

Evaluation of Salivary Flow Rate, pH and Buffer in Pre, Post & Post Menopausal Women on HRT

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ABSTRACT

Background: Climateric is considered to be a natural phase of life which by definition is the period of life starting from decline in ovarian activity until after the end of ovarian function. It is accompanied by various health consequences that include the changes in saliva too. This study was carried out to evaluate the salivary flow rate, pH, buffering capacity in pre-menopausal, post-menopausal and post-menopausal women on HRT.

Aims and objectives: (1) To evaluate the salivary flow rate, pH of resting saliva and stimulated saliva and buffer capacity of stimulated saliva in pre-menopausal, post-menopausal and post-menopausal women on Hormone Replacement Therapy (HRT). (2) To compare the above salivary findings between pre-menopausal, post-menopausal and post-menopausal women on HRT.

Materials and Methods: The study was carried out on 60 patients. These patients were divided into three groups of 20 patients: Group 1: Pre-menopausal women (control), Group 2: post-menopausal women (case), Group 3: post-menopausal women on HRT (case).

The control group consisted of 20 women volunteers, having regular ovulatory menstrual cycles with no known systemic illness and deleterious habits and Group 2 consists of 20 post-menopausal women and Group 3 will consist of 20 post-menopausal women on HRT.

After clearing the mouth by swallowing, stimulated saliva was collected after chewing paraffin for 10 mins in to a glass centrifuge tube graded in 0.1 mL increments up to 10mL. In rare cases the collection time will be reduced or extended (5-15 min), salivary flow rate will be determined as ml/min, immediately after collection, pH was determined by dipping pH test paper directly into the sample of oral fluid, salivary buffer capacity was determined by using saliva check buffer kit (GC corporation).

The data obtained was statistically evaluated using chi-square test, fisher exact test ANOVA analysis.

Results: In our study we found salivary flow rate significantly lower in the post-menopausal women in comparison with the menstruating women and also there was improvement in the flow rate in individuals who were on HRT, it was also observed that salivary pH of the post-menopausal group was significantly lower than that of the control group, statistically significant difference in buffer capacity values was found between the groups however buffer capacity values were higher in the post-menopausal group than the control group.

Conclusion: From the above study it is clear that post-menopausal women will present with oral discomfort, while HRT can improve the same. Hence our role as physicians and health care providers is to incorporate preventive dental health care in post-menopausal women and clearly inform patients about both the benefits and the limitations of HRT.

INTRODUCTION

Health needs of women are different mainly because of the distinct changes that occur over their lifetime. This is because hormonal fluctuations throughout the woman's life which can affect many oral tissues. Menopause is accompanied by a number of characteristic changes; some of which occur in the oral cavity [1]. It has been shown that estrogen can affect many oral tissues such as salivary glands, temporomandibular joints, oral mucosa and jawbones, function of taste buds and neural networks. Saliva plays an essential role in maintaining oral health. Alterations in salivary function may lead to impairment of oral tissues and have a large impact on the patient's quality of life. Previous investigations have shown that, salivary pH, buffering capacity and flow rate play important roles in the oral mucosal defence. When the salivary flow rate is reduced, susceptibility to various oral diseases is enhanced. The number of women using hormone replacement therapy (HRT) to cope with the hormonal changes is increasing there is no doubt that many women clearly benefit from the use of HRT, which may also have implications in the oral cavity, as saliva is essential for the maintenance of oral health and menopause is associated with salivary changes and higher prevalence of oral

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symptoms, the number of women receiving HRT is also increasing [2-4]. Accordingly, the present aim of the study was to investigate the effect of menopause on saliva by evaluating the salivary flow rate, pH and buffer capacity of stimulated whole saliva among the regularly menstruating women, post-menopausal women, and post-menopausal women on HRT.

MATERIALS AND METHODS

The study was carried out on 60 patients of whom 40 were from various Obstetrics and Gynaecology Clinics in Bangalore for post-menopausal concerns, and 20 female patients who had regular menstrual cycles (as control).

Written Consent was taken from the patients selected.

The patients included in the study were:

1. Healthy women aged between 35 to 55 years of age.
2. Twenty Pre-menopausal women having regular menstrual cycles.
3. Twenty post-menopausal women with history of menopause more than one year.

- Twenty post-menopausal women undergoing hormonal replacement therapy more than 6 months.
- Patients without any deleterious habits such as smoking, chewing tobacco, alcohol etc.

Exclusion Criteria

- Individual who have systemic illness such as diabetes, chronic infections, xerostomia due to any salivary gland pathology etc.
- Individual who has undergone any prior hormonal treatment.
- Individual using any medicine on a regular basis that affects the salivary flow.

The control Group I - consisted of 20 women volunteers, having regular ovulatory menstrual cycles with no known systemic illness and deleterious habits,

Group II-consisted of Twenty post-menopausal women (case) and Group III-consisted of Twenty post-menopausal women on HRT (case).

Detailed history was obtained regarding the medical condition, usage of any drugs and any previous treatment with HRT. Also, history regarding the onset of attaining menopause was obtained in Group II and III patients. Group III patients with a history of taking HRT for more than six months were selected.

Dietary regimen and oral hygiene habits were noted along with any associated symptom of xerostomia.

Detailed examination of the oral cavity was done to rule out for any signs and symptoms associated with xerostomia and this was done by visually assessing the oral hydration by everting the lower lip and gently blotting the labial mucosa with a small piece of gauze and observing the mucosa under good light, droplets of saliva form at the orifices of the minor glands. If the time taken was greater than 60 seconds then resting flow was considered low and if the time was less than 60 seconds then resting flow was considered normal.

Visual assessment of the consistency was done by asking the patient to expectorate the resting saliva in the calibrated saliva collection cup provided by the manufacturer if the expectorated saliva had, sticky frothy residues or frothy bubbly form then considered as increased viscosity, if it had clear watery form then considered as normal viscosity.

Collection of Saliva

The salivary samples were taken from the subjects under standard conditions, between 10 am to 12 noon, at least 1 hour after breakfast, with the patient being seated and leaning slightly forward. Saliva check buffer™ manufactured by General corporation, Batch number 20060601 (2 & 3) was used to check the flow rate, pH and buffer capacity as per the instructions given by the manufacturer.

Assessment of the pH

After assessing the consistency the pH of the saliva was determined by dipping the pH test strip provided in the kit in to the sample of resting saliva collected for 10 seconds and then the color of the strip was checked with the testing chart provided with the saliva check kit. Accordingly the pH of the saliva was determined and given highly acidic, moderately acidic or healthy saliva comparing with the chart and the color of test strip, if intermediate color was seen in the strip then a higher value was assigned.

Determination of the quantity of stimulated saliva

The subject was asked to clear the mouth by swallowing the residual saliva, and was instructed to chew the piece of paraffin wax for 30 seconds to stimulate the salivary flow. After 30 seconds the subject was made to expectorate in to the spittoon, further continuation of chewing for a further 5 minutes was done and the

stimulated saliva was collected at regular intervals in the calibrated cup provided by the manufacturer.

The quantity of the saliva was measured by checking the ml markings on the side of the cup and noted down at 5 minutes, if the quantity was Less than 3.5 ml then considered very low, Between 3.5 – 5 ml was considered as low and more than 5 ml was considered normal.

Assessment of Buffering Capacity of the Stimulated Saliva

The buffering capacity of the saliva was assessed by removing a buffer test strip from the foil package and then placed over an absorbent tissue with the test side up.

Using a pipette sufficient saliva was drawn from the collection cup and one drop on each of the 3 test pads was dropped and immediately turned the strip 90 degree to soak up the excess saliva on the absorbent tissue to prevent the excess saliva from swelling up the test pad and possibly affecting the test results.

The change in the test pad was noted after 2 minutes and the final result was calculated by adding the points according to the final color of each pad. The test pad color at 2 minutes was given values according to the conversion table which included, four points was assigned if it was green, three points if it was green/blue, two points if blue and one point if it was red/blue, Zero point if it was red and where a color pad gave an unclear result an intermediate score was given.

Later the sum of the Three pads was calculated and results were interpreted with the combined total as 0-5 then the salivary buffer capacity was considered very low, 6-9 was considered as low, 10-12 was considered to be normal/ high.

All the values of salivary flow rate, pH, buffer capacity among the three groups of menstruating, post-menopausal and post-menopausal women on HRT were obtained and noted down in a chart and later subjected for statistical analysis.

STATISTICAL METHODS

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean±SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of 2x3, 3x3 Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistical Software

The Statistical software namely SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables, etc.

RESULTS

[Table/Fig-1-5] Shows.

Age in years	Group I		Group II		Group III	
	No	%	No	%	No	%
35-39	9	45.0	0	0.0	0	0.0
40-44	10	50.0	0	0.0	0	0.0
45-49	1	5.0	4	20.0	7	35.0
50-54	0	0.0	16	80.0	13	65.0
Total	20	100.0	20	100.0	20	100.0
Mean ± SD	39.60±2.95		51.40±1.96		49.75±1.92	

[Table/Fig-1]: Age distribution

Viscosity	Group I		Group II		Group III	
	No	%	No	%	No	%
Normal	20	100.0	4	20.0	15	75.0
Increased	0	0.0	16	80.0	5	25.0
Total	20	100.0	20	100.0	20	100.0
Inference	Viscosity is significantly increased in Group II (80.0%) followed by Group III (25.0%) when compared to Group I with $p < 0.0001^{**}$					

[Table/Fig-2]: Comparison of viscosity between three groups

Salivary pH	Group I		Group II		Group III	
	No	%	No	%	No	%
<7.0	5	25.0	20	100.0	14	70.0
7.0	8	40.0	0	0.0	6	30.0
>7.0	7	35.0	0	0.0	0	0.0
Total	20	100.0	20	100.0	20	100.0
Inference	Salivary pH less than <7.0 is significantly predominant in Group II and III with $p < 0.0001^{**}$					

[Table/Fig-3]: Comparison of Salivary pH between three groups

Salivary flow	Group I		Group II		Group III	
	No	%	No	%	No	%
4 ml	0	0.0	11	55.0	2	10.0
5 ml	0	0.0	9	45.0	18	90.0
>5 ml	20	100.0	0	0.0	0	0.0
Total	20	100.0	20	100.0	20	100.0
Inference	Incidence of Salivary flow >5.0 ml significantly associated with Group I while <5 ml is significantly associated with Group II and Group III with $p < 0.001^{**}$					

[Table/Fig-4]: Comparison of Salivary flow between three groups

Buffer capacity	Group I		Group II		Group III	
	No	%	No	%	No	%
7-10	9	45.0	19	95.0	10	50.0
>10	11	55.0	1	5.0	10	50.0
Total	20	100.0	20	100.0	20	100.0
Mean \pm SD	11.10 \pm 1.02	8.45 \pm 1.39	10.55 \pm 1.43	100.0	20	100.0
Inference	Buffer capacity is significantly more in Group I (11.10), followed by Group III (10.55) and less in Group II(8.45) with $F=23.298$; $p < 0.0001^{**}$					

[Table/Fig-5]: Comparison of buffer capacity between three groups

DISCUSSION

Oral discomforts such as burning sensations have long been reported to be strongly associated with the menopause [5-7]. Menopause is accompanied by a number of characteristic changes; some of which occur in the oral cavity. It has been shown that estrogen can affect many oral tissues such as salivary glands, temporomandibular joints, oral mucosa and jawbones. Changes in the function of taste buds and neural networks have been noted to occur following menopause [8-12].

The high prevalence of oral discomfort in women at menopause was reported by Ferguson [2] and Wardrop [13] and noticed these complaints might be due to the hormonal alterations taking place at menopause causing vasomotor, neurological, and psychological changes.

Wardrop[13] also reported menopausal women with oral discomfort were relieved of symptoms after systemic HRT.

Saliva plays an essential role in maintaining oral health. Alterations in salivary function may lead to impairment of oral tissues and have a large impact on the patient's quality of life. A higher incidence of dental caries, oral mucositis, dysphagia, oral infections and altered taste has been reported in individuals with reduced salivary flow 5

all though, there are many studies showing controversy over the effect of menopause on saliva.

As saliva is essential for the maintenance of oral health and the number of women receiving HRT is increasing, it is important to assess the extent to which menopause and HRT affect saliva.

In the present study, the salivary flow rate, pH, and buffer capacity were evaluated in pre-menopausal, post-menopausal and post-menopausal on HRT.

Studies have shown the average age of women attaining menopause being 50 years though it also states that women may attain early menopause in certain conditions where the women are thin in stature and women who smoke attain early menopause than their counter parts, also racial and geographic variations are seen. In our study we came across women who had attained menopause in the range of 47-50 years.

During the study we also observed that few individuals also had complaints of oral burning sensation, halitosis and some with complaints of decreased taste acuity which has been also discussed in the study done by volpe [3] and other studies [1,2,14-16].

Changes in the salivary viscosity was also noted with increased consistency in the post-menopausal individuals than in the pre-menopausal and individuals on HRT.

Previous studies on the topic of the effect of menopause on salivary flow rate have revealed diverse results. Some studies have reported no change in salivary flow rate after menopause [5,7]. While other studies have shown lower flow rates in post-menopausal women [6,17,18]. The present study has demonstrated that salivary flow rate decreases after menopause, and was found to be statistically significant.

In the literature the data on the effect of menopause on salivary flow rate are confusing. Kullander and Sonesson [19] and Laine and Virtanen [20] reported lower secretion rates in menopausal women than menstruating women, while studies by Ship and co-workers [21] and Ben Aryeh [3], did not find any significant changes.

Several other studies [20,22-24] found menopausal women receiving HRT showed higher saliva flow rates than untreated controls. In our study we too found salivary flow rate significantly lower in the post-menopausal women in comparison with the menstruating women and also there was improvement in the flow rate in individuals who were on HRT.

Review of literature and the results from our study shows estrogen treatment has a beneficial effect on salivary flow rate. However, Ship [21] found no difference in flow rates between menopausal women receiving HRT and not receiving HRT, where they used lower dosages of estrogen. Estrogens vary greatly in their biological activities, which partly may explain the conflicting results.

In our study it was also observed that salivary pH of the post-menopausal group is statistically significantly lower than that of the control group. However, the study done by Yağın [25] disagrees with the findings, which was performed using unstimulated saliva. Observations by Laine and Virtanen [26] showed no significant changes in pH but disagrees with Sewon [23] who showed pH values increased during HRT.

We could not find any study on the effect of menopause on salivary buffer capacity in the literature. However some authors have studied salivary buffer capacity during HRT [20,23,27,28]. In the present study, statistically significant difference in buffer capacity values was found between the groups however buffer capacity values were higher in the post-menopausal group than the control group.

The observations from our study could be attributed to the oral symptoms presented by post-menopausal women which consisted

of burning sensation, halitosis and altered taste sensation, where as significant improvement in the pH, flow rate and buffer capacity seen in the group who were on HRT.

From the above studies it is clear that post-menopausal women will present with oral discomfort, while HRT can improve the same. Hence our role as physicians and health care providers is to incorporate preventive dental health care in post-menopausal women and clearly inform patients about both the benefits and the limitations of HRT (which includes risk of development of endometrial hyperplasia, breast cancer, deep vein thrombosis) if taken for longer duration and above all taking into account patients' preferences and concerns.

CONCLUSION

The results of the study showed significant changes in the pH, buffer capacity and decreased salivary flow rate in post-menopausal women than with the control group of regularly menstruating women and the post-menopausal group on HRT.

It was further noted that individuals on HRT had fewer oral symptoms when compared to the post-menopausal women who were not on HRT.

Further evaluation of individuals on long term HRT and its benefits on oral tissues with evaluation of its adverse effects considering the racial and environmental conditions among larger population groups could be undertaken, due consideration should also be emphasized on the salivary changes due to aging and changing trends of HRT with newer substitutes of bio-identical hormone replacement therapy in the current modality to deal with post-menopausal symptoms.

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