

Relationship between Levels of Salivary Cortisol and Presence of Xerostomia in Postmenopausal Women – A Comparative Study

Dr. Shivangi Singh¹, Dr. Smiti Rekha Das², Dr. Nidhi Puri³, Dr. Upasana Sethi Ahuja⁴, Dr. Akshay Rathore⁵, Dr. Manu Dhillon⁶

^{1,5}Reader, Department of Oral Medicine and Radiology, I.T.S Dental College, Muradnagar, Ghaziabad, U.P., India;

²PG Student, Department of Oral Medicine and Radiology, I.T.S Dental College, Muradnagar, Ghaziabad, U.P., India;

³Associate Professor, Department of Oral Medicine and Radiology, I.T.S Dental College, Muradnagar, Ghaziabad, U.P., India (Corresponding author);

⁴Professor & Head, Department of Oral Medicine and Radiology, I.T.S Dental College, Muradnagar, Ghaziabad, U.P., India;

⁶Professor, Department of Oral Medicine and Radiology, I.T.S Dental College, Muradnagar, Ghaziabad, U.P., India

¹E-mail; shivangi1087@gmail.com;

²E-mail; smitirekha31@gmail.com;

³Email; drnidhipuri16@gmail.com;

⁴Email: upasanasethi03@gmail.com;

⁵Email; dr.akshayrathore@its.edu.in;

⁶Email; drmanudhillon@yahoo.co.in

ABSTRACT:

Introduction: Saliva is a mucoserous exocrine secretion produced by three pairs of major salivary glands as well the minor salivary glands. A low salivary flow rate leads to oral discomfort including dry mouth and burning sensation in many elderly individuals, especially menopausal women.

Objective: To compare the stimulated and unstimulated whole salivary cortisol levels of menopausal women with xerostomia.

Methods: A case-control study was carried out on 240 selected post-menopausal women with/without feeling of xerostomia. Xerostomia Inventory score was used as an index of dry mouth severity. Stimulated and unstimulated whole saliva cortisol concentration was measured by ELISA. **Results:** Statistical analysis by Student's *t*-test and Pearson correlation was used. The mean cortisol concentration, XI grade and XS criteria showed no significant difference in case group whereas mean value of stress score showed highly significant difference. The XI grade, stress score and salivary cortisol concentration showed highly significant difference in the control group and Xerostomia Selection Criteria showed no statistically significant difference.

Conclusion: From present study we conclude that there is no significant difference in salivary cortisol level in stimulated and unstimulated salivary case group with a subjective feeling of dry mouth than in control group.

Keywords: Unstimulated saliva, stimulated saliva, salivary cortisol, xerostomia, post-menopausal women, stress.

Introduction

Saliva is a muco-serous exocrine secretion consisting of a variety of electrolytes which is produced by three pairs of major glands namely the parotid, submandibular and sublingual and hundreds of minor glands located beneath the epithelium. ***Total volume of saliva secreted normally amounts to 1-2 L per 24 hours.*** In humans, only the minor salivary glands secrete saliva spontaneously, at day time and rest of the secretion is mainly from submandibular glands but with respect to ***stimulated secretion, parotid becomes more dominant*** than other salivary glands.¹ The whole unstimulated saliva flow rate is estimated to be approximately 0.3-0.4 ml per minute. The flow rate of stimulated saliva is approximately 1-2 ml/min. A salivary flow rate, below <0.7 ml/min for stimulated saliva and less than 0.1 ml/min for unstimulated whole saliva is considered as ***hypo salivation (or) true oral dryness.***

A low salivary flow rate due to impairment of salivary gland function may lead to oral discomfort including dry mouth, altered taste and burning sensation; which are major complaints for a lot of elderly individuals, ***especially menopausal women.***² Xerostomia or dry mouth is a subjective feeling of oral dryness, which should be defined by the objective measurement of saliva flow rate.³

Menopause is defined as the absence of a menstrual period for 12 consecutive months that results from reduced secretion of the ovarian hormones oestrogen and progesterone, which takes place as the finite store of ovarian follicles is depleted.⁴ ***The transition to menopause usually begins when women are in their mid to late 40s and can last for several years.***⁵

Many clinical manifestations after menopause have been attributed; such as perspiration, anxiety, palpitations which are described as 'hot flush' and night sweats. Vasomotor symptoms have variability in duration, frequency, triggered by warm environments, hot food or drinks, stress and usually last for less than 5 min. Other common symptoms of menopause includes ***oral dryness, burning sensations of the tongue and oral mucosa***, vaginal dryness, itching and dyspareunia caused by reduced concentrations of hormones like estrogen and androgens.⁶

The imbalance in the hormonal physiology leads to increase in level of stress in menopausal women. Stress refers to a non-specific response that lead to a reaction in the brain (perceived stress), activating the physiological fight or flight response in the body. ***The role of stress as a causative factor of low salivary flow rate has been documented in literature.***

There is a significant correlation between the severity of dry mouth and the concentration of salivary cortisol level and also its output in menopausal women. Cortisol enhances the transportation of calcium from extracellular fluids to the cells. After menopause, adrenal steroid secretion provides a low level of estradiol in response to adrenocorticotrophic hormone (ACTH). Cortisol is released under the influence of hypophyseal ACTH.⁴

The serum and salivary cortisol levels have been shown to be a reliable indicator of stress. Salivary cortisol is one of the accurate reflection of the free, biologically active portion of cortisol in the blood. Various studies have shown that ***salivary cortisol measurements are reliable reflection of levels of serum cortisol.***

There has been noted a significant difference in salivary cortisol levels between stressed individuals and healthy subjects. Thus, salivary cortisol levels (stimulated or unstimulated) could prove to be a ***non-invasive biological marker for assessing the stress induced changes like xerostomia related to menopause;*** which would help in better management of these patients by ***multidisciplinary inter-professional approach.*** Previous studies in literature have compared the relationship of either stimulated or unstimulated whole salivary flow rate in post-menopausal women with or without oral dryness feeling but no studies have been attributed yet to assess both

stimulated and unstimulated in terms of cortisol levels and stress association. ***Keeping in view the above mentioned points, the present case control study was planned with an aim to assess both the stimulated and unstimulated salivary cortisol levels in xerostomic post-menopausal women.***

Methodology

The study was approved by the Ethical Committee of I.T.S Dental College, Ghaziabad and informed consent was obtained from all the participants. Menopausal women who did not have menstrual cycle for at least 12 months and complained of xerostomia were included in the study. Post menopausal females having history of smoking, took xerogenic medical agents, had underlying systemic disease, under corticosteroid or hormone replacement therapy, oral candidiasis or any unfavorable oral health condition such as poor oral hygiene, local inflammation and periodontal disease, were excluded from the study.

Based on these exclusion criteria and with the help of a questionnaire related to symptoms associated with xerostomia (Table. 1), a total of 240 post-menopausal women (age range 50 – 75years) were selected and those who had at least one positive answer were included in the case group and those with negative answer were included in control group. Depending on the method of saliva collection, the case group was further uniformly subdivided into two groups, stimulated unstimulated case groups with 60 patients each. Similarly, the control group was also subdivided into stimulated and unstimulated control group.

Another questionnaire (Table. 2) was completed by both groups to assess the severity of oral dryness feeling. The answer was graded as score of 1 for Never, 2 for hardly, 3 for occasionally, 4 for fairly often, and 5 for very often. ***Scores were summed, resulting in a Xerostomia Inventory Score (XI) for each individual.***

Sheldon Cohen perceived stress questionnaire was completed by both groups in order to assess the level of stress prior to saliva collection. Each answer was graded as 0 for never experiencing stress, 1 for almost negligible, 2 for sometimes, 3 for fairly often, and 4 for patients experiencing stress very often.

Saliva Collection: Stimulated and unstimulated whole saliva for both the case and control group was collected under resting conditions in a quiet room to reduce any stressful condition. The time for saliva collection was kept between 9 am – 11am and 90 minutes after the last intake of any solid or liquid meals. For stimulated saliva collection, pre stimulation was accomplished by chewing a piece of standard size paraffin wax for 60 seconds. The participants in all the groups were asked to swallow the initial saliva pooled in the mouth and then remaining saliva was collected for about 5 minutes into a pre-weighed dry, deionized and sterilized plastic tube. The saliva flow rate was calculated by subtracting the empty tube weight from the saliva filled one in gm/min which is equivalent to ml/min. Centrifugation of the samples was done for clarification and stored at -20° C for determination of cortisol.

Cortisol Analysis of saliva:

Analysis of cortisol concentration was done by ELISA technology using commercially available kit (WELDON BIOTECH).

Statistical analysis:

For statistical analysis, the data were presented as a mean and standard deviation and two tailed student's unpaired t –test was used. Pearson correlation analysis was used to identify any correlation between XI score and the salivary cortisol concentration; p value < 0.05 was considered as statistically significant.

1. Do you feel dryness in mouth when eating a meal?
2. Do you feel difficulty swallowing any foods?
3. Do you have to sip liquids to aid in swallowing dry foods?
4. Does the amount of saliva in your mouth seem to be less most of the time?
5. Do you feel dryness in your mouth at night or on waking?
6. Do you feel dryness during the daytime?
7. Do you feel the need to chew gum or use candy to relieve oral dryness?
8. Do you usually wake up thirsty at night?
9. Do you encounter any problem tasting food?
10. Do you feel burning sensation in tongue?

Table 1 : Selection of subjects with xerostomia questionnaire

- 1) Sipping of liquids help swallow food.
- 2) My mouth feels dry while eating a meal.
- 3) I get up at night to drink.
- 4) My mouth feels dry.
- 5) I have difficulty in eating dry foods.
- 6) I suck sweets or cough lozenges to relieve dry mouth.
- 7) I have difficulty swallowing certain foods.
- 8) The skin of my face feels dry.
- 9) My eyes feel dry.
- 10) My lips feel dry.
- 11) The inside of my nose feels dry.

Table 2: The xerostomia inventory (XI) questionnaire

Results:

Two tailed unpaired Students t – test was used to compare the mean salivary cortisol level, stress score, XI grade and xerostomia selection criteria between the stimulated case & control groups and unstimulated case & control group. The results showed highly significant difference between case and control subgroups in the both stimulated and unstimulated groups in respect to all the above-mentioned parameters that were analyzed. (Table 1&2 and Figure1).

	Group	N	Mean	Std. Deviation	p-value
Cortisol	Stimulated Control	60	3.7183	1.47849	0.000
	Stimulated Case	60	7.4355	2.35793	
Stress score	Stimulated Control	60	1.28	0.555	0.000
	Stimulated Case	60	3.17	0.827	
Xerostomia Inventory Grade	Stimulated Control	60	1.45	0.622	0.000
	Stimulated Case	60	3.78	0.691	
Xerostomia Selection Criteria	Stimulated Control	60	1.67	0.475	0.000
	Stimulated Case	60	1.18	0.390	

Table 1: The mean value of the parameters of the stimulated case and control group. p value of all the parameters is below 0.05.

	Group	N	Mean	Std. Deviation	p-value
Cortisol	Unstimulated Control	60	4.5783	1.96811	0.000
	Unstimulated Case	60	7.2050	3.12299	
Stress score	Unstimulated Control	60	1.65	.659	0.000
	Unstimulated Case	60	2.80	1.054	
Xerostomia Inventory Grade	Unstimulated Control	60	1.90	.951	0.000
	Unstimulated Case	60	3.73	1.177	
Xerostomia Selection Criteria	Unstimulated Control	60	1.60	.494	0.000
	Unstimulated Case	60	1.23	.427	

Table 2: The mean value and p – value of the parameters of the unstimulated case and control group. p value of all the parameters is below 0.05

On inter case group comparison, the results showed no significant difference in respect to cortisol concentration, XI grade, xerostomia selection criteria. But a highly significant difference in respect to stress score was observed where stimulated saliva in case group showed higher stress score (cortisol levels) as compared to stimulated saliva. (Table 3 and Figure 1). However, in the inter control group comparison, the mean value of cortisol concentration, XI grade and stress showed highly significant difference with higher values of all the parameters in unstimulated saliva. (Table 4 and Figure 1)

	Group	N	Mean	Std. Deviation	p - value
Cortisol	Unstimulated Case	60	7.2050	3.12299	0.649
	Stimulated Case	60	7.4355	2.35793	
Stress score	Unstimulated Case	60	2.80	1.054	0.036
	Stimulated Case	60	3.17	.827	
Xerostomia Inventory Grade	Unstimulated Case	60	3.73	1.177	0.777
	Stimulated Case	60	3.78	.691	
Xerostomia Selection Criteria	Unstimulated Case	60	1.23	.427	0.504
	Stimulated Case	60	1.18	.390	

Table 3 : : Inter – Group comparison of all parameter between unstimulated and stimulated

	Group	N	Mean	Std. Deviation	p - value
Cortisol	Unstimulated Control	60	4.5783	1.96811	0.008
	Stimulated Control	60	3.7183	1.47849	
Stress score	Unstimulated Control	60	1.65	0.659	0.001
	Stimulated Control	60	1.28	0.555	
Xerostomia Inventory Grade	Unstimulated Control	60	1.90	0.951	0.003
	Stimulated Control	60	1.45	0.622	
Xerostomia Selection Criteria	Unstimulated Control	60	1.60	0.494	0.453
	Stimulated Control	60	1.67	0.475	

Table 4: Inter – Group comparison of all parameter between unstimulated and stimulated control group.

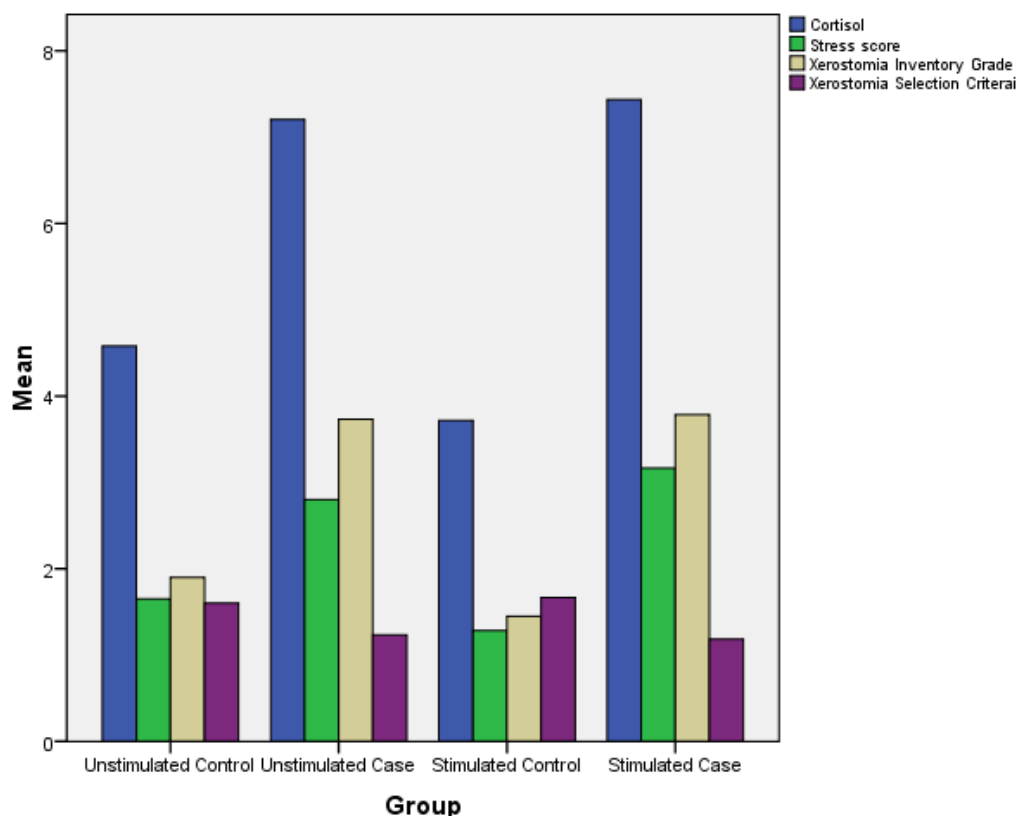


Figure 1: The comparison of stimulated and unstimulated group according to all the parameters.

The salivary flow was compared between both the case and control group. The mean salivary flow rate for unstimulated case group was 0.0470 whereas for stimulated case group the mean value obtained was 0.2383. The difference in salivary flow rate for case and control group for both stimulated and unstimulated saliva was found to be highly significant with higher flow rate in control group.(Table 5).

	Group	N	Mean	Std.Deviation	p value (2- tailed)
Salivary flow rate	Unstimulated Control	60	0.4017	0.15567	0.000
	Unstimulated Case	60	0.0470	0.02389	
	Stimulated Control	60	4.2100	1.57767	0.000
	Stimulated Case	60	0.2383	0.11363	

Table 5: Comparison of salivary flow rate in the unstimulated and stimulated group

On inter group comparison, the mean salivary flow rate for unstimulated case group was obtained as .0470 and .2383 for stimulated case group. However, for unstimulated control the

mean value was obtained as 0.4017 and 4.2100 for stimulated control group. There is statistically significant between the groups. (Table 6 and Figure 2).

	Group	N	Mean	Std.Deviation	p value (2- tailed)
Salivary flow rate	Unstimulated Case	60	0.0470	0.02389	0.000
	Stimulated Case	60	0.2383	0.11363	
	Unstimulated Control	60	0.4017	0.15567	0.000
	Stimulated Control	60	4.2100	1.57767	

Table 6: The inter case and control group comparison of salivary flow rate

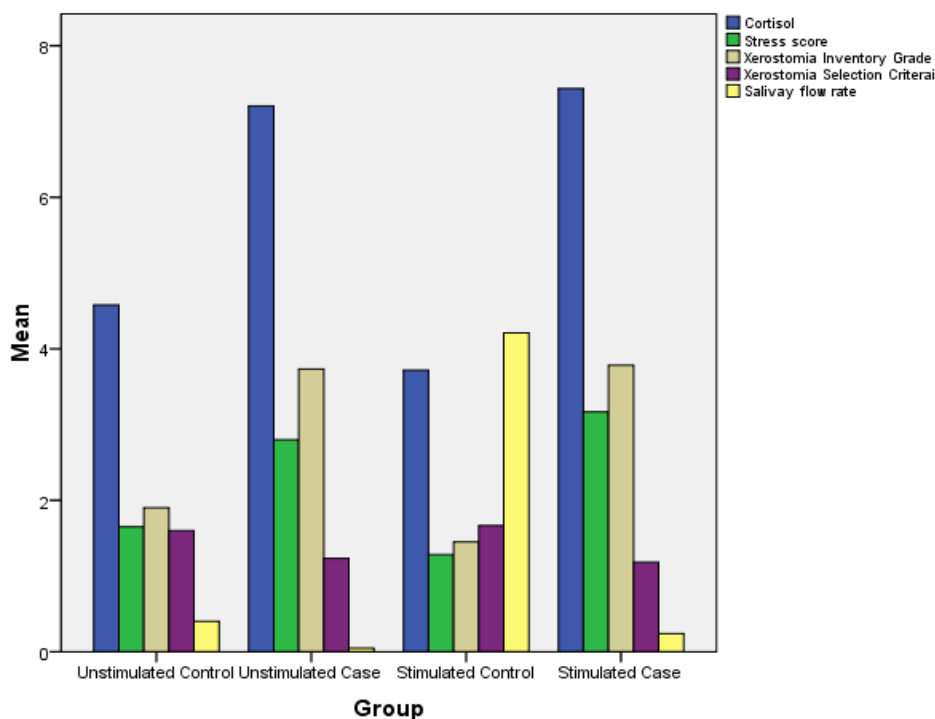


Figure 2: The comparison of salivary flow rate between the inter case and control group

The *Pearson correlation* was performed between the unstimulated and stimulated case and control group to assess the relationship between the salivary cortisol concentration and the severity of xerostomia. A significant positive correlation was found between unstimulated

control cortisol and severity of xerostomia ($r = 0.203$, $p = .119$). Similarly, a positive correlation was also found between stimulated control cortisol and severity of xerostomia ($r = .147$, $p = 0.261$). However, a negative correlation was found between unstimulated case cortisol and severity of xerostomia and also in between stimulated case cortisol and severity of xerostomia ($r = -.114$, $p = 0.387$) ($r = -.291$, $p = 0.024$) respectively.

DISCUSSION

Menopause is a complex physiological and psychological process involving various biological and endocrinal variations mostly associated with reduced ovarian estrogen and progesterone production of the individual which leads to increase in oral symptoms such as oral dryness and burning mouth syndrome, of which the most consistent oral symptom observed is oral dryness^{7,8}. Presence of sex hormone receptors have already been identified in the oral mucosa and salivary glands and decline in the level of these hormones in post-menopausal women is one of the primary reason of oral symptoms⁶.

In the present study, prevalence of oral symptoms was assessed and was found to be significantly greater in post-menopausal women (43%) than in control group (6%). Also stimulated and unstimulated salivary flow rates was compared between post-menopausal and control groups and highly significant difference was found with higher flow rate in control groups. In a study conducted by Parvinen et al (1982) and Narhi et al (1992) it was concluded that xerostomia is clearly associated with post – menopausal women. However, Ship et al observed in their study that healthy postmenopausal women, did not present a deterioration in glandular function nor a reduction in salivary flow rates and this is not in accordance with the present study.

In the literature, there are many studies where comparison between the either stimulated or unstimulated salivary flow rate with control group was carried whereas in the present study both the stimulated and unstimulated salivary flow rate has been compared with the control group. Cortisol plays an important role in mobilizing stress and is significantly related to epinephrine and norepinephrine levels. During menopause, due to diminution in the levels of serum estrogen, production of transcortin in the liver is reduced which disturbs the balance of free and bound plasma cortisol. In response, the HPA axis may try to recover the disturbed feedback loop by releasing more cortisol from the adrenal cortex thus, it is expected that the cortisol concentration shows a significant elevation in saliva.²

Various studies have shown increased in levels of cortisol in women experiencing menopausal transition. The present study also indicated no significant difference in salivary cortisol level in between stimulated and unstimulated salivary group in postmenopausal women. Shekar V et al in 2014, investigated the salivary cortisol level in 40 patients and found no statistically significant difference in the cortisol levels of unstimulated saliva of the experimental and control groups. This is in accordance with the present study.

A study conducted by Woods NF et al showed that a rise in cortisol level in women experiencing menopausal transition is related to significantly more severe hot flashes⁹. Many studies have been done to investigate the higher levels of salivary cortisol in post-menopausal women with xerostomia. A study conducted by Al-Kholy E.A. in 2014 found that mean salivary cortisol is higher in unstimulated saliva and this is in accordance with our study. In 2006, Gomez et al did not find significant difference in unstimulated salivary cortisol between menopausal women with feeling of oral dryness and women without oral dryness.

Also, the severity of Oral Dryness (Xerostomia Inventory score) was more in unstimulated group, when compared with the stimulated group. Another study conducted by F Agha-Hosseini on 70 selected menopausal women and found a significant positive correlation between the xerostomia score and the concentration of unstimulated whole saliva cortisol.²

Stress refers to a non-specific response that lead to a reaction in the brain (perceived stress), activating the physiological fight-or-flight response in the body. The role of stress as a causative factor of low salivary flow rate has been documented in literature whereas according to some authors stress has no role in decreasing salivary flow rate. High levels of stress could result in biological, psychological, and social problems and even serious harms to people. Psychological processes are often accompanied by disturbed oral sensations which can result in a sensation of dry mouth during a period of acute stress.¹⁰ The principal role of cortisol during the stress response is to restrain the effectors of the stress response. The cortisol in saliva is a potential biomarker of stress among menopausal women as it results in damage to the physical and mental well being of an individual.

In the present study, the level of stress was assessed and was found to be higher in stimulated and unstimulated case group than the control group. On comparing both the control groups and, it was found that the score was higher in case of unstimulated group. Similarly, on comparing the case groups, higher score was seen in unstimulated group.

Based on the results obtained from the present study it appears that there is no significant difference in salivary cortisol level in unstimulated salivary group with a subjective feeling of dry mouth than in stimulated group. Also, this study clearly indicates that salivary cortisol may be used as an aid to diagnose their situation in conjunction with clinical diagnosis.

CONCLUSION

From the results of the present study we can conclude that there is no significant difference in salivary cortisol level in between the stimulated and unstimulated salivary case group with a subjective feeling of dry mouth than in control group. The stress score showed highly significant difference between the control groups compared to case groups. The salivary flow rate showed highly significant difference in control groups compared to case groups which suggests a relationship between feeling of dry mouth and the flow rate in postmenopausal women. The cortisol in saliva is a biomarker of stress among postmenopausal women and evaluation of which could be done on regular basis.

CLINICAL RELEVANCE

The transition to menopause have many clinical manifestations such as dry mouth, burning sensation, hot flashes, perspiration and anxiety. Serum cortisol assessment is an invasive procedure and postmenopausal women suffer from anemia. There is a significant correlation between the severity of dry mouth and the concentration of salivary cortisol level and also its output. Salivary cortisol is a non-invasive biomarker and is correlated to stress in postmenopausal women. Assessment of cortisol and later can help clinicians in treating symptoms of dry mouth, anxiety.

REFERENCES

- 1) Delporte C. Role of aquaporins in saliva secretion. *OA Biochemistry* 2013; 1(2):14.
- 2) Hosseieni F, Dizgah I, Mirjalili N. Relationship of Unstimulated Whole Saliva Cortisol Level with Severity of Oral Dryness Feeling in Menopausal Women. *Australian Dental Journal*. 2011 ; 56(2):171-4.
- 3) Perlman, Barry; Kulak, David; Goldsmith, Laura T.; Weiss, Gerson The etiology of menopause: not just ovarian dysfunction but also a role for the central nervous system, *Global Reproductive Health: June 2018 - Volume 3 - Issue 2 - p e8*.
- 4) Nelson HD, Haney E, Humphrey L, Miller J, Nedrow A, Nicolaidis C, Vesco K, Walker M, Bougatsos C, Nygren P. Management of Menopause-Related Symptoms.
- 5) Al-Kholy EA et al., The relationship between salivary levels of cortisol, chromogranin A (CgA) and xerostomia in post-menopausal women, *Tanta Dental Journal* (2014).
- 6) Minicucci EM, Pires RBC, Vieira RA, Miot HA, Spoto MR Assessing the impact of menopause on salivary flow and xerostomia. *Australian Dental Journal* 2013; 58: 230–234
- 7) Rukmini JN, Sachan R, Sibi N, Meghana A, Malar CI. Effect of Menopause on Saliva and Dental Health. *J Int Soc Prev Community Dent*. 2018; 8(6): 529–533.
- 8) Suri V, Suri V. Menopause and oral health. *J Midlife Health*. 2014; 5(3): 115–120.
- 9) Woods NF, Mitchell ES, Smith-Dijulio K. Cortisol levels during the menopausal transition and early postmenopause : observations from the Seattle Midlife Women's Health Study. *Menopause*. 2009 Jul-Aug; 16(4):708-18.
- 10) Ben Aryeh H, Gottlieb I, Ish-Shalom S, David A, Szargel H, Laufer D. Oral complaints related to menopause. *Maturitas* 1996;24:185–189.