EVALUATION OF THE EFFECTS OF MENOPAUSE ON SALIVA

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ABSTRACT:

Background & Objective: Menopause leads to physiological changes that take place due to decreased production of estrogen by ovary in women. The study was carried out to know the effect of menopause on saliva by evaluating the unstimulated salivary flow rate, viscosity, pH and stimulate salivary flow rate and buffering capacity in menopausal females.

Method: The study was conducted on 50 healthy post-menopausal women (group I) and 30 pre-menopausal women (control- group II), 20 males of similar age group as group I (control- group III) respectively, who attended outpatient department of Oral Medicine and Radiology of Ahmedabad Dental College and Hospital. Data were collected through a questionnaire and oral examination. Samples of the stimulated and unstimulated saliva were taken. A standard size paraffin wax was chewed by participants for stimulated saliva collection. Flowrate was measured by observing saliva at orifices of the minor glands after some time. Viscosity was measure by visual examination. pH was measured using strips. Buffering capacity was measured using buffer test strips.

Result and Interpretation: A notable decrease in the unstimulated salivary pH and salivary flow rate, and viscosity as well as stimulated salivary flow-rate and buffering capacity was found in menopausal females compared to the control group.

Keywords: Post menopause, Salivary flow rate, Salivary pH, Oral changes

INTRODUCTION

The WHO defines menopause as “the permanent cessation of menstruation due to loss of ovarian follicular activity.” During the 5th decade of women life, a physiological process “Menopause” happens, which demands permanent cessation of menstruation. These physiological changes take place due to declining estrogen production by ovaries in women advancing towards menopause.[1]

It has been observed that life expectancy of women has increased significantly during the last decade, and most women spend one third of their lives after menopause.[2] For centuries, instabilities of temperament and behavior have been accompanying with reproductive endocrine system variation in womankind. Long-standing concerns of variations in ovarian hormonal intensities include morbidities interrelated with age such as vascular diseases, osteoporosis, complications linked to memorization, urinary incontinence, and skin aging.[3]

Saliva is a critical fluid in maintaining oral health. Alterations in salivary function may lead to impairment of oral tissues and have large impact on the patient’s quality of life. Oral discomfort including dry mouth, altered taste and burning sensation are common chief complaints encountered in dental clinics. The etiology of oral discomfort in menopausal women has been related to alterations in the quantity or the quality of saliva. A higher incidence of dental caries, oral mucositis, dysphagia, oral infections and altered taste has been reported in individuals with reduced salivary flow.[4]

Sex steroid hormones, especially estrogen, appear to play a significant role in the physiology of the oral cavity. The decrease in estrogen levels during menopause affects the oral epithelial maturation process, leading to thin and atrophic epithelium [5]. It has been shown that hormone replacement therapy (HRT) can reduce oral discomfort in postmenopausal women, further suggesting a role of female sex hormones in the maintenance of oral tissues [6 - 8]

The present study was conducted to evaluate effect of menopause on different parameters of saliva like flow rate, viscosity, pH of unstimulated saliva and flow rate and buffering capacity of stimulated saliva.

MATERIAL AND METHODS

The present clinical study was undertaken in the patients attending the outpatient department of Oral Medicine and Radiology, Ahmedabad Dental College and Hospital in 18 months.

A total of 100 subjects were selected from OPD of the department of Oral Medicine and Radiology of Ahmedabad Dental College and Hospital for this study on saliva. They were divided into three groups. Group I which is the study group contained 50 females of postmenopausal age (45 – 55 years). Group II and Group III were control Groups. Group II contained 30 females of reproductive age (25 – 35 years) and Group III contained 20 males of same age of case group (45 – 55 years).

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Inclusion Criteria:

1. Postmenopausal females of comparatively good or fair oral hygiene
2. Subjects should be otherwise healthy and not taking any hormonal therapy
3. Subjects with cessation of menstruation at least for one year

Exclusion Criteria:

1. Subjects having poor oral hygiene
2. Subjects having any major systemic disease
3. Subjects on hormonal therapy
4. Subjects having any oral habit (tobacco, alcohol, etc.)

Subjects fulfilling the above criteria were thoroughly explained about the study procedure in the language that they understand, with written and informed consent, detailed history, oral examination was done- to evaluate the effects of menopause on saliva.

Saliva Testing:

- **Saliva Collection:** For saliva collection subjects were told not to eat or drink for 1 hour before each sampling. Unstimulated and stimulated saliva were collected as mentioned below.
- **Unstimulated Saliva Collection:** Subjects were instructed not to expectorate or swallow saliva for 1 minute and then expectorate any pooled saliva into the collection cup.
- **Stimulated Saliva Collection:** Stimulated whole saliva was collected by chewing on a standardized block of paraffin. Instructions were given to chew for 1 minute and thereafter to spit out or swallow any saliva produced. Saliva secreted during this 1 minute was not taken for study. The subjects were then asked to continue chewing paraffin and saliva was collected into the collection cup five times at regular interval of one minute each.
- Unstimulated saliva was tested for unstimulated salivary flow rate, salivary viscosity and unstimulated salivary pH. Stimulated saliva was tested for stimulated salivary flow rate and buffering capacity.

Unstimulated Saliva Testing:

Unstimulated salivary flow rate:

- **Method**: Lower lip was gently everted. The labial mucosa blotted with a small piece of gauze, and the mucosa was observed under a good light source. Droplet of saliva formed at orifices of the minor glands after sometime. Interpretation was done as below.

- **Interpretation:**

Time for the droplets of saliva to appear	Hydration

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Less than 60 sec.	Low
Greater than 60 sec.	Normal

- **Salivary viscosity**
  - **Method**: Viscosity was determined by examining the unstimulated saliva in collection cup by visual examination.
  - **Interpretation:**

Saliva	**Viscosity**
Sticky frothy	High
Frothy bubbly	Intermediate
Watery clear	Normal

- **Unstimulated salivary pH**
  - **Method:** A pH test strip was placed into the sample of unstimulated saliva for 10 seconds, and then the colour of the strip was observed. This was compared with the testing chart.
  - **Interpretation:**

Colour on the saliva testing chart		**pH**
Red	5.0 – 5.8	Highly acidic
Yellow	6.0 – 6.6	Moderately acidic
Green	6.8 – 7.8	Healthy saliva

- **Stimulated Saliva Testing:**
- **Stimulated salivary flow rate**
  - **Method:** Quantity of stimulated saliva in collection cup was noted and results were interpreted as follows.
  - **Interpretation:**

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Quantity of saliva after 5 minutes	Flow rate
< 3.5 ml	Very low
3.5 – 5.0 ml	Low
> 5.0 ml	Normal

❖ **Buffering capacity of saliva**

- **Method**: Buffer test strip containing three test pads was removed from the foil sealed packet and placed onto an absorbent tissue paper with the test side up.Using a pipette, sufficient saliva was drawn from the collection cup, and dispensed one drop onto each of the three test pads. The strip was immediately turned 90 degrees to soak up any excess on the absorbent tissue. This prevented excess saliva from swelling on the test pad and affecting the accuracy of the test result. The test pads began to change colour immediately and after 2 minutes the final results were available.Points were allocated to each test pad based on colour and a combined total for the 3 test pads determined the buffering capacity.
- **Results after 2 minutes:**

Test pad colour	Points
Green	4
Green/blue	3
Blue	2
Blue/Red	1
Red	0

- **Interpretation:**

Combined total	buffering capacity of saliva
0 – 5 points	Very low
6 – 9 points	Low
10 – 12 points	Normal

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After salivary testing all the data obtained were recorded on proforma prepared specially for study. The data was analysed statistically using Chi-Square Tests.

OBSERVATION AND RESULTS:

TABLE I showing comparison of unstimulated salivary flow rate between subjects of group – I, group – II and group – III

UNSTIMULATED SALIVARY FLOW RATE	GROUP – I	GROUP – II	GROUP – III
LOW	31 (62%)	7 (23.33%)	3 (15%)
NORMAL	19 (38%)	23 (76.66%)	17 (85%)
TOTAL	50 (100%)	30 (100%)	20 (100%)
Overall p value **<0.0001**			
p value of Group I & Group II **=0.001**			
p value of Group I & Group III and Group I & Group II+III **< 0.0001**			
p value of Group II & Group III **= 0.470**			

Table I shows comparison of unstimulated salivary flow rate among subjects of Group I, Group II and Group III. Out of 50 subjects of Group I, unstimulated salivary flow rate was low in 31 (62%) and normal in 19 (38%) subjects. Out of 30 subjects of Group II, unstimulated salivary flow rate was low in 7 (23.33%) and normal in 23 (76.66 %) subjects. Out of 20 subjects of Group III, unstimulated salivary flow rate was low in 3 (15%) and normal in 17 (85%) subjects. p value of Chi Square Test among Group I, II & III was < 0.0001 which indicated highly significant difference of unstimulated salivary flow rate among these groups. p value of Chi Square Test of Group I & Group II was 0.001 which was significant and that of Group I & Group III and Group I & Group II+III was < 0.0001 which was highly significant. p value of Chi Square Test of Group II & Group III was 0.470 which indicated non-significant difference of unstimulated salivary flow rate among these groups.

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TABLE II showing comparison of unstimulated salivary viscosity between subjects of group – I, group – II and group – III			
UNSTIMULATED SALIVARY VISCOSITY	**GROUP- I**	**GROUP- II**	**GROUP-III**
HIGH	13 (26%)	2 (6.66%)	0 (0.00%)
INTERMEDIATE	29 (58%)	13 (43.33%)	10 (50%)
NORMAL	8 (16%)	15 (50%)	10 (50%)
TOTAL	50 (100%)	30 (100%)	20 (100%)
Overall p value **= 0.001** p value of Group I & Group II = **0.002** p value of Group I & Group III = **0.0003** p value of Group I & Group II+III **< 0.0001** p value of Group II & Group III = **0.485**			

Table II shows comparison of unstimulated salivary viscosity among subjects of Group I, Group II and Group III. Out of 50 subjects of Group I, unstimulated salivary viscosity was high in 13 (26%), intermediate in 29 (58%) and normal in 8 (16%) subjects. Out of 30 subjects of Group II, unstimulated salivary viscosity was high in 2 (6.66%), intermediate in 13 (43.33%) and normal in 15 (50%) subjects. Out of 20 subjects of Group III, unstimulated salivary viscosity was intermediate in 10 (50%), normal in 10 (50%) and none had high salivary viscosity. p value of Chi Square Test among Group I, II & III was 0.001 which indicated significant difference of unstimulated salivary viscosity among these groups. p value of Chi Square Test of Group I & Group II was 0.002 and Group I & Group III was 0.0003 which were significant and that of Group I & Group II+III was < 0.0001 which was highly significant. p value of Chi Square Test of Group II & Group III was 0.485 which indicated non-significant difference of unstimulated salivary viscosity among these groups.

TABLE III showing comparison of unstimulated salivary pH between subjects of group – I, group – II and group – III			
UNSTIMULATED SALIVARY pH	**GROUP – I**	**GROUP – II**	**GROUP – III**
HIGHLY ACIDIC	0 (0.00%)	0 (0.00%)	0 (0.00%)
MODERATELY ACIDIC	37 (74%)	7 (23.33%)	11 (55%)
HEALTHY	13 (26%)	23 (76.66%)	9 (45%)
TOTAL	50 (100%)	30 (100%)	20 (100%)
Overall p value **< 0.0001** p value of Group I & Group II and Group I & Group II+III **< 0.0001** p value of Group I & Group III = **0.122** p value of Group II & Group III = **0.222**			

Table III shows comparison of unstimulated salivary pH among subjects of Group I, Group II and Group III. Out of 50 subjects of Group I, unstimulated salivary pH was moderately acidic in 37 (74%), healthy in 13 (26%) and none had highly acidic pH. Out of 30 subjects of Group II, unstimulated salivary pH was moderately acidic in 7 (23.33%), healthy in 23 (76.66%) and none had highly acidic pH. Out of 20 subjects of Group III, unstimulated salivary pH was moderately acidic in 11 (55%), healthy in 9 (45%) and none had highly acidic pH. p value of Chi Square Test among Group I, II & III was < 0.0001 which shows highly significant difference in pH of unstimulated saliva among these groups. p value of Chi Square Test of Group I & Group II and Group I & Group II+III were < 0.0001 which were highly significant. p value of Chi Square Test of Group I & Group III was 0.122 and Group II & Group III was 0.222 which indicated non-significant difference of unstimulated salivary pH among these groups.

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TABLE IV showing comparison of stimulated salivary flow rate between subjects of group – I, group – II and group – III			
STIMULATED SALIVARY FLOW RATE	**GROUP- I**	**GROUP- II**	**GROUP-III**
VERY LOW	6 (12%)	1 (3.33%)	0 (0.00%)
LOW	32 (64%)	6 (20%)	0 (0.00%)
NORMAL	12 (24%)	23 (76.66%)	20 (100%)
TOTAL	50 (100%)	30 (100%)	20 (100%)
Overall p value **< 0.0001** p value of Group I & Group II, Group I & Group III and Group I & Group II+III < **0.0001** p value of Group II & Group III = **0.066**			

Table IV shows comparison of stimulated salivary flow rate between subjects of Group I, Group II and Group III. Out of 50 subjects of Group I, stimulated salivary flow rate was very low in 6 (12%), low in 32 (64%) and normal in 12 (24%) subjects. Out of 30 subjects of Group II, stimulated salivary flow rate was very low in 1 (3.33%), low in 6 (20%) and normal in 23 (76.66%) subjects. Out of 20 subjects of Group III, stimulated salivary flow rate was normal in 20 (100 %) and none had low & very low stimulated salivary flow rate. p value of Chi Square Test among Group I, II & III was < 0.0001 which shows highly significant difference in flow rate of stimulated saliva among these groups. p value of Chi Square Test of Group I & Group II, Group I & Group III and Group I & Group II+III was < 0.0001 which was highly significant. p value of Chi Square Test of Group II & Group III was 0.066 which indicated non-significant difference of stimulated salivary flow rate among these groups.

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TABLE V showing comparison of buffering capacity of stimulated saliva between subjects of group – I, group – II and group – III			
BUFFERING CAPACITY OF STIMULATED SALIVA	**GROUP – I**	**GROUP – II**	**GROUP – III**
VERY LOW	4 (8%)	1 (3.33%)	0 (0.00%)
LOW	29 (58%)	7 (23.33%)	5 (25%)
NORMAL/HIGH	17 (34%)	22 (73.33%)	15 (75%)
TOTAL	50 (100%)	30 (100%)	20 (100%)
Overall p value = **0.002** p value of Group I & Group II = **0.003** p value of Group I & Group III = **0.007** p value of Group I & Group II+III < **0.0001** p value of Group II & Group III = **0.710**			

Table V shows comparison of buffering capacity of stimulated saliva between subjects of Group I, Group II and Group III. Out of 50 subjects of Group I, buffering capacity of stimulated saliva was very low in 4 (8%), low in 29 (58%) and normal in 17 (34%) subjects. Out of 30 subjects of Group II, buffering capacity of stimulated saliva was very low in 1 (3.33%), low in 7 (23.33%) and normal in 22 (73.33%) subjects. Out of 20 subjects of Group III, buffering capacity of stimulated saliva was low in 5 (25%), normal in 15 (75%) and none had very low buffering capacity of saliva. p value of Chi Square Test among Group I, II & III was 0.002 which shows significant difference in buffering capacity of stimulated saliva among these groups. p value of Chi Square Test of Group I & Group II was 0.003 & that of Group I & Group III was 0.007 which were significant. p value of Chi Square Test of Group I & Group II+III was <0.0001 which was

highly significant. p value of Chi Square Test of Group II & Group III was 0.710 which indicated non-significant difference of buffering capacity of stimulated saliva among these groups.

DISCUSSION

Menopause is a physiological process which typically occurs in females in the fifth decade of life, and involves permanent cessation of menstruation at least for one year. Many physiological and psychological changes, most of which are due to decreased ovarian oestrogen production, take place in females approaching the menopause. Various systemic signs and symptoms of menopause include hot flushes, profuse sweating, atrophic epithelial changes in genital and urinary systems, vaginal dryness, decreased sexual desire, hair thinning, loss of elasticity and wrinkling of skin, dementia, osteoporotic changes in bone and psychological changes like headache, insomnia, dysphagia, depression. Various oral symptoms include dry mouth, dental caries, burning sensation of the mouth, gingivitis, halitosis and altered taste sensation. [9,10] These changes in the oral cavity are due to alterations in salivary function that may lead to impairment of oral tissues and have a large impact on the patient's quality of life. [10,11]

In menopausal females these changes can be due to hormonal alterations or aging process. [12,13] In order to prove that salivary changes in menopausal females are because of hormonal alterations and not aging process; reproductive females and males of the same age as menopausal females were included in the present study as a part of control group. [14,15,16]

Various local and systemic factors also have effects on saliva. Patients with poor oral hygiene may have caries and periodontal disease, which may alter the quality and quantity of saliva in the mouth. [17] Thus, the study was conducted only on subjects with fair or good oral hygiene. Any habits like tobacco/alcohol may alter the quantity and quality of saliva. So, subjects with habits were not included in the study to avoid any inaccuracy in the results. Systemic diseases and conditions like diabetes, hypertension, HIV, hepatitis or depression may alter the salivary secretion. [18-21] Hence, subjects with any systemic diseases were excluded from the study.

The number of females using hormone replacement therapy (HRT) is increasing in modern times to cope up with the hormonal changes occurring during menopause. There is no doubt that many females 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. [15,16] The females on HRT were excluded from the study.

Thus, the present study was conducted under standardized conditions to evaluate the effects of menopause on different parameters of saliva like unstimulated flow rate, viscosity, pH and stimulated flow rate & buffering capacity among menopausal females as well as reproductive age females and males of the same age as menopausal females.

When, in the present study, unstimulated salivary flow rate, viscosity, pH and stimulated salivary flow rate & buffering capacity were compared between females of menopausal age group and reproductive age group; significant difference was found between two groups.

Unstimulated salivary flow rate was found to be low in menopausal females compared to reproductive age females. This was in accordance with the previous study done by Mahesh DR et al (2014) and Dural S et al (2006). [15,16] Majority of the menopausal females had intermediate/high salivary viscosity whereas majority of reproductive age females had normal/intermediate viscosity. This was in accordance with the previous study done by Mahesh DR et al (2014). [16] Majority of menopausal females had moderately acidic pH whereas majority of reproductive age females showed normal pH values. This was in accordance with study done by Dural S et al (2006) and Mahesh DR et al (2014) and Bhat S et al (2010). [14-16]

When, in the present study, stimulated salivary flow rate was compared between menopausal females and reproductive age females; stimulated salivary flow rate was found to be low in menopausal females compared to reproductive age females. This was in accordance with the previous study done by Dural S et al (2006) and Mahesh DR et al (2014) and Agha-Hosseini F et al (2007). [15,16,22] While comparing buffering capacity of stimulated saliva in menopausal and reproductive age females, majority of menopausal females had low buffering capacity compared to reproductive age females. This result was in accordance with the previous study done by Mahesh DR et al (2014). [16]

Oestrogen levels are normal in reproductive females compared to reduced levels in menopausal females. So above mentioned changes in the saliva may be due to hormonal changes in the menopausal females, but aging can also be considered as one of the factors in menopausal females while comparing to females of reproductive age. In order to exclude aging as a factor responsible for salivary changes; males of the same age group as menopausal females were also included in the study.

Unstimulated salivary flow rate, viscosity, pH and stimulated salivary flow rate & buffering capacity of menopausal females were compared with males of same age and significant difference was found in some of the parameters. The unstimulated salivary flow rate of menopausal females was found to be significantly low compared to the males of same age. This was in accordance with the previous study done by Mojabi KB et al (2007) and Alani SH (2012). [12,13] Unstimulated salivary viscosity was low/intermediate in majority of menopausal females compared to males of same age who had normal/intermediate viscosity. There was no significant difference found in pH of these two groups. This result could be age related and not hormone dependant.

When, stimulated salivary flow rate and buffering capacity of menopausal females were compared with males of the same age, salivary flow rate was found to be low in menopausal females compared to the males of same age. Buffering capacity was found to be significantly low in menopausal females compared to males of the same age. These findings can be related to the

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hormonal alterations that occur during the period of menopause in females and not associated with aging.

Thus, in the present study when, salivary parameters of menopausal females and reproductive females were compared significant difference was found between these two groups. These differences could be because of hormonal changes and/or aging. Also, salivary parameters of menopausal females were compared with males of the same age of menopausal females, which showed significant difference in all salivary parameters except for pH. This indicated that difference could be because of hormonal changes occurring in females during menopause and not due to aging. Hence, it can be proved that the salivary changes in the menopausal females are mainly due to hormonal changes and not because of the aging process.

CONCLUSION:

Menopause also known as "the change" or "change of life," is a normal part of a female's life. It is a point of time of life when permanent cessation of menstruation occurs. This is the time when females might be experiencing menopausal symptoms, also called the menopausal transition. Menopausal symptoms also include alterations in salivary function that may lead to impairment of oral tissues and have a large impact on the female's quality of life. A higher incidence of dental caries, oral mucositis, dysphagia, oral infections and altered taste has been reported in individuals with altered salivary function.

The present study was undertaken to establish the effect of menopause on saliva and dental health. This study was conducted under standardized conditions to evaluate the effects of menopause on different parameters of saliva like unstimulated flow rate, stimulated flow rate, viscosity, pH and buffering capacity by collecting unstimulated and stimulated saliva from healthy subjects. At the end of the study following results were obtained.

- Unstimulated salivary flow rate is low in menopausal females
- Unstimulated salivary viscosity is mostly intermediate or low in menopausal females
- Unstimulated salivary pH is moderately acidic in menopausal females
- Stimulated salivary flow rate is low in menopausal females
- Stimulated salivary buffering capacity is low in menopausal females

In our study, salivary parameters were compared among menopausal females, reproductive females and males of the same age as menopausal females. The results suggested that there is a marked difference in the salivary parameters in menopausal females. This infers that these changes in salivary parameters are due to hormonal changes during menopause and not aging process. These changes in turn affect the oral hygiene of menopausal females.

Thus, to deliver high quality care, dental practitioners should have knowledge about menopause and its oral manifestations as a possible risk factor for increasing oral health problems occurring due to salivary changes. Current demographic trends in the Indian female population underscore this need. For example, the menopausal patient who comes to the dental clinic with complains of oral discomfort or loose teeth may not understand the aetiology of her dental concerns. A knowledgeable dental practitioner therefore, could advise that the conditions are possibly menopause-related and can play a vital role in meeting the oral health needs by early diagnosis, treatment planning, and patient education.

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CONFLICT OF INTEREST:

There are no conflicts of interest.

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