Effect of obstructive sleep apnea on carotid artery intima media thickness related to inflammation

Abstract

Purpose: Obstructive sleep apnea hypopnea syndrome (OSAHS) is an independent risk factor for atherosclerosis. To ascertain the effect of OSAHS on the development of atherosclerosis in Chinese OSAHS patients, we evaluated markers of atherosclerosis as well as vascular endothelial function and inflammation.

Methods: Chinese men with polysomnography-diagnosed OSAHS were subgrouped into mild-moderate (n = 28) and severe (n = 54) OSAHS groups on the basis of apnea hypopnea index (AHI) scores. The control group was made up of 30 healthy men. Atherosclerosis was assessed by carotid artery intima-media thickness (IMT) of both sides, flow-mediated dilation (FMD), and inflammation by interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein-1 (MCP-1) levels.

Results: Linear regression analysis was used to identify significant associations among risk factors and carotid IMT. The following parameters were significantly higher in patients with severe OSAHS than in the control group: waking triglycerides, total cholesterol, low-density lipoprotein cholesterol, apolipoprotein B, blood uric acid, blood glucose, IL-6 and hs-CRP. FMD in severe OSAHS patients was lower than in the control group. AHI score, waking hs-CRP, waking oxidized low-density lipoprotein, blood glucose, and vascular endothelial growth factor (VEGF) level were positively associated with IMT.

Conclusions: In Chinese male patients with severe OSAHS, the significantly higher carotid IMT and levels of inflammatory factors (IL-6 and hs-CRP) and lower FMD suggest that arterial endothelial damage and inflammation may play important roles in the development of atherosclerosis in OSAHS patients.
Patients with the most common sleep disorder, obstructive sleep apnea hypopnea syndrome (OSAHS), experience repeated occurrences of airflow cessation (apnea) and reduction (hypopnea) during sleep [1]. The prevalence of OSAHS is between 2% and 10% worldwide [2]. In China, OSAHS has been shown to affect 3.63% of people older than 30 years [3, 4]. Increasing evidence suggests that OSAHS is an independent risk factor for hypertension, coronary heart disease and stroke [5-7]. Because atherosclerosis has a pathophysiological basis in cardiovascular disease, it is important to understand how OSAHS and atherosclerosis may be related [8, 9].

Various studies have shown that an increase of intima-media thickness (IMT) of the common carotid artery (CCA) is an early marker of cardiovascular risk [10, 11]. Increased IMT is also a characteristic of patients with OSAHS [12, 13]. OSAHS and atherosclerosis share the same risk factors: advanced age, gender, overweight, alcohol consumption and smoking [1-6]. In addition, the intermittent hypoxia experienced in OSAHS induces oxidative stress, inflammation and endothelial damage, which are related to a higher risk of atherosclerosis [14-16]; however, it is unclear whether these factors are related to atherosclerosis in Chinese OSAHS patients. In addition, for these patients, the effect of the disease on the carotid IMT has not been fully investigated.

To clarify the effect of OSAHS on atherosclerosis, we evaluated markers of atherosclerosis, vascular endothelial function, and inflammation in Chinese patients with OSAHS. Specifically, we evaluated the IMT, flow-mediated dilation (FMD) and serum concentrations of interleukin 6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), and monocyte chemoattractant protein-1 (MCP-1).

Materials and Methods

Patients

The experimental protocol was established according to the ethical guidelines of the Helsinki Declaration and was approved by the First Hospital of China Medical University. All participants provided written informed consent for inclusion in the study. Eighty-two men (25–60 years of age) with OSAHS were recruited from the Department of Respiration from January 2009 to October 2012. The patients were diagnosed by Level I polysomnography (PSG). The severity of OSAHS was judged by the apnea hypopnea index (AHI) score, which was determined by counting the total episode of hypopnea and apnea during every hour during sleep. The minimum criteria for a diagnosis of OSAHS is AHI ≥5, and mild, moderate and severe OSAHS are defined as 5 ≤ AHI <15, 15 ≤ AHI <30, and AHI ≥30, respectively [17].

Accordingly, the OSAHS subjects were divided into two groups according to the AHI score (mild-moderate, 5 ≤ AHI <30, n = 28 and severe, AHI ≥30, n = 54). Patients with inflammatory disease, abnormal thyroid function, chronic obstructive pulmonary disease, heart failure, chronic insomnia, central sleep apnea, hypertension and diabetes were excluded. Thirty healthy men without a history of cardiac disease, atherosclerosis or systemic hypertension and having normal findings on physical examination, chest roentgenography, electrocardiography, and echocardiography, comprised the normal control group during the same period. Neck, hip and waist circumferences were measured, and the percent of persons who smoke was recorded.

Anthropometric measurements

Neck circumference was measured at the upper edge of the horizontal surface of the cricothyroid membrane perimeter. Waist circumference was measured at the midpoint between the lowest rib and the iliac crest. Hip circumference was measured at the femoral trochanter.

Polysomnography diagnosis

All patients were examined using a PSG monitoring system (Polysmith, Neurotronics, USA) for ≥7 h in the Respiration Sleep Monitoring Center. The start time of monitoring was based on patient-recorded bedtimes for the previous 3 days. Alcohol, coffee, sedatives and hypnotic drugs were forbidden on the monitoring day. No patient had suffered from a respiratory tract infection during the 2 weeks before monitoring.

The following PSG parameters were recorded: electroencephalogram, electrocardiogram, electrooculogram, oronasal distribution of respiratory airflow, chin electromyogram, snoring, posture and pulse oxygen saturation (SpO2). Hypopnea was defined as nasal airflow decreased >50% with SpO2 decreased >3% for ≥10 s, or nasal airflow decreased >40% with SpO2 decreased >4% for ≥10 s. Apnea was defined as nasal airflow decreased by >90% for ≥10 s (i.e., a cessation).

Blood sample collection and biochemical analysis

Blood samples from the antecubital vein were obtained before patients fell sleep (≥5 h after dinner) and immediately after waking up the next day (i.e., after a 12-h overnight fast). Serum
glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (ApoA), apolipoprotein B (ApoB) and blood uric acid (BUA) were determined using an automatic biochemical analyzer (Hitachi 7600, Japan). Oxidized LDL, hs-CRP, IL-6, VEGF and MCP-1 were measured with enzyme-linked immunosorbent assays, in accordance with the manufacturer’s instructions.

**Vascular endothelial function test**

A TOSHIBA 6000 color ultrasonic diagnostic system was operated using the guidelines of the International Brachial Artery Reactivity Taskforce [18] by one person who was blinded to the study. After patients had rested for at least 10 min in the supine position, an ultrasound probe was positioned at the brachial artery 2–5 cm above the elbow. The longitudinal section of brachial artery was used to show clearly both the anterior and posterior artery walls. Systolic and diastolic diameters were determined by averaging two measurements of two cardiac cycles.

**Flow-mediated dilation (FMD)**

After baseline diameters were determined by high-resolution B-mode ultrasonography with a 7.5 MHz transducer, a blood pressure cuff was placed on the forearm and inflated to 50 mmHg above the baseline systolic blood pressure for 5 min. The arterial diameter during peak hyperemia was determined within 1–3 min after release of the blood pressure cuff. The arterial diameter was also determined after 30 min of rest. The percent of FMD was determined by the following formula: (diameter during peak hyperemia – baseline diameter) / baseline diameter × 100%.

**Measurement of carotid IMT**

The carotid IMT was determined by high-resolution B-mode ultrasonography with a 7.5 MHz transducer at the CCA and proximal internal carotid arteries (i.e., bulb-ICA). The carotid IMT was defined as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. The far walls of both sides of the right and left CCAs and the carotid bifurcation were analyzed. The mean IMT was calculated as the average of six measurements (excluding sites of plaque) on the right and left sides during end diastole.

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**Statistical analyses**

Data are presented as mean ± standard deviation (SD) values. SPSS for Windows, version 11.5 (SPSS, Chicago, IL, USA) was used for all statistical analyses. The Student’s paired t-test and one-way analysis of variance were used for statistical analyses where appropriate. Multivariate correlation analysis was used to analyze the association between risk factors and carotid IMT in OSAHS patients. P < 0.05 was considered statistically significant.

**Results**

**Baseline characteristics of patients**

Compared with participants in the control and mild-moderate OSAHS groups, patients with severe OSAHS had significantly larger neck, hip and waist circumferences (all P < 0.05, Table 1). The percentage of smokers, age and BMI did not differ among the groups (P > 0.05, Table 1).

**Serum glucose and lipid levels in OSAHS patients**

Patients with severe OSAHS had significantly higher TG, TC, LDL-C, apolipoprotein B, uric acid and serum glucose levels than controls, both at bedtime and at waking (all P < 0.05, Table 2). In patients with severe OSAHS, waking HDL-C was significantly lower and waking oxidized LDL was significantly higher than in the control group (all P < 0.05, Table 2). Compared with that of patients with mild-moderate OSAHS, the waking TC level was higher in patients with severe OSAHS. Compared with that of the control group, the waking oxidized LDL level was higher in patients with mild-moderate OSAHS (P < 0.05, Table 2).

**Vascular endothelial function in OSAHS patients**

The baseline brachial artery IMT, systolic and diastolic diameters of the brachial artery during FMD also were similar in the control and patient groups, and the systolic and diastolic diameters of the brachial artery for IMT were similar (P > 0.05, Table 3). In patients with severe OSAHS, the FMD was significantly lower and the VEGF level was significantly higher than in the control group (P < 0.05, Table 3).

**Carotid IMT of OSAHS patients**

The IMTs of the CCAs in both patient groups were thicker than that in the control group (P < 0.05, Table 4). Atherosclerotic plaques were found in the CCAs in both patient groups. Compared with the control group, the carotid
TABLE 1. Baseline characteristics of the control group and OSAHS patients

<table>
<thead>
<tr>
<th>Subject, n</th>
<th>Control group*</th>
<th>Mild-moderate†</th>
<th>Severe‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>30</td>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.7 ± 6.6</td>
<td>44.9 ± 8.3</td>
<td>45.9 ± 9.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 3.6</td>
<td>27.3 ± 4.1</td>
<td>27.9 ± 4.8</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>37.01 ± 3.12</td>
<td>37.33 ± 3.78</td>
<td>39.75 ± 3.21 ab</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>103.54 ± 9.18</td>
<td>104.83 ± 7.98</td>
<td>108.88 ± 8.73 ab</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.32 ± 9.75</td>
<td>100.56 ± 8.78a</td>
<td>102.99 ± 8.62 ab</td>
</tr>
<tr>
<td>Smoking, n</td>
<td>8</td>
<td>7</td>
<td>12</td>
</tr>
</tbody>
</table>

*P < 0.05, compared with control group; †P < 0.05, compared with patients with mild-moderate OSAHS.

TABLE 2. Circadian changes in blood glucose, blood lipids, and blood uric acid

<table>
<thead>
<tr>
<th>Subject, n</th>
<th>Control group* (n=30)</th>
<th>Mild-moderate† (n=28)</th>
<th>Severe‡ (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bedtime</td>
<td>Waking</td>
<td>Bedtime</td>
</tr>
<tr>
<td>TG (μmol/L)</td>
<td>2.05 ± 0.74</td>
<td>1.94 ± 0.41</td>
<td>2.26 ± 0.73</td>
</tr>
<tr>
<td>TC (μmol/L)</td>
<td>4.53 ± 1.58</td>
<td>4.42 ± 1.47</td>
<td>4.46 ± 1.96</td>
</tr>
<tr>
<td>HDL-C (μmol/L)</td>
<td>1.21 ± 0.19</td>
<td>1.25 ± 0.24</td>
<td>1.08 ± 0.29</td>
</tr>
<tr>
<td>LDL-C (μmol/L)</td>
<td>1.73 ± 0.41</td>
<td>1.86 ± 0.39</td>
<td>2.35 ± 0.49</td>
</tr>
<tr>
<td>Apolipoprotein A (μmol/L)</td>
<td>1.23 ± 0.07</td>
<td>1.26 ± 0.12</td>
<td>1.13 ± 0.13</td>
</tr>
<tr>
<td>Apolipoprotein B (μmol/L)</td>
<td>0.63 ± 0.14</td>
<td>0.63 ± 0.18</td>
<td>0.91 ± 0.13</td>
</tr>
<tr>
<td>Uric acid, (μmol/L)</td>
<td>358.56 ± 41.67</td>
<td>362.58 ± 33.27</td>
<td>376.78 ± 45.67</td>
</tr>
<tr>
<td>Serum glucose (μmol/L)</td>
<td>5.17 ± 0.78</td>
<td>5.11 ± 0.23</td>
<td>6.87 ± 3.17</td>
</tr>
</tbody>
</table>

* n = 30; † n = 28; ‡ n = 54; a,b P < 0.05, compared with control group; c,d P < 0.05, compared with patients with mild to moderate OSAHS.
HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol.
TABLE 3. Measurements during FMD of the brachial artery

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mmHg)</th>
<th>Diameter (mm)</th>
<th>Hyperemia diameter (mm)</th>
<th>FMD diameter (%)</th>
<th>Brachial artery IMT (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Systolic</td>
<td>Diastolic</td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>136 ± 8</td>
<td>133 ± 8</td>
<td>4.14 ± 0.32</td>
<td>4.31 ± 0.43</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>89 ± 6</td>
<td>95 ± 9</td>
<td>4.13 ± 0.42</td>
<td>4.32 ± 0.32</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Systolic</td>
<td>4.54 ± 0.45</td>
<td>4.51 ± 0.52</td>
<td>4.38 ± 0.52</td>
<td>4.59 ± 0.53</td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
<td>4.69 ± 0.39</td>
<td>4.63 ± 0.47</td>
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</tbody>
</table>

* n = 30; † n = 28; ‡ n = 54; § P < 0.05, compared with the control group

TABLE 4. Baseline structural and functional measurements of the brachial artery

<table>
<thead>
<tr>
<th></th>
<th>OSAHS severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group*</td>
</tr>
<tr>
<td></td>
<td>(n=30)</td>
</tr>
<tr>
<td>IMT of CCA (mm)</td>
<td>0.73 ± 0.16</td>
</tr>
<tr>
<td>Blood flow velocity of CCA</td>
<td>1.01 ± 0.24</td>
</tr>
<tr>
<td>Plaque of CCA</td>
<td>1/30</td>
</tr>
<tr>
<td>IMT of carotid bifurcation (mm)</td>
<td>0.67 ± 0.18</td>
</tr>
<tr>
<td>Plaque of carotid bifurcation</td>
<td>1/30</td>
</tr>
</tbody>
</table>

* n = 30; † n = 28; ‡ n = 54; § P < 0.05, compared with control group.

TABLE 5. Circadian changes in IL-6, hs-CRP, MCP-1, and VEGF

<table>
<thead>
<tr>
<th></th>
<th>OSAHS severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group*</td>
</tr>
<tr>
<td></td>
<td>(n=30)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.87 ± 0.39</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>1.03 ± 0.47</td>
</tr>
<tr>
<td>MCP-1</td>
<td>31.38 ± 23.27</td>
</tr>
<tr>
<td>VEGF</td>
<td>71.43 ± 21.67</td>
</tr>
</tbody>
</table>

* n = 30; † n = 28; ‡ n = 54; § P < 0.05, compared with bedtime; † P < 0.05, compared with control group; \| P < 0.05, compared with mild-moderate OSAHS.
bifurcation IMT of patients with severe OSAHS was significantly thicker and associated with atherosclerotic plaques (P < 0.05, Table 4).

**Serum levels of IL-6, hs-CRP, and MCP-1 in OSAHS patients**

In patients with both mild-moderate and severe OSAHS, the waking serum levels of IL-6, hs-CRP, MCP-1 and VEGF were significantly higher than those at bedtime (all P < 0.05, Table 5). In patients with mild-moderate OSAHS, the waking serum levels of IL-6, hs-CRP, MCP-1 and VEGF were significantly higher than the corresponding levels in the control group (all P < 0.05, Table 5). In patients with severe OSAHS, the bedtime serum levels of IL-6, hs-CRP, MCP-1 and VEGF were significantly higher than the corresponding bedtime levels in the control group, whereas the corresponding waking levels in the severe OSAHS group were significantly higher than those in either the control group or the mild-moderate OSAHS group (all P < 0.05).

**Correlation between risk factors and carotid IMT in OSAHS patients**

Multivariate regression analysis showed that in patients with severe OSAHS, the AHI score, REM AHI, % total sleep time spent below 90 % SaO2, minimum SpO2, and serum glucose, hs-CRP, IL-6 and VEGF levels at bedtime and waking were positively correlated with the IMT (all P < 0.01, Table 6). In addition, the HDL levels at bedtime and waking were negatively correlated with the IMT (P < 0.01).

**Associations between inflammatory cytokines and minimum SpO2 in OSAHS patients**

Multivariate regression analysis showed that in patients with severe OSAHS, hs-CRP and VEGF levels at bedtime and waking were negatively correlated with the minimum SpO2 (all P < 0.01, Table 6). Also, the hs-CRP, VEGF and MCP-1 levels at bedtime and waking were negatively correlated with the AHI score and REM AHI, and IL-6 levels at waking were negatively correlated with the AHI score and REM AHI (all P < 0.01).

**Discussion**

OSAHS patients experience repeated episodes of apnea and hypopnea during sleep, which causes nocturnal hypoxemia and hypercapnia[1, 2]. Previous studies found that advanced age, gender, overweight, family history, alcohol consumption and smoking are all risk factors for OSAHS [1-6]. In the present
study, we also found that neck, hip and waist circumferences were significantly higher in patients with severe OSAHS than in both controls and patients with mild-moderate OSAHS, which indicates that local fat deposition was more pronounced in patients with severe OSAHS. Deposition of fat in the neck can increase oropharyngeal congestion and aggravate apnea and hypopnea [19]. In the present study, patients with severe OSAHS had significantly higher TG, TC, LDL-C, ApoB, blood uric acid and serum glucose levels than the control group. Moreover, the waking TC of patients with severe OSAHS was much higher than that of patients with mild-moderate OSAHS. These results imply that increased local fat deposition and hyperlipidemia are common in OSAHS. In addition, these parameters are regarded as risk factors for cardiovascular diseases, which suggests that OSAHS may be closely associated with atherosclerosis [14-16].

Previous studies have indicated that OSAHS is an independent risk factor for a variety of cardiovascular diseases, especially atherosclerosis [20, 21]. In the Chinese population, the majority of female patients with obstructive sleep apnea are postmenopausal women. Postmenopausal women often have very low levels of estrogen, which have a marked effect on atherosclerosis in female patients; therefore, we did not enroll female patients in the study.

In the present study, the CCA IMT of patients with OSAHS of differing severity was thicker than that in the control group. Atherosclerotic plaques were also found in the CCA of patients with OSAHS. Compared with the control group, the IMT of the carotid bifurcation of patients with severe OSAHS also was thicker, with the presence of atherosclerotic plaques. Our results suggest that severe OSAHS may accelerate atherosclerotic progression in Chinese patients, a finding that is consistent with those of previous studies in other populations. In one study of 102 Turkish patients [22], the walls of the CCA were thicker and the intima-media thickening ratios were higher in patients with severe OSAHS than in patients with mild-moderate OSAHS. Ciccone et al. [23] found, in a study of 80 Italian patients, that the carotid IMT was higher in OSAHS patients than in control subjects, and that moderate-severe OSAHS patients had a significantly higher carotid IMT than patients with mild OSAHS. Fox et al. reported that Canadian OSAHS patients, who did not have a history of additional risk factors for cardiovascular disease, had a higher carotid IMT than individuals in a BMI-, age- and gender-matched community-based cohort [24]. Iannuzzi et al. [25] found that the IMT was not associated with the AHI score, although the carotid IMT was much thicker in obese children with OSAHS than in children without OSAHS. The effect of OSAHS on IMT should be clarified by cohort-matched clinical studies with larger sample groups.

The exact relationship between OSAHS and atherosclerosis remains unclear, and to elucidate the possible mechanism underlying the promotion of atherosclerosis by OSAHS, we evaluated vascular endothelial function by FMD. Although brachial artery diameters during FMD did not differ among the control and OSAHS groups, FMD was significantly lower and VEGF was much higher in the severe OSAHS group than in the control group. Studies have shown that endothelial damage plays a key role in the development of atherosclerosis [26, 27]. VEGF is present in atherosclerotic plaques of the coronary artery and acts as an important angiogenic factor [28, 29]. In the present study, linear regression analysis showed that VEGF was positively correlated with the IMT in patients with severe OSAHS. These results suggest that vascular endothelial dysfunction is a link between OSAHS and atherosclerosis. It may be that intermittent hypoxemia increases factors such as inflammation, VEGF and oxidative stress, causing endothelial damage and atherosclerosis [14-16].

We also analyzed the role of inflammation in the effect of OSAHS in atherosclerosis. In the severe OSAHS group, bedtime IL-6, hs-CRP and MCP-1 levels were significantly higher than those of the control group, and waking IL-6, hs-CRP and MCP-1 levels were significantly higher than those of both the control group and the mild-moderate OSAHS group. These results suggest that inflammation is positively correlated with the severity of OSAHS. Previous studies found that the serum concentrations of hs-CRP and IL-6, which have been shown to be independent predictors of future cardiovascular events [(30, 31), are increased in OSAHS and decreased after treatment [32-34].

The present study showed that the AHI score, minimum SpO2 and serum concentrations of glucose, hs-CRP, IL-6 and VEGF at bedtime and waking are positively correlated with IMT in patients with severe OSAHS, whereas bedtime and waking hs-CRP, VEGF and MCP-1 levels are negatively correlated with AHI score. These results show that inflammation plays an important role in the pathogenesis of atherosclerosis in OSAHS. It may be that repeated hypoxia, as the major characteristic of OSAHS, induces an inflammatory reaction, leading to increased levels of inflammatory mediators [14-16].
Limitations

Potential limitations of the present study include the small population size and cross-sectional design that preclude meaningful multivariate analysis to determine whether changes in vascular endothelial damage and inflammation are independently linked to atherosclerosis progression. Such an analysis as part of a larger study will help elucidate the mechanism linking OSAHS and atherosclerosis.

Conclusion

In male Chinese patients with severe OSAHS, the carotid IMT was thicker, serum concentrations of inflammatory factors (IL-6 and hs-CRP) were higher, and FMD was lower than in healthy control individuals. Vascular endothelial damage and inflammation may play important roles in the pathogenesis of atherosclerosis in OSAHS.

Financial support

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Author Contributions

Delei Kong designed the study, performed experiments and wrote the manuscript. Zheng Qin acquired the data and participated in the data analysis. Wei Wang presented the findings and drafted and revised the manuscript. Jian Kang designed the study and drafted and revised the manuscript. All authors read and approved the final manuscript.

References