·Clinical Research·

Comparative study of bacterial status from conjunctival sac of the elder Qiang minority and Han people with dry eye in Sichuan, China

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Abstract

• AIM: To compare the status of bacteria in the conjunctival sac from the elder Qiang minority and Han people with dry eyes in Sichuan, China.

• METHODS: Total of 54 elder Qiang people with dry eyes (108 eyes) were examined by cluster sampling. In the similar habitation region of Han people, 80 (160 dry eyes) Han people were analyzed as the control group. The bacteria was separated from the inferior palpebral conjunctiva, then inoculated on blood plate for 48 hours and identified.

• RESULTS: Totally 24 strains of bacteria were cultured in either Qiang minority or Han c populations with 3 strains of them existed in both ethnic groups. The commonest bacteria in conjunctival sac in two ethnic groups were non-pathogenic bacterium. The composition of *Corynebacterium* in Han people (54.1%) was significantly higher than that in Qiang minority (27.4%) (χ^2=11.6721, P=0.0006). The percentage of *Sphingomonas Paucimobilis* in Qiang people was higher than that in Han people (χ^2=18.6442, P=0.0000). However, there was no significant difference between Qiang minority and Han people either in bacterial positive rate in conjunctival sac, or the composition of bacteria species and strains, or the composition of *staphylococcus epidemids* between two ethnic populations.

• CONCLUSION:There was no significant difference of bacterial positive rate in conjunctival sac from the elder of Qiang minority and Han people with dry eye, but the species of bacteria were different.

• KEYWORDS: conjunctival sac; bacteria; dry eye
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INTRODUCTION

Dry eye is a common disease in the middle age and elder people, especially in the elder women who is susceptible to the environment factors. There are some normal flora existed in the conjunctival sac, which is exposed to the outside and susceptible to environmental factors. Few reports in china can be found about the relationship between dry eye and conjunctival sac, and there is no report on the bacteria status from the conjunctival sac of ethnic minority people in China, neither from ethnic Qiang minority (one of the 55 minorities in China). Qiang minority with a population of about 200 000 are mainly in the upper reaches of Minjiang River in Sichuan Aba Tibetan and Qiang Autonomous region (including Prefecture of Maoxian, Wenchuan, Li County, Black water, Songpan) and Beichuan Qiang Autonomous County in Mianyang City. Among those areas, Beichuan Qiang Autonomous County in Mianyang City is the largest region for the Qiang minority settlements.

It is unknown whether the different ethnic people with dry eye will exhibit a different bacteria state in the conjunctival sac or not. The purpose of the study is to compare the bacteria in the conjunctival sac between the elder Qiang minority and Han population with dry eye in Mianyang city, Sichuan, China. However, as in Qiang minority area the proportion of Han people is quite low, we have to select the

Han people in Fucheng district of Mianyang city, for it is the nearest area to Beichuan Qiang Autonomous County and shows a similar geographic and environment condition to Beichuan. In this study, the bacteria in the conjunctival sac from the elder people of Qiang minority and Han were compared.

MATERIALS AND METHODS

Subjects In the early November, 2009, the cluster random sampling method was used in Beichuan Qiang Autonomous County and Mianyang Fucheng District. The elder Qiang minority (over 40 years old) in Beichuan Qiang Autonomous County, Mianyang city was surveyed and compared with the elder Han people with dry eye in Fucheng District, Mianyang city.

Methods

Sampling methods Before the survey, we got the full supports from local governments and correlated constitutions. The standard training was given for one week to the team how to carry on the saccus conjunctiva secretion selection vaccination by the specialist from laboratory medicine department. The pilot study was conducted outside the study area. Totally 216 individuals from another county nearby were examined and the results were excluded from this study. Pilot testing showed that the questionnaire was easily administered, understandable,and culturally acceptable. A team of ophthalmologists, administrative personnel, and enumerators from the villages and the medical workers of the township clinics (they also acted as translators) performed the data collection. The team was equipped with two vehicles, Snellen E chart, two slit lamp microscopes (66 Vision-Tech Co., Suzhou, YZ-5E, Crane, China), a binocular indirect ophthalmoscope (Keeler, Windsor, UK) with a Volk 20D lens, a Perkins hand-held tonometer (Clement-Clarke Ltd., Essex, UK), and two power generators for locations without electricity.

The questionnaire of Lü *et al* [1] was used in this study. A door to door visit was made by the team and a questionnaire was completed about demographic data (name, age, gender, locality, economic situation, medical history, and education), lifestyle (smoking, alcohol intake), and dry eye symptoms. The questions regarding dry-eye symptoms consisted of six items: (1) Did your eyes ever feel dry? (2) Did you ever feel a gritty or sandy sensation in your eyes? (3) Did your eyes every have a burning sensation? (4) Were your eyes ever red? (5) Did you notice much crusting on your lashes? (6) Did your eyes ever get stuck shut?

Participants who reported one or more of the six dry eye symptoms often or all the time were identified.

Dry eye criteria and eye examination Criterion of dry eye [2] includes: Patient's symptom of dry eye, positive Schirmer I test (5mm/5min); positive tear-film breakup time test (BUT) (<10s); positive corneal fluorescent staining score (FSS) (score>1). The patient was diagnosed as dry eye with any of the above symptoms, two positive tests and medical history.

The participants underwent eye examinations, which included measurement of best-corrected visual acuity, observation for signs of dry eyes, slit-lamp assessment of anterior segment and lens, and fundus evaluation. Ophthalmologists conducted the eye examinations according to a standardized protocol. The dry eye examinations included BUT, FSS, Schirmer test (ST) and slit-lamp assessment of anterior segment. The ophthalmologists performing the eye examinations were masked as to dry eye information from the questionnaire. All the surveyed participants had no history of topical use of antibiotics eye drops within three days.

Schirmer Ⅰ test A 5mm ×35mm filter strip (Tianjin Jingming New Technological Development Co., Ltd., Tianjin, China, range of wetting was from 0-30mm) was then placed temporally in both eyes for 5 minutes. The participant was encouraged to close his/her eyes but allowed to blink normally. After 5 minutes, the strip was removed, and the amount of wetting was recorded. Tear soaked filter paper wet length <10mm, suspected positive for dry eye; <5mm is positive.

BUT BUT was measured by applying a slightly moistened fluorescent strip to the inferior fornix while the participant looked up. The participant was asked to blink several times and then hold the eyes open. The tear film was then examined with the aid of the cobalt blue filter on the slit lamp while the participant refrains from blinking. The time that elapses before the first dry spot or line appears in the corneal fluorescent layer is the tear film break up time. BUT was repeated three times for each eye, and the average time was recorded.

FSS The fluorescent sodium paper was placed in the conjunctival sac and the corneal staining situation was examined under the slit-lamp. The grading method was stipulated as 0: no staining; 1:1-5 spots staining; 2:5-10 spots staining; 3: more than 10 spots staining in each quadrant. And each quadrant staining was classified moderate, medium and severe 3 level.

Conjunctival secretion collection and culture With the platinum wire loops, the conjunctival secretion was taken to inoculate on the blood agar culture medium (An Tulv Bio-engineering Limited Company, Zhengzhou, China). The secretion was cultured at 35℃ for 48 hours. If the microorganism was found from the culture, then it was further vaccinated and separated by blood agar culture medium. The detection and identification of microorganism was done in the lab department of Sichuan Provincial People's Hospital through VITEK2-compact automatic microorganism analyzer, on the Egyptian API appraisal board.

Statistical Analysis The collected data were entered into a computer processing system by designated professionals performing the same tasks to ensure consistency. SPSS Version15.0 (Statistical Package for Social Sciences, Inc,

Chicago, Illinois, USA) was used for statistical analysis. $P<0.05$ was considered to be statistically significant. The positive rate and the composition were compared by Chi-square test. The kinds of bacteria were analyzed by Wilcoxon rank sum test.

RESULTS

Demographic Information Dry eye was diagnosed in 54 patients (108 eyes) out of 210 Qiang minority persons surveyed in this study and conjunctival secretion was cultured for bacteria. Among them, there were 17 male (34 eyes), and 37 female (74 eyes). The age of patients ranged from 41-84 years, and the average age was 60.2 years.

Dry eye was diagnosed in 80 patients (160 eyes) out of 258 Han persons surveyed in this study and conjunctival secretion was cultured for bacteria. There were 40 males (80 eyes) and 40 females (80 eyes). Age of patients ranged from 41-85 years, and the average age is 63.7 years.

Positive Conjunctival Culture Rate for Bacteria in the elder Qiang Minority and Han People with Dry Eye There was no significant difference between the positive culture rate in Qiang minority and Han people (Table 1).

Composition of Single Bacteria and Multi-bacteria in the Conjunctival Sac from Qiang Minority and Han People with Dry Eye In Qiang minority and Han people, majority were the single bacteria colony, and small proportion were multi-bacteria colony (Table 2). There was no significant difference between Qiang and Han people in the single and multi-bacteria.

Species of Bacteria Cultured from the Conjunctival Sac in Qiang Minority and Han People In Qiang minority, 12 species of bacteria were cultured, and in Han people 15 species of bacteria were found (Table 3). There was no significant difference between Qiang and Han people (Wilcoxon rank sum test).

Gram Positive and Gram Negative Composition from Qiang Minority or Han People Totally 398 strains of bacteria were isolated, and 73 strains cultured from Qiang minority, 111 strains from Han people (including 1 fungus--*candida kruseic*). Both types of organisms were isolated significantly more often in Qiang minority when compared to Han people (Table 4).

Bacterial Strains in Qiang Minority and Han People

Distribution of conjunctive bacteria in Qiang minority and Han people with dry eye *Corynebacterium,* and *Staphylococcus epidermidis* were isolated more often in both populations (Table 5).

Comparative analysis of dominant bacteria in Qiang minority and Han In both populations *Corynebacterium* species and *Staphylococcus epidermidis* were shown as the dominant bacteria, however *Sphingomonas* in Qiang minority showed a high proportion up to 17.8%, while in Han none were isolated(Table 6).

Table 1 Percentage of bacterial positive culture from conjunctiva in Qiang and Han people with dry eye

Groups	*n*	Positive	Negative	Positive rate %
Qiang	108	62	46	57.4
Han	160	98	62	61.2
Total	268	160	108	59.7

X^2= 0.396, P =0.529.

Table 2 Composition of single bacteria and multi-bacteria in the saccus conjunctival from Qiang and Han with dry eye *n*(%)

Groups	Single bacteria colony	Multi-bacteria colony	Total
Qiang	52(83.8)	10(16.2)	62
Han	86(87.7)	12(12.2)	98
Total	138(86.2)	22(17.2)	160

X^2=0.483, P= 0.487.

Table 3 The number of bacteria species from Qiang and Han

Groups	Positive eyes	Number of species
Qiang	62	12
Han	98	15

Z=-0.273. P =0.785.

Table 4 Gram positive and negative composition from Qiang minority and Han Strains(%)

Groups	*n*	G +ve	G -ve
Qiang	73	58(79.5)	15(20.5)
Han	110	103(93.6)	7(6.4)

X^2= -08347,P= 0.004.

DISCUSSION

In the previous publications we have reported the bacteria state of the conjunctival sac from patients with dry eye and normal population, which showed a similar bacteria distribution between the normal eye and dry eye in either Qiang minority or Han population, even in other ethnic population [3-5]. In this study we observed the floras of conjunctival sac from a different view by comparing conjunctival bacterial conditions of two different ethnic populations in elder patients with dry eye. We found that there were no significant difference between the elder Qiang minority and Han people with dry eye in conjunctival bacterial positive rate, composition between single strain and multiple-strains, number of bacteria species in conjunctival sac. Owing to restrictions of materials and culture method, anaerobic bacteria and most fungi could not be cultured on the blood plate. However, *candida kruseic* isolated form conjunctival sac from Han people with dry eye was found growing on blood plate, although it belongs to fungus species. It is interesting to note that being as the universally acknowledged and commonest infectious pathogens of ocular surface diseases, we only found 3 strains of *aura staphylococcus* in Han, 3 strains of *streptococcus pneumonia* in Qiang minority, 2 strains of *pseudomonas aeruginosa* in Qiang minority, and none mo-double bacilli in this study. It is rationally to postulate that these pathogenic bacteria strains were "transient flora", if those pathogenic bacteria were omitted, we could find that the bacteria in conjunctival sac from the elder Qiang minority and Han with dry eye were almost non-pathogenic

Table 5 Conjunctive bacteria distribution in Qiang minority and Han with dry eye

Bacteria	Qiang		Han	
	Strains	Percentage(%)	Strains	Percentage(%)
Corynebacterium	20	27.4	60	54.1
Staphylococcus epidermidis	24	32.9	29	26.1
Intracellular bacteria Sphingomonas	13	17.8	0	0
Staphylococcus xylose	4	5.4	0	0
Streptococcus pneumoniae	3	4.1	0	0
Proteus	0	0	4	3.6
Micrococcu	0	0	3	2.7
Micrococcus luteus	2	2.7	0	0
Aeromonas short defects	2	2.7	0	0
Wolfowitz Staphylococcus	0	0	2	1.8
Staphylococcus haemolyticus	0	0	2	1.8
Staphylococcus aureus	0	0	2	1.8
Corynebacterium diphtheria	1	1.4	1	0.9
Staphylococcus squirrel	1	1.4	0	0
Strain variation Cook	1	1.4	0	0
Markov Corynebacterium	1	1.4	0	0
A*djacent particles Streptococcus*	1	1.4	0	0
Coriolis Staphylococcus	0	0	1	0.9
Streptococcus angina	0	0	1	0.9
Road Deng aureus	0	0	1	0.9
Staphylococcus heads	0	0	1	0.9
Alcaligenes faecalis	0	0	1	0.9
Krusei	0	0	1	0.9
Total	73	100	111	100

Table 6 Dominant bacteria in Qiang minority and Han

Groups	Total positive eyes	*Corynebacterium*		*Staphylococcus epidermidis*		*Intracellular bacteria Sphingomonas*	
		Positive eyes	Positive rate	Positive eyes	Positive rate	Positive eyes	Positive rate
Qiang	73	20	27.4	24	32.9	13	17.8
Han	87	60	54.1	29	26.1	0	0
X^2		11.6721		0.6771		18.6442	
P		0.001		0.411		0.000	

The above results indicated that there were significant differences in dominant bacteria of *Corynebacterium* and *Intracellular bacteria Sphingomonas* between two populations. The positive rate of *Corynebacterium* in Qiang minority was significant higher than that in Han, Intracellular bacteria *Sphingomonas* is one of dominant bacteria in Qiang minority with dry eye while in Han it does not exist. There was no significant difference in *Staphylococcus epidermidis* between both populations.

bacteria. Nevertheless, it showed that both the non-pathogenic bacteria and transient flora constitute the ocular surface bacteria in patients with dry eye.

Although the non-pathogenic bacteria between the Qiang minority and Han did not show significant difference, the differences of conjunctival floras between two ethnic populations could still be seen from this study. From Table 1, the composition of gram negative bacteria from Qiang minority with dry eye was significant higher than that from Han people. Furthermore, the distribution of bacteria species was also different (Table 2). Bacteria in conjunctival sac of Qiang minority and Han people with dry eye consisted of 24 species, among which only *corynebacterium, staphylococcus epidermidis,* and *corynebacterium diphtheria bacteria* could be found in two populations, while the remaining 21 species of bacteria existed either in Han or in Qiang minority (Table 5). Additionally, through analysis of dominant bacterial strains which occupied 3/4 of conjunctival bacteria of the two ethnic populations, it revealed that *corynebacterium* was dominant in Han people with a proportion up to 54.1%, almost twice as that from Qiang minority (27.4%); meanwhile the difference of *staphylococcus* composition was not statistically significant, but it ranked first in Qiang minority as a dominant strain, while *aphingomonas*

paucimobilis was in high proportion in Qiang minority but none was found in Han people.

The differences could probably be explained by the theory of microbiology; it means microecological environment of human conjunctival sac had an ability of "microecological succession". External environment (natural and social) and host internal environment could bring genetic characteristic changes in microbial communities resulting in replacement between dominant species and non-dominant species, including pathological succession, that is, when human or animals were in a pathological state, the normal flora bacteria of some specific parts would have subsequent changes [6]. The surveyed people in this study came from close administrative regions with similar geographical locations and climates, which ensured the comparison was homogeneous in location and geography. Thereby, the differences between the two groups could be speculated from the differences of living environments, living habits, or probable instinct genetic characteristics of different ethnic groups. Taking *sphingomonas paucimobilis* as an example, *pseudomonas paucimobilis* was reclassified into *sphingomonas paucimobilis* when *sphingomonas* was newly established according to 16S rRNA sequence analysis, cells containing special components (sphingosine, coenzyme Q10, and other features by Yabuuchi *et al* [7] in 1990. These bacteria possessed a special metabolic mechanism to efficiently adjust its growth and resistance to adverse environmental changes such as tolerance to poor nutrition, cold, ultraviolet radiation; consequently they behaved in a strong vitality and distributed widely in the nature [8]. Some membranes of these strains also had polymer channels and large plasmids, which could degrade polymeric organic pollutants. The bacteria were commonly presented only in Qiang minority with dry eye but none in infectious conjunctival sac; it might be related to the difference of habitat. However it needs further investigations. This phenomenon also indicated that the interactions from natural healthy microecological environment to the human ocular surface -even to some unhealthy dry eye with a local decreased resistance (were protective and repairable.

Although coagulase-negative staphylococci such as *staphylococcus epidermidis* have been as the main pathogenic factor of ocular surface infections in recent years [9,10], it is still thought to be a widespread resident bacteria in ocular surface other than a pathogenic one [11]. It is very interesting that Lai *et al* [12] found *staphylococcus epidermidis* may produce a beneficial effect to the epidermal tissue and its lipoteichoic acid could inhibit the dermal inflammation. It is because of these normal floras of bacteria regularly settled in the ocular surface, probably through promoting microeubiosis and internal environment stability even in the dry eye that the eye could still remain in non-infected state. From this perspective, in order to maintain the normal ocular surface microenvironment, even as in the patients with dry eye, as long as no signs of infection or no ocular surgery to destroy the microenvironment, it is unnecessary to use anti-bacterial eye drops to avoid breaking ocular surface microenvironment equilibrium.

This is the first time in China to compare the bacteria state from the conjunctival sac in the elder Qiang minority and Han people with dry eye. It demonstrated the bacteria state in conjunctival sac in elder Qiang minority and Han people. In this study we could conclude that there was no significant difference between Qiang minority and Han people in either bacterial positive rate in conjunctival sac, or the composition of bacteria species and strains, or the composition of staphylococcus epidermidis between two groups, but the Gram positive or negative species of bacteria and composition were different between two ethnic populations.

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