

# A Comparative Study Of Serum And Salivary Cortisol Levels In Oral Potentially Malignant Disorders And Oral Malignancy

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## ABSTRACT

### Introduction:

Oral malignancy is a bane for the human race which mostly affects people in the autumn of their lives. It accounts for 1-2% of all malignancies worldwide. The main incumbrance comes from the fact that most of the patients undergo diagnosis at a much later stage; i.e. in Stage III or IV. Delayed diagnosis causes a surge in mortality rate; with an average of a minimum of 5-year survival rate in 20-25% of the patients. Provided the fact that cancer diagnosis is immoderate for most people, they generally avoid it. This in turn drastically increases morbidity and mortality in those subjects.

### Objective:

The impetus of this study is to establish a relationship between and to compare the serum cortisol and salivary cortisol in subjects of oral pre-malignant and malignant lesions, as psychological stress is a very common occurrence in these patients. Also, the fact that undergoing a simple blood investigation is much more affordable and less invasive, hence comparing serum and salivary cortisol levels can prove to be a useful tool in early cancer detection.

### Materials and Methods:

Our study consisted of 15 patients with oral squamous cell carcinoma, 15 patients with oral pre-malignant lesions and 15 healthy patients as control. Non-stimulated whole saliva and serum were collected from all these patients during a fixed interval of time during the day (from 9:00 AM to 10:00 AM). Serum and saliva were measured for cortisol levels using the auto-analyzer machine.

### Results:

Data regarding malignancies demonstrated significant upregulation in the serum cortisol level compared to the PMD group ( $p=0.02$ ) and healthy controls. Whereas, the PMD group showed a significant upregulation of salivary cortisol levels compared to the malignant group and control ( $p=0.02$ ).

### Discussion:

Psychological stress and hence cortisol level plays an important role in understanding the development of oral PMDs & malignancies. Cortisol can thus function as an important bio-marker in understanding the relationship between the two.

### Conclusion:

Through this study, it was observed that there is a distinct relation between the salivary & serum cortisol levels in patients with potentially malignant & malignant lesions. Salivary cortisol was found to be high in the potentially malignant group whereas, serum cortisol level was high in the malignant group.

**Keyword:** Cortisol, Psychological stress, Oral Potentially-malignant lesions, Oral Malignancies, Squamous cell carcinoma.

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## I. INTRODUCTION:

An Oral precancerous lesion is a morphologically altered tissue in which oral cancer is more likely to occur than in its normal counterpart. An Oral precancerous condition is a generalized state associated with a significantly increased risk of cancer'. In recent times, the term Oral Potentially Malignant Disorders (PMD) has gained more acceptance for the above categories of diseases.

Oral Potentially Malignant Disorders are defined by WHO 2005 as "The risk of malignancy being present in a lesion or condition either at time of initial diagnosis or at a future date."

Cancer development is multifactorial. Several aspects like fear, depression, stress, etc. play important role in the mechanism of cancer development and its progression. [1]

It has been reported that chronic exposure of cancer cells to the stress hormone Cortisol increases the production of free radicals. This brings about damage to the DNA. Self-regulating anticancer mechanisms like DNA repair and Apoptosis get impeded. Elevated long-term stress stimulates the production of various growth factors including Insulin-like growth factor-1 (ILGF-1), Fibroblast Growth Factor (FGF), Vascular endothelial growth factor (VEGF) that can promote tumor cell growth. [2]

Cortisol is known as the “Stress Hormone” of the body, which is involved in the physiological regulation of stress. Both psychological and physical stress induces cortisol secretion, which is further regulated by feedback mechanisms. [3]

The majority of cortisol in plasma is found as cortisol bound to proteins; whereas cortisol is found in the free form in saliva.

Thus, the intention of the study was to analyze and compare the serum and salivary cortisol levels in patients with Oral PMDs, Oral malignancies and healthy controls and to judge their association with physiological and psychological constraints.

## **II. MATERIALS AND METHOD:**

This study was approved by the Ethical Committee of the Institution. The study was a randomized cross-sectional study that took tenure of one year and six months to complete (January 2022 to June 2023). It consisted of 45 subjects who were shortlisted after examining 127 subjects out of whom 82 subjects were eliminated.

The 45 subjects were grouped equally under three categories:

- a. Patients diagnosed with Oral Potentially Malignant Disorders (15 subjects)
- b. Patients diagnosed with Oral Malignant Disorders (15 subjects)
- c. Healthy subjects as Control (15 subjects)

### **Inclusion Criteria:**

Subjects within the age group of 23 to 73 were taken into the study. There were 6 female subjects out of the total 15 subjects in each of the 3 categories.

In the first category, there is one patient with Pre-leukoplakia, one patient with Smoker’s Palate (Type III), two patients with OSMF, one patient with OSMF and leukoplakia, four patients with Oral Lichen Planus and six patients with leukoplakia.

In the second category, there is one patient with Basal Cell Carcinoma, two patients with Verrucous Carcinoma and 12 patients with Squamous Cell Carcinoma.

### **Exclusion Criteria:**

For this study, subjects with the following conditions were excluded:

1. Patients with AIDS
2. Patients undergoing radiotherapy
3. Patients who are chronic alcoholics
4. Patients with blood dyscrasias
5. Patients with endocrinal, metabolic and autoimmune conditions
6. Patients with systemic illness
7. Patients taking corticosteroids or any other immunosuppressive drugs

### **Sample Collection:**

Since the concentration of Cortisol is higher in the morning hours, therefore the serum and salivary cortisol samples were collected from the subjects between 8:00 A.M. to 9:00 A.M. to avoid diurnal variation.

#### **A. Collection Of Salivary Sample:**

Morning unstimulated whole saliva was collected. The subjects were instructed to abstain from taking food, water, chewing gums, having snacks, especially with spices, drugs causing changes in salivary flow and brushing at least two hours before the predetermined sample collection time. The subjects were made to rinse their oral cavity for 20 seconds before collecting the sample. They were asked to sit in an upright but comfortable position and tilt their heads down in a slightly forward position so that the saliva would pool in the floor of the mouth. All the samples were collected in an air-conditioned room to avoid temperature fluctuations. The subjects were asked to expectorate the intraorally pooled saliva into 5ml sterile containers. The samples were then centrifuged at 3500 rpm for 10 min. 2ml of supernatant clear fluid was decanted and placed at -20°C until analysis was performed.

**B. Collection of Blood Samples:**

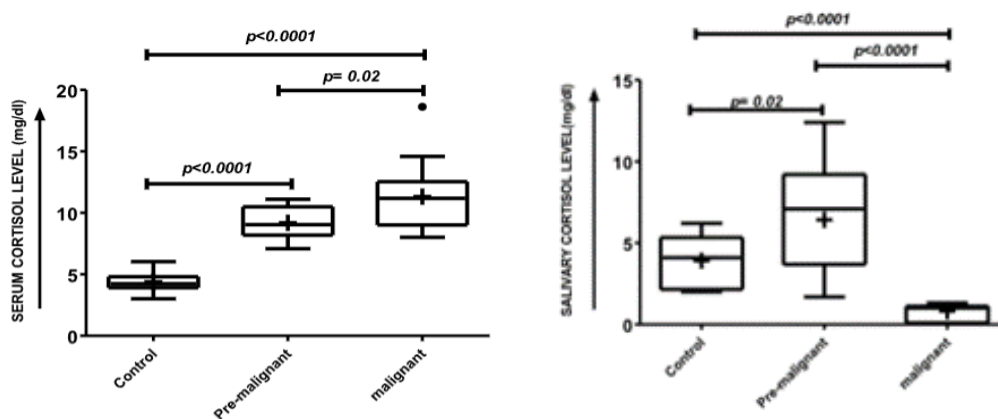
Blood samples were drawn after collecting the salivary samples to circumvent the increase in stress-induced plasma cortisol levels. 5ml of venous blood sample was collected in a sterile test tube. It was then centrifuged at 3500 rpm for 10 minutes. 2 ml of supernatant was decanted and used to detect cortisol levels.

**Analysis Of Sample:**

All the samples (both saliva and serum) were analyzed using the AccuBind Universal ELISA Kit, LDx R1 (TOSHO India, Pvt Ltd), which uses Streptavidin-coated plates. 25 microliters of sample were analyzed in each well. The optical density generated from each well was read at 450 nm on a microplate reader within 80 minutes. The Cortisol AccuBind Microplate ELISA Test System has a sensitivity of 62.5 pg. This is equivalent to a sample containing a concentration of 0.25 ug/dl. The sensitivity was ascertained by determining the variability of the 0 Hg/dl serum calibrator and using the 2g (95% certainty) statistic to calculate the minimum dose.



**STATISTICAL ANALYSIS:**



The obtained data were subjected to statistical analysis performed through the SPSS Statistics for Windows, Version 19.0.

Data showed significant upregulation of the serum cortisol level in both potentially malignant and malignant conditions to the healthy control ( $p < 0.0001$  for both). Data regarding malignancies demonstrated significant upregulation in the serum cortisol level compared to the potentially malignant group ( $p = 0.02$ ). Whereas, an alteration was found in the expression of salivary cortisol levels. The Potentially malignant group was found to have higher salivary cortisol levels compared to the healthy as well as the malignant group.

Salivary cortisol level was significantly less in the case of the malignant compared to the control and potentially malignant groups ( $p < 0.0001$  for both). Moreover, the potentially malignant group showed a significant upregulation compared to the control ( $p = 0.02$ ).

### III. DISCUSSION:

The above study shows how intricately stress is related to both oral potentially malignant and oral malignant lesions. Stress causes elevated secretions of stress hormones catecholamines, cortisol and epinephrine.[4] [5] Cortisol, being the most prominent component of a stress response. It is increased not only in oral PMDs and Oral malignancies but also in other malignancies elsewhere in the body.

This study has been devised to measure the amount of cortisol present in morning saliva and serum and co-relate them with the lesion (either PMD or malignancy) that the patients have. The level of cortisol also gives an idea about the stage and severity of the condition.

According to Paleri et al.,[6] escalated cortisol levels are a consistent feature in head and neck oncology patients from the time of diagnosis to 6 months after that.

Cytokines like Interleukin-1,2 & 6 have a stimulatory effect on the neuroendocrine system. These effects in addition to the psychological stress induce chronic hypercortisolemia in malignancy patients. [7] As per Bernabe et al. 2011, Interleukin-6 has an important role in angiogenesis, attachment and invasion of tumor cells. There are studies that prove that chronic stress is associated with tumor proliferation because Interleukin-6 invigorates proliferation and bony invasion of malignant cells. [7,8,9,10]

A study reveals that nicotine is the main component of cigarette smoke and can affect HPA axis, thereby disrupting cortisol secretion which attenuates the cortisol response to acute psychosocial stressors. [11]

In this study, we have found that there is a significant elevation in the serum cortisol level of patients with oral malignancies. On the other hand, we found that salivary cortisol level was less in these patients as compared to patients with PMDs and healthy controls. As for the patients with PMDs, an escalated level of salivary cortisol has been noted in comparison to the oral malignancy group and healthy controls.

For a complete evaluation of the HPA axis, cortisol rhythms should be measured during 24 hours as it is important to identify the fluctuations of cortisol levels over a day. S this is a limitation of this present study, where saliva and serum were collected from the subjects only during the morning hours.

Though the sample size was not very large, we received a significant result. We can consider it as a pilot study, thus opening up the scope to delve deeper into this particular topic in the near future, with a bigger sample size.

A similar study performed by Priyanka Sharma et al. showed the relationship between cortisol levels (serum & salivary) in patients with premalignant disorders and oral squamous cell carcinoma. This particular study helped us a lot to form a proper idea about how to conduct our own study.

### IV. CONCLUSION:

Through this study, we can safely conclude that physiological and psychological stress has a great impact on the development and progression of oral malignancies and oral PMDs. The greater the level of stress, the greater are the chances of an individual developing the above ailments in various forms. While diagnosing and treating a cancer patient we must keep the stress factor in mind and try to alleviate it as much as clinically and socially achievable. This will not only have a positive impact on the disease but will also improve the quality of life.

### REFERENCES:

- [1]. Thaker PH, Lutgendorf SK, Sood AK. The Neuroendocrine Impact Of Chronic Stress On Cancer. *Cell Cycle*. 2007;6:430–3. [PubMed] [Google Scholar]
- [2]. Denaro N, Tomasello L, Russi EG. Cancer And Stress: What's Matter. From Epidemiology: The Psychologist And Oncologist Point Of View? *J Cancer Ther Res*. 2014;3:6. [Google Scholar]
- [3]. 12. Rödström PO, Jontell M, Hakeberg M, Berggren U, Lindstedt G. Erosive Oral Lichen Planus And Salivary Cortisol. *J Oral Pathol Med*. 2001;30:257–63. [PubMed] [Google Scholar]
- [4]. Bernardi L, Valle F, Coco M, Calciati A, Sleight P. Physical Activity Influences Heart Rate Variability And Very-Low-Frequency Components In Holter Electrocardiograms. *Cardiovasc Res*. 1996;32:234–7. [PubMed] [Google Scholar]
- [5]. Bernardi L, Valle F, Coco M, Calciati A, Sleight P. Physical Activity Influences Heart Rate Variability And Very-Low-Frequency Components In Holter Electrocardiograms. *Cardiovasc Res*. 1996;32:234–7. [PubMed] [Google Scholar]
- [6]. Paleri V, Wight RG, Silver CE, Haigentz M, Jr, Takes RP, Bradley PJ, Et Al. Comorbidity In Head And Neck Cancer: A Critical Appraisal And Recommendations For Practice. *Oral Oncol*. 2010;46:712–9. [PubMed] [Google Scholar]
- [7]. Bernabé DG, Tamae AC, Biasoli ÉR, Oliveira SH. Stress Hormones Increase Cell Proliferation And Regulates Interleukin-6 Secretion In Human Oral Squamous Cell Carcinoma Cells. *Brain Behav Immun*. 2011;25:574–83. [PubMed] [Google Scholar]
- [8]. Okamoto M, Hiura K, Ohe G, Ohba Y, Terai K, Oshikawa T, Et Al. Mechanism For Bone Invasion Of Oral Cancer Cells Mediated By Interleukin-6 In Vitro And In Vivo . *Cancer*. 2000;89:1966–75. [PubMed] [Google Scholar]
- [9]. Heikkilä K, Ebrahim S, Lawlor DA. Systematic Review Of The Association Between Circulating Interleukin-6 (IL-6) And Cancer. *Eur J Cancer*. 2008;44:937–45. [PubMed] [Google Scholar]
- [10]. Chakravarti N, Myers JN, Aggarwal BB. Targeting Constitutive And Interleukin-6-Inducible Signal Transducers And Activators Of Transcription 3 Pathway In Head And Neck Squamous Cell Carcinoma Cells By Curcumin (Diferuloylmethane) *Int J Cancer*. 2006;119:1268–75. [PubMed] [Google Scholar]
- [11]. Kirschbaum C, Wüst S, Strasburger CJ. 'Normal' Cigarette Smoking Increases Free Cortisol In Habitual Smokers. *Life Sci*. 1992;50:435–42. [PubMed] [Google Scholar]