Original Article

Prevalence of vitiligo and associated comorbidities in adults in Shanghai, China: a community-based, cross-sectional survey

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Background: The prevalence of vitiligo has been reported to range from 0.1% to 8% worldwide, and vitiligo has been linked to some autoimmune and non-autoimmune diseases. This study aimed to estimate the prevalence of vitiligo and associated comorbidities in adults in Shanghai.

Methods: A community-based cross-sectional survey was conducted among 9,114 adults (4,288 males) in a community of Shanghai between October 2009 and January 2010. Face-to-face interviews were conducted at the home of each participant, and all respondents had their skin examined by dermatologists. The risks of comorbidities associated with vitiligo were evaluated by multiple logistic regression analysis.

Results: The estimated prevalence of vitiligo was 0.91%, and the standardized (age-adjusted) prevalence was 0.67%. Prevalence increased with age from 0.20% in 18–30 years to 1.59% in the 71–80 years age group. The presence of vitiligo was associated with increased risks of atopic dermatitis [adjusted odds ratio (aOR) =2.49; 95% confidence interval (95% CI): 1.46–4.23], urticaria (aOR =1.83; 95% CI: 1.11–3.04). and coronary heart disease (aOR =1.88; 95% CI: 1.03–3.41), although the association with coronary heart disease was only identified in subjects who were aged ≥60 years or overweight.

Conclusions: The prevalence of vitiligo in Shanghai was comparable to that seen in previous studies and increased with age. Vitiligo was associated with increased risks of atopic dermatitis, urticaria, and coronary heart disease in adults.

Keywords: Vitiligo; prevalence; comorbidity; coronary heart disease; epidemiologic studies

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Introduction

Vitiligo is an acquired, progressive depigmentation disorder characterized by the appearance of white macules on the skin, hair, or mucosa (1). The disease is classified into three different forms based on lesion distribution: non-segmental, segmental, and mixed (2). The etiology of vitiligo remains not fully clear, multiple mechanisms are involved including genetic predisposition, environmental

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triggers, neural mechanisms, oxidative stress and altered inflammatory and autoimmune response. It has been suggested that environmental factors such as ultraviolet rays and chemicals, act upon genetically predisposed individuals to help trigger and exacerbate vitiligo partly by inducing the gene damage or melanocyte stress (3). Several studies have established oxidative stress is involved in vitiligo onset, while autoimmunity contributes to the disease progression. Melanocytes under oxidative stress release danger signals important for the activation of the immune system or was triggered to apoptosis. Keratinocytes also involved in melanocyte destruction by secreting cytokines to recruit autoreactive T cells under oxidative stress. Although different mechanisms may play a role, the autoimmune hypothesis is currently considered as the main pathway. The cytotoxic T lymphocytes recruited through their CXCR3 are now proved to be involved in the final pathways of melanocyte destruction (3,4). Vitiligo remains difficult to treat and requires longitudinal care, and the treatment options include topical and systemic immunosuppressants, phototherapy and surgery (5). Although the disease is biologically benign, the alteration in appearance can exert a substantial psychologic burden and have a negative impact on quality of life (QOL) (6).

Previous studies have reported a wide range of estimates for the prevalence of vitiligo worldwide, ranging from 0.1% to 8% (7-9), and between 0.09% and 1.9% in different regions of China (10,11). A meta-analysis of more than 50 studies estimated the worldwide prevalence to be between 0.5% and 2% (12). Previous research has indicated a higher prevalence of vitiligo in hospital-based studies than in community- or population-based studies (9), indicating that such studies may overestimate the prevalence of vitiligo.

Since melanocytes are also located in areas other than the skin, vitiligo has been reported to not only target the skin but also potentially trigger the development of generalized syndromes with different etiologies and pathogeneses. In particular, vitiligo has been linked with autoimmune diseases such as thyroid disorders, alopecia areata, type I diabetes mellitus, and rheumatoid arthritis (8,13,14). Other studies have also associated vitiligo with non-autoimmune diseases such as psoriasis, atopic dermatitis, anemia, and dyslipidemia (8,15). However, the association between vitiligo and metabolic disorders, especially common chronic diseases of hypertension, diabetes mellitus, obesity, and coronary heart disease, was rarely explored. A case-control study has indicated that a family history of cardiovascular disease was a significant predictor for psoriasis and vitiligo (16). However, these associations have not been confirmed, and little is known about how they are impacted by age and body mass index (BMI) and whether there is an increased risk of cardiovascular diseases in patients with vitiligo.

We performed a population-based survey in Shanghai, the economic center of China, to estimate the prevalence of vitiligo and associated comorbidities, including metabolic disorders with a focus on cardiovascular diseases. The results may facilitate the management of vitiligo and its comorbidities. We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1738).

Methods

Study design and study population

The present study was a secondary analysis based on a cross-sectional, population-based study carried out in the urban-rural Beixinjing community of Shanghai (17) from October 2009 to January 2010 which aimed to investigate the prevalence of chronic diseases, with a focus on skin disease. Participants were required to be ≥18 years of age and have been living in Shanghai for more than one year and were recruited by cluster sampling. Statistics from seven randomly selected neighborhood committees were used for the analysis. Residential apartment buildings were taken as the survey unit (125–155 people), and 85 residential buildings were randomly selected.

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Shanghai Huashan Hospital affiliated to Fudan University (NO:2009-164), and informed consent was obtained from all participants.

Collection of clinical data

The selected apartments were visited in person by a team consisting of trained dermatologists from Shanghai Huashan Hospital affiliated to Fudan University and local general practitioners who explained the nature of the study and determine whether the residents were willing to participate. If necessary, an appointment was scheduled for a later visit.

Residents who agreed to participate were helped to fill out a questionnaire collecting the following information: date of birth, gender, height, weight, history of skin diseases and common diseases, and family history of skin diseases (the

diseases of interest included psoriasis, lupus erythematosus, atopic dermatitis, alopecia areata, urticaria, hypertension, diabetes mellitus, coronary heart disease, gout, and obesity). After completion of the questionnaire, each participant underwent a complete skin examination in privacy and was diagnosed by at least two dermatologists (18).

Clinical assessment

Since vitiligo is a clinical diagnosis made on the basis of the signs present at the time of examination, the diagnosis was made or confirmed by the dermatologists during the visit. Both primary and secondary lesions were assessed, and when present, vitiligo was classified into focal vitiligo, generalized vitiligo, universal vitiligo, acrofacial vitiligo, or segmental vitiligo (18,19). All vitiligo patients received a questionnaire about detailed data of vitiligo to complete. The World Health Organization QOL assessment scale (WHOQOL-BREF) (20) was used to evaluate the QOL of each participant.

Statistical analysis

Data were entered in EpiData 3.1 (EpiData Association, Odense, Denmark), and all analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC, USA). The age-adjusted prevalence of vitiligo was calculated using the population of Shanghai from the 2010 Shanghai Population Census (17). Participants with incomplete questionnaire were excluded. Logistic regression analyses with adjustment for age, gender, BMI, education level, marital status, drinking status, and smoking status were used to investigate the associations of various comorbidities with vitiligo, including psoriasis, lupus erythematosus, atopic dermatitis, alopecia areata, urticaria, hypertension, diabetes mellitus, coronary heart disease, gout, and obesity (not adjusted for BMI). The age of participants was categorized according to its distribution and BMI was categorized by commonly used standards. Crude odds ratios (ORs), adjusted ORs (aORs), and 95% confidence intervals (95% CIs) were calculated and stratified analyses were performed based on age (18–39, 40–59, and ≥60 years) and BMI (<24 and ≥24 kg/m^2).

Results

Baseline characteristics and prevalence of vitiligo

Among 12,488 adults (aged ≥18 years), 3,374 were excluded from the study (430 questionnaires not returned, 20 questionnaires rejected, and 2,924 questionnaires incomplete), leaving 9,114 respondents (4,288 males and 4,826 females) who completed questionnaires included in the final analysis, corresponding to a response rate of 73%.

Vitiligo was observed in 83 participants, and the baseline characteristics of participants with and without vitiligo are compared in *Table 1*. The prevalence of vitiligo was different among different age groups (P=0.003) and marital status groups (P=0.021), with a lower prevalence in younger participants and those who were unmarried. However, the prevalence did not differ according to other characteristics.

The overall estimated prevalence of vitiligo was 0.91% (95% CI: 0.73–1.13%), with 1.03% (95% CI: 0.75–1.38%) in males and 0.81% (95% CI: 0.58–1.10%) in females. When both genders were considered together, the prevalence of vitiligo increased progressively with increasing age from 0.20% in those aged 18–30 years to 1.59% in those aged 71–80 years (*Table 2*), and similar increases seen with older age were also observed in male and female subgroups (*Table 2*).

Clinicoepidemiologic profile of vitiligo

The clinical features of vitiligo in the 83 study participants are summarized in *Table 3*. The most common type was generalized vitiligo (30/83; 36.1%), while universal vitiligo was the least common (7/83; 8.4%). Focal vitiligo was seen in 22 patients (26.5%), 14 patients (16.9%) had acrofacial vitiligo, and 10 patients (12.0%) had segmental vitiligo. The age at onset of vitiligo averaged 44.9±15.7 years but varied widely from 21–79 years, with 38.9% of the participants becoming symptomatic at 20–39 years of age, and the most common primary symptomatic locations were the trunk (34/83, 40.96%) and face (28/83, 33.73%). Symptom onset occurred after psychological trauma in 16 participants (19.28%) and after sun exposure in 6 (7.23%). Overall, 78.3% (65/83) of vitiligo patient underwent treatment at the time when the survey was conducted. A positive family history of vitiligo was reported in 10 participants (12.05%) with a first-degree relative in four cases, second-degree relative in three cases, and other relative in three cases). More than half the participants with vitiligo (57.6%) reported a negative impact of their skin condition on their QOL, and 34.9% described a mild-to-serious impact on their QOL (*Table 3*). Individuals with vitiligo on their face reported a more negative impact on their QOL.

Table 1 Baseline characteristics of the study population

Characteristic	Vitiligo (n=83)	No vitiligo (n=9,031)	P
Gender			0.274
Male	44 (1.03%)	4,244 (98.97%)	
Female	39 (0.81%)	4,787 (99.19%)	
Age (years)			0.003
18–30	3 (0.20%)	1,525 (99.80%)	
31–40	4 (0.41%)	980 (99.59%)	
41–50	9 (0.81%)	1,109 (99.19%)	
51–60	28 (1.09%)	2,544 (98.91%)	
61–70	15 (1.19%)	1,242 (98.81%)	
71–80	17 (1.59%)	1,054 (98.41%)	
≥81	7 (1.20%)	577 (98.80%)	
Body mass index (kg/m^2)			0.225
<18.5	2 (0.30%)	660 (99.70%)	
18.5–23.9	54 (1.05%)	5,100 (98.95%)	
24.0–27.9	22 (0.87%)	2,521 (99.13%)	
≥28.0	5 (0.66%)	750 (99.34%)	
Education			0.380
Primary school and below	15 (1.21%)	1,228 (98.79%)	
Junior high school	20 (0.81%)	2,444 (99.19%)	
Technical secondary school/high school	29 (1.02%)	2,815 (98.98%)	
Junior college	12 (1.07%)	1,111 (98.93%)	
University and above	7 (0.52%)	1,339 (99.48%)	
Unknown	0 (0.00%)	94 (100.00%)	
Marital status			0.021
Unmarried	3 (0.23%)	1,292 (99.77%)	
Married	72 (1.02%)	7,013 (98.98%)	
Other	8 (1.09%)	726 (98.91%)	
Smoking			0.843
Often (1 cigarette/day for ≥6 months)	16 (0.84%)	1,900 (99.16%)	
Occasionally (<1 cigarette/day)	1 (0.61%)	162 (99.39%)	
Never	66 (0.94%)	6,964 (99.06%)	
Alcohol consumption			0.258
Yes	6 (0.59%)	1,006 (99.41%)	
No	77 (0.95%)	8,020 (99.05%)	

Data are presented as n (%).

Table 2 Prevalence of vitiligo in adults in Shanghai stratified according to age and gender

Variable	Age (years)							
	18–30	31–40	41–50	51–60	61–70	71–80	81–	Total
Men								
No.	2 [721]	1 [441]	3 [545]	16 [1,210]	8 [620]	9 [500]	5 [251]	44 [4,288]
%	0.28	0.23	0.55	1.32	1.29	1.80	1.99	0.68[a]
Women								
No.	1 [807]	3 [543]	6 [573]	12 [1,362]	7 [637]	8 [571]	2 [333]	39 [4,826]
%	0.12	0.55	1.05	0.88	1.10	1.40	0.60	0.65[b]
Total								
No.	3 [1,528]	4 [984]	9 [1,118]	28 [2,572]	15 [1,257]	17 [1,071]	7 [584]	83 [9,114]
%	0.20	0.41	0.81	1.10	1.20	1.59	1.20	0.67[c]

[a]Standardized prevalence based on the population of Shanghai according to the 2010 Shanghai Population Census. Standardized prevalence: [a]0.68%, [b]0.65%, [c]0.67%.

Table 3 Clinical features of vitiligo in adults in Shanghai

Clinical feature	n (%)
Symptomatic location at onset	
Trunk	34 (40.96)
Face	28 (33.74)
Extremity	21 (25.30)
Age at onset	
<50 years	54 (65.06)
≥50 years (late onset)	29 (34.94)
Inducing factor	
Psychologic trauma	16 (19.28)
Physical injury	8 (9.64)
Sun exposure	6 (7.23)
Not known	53 (63.85)
Quality of life	
No impact	36 (43.37)
Slight impact	18 (21.69)
Mild to severe impact	29 (34.94)

Comorbidities associated with vitiligo

When compared with participants who did not have vitiligo, those with vitiligo had an increased risk of atopic dermatitis (adjusted odds ratio, aOR =2.49; 95% CI: 1.46–4.23), urticaria (aOR =1.83; 95% CI: 1.11–3.04), and coronary heart disease (aOR =1.88; 95% CI: 1.03–3.41). However, no association was observed with diabetes mellitus, hypertension, psoriasis, alopecia areata, gout, or obesity (*Table 4*).

When we stratified the analyses by age (*Table 5*), the risk of atopic dermatitis and urticaria in participants with vitiligo were broadly similar between the different age groups, although the associations in most age groups were not significant (possibly due to the reduction in sample size and hence loss of statistical power). Notably, an increased risk of coronary heart disease was only observed in participants aged ≥60 years (aOR =2.25; 95% CI: 1.13–4.47) and not in those aged 40–59 years (aOR =0.97; 95% CI: 0.23–4.15).

When the analyses were stratified by BMI (*Table 6*), the increased risks of coronary heart disease (aOR =2.45; 95% CI: 1.02–5.88) and urticaria (aOR =2.70; 95% CI: 1.19–6.12) with vitiligo remained in the overweight group (BMI ≥24 kg/m^2), whereas these associations were not observed in the non-overweight group (BMI <24 kg/m^2). The increased risks of atopic dermatitis with vitiligo were similar in both overweight (aOR =2.23; 95% CI: 0.88–5.61) and non-overweight groups (aOR =2.67; 95% CI: 1.39–5.11).

Discussion

A notable finding of the present study was that the estimated prevalence of vitiligo in adult residents of Shanghai was 0.91%, and the standardized prevalence was 0.67%, and the

Table 4 Logistic regression analysis of chronic comorbidities associated with vitiligo in adults in Shanghai

Comorbidity	Vitiligo (n=83)	No vitiligo (n=9,031)	Crude OR (95% CI)	Adjusted OR (95% CI)
Atopic dermatitis	18 (21.69%)	917 (10.15%)	2.45 (1.45, 4.15)	2.49 (1.46, 4.23)[#]
Urticaria	21 (25.30%)	1,341 (14.89%)	1.94 (1.18, 3.19)	1.83 (1.11, 3.04)[#]
Coronary heart disease	15 (18.07%)	750 (8.31%)	2.44 (1.39, 4.28)	1.88 (1.03, 3.41)[#]
Alopecia areata	2 (2.40%)	120 (1.33%)	1.85 (0.45, 7.62)	1.61 (0.39, 6.69)[#]
Psoriasis	2 (2.41%)	105 (1.17%)	2.10 (0.51, 8.63)	1.86 (0.45, 7.72)[#]
Diabetes mellitus	7 (8.43%)	641 (7.10%)	1.21 (0.55, 2.62)	0.89 (0.40, 1.96)[#]
Hypertension	27 (32.53%)	2,239 (24.81%)	1.46 (0.92, 2.32)	1.00 (0.61, 1.65)[#]
Gout	2 (2.41%)	206 (2.29%)	1.06 (0.26, 4.33)	0.80 (0.19, 3.30)[#]
Obesity	5 (6.02%)	750 (8.30%)	0.71 (0.29, 1.75)	0.66 (0.26, 1.63)[§]

Data are presented as n (%). [#]Adjusted for gender, age, body mass index, education level, marital status, smoking status, and alcohol consumption. [§]Adjusted for gender, age, education level, marital status, smoking status and alcohol consumption. OR, odds ratio; 95% CI, 95% confidence interval.

Table 5 Logistic regression analysis of chronic comorbidities associated with vitiligo stratified by age group

Comorbidity	Adjusted odds ratio (95% confidence interval)		
	18–39 years	40–59 years	≥60 years
Atopic dermatitis[#]	1.58 (0.19, 13.21)	2.26 (0.98, 5.24)	3.10 (1.48, 6.50)
Urticaria[#]	2.88 (0.55, 15.10)	1.64 (0.76, 3.53)	1.90 (0.91, 3.97)
Coronary heart disease[#]	–	0.97 (0.23, 4.15)	2.25 (1.13, 4.47)
Hypertension[#]	–	0.73 (0.31, 1.71)	1.23 (0.64, 2.34)
Diabetes mellitus[#]	–	0.99 (0.23, 4.20)	0.85 (0.33, 2.20)
Alopecia areata[#]	–	–	4.38 (0.99, 19.36)
Gout[#]	–	–	1.26 (0.30, 5.38)
Obesity[§]	–	0.59 (0.14, 2.49)	0.78 (0.24, 2.55)

[#]Adjusted for gender, age, body mass index, education level, marital status, smoking status, and alcohol consumption. [§]Adjusted for gender, age, education level, marital status, smoking status, and alcohol consumption.

prevalence of vitiligo increased with age. The present study revealed that vitiligo was associated with increased risks of atopic dermatitis, urticaria, and coronary heart disease but not diabetes mellitus, hypertension, psoriasis, alopecia areata, gout, and obesity. The most important and novel finding is the association of vitiligo with coronary heart disease, especially in elderly (aged ≥60) and overweight (BMI ≥24 kg/m^2) participants. The present study provides new insights into the clinical features of vitiligo in a Chinese population.

Previous studies carried out in China have reported differing estimates of the prevalence of vitiligo, ranging from 0.09–1.9% (10,11). Nonetheless, our estimated prevalence of 0.91%, with standardized prevalence of 0.67%, is consistent with these previous studies, and is very comparable to the study of six cities from China with the prevalence of 0.56% from 17,345 subjects (10). When compared with populations with a similar skin-phototype from other Asian countries, the prevalence of vitiligo in the present study was between that reported in Japanese (1.68%) (21) and Korean populations (0.13%) (8). Patients' knowledge about vitiligo, attention to the disease, and degree of adaptability may contribute to regional differences (8), and hospital-based studies may overestimate

Table 6 Logistic regression analysis of chronic comorbidities associated with vitiligo stratified by body mass index

Variable	Adjusted odds ratio (95% confidence interval)[#]	
	BMI ≥24 kg/m^2 (n=3,298)	BMI <24 kg/m^2 (n=5,816)
Atopic dermatitis	2.23 (0.88, 5.61)	2.67 (1.39, 5.11)
Urticaria	2.70 (1.19, 6.12)	1.53 (0.80, 2.92)
Coronary heart disease	2.45 (1.02, 5.88)	1.44 (0.62, 3.34)
Hypertension	1.30 (0.60, 2.85)	0.84 (0.43, 1.64)
Diabetes mellitus	1.09 (0.37, 3.23)	0.71 (0.22, 2.34)
Gout	2.05 (0.47, 8.94)	–
Alopecia areata	2.70 (0.35, 20.92)	1.25 (0.17, 9.26)
Psoriasis	2.69 (0.35, 20.65)	1.45 (0.19, 10.85)

[#]Adjusted for gender, age, education level, marital status, smoking status and alcohol consumption. BMI, body mass index.

prevalence, further contributing to variability between reports (9,21). Our finding that the prevalence of vitiligo increased with age is in agreement with the those of others (7,9,10) and consistent with the nature of a chronic disease. The average age of vitiligo onset in our participants was similar to that reported by Wang *et al.* (10), whereas a younger age of onset was reported by Boisseau-Garsaud *et al.* (22) and Handa and Kaur (23) in hospital-based patients. The apparent discrepancy may lie in the fact that young people may have more concern for their appearance and visit the hospital more frequently for dermatological care.

According to the recent studies, inflammatory/immune alteration is the main cause leading to the vitiligo, especially at the progressive stage. There is increasing evidence that vitiligo is associated with thyroiditis and other autoimmune diseases (8,13,14). Consistent with previous research (24-26), the results of the present study indicated that atopic dermatitis and urticaria were associated with vitiligo, which would support the involvement of an abnormal inflammatory/immune response in its pathogenesis. However, we found no association between psoriasis and vitiligo, which was different from previous investigations (27,28). The disagreement may be due to the small number of patients with vitiligo and low prevalence of psoriasis in the present study, which would have limited the statistical power.

The most important and novel finding of this study was the increased risk of coronary heart disease in patients with vitiligo (especially in elderly or overweight participants), which has never been reported. A previous study reported that a family history of cardiovascular disease was a significant predictor of psoriasis and vitiligo comorbidity (16). Moreover, there is increasing recognition that metabolic syndrome may also be related to vitiligo (29). And children with vitiligo were found to have a higher ratio of low-density lipoprotein to high-density lipoprotein, which may explain the development of metabolic syndrome and cardiovascular disease (30).However, the mechanisms underlying these associations remain unclear. Homocysteine can inhibit tyrosinase and increase the risk of cardiovascular disease in patients with metabolic syndrome (31), and may be increased in patients with vitiligo (32), although not all studies have demonstrated this (33). Moreover, the unexpected regression of vitiligo in a patient treated with high-dose simvastatin (34), commonly used in dyslipidemia and cardiovascular diseases, suggests that lipid profile abnormalities may be relevant to its occurrence and severity, and indicates statins as immune-modulating medications, which not only improve lipid profile but also may play a role in the treatment of vitiligo patients. It is possible that oxidative stress and inflammatory processes, which play a vital role in the pathogenesis of the metabolic syndrome, coronary heart disease and the vitiligo. Excessive ROS in vitiligo patients is known to be responsible for lipid peroxidation, which is significantly elevated in plasma of patients with coronary heart disease (31,35). As all of these findings support those of the present study, irrespective of the underlying mechanisms, coexisting diseases should be borne in mind during the management of patients with vitiligo. Future studies on lipid disorders in patients with vitiligo may contribute new insights into the pathogenesis and treatment of vitiligo.

An important strength of the present study is that it was a large-scale, community-based study, which enabled us to obtain better assessments of the true prevalence of vitiligo, and the clinical features and associated comorbidities in adults in Shanghai. To our knowledge, this is the first study to demonstrate an increased risk of coronary heart disease in patients with vitiligo, which may give new insights into the mechanisms contributing to the pathogenesis of vitiligo. However, this study also has some limitations. First, the number of patients with vitiligo was small, so our analysis may have been underpowered to detect some true associations between vitiligo and associated comorbidities (possibly underestimated). Second, this was a cross-sectional

study, so no conclusions can be drawn regarding cause and effect. Third, the population sampled was from a region of Shanghai, so the results may not be generalizable to other regions of China or other countries. Fourth, since the survey was conducted during 2009–2010 and the present study was a secondary analysis, a more recent classification for the diagnosis of vitiligo (18) was not used.

Conclusions

The prevalence of vitiligo in adults in Shanghai was comparable to that reported in other studies. The present study shown an increased risk of atopic dermatitis, urticaria and coronary heart disease in adults with vitiligo, with the association of vitiligo with coronary heart disease in elderly and overweight participants the most important finding. The results may bring new insights into the study of vitiligo.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Shanghai Huashan Hospital affiliated to Fudan University (NO:2009-164), and informed consent was obtained from all participants.

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