

## Study Protocol

# **Correlation of Salivary Cortisol Levels with Anxiety in Type II Diabetes Mellitus Patients before and after Complete Denture Rehabilitation - An In vivo Study**

### **Abstract:**

**Background:** Complete edentulism constitutes health care burden, resulting in alterations in physical, psychological and emotional behavior. Also, a reduction in quality of life is expressed by patients, raising their levels of stress and anxiety. Stressful situations cause increased blood pressure and heart rate resulting in the secretion of cortisol hormone, which stimulates gluconeogenesis and glycogenolysis causing hyperglycemia, which may favor the occurrence of Diabetes. As the diabetic's population is rapidly rising, Prosthodontists are certain to see a greater number of diabetic edentulous patients. Thus, there is a need to assess the relationship of complete denture rehabilitation with the generic health of edentulous patients which can be done by checking the stress levels, and their effects on systemic health.

**Objectives:** To determine anxiety levels and Salivary Cortisol levels in Type II Diabetes Mellitus patients and compare and correlate them before and after rehabilitation with Complete Dentures. **Methodology:** It will be a cross-sectional study, carried out on edentulous diabetic patients visiting the Department of Prosthodontics of Sharad Pawar Dental College and Hospital. With consent for participation, Glycated Hemoglobin and anxiety levels will be determined, along with fabrication of complete dentures. Patients will be assessed at two stages; before and 1 month after complete denture rehabilitation. **Results:** The research has been approved by the Institutional Ethical Committee. The results expected are the significant reduction in anxiety levels after Complete Denture Rehabilitation and thus, a definitive correlation between anxiety and salivary cortisol levels. **Conclusion:** Adaptation of patients to their new set of Complete dentures can be improved by their clinicians by acquisition of the knowledge of relationship of the general health of the patient and correlating it to the dental rehabilitation.

**Keywords:** Salivary Cortisol, Complete Denture, Diabetes Mellitus.

### **Introduction:**

Complete edentulism constitutes a huge burden on global health care. According to World Health Organization (WHO) criteria, people with no teeth are considered physically impaired, disabled; due to their inability to eat and speak effectively or considered handicapped; as they tend to avoid eating and speaking in public. Failing to do these necessary tasks of life, seldom, resulting in alteration in physical, psychological and emotional behavior. Also, a reduction in quality of life is expressed by patients, raising their levels of stress and anxiety. [1]

Stressful circumstances can induce cardiac events, which actively take part in vigilance alteration and are susceptible to neuro-hormonal consequences. These mainly result in

outcomes like inflated heart rate, rise in blood pressure, and high action of the hypothalamic axis coupled with the immune cells stimulation resulting in the secretion of several hormones; particularly cortisol, well thought as the stress hormone.[2]

Cortisol is a cardinal regulator of the physiologic stress response, through a negative feedback mechanism.[3] Cortisol results in the blood flow with increased amounts of glucose. It stimulates gluconeogenesis and also elevates glycogenolysis in liver, by potentiating the effects of epinephrine. Thus, it results in large amount of glucose into the blood stream within minutes.[2]

Excess of gluconeogenesis and glycogenolysis causes hyperglycemia, favoring the occurrence of Diabetes and other disorders of glucose metabolism.[4] The prevalence of diabetes is rapidly rising throughout the world, it is expected to become one of the world's main killers within the next 25 years.[5] India is deemed as the Diabetic capital across the globe. The edentulous patients had 1.82 times greater risk of having Diabetes than the dentate patients. With growing life expectancy, the Prosthodontists are certain to see a greater number of Diabetic patients.[6]

It is thus, obligatory to assess, from the functional and esthetic viewpoint, the relationship of complete denture rehabilitation with the generic health of edentulous patients. It can be done by checking the stress levels with the help of hormonal markers and their effects on systemic health.[1]

There is a paucity of studies on edentulism and anxiety among Type II Diabetes Mellitus. Edentulism leads to anxiety and increased anxiety results in stress leading to uncontrolled Diabetes and vice versa. So, there is a need to find the correlation among these determinants.

The Rationale of this study is to determine the correlation between the salivary cortisol level, which is a stress bio-marker, to the anxiety of Diabetic patients rehabilitated with Complete Dentures.

The study aims to Correlate Salivary Cortisol levels with Anxiety in Type II Diabetes Mellitus patients before and after rehabilitation with Complete Dentures, with the following objectives;

- 1.To determine Salivary Cortisol levels in Type II Diabetes Mellitus Patients before and after rehabilitation with Complete Dentures.
- 2.To analyze anxiety levels using anxiety scale in Type II Diabetes Mellitus Patients before and after rehabilitation with Complete Dentures.
- 3.To compare and correlate levels of Salivary Cortisol with anxiety scale in Type II Diabetes Mellitus patients before and after Complete Denture Rehabilitation.

### **Methods:**

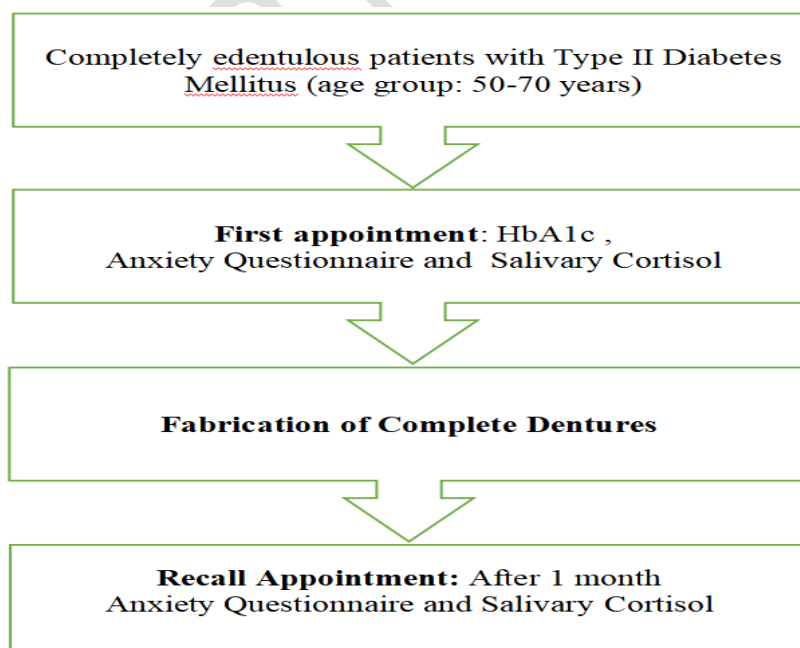
(Figure 1)It will be a Hospital based cross-sectional study, carried out at Department of Prosthodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha, a part of Datta Meghe Institute of Medical Sciences (Deemed University).A total of 40 completely edentulous patients with Type II Diabetes Mellitus, based on the glycemc hemoglobin levels, (age group: 50-70 years) will be selected from the Out Patient Department (OPD) of the Department of Prosthodontics over a period of 2 years. All the patients will be provided written consent before their participation in the study. Patient's Glycaemic Haemoglobin will

be checked in the first appointment. Maxillary and Mandibular Complete dentures will be fabricated for each patient in the department.

Inclusion criteria: Age 50 to 70 years completely edentulous patients and willing to participate in the study who are diagnosed with type II Diabetics for at least a period of three years.

Exclusion criteria: Patients who have other systemic disorders, skeletal disorders, metabolic disorders and other endocrinal disorders will not be included in the study.

- I. Selection of patients: This will be an In Vivo of study and a total of 30 completely edentulous Type II Diabetes Mellitus patients will be selected for the study who are willing to participate and who have signed the consent form. Routine blood and urine examination will be done to rule out any skeletal, metabolic, endocrinal and systemic disorder. Diabetic Type II patients Glycaemic Haemoglobin will be checked. Maxillary and Mandibular Complete dentures will be fabricated for each patient in the department.
- II. Determination of anxiety using dass-21 scale: The scale is a questionnaire based on 21 questions with 3 divisions measuring depression, anxiety and stress, each comprising of 7 questions. Patients have to self-report by reading each statement and mark circle on a number 0,1,2 or 3 based on the application of the statements over the past week.
- III. Collection of saliva and determination of salivary cortisol levels: Each patient will be directed not to drink, eat or perform oral hygiene before and during the procedure. The patients will then be asked to rinse their mouth thoroughly with water 15 minutes prior to collection. Collection of whole saliva will be done by allowing the saliva to accumulate in the floor of mouth and then spitting into a graduated plastic container. Saliva is collected and stored at -20 degree Celsius until it is processed using the commercial available Salivary Cortisol ELISA Test Kit. The DASS and salivary cortisol levels will be measured at two stages; before and a month after complete denture insertion.



**Fig 1. Workflow for the Study Protocol**

Sample Size		Study Parameters	
Group 1	19	Mean, group 1	9.35
Group 2	19	Mean, group 2	16.84
<b>Total</b>	<b>38</b>	Alpha	0.05
		Beta	0.2
		Power	0.8

**Fig 2. Sample Size Calculation**

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(8.3^2 + 8.3^2/1)(1.96 + 0.84)^2}{7.49^2}$$

$$n_1 = 19$$

$$n_2 = K * n_1 = 19$$

$\Delta = |\mu_2 - \mu_1|$  = absolute difference between two means  
 $\sigma_1, \sigma_2$  = variance of mean #1 and #2  
 $n_1$  = sample size for group #1  
 $n_2$  = sample size for group #2  
 $\alpha$  = probability of type I error (usually 0.05)  
 $\beta$  = probability of type II error (usually 0.2)  
 $z$  = critical Z value for a given  $\alpha$  or  $\beta$   
 $k$  = ratio of sample size for group #2 to group #1

### Results:

The data thus obtained will be tabulated for further statistical analysis. Data will be analysed using descriptive and analytical statistical methods. Statistical significance of the mean differences between the measurements in the interval of 1 months will be tested using student paired t-test.

Analytical and descriptive statistics will be done and represented in mean and standard deviations. The normality of data will be tested by Shapiro-Wilk test. If the data followed normal distribution parametric tests (independent sample t-test) will be used and if the data does not follow normal distribution non-parametric test (Mann-Whitney U test) will be used. The level of significant will be kept at  $p < 0.05$ .

As of now, the research has been approved by the Institutional Ethical Committee. Data collection has begun and will be continued over a year. The results expected are the significant reduction in anxiety levels after Complete Denture Rehabilitation and thus, a definitive correlation between anxiety and salivary cortisol levels.

### Discussion:

The objective of the study is to determine the correlation between the salivary cortisol level, which is a stress bio-marker, to the anxiety of Diabetic patients rehabilitated with Complete Dentures. The successful result of conventional complete denture treatment can be characterized by using both the subjective and objective criteria. Denture satisfaction determinants may include quality of denture, condition of the oral tissue, the attitude and personality of the patient, dentist-patient relation, and socioeconomic factors.

In addition to that there are several studies by various authors proposing that completely edentulous patients are at high risk for development of comorbidities which might include Diabetes, Cardiovascular disorders, Cancer, Asthma, Dementia, but whether these conditions are casual, i.e., the disease has happened due to growing age or causal, i.e., as a outcome of edentulism, has not been clearly appreciated.[7]

Gallagher P (2006) collected saliva by using various methods in 26 volunteers and assessed the relation between plasma levels and saliva and reported strong correlation.<sup>3</sup> Srivastav R et al also reported positive significant correlation between dental anxiety with denture satisfaction.[8]

Kansal and Goyal in the review on Type 2 Diabetes Mellitus and Prosthodontic implications mentioned about detailed medical history and measurement of glycated Hb values at initial appointments and use of stress reduction protocol during the treatment.[9] F.C. Veronez, used Oral Health Impact Profile (OHIP-14) questionnaire and reported that lower complete dentures has larger effect on the patient's quality of life than the upper complete dentures.[10]

Andreotti et al conducted a study on patient's rehabilitated with Implant supported Prosthesis and did not find any significant improvement on salivary flow and anxiety.[11] Goiato et al reported positive correlation between blood glucose and blood pressure and cortisol levels and concluded that complete denture rehabilitation is advantageous in reduction of salivary cortisol levels.[2]

Leybyuk et al conducted the research with the objective to study dynamic changes in the activity of stress factor indicators, in patients after prosthodontic rehabilitation with complete removable laminar dentures. The stress factors compared were namely salivary cortisol, glucose and alpha-amylase levels in diabetic Patients of age group 45-74 years were considered for study and were divided into 2 groups; one with diabetes mellitus and the other control. The study material was the saliva of patients from both the groups. In patients with diabetes mellitus, changes in saliva composition indicated a significant reduction in the adaptation mechanisms, which further complicates and significantly prolongs the adaptation periods to complete removable laminar dentures.[12]

Vassandacoumara and Daniel stated that salivary cortisol levels are higher in immunocompromised patients with oral lesions like oral lichen planus and recurrent aphthous stomatitis.[13] The results of their study suggested that anxiety and depression play a significant role in the disease pathogenesis. A comprehensive and holistic treatment approach is required for patients with psychosomatic diseases. In contrast to their study, Nadendla *et al.* reported a positive correlation between salivary cortisol and anxiety in their recurrent aphthous stomatitis group, and Shah *et al.* reported a positive correlation between salivary cortisol and depression in their oral lichen planus patients [14, 15].

A number of related studies on diabetes were reported [16-19]. Miller et al. has mentioned the concern of dental rehabilitation in patients suffering from diabetes mellitus. With the several possible oral manifestations of Diabetes Mellitus and risk of diabetic emergency intraoperatively, it is crucial for the dentists to recognize and appreciate the impact of the disorder on dental care. The health care team can work effectively with the thorough understanding to provide excellent oral health care to diabetic patients.[20-22].

**Conclusion:**

The stress-biomarker, Cortisol levels can be determined in Diabetes Mellitus Type II patients considered for Complete Denture rehabilitation which in turn will also improve the overall general prognosis of the treatment. Adaptation of patients to their new set of Complete dentures can be improved by their clinicians by acquisition of the knowledge of relationship of the general health of the patient and correlating it to the dental rehabilitation.

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