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Evaluation of salivary cortisol level in patient's pre and post insertion of removable partial and complete dentures

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Abstract

The aim of our study was to analyze the stress and anxiety in patients undergoing routine denture insertion, by the assessment of cortisol in saliva, which can be considered as one of the major hormones that is released during stressful events. Stress is defined as an organism's total response to an environmental condition or stimulus, also known as a stressor**.** Cortisol is a stress hormone produced by the adrenal glands. It is one of the stress hormones, and is produced at higher levels in individuals who are prone to stress. Aside from contributing factors such as work and emotions, it is also produced at greater levels by people who work early morning shifts. After obtaining the ethical approval and the patients were consented for their role in this study, we examined 22 patients (8 males, and 14 females).The age ranged from 22-74 with mean age of 59.6 & 42.6. I collected two salivary samples from each patient using spitting methods; one hour pre and one hour post insertion of removable partial and complete dentures (R.P.D. & R.C.D.). The samples were assessed and analyzed using ELISA test. Statistical analysis was performed using SPSS statistical software (version 19).ANOVA and t-test was applied to obtain the significance and correlation between the examined samples. Statically significant association regarding the difference of mean (post and pre using of denture) of cortisol level regarding the gender of the patients, where the difference of mean of female was higher than that of male patients (0.2, 0.14) respectively, p =0.001.My study revealed that salivary cortisol is within its highest levels after insertion of R.P.D. & R.C.D. in one hour. Significant association regarding the age was reported and the R.C.D group has had higher mean than that of R.P.D group.

Keywords: salivary cortisol, stress, anxiety, R.P.D., R.C.D., ELISA.

Introduction

Saliva is the watery fluid that is secreted into the mouth by the salivary glands. In many animals, including humans, it contains the enzyme amylase, which breaks down carbohydrates. Saliva also contains mucus, which lubricates food for swallowing, and various proteins and mineral salts (1).

Stress is nonspecific response of the body to any demand made upon it… and described it as a "general adaptation syndrome", i.e., a nonspecific means by which the body defends itself against damage caused by a noxious stimulus. This syndrome includes the physiological secretion of the pituitary hormones that activates

the cortex of the adrenal glands. Moreover, regardless of the "stressor" (the stimulus that result in the individuals' stress reaction, be it wound or psychological assault). The body responds with the same nonspecific type of reaction (2).

The diagnostic use of saliva salivary diagnosis is an increasingly important field in dentistry (3). A growing number of drugs, hormones and antibodies can be reliably monitored in saliva, which is an easily obtainable, non-invasive diagnostic medium (4, 5). Thus, salivary diagnosis is anticipated to be particularly useful in cases where repeated samples of body fluid are needed but where drawing blood is impractical, unethical, or both. Salivary concentrations of drugs and hormones also represent the free fractions of serum in many instances, with good correlations with the respective total concentrations in serum (6, 7 and 8). Assays of steroid hormones from saliva are widely used and well validated (9), providing an unstressful sampling instead of venipuncture. Multiple specimens of saliva for steroid hormone analysis can be easily collected by the patient, at home, to monitor stress and other diurnal variations (7). Stimulated saliva is secreted in response to either masticatory or gustatory stimulation, or to other less common stimuli such as activation of the vomiting center. A wide variation among individuals has been found (10). Men have higher flow rates than women (11). The factors affecting the flow of stimulated saliva are nature of stimulus, vomiting, smoking, gland size, gag reflex, olfaction, unilateral stimulation, and food intake (12).

Salivary cortisol has been measured since 1978 (14). Good correlation coefficients have been reported between salivary cortisol and plasma free cortisol concentrations, with correlations of r =0.80 or even r = 0.90 (13). Since the salivary cortisol assay is technically much simpler than any procedure for determining the free level, this makes the salivary assay useful in any situation in which cortisol binding is disturbed. Some investigators have found that salivary cortisol is a better measure of adrenal cortical function than serum cortisol and is particularly useful in studies with children (4).

Saliva testing for cortisol is a diagnostic procedure that measures cortisol levels by using a saliva specimen. This is relatively new technology as typically the blood or serum samples are used to measure cortisol levels in human body. Cortisol is a steroid found in the body and is the major glucocorticoid secreted by the adrenal cortex. It is more commonly known as the stress hormone, which plays a role in the regulation of the stress.

Salivary cortisol is frequently used as a biomarker of psychological stress. However, psychobiological mechanisms, which trigger the hypothalamus-pituitary-adrenal axis (HPAA), can only indirectly be assessed
by salivary cortisol measures.

Salivary cortisol was first introduced to psychobiological stress research almost two decades ago. Among the pioneers to use this method; Stahl and Dorner (15). They were able to show that cortisol levels can increase manifold within short periods after onset of stimulation. After stimulation by adreno-cortico-tropin (ACTH), the adrenal cortex synthesizes. Cortisol is a lipophilic steroid with low molecular weight (MW ~362 Dalton).

Following ACTH binding to membrane receptors on cells of the adrenal cortex, cortisol is synthesized and released into the blood stream. Up to 95% of the secreted cortisol will be bound to large proteins (CBG, albumin) and carried throughout the body in the blood. Since the vast majority of cortisol actions rely on binding to its cytosolic mineralocorticoid and glucocorticoid receptors, only the small fraction of unbound 5%, i.e., free cortisol is thought to be biologically active. Due to its low molecular weight and lipophilic nature, unbound cortisol enters cells by passive diffusion which makes it feasible to measure the free cortisol fraction in all bodily fluids (16).

In three decades ago (Mason, 1968), there are certain situation markers which can be regarded as crucial for induction of a cortisol stress response under such circumstances. Novelty, unpredictability, uncontrollability and (probably most important) anticipation of negative consequences in a given situation will lead to significant rises of salivary cortisol levels in most studysubjects(17). Also a number of psychological variables were found to be closely associated with the individual salivary cortisol stress response. For example, acute social support can buffer against the consequences of stress exposure in men while the opposite may be true for women (18). The cortisol stress response is rarely found to consistently correlate with certain personality traits when the subjects are exposed to stress once (19). In dentistry the acute effect of stressful events during the dental treatment can have a strong implication on our patients which reflects both the psychological and physiological components of stress; grave consequences can be related to anxious occasions during the course of the treatment which will influence the dentist and the patient's wellbeing. The increased metabolic demands throughout the stressful situations will have a dramatic impact on the patient general health especially on the medically compromised (20).

The concentration of salivary cortisol is independent of saliva flow rate. This means that under conditions of reduced saliva flow (e.g., induced by anxiety) as well as in times of maximal stimulation of saliva glands the concentration of cortisol found in saliva closely reflects the levels of unbound cortisol in blood (21, 22). While the determination of free cortisol in blood is time consuming and special technical expertise is required, the analysis of free cortisol in saliva is rather uncomplicated because no CBG is present here and commercial assay kits can be adapted for use with saliva instead of serum or plasma. Salivary cortisol is thus an inexpensive, yet valid and reliable measure of the bioactive cortisol in the body.
Cortisol is a rather stable molecule in saliva. Although it is recommended to readily store samples at -20EC to avoid molding, saliva can be kept at room temperature for at least four weeks without a significant drop in cortisol levels (23). This allows the collection of samples in a wide spectrum of field studies spanning from 'mundane' settings, e.g. at home or work (24).

A denture is a removable replacement for missing teeth and the tissues connected to those teeth. It is made of acrylic plastic and sometimes porcelain and metal materials. A denture closely resembles natural gum tissue and teeth. Complete dentures replace all of the teeth,

while partial dentures fill in the spaces created by missing teeth and prevent other teeth from shifting position. Dentures are not just for elderly patients. Patients of any age may lose some or all of their teeth and may require a denture of some sort. Because teeth are a permanent part of the body, tooth loss can have an emotional impact on some people. It is important to talk to your dentist about any fears, anxiety, or other emotions you are feeling about tooth loss. New denture wearers need time to get accustomed to their new "teeth," because even the best-fitting dentures will feel awkward at first. While most patients can begin to speak normally within a few hours, many patients report discomfort with eating for several days to a few weeks. In addition, denture wearers often notice a slight change in facial appearance, increased salivary flow or minor irritation or discomfort (25).

Every dental prosthetic treatment is associated with the placement of a foreign object (the prosthesis) in the mouth of the patient. As a direct consequence of such placement, the burden on the oral cavity tissues will be increased (26). A properly designed and fabricated RPD is of paramount importance both for the functional and biological requirements (27).

Elderly persons with health problems and physical limitations have reduced independence. Since poor functional ability is a known risk factor for disease among older populations, including oral disease, salivary defense factors and the stress hormone cortisol are significant markers for functional dependence (28).

Patients wearing complete dentures were subjected to experimental stress. Those subjects with persistent generalized soreness of the mucous membrane underlying the dentures exhibited a prolonged form of muscle activity associated with the stress, while normal subjects relaxed progressively during the experiment. The prolonged response associated with the stress may be an etiologic factor in the chronic soreness of the mucous membrane (29).

Stress, Pain, and Behavior in Dental Care presents the many different behavioral aspects of dental treatment, including specific dento-related behavioral dysfunctions (fear, anxiety and phobia, excessive gagging reflex, orofacial pain). Special attention is given to the specific problems of elderly dental patients, including possible problems in adapting to dentures (30).

Material & Methods

Salivary cortisol measurement would appear to be the measurement of choice in human stress where individual stress factors are to be identified and studied. The elevation of salivary cortisol levels can be the result of both physiological and psychological stress. The acute physic stress (hypoglycemia) the psychological acute stress (acute anxiety, depression, alcoholism) induces the secretion of adreno-cortico tropic hormone (ACTH) and rise in salivary cortisol.

The sample consisted of (22) patients (14 females & 8 males) who were admitted to the department of prosthodontics; Faculty of Dentistry/ Al-Mustansiriya University; seeking for fabrication of new removable partial and complete dentures (wearing dentures for the first time). The age range of the participants was between (22 & 74) years, with the mean age of 59.6 years for removable complete

dentures and 42.6 years for removable partial dentures.

All participants were examined by prosthodontist with exclusion for the patients with medical problems and the patients who wear an old denture previously. The removable partial and complete dentures were fabricated in a conventional method following the manufacturer's instruction using heat cured acrylic resin material (primary & final impressions, bite registration, try in, flasking, deflasking, finishing & polishing).

At the day of denture's insertion; the unstimulated saliva samples were collected from the participants using spitting method at two times interval for each participant; 1 hour pre insertion and one hour post insertion after midday. The participants were given written instructions beforehand regarding the saliva collections. Participants were told not to eat, drink or smoke for 1 h before each sampling. The saliva samples were always collected in restful and quiet circumstances. The saliva was collected from the patient within 10 minutes, put in plain tubes after collection of sufficient sample size and keeping the samples in cold then determines the plate layout of ELISA test (SALIMETRICS) by remove strips, labeled them, then 24 ml of assay diluents were added; 25 µL of standards, controls & unknowns were added into appropriate wells in duplicates, followed by adding of 25 µL into two wells to serve as the zero value and 25 µL of assay diluents were added into each well. A lake 1:1600 dilution of conjugate by adding 15 ml of the conjugate to the 24 ml of assay diluents prepared in above step; immediately mix the diluted conjugate solution and pipette 200 ml into each well using multichannel pipette, then mix the plate for five minutes at 500 rpm and incubated at room temperature for additional 55 minutes; washed the plate four times with washing buffer, then 200 µL of TMB solution were added for each well, mixing for 5 minutes at 500 rpm by using rotator, then incubated the plate in dark at room temperature for additional 25 minutes, then 50 µL of stop solution were added, mixing for 3 minutes. Lastly, read the results within 10 minutes; read at optical density 450 nm and calculate the results. The results were statistically analyzed using SPSS 19.

Result

Table -1 Demonstrated the descriptive criteria including the gender and the mean age of the patients, where the patients divided into two groups according to the type of denture was applied, the number and percentage of each gender and the mean age of patients in each group were described also the table showed that there was a significant association regarding the age was reported between two groups and the R.C.D group has had higher mean than that of R.P.D group.

Table -2 Revealed that there was a statistically significant association between pre and post using of denture for all patients according to the paired sample T –test results, where the results showed that the mean value of cortisol post using of denture is higher than pre-using statues. The results also proved there was a strong positive correlation with significant association ($p=0.001$) between the mean value of cortisol pre and post using of denture($R=0.9$) as seen in table-3- and figure-1-.

The results of paired sample T-test for mean value of cortisol of each type of denture was applied showed there was a significant association between pre-using and post-using of denture in

both types of dentures were applied (p=0.001,0.001)respectively as seen in table-4-.The results also revealed a statistically significant strong positive correlation between pre and post using of dentures in both types of dentures(R.C.D and R.P.D) ,(R=0.8,0.7)respectively as seen in table-5- and figures 2,3. As well as, the results revealed that the level of salivary cortisol was increased post using R.C.D. rather than R.P.D. (0.54, 0.24) respectively.

Table-6 Demonstrated that there was a statically significant association regarding the difference of mean (post and pre using of denture) of cortisol level between R.C.D and R.P.D groups, where the difference of mean of R.C.D group was higher than that of R.P.D group (0.26, 0.10) respectively, p =0.001.

Table-7 Demonstrated that there was a statically significant association regarding the difference of mean (post and pre using of denture) of cortisol level regarding the gender of the patients, where the difference of mean of female was higher than that of male patients (0.2, 0.14) respectively, p =0.001,when we tested the association regarding the gender according to the type of denture was used, the same significant association was reported in both types of dentures between the female and male patients and the mean value of difference of female patients was higher than that of male in both types of dentures were used as seen in table 8.

Discussion

Salivary cortisol has become an alternative tool for basic scientists and clinicians. Increasing interest in salivary cortisol assessment is due to a number of significant advantages: the stress-free ease collection, the absence of special handling or storage procedures, the correlation with cortisol levels in blood, not depending on flow rate, smaller sample aliquots, the possibility of a dynamic study, greater sensitivity and non- invasive collection procedure and a good cooperation with patients (31-33).

Discussion Dental anxiety has been ranked fifth among commonly feared situations and that will affect the patient obedience to treatment, avoid dental visits, and anxiety usually generates stress that can create significant problems especially for those who are medically compromised (34-36). The saliva is an excellent reservoir for cortisol and can be used as a diagnostic method for cortisol assessment (37).

The extent and direction of movement of removable denture (RDs) during their function are influenced by the nature of the supporting structures and the design of the prosthesis. The service expectancy of a RDs will be proportional to the degree of control of various stresses-induced by it. This is such an important factor in the success of this particular type of prosthesis that it should be emphasized by analyzing each stress and suggesting clinical and construction procedures for bringing about the most effective control. Functional stress stimuli, within certain limits, are necessary for the maintenance of the supporting structures. Beyond an optimal amount, which may vary to a considerable degree, stress may become an irritant, however, and may actually cause retrogressive changes to begin. The principal stresses, which are induced by RD, are stresses:

i. Resulting from an inaccurate appliance design;

ii. Stresses caused by an inaccurate appliance size;

iii. Stresses, which may cause impingement of the gingival structure (38).

This research tackled the relation of stress to partial and complete dentures by measuring the cortisol levels in saliva; the study adopted the use of ELISA for sample analysis and interpretation. The correlation between cortisol levels and stress is related mainly to the anticipation of physical harm; people generally showed increased cortisol levels when they participate or exposed to a potentially harmful situations or when they feel threaten, this basic instinct is reflected into dentistry in a dramatic way because the majority of patients attending dental clinics have some degree of anxiety and stress (39-41). The majority of dental treatments can have an influence on patient's anxiety and stress levels.

In this study we found significant age- and sex-differences in salivary cortisol levels. Levels were generally higher in women than men and in older subjects compared to younger. Furthermore, age and salivary cortisol were significantly inversely associated in women. This age-related increase in cortisol levels is supported by findings from several other studies (42-47).

The results obtained confirm that psycho emotional disorders may be one of the etiological factors of increase of salivary cortisol. It is also considered as a "stress hormone", because in stressful situations its levels in the saliva considerably grow (48, 49).

This study provides evidence for age-related increases in cortisol levels. Also we found significant variation in saliva cortisol with age and gender with older subjects having higher mean cortisol than younger subjects and highest mean cortisol occurring in older women. My findings of higher cortisol levels with increasing age and in particular higher levels of cortisol in women with age support those of Van Cauter et al. who found a 20-50% increase in salivary cortisol with ageing. Two studies analyzing salivary cortisol also support our findings. Nicolson et al demonstrated an increase in basal cortisol in older aged healthy subjects and Seeman et al. The reasons for this distinct age and gender pattern are not clear however mechanisms suggested include that HPA (hypothalamus pituitary adrenal) resiliency may decline with age leading to a loss of decline in cortisol following response to challenges of the HPA axis. It is now known that the rate of recovery of the HPA axis from a stimulus is most affected with ageing with corticotropic responsiveness increased in older women more than similar aged men in response to CRH (corticotropic releasing hormone) challenge (Greenspan) or to a driving challenge (Seeman) (50-54).

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Table-1 Descriptive criteria regarding age and gender according to the type of denture.

p-value	Type of denture					
	**R.P.D		*R.C.D			
	%	No.	%	*****No.		
Chi-square 0.7	57.1%	8	42.9%	6	***F	Gender
	50.0%	4	50.0%	4	****M	
T-test 0.04	Standard Deviation	Mean	Standard Deviation	Mean	Age	
	12.6	42.6	11.9	59.6		

*R.C.D.: Removable Complete Denture. **R.P.D.: Removable Partial Denture. ***F: Female. ****M:Male. *****No.: Number.

Table-2 Mean of salivary cortisol pre and post using of dentures for all patients.

P-value	Standard Deviation	No.	Mean	
0.001	0.09	22	0.19	salivary cortisol pre-using of denture
	0.17	22	0.37	salivary cortisol post-using of denture

Table-3 Correlation between the mean of salivary cortisol pre and post using of dentures for all patients.

p-value	Correlation	No.	
0.001	0.9	22	salivary cortisol pre-using of denture & salivary cortisol post-using of denture

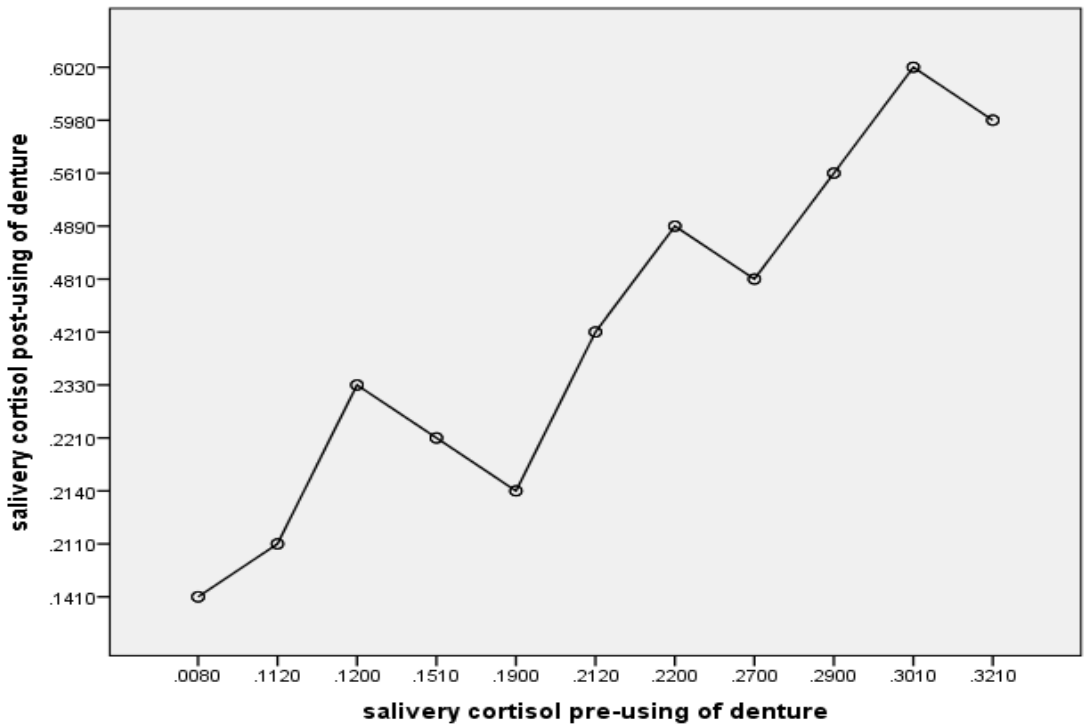

Fig-1 Correlation between the mean of salivary cortisol pre and post using of dentures for all patients.

Table-4 Mean of salivary cortisol pre and post using of denture according to the type of denture

p-value	Standard Deviation	No.	Mean	Type of denture	
0.001	0.03	10	0.28	salivary cortisol pre-using of denture	R.C.D
	0.054	10	0.54	salivary cortisol post-using of denture	
0.001	0.06	12	0.13	salivary cortisol pre-using of denture	R.P.D
	0.08	12	0.24	salivary cortisol post-using of denture	

Table-5 Correlation between the mean of salivary cortisol pre and post using of dentures according to the type of dentures.

Significant	Correlation	No.	Type of denture	
0.002	0.8	10	salivary cortisol pre-using of denture & salivary cortisol post-using of denture	R.C.D
0.005	0.7	12	salivary cortisol pre-using of denture & salivary cortisol post-using of denture	R.P.D

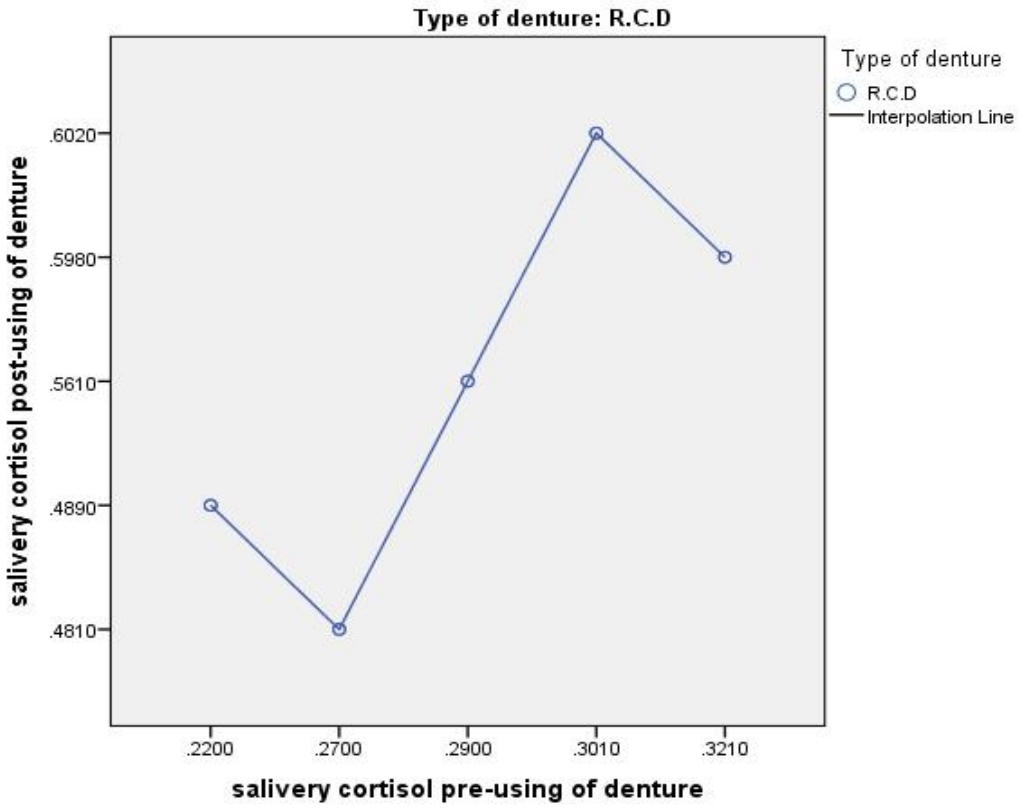

Fig-2 Correlation between the mean of salivary cortisol pre and post using of R.C.D dentures.

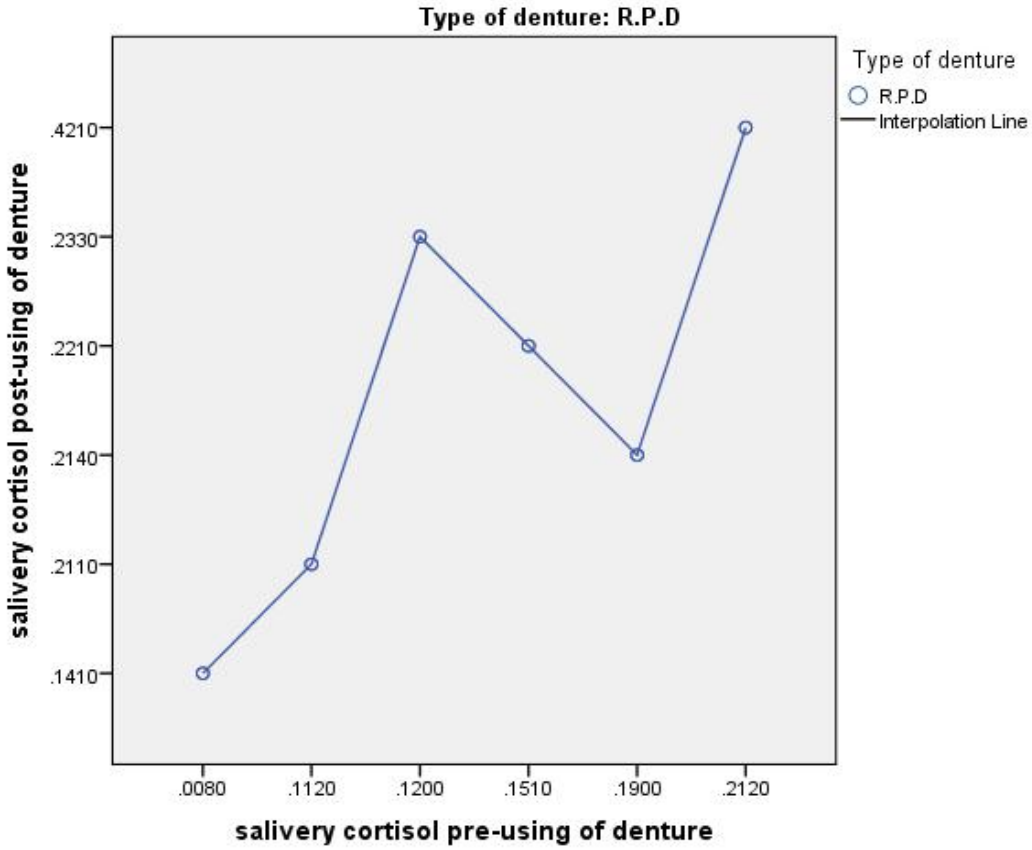

Fig-3 Correlation between the mean of salivary cortisol pre and post using of R.P.D dentures.

Table-6 Difference of mean of salivary cortisol (pre and post using of denture) for two types of dentures.

P-value T=7.5	Standard Deviation	Mean	No.	Type of denture	
0.001	0.03	0.26	10	R.C.D	Difference of mean pre and post using of denture
	0.05	0.10	12	R.P.D	

Table-7 Difference of mean of salivary cortisol (pre and post using of denture) according to the gender of patients.

P-value T=7.5	Standard Deviation	Mean	No.	Gender	
0.001	0.08	0.20	14	F	Difference of mean(post-pre using of denture)
	0.10	0.14	8	M	

Table-8 Difference of mean of salivary cortisol (pre and post using of denture) regarding the gender of patients according to the type of denture.

p-value	Standard Deviation	Mean	No.	Gender	Type of denture	
T=2.8 0.02	0.01	0.28	6	F	Difference of mean(post-pre using of denture)	R.C.D
	0.03	0.24	4	M		
T=3.6 0.004	0.04	0.13	8	F	Difference of mean(post-pre using of denture)	R.P.D
	0.02	0.04	4	M		