a significant difference was showed as **Table 1** (*t*=2.420, *P*=0.017; *t*=3.381, *P*=0.001).

Ocular surface vital staining score

The mean fluorescein score of eyes in CL group was significantly higher than that of the control group. Moreover, statistically significant differences were observed between the two groups (χ^2=20.000, *P*<0.001) (**Table 2**).

Mucin protein level

Regarding the mucin protein level in two groups, the mean protein quantity of MUC 1 in CL wearers (1.1612±0.2119 ng/ml) decreased in comparison with control group (1.2369±0.1825 ng/ml), there was a significant difference between the two groups (*t*=2.098, *P*=0.038). The average protein level of MUC5AC in CL group was 1.6731±0.2457 ng/ml, which was less than that of control group (1.7892±0.2461 ng/ml, *t*=2.586, *P*=0.011) (**Figure 1**).

MUC1, MUC5AC mRNA expression

The comparison of MUC1 and MUC5AC mRNA expression between CL wearing group and control subjects indicated that both the mRNA expression of MUC1 and MUC5AC in the CL group (1.3944±0.1212 and 1.622±0.1988

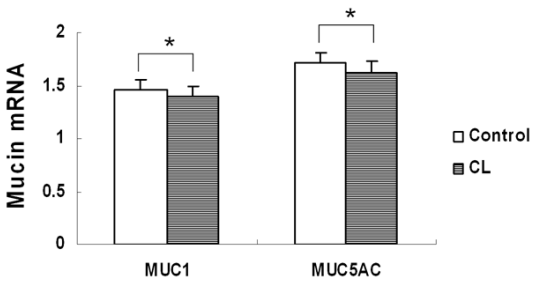

Figure 2. MUC1 and MUC5AC mRNA expression in different groups. Both the expression of MUC 1 and MUC5AC mRNA in eyes with CL were significantly down-regulated compared with control eyes ($P<0.01$). Statistically different data are indicated by asterisks, *represent $P<0.05$ compared with controls.

respectively) were down-regulated compared with control group (1.4630±0.1028 and 1.7056±0.1638 respectively). As demonstrated in **Figure 2**, significant changes were detected both in the expression of MUC1 ($t=3.343$, $P=0.001$) and MUC5AC ($t=2.514$, $P=0.013$) between the two groups.

Discussion

In our study, the age of majorities who accepted CL was 25-40 years old with high education level, and most of them came from cities. More young individuals prefer to choose CL as the vision correction for refractive errors since they sometimes subject to the disturbance of glasses or poor vision during work or sports, therefore the CL makes people avoid wearing glasses, at the same time helps them gain a good vision, and brings them conveniences as well. Less patients whose age over 40 years were the candidates of CL wearers as they concern much about the complications caused by CL such as dry eye, uncomfortable feeling and the potential damage to the ocular surface.

Tear film covers the ocular surface and plays integral role in maintaining the clarity of the reflective medium. The three major components of the tear film including lipid layer, aqueous layer and mucin protein layer act as a dynamic unit, dysfunction of any of these layers can result in ocular surface problem such as dry eye. Mucin layer is the innermost part of the tear film and functions as a barrier against the invasion of the micro-organism, external pathogens and foreign bodies [4]. Goblet cell is a kind of mucous gland and belongs to the conjunctiva epithelial cells, one of its main function lies in secreting gel-forming mucin MU5CAC, which is regarded as the prominent component of the mucin layer of tear film. The membrane-associated mucins including MUC1 and MUC4 come from the secretion of conjunctiva and corneal epithelial cells [5-7]. The abnormal secretion of mucin protein poses an impact on the corneal and conjunctiva, causes the uncomfortable feeling of ocular surface and even arises reduced visual acuity. Therefore, mucin layer is essential to maintain the metabolism and stability of tear film by forming a smooth interface.

The parameters in our study associated with the stability of ocular surface such as basic tear secretion and TBUT decreased. Regarding the outcome of corneal fluorescein staining, we found more obvious corneal staining presented in CL wearers compared with the control group. Additionally both the mucin (MUC1 and MUC5AC) protein level and mRNA expression exhibited lower in the CL wearer group. Our findings from the present study demonstrate that long-term CL wearing in Qinghai area has a negative impact on ocular surface.

The altitude in Qinghai ranges from 1900-4800 with the strong violet radiation. Ultraviolet radiation (UV) is classified into three types including A, B, and C categories. The ozone layer is a thin layer in the atmosphere that protects creatures from harmful UV rays. The thickness of ozone layer at high altitude is even thinner, so more UV rays get through to Earth's surface. Ocular surface including tear film is the first barrier between the out environment and eye, and therefore can be influenced by UV rays sensitively. Problems of ocular surface such as ptergium and dry eye caused by strong violet radiation, dry climate condition, more evaporation and hypoxia have been reported [8, 9]. The detail mechanism involved in the alteration of the stability of the tear film and the expression of mucin protein in CL wearers in our study is uncertain, whereas, the potential mechanisms by which this occurs including increased tear evaporation, inflammatory reaction, and increased osmolarity might be presumed.

Additionally, 80% oxygen that the corneal needs originates from the tear film. Oxygen is an indispensible part among nutritions that corneal

requires to perform its normal function and maintain its transparency. CL could be a barrier to oxygen transportation into the eye and produces a mechanical press on corneal, accordingly, the presence of CL reduces the biochemical changes across the ocular surface and alters the structure of tear film as well as evaporation rate [10]. Corneal hypoxia due to CL could increase susceptibility of microbial invasion [11]. Additionally, CL also has an impact on the tear film lipid layer which forms a thinner pre-lens lipid layer, increases lipid degradation, and leads to the alteration of certain lipid components such as fatty acids and cholesterol esters [12]. Consequently, without the enough protection of lipid layer, the aqueous layer evaporates quickly, which may influence the role of lipid in maintaining the structural stability of the tear film.

In our study, the instability of the ocular surface together with the decrease of mucin gene secretion implies that environmental factors such as hypoxia, UV radiation, high evaporation and low humidity in Qinghai may slow the metabolism of ocular surface. The disturbance of the stability of ocular surface in turn reduces the secretion of mucin protein, mucin layer can therefore not form regularly. Thus, wearing CL at such area may exacerbate this scenario.

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Disclosure of conflict of interest

None.

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