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Evaluation of salivary total antioxidant capacity and malondialdehyde in pre-menopausal and post-menopausal women

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Menopause is a natural and expected stage of development that occurs in the life of a woman and is characterized by the permanent end of menstruation. Ovarian dysfunction is a result of permanent alterations to the ovaries' reproductive and hormonal activities. The reproductive system of a woman is not the only thing that is impacted by fluctuations in hormone levels. Hormones have powerful influence on the development of the skeleton and the mouth cavity, as well as on the integrity of these structures. The oral cavity is significantly affected by them. Different stages of a woman's life, such as puberty, menstruation, pregnancy, and menopause, each have their own unique impact on the oral health. Oxidative stress is a condition that occurs when there is an imbalance between oxidants and antioxidants, with the former making it possible for living cells to sustain damage. Lipid peroxidation and the subsequent breakdown products, such as malondialdehyde (MDA), are formed, and these compounds can be observed in biological fluids.
Total antioxidant capacity is a term that is frequently used to refer to the antioxidant activity of the "nonspecific" antioxidant pool.
The objective: **to assess of salivary total antioxidant capacity, salivary malondialdehyde, salivary pH in pre-menopausal and post-menopausal women and correlation between pH and salivary biomarkers.**
Materials and methods**. A case-control study was conducted in 80 healthy women. The study included 80 females between the ages of 40 and 55 years; specifically, 40 persons were premenopausal and 40 women were postmenopausal.**
In the saliva of the patients, the total antioxidant capacity (TAC) and MDA level were determined using the immunoassay method, and the pH level was also studied. Data description, analysis and presentation were performed using Statistical Package for social Science (SPSS version 22, Chicago, Illinois, USA).
Results**. The findings indicated that the pH value in the post-menopausal group was significantly higher than that of the pre-menopausal group. In comparison to the post-menopausal group, the pre-menopausal group exhibited a significantly higher level of TAC. While MDA level was significantly greater in the post-menopausal group than in the pre-menopausal group.**
A significant correlation of salivary pH with biomarkers of TAC and MDA of saliva was found, but there was no significant correlation between MDA and TAC in premenopausal women. On the other hand, there is a non-significant negative correlation between salivary TAC and pH level, as well as an insignificant correlation of MDA with pH and salivary TAC in postmenopausal patients.
Conclusions**. There are differences in salivary total antioxidant capacity and malondialdehyde level between pre-menopausal and post-menopausal women.**
Keywords: *post-menopause, pre-menopause, total antioxidant capacity, malondialdehyde, antioxidant.*

Оцінювання загальної антиоксидантної здатності та рівнів малонового діальдегіду у слині у жінок у пременопаузі та після менопаузи

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Менопауза – це природний і очікуваний період, який настає у житті жінки і характеризується безповоротним припиненням менструації. Дисфункція яєчників є результатом постійних змін репродуктивної та гормональної діяльності яєчників. Репродуктивна система жінки – це не єдине, на що впливають коливання рівня гормонів. Гормони справляють потужний вплив на розвиток скелета і ротової порожнини, а також на цілісність цих структур.
Порожнина рота також зазнає їхнього впливу. Різні етапи життя жінки, такі, як статеве дозрівання, менструація, вагітність і менопауза, мають свій власний унікальний вплив на здоров'я ротової порожнини. Оксидантний стрес – це стан, який виникає, коли існує дисбаланс між оксидантами та антиоксидантами, причому перше дозволяє живим клітинам зазнавати пошкоджень. При цьому посилюються процеси перекисного окиснення ліпідів і утворення продуктів їхнього розпаду, таких, як малоновий діальдегід (МДА), і ці сполуки можна спостерігати у біологічних рідинах.
Загальна антиоксидантна здатність – це термін, який часто використовують для позначення антиоксидантної активності «неспецифічного» пулу антиоксидантів.
Мета дослідження: оцінювання загальної антиоксидантної здатності слини, рівня малонового діальдегіду слини, pH слини у жінок у пременопаузі та після менопаузи і кореляції між pH та біомаркерами слини.
Матеріали та методи. Дослідження типу «випадок–контроль» було проведено у 80 здорових жінок. У дослідженні взяли участь 80 жінок у віці від 40 до 55 років; зокрема 40 пацієнток були у пременопаузі і 40 осіб – у постменопаузі.
У слині пацієнток визначали загальну антиоксидантну здатність (ЗАЗ) та рівень МДА за допомогою імуноферментного методу, а також вивчали рівень pH. Опис, аналіз і подання даних проводили за допомогою Statistical Package for Social Science (SPSS, версія 22, Чикаго, Іллінойс, США).

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Результати. Показник pH слини у жінок після менопаузи був значно вищий, ніж в осіб до менопаузи. Також встановлено значно вищий рівень ЗАЗ у жінок до менопаузи щодо показника осіб після менопаузи. Рівень МДА був значно вищий у групі жінок після менопаузи, ніж у групі жінок до менопаузи.
Виявлено значну кореляцію pH слини з біомаркерами ЗАЗ і МДА слини, але не було істотної кореляції між МДА і ЗАЗ у жінок до менопаузи. З іншого боку, існує незначуща негативна кореляція між ЗАЗ слини та pH, а також незначна кореляція МДА з pH і ЗАЗ слини у пацієнток після менопаузи.
Висновки. Установлено відмінності у загальній антиоксидантній здатності слини та рівнях малонового діальдегіду у слині між жінками у пременопаузі та після менопаузи.
Ключові слова: *постменопауза, пременопауза, загальна антиоксидантна здатність, малоновий діальдегід, антиоксидант.*

Menopause is an unavoidable biological process that is present in the lives of all women. Once their reproductive years are over, the majority of them live a third of their life in this specific state. This is defined by the World Health Organization as the final menstrual period, which typically happens between the ages of 45 and 55 [1, 2]. Additionally, menopausal women have changes in their oral health, including xerostomia, burning mouth syndrome, dysesthesia, and changes in taste, atrophy of the gums and jaw bones, periodontitis, and an increased incidence of dental caries [3].

Women who are transitioning into menopause are also at risk for developing depressive mood, anxiety, and cognitive and memory impairment due to fluctuations in estrogen levels. This is because estrogen has the ability to affect the synthesis, release, and metabolism of neurotransmitters in the central nervous system [4]. Antioxidants are found in all bodily fluids and prevent oxidation while also protecting cells from damaging oxidants and reactive oxygen species (ROS). Antioxidants, which have numerous health benefits, are grouped into three types: chain-breaking, preventive, and enzymes, as well as ROS regulation. Antioxidant defense mechanisms eliminate free radicals for a healthy aerobic lifestyle [5].

Oxidative stress refers to an inequilibrium between oxidants and antioxidants, with a bias towards oxidants. This imbalance results in a disturbance of redox signaling and regulation, as well as potential harm to molecules [6, 7]. Malondialdehyde (MDA), the most prominent result of lipid peroxidation, can be utilized as an indicator of oxidative stress [8].Total antioxidant capacity (TAC) is the main antioxidant analysis method for biological material [9].

The objective: to assess of salivary total antioxidant capacity, salivary malondialdyde, salivary pH in pre-menopausal and post-menopausal women and correlation between pH and salivary biomarkers.

MATERIALS AND METHODS

This study is a case control study was performed in Baghdad city form December 2023 until April 2024. The study was authorised by the ethical committee collage of Dentistry in university of Baghdad. Reference number: 900, project number 900824, date; 4-2-2024. All the subject received detailed information concerning the nature of the study and the procedures involved.

Patient Selection: This case–control study was accomplished in the Karkh Health Department Al-Nour Specialized Dental center, microbiology laboratory of Dentistry College / University of Baghdad, and a private microbiology laboratory. The samples consisted of 80 females aged between 40–55 years; the study included (40) pre and (40) postmenopausal women.

Inclusion criteria for the case group were as follows: Postmenopausal women who have been in menopause for at least a year. Menopausal status was self-reported, and the duration of menopause is determined from the day of diagnosis.

The excursion criteria in both groups included complete edentulism, intraoral lesions, dry mouth, Cigarette smoking, intake of antioxidant supplements, which may include vitamin and iron supplements, usage of corticosteroid medicines applied topically over a period of at least three weeks, using any kind of hormone replacement treatment or having a known systemic disease, like chronic kidney disease, diabetes, or heart disease.

Sample collection: Unstimulated saliva was collected from participants between 9 and 11 a.m. The subject was asked to avoid drinking or eating for three hours before the saliva collection procedure, and then asked to wash their mouth with distilled water for one minute and relax for five minutes directly before starting saliva collection; subjects were asked to spit saliva into sterilized cups with graduations [10].

Biomarker analyses of oxidative stress in saliva. The salivary TAC, MDA were determined by ELISA (BT lab, China).

Ethical approval

The study was authorised by the ethical committee collage of Dentistry in university of Baghdad. Reference number: 900, project number 900824, date; 4-2-2024.

RESULTS AND DISCUSSION

Menopausal transition (premenopausal) is a period in the aging process of women that marks the transition from the reproductive stage to the non-reproductive stage. It lasts 4–7 years [11]. The duration of menopause typically spans from around 47 years of age until its completion, and can be categorized into an initial and later stage according to the Stages of Reproductive Aging Workshop [12].

The present study was conducted to compare the oxidant-antioxidant status (MDA and TAC) between pre-menopausal and post-menopause women. In the current study, 80 participants were involved with an age range between (40–57) years; they were categorized into two groups: 40 as the study group with post-menopausal women with an age range between (47–57) years and 40 as the control group pre-menopausal women with an age range between (40–47) years, the mean age of the study group was (52.525±2.810), and the mean age of the control group was (43.050±2.171).

Statistically significant differences were observed concerning the age for the groups ($p \leq 0.05$), as shown in Table 1.

This group of menopausal women meets the inclusion criterion of having gone through menopause for at least two years, which aligns with the global average age of menopause [13], which falls between 40 and 57 years old [14]. These findings were consistent with those of earlier investigations [15–17]. The results in Table 2 shown the mean value of salivary pH in groups. The result revealed that the pH value in post-menopause was higher than the pre-menopause group with significant differences.

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Table 1

Distribution of groups study according to age (years)

	Groups		T test	P value
	Pre-Menopause	Post-Menopause		
Minimum	40.000	47.000	16.874	0.000 Sig.
Maximum	47.000	57.000		
Mean	43.050	52.525		
±SD	2.171	2.810		

Table 2

Distribution of post-menopause group and pre-menopause grous according to salivary pH

	Groups		T test	P value
	Pre-Menopause	Post-Menopause		
Minimum	6.800	4.700	22.339	0.000 Sig.
Maximum	7.500	6.100		
Mean	7.047	5.930		
±SD	0.193	0.250		

Table 3

Salivary levels of Total Antioxidant Capacity

	Groups		T test	P value
	Pre-Menopause	Post-Menopause		
Minimum	0.834	0.619	10.162	0.000 Sig.
Maximum	1.371	0.881		
Mean	1.004	0.755		
±SD	0.141	0.063		

Table 4

Salivary levels of Malondialdehyde

	Groups		T test	P value
	Pre-Menopause	Post-Menopause		
Minimum	0.501	0.890	17.098	0.000 Sig.
Maximum	0.656	1.371		
Mean	0.589	1.033		
±SD	0.043	0.158		

Evaluation of salivary pH levels led to the conclusion that the salivary pH value of Post-menopausal women (5.930±0.250) was lower than that of pre-menopausal women (7.047±0.193). These results are in accordance with the study of salivary pH using a pH-meter by Rosita Aisyah, J. N. Rukmini [15, 18]. Reduced salivary flow rate, which regulates salivary pH, leads to a lower release of bicarbonate content at low flow rates, resulting in a reduction in salivary pH. The salivary glands undergo hypofunction as a consequence of hormonal changes and physiological ageing, which leads to a reduction in saliva volume and pH in menopausal women [19].

Oxidative stress is characterized by an imbalance between oxidants and antioxidants, with the oxidants having a greater influence. This imbalance can result in a disturbance of redox signaling and control, as well as molecular damage [20]. Postmenopausal women experience an elevation in molecular oxidation that coincides with the natural aging process [21, 22]. Excessive oxidative damage can lead to an increased likelihood of developing multiple chronic degenerative diseases, which can be further exacerbated during menopause, post-menopause, and the aging process due to heightened oxidative stress [3, 23]. Table 3 demonstrated that the mean level of TAC was higher in pre-menopause group when compared to post-menopause with significant differences. In the present study, TAC was significantly increased in post-menopause women, which could be explained as an antioxidant defense mechanism capable of neutralising excess oxidant stress markers.

Similarly V. J. Victorino et al., G. Sotoudeha and M. Abshirinib, A. Montoya-Estrada et al., observed an increase in TAC in postmenopausal compared with premenopausal women [24,3,25] but F. Zovari et al. [2] found that the mean saliva TAC in the post-mennopause was not significantly different compared with the mean saliva TAC in the pre-menopause. When all of these factors are considered, it is reasonable to draw the conclusion that menopausal women, particularly those who experience symptoms that are both frequent and severe, are more susceptible to oxidative stress and the damage that is associated with it. The mean level of MDA was higher in post-menopause group when compared to pre-menopause with significant differences Table 4.

The current findings revealed that postmenopausal women had greater saliva MDA levels than premenopausal women. According to similar studies Z. Fatemeh et al., and S. S. Saleh [2, 26]. Oxidative stress arises when the antioxidant system cannot adequately deal with the ROS and free radicals created by living organisms. Consequently, estrogens possess the ability to scavenge free radicals, which enables them to act as antioxidants and prevent the production of ROS or neutralise superfluous ROS [27].

Estrogen insufficiency following menopause results in heightened production of ROS and free radicals. These substances induce oxidative harm to biomolecules like lipids, proteins, and DNA, thereby disrupting regular physiology and metabolism. ROS is difficult to quantify directly due to its transient and unstable character. As a result, their ability to produce lipid peroxidation has been utilised as an indirect indicator. The end result of lipid peroxidation is malondialdehyde [28].

The results of the correlation between pH and salivary biomarkers are clearly shown in Table 5. Salivary TAC level with pH and salivary MDA with pH showed a significant correlation p=0.038, p=0.042 consequently but there is no significant correlation between MDA and TAC P=0.836 in pre- menopause group. On the other hand there is non-significant negative correlation between salivary TAC and pH (p=0.897), as well as salivary MDA with pH and salivary TAC also showed non-significant correlation (p=0.204), (p=0.734) consequently in post-menopausal group.

Based on the analysis of correlation salivary pH and salivary biomarkers in our study showed salivary TAC level with pH showed a significant correlation and salivary MDA with pH also showed significant correlation but there is no significant correlation between MDA and TAC in pre- menopause

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Table 5

Correlation between pH and salivary biomarkers

Groups		Total Antioxidant Capacity		Malondialdehyde	
		R	P	R	P
Pre-Menopause	pH	-0.329	0.038	-0.323	0.042
	Total Antioxidant Capacity			-0.034	0.836
Post-Menopause	pH	-0.021	0.897	-0.205	0.204
	Total Antioxidant Capacity			0.055	0.734

group. On the other hand there is non-significant negative correlation between salivary TAC and pH, as well as salivary MDA with pH and salivary TAC also showed non-significant correlation consequently in post-menopausal group.

There is no previous studies that analysis the correlation of salivary pH and salivary biomarkers. This may be explained due to changes oral cavity condition, salivary changes during menopause might have an immediate impact on a woman's oral health. Estrogen receptors are located in both the oral mucosa and the salivary glands. Both the oral cavity's microbiota and the hormone estrogen will be directly impacted by the decline in estrogen.

Estrogen insufficiency might impair the development process of the oral mucosal epithelium .The reduction in both saliva volume and pH resulting from menopause will have an impact on salivary function. Alterations in salivary function can result in harm to oral tissues and significantly affect one's quality of life [29]. Symptoms of dry mouth include a decrease in secretion and flow rate of saliva, as well as changes in the content of saliva [30].

Xerostomia commonly leads to gingival diseases characterized by easy bleeding and ulceration, namely in the form of menopausal gingivostomatitis. Menopause's effects on salivary volume and antibacterial content will also make caries more likely [31].

CONCLUSIONS

The current study compared salivary TAC and MDA between premenopausal and postmenopausal females. Premenopausal women had significantly higher levels of salivary TAC, whereas postmenopausal women had significantly higher levels of salivary MDA. According to the findings, estrogen deficiency leads to increased oxidative stress and impaired antioxidant defense following menopause.

This may result in possible oxidative damage to the cells, which could trigger the onset of a number of diseases, including depression, vasomotor disturbances, diabetes, hypertension, and cardiovascular disease.

Conflict of Interest. The authors report no conflict of interest.

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