Abstract: This study aimed to examine the role of stress in the occurrence of recurrent aphthous stomatitis (RAS) and oral lichen planus (OLP), as well as to analyze the efficacy of saliva, urine, and serum cortisol levels as markers of stress. This study included 30 subjects with RAS (Group A), 30 with OLP (Group B), and 30 controls (Group C). The serum, salivary, and urinary cortisol levels were measured using electro-chemiluminescence immunoassay. The results were analyzed using the independent t-test, and a statistically significant difference ($P = 0.000$) was observed between the study and control groups. Further analysis was done using ANOVA and post-hoc tests. The results of this study suggest that RAS and OLP patients had higher cortisol levels than controls, indicating that stress acts as a co-factor in the pathogenesis of RAS and OLP. Although urinary cortisol level was found to be the best indicator of stress, saliva can also be used as a reliable marker.

Keywords: cortisol; RAU; OLP.

Introduction
Cortisol (21 carbon glucocorticoid), also known as the stress hormone (1), is secreted by the adrenal cortex and is used to assess stress and anxiety in human beings. It also influences metabolism, immunoregulation, vascular responsiveness, cognition, behavior, and pathological conditions, such as inflammatory autoimmune disorders (2). Persistent anxiety problems causes changes in the Hypothalamus-pituitary-adrenal axis thereby increasing cortisol levels (1). Cortisol production exhibits an ACTH-dependent circadian rhythm, achieving peak levels early in the morning and the lowest levels at night (1).

The cortisol hormone levels are used as an indicator for stress evaluation. It is primarily excreted in free form in urine. Salivary cortisol levels are also a reliable indicator of free cortisol or biologically active cortisol, while serum cortisol measurement assesses total cortisol levels (1,3,4). Stress modifies the immune response in autoimmune conditions (5,6), that can be assessed using serum, saliva, and urine cortisol levels (7). Previous studies have suggested that psychological disturbances, such as stress and anxiety, may play a role in the onset and recurrence of recurrent aphthous stomatitis (RAS) (7,8). Moreover, oral lichen planus (OLP) may also be observed 1-2 weeks after severe emotional stress (9-14). Currently, there are no studies that compare the efficacy of the three markers, namely, salivary, serum, and urinary cortisol levels. The aim of this study was to assess the association between stress RAS and OLP by measuring cortisol levels in urine, saliva, and serum.

Materials and Methods
This study included 30 subjects with a clinical diagnosis of RAS (Group A), 30 subjects with a clinical diagnosis of OLP (Group B), and 30 controls (Group C). Patients with chronic RAS and OLP and a history of repeated episodes associated with emotional stress were selected. Pregnant or lactating women, hypertensives, diabetic patients, individuals suffering from renal or endocrinal problems, patients with psychiatric diseases, those on oral contraceptives and corticosteroids, and patients with
oral lichenoid reactions resulting from chewing arecanuts, gutkha, or tobacco and smoking were excluded from the study. Informed consent was obtained from all participants, and there were no financial implications for them. This study was approved by the Institutional Ethics Committee (Ref: SBDCECM 105/13/35).

A detailed history was collected, and a clinical examination was carried out on all subjects. All the details were recorded in a structured form. Of the 30 OLP patients, 11 had only oral lesions and 19 had both skin and oral lesions. Of 19 oral and skin lesions, 16 were reticular, 2 were erosive, and 1 was linear. Of the 11 only oral lesions 7 were reticular 2 were erosive and 2 were linear. The patient's serum was collected using tubes containing separating gel. Saliva was collected by the spitting method. Urine sample was taken from the 24-h urine samples collected in clean containers without preservatives. The samples were centrifuged at 2,500 rpm for 5-10 min, and cortisol estimation was carried out before 10 am (to account for diurnal variations in cortisol levels) with a cortisol assay kit (Fig. 1) using an electrochemiluminescence immunoassay (ECLIA) (Fig. 2).

**Results**

The RAS group comprised 14 males and 16 females, while the OLP group comprised 13 males and 17 females. The control group had 15 males and 15 females. Among the 30 RAS patients, 18 were in the 15-30-year age group, 5 were in the 31-45-year age group, and 7 were above 45 years of age. Of the 30 OLP patients, 8 were in the 15-30-year age group, 12 were in the 31-45-year age group, and 10 were above 45 years of age. Among the controls, 13 were in the 15-30-year age group, 7 were in the 31-45-year age group, and 10 were above 45 years of age. The mean age of patients was approximately 33.9 years in the RAS group and 39.9 years in the OLP group (both males and females). The most common site for RAS was the labial mucosa. Reticular pattern was found to be the most common type of OLP affecting the buccal mucosa of the oral cavity. A comparison of cortisol levels in serum, saliva, and urine between the cases (RAS and OLP) and controls was carried out using the independent t-test. A P value of <0.05 was considered statistically significant.

The mean serum cortisol level was 18.7393 in RAS cases and 10.4680 in the controls, with a mean difference of 8.27133 between the two groups \((P = 0.000)\) (Table 1). The mean salivary cortisol level was 0.5890 in the RAS cases and 0.2007 in the controls, with a mean difference of 0.38833 between the two \((P = 0.000)\). The mean urinary cortisol level was 149.97 in the RAS cases and 45.77 in controls, with a mean difference of 104.200 between the two \((P = 0.000)\). Serum, salivary, and urinary cortisol levels in the RAS and control groups were compared using ANOVA and post-hoc tests, and urinary cortisol was found to be the best indicator for estimating stress levels, followed by saliva and serum.

The mean serum cortisol level in OLP cases was 20.8720 and 10.4680 in controls, with a mean difference of 10.404 between the two \((P = 0.000)\) (Table 2). The mean salivary cortisol level was 0.525 in OLP patients and 0.201 in controls, with a mean difference of 0.3240 between the two \((P = 0.000)\). The mean urinary cortisol level in OLP cases was 262.57 and 45.77 in controls, with a mean difference of 216.800 between the two \((P = 0.000)\).

The serum, salivary, and urinary cortisol levels of OLP and controls were compared using ANOVA and post-hoc tests, and urinary cortisol was found to be the best indicator for estimating stress levels, followed by saliva and serum.
Discussion

The aetiopathogenesis of RAS is complex. In addition to genetic predisposition, viral and bacterial infections, food allergies, vitamin and microelement deficiencies, systemic diseases (e.g., Crohn’s disease, Celiac disease), increased oxidative stress, hormonal defects, mechanical injuries, and psychological disturbances, such as stress and anxiety, play an important role in the incidence of RAS (6). OLP is immunologically mediated, and stress acts as a co-factor in exacerbation of the disease (15). Cortisol, the stress hormone, is secreted by the adrenal cortex and is a reliable indicator of a patient’s stress levels. In this study, salivary and serum samples were collected between 9 and 10 am to account for diurnal variations in cortisol levels (1). The reticular type of OLP was the most common in this study, which is in accordance with previous studies. However, few studies have also reported that the erosive type was more common (3). When comparing the cortisol levels of the study groups and controls, significantly higher serum, salivary, and urinary cortisol levels were observed in the RAS and OLP groups compared to the control group. These results suggest that a higher level of psychological stress was present in the study group compared to the control group, indicating that psychological stress may play a role in the manifestation of RAS and OLP. Our findings are in agreement with previous studies (1,2).

Our study also confirmed that urinary cortisol was the best indicator of an individual’s stress levels. Estimation of cortisol levels in the serum and saliva is technique-sensitive, whereas 24-h urine samples provide an accurate assessment as they reflect the cortisol levels realistically by accounting for variations in the emotional status of a patient over 24 h.

This present study aimed to examine the role of stress in the occurrence of RAS and OLP. A significant rise in the cortisol levels in serum, saliva, and urine in RAS and OLP patients with that of normal individuals was observed. Therefore, the measurement of cortisol in serum, saliva, and urine reflects an individual’s response to stress and appears to be a promising parameter in the investigation of RAS and OLP. Hence, supportive psychological management should be provided along with conventional treatment in RAS and OLP patients to increase their ability to cope with stress. All three parameters, serum, salivary, and urinary cortisol levels, were helpful in evaluating stress in RAS and OLP patients, although urinary cortisol was found to be the best indi-
cator of stress.

The present study involved a small sample size, and the results must be confirmed by larger, longitudinal, population studies. Further research can be directed at assessing psycho-immune interactions as these may represent various ways in which the psychological status of an individual may affect immune status homeostasis of OLP and RAS.

Conflict of interest
The authors declare no conflicts of interest. No external source of funding was obtained for this study.

References