



Correlation of the Protein Expression of Salivary Cortisol, Serum Vitamin B12 and Interleukin 1 Beta in Patients with Recurrent Aphthous Stomatitis

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Authors' contributions

This work was carried out in collaboration among all authors. 'All authors read and approved the final manuscript.

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ABSTRACT

This study will help in determining which molecules participate in the ulceration with an integrated approach combining external and internal factors. Recurrent aphthous stomatitis (RAS) is a common condition affecting the oral mucosa. Clinically it is manifested through recurrent painful lesions. A total of 28 subjects were recruited for this systematic case-controlled study (14 Control and 14). Whole unstimulated saliva was used for measuring Cortisol and IL-1 β levels. Patients with RAS lesions exhibited an elevated salivary cortisol level as against their healthy counterparts. The mean value for salivary cortisol among RAS patients was determined to be 16.2 ± 2 nM/dL as against the control group which had a mean salivary cortisol of 12 ± 1 nM/dL. Overall, this study helps provide insights into the correlation of the different biomarkers and RAS patients which can eventually lead to better therapeutic options and prevent recurrence in patients.

Keywords: Recurrent aphthous stomatitis; mucosal disease; herpetiform ulcers; cytokines.

1. INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a common condition affecting the oral mucosa. Clinically it is manifested through recurrent painful lesions [1].

Although one of the most common oral mucosal disease affecting a significant proportion of population, there is little known about its aetiology.

RAS is a well-documented chronic inflammatory disease with more than one causative agents [2]. They present as small, ovoid, painful lesions, which are marked by red borders and yellow centres. The pain in the lesions is exacerbated with direct pressure or trauma on the lesion.

Clinical presentations of RAS are classified as Minor RAS, Major RAS and herpetiform ulceration. Minor RAS is the most widely occurring form. Major RAS is similar to minor RAS however larger in diameter. Herpetiform ulcers constitute only a small proportion of RAS cases. They are marked by several small ulcers occurring at the same time and may coalesce to form a larger ulcer [3,4]. These oral ulcers last anywhere between 7 to 21 days and heal automatically. The rate and frequency of recurrence are variable.

The aetiology and pathogenesis of the condition remains ambiguous and there could be a multifactorial basis for the establishment of ulcers. Some of the known causes and triggers could be local trauma, vitamin and micronutrient deficiency, infectious agents, increased oxidative stress, genetic predisposition, food allergies, hormonal systemic diseases and stress and anxiety [5].

While they are clinically classified as idiopathic ulcerations, a systematic enquiry and study into the various triggers can help propose new modes of treatment. There is no categorical or definite treatment available currently. Management of RAS is based on frequency, size and number of lesions [6]. Treatment is done symptomatically to relieve pain and reduce frequency of recurrence [2]. Thus there is an imminent need for alternative therapeutic interventions.

It is believed that there are multifactorial causes for RAS however there have not been studies

that correlate more than one factor with the occurrence of RAS. We hypothesized the multifactorial nature of RAS and the study analysed three different kinds of biomarkers.

Here we examine RAS by measuring the amounts of salivary cortisol, serum Vitamin B12 and Interleukin -1 - β in patients and healthy controls. Cortisol, the stress hormone is a well-established biomarker for stress and anxiety [7,8]. Similarly the association between Vitamin B12 and RAS ulcers has been proposed and Vitamin B12 supplements as possible therapy have been suggested [9]. Autoimmunity and a range of inflammatory factors have been implicated in the onset and progression of RAS. Cytokines are important factors in inflammation and lesions of RAS are often associated with a cytokine cascade [10]. Hence this study additionally investigated the correlation of IL-1 β . The aim of this study was to determine which molecules participate in the ulceration with an integrated approach combining external and internal factors.

2. MATERIALS AND METHODS

A total of 28 subjects were recruited for this systematic case-controlled study (14 Control and 14). The participants of the study were of the age group 23 to 29. 68% of participants were female and 32% were male (Table1). Samples from patients in the study were taken within 1 month of presentation of the lesion.

2.1 Sample (Serum and Saliva) Collection and Biomarker Quantification

Whole unstimulated saliva was used for measuring Cortisol and IL-1 β levels. Serum samples retrieved from 2mL of blood were used to estimate Vitamin B12 levels in the participants of the study. Both Serum and saliva samples were centrifuged at 3000rpm and the supernatant was used for analysis [11].

Salivary cortisol was analysed using the competitive Enzyme linked immunosorbent assay (ELISA, DiaMetra®). The tests were conducted in triplicates to ensure statistically significant values. Vitamin B12 and IL-1 β was measured using the respective ELISA kits from Abbkine. The ELISA protocols were followed according to manufacturer's instructions.

2.2 Statistical Analysis

Statistical analysis was performed using SPSS Software Version 23.0. Values were presented as mean, standard deviation and 95% confidence interval values. A t-test was used to compare control and patient groups. A level of significance was fixed at <0.05 for the comparisons made.

3. RESULTS

Patients with RAS lesions exhibited an elevated salivary cortisol level as against their healthy counterparts. The mean value for salivary cortisol among RAS patients was determined to be

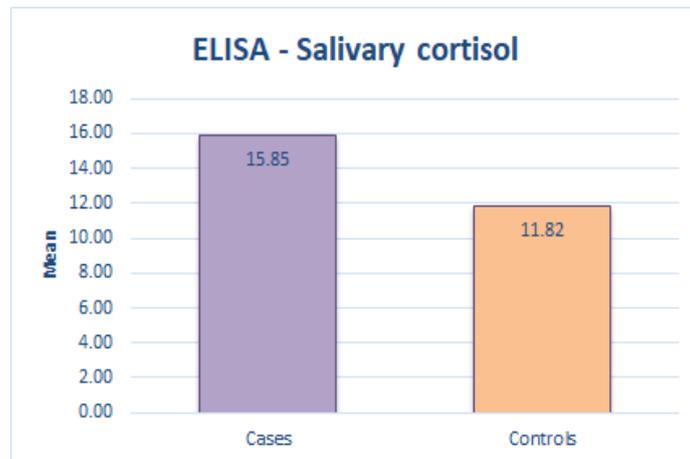
16.2±2nM/dL as against the control group which had a mean salivary cortisol of 12 ±1nM/dL.

In contrast, serum levels of Vitamin B12 was lower in RAS patients in comparison to the control group. The Vitamin B12 among the control group was 26.5 ±22.4 nM/dL. Patients with RAS showed a starkly lower serum Vitamin B12 value of 9.5 ± 2.6nM/dL. These results are in accordance with the hypothesis and studies that show patients with RAS show a Vitamin B12 deficiency [9].

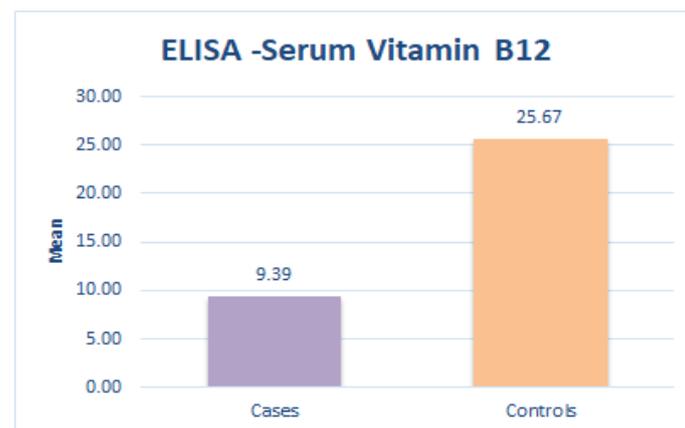
Graphs 1 and 2 shows the mean value percentage of salivary cortisol and serum vitamin B12 among case and control group respectively.

Table 1. Case and control ratio of male and female

| | Male | Female |
|---------|------|--------|
| Case | 7 | 39 |
| Control | 25 | 29 |



Graph 1. ELISA-salivary cortisol ratio



Graph 2. ELISA-serum vitamin B12

4. DISCUSSION AND CONCLUSION

RAS can frequently affect patients with repeated episodes of lesions and pain, thereby affecting the quality of life. The aetiology of RAS remains elusive. This study evaluated three different biomarkers that are potentially involved in the progress of RAS. Currently all therapies provide symptomatic relief but none offer complete remission.

As mentioned earlier, the aetiology is complex with an interplay of genetic, stress, nutritional and immunological factors

Stress has been consistently attributed as a key factor. Cortisol is an important hormone playing a role in glucose metabolism and stress response. In stressful conditions the Hypothalamus-pituitary axis and increases secretion of corticosteroids [12]. Several recent studies show that salivary cortisol is a reliable bio marker for stress. Although there are several studies that hypothesized elevated salivary cortisol in RAS patients, our work provides statistically significant proof for the correlation of cortisol in patients with active RAS.

Several studies have proposed the relationship between RAS and Vitamin B12. However there has been a lack of reliable experimental evidence to substantiate this. Experiments have shown multivitamin supplements, specifically Vitamin B12 and B9 have improved mood and alleviated stress [13]. Vitamin B has been shown to reduce stress by clearing Homocysteine (HCy). Homocysteine is a product of methionine metabolism and is also elevated during oxidative stress. Reduction in homocysteine levels through Vitamin B supplements have a proven effect in improved mood [14]. B12 has a direct impact on neurotransmitters through the synthesis of S-adenosyl-methionine [15]. Our data shows reduced Vitamin B12 levels in patients with RAS as compared to healthy controls.

The third biomarker in investigation was Interleukin-1 β (IL-1 β). In RAS there is an increased oxidative stress in the blood and a release of several pro-inflammatory cytokines. Polymorphisms on the IL-1 β gene have been linked to increased risk of RAS [16]. In addition to playing the role in inflammatory process, IL-1 β has been shown to influence secretion of cortisol, interacting with HPA axis at multiple levels [17,18]. Thus it is reasonable to conclude that IL-1 β could be potentially a valuable marker.

However our studies on IL-1 β remain inconclusive. We did not observe any noteworthy difference in IL-1 β levels between healthy individuals and RAS patients. For a conclusive result, it would require an improvement on the current experimental protocol and a larger sample size.

While the factors that can trigger and lead to progression of RAS has been regarded as multifactorial, stress has been a precipitating factor [8]. The aim of this study was to integrate more than one factor to provide a comprehensive correlation study. With increased sample size, improvement in protocols and replications such experiments will help provide greater clarity on the various triggers and molecules participating in RAS. Overall this study helps provide insights into the correlation of the different biomarkers and RAS patients which can eventually lead to better therapeutic options and prevent recurrence in patients.

CONSENT

As per international standard or university standard, participant's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was conducted in accordance with the institutional ethics protocols.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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