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RECURRENT APHTHOUS STOMATITIS AND ORAL MICROFLORA: IS THERE A RELATIONSHIP?

1. Anbari Fahimeh
Assistant professor, oral medicine department, School of dentistry, Shahid Beheshti University of medical sciences, Tehran, Iran
Fahimeh.anbari@gmail.com

2. Azimi Hosseini Sedighe
Associate professor, oral medicine department, School of dentistry, Shahid Beheshti University of medical sciences, Tehran, Iran
S_azimi2002@yahoo.com

3.Yazdani Zahra
Private denist, Esfahan, Iran
Zahra.yazdanii@gmail.com

4. Faghihi Banafsheh
Oral medicine specialist, Private practice, Tehran, Iran

5. Fallah Fatemeh
Professor, Department of Microbiology, School of Medicine & RICH, Pediatric Infections research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
fafallah@sbmu.ac.ir

Corresponding Author:
Name: Zahra Yazdani
Email: Zahra.yazdanii@gmail.com
Phone number: +989393581755
Address: Jandaq clinic, Jandaq, Esfahan, Iran

Abstract

Background and aim: Recurrent Aphthous Stomatitis (RAS) is one of the most common lesions in oral mucosa and it is unique to oral mucosa. Since it is a painful condition and cause eating and speaking disturbances, study on its etiology may be helpful in current modalities which are mainly palliative. Microbial factor as etiological factor for these lesions have been suspected. Studies did not show definite results. The aim of this study was to evaluate the relationship between oral microflora (subtypes of Streptococcus, Staphylococcus, Neisseria, Prevotella) and RAS.

methods & materials: this study is an analytical case control study. Totally 21 patients with RAS referred to the department of oral medicine of Shahid Beheshti dental school that 21 patient with no history of RAS participated in study as control group inclusion and exclusion criteria were placed in case group.Samples were taken during RAS (around the lesion) and emission periods and also in control group from the same area. Samples sent to laboratory for microbial culture as soon as possible.

Results: In case group during RAS and remission period relationship between presence of prevotella melaninogenicus (P=0.003), Neisseria lactamica (P=0.013) Neisseria mucosa (P=0.043), Neisseria subflava (p=0.031), Neisseria sapraphaga (P=0.014), Streptococcus viridance (P=0.001) , Streptococcus mutans (P=0.002) and staphyloccus aureus (P=0.46) was significant .In comparison with case and control group the relationship of prevotella melaninogenicus (P=0.001), Neisseria lactamica (p=0.004) Neisseria mucosa (p=0.045), Neisseria sapraphaga (p=0.03), Streptococcus viridans (P=0.001) and streptococcus mutans (P<0.001) were significant.

Conclusions: According to our findings we can claim that some subgroups of Prevotella, Neisseria and streptococcus may be related to developing aphthous stomatitis.

Keywords: Aphthous stomatitis, Neisseria, Prevotella, Staphylococcus, Streptococcus

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is one of the chronic, inflammatory diseases of the oral mucosa. The most characteristic symptom of the disease is the recurrent onset of single or multiple painful erosions and ulcers that appear mainly on unattached oral mucosa [1, 2]. Occasionally the lesions may also be observed on keratinized palatal and gingival mucosa. The prevalence of RAS in the general population is between 5 - 25 %. Differences have been reported depending on the ethnic origin of the population as well as the studies design or methodology.

Local trauma, genetic factors, nutritional deficiencies, viral and bacterial infections, and immune or endocrine disturbances implicated as etiological factors of frequent oral ulcerations. RAS is classified in three groups depends on the clinical features: minor (>70% of cases), major (10%), and herpetiform (10%). These subtypes differ in morphology, distribution, severity, and prognosis [3].

This disease is restricted to oral mucosa and the most prominent feature is recurrent painful oral ulcers. Due to the lack of specific diagnostic tests for RAS, clinical features are important in diagnosis. Despite these clear clinical features, its certain etiology is still unclear and therefore, more treatments are palliative [5]. The main symptom of RAS is the severe pain which can change the patients' eating and talking patterns efficiently [6]. Researchers believed that all three forms are part of the certain disease and share common etiology [7].

A local microbial background for RAS can explain why only the oral mucosa is affected in patients with RAS [8]. For the first time, Gray Kowsky et al considered the pleomorphic alpha hemolytic Streptococcus (*S. sanguinis*) as the cause of RAS. This microorganism has been separated from lesions of typical aphtous ulcers repeatedly and in most of the aphtous lesions, isolated microorganism was histologically similar to L form of Streptococcus [9].

New studies have shown that RAS is the results of autoimmune reaction against epithelium. It has been suggested that this autoimmune disease is a cross immune reaction which is activated by heat shock proteins that have been released from oral bacteria and affect the peptides of oral mucosa. Therefore, several antibiotics were tried up to now to decrease the severity of these lesions, such as broad spectrum antibiotics (tetracycline, cephalexin) or antimicrobial mouthwash (listerin, chlorohexidine, sucralfate and myrtle) [10].

Some studies have found that there is some cross- reactivity between the streptococcal 65-kDa heat shock protein (hsp) and the 60-kDa human mitochondrial hsp. It has thus been suggested that there is a molecular basis for earlier work suggesting a link between RAS and Streptococcus sanguinis, because monoclonal antibodies to the part of the 65-kDa hsp of mycobacterium tuberculosis react with S. sanguinis. Thus, RAS may be a T-cell-mediated response to antigens of S. sanguinis that cross-react with the mitochondrial hsp and induce oral mucosal damage This theory is still unproven [11].

Enhancement of T cell response against *S. mutans* in the recurrence of aphtous was studied by Sun A and Chin J. They observed that this response in the stage of aphtous recurrence was very higher than the remission stage and it was also higher in cases group compared to control group [12].

The relationship between *S. oralis* and aphtha was evaluated by Lennon and P Rlyggle but they found no significant relationship [13].

Safronova LA evaluated the oral microflora of the children with aphthae and compared them

with control healthy group. They demonstrated that the count of microorganisms in the mouth of children with RAS were higher and the spectrum was wider than control group [15] .Noha and Lesley investigated the salivary and oral mucosal microbial communities in Behcet syndrome and RAS .There was increased colonization with Rothia denticariosa of the non-ulcer sites of BS and RAS patients. Oral mucosa of healthy control were more highly colonized with Neisseria and Veillonella compared to all studied groups [15].

Campos and Marchini conducted a research about the bacterial variations in RAS. They isolated several bacterial species from RAS patients which were not existed or had lower level in control participants and reported that RAS microflora was differed from healthy human that can be used as new gate for analysis of this lesion [16].

So far, controversial results regarding the effect of the bacteria in the induction of RAS were reported. In the present study, the relationship between oral microflora and oral aphtous lesions was studied by evaluating the specific microbial species and their counts.

MATERIALS AND METHODS

This study was performed as an analytical case control study. a total of 42 peoples were included in the study. (21 in case group and 21 in control group)

Ethical committee of Shahid Beheshti university of medical sciences approved the study (IR.SBMU.DRC.REC.1388.506), informed consent was obtained from all the patients based on Helsinki declaration and they were assured to can quit the study in case of dissatisfaction, and their identity will be preserved over the study.

After clinical examination and exact medical history and confirmation of aphtous in them by two oral disease specialists, they were enrolled in the study. Patients were 18-70 years old.

Patients with aphtous related syndrome (Behçet's disease, Reiter's disease and Crohn's disease), smoking habits, Herpes simplex, lichen planus, systemic diseases or any undiagnosed untritibutedeficiency were excluded. At first, the O'leary index was evaluated by indicator tablets [17].

From 21 aphtous patients, sampling with sterile swap was performed from area around the lesions and placed in thioglycolate transport medium.Samples were cultured in laboratory immediately after obtaining.

We used blood agar, eosin methylene blue and chocolate agar as primary culture.After 24 hours of incubation the colonies were examined by slide preparation and Gram staining. According to microbiological diagnostic table, differential diagnostic tests and secondary culture for genus-species confirmation were performed. CFU of diagnosed colonies by preparation of 0.5 suspension of McFarland on the Muller Hinton agar plat was performed. If bacteria were fastidious, all analysis were performed on blood agar.

After 24 hours incubation, the obtained value of colonies were multiplied in dilution factor and then CFU was calculated [18].

From these patients, sampling from same area was performed after two weeks after recovery and all above mentioned steps were done for them. Also, similar sampling was performed from 21 healthy participant and culturing steps were followed as same as patients group.

Chi-square or Fisher exact test were used for comparison of patient and healthy groups. The McNemar analysis was performed for comparison between relapse and remission in aphtous patients. Distribution of logarithm of isolated bacterial number in relapse and remission was compared by Wilcoxon test and distribution of logarithm of isolated bacterial number in patient and control was compared by Mann-Whitney test.

RESULTS

In this study, 42 participants were evaluated which 21 of them had aphthae and 21 of them was control group. From 42 patients, 17 ones were woman (40.5%) and 25 ones were man (59.5%).

In patient groups, 12 men and 9 women and in control group, 5 men and 16 women were existed. The age range of participant in case group was 18-60 years with mean of 32 years and in control group was 22-50 years with

mean of 31 years. In 42 patients, oral health index (OIH) was in the unique range (60-100%) and the area of sampling in all of them was buccal or labial mucosa. Therefore, two groups were matched about age, sex, OIH, and sampling area.

Totally, 13 subgroups from 4 types of mentioned bacteria include Prevotella, Neisseria, Streptococcus and Staphylococcus were isolated from the culture media of case and control groups.

According to the findings of comparison of aphtous patients in relapse and remission, the relationship between existence of P. melaninogenicus (p=0.003), N. lactamica (p=0.013), N. mucosa (p=0.043), N. subflava (p=0.013), N. sapraphaga (p=0.014), S. viridinis (p=0.001), S. mutans (p=0.002), and S. aureus (p=0.046) and occurrence of the aphthae were significant.

Patient in case group were compared with control group in the recurrence status. Totally, in comparison of patients with aphthae and control ones, P. melaninogenicus (p=0.009), N. lactamica (p=0.004), N. mucosa (p=0.045), N. sapraphaga (p=0.03), S. viridinis (p=0.001) and S. mutans (p=0.000) shown significant relationship with occurrence of lesions.

DISCUSSION

This study showed an association between RAS and imbalances of the oral mucosal microbiome. Numerous reviews have appraised the vigorous of studies attributing an infectious basis (viral and bacterial) to the etiology of RAS [19]. This study suggests that imbalances of the oral mucosal microbiome, rather than individual infectious pathogens, may be implicated in the etiopathogenesis of RAS, as proposed previously [20]. Recent evidence implicated that mucosal microbiome changes may play a role in the etiology of chronic mucosal inflammatory conditions [21] .Investigation on mucosal microbiome in patients with RAS showed increased porphyromonadaceae and Veillonellaceae in ulcerated sites when compared with healthy control.Bacteridetes and bacteroidales were increased in healthy sites of RAS patients when compared with healthy control [22].

In the study of yun –jikim on the bacterial communities of the oral mucosa and saliva from RAS patients with active lesions, the RAS patients' mucosal microbiota, showed increased inter-subject variability. Particularly, decreased Streptococcus salivarius and increased Acinetobacter johnsonii in the mucosa were associated with RAS risk. A. johnsonii substantially inhibited the proliferation of gingival epithelial cells and showed greater cytotoxicity against the gingival epithelial cells than S. salivarius [23].

In the study of Bankvall et al. Buccal swabs were obtained from non-ulcerative areas of the patients who had lesions at the time of sampling, and 60 healthy age and sex matched controls. The microbiota of the non-inflamed buccal mucosa differed between patients and controls. The differences were most pronounced in patients who presented with lesions during sampling, suggesting that a disturbance in the normal buccal microbiota triggers the presence of lesions or that presence of lesions alters the microbiota. Three species, S. sanguinis, H. pylori and S. oralis that were presented in the microbiota, differed in abundance between the patients with RAS and controls [24].

In the Sun A, et al's study aphtous patients in relapse and remission were compared together .In addition to the culture, PCR also was used in this study. Streptococcus subgroups included S. mutan, S. sanguinis, S. oralis, S. gordonni, S. mitis, which were higher activated blood mononuclear and T cells in recurrence compared to remission ($p<0.05$). Results of this study was similar to our findings and relationship of S. mutanand S. viridiniswere significant. [25].

In the present study, the significant relationships of P.melaninogenicus (52.4%), N. lactamica (51.7%), N. mucosa (33.3%), N. saprafaga (38.1%), S. viridinis (95.2%) and S. mutans (57.1%) were detected.

In the study of Marchini and Campos P.melaninogenicus was only seen in aphtous patients (with prevalence of 16%) and not seen in control group. it has been noted that because of Prevotella was not detected in the healthy subjects, therefore, it can be a stimulator of induction of aphtous lesions [16]. In our study,

P.melaninogenicus (the only subgroup of Prevotella which existed in the oral microflora) were isolated from 52.4% of aphtous patients and 14.3% of control subjects. The studies of Donatsky and Safranova LA were confirmed the relationship of Neisseria and oral aphtous lesions [14,26]. In the study of Safranova LA, which performed on the children with age range of 7-15 years, the samples first cultured and then applied for PCR and results were in line with our findings, although we used only the culture method. In the study of Donatsky also the culture method was used and the relationship of Neisseria with aphthae was significant [28]. In the present study, subgroups of Neisseria also were evaluated separately and relationships of species of lactamica, mucosa, and saprafaga were significant, but the relationship of subflova, menengitidis, and cika were not significant.

In the present study, the relationship of existence of S. viridinis and S. mutans with occurrence of aphthae was significant. In the study of Safronova which was performed on the children of 7-15 years, all streptococcus subgroups were higher in aphthous patients except S. mitis that shown lower count in aphtous patients [14]. Also, in the study of Marchini and Campos all subgroups of Streptococcus were higher in aphthous patients exceptfor S.mitis and S. pneumonia (33.4% in aphtous patients and 46.4% in healthy subjects) [16]. These results are disagreed with findings of Greanspan and Hoover which isolated the S. mitis from aphtous patients [26]. In our study, the relationship of S. pyogen and streptococcus groups were not significant. These differences may be due to variations in the sampling strategies, in Marchini and Suns' studies the swabs were applied directly on the lesions and the swabs might have been infected secondarily. While in our study, samples were obtained from the periphery of the lesions.

Also, in the Marchini study, firstly swabs were soaked in a solution, that based on authors comments, this can be changed microflora [16].

It is necessary to mention that nutritional deficiencies were not evaluated in none of the studies for exclusion of participants (in 20% of cases, deficiencies are the etiology of the disease). Oral hygiene indices of two groups were not matched that can have a high impact on microbial differences.

CONCLUSION

Based on the present study, the existence of some subgroups of Streptococcus, Prevotella, and Neisseria in the oral cavity may be related to higher possibility of RAS occurrence.

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