ORIGINAL ARTICLE

Determination of levels of salivary IgA subclasses in patients with minor recurrent aphthous ulcer

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ABSTRACT

Context: Recurrent Aphthous Ulcer (RAU) is an inflammatory disease characterized by recurrent, painful oral ulcers. It is of multifactorial etiology. Salivary immunoglobulins have important role in the protection of mucosal surfaces. **Aim:** The aim of this study was to determine salivary immunoglobulin A1 (IgA1) and IgA2 in acute and remission phases of the disease. **Materials and Methods:** Thirty clinically confirmed cases of RAU and 30 age- and sex-matched controls were included in the study. After detailed case history and thorough clinical examination, 2 mL of saliva was collected in both acute and remission phases of the disease. The obtained saliva samples were subjected to quantification of IgA1 and IgA2 levels using RID kit. **Results:** The mean IgA2 level was significantly higher (P<.001) in both acute and remission phase of the study group. The mean IgA1 level also showed a significant increase in the acute phase compared to remission as well as controls (P<.05). Females exhibited a higher level in acute phase for IgA1 and in both phases for IgA2 (P<.05). **Conclusion:** The results associated with clinical observations suggest that acute phase is characterized with increase in IgA2 that might reflect increased immune response as a possible result of the microbial stimulation seen in the acute phase in comparison to the remission period. IgA plays an important role in the pathogenesis of RAU and it can be used as a parameter to assess the mucosal immune status

Key words: Radial immunodiffusion, recurrent aphthous stomatitis, recurrent aphthous ulcer, salivary IgA subclasses, secretory IgA

INTRODUCTION

Recurrent aphthous ulcers (RAU) are the most common oral disease characterized by the repeated development of painful ulcers with unknown etiology and an incidence of up to 20% of the general population. A large variety of local, hematological, gastrointestinal, immunologic, genetic, nutritional, and medication reactions have been identified as the probable trigger factors of RAU.[1,2]

IgA is the principal immunoglobulin isotype found in the external secretions including saliva and tears. Mucosal infections can induce specific salivary IgA immunological responses in the absence of detectable serum antibody; hence, IgA plays an important role in defence against infections.[3,4] Two subclasses of IgA are found in humans; IgA1 and IgA2 based on the antigenic differences.[5,6] Of the two subclasses IgA2 predominates in secretions like saliva.[7] There is a relatively high proportion of IgA2 in saliva because of the preferential transport of IgA2 produced in the distant tissues into saliva, and partly on the relatively increased number of IgA2 containing plasma cells in exocrine tissues.[8] The data regarding the levels of salivary IgA subclasses in RAU is still lacking. Most of the findings have been limited to the determination of total IgA either in serum or saliva. In the light of these factors this study was undertaken to investigate the possible alterations in salivary IgA1 and IgA2 in patients with recurrent aphthous ulcerations.

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MATERIALS AND METHODS

Thirty clinically confirmed untreated (with steroids) cases of RAU and 30 age and sex matched controls were included in the study. After a detailed case history and thorough clinical examination, 2 mL of saliva was collected in both acute and remission phases of the disease. The obtained saliva samples

were subjected to quantification of IgA1 and IgA2 levels using the RID kit.

Collection of saliva samples

The patients were asked to rinse their mouth thoroughly with water. Unstimulated whole saliva was collected in a sterile bottle by asking the patient to expectorate into it gradually over a period of 5–10 min. Approximately 1–2 mL of saliva was collected.

The obtained saliva samples were stored in deep freezer at –20°C until further test was carried out. The salivary IgA subclasses levels were estimated using the RID Kit (Human IgA subclass NLCombikit, the Binding site, UK).

Determination of the IgA subclasses

Appropriate volumes (10 µL for IgA1 and 10 µL for IgA2) of nondiluted saliva as well as of appropriately diluted calibrator and control sample were placed into agarose gel immunodiffusion plates containing the appropriate monospecific antibody. The plates were then tightly closed, resealed in their original pouches and placed in the moist chamber at 37°C in the incubator. After 96 h incubation, the precipitin ring diameters were measured using a tripartisan ruler. The antibody concentrations were read from the reference table enclosed, and expressed in mg/L of saliva. The ring diameters of the calibrator and control serum were always in the expected range, that is, their concentrations were always within 10% of the concentrations stated on the vial label.

Statistical analysis

The obtained salivary IgA1 and IgA2 levels in both study and control group were tabulated and statistical analysis was calculated using Mann-Whitney U test and students paired and unpaired *t* test was done. Using SPSS 11 version package A *P* value of < 0.05 was taken as significant.

RESULTS

The results of this study show that there was a statistically significant increase in the levels of IgA1 in an acute phase ($P<0.001$) as compared to remission phase and controls. Since IgA1 is susceptible to the proteases, it appears that its levels diminish in the remission phase.

There was a significant rise in the IgA2 levels in both acute and remission phases of the disease as compared to controls ($P<0.001$).

A genderwise comparison showed that females had higher levels of IgA1 in an acute phase ($P<0.05$). For IgA2, levels were increased both in acute and remission phases as compared to males ($P<0.001$). This may be attributed to hormonal related altered immune response.

Tables 1 and 3 illustrate that the comparison between mean IgA1 levels in acute phase of the study group and controls was statistically significant ($P<0.001$).

The comparison between mean IgA1 levels in remission phase of the study group and controls. The statistical difference was not significant ($P>0.05$).

Tables 2 and 3 illustrate that the comparison between mean IgA2 levels in acute phase of the study group and controls was statistically significant ($P<0.001$).

The comparison between mean IgA2 levels in remission phase of the study group and controls was statistically significant ($P<0.001$).

Table 1: IgA1 levels in acute and remission phases in the study group

Diametric range	mg/L	No. of cases	Percentage
Acute phase			
4.5–5.0	922–1350	13	43.33
5.1–5.6	1440–1926	14	46.66
Remission phase			
4.0–4.2	541–691	24	80
4.3–4.5	768–922	6	20

Mean and s.d for IgA1 in the acute phase: Mean: 1430.86 mg/L; s.d: ±392.98. Mean and s.d for IgA1 in the remission phase: Mean: 684.8 mg/L s.d: ±197.46

Table 2: IgA2 levels in acute and remission phases in the study group

Diametric range	mg/L	No. of cases	Percentage
Acute phase			
6.1–8.0	252–500	14	46.66
8.1-10.0	514–833	16	53.34
Remission phase			
6.0–7.1	240–373	3	10
7.2–8.3	387–545	17	56.66
8.4–9.5	560–742	10	33.33

Mean and s.d for IgA2 in acute phase: Mean: 545.93 mg/L s.d: ±123.0. Mean and s.d for IgA2 in remission phase: Mean: 498.66 mg/L s.d: ±112.48

Table 3: IgA1 and IgA2 levels in controls

Diametric range	mg/L	No. of cases	Percentage
IgA1			
4.0–4.1	541–614	21	70
4.2–4.3	691–768	9	30
IgA2			
4.8–4.9	121–129	18	60
5.0–5.1	139–148	12	40

Mean and s.d for IgA1: Mean: 617.90 mg/L s.d: ±69.9. Mean and s.d for IgA2: Mean: 131.86 mg/L s.d: ±10.3

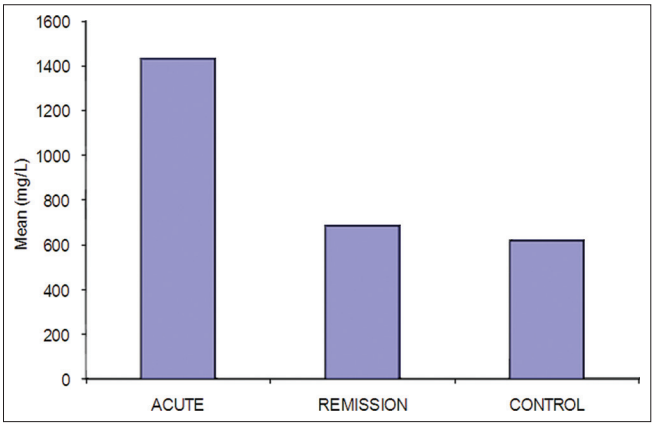
DISCUSSION

Recurrent aphthous ulceration or recurrent aphthous stomatitis (RAS) is an intriguing condition known to humanity since ages and was first described by Hippocrates (460–370 BC). He used the term "aphthai," a Greek word meaning small ulcer, to describe the disorders of the mouth.[9] Secretory IgA (s-IgA) help maintain the integrity of the oral surfaces by limiting microbial adherence to epithelial and tooth surfaces; by neutralizing enzymes, toxins, and virus; or by acting in synergy with other antibacterial factors such as lysozyme, lactoferrin, salivary peroxidase, and mucins. s-IgA may also prevent the penetration of antigens in the oral mucosa. It forms the first line of defence against pathogens in the oral cavity.[10] The literature search gives the evidence that levels of IgA in (serum/saliva) gets altered in oral mucosal diseases such as RAU.[8,11-13] Most of the studies have been limited to the estimation of the whole salivary/serum IgA levels that have given contradictory results. Hence, this study was aimed to evaluate and compare salivary IgA1 and IgA2 levels in patients with minor RAU.

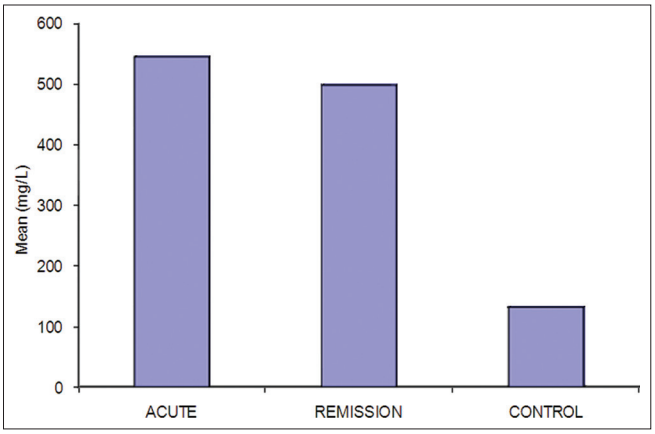
This study comprised of 60 subjects. Thirty subjects with RAU comprised the study group. Thirty age and sex matched healthy subjects were taken as controls. In our study 56.66% cases were females and 43.3% were males. The female predominance is consistent with other studies Jurge *et al*.[14] Minor RAU were the clinical type of aphthous ulcer included in this study. This is in accordance with study of Porter *et al*., who stated that minor aphthous stomatitis affects 80% of the population.[15] In this study, 41.7% (seven cases) were affected by RAU during the time of their menstruation. This finding substantiates the previous reports that menstruation may be a triggering factor for RAU.[16] This may be due to hormone induced alterations in the microbiota of oral mucosa leading to inflammation. It has also been related to the increased blood level of progesterone.[17] Out of 30 subjects, 15 cases, that is, 50% gave a positive history of stress. All 15 cases were college students preparing for summative examination. The high incidence of RAU seen in our study may be due to academic stress and anxiety faced by the students. Many studies have reported association of stress with RAU.[18,19] 16.66% of the cases had the habit of smoking. Earlier studies have reported an inverse relation between smoking and occurrence of the disease. The nicotine in tobacco has been found to be beneficial in RAU and its effects may result from influences on nerve functions, although these may also exert direct anti-inflammatory effects. The mechanism by which nicotine protects against RAU is still unknown.[16]

Numerous studies have reported an increase in the s-IgA levels at the acute period and its reduction in the periods of remission.[13,20,21] These results suggest a strong correlation between s-IgA and the lesion mechanisms in RAU. Conversely many authors found no differences in the levels of salivary IgA in either acute or remission phases of RAU.[22,23] The mean IgA1 and IgA2 levels in acute and remission phases showed a statistically significant increase (P<0.001) [Table 1]. The comparison of mean IgA1 levels in the study group in the acute phase and controls [Graph 1] showed significantly raised levels while the IgA1 levels in the remission phase (684.8 mg/L) returned to the values not different from those in healthy controls (617.9 mg/L) [Tables 1 and 3]. This finding is in contrast to a study Sistig *et al*., 2002 in which only IgA2 levels were significantly raised.[8]

On comparison of acute and remission phases of aphthous patients with controls, it was found that the mean IgA2 levels remained high in both the phases [Tables 2 and 3] (P<0.001). Graph 2 illustrates consistently raised IgA2 levels in both the phases, being highest in the acute phase (545.9 mg/L) compared to controls. The consistent rise in IgA2 levels in our study suggests that minute variations in IgA subclasses in RAU could not be elicited by simple determination of total IgA. The results of this study are compatible with the study of Sistig *et al*.,[8] with one difference that in our study IgA2 levels were raised both in acute and remission phases. They suggested that these changes could be related to the chronic antigen stimulation because of higher concentration of the specific microorganism that could be involved in the pathogenesis of RAU.



Graph 1: Mean values for IgA1 in acute, remission phases of study groups compared with control



Graph 2: Mean values of IgA2 in acute and remission phase in study groups compared with control

Changes in the IgA1 and IgA2 concentrations in saliva may be the result of different antigen stimulation in the oral cavity that preferentially induces either IgA1 or IgA2 antibody production from plasma cells. Furthermore, the IgA subclass ratio may change during an active immune response (Crago *et al*., Brown and Mestecky.[24,25]) IgA1 is less resistant to bacterial proteases than IgA2. Killian.[26,27] So the decrease seen in the IgA1 levels in the remission phase to the same as healthy controls could be due to its reduced sustainability because of its degradation by enzymes. A genderwise comparison in the levels of salivary IgA1 and IgA2 was made. The mean comparison of IgA1 levels in the acute phase between males and females showed statistically significant difference ($P<0.05$) with higher levels seen in females, that is, 1667.7 mg/L as compared to males 1121.15 mg/L. The mean IgA2 levels in both acute and remission phases in females showed a statistically significant increase ($P<0.05$) as compared to males. The reason for the increased levels in females as compared to males is not clear and further studies are required to confirm the present finding. The IgA subclasses levels in female patients did not show any association ($P>0.05$) with menstrual cycle. In a study on RAU, discrepant results were reported with regard to menstrual cycle and s-IgA.[13] Kazikawa *et al*. showed that salivary IgA levels were affected by menstruation.[28] In the current study, results are not conclusive as the sample size in the two categories of females is very small. Effect of microbiological load in the oral cavity has not been studied in this study. Some of the results are not conclusive as the study subgroups were small. Further studies taking large samples are required to establish the correlation beyond doubt.

CONCLUSION

Immunoglobulin A is an important parameter to assess the status of mucosal immune system that can be measured by noninvasive methods and without patient discomfort. This study was an attempt to determine the IgA subclasses levels in acute and remission phases of the disease RAU using RID kit. Considering the limitations of the current study following conclusion can be drawn

1. IgA subclass ratio may be changed during the active immune response, reflecting the generation of specific antibodies to various antigens of mucosal pathogens.
2. Determination of salivary IgA using RID is a reliable, quick and a noninvasive means to assess the immune status in patients with RAU.
3. IgA subclasses play an important role in the pathogenesis and defense mechanism of RAU.

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