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Anxiety and small intestinal bacterial overgrowth: associations with recurrent aphthous ulcers

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Abstract

Background Recurrent aphthous ulcer (RAU) is the most prevalent oral mucosal disease, yet its etiology remains unclear. Anxiety and depression have been linked to the onset of RAU, but research findings were contradictory. The association of intestinal diseases with RAU implies a potential role of gut microbiota in the development of this condition. This study aims to explore the correlation between the presence and severity of RAU and psychological factors, as well as gut microbiota dysbiosis.

Methods The Zung's self-rating anxiety scale (SAS), Zung's self-rating depression scale (SDS), and Pittsburgh sleep quality index (PSQI) were used to assess the participants' psychological status. The lactulose hydrogen-methane breath test was performed to detect the presence of small intestinal bacterial overgrowth (SIBO) in RAU patients. The long-term severity of RAU is quantified using the monthly number of ulcers. Compare the differences in outcomes between individuals with RAU and the healthy population, and explore the factors influencing the severity of RAU.

Results Forty-nine patients and 49 controls were included. The RAU group had significantly higher SAS scores ($t=2.18$, $p=0.034$), and SIBO positivity ($\chi^2=75.67$, $p<0.001$). Factors correlated with the monthly number of ulcers included SAS score ($r=0.52$, $p<0.001$), symptoms of anxiety ($r=0.42$, $p=0.004$), SDS score ($r=0.46$, $p=0.002$), PSQI score ($r=0.35$, $p=0.020$), and SIBO positivity ($r=0.42$, $p=0.005$). Multiple linear regression analyses indicated that anxiety and SIBO may influence the severity of RAU. Moreover, SAS score ($r=0.38$, $p=0.010$) and SDS score ($r=0.38$, $p=0.009$) exhibited correlations with SIBO.

Conclusions RAU patients are at a higher risk of anxiety and gut microbiota dysbiosis, which could potentially escalate the severity of RAU. The role of the brain-gut axis in the pathogenesis of RAU warrants further exploration.

Keywords Recurrent aphthous ulcers, Anxiety, Psychology, Gut microbiota dysbiosis, Small intestinal bacterial overgrowth

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Background

Recurrent aphthous ulcers (RAU) are characterized by the recurrent emergence of painful, round, shallow ulcers. These lesions feature well-defined erythematous margins and a yellowish-gray pseudomembranous center [1]. RAU exhibit a global prevalence ranging from 5 to 66% [2, 3], with an approximate rate of 20% in China [4]. Numerous factors influence the onset of RAU [1, 5], with many emphasizing the role of immune regulation [6]. Currently, the mainstream clinical treatment for recurrent RAU involves the use of immunomodulatory drugs. Different guidelines recommend different oral medications, mainly including glucocorticoids, thalidomide, and colchicine. However, the efficacy is not significant, primarily because RAU continues to recur after the discontinuation of these medications [7]. On the other hand, long-term use of glucocorticoids can induce immunosuppression and osteoporosis [5]. Thalidomide leads to drowsiness in about one-third of individuals, peripheral neuropathy in 17% of cases, and its teratogenic effects severely restrict its use in female patients [8]. Colchicine carries a risk of multi-organ damage and can penetrate the placental barrier. Exploring other triggers and treatment modalities for RAU is crucial to enhance long-term treatment outcomes and avoid adverse reactions associated with immune-modulating drugs. In recent years, systemic factors such as psychological and digestive abnormalities have received increased attention.

Anxiety and depression affect the hypothalamic-pituitary-adrenal (HPA) axis, resulting in increased levels of salivary and plasma cortisol [9]. This elevation leads to a rise in local leukocytes and inflammatory factors such as TNF-α and IL-1 [10, 11]. Furthermore, anxiety disrupts the balance between oxidants and antioxidants, contributing to oxidative stress and the onset and persistence of RAU [12]. Depression and stress may cause patients to habitually bite their oral mucosa, increasing local irritation and the likelihood of ulcer formation [13]. Previous researches have explored the impact of anxiety or depression on RAU, Polat [9] found a correlation between RAU and depression, instead of anxiety, using the Hamilton's anxiety/depression rating scale. Nevertheless, Mazzoleni [14] employed the same method and arrived at a completely opposite conclusion. Studies utilizing different assessment scales have also led to inconsistent findings [14, 15].

Sleep disorders directly affect the circadian rhythm of cortisol and growth hormone secretion [16], which influences not only fibroblast proliferation and keratinocyte migration but also T-cell differentiation [17]. Sleep deprivation has been found to increase levels of pro-inflammatory cytokines, cortisol, and adrenocorticotropic hormones, while disturbing the balance between oxidants and antioxidants [18]. These changes can contribute to the onset of RAU and impede the healing process. Fewer studies have explored the relationship between sleep and RAU, suggesting that short sleep duration and poor sleep quality may potentially impact the onset of RAU [19, 20].

On the other hand, in patients with RAU, a significant proliferation of Escherichia *coli* has been observed on the oral mucosa. Several studies indicated that RAU patients were more likely to exhibit dysbiosis in the gut microbiota [21]. Individuals infected with Helicobacter *pylori* [22] and those diagnosed with celiac disease [23] are at increased risk of developing RAU. This highlights a potential link between changes in gut microbiota and RAU. However, in clinical settings, it is notable that most RAU patients do not exhibit obvious gastrointestinal disorders; rather, they commonly experience symptoms such as abdominal discomfort and diarrhea.

The small intestinal bacteria are essential components of the gut microbiota and play a crucial role in maintaining a healthy microbial balance in the intestine. Small intestinal bacterial overgrowth (SIBO) primarily refers to alterations in the composition and/or increased abundance of bacterial species in the small intestine, leading to gastrointestinal symptoms such as abdominal pain, diarrhea, constipation, and bloating [24, 25].

The role of the brain-gut axis or microbiota-gut-brain axis is being increasingly uncovered. Immune cells in the intestines and brain continuously monitor environmental stimuli, triggering responses that provide insights into the body's physiological condition [26]. This may represent a research direction worthy of exploration in understanding the etiology of RAU. Based on previous research and extensive clinical observations, we crafted this study from clinical perspectives to examine the disparities in psychological and gastrointestinal conditions between patients with RAU and the general population, and the impact of these conditions on the severity of RAU.

Methods

Study design

This study was conducted in accordance with the 2013 revision of the Declaration of Helsinki and was approved by the Biomedical Ethics Committee of Peking University Stomatological Hospital with the approval number PKUSSIRB-20,216,201,420,013. Informed consents were obtained from all individual participants included in the study. The study design followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (https://www.strobe-statement.org), as shown in Supplementary Table S1.

The cases for this study were recruited from patients with RAU and their family members seeking treatment at the Department of Oral Mucosa, Peking University Stomatological Hospital, between June 2021 and June 2023. Data collection included participants' age, gender,

body mass index (BMI), cigarette use, alcohol use, stool characteristics according to the Bristol Stool Form Scale [27], and psychological status (self-perceived anxiety or depression). Patients were instructed to discontinue oral medications upon enrollment in the study, and for ulcer episodes, they were permitted to use topical medication Tong Ren Tang Oral Ulcer Powder (Tong Ren Tang Pharmaceutical Factory, Beijing Tong Ren Tang Co., Ltd., GYZ11020184, 3 g/bottle). The number of oral ulcers per day was recorded using a diary. After one month, a follow-up visit was conducted to assess anxiety, depression, and sleep quality of both patients and their family members. The severity of RAU in patients, as well as their oral hygiene status (periodontal probing depth and decayed-missing-filled tooth), and the presence or absence of SIBO were also examined.

Participants and recruitment

Patients

Inclusion criteria:

(1) Male or female individuals aged between 18 and 85 years; (2) Clinically diagnosed with RAU.

The diagnosis of RAU was based on medical history and clinical manifestations [28, 29]. The diagnosis was confirmed by experienced specialists in oral mucosal disease.

Exclusion criteria:

(1) Individuals unwilling or uncooperative with the tests; (2) Patients with other identified oral mucosal diseases; (3) Patients with systemic diseases, such as anemia, immunodeficiency diseases, autoimmune diseases, malignant tumors, severe cardiovascular and cerebrovascular diseases; (4) Patients taking steroids or immunosuppressive drugs; (5) History of severe allergies; (6) Family history of RAU; (7) Patients with traumatic ulcers or clear traumatic factors in the oral cavity.

Control

The control group consisted of healthy accompanying individuals without a history of RAU or systemic diseases, with preference given to spouses, in order to minimize the impact of different diet and living environments on psychological status and gastrointestinal function.

Psychological questionnaire

The anxiety and depression levels of the participants were assessed using Zung's self-rating anxiety scale (SAS) [30] and self-rating depression scale (SDS) [31]. Please refer to supplementary Tables 2 and Table 3 for detail information. A higher score indicates a higher level of anxiety or depression in the respondent. Recent research suggests setting the diagnosing threshold at 50 for SAS and 55 for SDS to achieve a balance between sensitivity and specificity [32, 33]. Scores above these thresholds indicate symptoms of anxiety or depression.

As sleep is related to emotions, we conducted a sleep quality assessment using the Pittsburgh sleep quality index (PSQI) on the participants (https://sleep.pitt.edu/instruments/#psqi). Higher scores indicate poorer sleep quality [34].

SIBO testing

Breath test is currently the most widely used method for SIBO detection in clinical practice [35]. Patients underwent a lactulose hydrogen-methane breath test following the guidelines set by the North American Consensus [36].

One month prior to the examination, no enemas or colonic washes should be performed; four weeks before the examination, no antibiotics or probiotics should be taken; one week before the examination, no laxatives, fiber supplements, or stool softeners should be used; forty-eight hours before the examination, high-carbohydrate foods and snacks should be avoided; and 12 h before the examination, no food should be consumed, including clear liquids, gum.

The lactulose hydrogen-methane breath test took place at the Sixth Medical Center of PLA General Hospital, using the QuinTron apparatus from the QuinTron Instrument Company in the USA. Initially, the patients' fasting H2 and CH4 levels were assessed before they were given 10 g of lactulose mixed with a cup of water. Subsequently, the levels of exhaled hydrogen and methane were tracked every 30 min over a total period of 210 min. Considering that patient cooperation (such as the duration of expiration and meeting the required volume of expiration) may influence test results, we had professional staff supervising subjects throughout the test to ensure compliance. Data analysis is automatically generated by the instrument, which is regularly maintained by trained professionals. The diagnostic criteria for SIBO included an increase in hydrogen level of ≥ 20 ppm from the baseline within 90 min and a methane level of ≥ 10 ppm detected during the test [37, 38]. Supplementary Figure S1 presented the cases categorized as SIBO-negative and SIBO-positive.

Evaluation of RAU severity

The severity of RAU was evaluated based on two aspects: the severity of long-term recurrent episodes experienced by the patient and the severity of the ulcers at the time of consultation.

Monthly Number of Ulcers [7]: This parameter represents the total number of oral ulcers per day over a month, reflecting both the frequency of occurrences and the quantity of ulcers during each episode.

The severity assessment at the time of consultation included:

Area of Ulcers: This measure is the sum of all oral ulcers' areas, determined by clinical practitioners using small-scale graduated ruler strips.

Pain: Pain levels were assessed using the Visual Analogue Scale (VAS). Patients self-rated their pain on a scale from 0 to 10, where 0 indicated no pain and 10 represented the highest pain level.

Study size

Given the lack of research on the correlation between SIBO and RAU, and recent studies indicating that RAU patients were more prone to anxiety without a significant correlation with depression [14]. We considered anxiety as a factor in calculating the sample size. Based on a recent study in a large sample of Chinese individuals [39]. Considering $\alpha = 0.10$, $\beta = 0.20$, and a 20% dropout rate, the calculated sample size per group was 49 cases.

Statistical methods

SPSS 26.0 software was used for statistical analyse, and $p < 0.05$ was considered statistically significant. The paired t-test, Wilcoxon non-parametric test, and. chi-square test or Fisher's exact test was used to assess differences between the two groups. For correlation analysis, different tests were employed based on the nature of variables. Various correlation coefficients such as Cramer's V, Kendall's tau, Pearson, Point-Biserial, and Spearman correlation coefficient were utilized. Multiple linear regression analysis was used, where variables with potential collinearity were not included simultaneously, and the p-values of the variables included in the equation were required to be less than 0.1.

Results

Participants

During the recruitment of participants from the outpatient department, a total of 297 RAU patients who met the inclusion criteria were identified. However, many patients declined SIBO testing as it required visiting another hospital. Consent for participation in this study was obtained from 49 patients and their families, and ultimately, SIBO testing was completed for 45 patients. Figure 1 showed a detailed flowchart of participant inclusion.

RAU patients were more prone to experiencing negative emotions and abnormal bowel movements

The average age of the RAU group was 43.55 ± 13.49 years, with males accounting for 46.94%. The BMI was 22.43 ± 3.33, with 4 individuals having a history of smoking and 10 individuals having a history of alcohol consumption. There were no significant differences observed between the RAU group and the control group in these characteristics.

Among the RAU patients, 40.82% had normal stool morphology (sausage-shaped), which was lower than the control group ($\chi^2 = 9.32$, $p = 0.002$). Additionally, 30.61% of RAU patients reported that their stool shape tended to be nuts-shaped or sausage-shaped but lumpy, which was significantly higher than the control group ($\chi^2 = 4.91$, $p = 0.027$); Fourteen (28.57%) RAU patients had pasty or watery stool, which was more than the control group with 8 individuals (16.33%), but the difference was not statistically significant.

In terms of negative emotions, 28.57% of RAU patients reported feeling anxious, which was significantly higher than the control group ($\chi^2 = 6.81$, $p = 0.009$). Although 5 RAU patients (10.20%) reported feeling depressed, which was higher than the control group with 2 individual (4.08%), the observed difference did not reach statistical significance. Table 1 showed more details.

RAU patients exhibited higher SAS scores and positive rates of SIBO

The SAS score in the RAU patient group was 40.83 ± 8.14, which was higher than the control group's score of 37.11 ± 7.16 ($t = 2.18$, $p = 0.034$). According to the SAS scoring, 5 RAU patients had symptoms of anxiety, which was more than the control group where no individuals showed ($p = 0.056$). There were no significant differences observed between the two groups in terms of SDS scores, symptoms of depression, and PSQI scores. See Table 2.

Out of the 45 RAU patients tested, 66.67% ($n = 30$) had positive SIBO results. Due to ethical considerations and the unique circumstances of the COVID-19 pandemic, the control group was not subjected to SIBO testing. A recent study conducted at the same center utilizing the same SIBO detection method included 50 participants in the healthy control group [40]. The average age was 40 ± 5.18 years, revealing a SIBO positivity rate of 18% among the healthy population, which was significantly lower compared to the RAU patient group ($\chi^2 = 23.18$, $p < 0.001$).

The severity of RAU was associated with psychological and SIBO conditions

Correlation analyses were conducted to examine the relationship between the severity of RAU and various factors. Factors correlated with the monthly number of ulcers included SAS score ($r = 0.52$, $p < 0.001$), symptoms of anxiety ($r = 0.42$, $p = 0.004$), SDS score ($r = 0.46$, $p = 0.002$), PSQI score ($r = 0.35$, $p = 0.020$), and SIBO positivity ($r = 0.42$, $p = 0.005$). The remaining variables exhibited a lack of statistically significant correlation with the monthly number of ulcers. No variables showed significant correlations with the severity of ulcers at the time

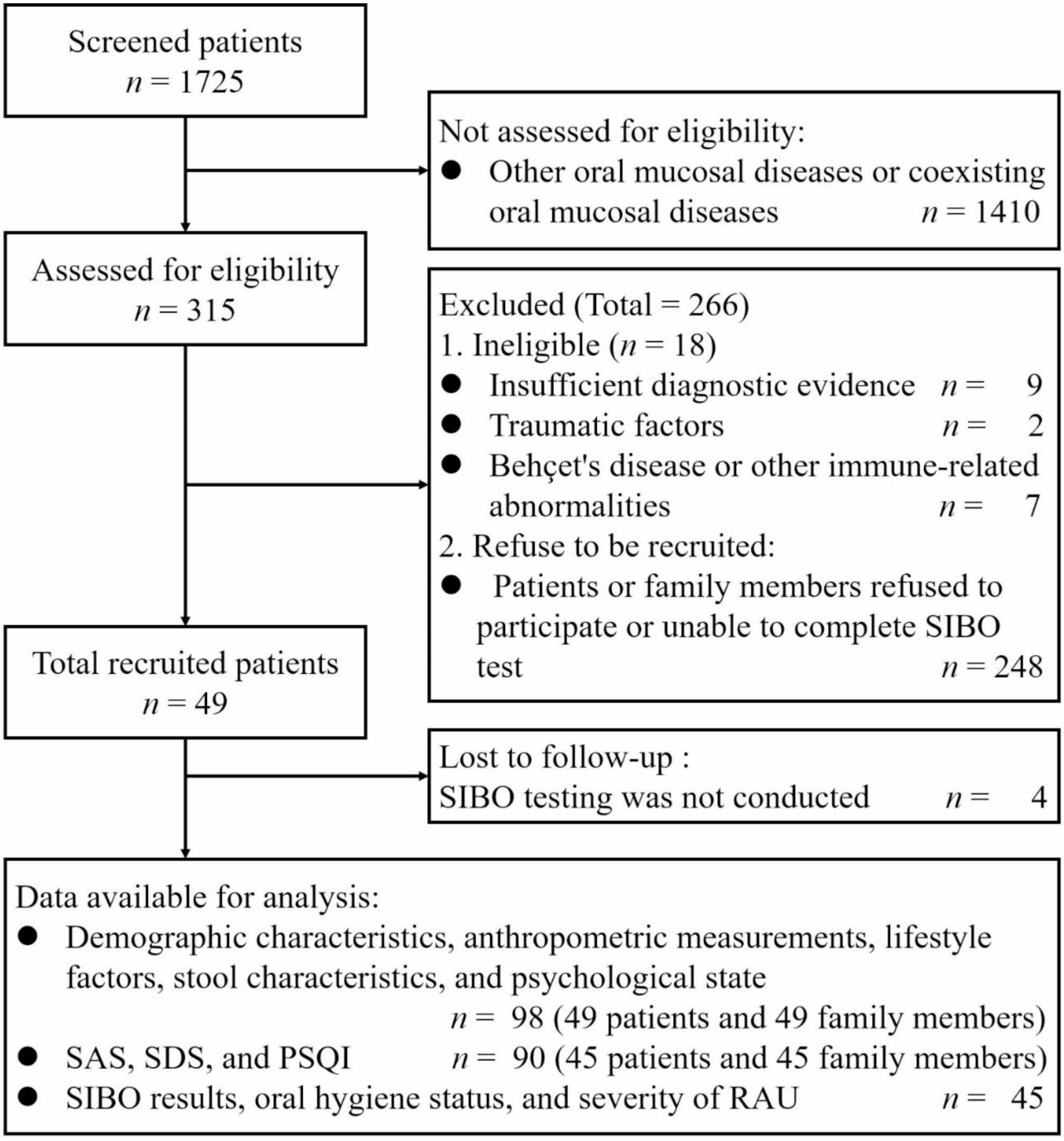

Fig. 1 Participants flowchart. A total of 98 participants were enrolled in the study, with 45 RAU patients undergoing SIBO testing. SAS, self-rating anxiety scale; SDS, self-rating depression scale; PSQI, Pittsburgh sleep quality index; SIBO, small intestinal bacterial overgrowth; RAU, recurrent aphthous ulcer

of consultation (area of ulcers and pain). Please refer to Table 3 for more details.

Anxiety and SIBO May influence the severity of RAU

The monthly number of ulcers was designated as the dependent variable. Age, gender, BMI, SDS score, PSQI score, and SIBO positivity were considered as independent variables. Two separate multiple linear regression analyses were conducted, with SAS score and symptoms of anxiety serving as the independent variables in each analysis.

In the model including SAS score and filtered variables, the variables ultimately included in the equation were SAS score ($B = 2.97$, $t = 2.57$, $p = 0.014$) and SIBO positivity ($B = 33.48$, $t = 1.70$, $p = 0.097$). In the model including symptoms of anxiety and filtered variables, the variables

Table 1 Demographic and clinical characteristics

	RAU patients (*N* = 49)	Control (*N* = 49)	*P* value
Age, y	43.55 ± 13.49	43.00 ± 15.27	0.881
Male, n (%)	23 (46.94%)	23 (46.94%)	>0.99
BMI	22.43 ± 3.33	22.28 ± 2.12	0.789
Cigarette use, n (%)	4 (8.16%)	10 (20.41%)	0.083
Alcohol use, n (%)	10 (20.41%)	13 (26.53%)	0.247
Sausage-shaped, n (%)	20 (40.82%)	35 (71.42%)	0.002
Nuts-shaped or sausage-shaped but lumpy stool, n (%)	15 (30.61%)	6 (12.24%)	0.027
Pasty or watery stool, n (%)	14 (28.57%)	8 (16.33%)	0.223
Self-perceived anxiety, n (%)	14 (28.57%)	4 (8.16%)	0.009
Self-perceived depression, n (%)	5 (10.20%)	2 (4.08%)	0.436

Data were present as the number of subjects (%) or mean ± standard deviation. The paired t-test, chi-square test, and Fisher's exact test was used to assess differences between the two groups. RAU, recurrent aphthous ulcer; BMI, body mass index

Table 2 Comparison of psychological and SIBO conditions between the RAU and control group

	RAU patients (*N* = 45)	Control (*N* = 45)	*P* value
SAS score	40.83 ± 8.14	37.11 ± 7.16	0.034
Symptoms of anxiety	5 (11.11%)	0	0.056
SDS score	40.58 ± 9.77	39.84 ± 6.68	0.683
Symptoms of depression	3 (6.67%)	0	0.242
PSQI score	7.18 ± 3.44	6.51 ± 2.70	0.379
SIBO positivity, n (%)	30 (66.67%)	9/50 (18.0%)	<0.001

Data were present as the number of subjects (%) or mean ± standard deviation. The paired t-test, Wilcoxon non-parametric test, chi-square test, and Fisher's exact test was used to assess differences between the two groups. RAU, recurrent aphthous ulcer; SAS, self-rating anxiety scale; SDS, self-rating depression scale; PSQI, Pittsburgh sleep quality index; SIBO, small intestinal bacterial overgrowth

ultimately included in the equation were symptoms of anxiety ($B = 91.20$, $t = 3.39$, $p = 0.002$) and SIBO positivity ($B = 37.65$, $t = 2.10$, $p = 0.042$). There was no significant collinearity among the independent variables in both equations (*variance inflation factor* = 1.17 or 1.07). As shown in Table 4.

Table 3 Analysis of factors correlated with the severity of RAU

	Monthly number of ulcers	Area of ulcers	VAS
SAS score	0.52**	0.13	0.51
Symptoms of anxiety	0.42**	0.08	0.06
SDS score	0.46**	0.05	-0.13
Symptoms of depression	0.28	0.04	-0.02
PSQI score	0.35*	0.2	0.22
SIBO positivity	0.42**	0.12	0.13
Gender	0.29	-0.13	-0.21
Age	0.02	-0.13	-0.17
BMI	-0.12	0.02	0.11
Cigarette use	0.01	-0.17	-0.13
Alcohol use	-0.06	-0.09	-0.05
Periodontal probing depth	0.13	-0.18	-0.12
Decayed-missing-filled tooth	0.13	-0.17	-0.06
Nuts-shaped or sausage- shaped but lumpy stool	0.09	0.03	-0.06
Pasty or watery stool	0.09	-0.19	-0.12
Topical medication	0.20	0.27	0.13

The data presented in the table represents correlation coefficients, and the choice of the test method depends on the variable types, including Cramer's V, Kendall's tau, Pearson, Point-Biserial, and Spearman correlation coefficients. *$p < 0.05$, **$p < 0.01$. RAU, recurrent aphthous ulcer; VAS, visual analogue scoring; SAS, self-rating anxiety scale; SDS, self-rating depression scale; PSQI, Pittsburgh sleep quality index; SIBO, small intestinal bacterial overgrowth; BMI, body mass index

The correlation between psychological and SIBO conditions

Correlation analysis revealed a significant correlation between SAS score ($r = 0.38$, $p = 0.010$) and SDS score ($r = 0.38$, $p = 0.009$) with SIBO.

Discussion

This study represents the first exploration of the involvement of the brain-gut axis in the onset of RAU, aiding in the elucidation of RAU's etiology. Our case-control study demonstrated that patients with RAU had a higher likelihood of experience anxiety and abdominal symptoms, as indicated by both subjective descriptions and objective evaluations. Moreover, the cross-sectional study suggested that both anxiety and SIBO might contribute

Table 4 Multiple linear regression

Factor		B	SE	β	t	*p*	VIF
SAS and filtered variables [a]							
	Constant	-108.37	46.84		-2.31	0.026	
	SAS score	2.97	1.15	0.37	2.57	0.014	1.17
	SIBO positivity	33.48	19.72	0.24	1.70	0.097	1.17
Symptoms of anxiety and filtered variables [b]							
	Constant	-95.30	37.55		-2.54	0.015	
	Symptoms of anxiety	91.20	26.93	0.44	3.39	0.002	1.07
	SIBO positivity	37.65	17.95	0.27	2.10	0.042	1.07

Multiple linear regression analysis. *B*: Unstandardized coefficient, *SE*: Standard error, *β*: Standardized coefficient, *VIF*: Variance Inflation Factor; [a] $R^2 = 0.26$, *Adjusted* $R^2 = 0.23$, $F = 7.52$, $p = 0.002$; [b] $R^2 = 0.33$, *Adjusted* $R^2 = 0.30$, $F = 10.35$, $p < 0.001$. SAS, self-rating anxiety scale; SIBO, small intestinal bacterial overgrowth

to an increased frequency and greater number of RAU occurrences. These results indicate that for patients with RAU, clinicians should pay attention to inquiring about abnormal bowel movements and psychological states, particularly anxiety symptoms. For patients with severe anxiety, referral to psychological outpatient services is recommended, while those with abnormal bowel habits should be screened for SIBO. Treating anxiety and SIBO can improve patient health and potentially alleviate or cure recurrent RAU. However, this necessitates further confirmation through large-scale cohort studies.

Factors influencing RAU mainly include local, systemic, immunologic, genetic, allergic, nutritional, microbial factors, hormone levels, and the use of immunosuppressive drugs [1, 5]. By implementing rigorous inclusion and exclusion criteria, ensuring age and gender matching between the two groups, selecting spouses as controls, and administering standardized topical medications, we were able to narrow down our investigation and concentrate on factors that are specifically associated with immunity, psychology, and bacterial influences on RAU.

Investigations solely focused on examining the relationship between anxiety or depression and RAU often reported significant correlations [15, 41, 42]. However, studies that simultaneously investigated both anxiety and depression predominantly supported anxiety's correlation with RAU rather than depression [9, 14, 43]. These findings aligned with our research results: the monthly number of ulcers correlated with SAS and SDS scores, but in multiple linear regression, factors influencing RAU did not include SDS scores. The reasons for this discrepancy may include population differences and sample sizes. Furthermore, anxiety may amplify negative emotions, leading patients with higher anxiety scores to also exhibit elevated depression scale scores. The correlation coefficient between symptoms of anxiety and RAU was significantly higher than that associated with depression. Therefore, we propose that anxiety is a more closely linked emotional factor to RAU.

In recent years, metagenomic association studies indicated that RAU patients were prone to experiencing dysbiosis in the gut microbiota, microbial functional impairments, and immune imbalances [21]. In the context of Crohn's disease, patients often exhibit elevated levels of Proteobacteria and inflammatory markers like IL-1β in their blood [44]. To date, inflammation is one of the few well-defined etiological factors for RAU, as IL-2, IFN-γ, and tumor necrosis factor-α (TNF-α) contribute to ulcer development [1]. Other studies observed that RAU patients exhibited significantly elevated serum inflammation levels compared to the general population [45].We utilized SIBO testing to assess gut microbiota dysbiosis, characterized by alterations in bacterial quantity or species in the small intestine [38], and found a significant correlation between gut microbiota dysbiosis and RAU.

The results of multiple linear regression indicated that the frequency and quantity of RAU might be influenced by anxiety and gut microbiota dysbiosis. Moreover, there was a correlation between anxiety, depression, and SIBO, suggesting that the brain-gut axis may have an impact on RAU. Recent studies suggested that cross-talk along the gut-brain axis played a regulatory role in inflammatory nociception, inflammatory responses, and immune homeostasis. In this process, inflammatory factors, such as interleukin-1β (IL-1β), IL-6, IL-17, and interferon gamma (INF-γ), released by intestinal immune cells were released systemically [26]. This suggests that the brain-gut axis could potentially influence the onset of RAU through inflammatory processes. On the other hand, the brain-gut axis may affect the oral mucosa's reparative capacity through a neuroendocrine-exocrine mode of peptide action [46]. To validate these findings, future research with larger sample sizes and investigations into the underlying mechanisms are warranted.

This study has several limitations that should be acknowledged. The latest research on the sensitivity and accuracy of breath testing for SIBO was a meta-analysis in 2020 that included 14 studies. The results indicated that the sensitivity of lactulose hydrogen-methane breath testing for detecting SIBO was 42%, with a specificity of 70.6% [47]. The studies included in the meta-analysis are dated and lack standardized criteria for positive diagnosis and patient preparation before testing. Addressing these issues, detailed testing protocols were outlined in the 2017 North American Consensus [36] and the 2020 American College of Gastroenterology clinical guidelines [48], ensuring that results obtained through adherence to these guidelines are more reliable. However, recent comments from experts in neurogastroenterology have raised concerns about the accuracy of breath tests for diagnosing SIBO and their correlation with disrupted gut microbiota [49]. While the aspiration of small bowel fluid for culture and bacterial count remains the prevailing gold standard for diagnosing SIBO, the convenience and reasonably accurate results provided by breath tests made them a more commonly utilized option in clinical settings [38].

As SIBO testing results may lack precision, conducting stool sample evaluations such as 16 S rDNA analysis, metagenomics, and metabolomics studies can accurately identify the bacteria causing disruption in the gut microbiota of RAU patients and the pathways affected. These approaches help provide support for accurate etiological exploration and treatment strategies.

Another important point to consider is that our findings are based on a Chinese population. Research outcomes may be influenced by race and genetics. The

higher prevalence of RAU in Asian populations may be associated with the race-specific HLA-B51 gene [50]. Furthermore, recent studies have found a decrease in the diversity and abundance of gut microbiota in Asian populations, predisposing them to intestinal-related diseases or chronic conditions [51]. Wilson [51] suggests a link to the Chinese diet being higher in fat and lower in carbohydrates, while Ang refuted this viewpoint, attributing the differences to racial variations [52]. Studies conducted in other regions may test the generalizability of these results.

Additionally, due to the observational design of this study, the findings indicate only correlation. Future prospective intervention studies are needed to establish causation.

Conclusion

RAU patients were more prone to experiencing anxiety, abnormal bowel movements, and SIBO. Anxiety and gut microbiota dysbiosis may contribute to more severe RAU. The brain-gut axis may play a role in the onset of RAU. In the future, large-scale multicenter prospective studies and basic research are needed to further investigate and confirm these findings.

Abbreviations

RAU	Recurrent aphthous ulcer
SIBO	Small intestinal bacterial overgrowth
BMI	Body mass index
SAS	Self-rating anxiety scale
SDS	Self-rating depression scale
PSQI	Pittsburgh sleep quality index
VAS	Visual analogue scoring
IL	Interleukin
INF	Interferon
TNF	Tumor necrosis factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-05998-0.

Supplementary Material 1

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Author contributions

Z.J. Liu, M.X. Lu, L.H. Cui, and H.W. Liu contributed to conception and design of the study. Z.J. Liu and M.X. Lu organized the database. Z.J. Liu and Y. Han performed the statistical analysis. S.F. Li, Q.Y. Guo, Y.T. Wang, X.Y. Liu, X. Wang, Z. Cheng, Q. Wang, and J. Jin screened possible subjects. J.L. Tang conducted SIBO testing. W. Wang analyzed the data. L.H. Cui verified the reports. Z.J. Liu wrote the first draft of the manuscript. Z.J. Liu and Y. Han prepared figures and tables. L.H. Cui, and H.W. Liu revised the manuscript critically for important intellectual content. All authors contributed to manuscript revision, and approved the submitted version.

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Data availability

Data is provided within the manuscript and supplementary information files.

Declarations

Ethics approval and consent to participate

This study was conducted according to the guidelines of the Declaration of Helsin, and approved by the Biomedical Ethics Committee of Peking University Stomatological Hospital with the approval number PKUSSIRB-202162014200013. Informed consents were obtained from all individual participants included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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