Assessment of serum cortisol levels in oral submucous fibrosis patients
K.Monisha¹, V. Vishnu Priya²*, R. Gayathri²

INTRODUCTION
Oral cancer is the sixth most common human cancer in the world with 5% of morbidity rate and 5-year mortality rate of about 50%.¹ Among oral cancer, oral submucous fibrosis (OSMF) is seen in the Indian subcontinent and Southeast Asia.² In India, the prevalence increased over the past four decades. The prevalence is seen commonly in males between 20 and 40 years of age.

OSMF is a premalignant disease of the oral mucosa. It is a progressive and chronic disease. The most common cause of OSMF is chewing areca nut.³ Areca nut has a high copper content. This acts as a mediator of OSMF by upregulating collagen production in oral fibroblasts. Areca nut is made up of alkaloid and flavonoid components. Four different alkaloids have been identified in areca nut, namely arecoline, arecaidine, guvacine, and guvacoline of which arecoline is the most potent agent and plays a major role in the pathogenesis of OSF by causing an abnormal increase in collagen production.

The flavonoid components such as tannins and catechins have been found to have some direct influence on collagen metabolism.⁴ Basic mechanisms involved in the pathogenesis of OSF can be divided into four steps as follows:

The occurrence of the chronic inflammation at the site of betel-quid placement, increased collagen synthesis,
collagen cross-linking, and decreased collagen degradation.

The extent and severity of this disorder are dependent on the amount of areca nut in the betel quid, duration, and frequency of this habit. OSMF involves the buccal mucosa, tongue, and soft palate. The affected mucosal surfaces appear pale, blanching marble-like with focal areas of atrophy, and erythema. Patients commonly present with trismus, burning sensation, and xerostomia; difficulties in speech, mastication, and swallowing are experienced at the advanced stage.[3]

A number of other etiological factors also play a role in the pathogenesis of this disease. Factors include areca nut chewing, ingestion of chilies, genetic and immunologic processes, and nutritional deficiencies. Various studies on fibroblast and keratinocytes support the chewing of areca nut as one of the most important risk factors for OSMF.

Genetic predisposition and nutritional deficiencies have also been implicated in the pathogenesis of oral submucous fibrosis.[6,7] It increases fibroblast proliferation and collagen formation by increasing the production of growth factors (platelet-derived growth factor, fibroblast growth factor, transforming growth factor-beta, and connective tissue growth factor), cytokinins (interleukin-1 [IL-1], IL-6, IL-8, and tumor necrosis factor-alpha), tissue inhibitor of metalloproteinase and also by reducing the matrix metalloproteinase production, and collagen phagocytosis.

Cortisol is a steroid hormone which is secreted by the adrenal glands; it responds the body to stress, bodily infections, and also regulates blood sugar levels. Lack of cortisol levels produces exhaustion, chronic fatigue, and endocrine-related diseases. Salivary cortisol measurement indicates free cortisol or biologically active cortisol in human serum and provides noninvasive and easy technique. Cortisol levels can also affect noncancerous patients through physical stress, emotional stress, and illness.[8]

A common syndrome that is associated with increased levels of cortisol is Cushing syndrome. Other causes are Addison’s disease, pituitary insufficiency, hypothalamic insufficiency, and congenital adrenal hyperplasia. Cortisol counteracts with insulin, acts as a diuretic, stimulates gastric acid, and renal hydrogen ion secretion. Serum cortisol levels are increased in oral cancer patients due to high stress, depression, and anxiety levels.

Cortisol “stress hormone” is used as an indicator in stress evaluation studies. Cortisol is the major glucocorticoid in humans and has a wide range of influences on metabolism, immunoregulation, vascular responsiveness, cognition, and behavior.[9] In stressful situations, there is an activation of the hypothalamus–pituitary–adrenal (HPA) axis, with release of cortisol, a hormone that shows a complex action on the metabolism of carbohydrates, proteins, and lipids, besides acting on inflammatory and immunological responses.[10]

It is believed that high anxiety level causes increase in serum and salivary cortisol level and has been used as an indicator in the stress, anxiety, and depression evaluation studies. In conditions involving pain and anxiety many metabolic and endocrine changes occur, and among these a rise in the levels of serum as well as salivary cortisol which is one of the most important physiological effects. Cortisol can be measured in urine, plasma, and saliva. Collection of serum cortisol is an invasive method as it involves collection of blood; patient may develop anxiety due to fear of withdrawing blood and may give false cortisol values compared to secretion of cortisol in saliva which is a noninvasive method and a reliable indicator of serum cortisol level. Thus, the use of saliva sampling which is a reliable, non-invasive, and easy procedure is advocated in the present study. Several studies have suggested the relationship between salivary cortisol level and stress, anxiety, and depression levels.[11] The prognosis of patients with elevated serum cortisol tended to be poorer (P = 0.06) than the prognosis of those with lower levels.[12]

The aim of the present study is to find if there is a significant increase in serum cortisol levels in OSMF patient compared to the control group.

**MATERIALS AND METHODS**

A total of 30 patients were recruited from the routine outpatient Department of Periodontics. The nature and rationale of the present study were explained to all the participants, and consent was obtained for the same. The study group was divided into three groups (n = 3) as follows:

- Group-1: Those patients without areca nut chewing habits and without OSMF.
- Group-2: Those patients with areca nut chewing habits without OSMF.
- Group-3: Those patients with areca nut chewing habits and with OSMF.

Group 1 (Control group), Group 2, and Group 3 (Study group).

**Method of Collection of Sample**

About 3–5 ml of blood was collected by venipuncture, and blood was centrifuged, and then, serum cortisol level was evaluated using cortisol ELISA kit.

The amount of cortisol present in the serum undergoes diurnal variation, with the highest levels being present.
early in the morning and lower levels at night, several hours after the onset of sleep. The highest levels are at about 6–8 am.

About 20 μl of the serum sample was added to their respective wells and 200 μl of cortisol-HRP conjugate to each well. Incubation is done for 1 h at 37°C. The wells are then washed well three times with 300 μl diluted washing solution. About 100 μl of TMB substrate solution is added to all wells and was incubated for 15 min at room temperature in the dark. About 100 μl stop solution was added to all wells in the same order at the same rate as for the TMB substrate solution. Absorbance of the sample at 450 nm is measured within 5 min of addition of the stop solution. The results are expressed as ng/ml.

RESULTS

From the Graph 1, the absorbance values of Group-1 are -249 ng/ml, Group-2–265 ng/ml, and Group-3–472 ng/ml.

Results are expressed as a mean ± standard error of the mean, \( n = 3; *P < 0.001 \), statistically significant as compared to Group 1 and Group 2.

DISCUSSION

OSMF stage has been noted to be associated with depression probably due to chronicity of the condition and critical weakening (restricted mouth opening, eating, gulping, talking troubles, and smoldering sensation in the mouth), which consequently affected the serum cortisol level that as many as 50% of cancer patients experience symptoms of depression. The negative impact of depressive symptoms in cancer patients takes many forms including reduced quality of life, poorer functional status, disruption of social relations, and poorer medical outcomes and possibly reduced survival time.[13]

Depression has an effect on the HPA axis, leading to increased production of corticotrophin-releasing hormone, and consequently increases in the serum cortisol level. In oral lichen planus (OLP), patients typically manifest elevated sense of anxiety and depression and higher susceptibility to psychotic disturbances. Such patients are reported to have high levels of cortisol in their saliva, which has confirmed the association of this pathology with stress.[14] OSMF and leukoplakia are the main premalignant diseases leading to oral carcinoma which is a very important matter of concern in the current scenario.[15] The present study shows that there is a significant increase in individuals with habit of areca nut chewing and with OSMF when compared to a patient with OSMF with no habit of chewing areca nut and healthy controls.

It shows that OSMF itself causes high inflammation in the body due to the malignant cells.

Along with OSMF, areca nut chewing habituation triggers the inflammation by losing oral hygiene and distracting the metabolic pathways.

During recent years, chronic physical illnesses and psychiatric disorders have been studied extensively.[16] It is suggested that individuals with chronic somatic diseases such as cancers, temporomandibular disorders, burninng mouth syndrome, OLP and recurrent aphthous stomatitis, asthma, arthritis, chronic obstructive pulmonary disease, and diabetes mellitus are at a relatively higher risk of developing psychological distress than physically healthy people. Psychological distress may manifest itself in many different ways, from having to make an extra effort to cope with illnesses, through emotional symptoms such as grief or anxiety that naturally accompany a fearful situation, to a clear-cut psychiatric disorder. Stress is one of the etiology or predisposing factors in many diseases. In course of time, personal habits such as gutkha, tobacco and betel nut chewing, pan chewing, smoking, etc., which lead to harmful effects on oral mucosa. Some diseases are considered as idiopathic; hence, diagnosis and treatment plan of such diseases always becomes controversial. OLP, OSMF, leukoplakia, and squamous cell carcinoma are the most common oral mucosal diseases known to human beings and constitute entities that deserve to be investigated as psychosomatic diseases. Studies have find that 20%–40% of cancer patients have significant levels of distress.[17]

When cortisol is released into the circulation, it leads to unsolicited effects throughout the body such as modification of certain growth factor levels, elevation of blood glucose levels, and containment of the inflammatory response.[18]

In a study done by Kanodia et al., the mean serum cortisol level of the study group showed a highly significant difference from the control.[19]

OSMF and its association with psychiatric morbidity have been noticed by Mubeen et al., Raja et al.,
and Arjun et al. Advanced OSMF stages have been reported to be associated with higher psychiatric morbidity by Mubeen et al. and Raja et al. [20-22]

CONCLUSION

From the study, it reveals that the habituation of chewing areca nut may cause the OSMF condition and increases inflammation which may leads to other metabolic disorder.

The present study also concludes there is a significant increase in serum cortisol levels in oral submucous patients compared to the control group.

REFERENCES


Source of support: Nil; Conflict of interest: None Declared