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# "Unveiling the Biofluid Omic" A Sialoanalysis in Postmenopausal Women

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#### **ABSTRACT**

The present research is intended to observe any additive effect of Diabetes and Hypertension on salivary flow rate, pH and salivary calcium levels in post menopausal women. A total of 75 subjects, divided into three groups of 25 each in which group 1 includes 25 female postmenopausal women without the history of diabetes or hypertension. Group 2 includes 25 female postmenopausal women with diabetes and Group 3 includes 25 female postmenopausal women with hypertension. In these subjects the salivary flow rate, pH and salivary calcium levels were measured using Spit method, pH strips and ARSENZO principle respectively. The results were analysed using ANOVA test and Pearson correlation coefficient formula. The results showed Statistically significant difference in USFR (p value <0.05), whereas for Salivary pH and calcium no statistical significance was found (p value >0.05). Conclusion: Compared to normal, a higher percentage of healthy menopausal women of the study group had been just a borderline away from hyposalivation while the > 90 % of diabetic and hypertensive group were severely hyposalivated. Also our aim to observe any shift in pH and salivary calcium levels were determined as both levels showed a decrease from normal levels in diabetic and hypertensive groups. Keywords: Postmenopausal, salivary flow rate, pH, calcium, Diabetes, Hypertension

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#### INTRODUCTION

The saliva is considered as a significant body-fluid and has been termed as the 'mirror of the body' by David T. Wong. The composition and behavior of saliva in health and disease has opened up a window in the field of sialo-diagnostics, sialo-analytics and sialonomics. The overall saliva produced by the salivary glands is defined as the 'whole saliva'. Though an individual is in a state of rest there is a constant rate of salivary flow into the oral cavity in order to maintain the integrity of the oral mucosal epithelium as well as in lubrication. This saliva produced is called the 'unstimulated saliva' which composes of a mixture of two – thirds of sub-mandibular gland saliva (mixed) and in addition to that one – thirds of a little amount of parotid and sub – lingual gland saliva [1]. In normal conditions a person produces 0.5 to 1.5 liters of whole saliva on a daily basis. The normal whole unstimulated flow rate of saliva (USFR) is approximately 0.3 –0.4 mL/min. USFR less than 0.1 mL/min is an indication of hyposalivation where there is objective evidence of decreased salivary flow rate. The patient has the symptom of Xerostomia or dryness of the mouth.

The prevalence of Type II Diabetes and Hypertension is very common in the population above the 4th and 5th decades in the global population. This is a common age for women to go through menopausal or post – menopausal changes. The lack of oestrogen production in menopausal women, a normal systemic condition brings about physiological changes in the quantity of saliva thus leading to Xerostomia and burning mouth [2]. Though several studies have evaluated the individual effects of Hypertension and Type II Diabetes on flow rates of saliva, there was a dearth in literature regarding the evaluation of the additional effect of going through menopause or being menopausal along with the presence of a systemic disease such as diabetes or hypertension on the unstimulated flow rate of saliva in women. Thus the current study was formulated with a research hypothesis as follows – 'There will be a difference in USFR when comparing post menopausal women without and with either diabetes and hypertension.'

## **MATERIAL AND METHODS**

A Total sample of 75 female subjects selected from the Oral medicine and radiology department have been reported. Of these total 75 subjects, group 1 includes 25 female postmenopausal women without history of

diabetes and hypertension. Group 2 includes 25 female postmenopausal women with diabetes and Group 3 includes 25 female postmenopausal women with hypertension. The study was commenced after obtaining the Institution Research review board and Institutional Ethics Committee approval (Ref.No: IECVDC/2022/22D067).

The female patients above 45 years of age with controlled diabetes or hypertension who attained menopause were included in the study. Exclusion Criteria are patients not willing for the procedure, irradiated patients, not sure of menopause history, with systemic diseases other than diabetes or hypertension and not under medication for diabetes or hypertension.

**Estimation of Salivary flow rate** - The patients were instructed to abstain from taking food, drinks or brush 1 hour before the sample collection and were asked to sit down on a dental chair in an upright position and to remain in a relaxed and resting condition. The saliva sample is collected between 9 am and 12 noon using the spit method for a period of 5 minutes. The patient was informed to pool up saliva in the mouth without swallowing and asked to spit it into a graduated test tube receptacle using a funnel for every 1 minute within the 5 minutes. The collected saliva was measured with the markings on the test tube followed by calculation of the unstimulated salivary flow rate (ml/min) as in (fig 1)

Unstimulated flow rate of saliva = Amount of saliva collected in the test tube

Period of collection (5 min)

**Estimation of salivary pH -** Salivary pH was measured using pH strips (HYDRION) with the pH range of 5.5-8.0 as in (fig 2)

# Estimation of salivary calcium

Salivary calcium estimation is based on principle ARSENZO. The collected unstimulated saliva was centrifuged at 3000rpm, and the supernatant obtained was evaluated using an Erba autoanalyzer Chem 5Xf or Ca as in (fig 3)

#### Statistical analysis

The 2 way ANOVA test was to used analyze the influence of the considered condition (menopause) and age on the USFR, pH, and Salivary calcium. The Pearson correlation coefficient formula was the tool used for inter – group and age related correlations. The data was analyzed utilizing SPSS software version 22. Statistical significance was observed for USFR (p value <0.05), whereas for Salivary pH and calcium no Statistical significance was observed (p value >0.05)

Table 1: Mean USFR and its association among the study groups

Groups	Mean USFR (ml/min)	Minimum (ml/min)	Maximum (ml/min)
GPM	0.31	0.06	1.20
GDM	0.20	0.02	0.42
GHT	0.23	0.01	0.70

p value - 0.05

Table 2: Mean Salivary Ph and its association among the study groups

Groups	Maximum	Minimum	Mean
GPM	8.0	6.0	6.7
GDM	7.6	5.5	6.5
GHT	7.4	5.5	6.4

p Value – 0.213

Table 3: Mean Salivary calcium and its association among the study groups

Groups	Maximum	Minimum	Mean
GPM	11.1	4.4	6.5
GDM	14.6	3.9	6.3
GHT	10.4	4.2	5.9

P value - 0.59

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Figure: 1 Shows graduated test tube and funnel



Figure 2; shows the salivary pH strips





Figure 3; shows the reagent and Erba autoanalyzer

#### RESULTS AND DISCUSSION

The term 'menopause' refers to the 'meno' - menstrual cycle and 'pause' - to stop. It is a physiological process occurring around the 4th and 5th decade of a woman's life. It was defined as ' the cessation of menstruation permanently due to ovarian follicular activity loss.' by the World Health Organization (W.H.O). The changes in a menopausal body are excessive perspiration, hot flashes, osteoporosis, cardiovascular disorders as well as changes in the skin and uro- genital bacterial imbalance. The causative is the decreased circulating levels of steroid hormones - estrogen and progesterone[3]. The cells of the salivary glands and oral mucosa possess receptors specific to both the above hormones are thereby believed to influence the quality and quantity of saliva. Histopathological sections of the buccal mucosa and salivary glands obtained by Liemola - Virtanen et. al have observed estrogen receptors on the surface of the specimens thus confirming their role in female oral homeostasis.<sup>3</sup> The saliva is an indicator of whole systemic health and disease. The saliva's volumetric secretion is controlled by the parasympathetic innervations while the composition is by the sympathetic counterpart [4]. Women in and around the period of menopause as well as post - menopause are bound to face an alteration in the oral environment and the mucosal integrity leading to discomfort due to several factors. Among the various oral disorders associated with menopause, Xerostomia is most commonly observed. Xerostomia is a subjective complaint of dry mouth which occurs due to the underlying objective reason of hyposalivation. Several studies revealed that the prevalence of Xerostomia is greater than 30% among the population above 60 years of age, the main

reason being systemic diseases and the administration of xerogenic polypharmacotherapy have been found to hinder the signal transmission at the neuro - glandular junction. Among all the systemic diseases the most chronic and prevalent ones during the age of menopause are arterial hypertension and type II diabetes mellitus. These diseases are said to cause disruption in oral homeostasis by altering the type of saliva, its pH, buffering capacity and also lead to disruption in salivary anti - oxidant levels [5]. The effect of hypertension on flow levels of saliva is more of a local factor i.e, anti – hypertensive poly-pharmaceuticals (prescription of several medical drugs) such as ACE inhibitors, ß – adrenergic blockers, diuretics and drug combinations. On the other hand, the influence of diabetes on salivary flow is that of a systemic one caused as an end result of neuropathy, polydypsia and polyuria.<sup>5</sup> Also studies conducted by Minicuci and Yalcin et al [6] stated their observation of a strong correlation between low salivary flow rate in women with menopause. The observations regarding the diabetic and hypertensive group were similar to a study conducted by Bernardi L. et. al 7 where he compared flow rates of saliva of diabetics with those who are hypertensive. He presented that the diabetic group had a significant lower USFR when compared to the normal group. However his observation of hypertensive groups to have normal limit of saliva compared to the present study is contrary. The findings of Djukic et. al [8] regarding the decrease in salivary flow rate due to anti - hypertensive medication is similar to the current study but contradictory to the statement by Dodds et. al [9] where cases of isolated hypertension were not associated with reduced flow. The normal range of unstimulated flow rates of saliva is 0.3 - 0.4 ml/ min. The mean values achieved in the study were correlated to the normal range. A larger percentage of the sample were found to be hyposalivated that is with USFR less than 0.3 ml/ min. The correlation of the three conditional groups and age with the normal range of USFR was found to be significant. However a larger percentage of healthy women were found to have normal USFR. This was similar to the research conducted by Agha Hosseini et al, [2] where they stated no reduction in salivary flow rate in post menopausal women. Contrary to this Gill et. al [10] reiterated the undeniable reduction in the USFR -- observed in their study sample. The observed mean USFR were comparatively to the lesser limit thus indicating a relation with menopause. In salivary Ph estimation of the present study showed that Ph of saliva of post menopausal women with diabetic or hypertensive is lower than the normal group and these results are similar to Saluja p.et al [11]. In this study salivary calcium levels are less in GDM and GHT compared to GPM similar to study done by Al-Abdaly, M.M.et al [12] but there is no much significance between the groups.

#### CONCLUSION

Compared to normal, a higher percentage of healthy menopausal women of the study group had been just a borderline away from hyposalivation while the > 90 % of diabetic and hypertensive group were severely hyposalivated. Also our aim to observe any shift in pH and salivary calcium levels were determined as both levels showed a decrease from normal levels in diabetic and hypertensive groups. This study confirms the importance of evaluating and focusing on the oral needs of menopausal women with systemic diseases and provide them the necessary therapeutic support (salivary substitutes, Vit C supplements etc) to improve their quality of life and prevent hyposalivation associated dental problems.

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