Salivary tumor marker CA15-3 and selected elements in relation to oral health status among a group of breast cancer women

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ABSTRACT

Background: Breast cancer is the commonest type of malignancy worldwide and in Iraq. It is a serious disease that affects the general health and cause systemic changes that affect the physical and chemical properties of saliva leading to adverse effects on oral health. This study was conducted to assess the tumor marker CA15-3 and selected elements in saliva and their relation to oral health status among breast cancer patients compared to control group.

Materials and Methods: The total sample consisted of 60 women aged 35-45 years. 30 women were newly diagnosed with breast cancer before taking any treatment and surgery (study group) and 30 women without clinical signs and symptoms of breast cancer as a control group. Dental caries was recorded using DMFS index of WHO1987, and periodontal parameters which include plaque index (PII), calculus index (CaII), gingival index (GI), and Ramfjod index for the loss of periodontal attachment (CAL) were recorded. Stimulated salivary samples were collected and salivary flow rate, salivary CA15-3 and selected elements were determined.

Results: Caries experience (DMFS) was higher among the study group compared with the control group but the difference was statistically not significant. The mean values of plaque index, calculus index, gingival index and loss of attachment were higher among the study group than the control group with a highly significant difference($P<0.01$). The concentrations of the tumor marker CA15-3 in saliva of breast cancer patients were highly significantly higher than that of the controls. The salivary flow rate was observed to be lower among study group compared to control group with a highly significant difference ($P<0.01$). Higher concentrations of salivary phosphorus, copper and total proteins were recorded among study group compared to control with statistically highly significant differences concerning phosphorus and copper. While salivary calcium and zinc were lower among study group compared to control group with statistically highly significant difference concerning zinc

Conclusions: This study showed that the breast cancer patients had poor oral hygiene and higher rates of periodontal diseases and dental caries. In addition, the results of this study could support the concept that salivary concentrations of CA15–3 might serve to be used in the detection of breast cancer and/or the post-operative follow-up of patients under treatment for carcinoma of the breast.

Key words: Breast cancer, Oral health, CA15-3. (J Bagh Coll Dentistry 2015; 27(3):124-129).

INTRODUCTION

Breast cancer is the most common cancer affecting women in the world today that it has become a major health problem in the developed world. It is the leading cause of cancer related death for women aged between 35 and 55 years worldwide [1,2]. Most commonly originate from the inner lining of milkducts or the lobules that supply the ducts with milk. According to the latest Iraqi Cancer Registry, breast cancer account for approximately one third of the registered female cancers in Iraq, indicated that the breast cancer is the leading cancer site among females [3,4].

As breast cancer affects general health, it has also an effect on oral health, and the oral diseases were found to have an effect and role in initiating breast cancer as some studies found that dental caries and periodontal diseases have a role in carcinogenesis and may predict cancer risk [5-7].

Human saliva is an important biological fluid that plays a critical role in the maintenance of oral and dental health [8].

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Saliva through its physical properties like flow rate, and chemical composition that include inorganic and organic components affects oral health [9].

Salivary diagnostics have been developed to monitor oral diseases such as periodontal diseases and dental caries [10]. Saliva can also be used as a diagnostic fluid in medicine and it has long been recognized as a mirror of the body's health, therefore a large number of salivary biomarkers for different diseases including breast cancer was detected [11,12]. Carcinoma antigen CA15-3 (MUC1) is a glycoprotein which is found on the surface of cancer cells and sheds into the blood stream, and this tumor marker was found in the saliva of women diagnosed with breast cancer [13].

As there is no previous study that investigate the tumor marker CA15-3 and the salivary elements (Ca, P, Zn, Cu, total proteins) and their relation to oral health status among breast cancer patients so this study was conducted.

MATERIALS AND METHODS

The studied sample consisted of 30adult women with breast cancer aged 35-45 years. The age was recorded according to the last birthday

(WHO, 1997) [14]. They were patients attending the Breast clinic in Al-Kadhemyia teaching Hospital. All The patients were examined clinically and also by mammography, ultrasonography and biopsy taking (fine needle aspiration, excisional biopsy), and a physician approved the breast tumor. The sample include patients with stages II and III before taking any treatment .While the control group composed of 30 women didn't have any clinical signs of breast cancer matching in age with study group. Both study and control groups were married, having children, with history of breast feeding, non smokers, shouldn't take any medication or nutritional supplements and with no history of medical problem.

Caries experience was recorded using plane mouth mirror and dental explorer according toDecayed, Missing and Filled Surfaces index (DMFS)[15].Oral hygiene was assessed by using plaque index(PlI) of Silness and Loe [16] and calculus index (CalI) of Ramfjord [17], Gingival inflammationwas assessed by using the gingival index (GI) by Loe and Silness [18] and measurement for the loss of periodontal attachment (CAL) wasmade by using calibrated periodontal probe (William's probe) following the criteria of Ramfjord [17].

The collection of stimulated saliva from subjects was performed following instruction cited by Tenovuo and Largerlof [19]. Saliva was collected between 9.00 and 11.30 Am after the examination of dental plaqueby asking each individual to chew Arabic gums of uniform size for one minute then to remove all saliva by expectoration and this was continued for 10 minutes, then the salivary volume wasestimated by measuring cylinder and the rate ofsecretion was expressed as milliliter per minute (ml/min). Each salivary sample was centrifuged at 4000 r.p.m for 10 minutes then the clear supernatants were stored at (-20°C) in a deep freeze till the time of biochemical analysis.

Chemical analyses of the elements Ca, Cu and Zn ions were determined using air-acetylene atomic absorption spectrophotometer (Buck scientific, 210 VGP, USA) according to instrumental manufacturer's specification[20],while inorganic phosphorous and total proteins were determined colorimetrically by using readymade kits (Biomaghreb, Tunisia) for phosphorous and (SYRBIO) for total proteins. CA15-3 was determined using (CUSABIO, CSB-E04772h) ELISA kit and the assay determined by ELISA method.

Analysis of data was carried out using SPSS (version19). Statistical tests used were Student's t-test and Pearson's correlation coefficient. The confidence limit was accepted at 95%, $P< 0.05$ was regarded as statistically significant and $P< 0.01$ was regarded as highly significant.

RESULTS

Clinical examination showed that all subjects in both groups were affected by dental caries. Caries experience (mean and standard deviation) among breast cancer and control groups are shown in Table (1). Results revealed that caries experience represented by DMFS and decayed surfaces was higher among breast cancer group compared to control group, but the difference was statistically not significant ($P>0.05$). Results also showed higher mean values of plaque index, calculus index, gingival index and clinical attachment loss among the study group than the control group with statistically highly significant difference (Table 2).

The concentrations of the tumor marker CA15-3 in saliva of breast cancer patients were highly significantly higher than that of the controlsas shown in Table (3). The correlations between salivary CA15-3 and dental caries and periodontal diseases were weak and statistically not significant as shown in Table (4).

The salivary flow rate was lower among breast cancer group than control group with highly significant difference between them.The concentrations of salivary phosphorus, copper and total proteins were higher among study group compared to control with statistically highly significant differences concerning phosphorus and copper, while salivary calcium and zinc were lower among study group compared to control group with statistically highly significant difference concerning zinc, these results are shown in Table (5).

Table (6) illustrates the Pearson's correlation coefficientbetweensalivary variables and caries experience.Analysis among breast cancer group revealed weak negative not significant correlation between salivary flow rate and DS, while the relation was weak positive with DMFS. In control group the correlation was weak negative not significant for DMFS but was significant for DS. Also the results showed that all the correlations between the caries experience and salivary constituents in the study and control groups were weak and not significant except the correlation with total proteins in the control group was significant positive.

Table 1: Dental caries experience (mean± S.D.) among study and control groups

Variable	Study group		Control group		Statistical test	
	Mean	± SD	Mean	± SD	t-test	P-value
DS	8.80	8.27	6.57	4.65	1.29	0.20
DMFS	34.03	14.12	28.37	12.84	1.62	0.11

Table 2: Oral hygiene and periodontal indices among study and control groups

Variable	Study group		Control group		Statistical test	
	Mean	± SD	Mean	± SD	t-test	P-value
PI	1.72	0.21	1.24	0.17	9.75	0.00**
CalI	0.84	0.30	0.52	0.17	5.21	0.00**
GI	1.27	0.19	0.89	0.20	7.88	0.00**
CAL	1.37	0.67	0.30	0.21	8.24	0.00**

Table 3: Salivary CA15-3 (U/ml) among study and control groups

Variable	Study group		Control group		Statistical test	
	Mean	±SD	Mean	±SD	t-value	P-value
CA15-3	8.84	1.70	3.37	1.05	14.99	0.00**

Table 4: Correlation coefficients of salivary CA15-3 with caries experience, gingival index and loss of attachment among study and control groups

Oral variables		Study group			Control group		
		CA15-3			CA15-3		
		r	P	Sig.	r	P	Sig.
caries experience	DS	0.19	0.32	NS.	-0.19	0.32	NS.
	DMFS	-0.05	0.79	NS.	-0.33	0.08	NS.
GI		0.01	0.96	NS.	-0.06	0.77	NS.
CAL		0.12	0.53	NS.	-0.05	0.82	NS.

Table 5: Salivary variables among study and control groups

Salivary variables	Study Group		Control Group		Statistical test	
	Mean	± SD	Mean	± SD	t-test	P-value
Salivary flow rate	0.75	0.27	1.25	0.29	-7.11	0.00**
Ca(mg/dl)	1.89	1.04	2.34	0.89	-1.77	0.08
P (mg/dl)	9.70	2.84	7.72	2.00	3.13	0.00**
Zn(µg/dl)	2.61	0.79	4.72	0.93	-9.45	0.00**
Cu(µg/dl)	4.55	0.84	2.59	0.30	12.03	0.00**
TP(mg/dl)	83.07	10.90	79.38	15.95	1.05	0.30

Table 6: Correlation coefficients of salivary variableswith dental caries

Variable	Study group				Control group			
	DS		DMFS		DS		DMFS	
	r	P	r	P	r	P	r	P
Salivary flow rate	-0.11	0.56	0.15	0.42	-0.36	0.05*	-0.31	0.1
Ca	-0.08	0.69	-0.02	0.91	-0.06	0.07	-0.06	0.75
P	-0.17	0.37	-0.26	0.16	0.06	0.76	-0.02	0.92
Zn	-0.1	0.59	0.35	0.06	0.23	0.22	0.33	0.08
Cu	-0.24	0.19	-0.13	0.48	0.13	0.49	0.09	0.64
TP	0.14	0.45	0.23	0.21	0.39	0.03*	0.41	0.03*

DISCUSSION

Breast cancer affects general health like other cancers in any part of the body [21], as well as it affects oral health [5,22].

Saliva as a diagnostic fluid offers some distinct advantages over serum in diagnosing diseases [23,24]. From a logistical perspective, the collection of saliva is safe, noninvasive, and simple, and it may be collected repeatedly without

discomfort to the patient,because of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses, such as cancer, is of great interest for most salivary researchers. The tumor marker CA15-3 (MUC1) is a high-molecular-mass glycoprotein, that expressed at the luminal surface of most secretary epithelial [25], it is the best and the most extensively used tumor marker in breast cancer as its expression greatly increases in most breast carcinomas [26], the data of the present study showed that the level of the tumor marker CA15-3 was higher among breast cancer group with a highly significant difference, this result agreed with that reported by Streckfus [27] in stimulated whole saliva and also Agha-Hosseini [13]found the same result in unstimulated saliva. The correlations between CA15-3 and DMFS, GI and CAL were weak not significant and may indicate that CA15-3 has no effect on oral health, but further studies are required concerning the effect of CA15-3 on dental caries and periodontal diseases.

Data of the present study showed that caries experience represented by DMFS and Ds components among breast cancer group was higher than that of control group but the difference was statistically not significant, this result agreed with that reported by Kanan [22] and Tojal et al. [5], the increased caries experience among breast cancer group could be attributed to the reduction in the salivary flow rate as the flow rate in this study was lower among breast cancer group with a highly significant difference and the correlation with DS in the breast cancer group wasinverse not significant,as the salivary flow rate play an important role in relation to dental caries because of the washing action of saliva as well as its protective constituents that increased with increasing flow rate [8,28]. The other important factor that may affect caries experience in saliva is its constituents [29]. In the present study, although statistically not significant, inverse correlations were recorded between caries-experience (DMFS) and salivary elements (Ca and P) in both groups, this could indicate the importance of calcium and phosphorus as their presence in saliva may greatly affect remineralization and increase resistance of outer enamel surface to acid dissolution [9,29].

Measurement of salivary zinc showed alower mean value recorded in study group than control group with statistically highly significant difference,This reduction may be due to the reduction of zinc levels in serum of patients with breast cancer [30,31]. In additionnon-significant negative weak correlation between salivary zinc and DS in study group was recorded, these findings can explain the higher caries experience among the study group because it was found that zinc have a role in tooth mineralization, and accumulation of zinc quantities on enamel surface made teeth more caries resistance [32]. Increased susceptibility to dental caries in zinc-deficient animals might be mediated by alterations in salivary proteins that are associated with the maintenance of tooth structure [33]. While for copper ions in saliva the results of the present study showed higher concentration level in breast cancer group,this result may be attributed to the high levels of copper in serum of breast cancer patients [30,31],beside that current study showed non-significant inverse weak correlation between copper and DMFS and DS fraction, this inverse relation can be attributed to copper's ability to inhibit bacterial growth because the divalent metal ions properly inhibit glycolysis in dental biofilm and its antibiofilm effect related to antimicrobial activity and displacement of Ca ions from pellicle and microbial surface and change of microorganism adherence [34], and also its ability to directly inhibit acid dissolution of enamel [35].

Total protein in the current study showed a higher level in breast cancer group than control group with no significant difference between them, a not significant positive correlation was found between total protein and DMFS/DS in breast cancer group, and a significant positive relation was found in control group. These results can be attributed to the fact that some salivary proteins are essential source of nutrient for bacteria and encourage bacterial aggregation [36], this may involve cariogenic bacteria as well, which in turn may increase the risk to dental caries.

Another explanation for the increased caries experience among breast cancer group is the poor oral hygiene as reported in the present study by highly significantly higher plaque and calculus accumulation among breast cancer group, as dental plaque was found to be the primary etiological factor in dental caries pathogenesis [8,37].

In the present study the results showed that the study group has a higher mean value of gingival inflammation and clinical attachment loss than control group with a highly significant difference between them this could be attributed to the poor oral hygiene as indicated by the higher plaque and calculus accumulation among the study group than control group with a highly significant difference between them, these results agreed with those reported by Kanan [38]. Since

poor oral hygiene plays an important role in the etiology and progression of periodontal disease [39,40]. Saliva may affect periodontal diseases through its physiochemical properties [24], this can be explained by that the salivary flow rate may play an important role in relation to plaque accumulation since decrease of salivary flow rate lead to decrease of washing action of saliva as well as the protective constituents decreased with decreased flow rate [41].

The increased gingival inflammation of breast cancer group in the present study may be also attributed to the fact that alteration in estrogen and progesterone hormones level due to breast cancer [42,43] may affect the gingival tissues, as these tissues respond to increased levels of estrogen and progesterone by undergoing vasodilatation and increased capillary permeability also there is an increased migration of fluid and white blood cells out of blood vessels. The other effect of increase progesterone levels are alterations in the existing microbial populations, howeverthe levels of Gram-negative anaerobic bacteria increase as a result of the high concentration of hormones available as a nutrient for growth [43,44].

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الخلاصة

خلفية الموضوع :سرطان الثدي هو من أكثر الأورام السرطانيةشيوعاً لدى النساء في جميع أنحاء العالم وفي العراق. و يعتبر من الامراض الخطيرة التي تؤثر على الصحة العامة اضافة الى تاثيره على صحة الفم وخاصة على خصائص اللعاب الفيزيائية والكيميائية والتي تؤثر سلبا على صحة الفم. وقد اجريت هذه الدراسة لتقييم المؤشر الورمي CA15-3 وعناصر مختارة في اللعاب وعلاقتهم بصحة الفم والاسنان ضمن مجموعه من النساء المصابات بسرطان الثدي ومقارنتهم مع المجموعة الضابطة.

المواد والطرق : شملت الدراسة مجموعة من النساء وعددهن٦٠ باعمار تتراوح بين ٣٥- ٤٥ سنة من المراجعات لمستشفى الكاظمية التعليمي,٣٠امرأة (المجموعة تحت الدراسة) شخصت حديثا اصابتهن بسرطان الثدي قبل البدء بالعلاج و ٣٠ امرأة اخرى(المجموعة الضابطة) تشتمل على نفس المواصفات ولكن ليس لديهم اي علامات او اعراض سريرية على الاصابة بسرطان الثدي.وكان التشخيص و حساب شدة تسوس الاسنان من خلال مقياس (تسوس، قلع، حشوة) (DMFS) حسب منظمة الصحة العالمية للعام (١٩٨٧)، وقد تم استخدام مقياس الصفيحة الجرثومية، التهاب اللثة، والقلح على الاسنان تبعا لتصنيف سلنس و لو (١٩٦٤)، ولو وسلنس (١٩٦٣)، ورامفورد (١٩٥٩) على التتابع وبالنسبة لمعدل تلف الأنسجة الرابطة فقد تم تحديده وفقا لمقياس رامفورد (١٩٥٩). تم جمع عينات من اللعاب المحفز حيث سجل مستوى جريان اللعاب ، ثم تم تحليل العينات كيميائيا لتحديد التراكيز لكل من:- المؤشر الورميCA15-3, الكالسيوم، الفسفور، الخارصين، النحاس، إضافة إلى البروتين الكلي.

النتائج : أظهرت النتائج أن متوسط قيمة تسوس الأسنان (DMFS) كان أعلى بين المجموعة تحت الدراسة من المجموعة الضابطة مع عدم وجود فرق معنوي بين المجموعتين. كما وجد ان القيمة المتوسطة للصفيحة الجرثومية, القلح, التهاب اللثة, تلف الأنسجة الرابطة أعلى بين مجموعة الدراسة مقارنة بالمجموعة الضابطة مع وجود فرق معنوي عال= إحصائيا. واظهرت النتائج ان مستوى المؤشر الورمي CA15-3 في لعاب النساء المصابات بسرطان الثدي كان اعلى من النساء في المجموعة الضابطة مع وجود فرق معنوي عال= احصائيا. وقد لوحظ انمعدل جريان اللعاب كان اقل بين مجموعة الدراسة مقارنة بالمجموعة الضابطة مع وجود فرق معنوي عال= احصائيا.وقد سجلت الدراسه تراكيز عالية لكل من الفسفور, النحاس و البروتين الكلي في لعاب النساء في المجموعة تحت الدراسة مقارنة بالمجموعة الضابطة مع وجود فرق معنوي عال= احصائيا بالنسبه للفسفور والنحاس. بينما كانت تراكيز كل من الخارصين والكالسيوم اقل في المجموعة تحت الدراسة مقارنة بالمجموعة الضابطة مع وجود فرق معنوي عال= بالنسبة للخارصين.

الاستنتاجات:اظهرت الدراسة ان النساء المصابات بسرطان الثدي لديهن نظافة فم واطئه و معدلات حدوث امراض لثة وتسوس اسنان عالية. ولقد اضافت نتائج هذه الدراسة دعما لمفهوم امكانية استخدام المؤشر الورمي CA15-3 الموجود في اللعاب للكشف عن مرض سرطان الثدي او متابعة المرضى اللاتي يتم علاجهن من سرطان الثدي بعد العمليه.