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Original Article

Risk factors of primary biliary cirrhosis in mid-west China

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Abstract: Background/Aims: PBC is an autoimmune liver disease with unclear etiology, although environmental and genetic interactions have been proposed in previous studies. We conducted a large case-control study in Mid-West China to indentify PBC risk factors and compare study findings with previous studies. Methods and material: Data of 512 PBC patients and 761 age-, sex-, residential location-matched controls were recorded by questionnaire. Questionnaire data on general, psychological, lifestyle conditions and medical history from all subjects and data on menstrual and reproductive features from females were collected and examined. Data from cases and controls were compared using logistic regressions, adjusting for age, sex and BMI. Results: Psychological stress, hair dye usage, allergy, urinary tract infection, surgery and the number of live births, abnormal pregnancy and pruritus during pregnancy were significantly associated with increased risk of PBC. However, outdoors exposure time (OR: 0.928; 95% CI: 0.879, 0.980) may be a protective factor in the development of PBC. Conclusions: This study confirms risk factors previously reported and first indentifies the association with psychological stress, outdoor exposure time and PBC. Further investigation is needed to examine these findings.

Keywords: Primary biliary cirrhosis, risk factors, Chinese population, psychological stress, outdoor exposure time

Introduction

Primary biliary cirrhosis or primary biliary cholangitis (PBC) [1, 2] is a chronic autoimmune disease with positive serum antimitochondrial antibodies (AMA) and the immune-mediated progressive destruction of intra-hepatic bile ducts [3]. Its pathogenesis remains largely unknown. It is hypothesized that PBC results from the broken of immune-tolerance triggered by interactions of genetic and environmental factors. High monozygotic twin concordance rate [4], family history of PBC [5, 6] and human leucocyte antigen genotype [7, 8] may support the role of genetic factors in conferring susceptibility to PBC. However, environmental factors may trigger susceptible individuals and finally lead to the onset of PBC. Evidence for contribution of environmental factors includes time-space clustering of PBC prevalence [9], urinary tract infection [10-12] and exposure to various chemicals such as smoking and hair dye [13].

After the first case-control study of PBC risk factors was published in 1982 [14], studied based on large cohorts were subsequently carried out, particularly during the last two decades. A variety of risk factors have been assumed in the development of PBC. Moreover, the psychological status has been identified as risk factors in several autoimmune diseases [15], while this association has not been reported in PBC.

There is considerable variation in the prevalence of PBC worldwide, being most common in Northern Europe. A study conducted in Austria stated that migrant races from Italian, Greek, British and other countries were more likely to develop PBC compared to the native population [16]. Another study in Southeast Asia with a very small sample size indicated a prevalence rate in Chinese subjects that was twice as high as that in the Malay population [17]. Considering these findings, the PBC prevalence among the Chinese might not be low. However, data from

Asia is rare. Therefore, we conducted a survey aimed at exploring the possible risk factors of PBC patients in Mid-West China.

Materials and methods

This case-control study was performed in Xijing Hospital of Digestive Diseases. This area includes the six provinces Shaanxi, Gansu, Qinghai, Ningxia, Shanxi and Henan.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Xijing Hospital committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

PBC cases

Five hundred and thirty-nine PBC patients who were consecutively referred to the Xijing Hospital of Digestive Diseases during October 2011 to May 2015 were invented to participate in this study. Four patients fulfilling the criteria of overlap syndrome of autoimmune hepatitis and 11 patients who refused to participate in the study were excluded. Additionally, the data of 3 patients diagnosed with PBC were undergoing hepatic encephalopathy and could not finish the interview by themselves and 9 PBC subjects were incomplete after two telephone interviews. Finally, 512 patients were included in the study. All PBC patients gave the oral informed consent.

All the PBC patients were diagnosed using the clinically accepted criteria published by the European Society of Liver Disease in 2009 [3].

Controls

We enrolled consecutive healthy controls from the Xijing Hospital Healthy Examination Register Database. The controls were matched based on gender, age±2 and residence location (province). After phone interviews, 946 volunteers from six provinces agreed to take part in the study. Excluding uncompleted data, there were 761, 80.4% volunteers were eventually included in controls. Any individuals presenting with potential liver damage, which generally came from liver biochemical tests, were excluded from the controls. All volunteers gave the oral informed consent.

Data collection

The questionnaire consists of 54-items and result from two discussions from 7 liver disease experts, 2 statistical experts and 1 epidemiological expert. Two dimensions comprised the entire questionnaire: part A and part B. Part A focused on general characteristics (name, age, educational and financial levels). Part B consisted of five components regarding psychological, lifestyle, the medical history and menstrual and reproductive conditions in females. The physicians were trained prior to conduct the one-on-one telephone interviews. Before the official interview, we conducted a retest study within two weeks in PBC cases. The test-retest reliability coefficient was 0.87.

For the psychological component, the Chinese 14-item perceived stress scales (CPSS) was applied to access the mental stress [18, 19]; the Satisfaction with life scale (SWLS) for the life satisfaction [20]; the Pittsburgh sleep quality index (PSQI) for the quality of sleeping [21]. In the CPSS, scores greater than 25 were identified as high pressure, and scores equal to or less than 25 as normal pressure. SWLS contains 5 questions with 7 options for each question; scores of 35 to 20 were classified as positive and scores of 19 to 5 as negative. In the PSQI, scores of 0-10 were defined as good quality sleeping, whereas scores of 11-20 were defined as poor quality sleeping.

Outdoor exposure time in our questionnaire is designed “how much time on average do you spend outdoors during sunlight hours in recent three months?”. According to local seasonal climate our study conducted, seasonality was defined as winter-spring from December to May and summer-autumn from June to November. Outdoor exposure time in this study included physical activity time but did not share the same definition.

Data analysis

After the Kologorov-Smirnov testing, normal distributed continuous variables were expressed as the mean ± deviation standard, and abnormal distributed variable was expressed as median, (P_{25}, P_{75}). Categorical variables were expressed as percentages. In the socio-demographic features section, the Chi-square test was used for the categorical variables and

Table 1. Socio-demographic features of PBC cases and controls

	Cases	Controls	*P* value
Gender (female/male)	449/63 (12.3%)	647/114	0.754
Age (yr)	49.35±12.15	49.41±13.00	0.935
Height (m)	1.62±0.06	1.62±0.06	0.566
Weight (kg)	58.31±10.17	59.49±9.78	0.040
BMI (kg/m^2)	22.23±3.16	22.63±3.08	0.027
Blood type			
A	141	214	0.739
B	129	203	
0	121	272	
AB	44	72	
Educational level			
Primary or secondary	339 (66.2%)	511 (67.1%)	0.728
College and post-college	173 (33.8%)	250 (32.9%)	
Financial condition (yuan/month)			
<1000	43 (8.4%)	52 (6.8%)	0.472
1000-2000	416 (81.3%)	620 (81.5%)	
>2000	53 (10.4%)	89 (11.7%)	

Note: PBC, Primary Biliary Cirrhosis. BMI, Body mass index.

Student' *t* test was used for the normally distributed continuous variables. And Spearman' rank was applied for correlation test. In the risk factors section, univariate logistic regression was applied to analyze all variables. Variables that were significantly associated with PBC were entered into the multivariate binary logistic regression analysis, and backward stepwise elimination was applied. Two models were applied in the multivariate logistic regression analysis: a model for psychological status, lifestyle factors and medical station and another model for menstrual, reproductive station in female only. Multivariate binary logistic regression analysis was applied to analyze the interaction between two items. Variables in logistic regression analysis were expressed with odds ratios (OR) and 95% confidence intervals (CI).

The statistical analyses were performed using SPSS software 19.0 (IBM, USA) and GraphPad Prism 5 (GraphPad Software, USA). In all the data analyses, the probability values were two-sided, and *p* values less than 0.05 were considered statistically significant.

Results

Socio-demographic features between the PBC cases and controls are showed in **Table 1**. The PBC patients appeared to weigh slightly less and have a lower BMI compared to the controls, *P*=0.027. The gender, age, height, financial condition and the educational level were similar between cases and controls.

The psychological status of the PBC patients and the controls is showed in **Table 2**. In total, 20.5% PBC patients suffered from sleeping difficulties, whereas only 14.5% in controls, *P*=0.005. Nearly 21.7% of the PBC cases reported high psychological pressure derived from home and work, whereas the percentage in controls was only 11.7%, *P*< 0.001. Negative satisfaction of life was more frequently reported by cases 12.3% vs. 7.6%, *P*=0.005.

Table 3 shows the relationship between PBC and lifestyle factors. PBC cases appeared to spend less time outdoors than controls, (1, 3) hours in cases and (1, 4) hours in controls, *P*=0.001. Furthermore, the outdoor exposure time was classified into three categories (Supplementary Figure 1). Correlation analyses between outdoor exposure time and 25-hydroxyvitamin D, season, pathological stage were shown in (Supplementary Table 2). The proportion of patients who used hair dye one or more times per year was 46.5% which was significantly higher compared to 34.3% of the controls. The history of active and passive smoking did not differ significantly between the two groups. The frequency of alcohol, tea and coffee consumption (defined as more than 1 time per week) were similar between cases and controls. The age of first dyeinghair and hair wave makeup and the proportion of hair wave or fingernail usage were not significantly associated with case status.

The medical conditions reported by the PBC and controls are showed in **Table 4**. PBC patients reported to have one or more inflammatory or autoimmune disease(s) more frequently than controls (38.3 vs. 22.2%, *P*<0.001). Similar association was observed in first-degree relatives of both cases and controls (15.6 vs. 7.0%, *P*<0.001). Among autoimmune diseases,

Table 2. Psychological status in PBC cases and controls

	Cases No, (%)	Controls No, (%)	Odds Ratio	95% CI†	*P* value
Quality of sleeping					
Bad (11-20 scores)	105 (20.5%)	110 (14.5%)	1.527	1.137, 2.050	0.005
Psychological stress					
High pressure (>25 scores)	111 (21.7%)	104 (13.7%)	1.749	1.302, 2.348	<0.001
Satisfaction of life					
Negative (5-19 scores)	63 (12.3%)	58 (7.6%)	1.701	1.168, 2.476	0.006

Note: PBC, Primary Biliary Cirrhosis. †, 95% Confidence interval.

Table 3. Lifestyle factors reported by PBC cases and controls

	Cases No, (%)	Controls No, (%)	Odds Ratio	95% CI†	*P* value
Outdoor exposure time (hour/day)	1, 3‡	1, 4‡	0.918	0.872, 0.967	0.001
Pollutes of both chemical and physical factors around the dwelling houses	89 (17.4%)	105 (13.8%)	1.315	0.966, 1.788	0.082
Active smoking					
≥1 time/week	72 (14.1%)	81 (10.6%)	1.374	0.978, 1.929	0.067
Passive smoking					
≥1 time/week	181 (35.4%)	250 (32.9%)	1.118	0.883, 1.415	0.355
Alcohol drinking					
≥1 time/week	78 (15.2%)	126 (16.6%)	0.906	0.666, 1.232	0.528
Coffee					
≥1 time/week	157 (30.7%)	219 (28.8%)	1.095	0.857, 1.398	0.470
Tea					
≥1 time/week	326 (63.7%)	469 (61.6%)	1.091	0.865, 1.376	0.461
Hair dye usage					
≥1 time/year	238 (46.5%)	261 (34.3%)	1.664	1.323, 2.093	<0.001
Wave hair					
≥1 time/year	130 (25.4%)	176 (23.1%)	1.131	0.872, 1.468	0.354
Use of fingernail dye					
≥1 time/month	92 (18.0%)	127 (16.7%)	1.094	0.814, 1.469	0.553

Note: PBC, Primary Biliary Cirrhosis. †, 95% Confidence interval. ‡, Outdoor exposure time expressed as quartile, (P_{25}, P_{75}).

multiple sclerosis (1.2 vs. 0.3%), thyroid disease (4.5 vs. 1.7%), urinary tract infection (9.0 vs. 2.6%) and rheumatoid arthritis (7.4 vs. 4.1%) were more commonly reported PBC cases. For chronic diseases, urinary tract infection (UTI) (9.0 vs. 2.6%) and thyroid disease (4.5 vs. 1.7%) differed significantly between the two groups. In the PBC cohort, 17.6% of the cases suffered from a history of allergies, whereas only 10.4% in controls, *P*<0.001. History of surgery was higher in PBC cases (39.3 vs. 27.1%, *P*<0.001). Among the surgery categories, the prevalence of cholecystectomy in the PBC cases was much higher than controls (12.7 vs. 8.0%, *P*<0.001). The vaccination rate in the previous 20 years was similar among the cases and controls (57.0 vs. 59.3%, *P*=0.428). Compared to the controls, the PBC patients reported a slightly higher prevalence of medication taken except for UDCA or other medication for PBC treatment (23.2 vs. 23.1%, *P*=0.962). No association was observed with either osteoporosis or bone fracture.

A total of 449 female PBC cases and 647 female controls were included in menstrual and reproductive analysis model (**Table 5**). The PBC cases reported more babies (2.37±1.26) compared to the controls (2.12±1.07), *P*=0.002. The PBC patients had a significantly higher prevalence of abnormal pregnancy than controls (12.0 vs. 3.6%, *P*<0.001). Among female PBC cases and controls, approximately 6.9 and 2.5% recalled pruritus during pregnancy, *P*<0.001. Patients with PBC reported having hormone replacement therapy more frequently than the controls (8.1 vs. 4.5%, *P*=0.014). No difference in age at menarche, proportion of

Table 4. Medical station reported by PBC cases and controls

	Cases No, (%)	Controls No, (%)	Odds Ratio	95% CI†	*P* value
Vaccination	292 (57.0%)	451 (59.3%)	0.912	0.727, 1.145	0.428
Long-term medications	119 (23.2%)	176 (23.1%)	1.006	0.772, 1.312	0.962
Allergy	90 (17.6%)	79 (10.4%)	1.841	1.329, 2.550	<0.001
Inflammatory or autoimmune disease	196 (38.3%)	169 (22.2%)	2.173	1.697, 2.781	<0.001
Sjogren's syndrome	15 (2.9%)	0	Incalculable		
Raynaud's syndrome	3 (0.6%)	0	Incalculable		
Systemic Lupus Erythematosus	1 (0.2%)	1 (0.1%)	1.487	0.093, 23.832	0.779
Scleroderma	3 (0.6%)	0	Incalculable		
Multiple sclerosis	6 (1.2%)	2 (0.3%)	4.500	0.905, 22.384	0.066
Urinary tract infection	46 (9.0%)	20 (2.6%)	3.657	2.136, 6.261	<0.001
Thyroid disease	23 (4.5%)	13 (1.7%)	2.706	1.358, 5.393	0.005
Rheumatoid arthritis	38 (7.4%)	31 (4.1%)	1.888	1.159, 3.076	0.011
Diseases above in familial members	80 (15.6%)	53 (7.0%)	2.474	1.714, 3.571	<0.001
Osteoporosis	70 (13.7%)	119 (15.6%)	0.854	0.631, 1.176	0.334
Bone fracture	65 (12.7%)	87 (11.4%)	1.127	0.800, 1.587	0.496
Surgery	201 (39.3%)	206 (27.1%)	1.741	1.371, 2.211	<0.001
Splenectomy	26	0	Incalculable		
Cholecystectomy	65 (12.7%)	61 (8.0%)	1.669	1.154, 2.413	0.007
Appendectomy	25 (4.9%)	29 (3.8%)	1.296	0.750, 2.239	0.353

Note: PBC, Primary Biliary Cirrhosis. †, 95% Confidence interval.

Table 5. Menstrual and reproductive station of PBC cases and controls

	Cases No, (%)	Controls No, (%)	Odds Ratio	95% CI†	*P* value
Mean age at menarche	14.75±1.92	14.85±1.97	0.973	0.906, 1.045	0.452
Age at first pregnancy	23.97±3.32	24.35±3.11	0.963	0.922, 1.006	0.090
Number of live births	2.37±1.26	2.12±1.07	1.209	1.075, 1.360	0.002
Mean age at menopause	48.90±3.88	48.91±4.16	1.000	0.953, 1.049	0.989
Years of menstruation	33.80±4.13	33.68±4.86	1.006	0.962, 1.052	0.790
Menstrual periods (irregular)	32 (7.1%)	39 (6.0%)	1.196	0.737, 1.941	0.468
Dysmenorrhea	52 (11.6%)	64 (9.9%)	1.193	0.810, 1.758	0.372
Use of oral contraceptives	61 (13.6%)	78 (12.1%)	1.147	0.801, 1.642	0.454
Abnormal pregnancies‡	54 (12.0%)	23 (3.6%)	3.709	2.241, 6.140	<0.001
Twin pregnancy	11 (2.4%)	11 (1.7%)	1.452	0.624, 3.379	0.387
Pruritus during pregnancy	31 (6.9%)	16 (2.5%)	2.925	1.580, 5.415	<0.001
Menopause	199 (44.3%)	292 (45.1%)	0.968	0.760, 1.233	0.791
Hormone replacement therapy	36 (8.1%)	29 (4.5%)	1.867	1.127, 3.092	0.015
Uterine-incision delivery	53 (11.8%)	40 (6.2%)	2.031	1.322, 3.121	0.001

Note: PBC, Primary Biliary Cirrhosis. †, 95% Confidence interval. ‡, Consist of miscarriage, pre-and post-mature delivery during pregnancy.

irregular menstrual cycles, menopause, years of menstruation, proportion of menopause or oral contraceptives, twin pregnancy history were found between the cases and controls. However, nearly 11.8% female PBC cases suffered from uterine-incision delivery while only 6.2% in controls, *P*=0.001 (**Table 6**).

Variables that were significantly associated with PBC were included in the multiple logistic regression analysis (**Table 6**). In the model of psychological status, psychological stress (OR: 1.478; 95% CI: 1.038, 2.105) was significantly associated with an increased risk of developing PBC. In the lifestyle factors model, hair dye

Table 6. Multiple logistic regression analysis

	β	OR†	95% CI‡	*P* value
Psychological status				
Psychological stress	0.391	1.478	1.038, 2.105	0.030
Lifestyle factors				
The outdoor time (hour/day)	-0.074	0.928	0.879, 0.980	0.007
Hair dye usage	0.497	1.644	1.258, 2.147	<0.001
Medical station				
Allergy	0.523	1.688	1.169, 2.437	0.005
Urinary tract infection	0.803	2.232	1.182, 4.214	0.013
Surgery	0.396	1.486	1.092, 2.023	0.012
Menstrual and reproductive station				
Number of live births	0.212	1.236	1.083, 1.411	0.002
Abnormal pregnancies	1.051	2.860	1.645, 4.973	<0.001
Pruritus during pregnancy	0.991	2.695	1.273, 5.707	0.010

Note: PBC, Primary Biliary Cirrhosis. †, Odds Ratio, adjusted for age, sex, BMI. ‡, 95% Confidence interval. Dependent factors: PBC, yes and no; Independent factors including quality of sleeping; psychological stress; satisfaction of life; the daily hours spent outdoors; hair dye usage; allergy; inflammatory or autoimmune diseases; the above diseases in familial members; thyroid disease; rheumatoid arthritis; urinary tract infection; surgery; cholecystectomy; the number of live births; hormone replacement therapy; abnormal pregnancies; pruritus during pregnancy; uterine-incision delivery.

usage was significantly associated with PBC onset. However, the daily hours spent outdoors (OR: 0.928; 95% CI: 0.879, 0.980) turned out to be a protective factor in the development of PBC. Among medical conditions, allergy, urinary tract infection and surgery were independently associated with PBC. In the female menstrual and reproductive station model, the number of live births (OR: 1.236, 95% CI: 1.083, 1.411), abnormal pregnancy (OR: 2.860; 95% CI: 1.645, 4.973) and pruritus during pregnancy were significantly associated with PBC.

Discussion

We conducted the first large single-center case-control study on the risk factors for PBC in China. Our findings confirm the association reported in previously studies between PBC and hair dye usage, chronic inflammatory or autoimmune diseases, diseases in relatives, history of surgery and allergy as well as the number of live births [22], pruritus during pregnancy and abnormal pregnancies in the female menstrual and reproductive. Furthermore, we indentify psychological stress using CPSS scale as a risk factor independently associated with PBC. However, results of this study suggest a possible prophylactic role of outdoor exposure time on the risk of developing PBC.

The increased prevalence of PBC has been discovered in certain geographical locations in Northern Europe and Northern America [23]. And the latitude of the five provinces in this study ranges from 31° to 42° north, shared almost the same geographical range. Our findings may be complementary to PBC risk factors studies. And we also did the comparison of risk factors from four previous published PBC case-control studies with our own (**Table 7**).

We did the first investigation of personality psychology and the results indicate that high psychological stress, bad quality of sleep and low satisfaction of life were more common in PBC cases. Furthermore, psychological stress was independently associated with the risk of PBC in multivariable modeling. Psychological stress has been assumed as precipitating factor in several autoimmune diseases such as Graves' disease, type 1 diabetes mellitus and rheumatoid arthritis [15, 24, 25]. One hypothesis in immune and nervous system stated that psychological stress is associated with increased secretion of the adrenocorticotrophic hormone (ACTH) which may determine the immune suppression [26]. And Firdaus S noted that chronic stress can suppress protective immune response and/or exacerbate the pathological immune response [27]. We did not found an association between psychological stress and pathological stage in PBC patients (Supplementary Table 1). However, the sequence of high psychological stress and PBC onset is not clear. Unfortunately, we cannot provide details of stressful life event or psychological stress domains in both PBC cases and controls.

Importantly, the PBC cases spent fewer hours outdoors than the controls. The previous study from our group indicated the lower amount of serum 25-hydroxyvitamin Din patients before PBC diagnosis [28]. The correlation analyses in present study indicated that the more outdoor

Table 7. Comparison of odds ratios from published PBC case-control studies

	Howel	Gershwin	Prince	Corpechot	Lammert	China
PBC cases	100	1032	318 epidemiological cases; 2258 foundation cases	222	522	512
Controls	223	1041	2438	509	616	763
Hair dye usage	NA	NS	OR=1.8†; OR=1.3‡	NS	NA	OR=1.6
Allergy	NA	NA	NA	NA	NA	OR=1.7
Urinary tract infection	NS	OR=1.5	OR=2.4†; OR=1.7‡	OR=2.7§	OR=1.5	OR=2.2
Surgery	NA	NA	NA	OR=1.9	NS	OR=1.5
Cholecystectomy	NA	OR=1.8	NA	OR=1.8	OR=2.6	OR=1.7
Number of live births	NS	NS	NS	NS	NS	OR=1.2
Abnormal pregnancies	NS	NA	NS	OR=2.0¶	NA	OR=2.9
Pruritus during pregnancy	NS	NA	NS; OR=2.1‡	OR=3.9	OR=2.0	OR=2.7

Note: PBC, Primary Biliary Cirrhosis. †OR compared to epidemiological cases; ‡OR compared to foundation cases. §Recurrent urinary tract infection; ¶Abortion or EUP, in this study the proportion of miscarriage did not differ significantly; NS, Not statistically significant. NA, Not available for analysis.

exposure time, the higher serum 25-hydroxyvitamin D level in PBC cases. A meta-analysis conducted by our group implied that TaqI gene polymorphism in Vitamin D receptor was significantly associated with PBC [29]. And serum Vitamin D deficiency is associated with disease severity in PBC [28]. Ultraviolet exposure can increase the serum Vitamin D level in human body. Outdoor exposure time did not differ significantly in different seasons both in PBC cases and controls. There was no statistical difference between PBC pathological stages and outdoor exposure time either. After interaction multiple logistic regression, outdoor exposure time and psychological stress did not show interaction influence (Supplementary Table 1). This finding has not been reported previously and further study will be needed to confirm this novel finding and examine the potential mechanism in PBC development.

PBC cases reported significant lower BMI than the controls at the time of questionnaire in this study. And this anthropometric difference was consistent with two prior studies [10, 11]. Nearly 52.6% of patients used hair dye more than once per year, whereas the proportion in controls was only 37.5%. This finding supported the hypothesis that exposure to various chemical components of hair dye may confer an elevated risk for PBC. Previous studies suggested that smoking or second-hand smoking [10, 11, 30], but our findings did not confirm a significant increased risk either in smoking history or history of passive smoking. We did not identify any association between alcohol drinking [12], coffee and tea intake and PBC in present study.

We examine the medical condition in PBC cases and controls. The observed high rate of theallergy, chronic inflammatory or autoimmune diseases and diseases in family members in PBC cases strongly support the hypothesis that genetic background is crucial in establishing predisposition to PBC. Similar to previous studies, our data indicated that PBC is mostly associated with multiple sclerosis, thyroid disease and rheumatoid arthritis [11, 31]. Urinary tract infection was independently associated with PBC, and this finding support the role of infectious agents, maybe through the molecular mimicry mechanism, in the development of PBC [32]. Patients with PBC reported use of long-term medication such as antihypertensive, antidiabetic drugs and hypolipidemics more frequently than controls. PBC cases reported significantly higher prevalence of surgery, and higher rate of cholecystectomy [10, 31]. The information on medical history is based on self-reported, and we are failed to confirm the responses by laboratory tests so that misclassification of exposure must be considered.

Finally, we investigate the menstrual and reproductive factors of both PBC and controls women. The association of number of live births and PBC is consistent with the result of Annarosa F [22] and supports the proposed role for fetal microchimerism in the pathogenesis of autoimmunity. There were three studies indicated that the number of live births was higher in PBC cases, but the difference did not reach the statistical level [10, 11, 33]. Similar to previous studies, PBC cases reported higher prevalence of pruritus during pregnancy and abnormalities of pregnancy which consist of pre- or post-mature delivery, miscarriage or abortion. We found that uterine-incision delivery was more common in PBC cases

when compared with controls. Nevertheless, the delivery method did not only depend on the special complications during pregnancy in China, this confounding factor should take into consideration in the interpretation of this finding. We did find that hormone replacement therapy was more common among PBC cases, but not significantly so in multivariable model. We did not find evidence of an association between oral contraceptives and PBC, while previous study suggested that longer use of oral contraceptive may be protective against PBC [10]. Data in this study indicate that age at menarche or menopause, menstrual period, dysmenorrheal, twin pregnancy and age at first pregnancy were not different between PBC cases and controls.

This observational study has some limitations. The majority of participants came from one tertiary medical center and the sample size in this present study was comparatively moderate. The selection bias of participant resource cannot be excluded and must be considered in the interpretation of the results. The relatively low rate of female smoking in China would tend to underestimate the association of smoking in PBC as previous studies reported. And the statistical method applied in this study may indicated the associated risk factors of PBC which need further investigation. Finally, this study suffers from the recall bias because of the questionnaire method applied during investigating process.

In conclusion, this case-control study reports on the association between various risk factors and PBC from a tertiary medical center. This present study strongly confirm formerly reported risk factors of PBC including hair dye use, surgery history, autoimmune or inflammatory disease, pruritus during pregnancy, number of live births and abnormal pregnancies. We have also identified psychological stress as a risk factor whereas outdoor exposure time as a protective factor. These findings have not been studied in current study of PBC risk factors and thus warrant further systematic investigation. Furthermore, study focus on interactions of genetic and environmental factors may investigate PBC risk factors more completely.

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Disclosure of conflict of interest

None.

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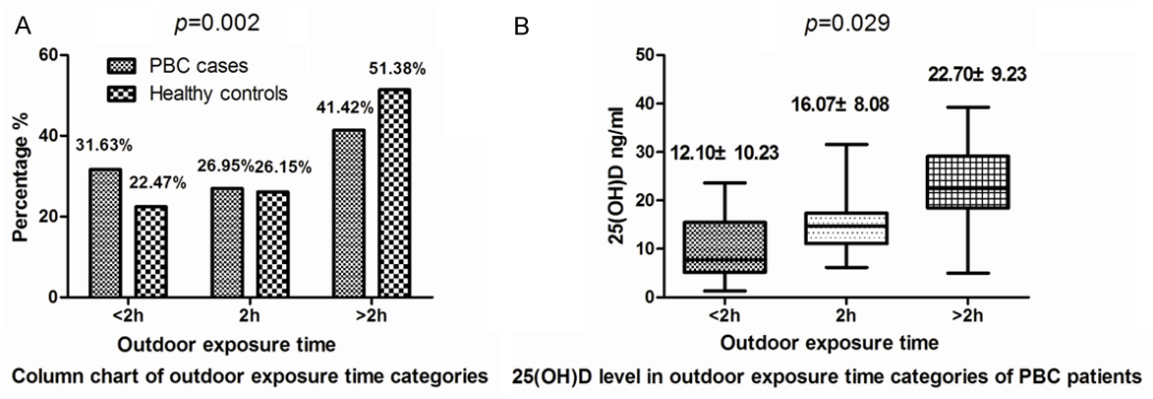

Supplementary Figure 1. Outdoor exposure time categories in two groups and 25(OH)D level in PBC patients. A: Column chart of outdoor exposure time categories. In category of less than 2 hours outdoor exposure time, there were 31.63% in PBC cases versus 22.47% in healthy controls; 26.15% of PBC cases versus 26.95% of healthy controls in category of 2 hours; and 41.42% of PBC cases versus 51.38% of healthy controls in category of more than 2 hours. Mann-Whitney U test was applied to analyze the distribution of these three categories between PBC cases and healthy controls, and *P*=0.002. B: 25(OH)D level in outdoor exposure time categories of PBC patients. The serum 25(OH)D level of PBC patients in category of less than 2 hours outdoor exposure time was 12.10±10.23, in 2 hours category was 16.07±8.08, and in more than 2 hours category was 22.70±9.23, *P*=0.029.

Supplementary Table 1. Correlation analyses between outdoor exposure time, psychological stress and 25-hydroxyvitamin D, pathological stage

	Correlation coefficient	*P* value
25-hydroxyvitamin D and Outdoor exposure time	0.445	0.006
25-hydroxyvitamin D and psychological stress	-0.152	0.336
Psychological stress and Outdoor exposure time	-0.030	0.617
Pathological stage and outdoor exposure time	0.053	0.231
Pathological stage and psychological stress	-0.083	0.060

Supplementary Table 2. Correlation analyses between outdoor exposure time, psychological stress and season, pathological stage

	Outdoor exposure time	r	*p* value	High psychological stress	r	*p* value
Season		0.073	0.219			
Winter-Spring	1, 3†					
Sumer-Autumn	1, 3†					
Pathological stage		-0.083	0.231		0.053	0.06
I, II	1, 3†			48 (9.38%)		
III, IV	1, 3†			61 (11.91%)		

Note: †Outdoor exposure time expressed as quartile, (P_{25}, P_{75}).