Non-negligible prevalence of focal lymphocytic sialadenitis in minor salivary glands of non-Sjögren's disease individuals

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Abstract

Objective

Focal lymphocytic sialadenitis (FLS) in minor salivary gland biopsy (MSGB) has long been regarded as a histologic hallmark of Sjögren's disease (SjD), but it can also occur in non-SjD individuals. This study aimed to define the prevalence of FLS in labial minor salivary glands of non-SjD individuals via both an autopsy study and a meta-analysis.

Methods

A total of 214 genotype-tissue expression (GTEx) volunteers was included in the autopsy study, and FLS in labial minor salivary gland was evaluated. A meta-analysis was also performed to comprehensively define the prevalence of FLS in labial minor salivary glands of non-SjD individuals.

Results

In the autopsy study of 214 GTEx volunteers, the frequency of FLS in labial minor salivary glands was 13.1%. GTEx volunteers aged 60 years and older demonstrated a greater prevalence of FLS compared to those volunteers younger than 60 years (20.3% vs. 9.7%, p=0.03). In the meta-analysis, a total of 8 eligible studies involving 917 labial minor salivary gland samples were included. The pooled prevalence of FLS in labial minor salivary glands of non-SjD individuals was 6.2%. In the subgroup analysis by gender, the pooled prevalence of FLS in labial minor salivary glands for female and male non-SjD individuals was 10.4% and 5.0%, respectively.

Conclusion

This study provides compelling epidemiological evidence for the considerably high prevalence of FLS in minor salivary glands of non-SjD individuals. The clinical significance of FLS should be cautiously considered when MSGB is used to confirm seronegative SjD.

Key words

Sjögren's disease, focal lymphocytic sialadenitis, autopsy, meta-analysis, minor salivary glands

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Introduction

Sjögren's disease (SjD) is a rheumatic disease characterised by pronounced immune cell infiltration in exocrine glands such as salivary and lacrimal glands (1, 2). SjD predominantly occurs in females, with a female/male ratio exceeding 10:1 and an estimated prevalence of approximately 10 individuals per 10,000 in the population (3, 4). Dysfunction of glands due to autoimmune damages can result in symptoms of dry eyes and mouth, which are the primary clinical features of SjD (1). SjD patients typically experience progressively worsening dryness of the eyes and mouth, but may also manifest involvement of other exocrine glands, affecting various organs and tissues such as skin, haematological system, muscles, lung, and kidney (5). SjD poses a challenging rheumatic disease in terms of both diagnosis and treatment (6, 7). Currently, there are several issues in the diagnosis of SjD, including diagnostic delay, misdiagnoses, and omissions (6, 8, 9). Many SjD patients already have severe glandular dysfunction or widespread systemic involvement by the time of diagnosis (8, 10). Early and accurate diagnosis of SjD remains extremely challenging in clinical practice, which has long been a clinical puzzle and research focus in the field of rheumatology (11, 12).

The diagnosis of SjD is primarily based on clinical dryness symptoms, serological autoantibodies, and histopathological examination of labial salivary gland tissues. Currently, SjD should fulfil these criteria in the 2016 classification criteria established by American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) (13). The 2016 ACR/EULAR classification criteria for SjD are primarily based on five parameters: positive anti-SSA/Ro antibodies, labial salivary gland biopsy showing focal lymphocytic sialadenitis (FLS), ocular staining score ≥5, Schirmer test ≤5mm/5min, and unstimulated whole saliva flow rate (UWS) ≤0.1ml/min. According to these criteria, the presence of FLS in labial salivary gland biopsy and positive anti-SSA/Ro antibodies are the major criteria, each scored 3 points, while the other three minor criteria are each scored 1 point. FLS, defined as a focus score no less than one inflammatory foci per 4 mm^2 of glandular tissue in minor salivary gland biopsy (MSGB), has long been regarded as a histologic hallmark of SjD (14). The identification of FLS through MSGB is deemed a key classification criterion, particularly in patients with negative anti-SSA/Ro antibodies (15, 16).

Some studies have suggested that FLS may occur in the absence of SjD. Previous studies have reported that none of the subjects without SjD showed focal lymphocytic infiltration in minor salivary glands (14, 17), whereas several later studies have found that some healthy subjects also could have FLS in minor salivary glands (18-20). Therefore, there is considerable heterogeneity in the findings of those studies on the proportion of FLS in non-SjD subjects, and the prevalence of FLS among non-SjD individuals has not been well defined. There is a necessity to perform a meta-analysis, which is crucial for synthesising evidence, increasing statistical power, and resolving discrepancies on the prevalence of FLS among non-SjD individuals. This study aims to define the prevalence of FLS in minor salivary glands of non-SjD individuals through both an autopsy study and a meta-analysis of all currently published studies.

Methods

Design and inclusion

A retrospective analysis of autopsy samples from genotype-tissue expression (GTEx) project (https://www.gtexportal.org/) was carried out. GTEx project is a public resource for studying tissue-specific gene expression, and samples were collected from non-diseased tissue sites (21). We included all available subjects with histopathology of minor salivary glands from GTEx. Histology images and demographic data of those included subjects were retrieved from GTEx project database in 2022. Microscopical examination of minor salivary glands from 214 subjects undergoing autopsy in GTEx project was analysed. The GTEx project has implemented exclusion criteria to ensure data qual-

ity, excluding individuals with previous radiotherapy, history of metastatic cancer, or high risk of exposure to hepatitis C virus (HCV), hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Despite the availability of age and gender data, comprehensive medical records or information regarding underlying diseases for the donors are not provided by the GTEx database (21).

Pathological assessment

In GTEx project, minor salivary glands were stained with haematoxylin & eosin (H&E) and then were evaluated under the microscope. For FLS assessment, we followed relevant recommendations for FLS assessment in the standardisation guideline of labial salivary gland histopathology proposed by Fisher *et al*. (22). Two pathologists with great expertise in the field evaluated the images, and the kappa values for intra-rater reliability and inter-rater reliability in the assessment of FLS were both over 0.90 in this study. Histological sections were examined, and of lymphocytes comprising more than 50 cells were counted. One inflammatory focus was defined as an aggregate of 50 or more mononuclear cell infiltrates, and focus score was defined as the number of inflammatory foci per 4 mm^2 of minor glandular tissues in MSGB (14, 23). The presence of at least one inflammatory focus comprising more than 50 mononuclear cell infiltrates in a representative section was considered as indicative of focal lymphocytic infiltration (14, 23). To reduce misestimation risk caused by the non-specific chronic sialadenitis (NSCS), lymphocytic infiltration that was adjacent to fibrosis, acinar atrophy or duct dilation but with no evidence of being adjacent to the normal parenchyma or normal appearing acini were not counted. For samples with at least one inflammatory focus, a focus score was then calculated. For samples without any inflammatory focus, the focus score was calculated as 0. FLS was defined as a biopsy with a focus score of no fewer than one inflammatory focus per 4 mm^2 of minor glandular tissues (13, 14). During the evaluation of the H&E-stained tissue sections, ectopic germinal centre-like structures were identified by a well-circumscribed immune cell infiltrate consisting of at least 50 mononuclear cells and the presentation of a lymphoid-like organisation which comprised a central dark zone and a peripheral light zone (24-27). This study mainly focused on FLS, and other non-focus score parameters such as fibrosis were not analysed in the present study. Besides, immunohistochemical parameters were also not assessed in this study.

Statistical analyses

Both categorical data of FLS and quantitative data of focus score were used in this study. Qualitative data or categorical data were shown as frequency and proportions, while quantitative data were shown as mean with standard deviation or median with interquartile range. Intergroup differences in categorical variables were analysed using the chi-square test or Fisher's exact test. Intergroup differences in quantitative variables were analysed using unpaired t test or ANOVA analysis. Statistical analyses were conducted using R (Version 3.6.1, The R Foundation for Statistical Computing) and GraphPad Prism (v. 8.0, GraphPad Software Inc., Boston, USA). *p*-values less than 0.05 were deemed to be statistically significant.

Meta-analysis

To comprehensively assess the prevalence of FLS among non-SjD individuals, we conducted a systematic review and meta-analysis to integrate all relevant studies on this topic. We performed a PubMed search for relevant studies, covering the period from the establishment of the database to December 2023, with no language restrictions. Additionally, we traced the references of relevant studies to identify additional potential literature. The search strategy included terms such as (Focus score OR Lymphocytic Foci OR Lymphocytic infiltration OR focal sialadenitis OR sialadenitis) AND (salivary glands OR salivary gland). This meta-analysis was conducted in adherence to the PRISMA guideline (28).

Inclusion criteria were as follows: 1) Clinical observational studies, such as cohort studies, cross-sectional studies, or case-control studies; 2) Participants included a minimum of 20 non-SjD individuals; 3) Reporting the prevalence of FLS in labial minor salivary glands, excluding lacrimal glands, submandibular glands, or parotid glands; 4) Using FLS definition criteria consistent with the current classification criteria for SjD; 5) Non-overlapping data reported when compared to other included studies; 6) Full-text reports were available for eligibility evaluation and data extraction. Studies not meeting these inclusion criteria were excluded.

Two independent researchers extracted data from the eligible studies, including author information, country, sample size, FLS assessment methods, and corresponding statistical data. Any discrepancy in the extracted data between the two researchers was resolved through group discussion. The quality assessment of studies was carried out using the Newcastle-Ottawa Scale (NOS). Studies were excluded if they had low quality (29).

The heterogeneity of the included studies was assessed using the I^2 statistic, with an I^2 exceeding 50% indicating significant heterogeneity (30). To mitigate the impact of heterogeneity on the pooled results, we employed the DerSimonian-Laird random-effects model for the meta-analysis (31). Statistical analysis was conducted using R (Version 3.6.1, The R Foundation for Statistical Computing). *p*-values less than 0.05 were considered to be statistically significant.

Results

Prevalence of FLS in labial minor salivary glands of GTEx volunteers

The age of 214 GTEx volunteers ranged from 21 to 70 years, and 150 of them were men (Table I). Among 214 labial minor salivary gland samples from GTEx project, 43 (20.1%) samples had at least one inflammatory focus (an aggregate of 50 or more mononuclear cell infiltrates), and 25 (11.7%) samples had at least two discrete inflammatory foci. The focus score of those 43 minor salivary gland samples was then calculated out. According to the FLS definition, a focus score of no less

Table I. Prevalence of FLS among 214 GTEx volunteers and different groups divided by age and gender.

Study	Number	FLS	Percentage (%)	*p*-value
Total	214	28	13.1%	
Gender				0.25
Women	64	11	17.2%	
Men	150	17	11.3%	
Age group divided by 10-year intervals				0.23
21-29	20	2	10.0%	
30-39	17	1	5.9%	
40-49	41	6	14.6%	
50-59	67	5	7.5%	
60-70	69	14	20.3%	
Age group divided by the 60-year threshold				0.03
21-59	145	14	9.7%	
60-70	69	14	20.3%	

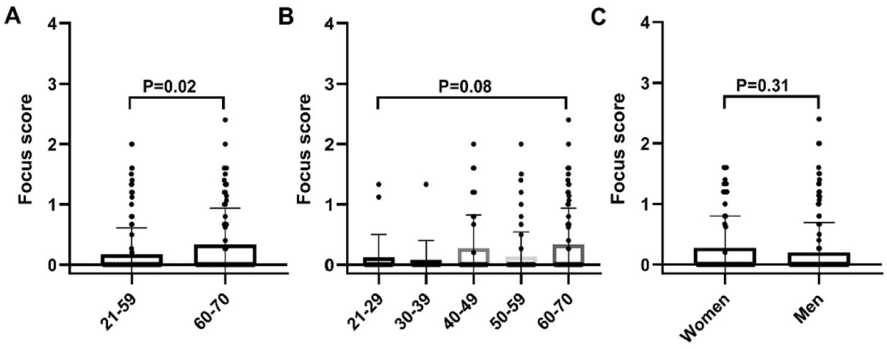

Fig. 1. Individual subject data for focus score in minor salivary tissues and comparisons between groups divided by age and gender.
A: Comparison of the focus score of minor salivary tissues between two age groups divided by the 60-year threshold;.
B: Comparison of the focus score in minor salivary tissues across five distinct age groups divided by 10-year intervals;
C: Comparison of the focus score in minor salivary tissues between groups divided by gender.

than one inflammatory focus per 4 mm^2 of glandular tissue in MSGB, FLS was found in 28 minor salivary gland samples. Therefore, in the autopsy study of 214 GTEx volunteers, the prevalence of FLS in labial minor salivary glands of non-SjD individuals was 13.1%. In those 28 samples with FLS, most focus lymphocytic infiltrates were found in the periductal area, which was a pattern similar to that of SjD patients. Ectopic germinal centre-like structures are relatively common in salivary gland tissues from SjD patients, and its presence in minor salivary gland tissues was assessed in this study. Among 28 minor salivary gland samples with FLS, only 5 samples (17.9%) had evidence of ectopic germinal centre-like structures, while the others did not have ectopic germinal centre-like structures.

The percentages of positive FLS findings in minor salivary tissues among GTEx volunteers with different ages were as follows: 10.0% for those aged 21–29 years, 5.9% for those aged 30–39 years, 14.6% for those aged 40–49 years, 7.5% for those aged 50–59 years, and 20.3% for those aged 60–70 years. Volunteers aged 60 years and older exhibited a higher percentage of positive FLS findings in minor salivary tissues compared to those younger than 60 years (20.3% *vs*. 9.7%, p=0.03; Table I). Therefore, there was no significant increase in the prevalence of FLS among GTEx volunteers until those aged 60 years and older, but GTEx volunteers aged 60 years and older demonstrated a greater prevalence of FLS compared to those volunteers younger than 60 years. Additionally, the focus score in minor salivary tissues was significantly higher in volunteers aged 60 years and older compared to those younger than 60 years (p=0.02) (Fig. 1).

Among 64 labial minor salivary gland samples from female volunteers, 11 samples had FLS, the prevalence of which in minor salivary glands of female GTEx volunteers was 17.2%. Among 150 labial minor salivary gland samples from male volunteers, 17 samples had FLS, the prevalence of which in minor salivary glands of male GTEx volunteers was 11.3%. There was no obvious difference in the percentage of positive FLS finding in labial minor salivary tissues between female and male GTEx volunteers (p=0.25). There was also no obvious difference in the focus score in labial minor salivary tissues between female and male GTEx volunteers (p=0.31) (Fig. 1-C).

Among those 28 subjects who had FLS in labial minor salivary glands, 25 had histology images of thyroid tissues, and signs of organ autoimmune involvement in those thyroid samples were further evaluated. Among those 25 thyroid samples, only 3 (12.0%) had focal lymphocytic deposits and possibly had Hashimoto's thyroiditis, while the left 22 samples (88.0%) did not have obvious lymphocytic aggregates likely Hashimoto's thyroiditis.

Meta-analysis of the prevalence of FLS in labial minor salivary glands of non-SjD individuals

Through a systematic literature search on the prevalence of FLS in minor salivary glands of non-SjD individuals in PubMed, 4505 abstracts were identified. After the screening of titles and abstracts, 4482 studies were excluded based on the irrelevance to FLS in non-SjD individuals, and 23 full-text articles were assessed for eligibility according to predefined criteria. Of these 23 full-text articles, 16 studies were excluded due to reasons such as using major salivary gland samples but not labial minor salivary glands, containing overlapping data from other included studies and lack of full-text report for eligibility assessment and data extraction. Those 16 studies which were excluded after full-text evaluation were shown together with reasons for exclusion in the Supplementary Table S1. Besides, owing to

Table II. Main characteristics of studies evaluating the prevalence of FLS in minor salivary glands of non-SjD individuals.

Study	Country	Participants	Outcomes by gender
Chisholm, 1970 (17)	UK	116 post-mortem subjects excluding neoplasm and had cytotoxic drugs within the last three months before death.	Yes
Takeda, 1986 (18)	Japan	190 postmortem subjects after excluding autoimmune diseases, connective tissue diseases, leukaemia, local infectious diseases, administration of steroid or cytotoxic drugs within the previous two months.	Yes
De Wilde, 1986 (28)	Netherlands	68 healthy volunteers who had undergone intraoral surgery for cosmetic or pre-prosthetic purposes and did not suffer from any systemic disease nor had they undergone radiotherapy or chemotherapy before surgery.	Yes
Segerberg, 1989 (29)	Finland	102 consecutive subjects undergoing medicolegal postmortem examination excluding infection or neoplasm.	Yes
Vered, 2001 (20)	Israel	120 biopsies of labial salivary glands obtained from autopsy subjects free of salivary gland tumours/diseases.	No
Radfar, 2002 (19)	USA	54 healthy volunteers who served as control subjects in various studies of salivary dysfunction.	No
Kikuchi, 2004 (30)	Japan	53 autopsy subjects who had no sicca symptom.	No
GTEx study	USA	214 non-diseased minor salivary glands from autopsy subjects.	Yes

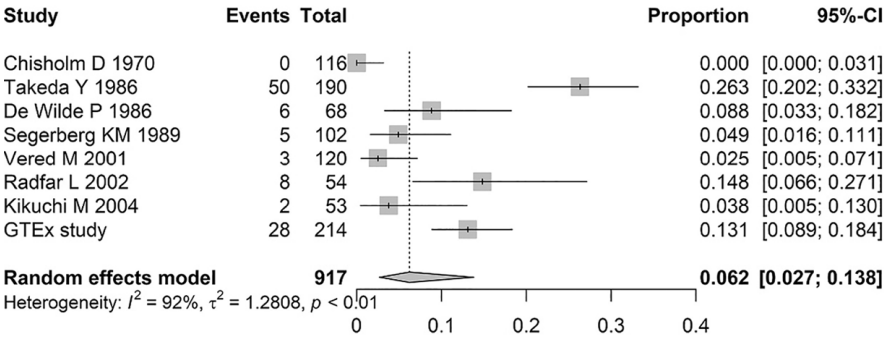

Fig. 2. Forest plot showing the pooled prevalence of FLS in minor salivary glands of non-SjD individuals.

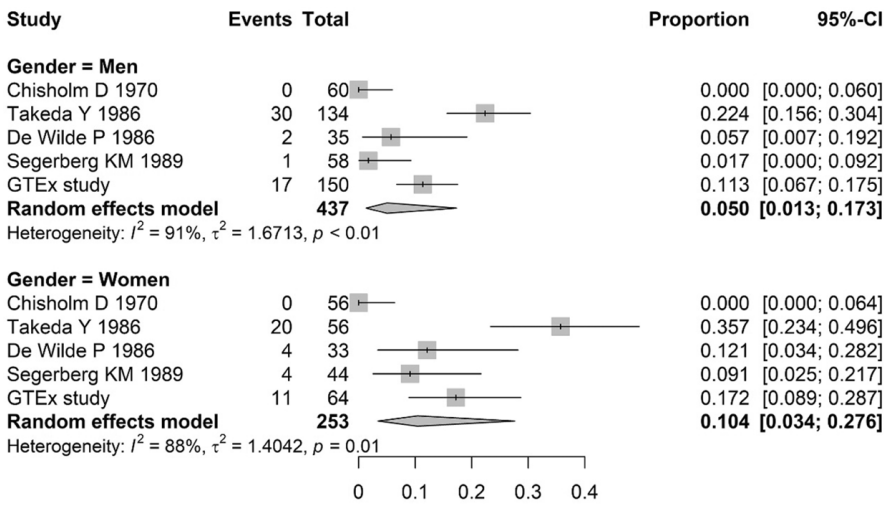

Fig. 3. Forest plot in the subgroup analysis of the prevalence of FLS in minor salivary glands of non-SjD individuals by gender.

the lack of full-text report for the study by Scott *et al*., we were unable to assess its eligibility and get possible data for data synthesis. Ultimately, 7 published studies with full-text reports met the inclusion criteria and were included into the meta-analysis (17-20, 28-30).

Table II shows the main characteristics of studies evaluating the prevalence of FLS in labial minor salivary glands of non-SjD individuals. The sample size of those 7 published studies ranged from 53 to 190. Finally, this meta-analysis included a total of 8 studies involving 917 labial minor salivary gland samples. Five of those 8 studies reported outcomes by gender. These studies varied in design but consistently reported on FLS detection in minor salivary glands, allowing for a meaningful data synthesis in meta-analysis. All studies had moderate or high quality according to NOS.

Through meta-analysis, the pooled prevalence of FLS in labial minor salivary glands of non-SjD individuals was 6.2% (Fig. 2). In the subgroup analysis by gender, the pooled prevalence of FLS in labial minor salivary glands for female and male non-SjD individuals was 10.4% and 5.0%, respectively (Fig. 3).

Discussion

FLS has long been described as suggestive of SjD, but it can also occur in non-SjD individuals. This study was carried out to define the prevalence of FLS in labial minor salivary glands of non-SjD individuals via both an autopsy study and a meta-analysis of relevant studies. The outcomes of this study have provided strong epidemiological evidence for the significantly

high prevalence of FLS in labial minor salivary glands of non-SjD individuals. The detection of FLS in labial salivary gland biopsies serves as a key pathological method for clinical confirmation of SjD (35). According to the 2016 ACR/EULAR SjD classification criteria, FLS and positive anti-SSA/Ro antibodies are the main indicators for SjD. Due to risk factors such as prolonged use of electronic devices and excessive use of eyes in study and work, the prevalence of symptomatic dry eye disease has increased in recent years, reaching around 10% (36, 37). There is a high risk of SjD misdiagnosis for those non-SjD individuals, who suffered from dry eye disease and exhibited FLS in labial minor salivary gland biopsies. As the prevalence of FLS in minor salivary glands of female adults is about 10% and the prevalence of dry eye disease is around 10% in the general population, approximately 1% of female adults may have both dry eye disease and FLS in labial minor salivary glands. However, these female adults also meet the current classification criteria for SjD, which leads to an estimated SjD prevalence of around 1% among female adults. However, the real prevalence of SjD among female adults reported by literatures is about 0.16%, which is significantly lower than the prevalence estimated above (4). This contradiction challenges our understanding of SjD as a rheumatic disease with a relatively low incidence. Accordingly, the utilization of FLS for the classification of SjD may potentially yield false-positive outcomes.

A major drawback of using MSGB for the classification of SjD is the poor reproducibility, with notable discrepancies in the histopathological evaluation of MSG biopsies among different institutions or pathologists (38-41). Furthermore, the identification of positive FLS through labial salivary gland biopsy exhibits a certain degree of randomness (42, 43). Because MSGB is an invasive examination, to mitigate potential harm to the patient, the labial salivary gland tissues from a single biopsy procedure are usually restricted in the current clinical practice. Besides, in a single biopsy procedure, SjD patients may present FLS positivity in only one or some of the 3-5 salivary gland samples obtained, while other salivary gland samples may not reveal FLS in the pathological analysis. The absence of FLS in a salivary gland may stem from either a genuine lack of FLS in that salivary gland or from the possibility that the selected pathological section failed to capture the presence of FLS (42, 43).

Relying on FLS in labial gland tissue as the primary pathological basis for identifying SjD poses challenges. On the one hand, it may lead to misdiagnosis, as individuals with occasional FLS but lacking typical evidence of autoimmunity in SjD may be wrongly diagnosed as SjD. On the other hand, some SjD patients, potentially in the early stages of the disease or due to the occasional nature of FLS detection in pathological examinations, may not exhibit FLS in labial gland biopsies, leading to delayed diagnosis or misdiagnosis as non-SjD individuals with dryness symptoms. Therefore, it is essential to identify more effective biomarkers for SjD and establish diagnostic tools with both high sensitivity and specificity to reduce the risk of misdiagnosis. A recent publication by van Ginkel *et al*. showed that 12% of patients with non-SjD sicca had a focus score of 1 or higher, and the diagnostic accuracy of the labial gland biopsy for SjD could be improved by the combination of focus score and other histopathological features such as presence of ectopic germinal centre-like structures and presence of plasma cell shift (44). Thus, histopathological features except for focus score may be used for the classification of SjD, and more future research on their roles in the classification of SjD is recommended.

The impact of age on the occurrence of FLS in minor salivary gland tissues has been debated for many years. There are several studies showing no obvious impact of age on the occurrence of FLS in minor salivary gland tissues. For instance, a study by Chisholm *et al*. analysed samples from 116 postmortem subjects but did not identify a statistically significant difference in the lymphocytic infiltration of the labial salivary glands between different age groups (17). Another study by De Wilde *et al*. analysed samples from 68 healthy volunteers and found that the volume percentage of lymphocytic foci was constant with age (32). The study by Radfar *et al*. analysed samples of 54 healthy volunteers and concluded that focus score in minor salivary glands of healthy, asymptomatic individuals did not correlate with age (19). The study by Kikuchi *et al*. analysed labial salivary glands from 53 asymptomatic elderly subjects with an average age of 84 years and reported that there was no obvious relationship between degree of lymphocytic infiltration and age in both major and minor salivary glands among the asymptomatic elderly (34). However, there are several other studies which support an obvious impact of age on the occurrence of FLS in minor salivary gland tissues. For instance, a study by Takeda *et al*. analysed samples from 190 postmortem subjects and reported that the focal lymphocytic infiltration had a tendency to be more prevalent in the groups over 40 years age compare with younger groups (18). The study by Segerberg-Konttinen *et al*. analysed 102 consecutive medico-legal post-mortem subjects and found that focal lymphocytic infiltration mainly occurred in individuals aged 50 to 96 years (33). A study by Syrjänen *et al*. analysed histological changes in labial minor salivary glands of 78 healthy individuals aged from 19 to 87 years and reported that inflammatory cell foci first appeared in the age group of 30-39 years and the focus score was higher in the older groups (45). In the present study, we analysed the prevalence of FLS among GTEx volunteers and found that there was no significant increase in the prevalence of FLS among GTEx volunteers until those aged 60 years and older. GTEx volunteers aged 60 years and older demonstrated a greater prevalence of FLS and a higher focus score in minor salivary gland tissues compared to those volunteers younger than 60 years ($p<0.05$). Therefore, the findings above suggest a possible correlation between the occurrence of FLS in minor salivary gland tissues and age.

The GTEx project excludes individuals with prior radiotherapy, metastatic cancer, or high-risk exposure to HCV, HBV, and HIV (46). Chronic virus infections such as HCV and HIV can cause oral manifestations including sialadenitis or sicca syndrome (47-49), and the exclusion of individuals with high risk of exposure to HCV/HBV/HIV in the GTEx project helps reduce the likelihood of including SjD patients and is helpful to the research topic of this study. Due to the lack of detailed medical history and laboratory evaluations, some SjD patients might still be included. However, given the extremely low prevalence of SjD, there may be few or only several cases who had been diagnosed to have SjD among those subjects in the GTEx project, and its impact on the results is likely minimal. Additionally, the results from our meta-analysis further validate the findings from the GTEx project.

We studied the digital data of minor salivary gland biopsies from GTEx project, but did not perform minor salivary gland biopsies exactly according to the standardised consensus guidance for the use of labial salivary gland histopathology in the classification of SjD. For instance, the surface area of the minor salivary gland and the size of the tissue samples were unable to be assessed with the digital data of minor salivary gland biopsies from GTEx project. This could cause some biases in the evaluation of the prevalence of FLS in minor salivary glands among volunteers from GTEx project.

Conclusion

This study provides compelling epidemiological evidence for the high prevalence of FLS in minor salivary glands of non-SjD individuals. FLS is considerably common but not rare in minor salivary glands of non-SjD individuals. Its clinical significance should be cautiously considered when MSGB is used to confirm seronegative SjD.

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