The relationship between salivary levels of cortisol, chromogranin A (CgA) and xerostomia in post-menopausal women

E.A. Al-Kholy a,*, M.A. Mahmoud a, F.A. EL Nomany a, E.A. EL Zamarany b

a Oral Medicine, Periodontology, Oral Diagnosis & Oral Radiology, Faculty of Dentistry, Tanta University, Egypt
b Clinical Pathology, Faculty of Medicine, Tanta University, Egypt

Received 3 March 2014; revised 15 June 2014; accepted 15 June 2014
Available online 27 September 2014

Abstract

Purpose: Menopause may be associated with some adverse changes, such as oral dryness (OD) feeling. The exact mechanisms that mediate sensation of OD in menopausal women have not been firmly established. The purpose of the present study was to assess the relationship of un-stimulated whole saliva cortisol and chromogranin A (CgA) levels with OD feeling in post-menopausal women.

Materials & methods: The present study was conducted on eighty selected post-menopausal women with/without xerostomia. Subjects were equally divided into two groups, forty patients each. Group I: (study group) constituted by 40 female subjects meeting the selection criteria and having xerostomia. Group II: (control group) constituted by 40 female subjects meeting the same criteria with the exception of the presence of xerostomia. Un-stimulated whole saliva cortisol and chromogranin A concentrations were measured by ELISA (enzyme-linked immunosorbent assay). Collected data of the present study was tabulated and statistically analyzed using the statistical software package.

Results: The means of salivary cortisol and chromogranin A concentrations were significantly higher with significantly decrease in the mean un-stimulated salivary flow rate (UWSFR) in group I (xerostomia group) compared to group II (control group) (P ≤ 0.001).

Conclusion: Significant associations between salivary cortisol and CgA levels and symptoms of oral dryness and reduced salivary flow rates were detected.

© 2014, Hosting by Elsevier B.V. on behalf of the Faculty of Dentistry, Tanta University.
Open access under CC BY-NC-ND license.

Keywords: Post-menopausal women; Salivary flow rate; Un-stimulated whole saliva; Xerostomia; Salivary cortisol; Salivary chromogranin A

1. Introduction

Menopause is defined as the permanent (after 12 months) cessation of menstruation resulting from the loss of ovarian function. The age at which physiological menopause appears is between 45 and 55 years, with an average of 52.5 years [1]. Utian, (1999) [2] classified menopause stages to: 1- Pre-menopause:
the reproductive years prior to the last menstrual period, 2- Peri-menopause: the time immediately around the menopause, often accompanied by longer cycles and heavier and prolonged bleeding. These menstrual irregularities are due to decline in ovarian follicular function, but no 12 consecutive months of amenorrhea (lack of menstruation) have yet occurred. This stage is often accompanied by hot flushes. The average age of peri-menopause is 45.1 years but it can start any time between ages 39 and 51, and can last between two and eight years (the average being five), 3- Post-menopause: a period of time where no menstruation has occurred in 12 consecutive months. The median age for this to happen is 51 years.

When a woman is post-menopausal, she has some additional long-term age-related health considerations such as the development of osteoporosis and cardiovascular disease. As a woman reaches the menopause stages of life, it is a good opportunity to assess overall health and lifestyle choices to address potential long-term health issues. The issue of menopause and post-menopausal health in women is of significance to society in general because of the universality of menopause, it affects all women and because of the unprecedented increase in the number of post-menopausal women [3].

Premature menopause is defined as occurring when a woman is less than two standard deviations below the median age for menopause in the referred population. Age of 40 yrs is often used as an arbitrary age below which the menopause is viewed as premature. There are primary and secondary premature ovarian failure [4].

The fall in hormones (estrogen and progesterone) levels at the post-menopause can cause a variety of symptoms such as hot flushes, night sweating, palpitation, headache, changes in the skin, brittle nails, hair loss, muscular aches, osteoporosis, Irregular menstrual periods, diminished sexual function, variable signs or symptoms which reflecting a depressed mood and oral discomfort [5—9]).

Oral discomfort is characterized by a burning sensation, sensation of oral dryness (xerostomia or dry mouth) and decreased saliva secretion (hypo-salivation or hypo-function) [10].

Xerostomia is a common oral concern for many patients. It is estimated that up to 10 percent of the general population experiences persistent oral dryness [11,12]. Xerostomia is more frequent with increasing age because the number of acini reduces and the amount of fatty and fibrous tissue increases, and over 25 percent of elders complain of daily dryness especially in post-menopausal women [13].

However, it does not necessarily relate to decreased salivary flow rate (hypo-function) in up to one third of cases. Although salivary gland failure may lead to OD, the subjective experience of xerostomia is not a reliable indicator of salivary gland hypo-function. It may also occur with the changes in the quality of saliva, while the amounts of saliva stay unchanged [14,15]. OD can lead to considerable difficulty in speaking, eating and tasting, and predispose mucosa to wounds, abrasion and infection [16—18].

Saliva can be considered a filtrate of the serum in as much as it is derived from the blood. It follows that the process of saliva production is linked to overall body fluid balance and that blood flow through salivary gland tissues (from branches of the maxillary and other arteries) has a major effect on the production of saliva. So, saliva is a good indicator of the plasma levels of various substances such as hormones and drugs and can therefore be used as a non-invasive method for monitoring plasma concentrations of medicines or other substances [19,20].

Total or whole saliva refers to the complex mixture of fluids from the salivary glands, the gingival fold (crevicular space), oral mucosa transudate, in addition to mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells, as well as traces of medications or chemical products [21].

So, the knowledge of normal salivary flow rate (SFR) is extremely important when treating dental patients. Early diagnosis and treatment of xerostomia and hypo-salivation will preserve the health of oral structures or tissues and lower the incidence of dental caries, fungal infections, and other oral diseases that could result from insufficient SFR. However, it receives little attention until its quantity diminishes or its quality becomes altered [22].

For 40 years, endocrinologists have used saliva as a supplementary sample matrix. Prior reviews, from Riad et al., (1982) [23] to Lewis, (2006) [24], have focused on salivary analysis of many steroids, although numerous reported studies have demonstrated that saliva monitoring is a useful alternative method for analyzing hormones of other biochemical origins.

In addition, numerous publications have described the use of salivary hormone analysis in many fields of clinical and basic research to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health [25,26].

Salivary biomarkers such as salivary chromogranin A (CgA) and salivary cortisol are being explored as a
means of monitoring general health and in the early diagnosis of disease. Disorders and diseases in which saliva may aid in diagnosis include but are by no means limited to human immune deficiency virus (HIV) and cancer [27,28].

Cortisol, released by adrenal glands, is the main glucocorticoid in the human hormonal system and is responsible for some critical homeostatic tasks, such as vascular reactivity. Cortisol follows a circadian rhythm with peak level at about 8.00 a.m. and its nadir in the late evening [29–31].

Chromogranin A (CgA) is an acidic protein that consists of 439 amino acids encoded on chromosome 14. CgA is the main member/element of the chromogranins family consisting of water soluble acidic glycoproteins which is a prohormone discovered for the first time in the secretory granules from adrenal medullary chromaffin cells and released into the circulation after splanchnic nerve stimulation together with catecholamine. The secretion granules from the chromaffin like cells of the medullo-infrarenal system store a complex mixture of the molecules which are today known as catecholamine, and additionally a large number of precursor proteins of the antimicrobial peptides like chromogranins, secretogranins and proencephalin A [32,33].

CgA was also recovered in biological fluids implicated in defense mechanisms like abscess fluids or saliva as CgA be released into saliva from salivary glands including submandibular gland [34].

Based on the above, the purpose of the present study was to investigate the salivary levels of cortisol and CgA in post-menopausal women with xerostomia and compared to those without xerostomia.

2. Materials & methods

The present study was conducted on 80 female subjects in the post-menopausal period selected from the outpatient Clinic of Oral Medicine, Periodontology, Oral diagnosis and Radiology Department, Faculty of Dentistry, Tanta University.

The present study was approved by the Research Ethical Committee of Tanta University. Written informed consent from patients was obtained. The subjects used for this study were females in the post-menopause period, age of 45–55 years, cessation of menstruation for at least 12 continuous months and with or without a complain of xerostomia. Patient having any of the following are not involved in the current study:

1 patients suffering xerostomia produced by specific causes such as:
a) Local inflammation, focal infection and fibrosis of the major salivary glands.
b) Autoimmune diseases e.g. Sjögren’s syndrome (primary and secondary) and Mikulicz’s disease.
c) Malnutrition e.g. anorexia, dehydration.
d) Alcoholism or smoking.
e) Systemic diseases as diabetes or hypertension or thyroid disease (hypo- and hyper-thyroidism) and late stage liver disease.
2 The use of hormonal replacement therapy (HRT) e.g. Estrogen or Estrogen/Progestin products.
3 The use of medications known to affect salivary gland flow rate such as diuretics, anti-spasmodic, expectorants, econgestants, systemic bronchodilator …etc.
4 Patients under treatment for xerostomia.
5 Past history of radiotherapy or chemotherapy as in cancer treatments.

To diagnose xerostomia, a questionnaire was applied to the patients to be answered by yes or no illustrating presence of xerostomia [35,36] (Table 1).

Response options yes/no. Subjects who had at least one positive response entered the study group, and those without any positive responses, formed the control group. Once xerostomia were reported, patients were questioned about duration (onset) of xerostomia and menopause [37].

The selected patients divided into two equal groups according to their complain of xerostomia

**Group I** (study group) 40 female subjects having xerostomia.

Table 1

<table>
<thead>
<tr>
<th>Questionnaire used for selection of subjects with xerostomia.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Does your mouth feel dry when eating a meal?</td>
</tr>
<tr>
<td>2 Do you have difficulties swallowing any foods?</td>
</tr>
<tr>
<td>3 Do you need to sip liquids to aid in swallowing dry foods?</td>
</tr>
<tr>
<td>4 Does the amount of saliva in your mouth seem to be reduced most of the time?</td>
</tr>
<tr>
<td>5 Does your mouth feel dry at night or on awakening?</td>
</tr>
<tr>
<td>6 Does your mouth feel dry during the daytime?</td>
</tr>
<tr>
<td>7 Do you chew gum or use candy to relieve oral dryness?</td>
</tr>
<tr>
<td>8 Do you usually wake up thirsty at night?</td>
</tr>
<tr>
<td>9 Do you have problems in tasting food?</td>
</tr>
<tr>
<td>10 Does your tongue burn?</td>
</tr>
</tbody>
</table>

Response options

Yes/no
Group II (control group) 40 female subjects not having xerostomia.

2.1. Clinical work

2.1.1. Materials

The salivary flow measurements for all patients were done according to Walsh, 2010 [38] (Fig. 1).

Determining un-stimulated whole salivary flow rates (UWSFRs) was done by the spitting or expectorating technique. The subjects were told to refrain from eating and drinking at least one hour prior to the examination time (between 9:00 a.m. and noon) for all patients to minimize any circadian rhythm effects and so to avoid variation in salivary flow rates. Before taking a sample of saliva, subjects were allowed to rest for 30–60 min the subjects were asked to sit in a quiet position with the head tilted forward, they were asked to rinse their mouth with water in order to eliminate any possible detritus and to obtain a clean sample [39].

Each sample was obtained by having the patient expectorate or spit all saliva into a graduated test tube through a glass funnel every 1 min, for 10 min. Once the sample had been obtained it was allowed to settle, placing the tube in a test tube rack, in order to achieve a better reading of the saliva volume. Then, volume of saliva was measured with milliliters (mL) and USFR was calculated by division volume on minutes (mL/min). USFR <0.1 mL/min are considered abnormally low and indicative of marked salivary hypo function, however, USFR \( \geq 0.1 \) mL/min to 0.3 mL/min are considered xerostomia. Then the sample was sent to the laboratory and was centrifuged at 3000 rpm at least once in order to separate the mucin and frozen at \(-20^\circ C\) to \(-30^\circ C\) until further analysis to find salivary cortisol & CgA concentrations (µg/dl) [40,41].

2.2. Laboratory work

2.2.1. Analysis of saliva

From each of the collected saliva sample, an equal volume (100 µL) was taken for measurement of salivary chromogranin A (CgA) level and salivary cortisol level using commercial kits [42] (Fig. 2).

2.2.1.1. DRG salivary cortisol ELISA

2.2.1.1.1. Assay principle. The DRG Salivary cortisol ELISA kit was based on the competition principle and the micro plate separation. An unknown amount of cortisol presented in the sample and a fixed amount of cortisol conjugated with horse-radish peroxidase competed for the binding sites of mouse monoclonal cortisol-antiserum coated onto the wells. After one hour incubation, the micro plate was washed to stop the competition reaction. After addition of the substrate solution the concentration of cortisol was inversely proportional to the optical density measured.

2.2.1.2. DRG salivary chromogranin A (CgA) ELISA

2.2.1.2.1. Assay principle. This ELISA was designed, developed and produced for the quantitative measurement of human CgA in saliva sample. The assay utilized the two-site “sandwich” technique with two selected antibodies that bonded to different epitopes of human CgA.

Assay standards, controls and patient samples were added directly to wells of micro plate that was coated with a polyclonal CgA antibody. After the first incubation period, the antibody on the wall of micro titer well captures human CgA in the sample and unbound proteins in each micro titer well were washed away.

Then a horseradish peroxidase (HRP) labeled monoclonal anti-human CgA antibody was added to each micro titer well and a “sandwich” of “monoclonal

---

1 (DRG International, Inc., USA. Cat. NO. SLV-2930) for salivary Cortisol.
2 (DRG International, Inc., USA. Cat. NO. SLV-4521) for salivary Chromogranin A (CgA).
antibody - human CgA — polyclonal antibody” was formed. The unbound monoclonal antibody was removed in the subsequent washing step.

For the detection of this immune complex, the well was incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric micro plate reader. The enzymatic activity of the immune complex bound to the CgA on the wall of the micro titer well was directly proportional to the amount of CgA in the sample.

2.3. Statistical analysis

The data of the present study was collected, tabulated and statistically analyzed using the statistical software package SPSS version 11.0 statistical package. For comparison of both mean variables for each group the student, s test was used.

3. Results

Regarding the mean value of the salivary volume taken during ten minutes in group I (study) compared to group II (control), there was a statistically significant decrease in the salivary volume in group I as the P value was ≤ 0.001 (Table 2 & Fig. 3). Comparing the mean value of the un-stimulated salivary flow rate taken during ten minutes in group I compared to group II, there was a statistically significant decrease in the un-stimulated salivary flow rate in group I compared to group II subjects (P ≤ 0.001) (Table 2 & Fig. 4).

Concerning the mean value of the salivary cortisol concentration that was recorded after laboratory work in group I Vs group II, there was a statistically significant increase in the salivary cortisol concentration in group I compared to group II subjects (P ≤ 0.001) (Table 2 & Fig. 5).

However, the mean value of the salivary CgA concentration recorded in group I compared to group II, there was a statistically significant increase in group I versus group II subjects (P ≤ 0.001) (Table 2 & Fig. 6).

Table 2
Comparison of the mean score and standard deviation (SD) of the clinical and laboratory results between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>2.1 ± 0.978</td>
<td>6.5 ± 1.16</td>
<td>≤0.001*</td>
</tr>
<tr>
<td>Duration (min)</td>
<td>10 0</td>
<td>10 0</td>
<td>0.996</td>
</tr>
<tr>
<td>UWSFR (ml/min)</td>
<td>0.21 ± 0.097</td>
<td>0.65 ± 0.116</td>
<td>≤0.001*</td>
</tr>
<tr>
<td>Cortisol. Conc (µg/dl)</td>
<td>18.5 ± 4.32</td>
<td>0.855 ± 0.308</td>
<td>≤0.001*</td>
</tr>
<tr>
<td>CgA. Conc (µg/dl)</td>
<td>5.02 ± 1.06</td>
<td>0.459 ± 0.181</td>
<td>≤0.001*</td>
</tr>
</tbody>
</table>

Statistically significant at 5% level.

Fig. 2. ELISA commercial kits of salivary cortisol and salivary chromogranin A.

Fig. 3. Comparison of the mean value of the salivary volume in group I versus group II.

Fig. 4. Comparison of the mean value of USFR in group I versus group II.

Fig. 6. Comparison of the mean value of salivary CgA concentration in group I versus group II.
4. Discussion

Xerostomia is a consequence of many oral and systemic diseases and is estimated up to 10% of the general population. Also, xerostomia is a complaint reported by a large number of post-menopausal women. So, early assessment of xerostomia may aid in recognition of treatment in post-menopause stage in which women are more exposed to systemic diseases.

The measurement of biomarkers in saliva has many advantages, because it is stress free and allow for frequent and rapid sampling, whereas diurnal rhythm, artificial changes due to food or drinking substances, and blood contamination are some of the disadvantages [43]. Accordingly, in our study, we used salivary biomarkers (cortisol and chromogranin A (CgA)) to investigate relationship between salivary biomarkers levels and xerostomia in post-menopausal women by un-stimulated condition.

In a study by Pedersen et al., (2002) [44] and Agha–Hosseini et al., (2010) [45], they reported significant lowering of stimulated whole salivary flow rate in post-menopausal women with xerostomia, compared with post-menopausal women without xerostomia. Although this is consistent with our study, the difference was in use of un-stimulated conditions to calculate salivary flow rate (SFR). Un-stimulated conditions were more accurate than stimulated conditions because of difference in type and degree of stimulation.

Our data indicated that un-stimulated whole salivary cortisol & CgA levels were increased with reduced un-stimulated whole salivary flow rate in post-menopausal women suffering from xerostomia (group I) compared to post-menopausal women not suffering from xerostomia (group II), this is in accordance with the findings of Shigeyama et al., (2008) [42] who also investigated the salivary levels of cortisol and CgA in subjects with xerostomia compared to control subjects without xerostomia also, their subjects aged were 50ys and over and added stimulated conditions with un-stimulated conditions. However, our study used aged ranged from 45 to 55ys and did not use stimulated condition.

The findings of the present study were different from study of Gómez et al., (2006) [37] who found that there was no significant association between un-stimulated salivary cortisol levels and post-menopausal women with xerostomia and no significant differences between post-menopausal women with xerostomia and post-menopausal women without xerostomia. This might be attributable to smaller sample size (n = 30) and unclear criteria for the diagnosis of xerostomia or dry mouth while our study was done on larger sample size (n = 80) so, criteria of xerostomia were clear by clinical examinations and group of questions illustrating presence of xerostomia which were explained in our method in this study.

On other hand, studies reported on males had revealed that males had higher secretion rates of un-stimulated saliva compared with females (post-menopausal women) at age 55ys and over. The differences were attributed to greater glandular mass in males, differences in hormonal patterns and differences in body mass index (BMI). Based on that, studies were found that xerostomia was more frequent with post-menopausal women with a range between 24% and 65.1% of them who complained of daily oral dryness or xerostomia [46–49]. This was going inside with our study but our study did not deal with male as, comparison was between post-menopausal women with xerostomia and others without xerostomia.

Other studies had indicated that salivary alterations might be directly related to age rather than disease and/ or drug use in post-menopausal women. This was
supported by studies of Rafael et al., (2004) [50] and Dodds et al., (2005) [51] who explained that age-related histological changes in the submandibular glands (used only once) included atrophy in both secretory acinar and ductal epithelia, accompanying structural changes in fibrous and elastic tissues as well as in the walls of arteries and veins. Accordingly, our exclusion criteria in this study that patients without systemic diseases or did not take any medications which might contribute to xerostomia.

The last opinion by Ship, (1999) [52] and Ship et al., (2002) [53] gave results conflicting with ours in that aging per se lead to a diminution in the capacity of the salivary glands to produce saliva. They found that many post-menopausal women complained of a dry mouth and had decreased salivary output, was explained in large as a reflection of systemic conditions and their treatment (drugs, chemotherapy and radiotherapy) rather than being normal squeal of aging. This was not the case in our study because our exclusion criteria included systemic diseases and treatments which caused xerostomia.

5. Conclusion

From the results of the present study we can conclude that un-stimulated whole saliva cortisol and chromogranin A levels are higher in post-menopausal women with xerostomia than in controls which suggests an association with symptoms of xerostomia and lower salivary flow rates. Additional studies of salivary biomarkers (cortisol & CgA) may lead to the development of a method of monitoring salivary glands functions in subjects suffering xerostomia and early treatment to prevent complications of xerostomia e.g hyposalivation which may result from systemic diseases.

References