Original Article

Association of Interleukin-4 in Patients with Recurrent Aphthous Stomatitis

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is a frequent ulcerative inflammatory disorder of the mouth. Various localized, systemic, and immunologic factors have quite a function in oral tolerance, although the etiology of RAS remains unexplained. The previous studies suggest that there is a significant association (interleukin-4—IL-4) level between in case and control group with RAS in saliva, and no significant difference between IL-4 rs 2243266 gene polymorphism with RAS. **Objectives:** To determine the level of IL-4 in saliva and the association of IL-4 gene polymorphism with the incidence of RAS. **Materials and Methods:** The subjects enrolled in the present study were (80) subjects of both genders. The age range was from 20 to 60 years. This study is carried out to detect the association of IL-4 level with RAS by enzyme-linked immunosorbent assay (ELISA) test and polymerase chain reaction (PCR) technique in Babylon Province, Iraq. The study sample consists of (80) subjects of both genders. The samples were divided into two groups including the patient group, (40) samples (female 21 and male 19) with RAS, and the control group (C) included (40) samples (20 females and males 20). **Results:** The results of the association between IL-4 level with RAS showed a significant association while the result of PCR sequencing found that there was no significant association of AG genotype of rs 2243266 with RAS with odd ratio 1.169 (0.54–2.54) 0.855 (0.39–1.86) and *P*-value 0.693. **Conclusion:** Females were more affected by RAS than males. There was a significant difference in IL-4 levels between the case and control groups.

Keywords: Gene polymorphism, incidence, interleukin-4 (IL-4), recurrent aphthous stomatitis

Introduction

Aphthous stomatitis, an inflammatory oral mucosal ailment, is common among people of many different racial and cultural backgrounds and geographic regions.[1] "Canker sore" is a common colloquial phrase for what is known as "recurrent aphthous stomatitis" (RAS) in the scientific literature. In most cases, RAS can cure on its own in 1–2 weeks. The severity, duration, and frequency of an aphthous sore make a big difference in the morbidity it can cause.[2] In addition to being a symptom of an autoimmune or inflammatory ailment, RAS or RAS-like lesions may be an indicator of an underlying disease or deficit.[3] Moreover, the inflammatory aspect of RAS is important to the readership of this paper, even if the etiology and pathophysiology of the illness are not entirely known and may include several causes. As a result, we will first go into RAS and then discuss certain systemic autoimmune disorders that cause mouth ulcers.[4] Well-defined edges and a necrotic center, which is shallow characterize an aphthous ulcer, which is the diagnostic hallmark of aphthous. A delicate grayish to white pseudomembrane or fibrous clot, likely the result of localized vasculitis, covers the ulcer base.

The lesions manifest as "erosions," but they spread across the epithelium and may even invade the connective tissue underneath reddened and maybe somewhat raised edges.[5] Drainage is not connected. Most cases of aphthous occur in the nonkeratinized mucosa of the cheeks, interior lips, mouth's floor, ventral side of the tongue, and soft palate. It is safe for keratinized tissues, so it will not harm your

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hard palate or associated gingiva.[5] Blisters, which are more common HSV (with herpes simplex virus) infections of the mouth, do not appear initially with RAS.[6] Inflammation of the preulcerative epithelium has been linked to a prodromal tickling feeling in the local mucosa experienced by some individuals.[7] With no underlying systemic illness or infection, aphthous stomatitis is not a feverish but painful condition. Sore throats and even earaches are common complaints when an ulcer is located near the oropharynx. The need for, and normality of, an extraoral exam.[6]

Since RAS typically manifests in young children, it is possible that a second biopsy of a mouth ulcer may be required to confirm the lesion's ongoing benign nature. The greatest incidence of ulcers occurs between the ages of 5 and 29.[8] Depending on their size, aphthous have been classified as either minor (below 1 cm in diameter, and often below 1 mm) or significant (over 1 cm in diameter and rarely several centimeters may affect keratinized gingiva). Aphthosis caused by RAS can manifest clinically in two distinct ways, depending on the lesion's morphology: as a "simple" form or a "complex" type.

The most common clinical manifestation of RAS is mild to moderate aphthosis. There has been a history of comparable ulcers that have healed without complications since childhood, and the ulcers typically heal within 5–10 days with no scarring.[9] When RAS is difficult to control, a systemic illness is more likely to develop.[9] RAS is diagnosed by a process of elimination involving a thorough patient history, system, and physical examination. The ulcer's current behavior and pattern must be discussed in the patient's medical history. The risk of developing RAS is elevated in people with a family history of the condition or who have just stopped smoking.[10] To determine the level of interleukin-4 (IL-4) in saliva and the association of IL-4 gene polymorphism with the incidence of RAS.[4]

Materials and Methods

Saliva was carried out with a sterile plain tube. Saliva was performed when the patients had the possibility to visit the clinic. The subjects enrolled in the present study were (80) subjects of both genders. The age range was from 20 to 60 years. Subjects were primarily dental clinic personnel, and students from the University of Babylon's College of Dentistry and specialized dentistry center in Babylon, Iraq. The study period was extended from January 2022 to April 2022. Control group (C) included (40) subjects who were healthy individuals while the patient group (P): included (40) subjects with RAS.

Exclusion criteria

Participants who met the following criteria were not included in the study: rheumatoid arthritis, diabetes mellitus, cardiovascular illness, renal disease, and hepatic disease. Inclusion criteria include all free healthy individual.

Human interleukin-4 ELISA kit

The assessment of human IL-4 enzyme-linked immunosorbent assay (ELISA) Kit produced by technique sandwich immunoassay with properties (Elabscience—USA).

Polymerase chain reaction, sequencing, and genotyping

The designed primer set for rs 2243266 genotyping and their properties is illustrated in Table 1. The exact position of reverse, forward primers, and the targeted polymorphism (rs2243266) on the IL 4 gene (NCBI nucleotide browser).

Statistical analysis

Statistical analysis was carried out using SPSS version 25.0 (SPSS, IBM Company, Chicago, IL). When *P* values were less than or equal to 0.05 considered as statistical significance, while *P* values were more than 0.05 considered as statistical nonsignificance.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 115 on 18-12-2021.

Results

In this study, the total number of subjects was (80) persons divided in to two groups, the first was the patients group (includes (21) females and (19) males) and the control group (includes (20) females and (20) males). The distribution of patients and control group was as follows: females 51.2% and the males 48.8% among all subjects in the study [Table 2].

Table 1: The designed primer set for rs 2243266 genotyping and their properties

	Sequence (5′ → 3′)	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3′ complementarity
GT66f	TCTGTAGCCTGGGATTCTGGT	Plus	21	132678042	132678062	60.27	52.38	3.00	0.00
GT66r	GCCCTTCGGTGGTATTAGAGAA	Minus	22	132678295	132678274	59.57	50.00	3.00	2.00
Product length				254					

Table 2: Distribution of patients and control group

		Frequency	%
Valid	Female	41	51.2
	Male	39	48.8
	Total	80	100.0

Table 3: Association of BMI, age, salivary IL-4 with recurrent aphthous stomatitis

Parameters	Mean difference	Std. error difference	*P*-value
BMI	−2.29800	1.10783	0.041
	−2.29800	1.10783	0.043
Age	0.07500	0.72866	0.918
	0.07500	0.72866	0.918
IL-4	83.97025	3.42945	0.000
	83.97025	3.42945	0.000

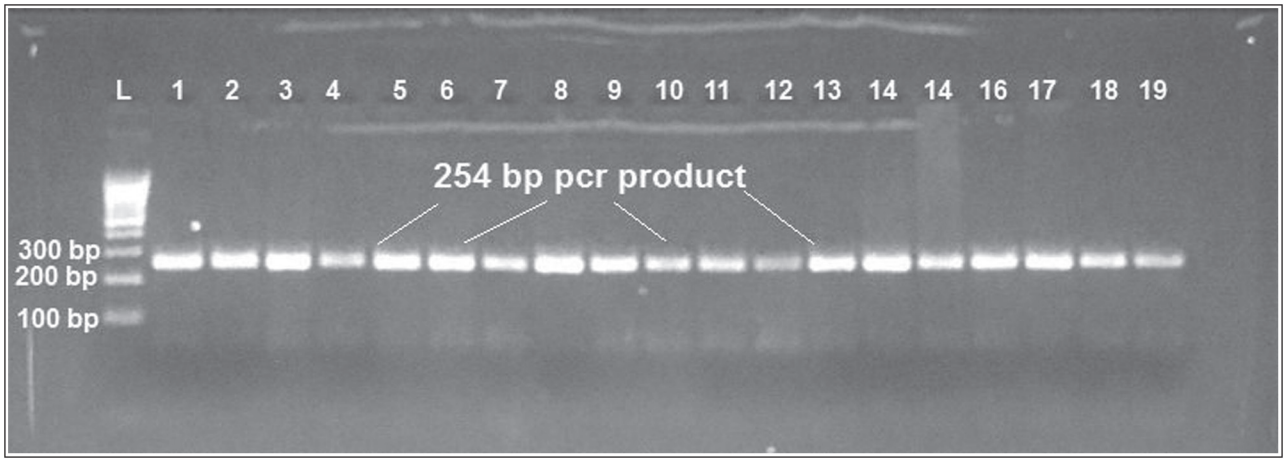

Figure 1: PCR gel electrophoresis of amplified IL-4 gene (254 bp), lane L DNA ladder, other lanes represent different samples

Association of body mass index, age, salivary (IL-4) with recurrent aphthous stomatitis

In this study and through the relationship between IL-4 with RAS by comparing them. According to the findings, there is a statistically substantial distinction among case and control group regarding IL-4 with RAS, as well as with BMI. The age and although the findings no significant statistically substantial link among RAS and other variables involved in this study as shown in [Table 3].

The polymerase chain reaction (PCR) product of the IL-4 gene (254 bp) was amplified in all samples, by gradient annealing temperature PCR, as shown in Figure 1.

Results showed genotyping of rs 2243266 by PCR-RFLP technique lanes L DNA ladder lane 15 AA genotying lanes 8,13,14,23 and 24 GA genotype;other lanes GG genotype [Figure 2].

Discussion

According to the findings of certain studies, a dysfunction in the innate immune system can lead to RAS[11] Additional research was conducted to investigate the connection of each genotype with the illness of RAS using a variety of heredity theories. Results showed zero significant difference of any genotype with RAS.[12,13] In the present study results showed that BMI for patients higher than control and this was incompatible with other study that reported that that blisters caused intense discomfort, made eating, chewing, and swallowing problematic, hampered communication and standard of living, and contributed to a person losing weight.

Regarding IL-4, results found that RAS sufferers had significantly higher IL-4 (inflammatory) levels of cytokines than baseline. Those results were compatible with the vast majority of previous studies that demonstrated an enhanced Th1 response in RAS patients.[14,15] There may be groups of afflicted individuals that start off RAS dysfunctional routes in diverse manners due to different alterations. Others have shown that certain RAS sufferers, notwithstanding the disease's Th1 nature, exhibit characteristics more typical of a Th2 immune reaction, including atopy and elevated levels of the antibodies immunoglobulin E and IL-4.[16,17]

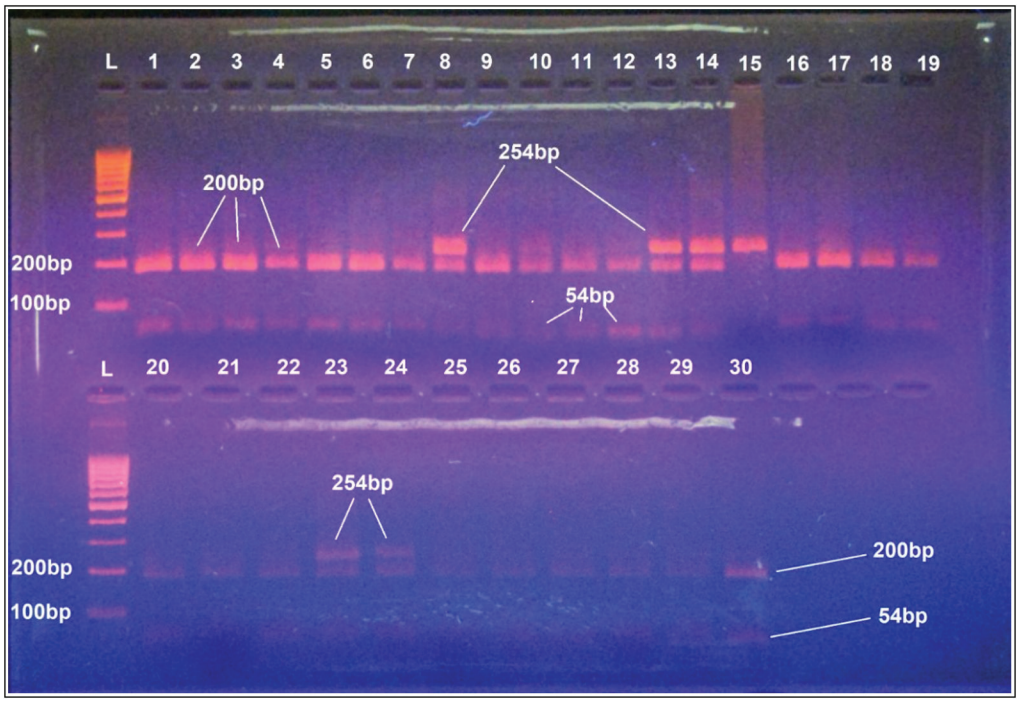

Figure 2: Genotyping of rs 2243266 by PCR-RFLP technique lanes L DNA ladder lane 15 AA genotying lanes 8, 13, 14, 23 and 24 GA genotype; other lanes GG genotype

The presence of the Th2 cytokine IL-4 in oral ulcer lesions shows that people with RAS are exposed to diverse antigenic stimuli.[14,18] Stimuli trauma, hormonal variables, and mental stress are all linked to RAS, in addition to the immune system's influence and family history. A plausible hypothesis is that many diseases are caused by areas of inflammation.[19,20] Radical O_2 and N_2 species created by phagocytic cells and leukocytes following inflammation are responsible for damage of DNA in proliferating cells.[21] Therefore, DNA in proliferative epithelium undergoes irreversible genomic modifications like genetic variations, losses, and translocations as a consequence of repetitive tissue destruction, renewal in the midst of exceptionally reactive N_2 and Oxygen molecules emitted from inflammatory cells.[22]

The findings demonstrated that there are zero discernible changes among the case sample and the control group. The present results were compatible with de Gallo *et al.*[23] who demonstrated that the prevalence of RAS is influenced by the population studied and diagnostic criteria according to the findings of this research, the occurrence of RAS was discovered to be greater in females in comparison to males[24] but this result was incompatible with.[25]

This discovery prompted some researchers to hypothesis that stress during a patient's life is a primary component in RAS, however variances owing to age should also be addressed pathogenesis of RAS in male and female stays uncertain.[26] The accumulation of certain subgroups of pro-inflammatory cytokines and T cells is central around one version of the theory that RAS is caused by a dysfunction in cell-mediated immunity.[27] A participant's risk of developing RAS can be increased by a number of variables, including hormonal changes, stress, family background, and trauma.[28-33]

Conclusions

Female were more affected by RAS than males. There was a significant difference of IL-4 level between the case and control groups. There was no significant association between studied IL-4 rs2243266 gene polymorphisms with RAS. There was no significant difference RAS between control and case according to gender and age, but there was a significant difference of BMI level between case and control groups.

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Conflicts of interest

There are no conflicts of interest.

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