·Clinical Research·

Comparison on conjunctival sac bacterial flora of the seniors with dry eye in Ganzi autonomous prefecture

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Abstract

• AIM: To compare the bacterial flora in palpebral conjunctiva of xerophthalmia seniors of Tibetan, Yi and Han, and analyze the differences and similarities of the bacteria.

• METHODS: The test subjects were selected from 2 Tibetan, 2 Yi and 3 Han populated places, respectively. Total 222 seniors (444 eyes) with dry eye were examined. Secretion was collected from the palpebral conjunctiva of the subjects and then inoculated onto a blood agar plate. After 48h of incubation, the bacteria were examined for the differences and similarities between different ethnics.

• RESULTS: There was no significant difference(P>0.05) of Gram stain characterization, dominant bacteria and number of the bacterial species present in oxrophthalmia patients among Tibetan, Yi and Han nationalities. The bacteria presented in all groups include *staphylococcus epidermidis*, *corynebacterium*, *micrococcus luteu*, *intracellular bacteria sphingomonas*, *pseudomonas aeruginosa*. The bacteria detected from the two of three ethnic groups were *staphylococcus aureus*, *staphylococcus haemolyticus*, *escherichia coli*, *kytococcus sedentarius*, *streptococcus angina*, *micrococcus lylae*, *and staphylococcus heads*. The incidence rate of bacteria-associated dry eye in Tibetan population was significantly lower than that of Han and Yi population.

• CONCLUSION: There is no significant difference in the bacteria flora of palpebral conjunctiva observed among dry eye elder populations of Tibetan, Yi and Han people. All of *staphylococcus epidermidis*, *corynebacterium*, *micrococcus luteu*, *intracellular bacteria sphingomonas*, *pseudomonas aeruginosa*, *staphylococcus aureus*, *staphylococcus haemolyticus*, *escherichia coli*, *kytococcus sedentarius*, *streptococcus angina*, *micrococcus lylae* and *staphylococcus heads* are common bacteria flora of the three nationalities inhibiting in this area.

• **KEYWORDS:** conjunctiva; bacteria; dry eye; Tibetan; Yi ethnic

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INTRODUCTION

Dry eye is a disease, a chronic lack of sufficient lubrication and moisture on the surface of the eye, resulted from various factors. Its symptoms include persistent dryness, scratchiness, and a burning sensation in eyes, blurred vision, instable tear film and superficial injury of eye and impair vision. Other symptom is increased tear osmotic pressure [1]. Obviously, dry eye can change the physiological and biochemical characteristics of ocular surface. A study result obtained by Solomon *et al* [2] indicates that the early prevention mechanism for superficial inflammation and injury of eyes is weakened by the change of tear quality and quantity. After compare dry eye of middle aged and senior Han people resided in Mianyang, Qiang people in Sichuan, with the healthy eye people of the same ethnic and inhibited at the same area, we found that the bacterial flora colonized in the palpebral conjunctiva of the patients with dry eye are

similar to those observed in healthy people [3]. Since dry eye is a disease easily affected by environmental factors, we studied the bacterial flora in the palpebral conjunctiva of middle aged and senior population of Tibetan, Yi and Han people inhibited in Ganzi Autonomous Prefecture, for uncovering the characteristics of bacterial flora found in the palpebral conjunctiva of dry eye patients. At the same time, we observed the bacteria flora from multi-angle analysis.

SUBJECTS AND METHODS

Subjects The residents of greater than 40 years old from two Tibetan residential areas (Guzan town and Jiulong town of Kangding County, conducted on March 2010), two Yi ethnic areas (Wanba and Sanya Yi areas of Jiulong County, conducted on March 2010) and three Han areas (Yaer, Guzan and Lucheng town of Jiulong County, conducted on October 2010) of Ganzi Autonomous Region, Sichuan, were screened, and those diagnosed as dry eye disease were selected as studying subjects. Following criteria were used for diagnosing dry eye disease [4]: symptoms, case history, results of Schirmer I test, tear break-up time (TBUT) and corneal fluorescein staining. Common symptoms of dry eye disease are lack of tear, discomfort of eye (sandy-gritty irritation), the situation get worse after prolonged periods of eye strain, and the patients have xerophthalmia, asthenopia, foreign body sensation, and so on. If positive test results are obtained from any two of above-listed diagnostic tests (Positive results identified: Schirmer I test $\leqslant$5mm/5min, TBUT $\leqslant$10s, corneal fluorescein staining $\geqslant$1), combined with the symptoms and case history, the incidence is diagnosed as dry eye. The subjects with dry eye symptoms suffering with lacrimal duct disease, acute and chronic eye infection diseases such as meibomitis-related keratoconjunctivitis and infectious keratoconjunctivitis, or current medicine use for eye treatment are excluded in the project.

Project team members All of the research team members are ophthalmologists received proper training before the survey started. All steps including subject registration, questionnaire completion, eye examination, palpebral conjunctiva secrets sampling and agar plate inoculation were conducted by designated team members. Bacterial culturing and identification were conducted by designated laboratory technicians of People's Hospital of Ganzi Autonomous Prefecture. The project manager was responsible for the quality control of entire project.

Methods

Questionnaire A slightly modified questionnaire of Lü and Qu [5] was used in this study. The contents include name, gender, age, nationality, towners or non-towners (based on the characteristics described in the data base of National Sixth Census in Ganzi Autonomous Region), case history, symptoms, signs of subject's self-description, life-style and living environment, *etc*.

Diagnosis of dry eye Visual acuity, slit-lamp examination, fundus examination, Schirmer I test, culture of the bacteria derived from palpebral conjunctiva, *etc*.

Criteria

TBUT We applied a fluorescein sodium ophthalmic strip to the inferior fornix of conjunctiva sac for several minutes. Under the slit lamp, we measured the time for tears to break up in the eye. We took the average value of total 3 measurements to evaluate the stability of one's tear film. If the value is greater than 5s and equal or smaller than 10s, tear film is unstable. If the value is equal or smaller than 5s, tear film is abnormal.

Schirmer I test A 5mm paper strip was placed (manufactured by Tianjin Jingming Company) inside the lower eyelid (conjunctival sac). The eyes closed for 5min. The paper is then removed and the amount of moisture is measured and recorded. >5mm or $\leqslant$10mm wetting of the paper after 5min means moderate dry eyes. $\leqslant$5mm wetting of the paper means severe dry eye.

Corneal fluorescein stain We placed a fluorescent sodium paper strip (manufactured by Tianjin Jingming Company) directly onto the ocular surface, then the paitents blinked several times to spread the dye around and coated the tear film covering the surface of the cornea. A slit lamp is then directed at the eye. Corneal healthy situation is assessed and graded based on the staining results of light, mediate or heavy stain in each quadrant, total 0-12 points. For example, the point for non-stain is 0, normal is <1, light stain is 1, more stain spots is 2, and blocking stain is 3.

Collecting and culturing of conjunctiva sac -derived secretes Sterilized inoculation loop was used collect a small amount of conjunctiva sac secretion and inoculated onto blood agar medium (manufactured by Antu Lvke Ltd). Then, the agar plates were kept in a specimen container (provided by the test laboratory of Ganzi Hospital) and transported at an ambient temperature (for over 24h) to Ganzi People's Hospital. Plates were examined after 48h incubation at 35℃. Negative result means that no bacterial growth was observed on the plate. The bacteria, if any, will be transferred onto another blood agar plate for isolation and purification. Finally, isolated bacteria were examined and identified using (The semi automatic microorganism analyzer Becton, Dickinson and Company, America). The bacterial ID penal used is BBL Crystal identifying Gram+ coccus, Enterobacter spp./nonfermentative bacteria. The bacteria that cannot be identified by bacterial ID penal are determined through Gram stain and NaOH stain, as well as cell phenotype observation under microscope, such as corynebacterium, bacillus subtilis and fungi.

Statistical Analysis The collected data were entered into designated computer processing system and analyzed using

Table 1 General conditions of middle aged and seniors with dry eye in Tibetan, Yi and Han nationalities

Group	Participants (*n*)	M (eyes)	F (eyes)	Age (a, $\bar{x} \pm s$)
Tibetan	78 (156)	38 (76)	40 (80)	52.77±10.90
Yi ethnic	70 (140)	36 (72)	34 (68)	54.00±11.77
Han nationality	74 (148)	38 (76)	36 (72)	53.46±10.93
Total	222 (444)	112 (224)	110 (220)	

statistical software SPSS 15.0. F-test is used to assess whether there was a significant difference in subjects' age, while χ^2-test was used to analyze the distribution of bacterial spices in the conjunctiva sac of different population, and Wilcoxon rank sum test is used for the number of bacterial species contained in the positive conjunctiva sac of each group. This research project was approved by the Medical Ethics Committee of Sichuan Provincial People's Hospital, Sichuan Provincial Academy of Medical Sciences. Before the investigation started, the subjects were informed of test methods and significance of the project, and gave their oral consent. All tests conducted are noninvasive.

RESULTS

General Information Totally 78 middle aged and seniors of Tibetan (156 eyes) with dry eye participated in this study, including 38 male (76 eyes), 40 female (80 eyes), age 40-78 (average age 52.77±10.9) years; for Yi minority, 70 middle aged and seniors (140 eyes) were involved in the study, including 36 male (72 eyes), 34 female (68 eyes), age 40-82 (average 52.8±11.77); for Han people, 74 middle aged and seniors (148 eyes) were joined in the study, including 38 male (76 eyes), 36 female (72 eyes), age 42-79 (average 53.46±10.93) years. F-test result revealed that there was no significant difference between the participants' age of three nationalities (F=0.849, P=0.429); there was no significant difference in rate of sex between different nationalities (χ^2=-0.144, P=0.930, Table 1).

Comparative analysis of positive ratio of conjunctiva sac bacteria detected in Tibetan, Yi and Han Nationalities with dry eye As illustrated in Table 2, the positive ratio of conjunctiva sac bacteria in three groups was Tibetan<Han <Yi, the data of later two groups were closer than other two groups. Therefore, Yi and Han nationalities were compared and found that there was no significant difference [$\chi^2_{(\text{Yi and Han})}$= 2.450, $P_{(\text{Yi and Han})}$=0.118]. If compare Tibetan with the combination of Han and Yi ethnic, some differences were confirmed [$\chi^2_{(\text{Tibetan-Yi and Han})}$=16.364, $P_{(\text{Tibetan and Yi - Han})}$=0.000]. This result indicates that the positive rate of conjunctiva sac bacteria in Tibetan with dry eye was higher than that of Yi and Han people.

Comparative analysis of conjunctiva sac bacteria distribution in Tibetan, Yi and Han people with dry eye Single and more than one bacteria coexist of conjunctiva sac bacteria in Tibetan, Yi and Han people with dry eye. Table 3 reveals that mainly one species of bacteria was detected in the conjunctiva sac of middle aged and senior Tibetan people with dry eye (χ^2=7.669, P=0.022). Coexisting phenomenon of conjunctiva sac bacteria in Yi minority was the least, and that of Tibetan and Han people was similar. Therefore, the composition of conjunctiva sac single bacterium growth in Tibetan and Han groups were compared first, there was no significant difference between two groups [$\chi^2_{(\text{Tibetan and Han})}$=0.078, $P_{(\text{Tibetan and Han})}$=0.7801]. When compare Yi minority with the combination of Tibetan and Han groups, result has significant statistical differences [$\chi^2_{(\text{Tibetan and Han-Yi})}$=7.561, $P_{(\text{Tibetan and Han-Yi})}$=0.006]. This result indicates that the coexist phenomenon of conjunctiva sac bacteria in Yi minority with dry eye was significantly less than that of Han and Tibetan groups (Table 3).

Table 2 Comparative analysis of the positive ratio of conjunctiva sac bacteria in Tibetan, Yi and Han people with dry eye

Group	Total (eyes)	Positive eye	Negative eye	Positive ratio (%)
Tibetan	156	75	81	48.08
Yi	140	101	39	72.14
Han	148	94	54	63.51
Total	444	270	174	60.81

Table 3 Single and more than one bacteria coexist of conjunctiva sac bacteria in Tibetan, Yi and Han people with dry eye *n* (%)

Group	Single bacterium	Coexist bacteria	Total
Tibetan	66 (88.00)	9 (12.00)	75(100)
Yi	99 (98.02)	2 (1.98)	101(100)
Han	84 (89.36)	21 (7.78)	270(100)
Total	249 (92.22)	21 (7.78)	270(100)

Analysis of the species of conjunctiva sac bacteria in Tibetan, Yi and Han people with dry eye Seven, 14 and 13 species of bacteria were isolated from 75, 101 and 94 eyes of Tibetan, Yi and Han people with positive conjunctiva sac bacteria, respectively. Due to the limitation of test condition in local hospital, each group has some bacteria unidentified. Even though the number of bacteria isolated from Tibetan people was less than other two groups, after Wilcoxon rank sum test, the number of bacterial species contained in the positive conjunctiva sac of three groups has no significant differences (H=2.332, P=0.312; Table 4).

Comparison of Gram stain characteristics of conjunctiva sac bacteria derived from Tibetan, Yi and Han people with dry eye No matter the subjects were the middle aged and seniors of Tibetan, Yi or Han people with dry eye , the majority of conjunctiva sac bacteria derived were Gram positive. The data listed in Table 5 indicates that there was no significant statistical difference between individual group (χ^2=2.096, P=0.351).

Table 4 Comparative analysis of the bacterial species detected from the conjunctiva sac of middle aged and seniors of Tibetan and Han groups with dry eye

Group	Eye with bacteria	Species identified	Non-identified bacteria
Tibetan	75	7	8
Yi	101	14	8
Han	94	13	7

Table 5 Comparison of Gram stain characteristics of conjunctiva sac bacteria derived from Tibetan and Han people with dry eye

Group	Bacteria (*n*)	G^+ *n* (%)	G^- *n* (%)
Tibetan	84	83 (98.81)	1 (1.19)
Yi	103	98 (95.15)	5 (4.85)
Han	104	101 (97.12)	3 (2.88)
Total	291	282 (96.91)	9 (3.09)

Table 6 Conjunctive bacteria distribution in Qiang minority and Han with dry eye strains(%)

Bacteria	Tibetan	Yi	Han
Staphylococcus epidermidis	39 (46.43)	39 (37.86)	46 (44.23)
Corynebacterium	27 (32.14)	32 (31.07)	33 (31.73)
Micrococcus luteus	5 (5.95)	1 (0.97)	5 (4.81)
Intracellular bacteria sphingomonas	1 (1.19)	2 (1.94)	1 (0.96)
Pseudomonas aeruginosa	1 (1.19)	1 (0.97)	1 (0.96)
Staphylococcus aureus	0 (0)	7 (6.80)	1 (0.96)
Staphylococcus haemolyticus	4 (4.76)	0 (0)	2 (1.92)
Escherichia coli	0 (0)	4 (3.88)	2 (1.92)
Kytococcus sedentarius	0 (0)	2 (1.94)	2 (1.92)
Streptococcus angina	0 (0)	2 (1.94)	1 (0.96)
Micrococcus lylae	0 (0)	1 (0.97)	1 (0.96)
Staphylococcus heads	0 (0)	1 (0.97)	1 (0.96)
Kocuria roseus	2 (2.38)	0 (0)	0 (0)
Lactococcus raffinolactis	0 (0)	2 (1.94)	0 (0)
Aerococcus urinae	0 (0)	0 (0)	1 (0.96)
Acinetobacter lwoffii	0 (0)	1 (0.97)	0 (0)
Unidentified Gram-positive bacteria	4 (4.76)	8 (7.77)	6 (5.77)
Unidentified Gram-negative bacteria	1 (1.19)	0 (0)	1 (0.96)
Total	84 (99.99)	103 (99.99)	104 (99.98)

Table 7 Comparative analysis of the dominant bacteria composition in the conjunctiva sac of middle aged and senior Tibetan and Han people with dry eye cfu (%)

Group	Total bacteria	*Corynebacterium*	*Staphylococcus epidermidis*	Other bacteria
Tibetan	84	27 (32.14)	39 (46.43)	18 (21.43)
Yi	103	32 (31.07)	39 (37.86)	32 (31.07)
Han	104	33 (31.73)	46 (44.23)	25 (24.04)
Total	291	92 (31.62)	124 (42.61)	75 (25.77)

Comparative Analysis of the Species of Conjunctiva Sac Bacteria Derived From Tibetan, Yi and Han People with Dry Eye

Composition and distribution of conjunctiva sac bacteria inhabited in Tibetan, Yi and Han people with dry eye From the data described in Table 6 it can be seen that total 16 species of the bacteria were isolated from the conjunctiva sac of Tibetan, Yi and Han people with dry eye. Only 4 of 16 species exist independently in the conjunctiva sac of one nationality, such as *kocuria roseus*, *lactococcus raffinolactis*, *acinetobacter lwoffii*, and *aerococcus urinae*. Five species including *staphylococcus epidermidis*, *corynebacterium*, *micrococcus luteu*, *intracellular bacteria sphingomonas and pseudomonas aeruginosa* were isolated from all three groups.

Comparative analysis of the dominant bacteria derived from the conjunctiva sac of Tibetan, Yi and Han people with dry eye The dominant bacteria existed in all three groups are *staphylococcus epidermidis* and *corynebacterium*. There was no significant difference in dominant bacteria between groups (χ^2=2.771, P=0.597; Table 7).

DISCUSSION

Sichuan Ganzi Tibetan Autonomous Prefecture is situated in the area bordering south-west highland along part of Tibetan, where the population density was 7 people per square kilometers, the lowest in Sichuan Province. Tibetan ethnic dominantly accounts for 78.29% of the total population. Yi ethnic mainly lives in 7 small towns of Jiulong County. Han people account for 18.24% , scattered all over different towns. There is no report on the flora status in the ocular surface from the Han and ethnic minority in China. In this study we mainly focused on the analysis of bacterial flora on the ocular surface of dry eyes, and influence factors including age, sex and living environment. All participants were middle aged or elder people, and gender ratios were similar. Majority of the participants were farmers or herdsmen. Examining, sampling and bacterial culturing process were consistently performed by one person. The

semi automatic microorganism analyzer and BBL Crystal were used for bacterial identification. Thus, the influences of gender, age, occupation, residential environment, sampling and culturing methods on the test results were minimized.

Sampling and comparing the ethnic and Han population with dry eye is important for understanding the bacteria flora in the ocular surface. A study on the conjunctival bacteria flora of inpatients conducted by Shi *et al* [6] reveals that the positive rate of conjunctival bacteria flora in different seasons varies. The incidence reaches the highest level during spring, while drops to the lowest level in winter. In our studies, Han people were not only from different Tibetan areas, but also were examined in different seasons. However, the result by Jiachu *et al* [7] did not get the conclusion that season has any influence on the bacteria flora of conjunctival sac among the Han people resided at Tibetan area. Therefore, we suspected that sampling from the Han people of three different Tibetan areas in designated two different seasons could reflect the Han condition of Tibetan area better than sampling from one settlement on March only.

Targeting Tibetan, Yi and Han people, the influencing factors, such as the lifestyle, living environment, genetic factors in different nationalities were compared. It indicated that the bacteria flora detected from the conjunctival sac of three nationalities had similarities, including the number of bacteria species detected from the infected eyes, Gram classification of the bacteria and the amount of dominant bacteria were not impacted by the differences of nationalities.

Five bacterial species were found in all nationalities examined, another seven species were observed from two nationalities. The result indicated that these 12 species of bacteria were the common bacteria existed on eye surface. In previously published two papers [8,9], we have analyzed conjunctival sac bacterial flora of dry eyes and heathy eyes in Tibetan and Yi nationalities of Ganzi Prefecture, middle aged and senior population of Han and Qiang nationalities resided in Mianyang, Sichuan province, and concluded that conjunctival sac bacterial flora of the subjects with dry eye were normal flora. Those common flora included *staphylococcus epidermidis, corynebacterium, micrococcus luteu, intracellular bacteria sphingomonas* and *pseudomonas aeruginosa*. Less common flora are *staphylococcus aureus, staphylococcus haemolyticus, escherichia coli, kytococcus sedentarius, streptococcus angina, micrococcus lylae*, and *staphylococcus heads*. Unfortunately, some bacteria in this research project were not identified into species due to the limitation of test condition. Therefore, the results of this study is incomparable to the outcomes that did in Mianyang city[3].

However, *staphylococcus epidermidis* and *corynebacterium* were observed from the conjunctival sac of all participants, while *micrococcus luteu, intracellular bacteria sphingomonas*, *staphylococcus aureus, staphylococcus haemolyticus, streptococcus angina* and *staphylococcus heads* were detected from Qiang minority and Han population. This result indicated that these bacteria were common resident flora of ocular surface. Normal flora in the habitat of origin usually are not pathogenic. After the first few years of life，the microbiota gradually colonize the mucosal and skin surfaces of the neonate and exert the greatest effect on the development of the immune system [10]. Resident microflora contains a number of components able to activate innate and adaptive immunity. Regulation of microflora composition offers the possibility to influence the development of mucosal and systemic immunity but it can play a role also in prevention and treatment of some diseases[11]. Staphylococcal species are the most frequently cultured normal inhabitants of the healthy human skin and have been hypothesized to serve a role in human health[12,13]. Lai *et al* [14] confirmed that *staphylococcus epidermidis* when inhabits on skin surface or the outermost layer of the original skin is not harmful to the body, and its lipoteichoic acid can effectively prevent the skin from inflammation. It become pathogenic only in the translocation onto foreign habitats or the place originally no bacteria colonized. Therefore, we hypothesized the large ratio of dominant bacteria on ocular surface and the regular constitution of above-mentioned normal flora built a microbial barrier that could resist allochthonous flora and maintain microeubiosis of ocular surface.

However, there still are some differences between different nationalities, for example, the ratio of the bacteria causing dry eye in Tibetan ethnic is obviously lower than that of Han people and Yi minority, the number of coexist bacteria in Yi minority is significantly less than those of Han people and Tibetan. *Staphylococcus aureus* is acknowledged as a pathogenic bacterium, and its appearance rate in Yi minority is higher than other two nationalities. In the publications of Jiachu *et al* [7] and Zhang *et al*[3], *staphylococcus aureus* was also detected from the conjunctival sac of the dry eye patients of Han people resided in Ganzi Prefecture and Mianyang City, respectively. Wang[15] compared normal flora in the conjunctival sac of general population inhabited in several countries, and found that the detection rate of *staphylococcus aureus* was relatively high. *Staphylococcus aureus* coagulase is able to convert liquid plasma fibrinogen into solid fibrin which can deposit on cell surface, hinder macrophage function, and resist host's defense mechanism, produce pollution. However, among dry eye population of Yi minority, this bacterium *staphylococcus aureus* does not cause infection. This phenomenon may be explained as follows: firstly, the micro-ecological succession existed in Yi minority, including living habits or their genetic factors, may result in *staphylococcus aureus* growth; secondly, under

balanced micro-ecological environment of ocular surface, *staphylococcus aureus* could also become a non-pathogenic bacterium.

In general, there is no significant difference in the bacteria flora of palpebral conjunctiva observed among dry eye elder populations of Tibetan, Yi and Han people. All of *staphylococcus epidermidis*, *corynebacterium*, *micrococcus luteu*, *intracellular bacteria sphingomonas*, *pseudomonas aeruginosa*, *staphylococcus aureus*, *staphylococcus haemolyticus*, *escherichia coli*, *kytococcus sedentarius*, *streptococcus angina*, *micrococcus lylae and staphylococcus heads* are common bacteria flora of the three nationalities inhibiting in this area.

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