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# Sociodemographic characteristics and risk factor analysis of *Demodex* infestation (Acari: Demodicidae)*

Ya-e ZHAO†[1], Na GUO[1], Meng XUN[1], Ji-ru XU[1], Mei WANG[2], Duo-lao WANG[3]

([1]*Department of Immunology and Pathogen Biology, Xi'an Jiaotong University College of Medicine, Xi'an 710061, China*)

([2]*Department of Dermatology, the Second Affiliated Hospital, Xi'an Jiaotong University College of Medicine, Xi'an 710004, China*)

([3]*Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK*)

†E-mail: zhaoyae@mail.xjtu.edu.cn

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**Abstract:** To identify sociodemographic characteristics and risk factor of *Demodex* infestation, 756 students aged 13–22 years in Xi'an, China were sampled for the school-based cross-sectional study. *Demodex* was examined using the cellophane tape method (CTP). The results showed that the total detection rate of *Demodex* was 67.6%. Logistic regression analysis revealed that five variables (gender, residence, sharing sanitary ware, frequency of face-wash per day, and use of facial cleanser) were found to be uncorrelated with *Demodex* infestation, whereas three variables (age, skin type, and skin disease) were found to be independent correlates. Students aged over 18 years had 22.1 times higher odds of *Demodex* infestation compared to those under 16 years and students aged 16–18 years also had 2.1 times higher odds compared to those aged 13–15 years. Odds of having a *Demodex* infestation for oily or mixed skin were 2.1 times those for dry or neutral skin. Students with a facial skin disease had 3.0 times higher odds of being infested with *Demodex* compared to those without. The inception rate of students with facial dermatoses increased in parallel with increasing mite count. The inception rates were 21.3%, 40.7%, 59.2%, and 67.7% in the negative, mild, moderate, and severe infestation groups, respectively ($\chi^2$=60.6, $P$<0.001). Specifically, the amount of infested mites and inception rate of acne vulgaris were positively correlated ($R^2$=0.57, moderate infestation odds ratio (OR)=7.1, severe infestation OR=10.3). It was concluded that *Demodex* prevalence increases with age, and *Demodex* presents in nearly all adult human. Sebaceous hyperplasia with oily or mixed skin seems to favour *Demodex* proliferation. *Demodex* infestation could be associated with acne vulgaris. The CTP is a good sampling method for studies of *Demodex* prevalence.

**Key words:** Students, *Demodex* infestation, Sociodemographic characteristics, Risk factor, Logistic regression analysis, Facial dermatosis

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## 1 Introduction

The *Demodex* mites, *Demodex folliculorum* (*D.f.*) and *Demodex brevis* (*D.b.*), are the most common permanent parasites found on humans (Desch and Nutting, 1972). These two *Demodex* species belong to the family Demodicidae of the superfamily Cheyletoidea of the subclass Acari. *D.f.* and *D.b.* are obligatory parasites in hair follicles and pilosebaceous glands. Mites have been retrieved from almost every area of human skin but have a predilection for the face. *Demodex* mites can be found in any age groups except newborns who are presumably infested soon after birth by direct contact (Bonnar *et al.*, 1991). The mite population varies with age. Some researchers reported that the infestation rate of *Demodex* could be higher than 90% in college students (Zhao *et al.*, 2006).

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*Demodex* has been associated with the development of pityriasis folliculorum, rosacea, perioral dermatitis, seborrheic dermatitis, pustular eruption, blepharitis, seborrheic alopecie, and other skin lesions although much controversy persists (Bonnar *et al.*, 1993; Forton and Seys, 1993; Damian and Rogers, 2003; Clyti *et al.*, 2005; EI Bassiouni *et al.*, 2005; Forton *et al.*, 2005; Anane *et al.*, 2007; Hsu *et al.*, 2009). Some authors consider them as mere passengers that can be practically found in normal adult skin or coincidentally in diseased skin (Kemal *et al.*, 2005; Okyay *et al.*, 2006). However, a growing number of case reports and epidemiological studies show that *Demodex* has an aversion for skin lesions of face. Those skin diseases badly deface the patients and bring them, especially the young, mental stress and psychological pain (Yu *et al.*, 2006). The symptoms can disappear under acaricidal therapy, and the skin conditions can heal. Moreover, it is also suspected that *Demodex* infestation may be one of the triggering factors of carcinogenesis in eyelid basal cell carcinomas (Erbagci *et al.*, 2003) and sebaceous adenoma (Dhingra *et al.*, 2009). The infestation of *Demodex* has therefore been recognized by many researchers as one of the important causes of skin diseases and has increasingly become a public health concern.

Fortunately, the role of *Demodex* mites as risk factors in rosaeca has been confirmed recently with odds ratio (OR)=7.57 (Zhao *et al.*, 2010). The objectives of this study were to assess the rate of *Demodex* infestation and to identify sociodemographic characteristics and risk behavior correlates of *Demodex* infestation among students aged 13 to 22 years in Xi'an, China.

## 2 Materials and methods

### 2.1 Study population

The study was an exploratory investigation and 812 students were randomly recruited using a stratified cluster random sampling method from one university and five middle schools in Xi'an, Shaanxi, China. These students, consisting of 420 university students from 21 classes, 130 senior high school students from 2 classes, and 262 junior high school students from 5 classes, were between 13 to 22 years old. The study was undertaken recently. Signed consent forms were collected from the participants after the study was fully explained to them.

### 2.2 Data collection

#### 2.2.1 Questionnaire

The information on age, gender, family address, telephone number, skin type, sanitary ware sharing (such as towel, wash-basin, soap, underwear, and bedclothes), frequency of face washing, and type of soap or cosmetics was collected in the study.

#### 2.2.2 *Demodex* examination

To reduce the omission factor, investigators, two post-graduates, were trained before the investigation, and the examining method and judgment criteria were unified. Investigators provided slides and tapes, and explained the examining method to students. Cellophane tape method (CTP) was employed for all the students. A positive diagnosis was made only after visualizing the *Demodex* mites (*D.f.* or *D.b.*) under microscope magnification.

CTP established by Li and Wang (1986), was used in our study for *Demodex* examination. The detailed steps were as follows. Firstly, wash the face with warm water. After the face dries up, paste 2 cm×5 cm cellophane tapes respectively on the forehead, the cheeks, the nose, and the chin before sleep at night. Secondly, remove the tapes from the face and press them onto the slides the next morning. Lastly, observe the tapes under an optical microscope (10×4). According to the count of mites, the degree of infestation was classified as mild (1–10 mites/5 slides), moderate (11–30 mites/5 slides) or severe (≥ 31 mites/5 slides).

The squeezing method (Chen *et al*., 1982) was only used in university students when comparing the efficiencies of the squeezing method and CTP. The steps were: squeeze nasolabial fold and wings of the nose with thumb to get some sebum, place the sebum on the slides, add a drop of liquid paraffin, place cover glass, and examine the slides under a microscope (OLYMPUS 043191).

### 2.3 Influencing factor of the detection rates

Influencing factors consist of the detection method (CTP and the squeezing method), checkpoints (the forehead, the cheeks, the nose, and the

chin), number of examination times (1, 2, 3), and whether a shower has been taken before examination. The study sample of the influencing factors was limited to university students.

### 2.4 Diagnosis of facial dermatosis

Investigators were one dermatologist and two post-graduates in the Pathogen Biology Department of Xi'an Jiaotong University College of Medicine. The latter were specially trained in the Department of Dermatology before the study. Dermatosis of the survey consisted of rosacea, acne vulgaris, blepharitis, pruritus of the external ear, and seborrheic alopecia. Primary diagnoses of facial dermatosis were made by the two post-graduates by examining signs and symptoms. Final diagnoses were made by the dermatologist, who has worked more than ten years in the Department of Dermatology of the Second Affiliated Hospital of Xi'an Jiaotong University, College of Medicine. If the same subject presented two or more diagnoses, the subject was counted separately in all his diagnoses in the questionnaire.

### 2.5 Statistical analysis

The main outcome of the study was the occurrence of *Demodex* infestation. The prevalence of *Demodex* infestation was calculated by the characteristics of participants. To control for the effect of age composition on the rate of infestation, the standardized prevalence rate was calculated. To identify the correlates of the occurrence of *Demodex* infestation, we used the logistic regression model. In addition, the receiving operating curve (ROC) for the estimated logistic regression model was used to assess the model's discriminating power. The correlation between the infectiosity of mites and the inception rate of five dermatoses was shown using a histogram. The relationship between the amount of infested mites and the inception rate of acne vulgaris was shown using a scatterplot.

## 3 Results

### 3.1 General information

In the questionnaire, 812 students of 13–22 years old were studied. In total, 756 (93.1%) participants who completed the questionnaire effectively were included in this study. The total infestation rate of *Demodex* mites was 67.6% (511/756). The infestation rate of *D.f.* was the highest (60.3%, 308/511), followed by the infestation rate of *D.b.* (30.7%, 157/511), and the mixed infestation rate was the lowest (9.0%, 46/511). The three infestation rates were significantly different ($\chi^2$=304.6, $P$<0.01).

### 3.2 Sociodemographic characteristics and logistic regression analysis

Table 1 shows sociodemographic characteristics of the students with the *Demodex* infestation and presents the prevalence rates of *Demodex* by the characteristics of the participants. Results from logistic regression analysis revealed that after controlling for covariates in the multivariate analysis, five variables (gender, residence, sharing sanitary ware, frequency of face-wash per day, and use of facial cleanser) were found to be uncorrelated with *Demodex* infestation, whereas three variables (age, skin type, and skin disease) were found to be independent correlates of *Demodex* infestation. Students aged ≥18 years had more than 20 times higher odds of *Demodex* infestation (OR=22.1, 95% confidence interval (CI) 12.5–39.1) compared to those aged 13–15 years and students aged from 16 to 18 years also had approximately two times higher odds of *Demodex* infestation (OR=2.1, 95% CI 1.2–3.5) compared to those aged from 13 to 15 years. *Demodex* infestation was more likely to attack students with oily or mixed skin (OR=2.1, 95% CI 1.4–3.2) compared to those with dry or neutral skin. Students with skin diseases had higher odds of *Demodex* infestation (OR=3.0, 95% CI 1.9–4.7) than those without skin diseases.

Fig. 1 shows the ROC curve for the final multivariate logistic regression model. The value for the area under the ROC curve was 0.86, suggesting that the estimated logistic model fitted the data very well in terms of specificity and sensitivity.

We also did exploratory interaction tests between age and type of skin, and age and presence of skin disease, but did not find any significant interactive effects.

### 3.3 Relationship between *Demodex* infestation and facial dermatosis

The inception rate of students with facial dermatoses increased in parallel with the increasing mite

**Table 1 Characteristics and results of logistic regression analysis of *Demodex* infestation (*n*=756)**

| Characteristics | Description | Distribution (%) | Prevalence of *Demodex* (%)* | OR (95% CI)** | *P*-value |
|---|---|---|---|---|---|
| Age (year) | 13~ | 31.5 | 33.6 (80/238) | 1.0 | |
| | 16~ | 15.6 | 50.9 (60/118) | 2.1 (1.2–3.5) | 0.004 |
| | 18–22 | 52.9 | 92.8 (371/400) | 22.1 (12.5–39.1) | <0.001 |
| Gender | Male | 51.3 | 58.9 (286/388) | 1.0 | |
| | Female | 48.7 | 59.0 (225/368) | 1.1 (0.7–1.6) | 0.710 |
| Residence | Urban | 43.0 | 41.0 (252/325) | 1.0 | |
| | Rural | 57.0 | 45.1 (259/431) | 1.1 (0.6–1.7) | 0.835 |
| Sharing sanitary ware | Unshared | 57.5 | 60.0 (347/435) | 1.0 | |
| | Shared | 42.5 | 57.3 (164/321) | 1.2 (0.7–1.9) | 0.551 |
| Frequency of face-wash per day | 1 | 13.0 | 58.0 (60/98) | 1.0 | |
| | 2 | 51.6 | 60.3 (257/390) | 1.1 (0.6–1.9) | 0.796 |
| | >3 | 35.4 | 58.0 (194/268) | 1.0 (0.5–1.9) | 0.971 |
| Use of facial cleanser | No | 30.3 | 60.2 (159/229) | 1.0 | |
| | Yes | 69.7 | 58.6 (352/527) | 0.7 (0.5–1.1) | 0.170 |
| Skin type | Dry/neutral | 50.5 | 54.6 (220/382) | 1.0 | |
| | Oily/mixed | 49.5 | 68.3 (291/374) | 2.1 (1.4–3.2) | <0.001 |
| Skin disease | No | 62.0 | 53.2 (266/469) | 1.0 | |
| | Yes | 38.0 | 71.2 (245/287) | 3.0 (1.9–4.7) | <0.001 |

* Prevalence of *Demodex* was standardized by age. ** OR: Odds ratio; 95% CI: 95% confidence interval

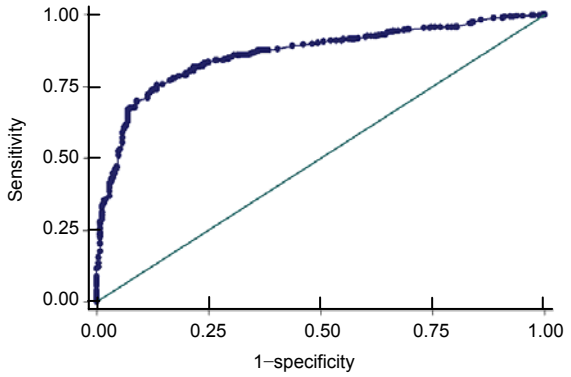


**Fig. 1 Receiving operating curve for the logistic regression analysis of *Demodex* infestation (*n*=756)**

count among the 756 students (Table 2, Fig. 2). There was a significant difference among inception rates of different groups. The inception rates were 67.7% in severe infestation group, 59.2% in moderate infestation group, 40.7% in mild infestation group, and 21.3% in negative group ($\chi^2$=60.6, $P$<0.001). The number of infested students followed the opposite pattern. Amongst 488 infested students, 378 were mildly infested, 76 were moderately infested, and 34 were severely infested. Meanwhile, *Demodex* infestation rates were significantly different between students with one of the five skin diseases and healthy students ($\chi^2$=51.3, $P$<0.001). Amongst 222 students with one of the five skin diseases, 83.8% (186/222) students had acne vulgaris. The inception rate of acne vulgaris and amount of infested mites were positively correlated, with a coefficient of determination $R^2$= 0.57 (Fig. 3). The risk of developing acne vulgaris was 3.6 times (95% CI 2.5–5.3) higher for people with *Demodex* infestation, especially in moderate infestation (OR=7.1, 95% CI 4.0–12.6) and severe infestation (OR=10.3, 95% CI 4.6–22.9).

### 3.4 Influencing factor of the detection rates of *Demodex*

The detection rate of *Demodex* of 400 university students was affected by the detection method, checkpoints, number of examinations, and whether a bath has been taken before examination (Table 3). The detection rate of CTP was much higher than that of the squeezing method. The detection rates on the cheeks were higher than that on the nose, the forehead, and the chin. The detection rate increased with the increased frequency of examination. The results also showed whether students took a bath or not before examination did not affect the detection rate.

**Table 2 Relationship between *Demodex* infestation and five skin diseases**

| Skin disease | Number of cases | | | | Infestation rate (%) |
|---|---|---|---|---|---|
| | Severe infestation | Moderate infestation | Mild infestation | Negative infestation | |
| Rosacea | 1 | 0 | 7 | 2 | 80.0 (8/10) |
| Acne vulgaris | 22 | 43 | 121 | 41 | 81.9 (186/227) |
| Blepharitis | 0 | 0 | 2 | 1 | 66.7 (2/3) |
| Pruritus of external ear | 0 | 0 | 8 | 3 | 72.7 (8/11) |
| Seborrheic alopecia | 0 | 2 | 16 | 10 | 64.3 (18/28) |
| Total | 23 | 45 | 154 | 57 | 79.6 (222/279) |
| None | 11 | 31 | 224 | 211 | 55.8 (266/477) |
| Inception rate | 67.6% (23/34) | 59.2% (45/76) | 40.7% (154/378) | 21.3% (57/268) | |

**Table 3 Influencing factors of the detection rate of *Demodex***

| Characteristics | Description | No. of subjects | No. of infestation | Prevalence (%) | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|
| Detecting method | Cellophane | 400 | 364 | 91.0 | 174.1 | <0.01 |
| | Squeezing | 400 | 136 | 34.0 | | |
| Checkpoint | Cheeks | 400 | 241 | 60.3 | 30.6 | <0.01 |
| | Nose | 400 | 202 | 50.5 | | |
| | Forehead | 400 | 197 | 49.3 | | |
| | Chin | 400 | 163 | 40.8 | | |
| Detecting times | 1 | 255 | 228 | 89.4 | 6.3 | <0.05 |
| | 2 | 255 | 236 | 92.6 | | |
| | 3 | 255 | 243 | 95.3 | | |
| Bath | Yes | 110 | 100 | 90.9 | 0.8 | >0.05 |
| | No | 290 | 271 | 93. 5 | | |

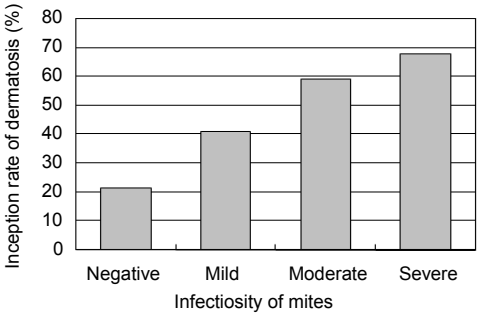


**Fig. 2 Correlation of dermatosis inception rate and infectiosity of mites**
Infectiosity of mites: negative (0); mild (1–10 mites/5 slides); moderate (11–30 mites/5 slides); severe (≥31 mites/5 slides). Inception rate of dermatosis (%): negative (21.3); mild (40.7); moderate (59.2); severe (67.7)

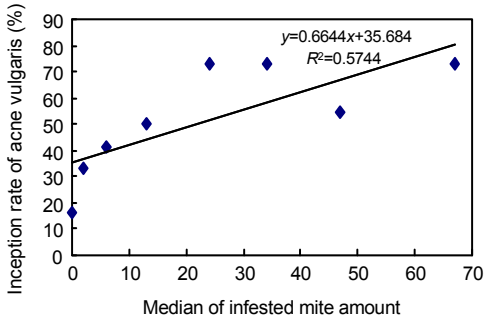


**Fig. 3 Scatterplot of the inception rate of acne vulgaris and amount of infested mites**
Amount of infested mites (median): 0 (0), 1–4 (2), 5–10 (6), 11–20 (13), 21–30 (24), 31–40 (34), 41–50 (47), 51–224 (67). Inception rate of acne vulgaris (%): 16.3, 33.1, 41.2, 50.0, 73.1, 72.7, 54.6, 72.7

## 4 Discussion

From literature searching and reviewing, we found that English literature about *Demodex* infestation mainly focused on case reports and case-control studies of skin diseases like rosacea, pityriasis folliculorum, and blepharitis, whereas few reported the sociodemographic characteristics of *Demodex* amongst the normal population. Most literature searched from MEDLINE (Meng *et al.*, 1990; Ji *et al.*, 2003; Yao, 2005; Wang and Zhang, 2006) was from mainland China, and a few were from Turkey (Aycan *et al.*, 2007; Yazar *et al.*, 2008). We found a large number of short papers (Meng *et al.*, 1990; Wang, 1990; Sun *et*

*al.*, 1997; Ji *et al.*, 2003; Yao, 2005; Wang and Zhang, 2006; Di *et al.*, 2007; Peng *et al.*, 2009) about the sociodemographic characteristics of *Demodex* in China Journal Net, although few of them conducted multivariate regression analysis. The sociodemographic characteristics and risk factor analysis of *Demodex* infestation were investigated in our study, in order to find out the major risk factor for *Demodex* infestation. The findings could provide guidance to clinic workers and help improve the diagnosis of skin diseases like rosacea, acne, blepharitis, and the treatment of *Demodex* infestation.

Study results about *Demodex* infestation differ with different investigation methods (Hou *et al.*, 1996; Nong *et al.*, 2008). The methods used to sample *Demodex* mites usually include CTP, skin scraping, skin pressurization, standard skin surface biopsy, skin biopsy, hair epilation, comedo extraction, etc.

Overall, skin biopsy (Moravvej *et al.*, 2007; Hsu *et al.*, 2009) and standard skin surface biopsy (SSSB) (Bonnar *et al.*, 1993; Forton and Seys, 1993, Forton *et al.*, 1998; 2005; Hsu *et al.*, 2009) are most frequently used in areas outside mainland China. Skin biopsy is a good method in observing damage by pathological section in depth of the skin, but is an invasive sampling method and needs local skin anaesthesia in examination. The SSSB is a convenient, quick to conduct sampling method, which analyzes the mites inside the infundibulum of the pilosebaceous follicles on 1 $cm^2$ during 1 min and appears to be an appropriate method for measuring *Demodex* density in facial skin diseases, but it is not a method to study the mite prevalence in the population (Forton, 2007). Thus the two methods are mostly applied in case reports and case-control studies, and rarely used in epidemiologic survey on the normal population.

CTP (Meng *et al.*, 1990; Wang, 1990; Sun *et al.*, 1997; Ji *et al.*, 2003; Yao, 2005; Di *et al.*, 2007; Peng *et al.*, 2009) and skin pressurization (Wang and Zhang, 2006; Cao *et al.*, 2009) are generally applied in mainland China. Skin pressurization, mostly applied in fast diagnosis of outpatients, is more convenient and quick to conduct, and there is no need to stay overnight compared with the CTP, but it is rarely used in epidemiologic surveys because it analyzes only a very small sample of the *Demodex* mites biotope with a low detection rate.

CTP is a convenient, non-invasive sampling method and makes use of the habit of *Demodex*, which moves and copulates at the follicular orifice and on the skin surface at night. It shares similar principles with SSSB, taking a superficial part of the skin, and enables comparisons to be made by different investigators. It can be reproduced and can measure *Demodex* density (Meng *et al.*, 1990). The only difference between the two detection methods is that CTP uses cellophane tape and acrylate glue, whereas SSSB uses slide and cyanoacrylate glue. Although the SSSB on 1 $cm^2$ during 1 min is probably much more sensitive than the CTP on 1 $cm^2$ during 1 min, the CTP is performed on five defined areas ($5\times10$ $cm^2$) for a night and sticks to the skin better than slides, especially on uneven areas of the face such as the nose. CTP examines a greater surface and increases the opportunity of mites moving to the skin surface. Therefore, the sensitivity of the CTP on *Demodex* prevalence is greater than SSSB. We consider the CTP as a more suitable method than others to be used in epidemiologic surveys.

Although the detection rate of *Demodex* with CTP is generally high, it can be affected by many factors (Huang and Yang, 2006), including checkpoint, area, times of examination, glutinosity of the tape, and whether or not the operation is correct. Past studies showed that detection rates on the cheeks were higher than those on the nose, the forehead and the chin (Meng *et al.*, 1990; Okyay *et al.*, 2006; Gao *et al.*, 2008). The detection rate increased with the increasing frequency of examination, number of checkpoints and areas (Wang, 2000; Zhao *et al.*, 2006). In our present study, we employed CTP (2 cm×5 cm per strip) on the cheeks, the nose, the forehead, and the chin of a subject as checkpoints for *Demodex* examination. The number of checkpoints we chose and the area of examination were larger. Moreover, this study was well designed and conducted, the omission was remarkably reduced and the compliance was very good, so the detection rate was higher than other similar studies (Hou *et al.*, 1996; Ji *et al.*, 2003; Yao, 2005; Di *et al.*, 2007; Nong *et al.*, 2008; Peng *et al.*, 2009). Therefore, the result of this investigation was expected to objectively reflect the true level of *Demodex* infestation among students aged 13 to 22 years.

Sociodemographic characteristics and logistic regression analysis results revealed that gender was

not statistically correlated with *Demodex* infestation. This result was in accordance with the conclusions of previous reports (Bonnar *et al.*, 1991; 1993; Zhao *et al.*, 2000; Wang and Zhang, 2006; Aycan *et al.*, 2007; Gao *et al.*, 2008; Liu *et al.*, 2008; Wang and Wang, 2008; Cao *et al.*, 2009; He *et al.*, 2009). Individual hygienic habits were not statistically correlated with *Demodex* infestation. This result corresponded to the conclusions of Yao (2005) and Xu and Zhang (2005), but contradicted Sun and Luo (2005), Wang and Zhang (2006), and Cheng *et al.*, (2008). One possible explanation is that washing face with soap or other kinds of facial cleanser can clean up the skin surface but may not effectively eliminate the parasite of *Demodex* in hair follicle and sebaceous glands. Furthermore, ordinary soap or other kinds of ordinary facial cleanser cannot clean off *Demodex*. Therefore, the frequency of washing face and whether using soap or other kinds of facial cleanser were not related to the reduction of *Demodex* infestation rate. In theory, sharing sanitary ware with other people can increase the chance of infestation by cross infestation, especially in a primary infected population, such as children, but the data from this study did not support the above theory.

According to the literature, the detection rate of *Demodex* infestation increased in parallel with the increase of age (Okyay *et al.*, 2006; Yang and Luo, 2006; Gao *et al.*, 2008). We had a similar result in our study. Over 90% of students above 18 years old were infected with *Demodex*. This finding was consistent with the results by Wang (1990) and Abd-El-Al *et al.* (1997). We may consider that the mite prevalence in the population have reached upper limit after 18 years old, because the skin is mature, and a great deal of follicular and sebaceous gland cells can provide *Demodex* with abundant nutrition. *Demodex* infestation could cause hyperkeratosis. Follicular orifices are easily blocked up. Therefore, large amounts of *Demodex* would parasitize and propagate in hair follicles.

This study also revealed that the detection rate in students with oily or mixed skin was much higher than that of students with dry or neutral skin. This indicated that people with oily or mixed skin were more easily infested with *Demodex* than others. This result corresponded to the conclusions of Peng *et al.* (2009), Wang and Wang (2008), and Cao *et al.* (2009). One possible explanation is that sebaceous glands in oily or mixed skin are more developed, which could provide plenty of nutrition that is suitable to the reproduction of *Demodex*. To worsen the condition, movement of chelae and claws of *Demodex* which parasitizes in pilosebaceous unit will stimulate sebaceous follicle and enhance the secretion. However, Forton *et al.* (2005) mentioned that, 30% patients of rosacea-like demodicosis subjectively claimed that they had dry skin. This was probably a false opinion. When the opisthosomas of the numerous *Demodex* mites protruding at the follicular orifice, which are easily blocked up, the sebum outflow will be reduced, so that the skin will manifest scales and the patients will feel dry skin (Crosti *et al.*, 1983). Basta-Juzbašić *et al.* (2002) also reported that *Demodex* was frequently found in patients treated with topical steroids after application of strong steroids during a long period can lead to a significant decrease in sebum excretion, probably because of the antiproliferative effect on the sebaceous cells.

The infestation rate among students with skin diseases was higher than that of students without skin diseases in our study, confirming similar results of previous reports in the literature (Purcell *et al.*, 1986; Bonnar *et al.*, 1993; Xiang *et al.*, 1993; Li, 1999; Basta-Juzbašić *et al.*, 2002; Forton *et al.*, 2005; Sun and Luo, 2005; Yao, 2005; Anane *et al.*, 2007; Aycan *et al.*, 2007; Moravvej *et al.*, 2007; Liu *et al.*, 2008; Wang and Wang, 2008; Cao *et al.*, 2009; Hsu *et al.*, 2009; Peng *et al.*, 2009). The results suggested that the *Demodex* infestation may be associated with the development of five skin diseases. When the presence of skin disease was treated as the dependent variable in the logistic regression analysis model, the presence of *Demodex* infestation became a significant correlate of skin disease, but a causal relationship could not be confirmed. We also fit a logistic model for the occurrence of *Demodex* infestation without the presence of skin disease and found that the effects of age and skin type were still statistically significant.

The inception rate increased with the mite count, confirming similar results found by Bonnar *et al.* (1993), EI Bassiouni *et al.* (2005), Moravvej *et al.* (2007) and Zhao *et al.* (2011). From Table 2, we concluded that the number of severely infested students was the fewest but the inception rate among the severely infested was the highest. The number of mildly infested students was the greatest but the

inception rate among the mildly infested was the lowest. This could explain why the mite prevalence in the population is high after 18 years old but the inception rate of *Demodex* infested patients is low. This finding indicated that the increase in the number of mites favors an inflammatory reaction, or may cause an impaired cutaneous immunological response to the parasites. However, some past literature (Meng *et al.*, 1990; Forton *et al.*, 2005; Zhang *et al.*, 2008) and our investigation also found that some people who were severely infested with *Demodex* did not show a dramatic clinical picture. Therefore, *Demodex* mite count as a sufficient aetiological condition for facial dermatosis needs to be investigated further. Mumcuoglu and Akilov (2005) reported an increased incidence of demodicidosis in patients with human leucocyte antigen (HLA) Cw2 and Cw4 alleles related to a decrease in natural killer cells. It is thus evident that HLA could play a role in resistance or susceptibility to demodicosis by regulating the end phase of the immune response.

Recently the role of *Demodex* mites as risk factors in rosacea has been confirmed (OR=7.57) (Zhao *et al.*, 2010), but the association between acne vulgaris and *Demodex* did not reach a unanimous conclusion. Acne vulgaris, the most common facial dermatosis among the young, was rarely reported to be related to *Demodex* infestation in English literature. Baysal *et al.* (1997) and Okyay *et al.* (2006) concluded that there was no connection between *Demodex* infestation and acne vulgaris, whereas much of the Chinese literature (Sun *et al.*, 1997; Yao, 2005; Di *et al.*, 2007; Wang and Wang, 2008; He *et al.*, 2009) showed a significant connection between the infestation and acne vulgaris. In our study, the inception rate of acne vulgaris and amount of infested mites were positively correlated, with a coefficient of determination $R^2$=0.57. The OR in students with acne vulgaris was 3.6, and was especially high for students moderately infested (OR=7.1) and severely infested (OR=10.3). This finding corresponds to multivariate logistic regression results of 290 clinic cases aged 14–26 years in Li *et al.* (2008) and OR of acne vulgaris and *Demodex* parasitism was 4.0. It shows that acne vulgaris could be associated with excessive *Demodex*, but the association between acne vulgaris and *Demodex* density needs to be investigated further.

Although one previous English paper with SSSB (Okyay *et al.*, 2006) concluded that there was no connection between acne vulgaris and *Demodex* infestation, the results and conclusions were doubtful because of the scarcity of English papers. We deduce that two major reasons may lead to the negative conclusion. One is that the SSSB, with a significantly smaller detected area (only 1 $cm^2$) than CTP (50 $cm^2$), has a high false negative. The other is that insufficient sample sizes may result in statistical bias. However, a more convincing association between *Demodex* density and acne vulgaris might be obtained by future studies, in which SSSB is used to measure the mite density (Forton, 2007), and a multi-checkpoint and large sample controlled study could be conducted to observe different *Demodex* densities between acne vulgaris patients and the control.

In addition, CTP can detect both *D.f.* and *D.b.* easily, whereas SSSB was rarely used for detection of *D.b.* (Forton *et al.*, 2005). Our study shows that the infestation rate of *D.f.* was the highest (60.3%, 308/511), followed by that of *D.b.* (30.7%, 157/511), and the mixed infestation rate was the lowest (9.0%, 46/511). This result was in perfect accordance with similar previous reports (Ayres, 1963; Yao, 2005; Okyay *et al.*, 2006; Aycan *et al.*, 2007; Cao *et al.*, 2009). A possible explanation for a lower detection rate of *D.b.* is that *D.b.* settles in the deeper sebaceous and meibomian glands, so it is not always possible to pick them out unless *D.b.* crawls onto the skin surface during night time.

The study has some limitations. Firstly, the results of this school-based study in Xi'an may not be representative of the entire group aged 13 to 22 years in all parts of China. Secondly, the cross-sectional design of this study means that the study may not provide direct epidemiological inference for causality and its results may be subject to unobserved confounding factors, which need further investigation.

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