Incidence of scoliosis among junior high school students in Zhongshan city, Guangdong and the possible importance of decreased miR-30e expression

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Abstract
Objective: We investigated scoliosis incidence among junior high school students in Zhongshan city, Guangdong, China and the expression of miR-30e among those with scoliosis.
Methods: A total 41,258 students were included. From July 2015 to December 2017, all students underwent screening including routine observation of the standing and sitting posture, Adam’s forward bend test, dorsal tilt angle measurement, and X-ray examination. Age, sex, height, weight, and body mass index (BMI) were recorded. Reverse transcription-quantitative polymerase chain reaction was used to assess miR-30e expression among students with scoliosis and 200 healthy students.
Results: Overall, 743 students were diagnosed with scoliosis, with an incidence rate of 1.80%. A total 646 (86.9%) students were diagnosed with idiopathic scoliosis, 38 (5.1%) with congenital scoliosis, and 59 (7.9%) with other scoliosis types. Compared with healthy students, height was significantly greater whereas weight and BMI were significantly lower among students with scoliosis and 200 healthy students.

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scoliosis, and expression of miR-30e was significantly lower. However, no significant difference was found in height, weight, BMI, and mean Cobb angle between high/low miR-30e groups.

**Conclusion:** The incidence rate for scoliosis was 1.80%. Compared with healthy students, those with scoliosis were taller, had lower weight and BMI, and miR-30e expression was significantly downregulated.

**Keywords**
Scoliosis, miR-30e, adolescents, Cobb angle, incidence, China

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**Introduction**
Scoliosis, a complex deformity of the spine, is defined as a Cobb angle >10° and vertebral rotation. Scoliosis is most commonly diagnosed as adolescent idiopathic scoliosis (AIS), which is defined as scoliosis developing between 10 years of age and the end stage of growth; the causes of AIS remain unknown. Studies have shown that nearly 3% of children age <16 years develop scoliosis. About 10% of those diagnosed with scoliosis need some form of treatment, and 0.3% to 0.5% of patients have progressive curves requiring surgery. Scoliosis can lead to a series of problems including disability, pain, and functional limitations, which result in decreased quality of life.

The mechanisms for how scoliosis develops remain unclear. In recent decades, many factors and genes have been found to be associated with scoliosis, such as leptin and BMP4. Among these factors, microRNA (miRNA), a kind of small endogenous RNA involved in many biological process and diseases, has also been found to play important roles in scoliosis. Based on our previous research in which we detected a series of miRNAs in scoliosis, we found that miR-30e might be abnormally expressed in individuals with scoliosis (unpublished); however, clinical evidence is still lacking in this regard.

In the present study, we evaluated junior high school students in Zhongshan city, Guangdong, China for the presence of scoliosis. We also investigated the expression of miR-30e in students with a diagnosis of scoliosis. Our study findings might provide additional clinical data regarding the prevalence of scoliosis as well as identifying novel study targets for the investigation of scoliosis development.

**Methods**

**Participants and measurement**
The present study included junior high school students in Zhongshan city, Guangdong. The study period was from July 2015 to December 2017. All enrolled students received routine screening, including observation of the standing and sitting posture. For students with trunk asymmetry, the Adam’s forward bend test was performed, and those with positive results underwent further testing. In these students, the dorsal tilt angle was measured and those with dorsal tilt angle ≥7° were considered positive. Finally, participants with positive results for both the Adam’s forward bend test and dorsal tilt angle test underwent X-ray examination. The diagnosis of scoliosis was made according to criteria of the Scoliosis Research Society. Students with Cobb angle >10° were considered to have scoliosis (Figure 1). Written consent was obtained from all students. The present study was approved by the...
ethics committee of ZhongShan Hospital of TCM.

Data collection and measurement

Demographic data such as age, sex, height, weight, and body mass index (BMI) were recorded. The Bjure formula was used to adjust height according to the Cobb angle. The adjusted height was calculated as measured height + \( \log^{-1}(0.011 \times X + 0.177) \), where \( X \) is defined as the maximum Cobb angle. The BMI for students with scoliosis was also calculated using adjusted height.\(^{14}\) In addition, the Cobb angle was recorded.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Blood samples were collected for all students diagnosed with scoliosis, and the expression of miR-30e was measured. Additionally, blood samples from 200 randomly selected healthy students were used as controls. To measure miR-30e expression, miRNA was extracted from serum samples using a mirVana miRNA isolation kit (Thermo Fisher Scientific, Waltham, MA, USA).\(^{15}\) RNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA

Figure 1. X-ray findings among students with scoliosis.
was converted into complementary DNA using a TaqMan MicroRNA Reverse Transcription Kit (cat no. 4440887; Thermo Fisher Scientific). Polymerase chain reaction (PCR) was performed with an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific). The following conditions were used for PCR: initial activation step at 95°C for 10 minutes and 35 cycles, denaturation for 40 cycles at 95°C for 15 seconds, annealing at 56°C for 25 seconds, and extension at 72°C for 30 seconds. U6 (cat no. 001973; Thermo Fisher Scientific) was used as an internal control. Relative miRNA levels were calculated using the $2^{-\Delta\Delta Cq}$ method.

**Statistical analysis**

All experiments were conducted in triplicate. Continuous data were expressed as mean ± standard deviation (SD). Rates were compared using the chi-square test. Comparisons between two groups were performed using the Student $t$-test. Statistical significance was defined as a P value < 0.05. All calculations were performed using IBM SPSS 20.0 (IBM Corp., Armonk, NY, USA).

**Results**

**Basic characteristics of all students and scoliosis incidence**

The present study included 41,258 junior high school students from Zhongshan city, Guangdong Province, China. A total 21,342 male and 19,916 female students were included, with mean age 13.3 ± 2.4 years. Among all participants, 2105 (5.1%) students had positive results for the Adam’s forward bend test, and 1238 (3.0%) students had positive results for the dorsal tilt angle test. Thus, 849 students who were positive in both tests underwent X-ray examination. Finally, 743 students were diagnosed as having scoliosis (Cobb angle >10°), with an incidence rate of 1.80%. Among all students with scoliosis, 646 (86.9%) were diagnosed with idiopathic scoliosis, 59 (7.9%) with congenital scoliosis, and 38 (5.1%) with other types of scoliosis including neuromuscular scoliosis and neurofibromatosis with scoliosis (Table 1).

**Body indices of students with scoliosis and healthy students**

As shown in Table 2, body indices of height, weight, and BMI were recorded for all 743 students with scoliosis and 200 healthy controls; the mean Cobb angle was calculated for participants with scoliosis. The results showed that height was significantly greater and weight was significantly lower in students with scoliosis than in healthy students (P < 0.05). BMI was also significantly lower in participants with scoliosis (P < 0.05). The mean Cobb angle was 21.2 ± 8.5° (11–74°). These results indicated that scoliosis might influence the height and weight of adolescents.

**Possible relationship of miR-30e with scoliosis**

We measured the expression of miR-30e in all students with scoliosis as well as in the
200 healthy controls. The findings showed that the expression of miR-30e was significantly lower in adolescents with scoliosis (Figure 2a, \( P = 0.000 \)). However, no significant difference was found for expression of miR-30e according to a diagnosis of idiopathic, congenital, or other types of scoliosis (Figure 2b). We then further divided students with scoliosis into high and low miR-30e groups, according to the mean value (0.290). However, no significant difference was found for height, weight, BMI, and mean Cobb angle between the two groups (Table 3), suggesting that miR-30e might be associated with the incidence of scoliosis; as yet, the underlying mechanisms of such an association remain unknown.

**Discussion**

Although many studies have demonstrated the influence of scoliosis on adolescents, epidemiologic studies of scoliosis with large sample sizes are still needed. Moreover,
very few studies have focused on the role of miRNA in the development of scoliosis. In the present study, we performed screening among 41,258 junior high school students in Zhongshan city, Guangdong, China. Our results showed a scoliosis incidence rate of 1.80%. We also demonstrated that students with scoliosis had greater height as well as lower weight and BMI than normal controls. Interestingly, we report for the first time that the expression of miR-30e was significantly downregulated in our participants with scoliosis.

The clinical significance of BMI in scoliosis has been reported by several authors. Gilbert et al.\textsuperscript{18} demonstrated a correlation between BMI and the curve magnitude in patients with scoliosis. Tarrant et al.\textsuperscript{19} showed that adolescents with idiopathic scoliosis might have low BMI. Sun et al.\textsuperscript{20} also found that lower BMI can be considered a predictive factor for bracing failure in patients with AIS. In the present study, we also found that among junior high school students in Zhongshan city, Guangdong, the BMI of those with scoliosis was significantly lower than that of healthy students, indicating that scoliosis might influence the growth of these adolescents. However, additional clinical evidence is needed to clarify understanding of this relationship.

Until now, several biomarkers or factors have been considered to be associated with the development of scoliosis. Among these, one typical factor is leptin. It has been found that patients with scoliosis might have significantly higher sOB-R levels and a lower free leptin index.\textsuperscript{21} One study also demonstrated that in individuals with AIS, the leptin receptor level was decreased and sensitivity to leptin was increased.\textsuperscript{22} It has recently been reported that miRNAs might have an important role in scoliosis. Ogura et al.\textsuperscript{11} reported that methylation of MIR4300HG, the host gene of miRNA MIR4300, was associated with progression of AIS. Garcia-Giménez et al.\textsuperscript{23} demonstrated that in AIS, differently expressed miRNAs have the potential to be used as diagnostic biomarkers. It was also reported that miR-143 and miR-491 were significantly downregulated in degenerative scoliosis and degenerative lumbar scoliosis, respectively.\textsuperscript{24,25} In 2019, Hui et al.\textsuperscript{26} analyzed miRNAs in AIS and found 2 upregulated and 42 downregulated miRNAs in bone marrow mesenchymal stem cells from patients with AIS. In our research, we demonstrated for the first time that miR-30e was downregulated in adolescents with scoliosis. However, the manner in which miR-30e influences scoliosis development requires further investigation.

In conclusion, we performed scoliosis screening among 41,258 junior high school students in Zhongshan city, Guangdong, China. We revealed an incidence rate for scoliosis of 1.80% in our study population. Students with scoliosis were taller and had lower weight and BMI than healthy students.

### Table 3. Body indices of students with scoliosis for high/low miR-30e groups.

| Variable                        | High miR-30e (n = 362) | Low miR-30e (n = 381) | P value |
|---------------------------------|------------------------|-----------------------|---------|
| Age, years (mean ± SD)          | 13.2 ± 1.5             | 13.1 ± 1.4            | 0.249   |
| Sex (male: female), n           | 139: 223               | 143: 238              |         |
| Height (adjusted), cm           | 161.2 ± 7.1            | 161.1 ± 6.8           | 0.879   |
| Weight, kg (mean ± SD)          | 44.6 ± 4.7             | 45.0 ± 5.8            | 0.336   |
| BMI (adjusted), kg/m\(^2\)      | 17.1 ± 2.2             | 17.1 ± 2.1            | 0.848   |
| Mean Cobb angle, ° (range)      | 20.7 ± 7.8° (11–74°)   | 21.6 ± 9.1° (11–70°)  | 0.127   |

BMI, body mass index.

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In addition, the expression of miR-30e was significantly downregulated in our participants with scoliosis. The findings of our study might yield useful clinical data regarding the prevalence of scoliosis as well as provide some novel study targets in further investigation of scoliosis development.

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

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