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ORIGINAL RESEARCH

Estimation of Salivary Nitric Oxide in Recurrent Aphthous Ulcer and Oral Lichen Planus Patients with Its Clinical Significance

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ABSTRACT

Aim: The present study was undertaken to study the role of nitric oxide in human saliva and its diagnostic/prognostic role in recurrent aphthous ulcers (RAU) and oral lichen planus (OLP).

Materials and methods: The study was carried out at outpatient department of Govt Dental College and Hospital, Aurangabad. Twenty cases with RAU, 15 with OLP and 30 healthy individuals were included in the study. The clinically diagnosed known cases of RAU and OLP were included after taking the detail case history and subjected to blood analysis for hemogram and biochemically salivary nitric oxide was estimated.

Results: The salivary nitric oxide levels were found to be increased significantly in RAU and OLP group, when compared with controls. Further, significantly increased levels have been observed in OLP group, when compared with RAU group ($p < 0.001$). The salivary nitric oxide levels were found to be increased significantly in minor RAU than major RAU and increased in erosive type of OLP than nonerosive type of OLP.

Conclusion: Thus, salivary nitric oxide can be treated as a diagnostic tool for the differential diagnosis of RAU and OLP.

Clinical significance: Nitric oxide plays an important role in modifying physiopathological processes of oral mucosal membrane so has diagnostic as well as prognostic value.

Keywords: Recurrent aphthous ulcer, Oral lichen planus, Nitric oxide.

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INTRODUCTION

Aphthous ulcers are very common with the incidence rate of 17 to 50%. Recurrent aphthous ulcers (RAU) are characterized by painful recurring ulceration of oral mucosa.[1] Oral lichen planus (OLP) is clinically recognized as persistent white striations and patches associated with mucosal atrophy and erosion.[2] The etiological factors associated with RAU and OLP are trauma, allergy, hereditary and psychosomatic stress.[3,4] Though various treatment modalities are available, still the symptomatic treatment is the best option available.[5] The immunological imbalance and psychological stress are considered to be the prime factors for the condition, like RAU and OLP. Further, the oxidative stress caused by nitric oxide has a definitive role in establishing the correlation between RAU and OLP.[6] Nitric oxide is pathological or patho- psychological free radical mediator of the number of diseases of CNS and PNS, cardiovascular system and kidneys. Its role in angiogenesis and tumor progression in head and neck cancer has been described by Emarnula Masini et al 1998.[7] Nitric oxide have been reported to be present in freshly secreted human saliva and it was suggested that nitric oxide plays an important role in modifying physiopathological processes of oral mucosal membrane.[2]

Therefore, the present study was undertaken to estimate salivary nitric oxide levels in RAU and OLP with view to ascertain its diagnostic as well as prognostic value.

MATERIALS AND METHODS

Thirty-five cases were studied inclusive of 20 cases of RAU and 15 cases of OLP and 30 healthy controls were included in the study for comparison. A thorough case history was recorded and clinically diagnosis of RAU and OLP was confirmed.

Under all aseptic precautions, the fresh saliva was collected in sterile bulb. The sample was subjected to biochemical analysis of nitric oxide by diazotization reaction with adoption of the principles of Griess Reagent.

The RAU cases were subdivided to major and minor RAU and OLP cases were divided into erosive and non-erosive type after clinical and histological examination.

RESULTS

The 30 cases in the control group comprised of equal number of males and females. However, 35 cases under study group were comprised of 24 males (68.5%) and 11 females (31.5%) in RAU and OLP. Male to females ratio was 2.1:1 (Table 1).

The patients in the age range of 11 to 20 years were four each in control and RAU group, 19, 08 and 03 in the age range of 21 to 30 years, 3, 4 and 6 in the age range of 31 to 40 years and 4, 4 and 6 cases in the age range above 40 years in control RAU and OLP groups (Table 2).

Salivary nitric oxide levels in various age range in all the groups and their comparison with the control group is as under Table 3. Table 4 shows comparison of salivary nitric oxide levels in control group with RAU and OLP.

Salivary nitric oxide levels found to be increased significantly in RAU and OLP group, when compared with control $p < 0.001$ which is statistically highly significant, all age ranges studied (Tables 5 and 6).

When comparision was made between RAU and OLP group, only in the age range of 21 to 30 years salivary nitric oxide levels were increased significantly, $p < 0.001$, in OLP when compared with RAU.

In the age range of 31 to 40 years, salivary nitric oxide levels were increased significantly, $p < 0.001$, in OLP group when compared with RAU.

In the age range above 40 years, the contrasting results were observed, i.e. salivary nitric oxide levels were increased significantly in RAU groups when compared with OLP groups.

The patients in the OLP groups were advised oral steroids along with the topical application for 15 days in four cases followed, salivary nitric oxide levels were found to be 181.0 ± 13.5 μgm%.

Three cases in OLP group turned up after 1 month who has applied topical medications without oral steroids. The salivary nitric oxide levels observed were 300.0 ± 4.0 μgm%. (Table 7).

Mean salivary post-treatment levels of nitric oxide in OLP group were found to be decreased significantly $p < 0.001$. When compared with mean pretreatment levels of salivary nitric oxide, statistical analysis was carried out by appling paired t-test (Figs 1 and 2).

Table 1: Sex distribution

Group	No. of cases	Age range (years)	Mean age (years)	Male	Female
Control	30	15-55	28.1	15	15
RAU	20	15-66	30.8	15	05
OLP	15	24-55	37.2	09	06
Total	65			39	26

Table 2: Age distribution

Group	No. of cases	Age (years)			
		11-20	21-30	31-40	>40
Control	30	04	9	03	04
RAU	20	04	08	04	04
OLP	15	–	03	06	06
Total	65	08	30	13	14

Table 3: Mean salivary nitric oxide levels observed in various groups

Group	No. of cases	Nitric oxide levels (μgm%)
Control	30	214.35 ± 1.42
RAU	20	339.7 ± 6.72
OLP	15	352.3 ± 12.0

Table 4: Comparison of salivary nitric oxide levels in control group with RAU and OLP

Group	No. of cases	Nitric oxide level (μgm%)	SD	t-value	p-value
Control	30	214.35 ± 1.42	–	–	–
RAU	20	339.7 ± 6.72	19.09	22.72	<0.001
OLP	15	352.3 ± 12.0	5.26	62.91	<0.001

$p < 0.000$, highly significant

Table 5: Comparison of salivary nitric oxide levels in observed in RAU group with OLP group with respect to age group

Groups	No. of cases	Nitric oxide level (μgm%)	SD	t-value	p-value
RAU	20	339.7 ± 6.72	–	–	–
OLP	15	352.3 ± 12.0	3.95	9.33	<0.0001

$p < 0.000$, highly significant

Table 6: Comparison of salivary nitric oxide levels observed

Groups	No. of cases	Nitric oxide level (μgm%)	SD	't'	p-value
Age range 11 to 20 years in all groups					
Control	04	213.6 ± 3.2	–	–	–
RAU	04	346.0 ± 3.0	3.10	60.38	<0.001
OLP	–	–	–	–	–
Age range 21 to 30 years in all groups					
Control	19	201.6 ± 0.1	–	–	–
RAU	08	328.3 ± 15.0	63.00	4.76	<0.001
OLP	03	393.0 ± 1.0	02.52	57.2	<0.001
Age range 31 to 40 years in all groups					
Control	03	201.6 ± 0.1	–	–	–
RAU	04	313.2 ± 15.0	3.00	43.59	<0.001
OLP	06	351.1 ± 31.8	26.9	07.24	<0.001
Age range above 40 years in all groups					
Control	04	215.7 ± 0.1	–	–	–
RAU	04	371.5 ± 5.5	3.88	75.14	<0.001
OLP	06	312.8 ± 3.2	2.53	82.60	<0.001

Table 7: Comparison of pre- and post-treatment levels of salivary nitric oxide in OLP group

Group	*No. of cases*	*Pretreatment levels (μgm%)*	*Post-treatment levels (μgm%)*	*SD*	*t-value*	*p-value*
OLP	10	359.71 ± 57.1	240.8 ± 74.4	68.84	8.6	<0.001

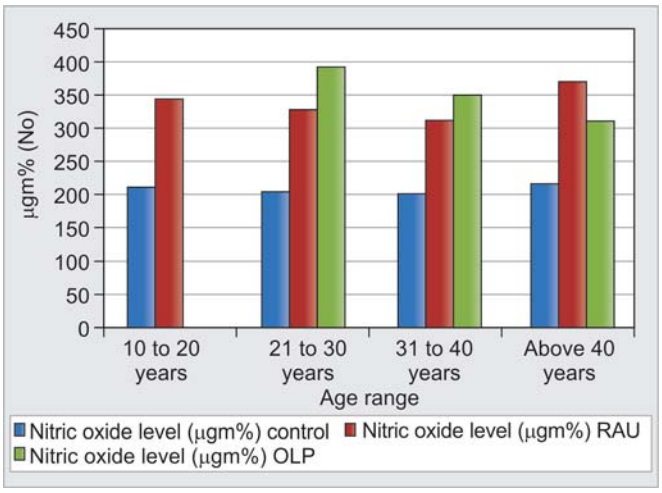

Fig. 1: Histogram-salivary (NO) levels in control, RAU and OLP groups

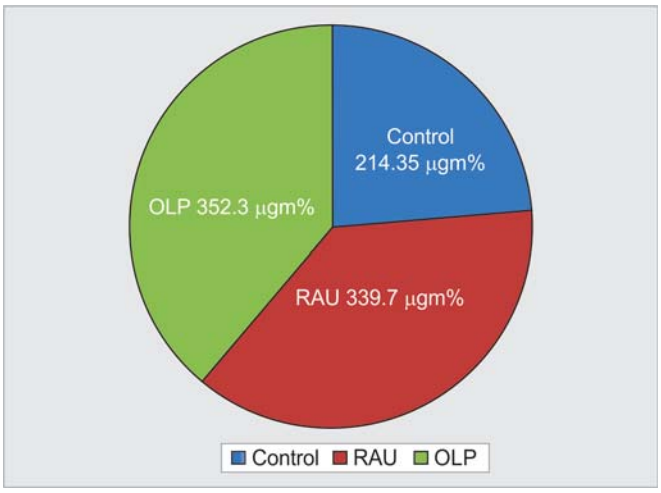

Fig. 2: Salivary nitric oxide levels in control, RAU and OLP groups

DISCUSSION

Common histological features of these diseases are characterized by basal cell destruction and presence of lymphocytic infiltrate. Immunologic and psychological disturbances have been considered in their etiology (Vincent et al 1992).[8]

The common etiologic factors especially on the psychological background and microscopic features were specifically selected for the study to evaluate salivary nitric oxide levels. It is believed that there is a specific correlation that exists between the psychological stress and salivary nitric oxide levels in RAU and OLP.

The RAU group revealed salivary nitric oxide levels of 346.0 ± 1.5, 332 ± 11.7, 313.2 ± 1.7 and 356. ± 4.3 μgm% in the age groups 11 to 20, 21 to 30, 31 to 40 and above 40 years respectively. Maximum levels were observed in age groups above 40 years, followed by 11 to 20 years. Prepubertal age or premenstrual age range and post-menopausal age range were more prone to the emotional stress and hormonal imbalance which results in the elevation of salivary nitric oxide levels. During the process of ageing, reactive oxygen species (ROS) generates in bulk quantities which results into the elevated nitric oxide levels in RAU.

Salivary nitric oxide levels observed in various age range in OLP group were 289.7 ± 3.75, 340.3 ± 1.3 and 312.4 + 5.5 μgm% in the age range 21 to 30, 31 to 40 and above 40 years respectively. The psychological stress or physiological stress causes excess nitric oxide release in the hypothalamic pituitary adrenal axis (HPA).[9,10] Also, the hormonal imbalance leads to the highest salivary nitric oxide concentration (Yashikwa J Yoshida 1997).

It has been reported that submandibular or parotid salivary glands did not show any gross difference in the concentration of nitric oxide. Therefore, the whole saliva was used for the quantitation of salivary nitric oxide.

At present, precise mechanism for the synthesis of nitric oxide is not fully understood, however, it is believed that following three mechanism seems to generate nitric oxide:

1. The process involving deamination of arginine to form ammonia which is then oxidized to nitric oxide (Hibbs et al 1987).
2. Formation of *N*-hydroxyarginine via NADPH tetra-hydrobiopterin-dependant mono-oxygenation (Marcletta et al 1988).
3. *N*-hydroxyaginine gives rise to hydroxyl amine which is converted into nitric oxide by the action of enzyme catalase (De Moster et al 1989).[10]

It is believed that nitric oxide is synthesized by variety of cells and tissues, including brain. Nitric oxide acts as a crucial mediator of various metabolic function and serves as retrograde messenger.[11] There is a correlation between immunostimulation and elevated nitric oxide synthesis. The cytotoxicity of activated macrophages against tumor cells was shown to be dependent on L-arginine and ultimately nitric oxide (Granger et al 1980).[10]

Nitric oxide induced by several kinds of stress has a significant role to produce ulcers. Thus, free radicals including nitric oxide, represent one route for the pathogenesis of erosion or ulceration.[12]

The physiological stimuli which release or generates nitric oxide is shear stress which might be one of the major

cause of the oral cavity diseases, like RAU and OLP (Calga L Gradine 1993).[2,10]

In OLP group, the patients were advised to take steroid along with topical applications. The pretreatment salivary nitric oxide levels were increased significantly $p < 0.001$ when compared with the post-treatment levels. Corticosteroids directly inhibits the generation of nitric oxide by inhibiting nitric oxide synthase activity.[10]

CONCLUSION

Thus in the present study, the diagnostic value of salivary nitric oxide in RAU and OLP was evaluated and prognostic value in OLP was also assessed. This parameter can also be used for the differential diagnosis of RAU and OLP. Still further studies are required to establish concrete relation between salivary nitric oxide and RAU and OLP.

CLINICAL SIGNIFICANCE

Nitric oxide have been reported to be present in freshly secreted human saliva and it was suggested that nitric oxide play an important role in modifying physiopathological processes of oral mucosal membrane. Therefore, salivary nitric oxide levels present diagnostic as well as prognostic value in RAU and OLP.

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