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Relation Between Salivary (Immunoglobulin A, Flow Rate and PH) level and Estrogen Receptor Expression Among Patients with Breast Cancer

Esra Hassan Abd-Ali Al-Hashemi *, Maha Jamal Abbas *, Haraa Khairi Al-Hadithi.**

**** Department of Basic Science, College of Dentistry, University of Mustansiriyah***
***** prevention Department, College of Dentistry, University of Mustansiriyah***
esrahassan2007@uomustansiriyah.edu.iq
maha_jamal2006@yahoo.com
haraakhairi@yahoo.com

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Abstract:

Estrogen receptors (ERs) are a group of proteins, either of nuclear or membrane type, that activated by estrogen hormone which expressed in majority of breast cancer patients. Breast cancer is the most frequent cancer among the women and account about one third of the registered female malignancies. The relation between salivary immunoglobulin A (SIgA), Salivary Flow Rate (SFR) and PH of saliva with sex hormone receptors expression yet not well understood where there were few researches that highlights the nature of relation. The reduction in the SIgA and SFR often makes oral tissue more prone for infection. The objectives of this study are to determine the relation between the SIgA, SFR, PH, and sex hormone receptor expression in patients with breast cancer.

study of cross sectional design was conducted from Jan. to Nov. 2016, samples of 3-5 ml of saliva collected from forty-five (45) female patients with breast cancer at Oncology Teaching Hospital. The patients were categorized according to estrogen receptor status expression depending on their medical records (26 positive and 19 negative expression). The sociodemographic characteristics and gynecological history including the age, Body Mass Index(BMI), menopause status also reported. The SIgA, measured by Enzyme Linked Immunosorbent Assay technique (ELISA), the SFR calculated by dividing the total collected saliva volume in milliliter by time in minute. The salivary PH measured by digital PH meter. Then the measured variables compared according to expression of estrogen receptor either positive or negative .The statistical analysis carried out by using independent student T- test and pearson correlation test were used to confirmed significant($p \leq 0.05$) was considered significant The results showed that the level of SIgA, SFR and salivary PH for group of ER+ ve expression were significantly lower than that of ER –ve expression where it was for ER+ve (136.8 , 155.7 and 5.8) while for ER-ve was (304.5, 248.5 and 6.1) respectively.also,the results revealed that a positive correlation between SIgA and SFR (r=0.7) while a negative correlation between the SIgA and PH (r = -0.3) . We could conclude that the patients with breast cancer of ER+ve expression had low level of SIgA also had reduction in SFR which make them more prone for oral infection

Key words: Estrogen receptor, Breast cancer, Salivary IgA

العلاقة بين المستوى اللعابي ل (كلوبيولين المناعي أ، مستوى تدفق اللعاب ، درجة الحموضة) مع مستقبلات هرمونات الاستروجين في مرضى سرطان الثدي

اسراء حسن عبد علي الهاشمي* مها جمال عباس**حراء خيري الحديثي**
****فرع العلوم الاساسية كلية طب الاسنان ، الجامعة المستنصرية***
**** فرع الاطفال والتقويم والوقائي / كلية طب الاسنان / الجامعة المستنصرية**

الخلاصة:

مستقبلات الاستروجين هي مجموعة من البروتينات اما من النوع النووي او الغشائي والتي يتم تنشيطها عن طريق هرمون الاستروجين وتظهر في غالبية المصابين بسرطان الثدي . سرطان الثدي هو السرطان الأكثر شيوعا بين النساء ويمثل

حوالي ثلث الأورام الخبيثة المسجلة للإناث وان العلاقة بين مستوى الكلوبيولين اللعاب المناعي نوع ا ، معدل و تدفق اللعاب و درجة حامضية اللعاب مع مستقبلات الهرمونات الجنسية (الاستروجين) غير مفهومة جيدا حيث لم يكن هناك الكثير من البحوث التي تسلط الضوء على هذه العلاقة .وغاليا يؤدي الانخفاض في مستوى الكلوبيولين اللعابي المناعي أ وقلة تدفق اللعاب يجعل الانسجة الفموية اكثر عرضة للاصابة اهداف هذه الدراسة هي تحديد العلاقة بين مستوى الكلوبيولين المناعي أ ، مستوى التدفق اللعابي ودرجة حموضة اللعاب مع مستقبلات الهرمون الجنسي (استروجين) لدى مرضى سرطان الثدي.

اجريت الدراسة بتصميم الدراسة العرضية للفترة من شهر كانون الثاني ولغاية شهر تشرين الثاني (2016) ،(5-3) مل من اللعاب تم جمعها من خمسة وأربعين (45) مريضة مصابة بسرطان الثدي في مستشفى الأورام التعليمي.و تم تقسيم المرضى باعتماد التعبير لمستقبلات هرمون الاستروجين التي تم توثيقها في سجلاتهم الطبية الى مجموعتين شملت الاولى مجموعة المرضى الايجابية التعبير لمستقبلات الاستروجين (26) مريضا و المجموعة الثانية شملت 19 مريضا بتعبير سلبي للاستروجين . وتم تسجيل معلومات المريض المتعلقة بالخصائص الاجتماعية والديموغرافية وتاريخ الأمراض النسائية والعمر ، مؤشر كتلة الجسم و تاريخ سن اليأس. اما المستوى اللعابي للاميونوكلوبيولين أ تم قياسه بواسطة تقنية فحص الامتصاص المناعي المرتبط بالإنزيم (ELISA) ، وتم حساب مستوى تدفق اللعاب عن طريق قسمة إجمالي حجم اللعاب المجمع في المليلتر في الدقيقة . وتم قياس درجة حموضة اللعاب بواسطة مقياس الحموضة الرقمي. ثم قارنت مستويات المتغيرات المقاسة وفقاً للتعبير عن مستقبلات هرمون الاستروجين سواء كانت موجبة أو سالبة و تم استخدام التحليل الإحصائي باستخدام اختبار T-test واختبار ارتباط Pearson واعتمد قيمة ($p \leq 0.05$) لتاكيد الاختلاف.

اظهرت النتائج أن مستوى الغلوبيولين المناعي أ و مستوى تدفق اللعاب ودرجة حموضة اللعاب في المجموعة الموجبة لمستقبلات الاستروجين (136.8، 0.7 ، 5.8) غلى التوالي كانت أقل من مستوياتها في المجموعة السالبة لمستقبلات الاستروجين وبفرق معنوي (304.5 و 1.4 ، 6.1) على التوالي.كذلك اكدت الدراسة بوجود علاقة طردية بين مستوى الاميمينوكلوبيولين أ ومستوى تدفق اللعاب بمعامل ارتباط7 .r=0 وعلاقة عكسية بين مستوى المينوكلوبيولين وحموضة اللعاب بمعامل ارتباط r= -0.3

نستنتج أن المرضى الذين يعانون من سرطان الثدي من مجموعة الموجبة لمستقبلات الاستروجين لديهم مستوى منخفض من امينوكلوبيولين أ اللعابي و قلة في مستوى تدفق اللعاب مما يجعلهام أكثر عرضة للامراض الفموية.

الكلمات الرئيسية: مستقبلات هرمون الاستروجين ، سرطان الثدي ، اميونوكلوبيولين أ

Introduction:

Breast cancer is common malignancy of females and second cause of death due to malignancy among females. The incidence of breast cancer differed all over the world, where many literatures linked this variation in incidence to cultural difference, so they suggest that etiological factors differed in their biological expression, thus they had an impact on disease outcome. [1].

The exact causative factor for cancer of breast are unknown, but the genetic and environmental factors are included. The mutations in genes; human epidermal growth factor receptor (HER_2), BRCA-1, BRCA-2 and P53 have been found associated to breast cancer [2]. The degeneration of extracellular matrix allowed to tumor cells to invade the local tissue and spreading to blood stream that result in development of secondary tumor in distant tissues [3]. The World Health Organization (WHO) documented that early detection of breast cancer by intensive screening programs with adequate therapy helps in minimizing the breast cancer mortality [4]. Status of hormone receptors, lymph node involvement, histological type and tumor size considered as prognostic factors in treatment of breast cancer [5, 6]. In 20%–30% of breast cancers. The HER_2/neu is overexpressed; therefore, it used as an indicator of patient relapse during the follow up period [7]. The substances that present in abnormal amount in blood, urine and tissue of some patient with malignancy usually used to predict the progression of tumor or relapse called tumor markers [8]. The estrogen receptor(ER), progesterone receptor (PR), HER_2, and proliferation marker Ki-67 used for several years to predict the prognosis of breast cancer and to guide its therapy [9].

The process of binding the antibody to antigen in biological tissue for localizing

the protein in the cells of the tissue section referred to as immunohistochemistry process [10]. It used in researches to determine the distribution and localization of specific biomarkers in the tissues [12].

The Immunohistochemical detection of ER, PR, HER-2 have prognostic and therapeutic role in breast cancer where the therapy individualized depending on the status of expression of these markers [11].

Saliva protects the oral tissues in many ways. Normal saliva flow and phosphate buffering system can maintain the ability of self-clearance and inhibition of a large number of acid-producing cariogenic bacteria from the oral cavity. An important saliva ingredient is a group of antibacterial proteins including immunoglobulin such as SIgA, immunoglobulin G (IgG), and immunoglobulin M (IgM)) and no immunoglobulins such as lysozyme, lactoferrin, lactoperoxidases, defensins, histatins, saliva peroxidase system, and lectin protein), which are closely related to local or systemic malfunction. These proteins play important roles not only in protecting the integrity of oral tissues, but also in providing clues for local and systemic diseases such as breast cancer (systemic inflammation) and oral cancers (local inflammation) [12]. Therefore, the use of saliva as a salivary marker has become a somewhat success story of translational research [13].

The saliva proteins can be affected by some physiological and pathological factors, such as psychological and hormonal status, ages, physical exercises, oral hygiene, drugs, and smoking [14]. Salivary SIgA is the primary means of measuring the "first line of defense" at the oral mucosal surface. It serves as an effector in mucosal immunity by suppression of submucosal invasion. Previous literatures have suggested an association between the levels of SIgA and risk of infection [15].

The reduction in salivary flow rate result in burning of mouth, dry mouth in addition to taste disturbance as well as the quality of saliva shows a shift towards a more aciodogenic microflora [16].

Aim of study

To assess the SIgA, SFR and pH level and studying their relation to breast tumor hormone receptors expression

Material and methods

Cross sectional study conducted between Jan to Nov. 2016 at Oncology Teaching Hospital-Medical city, Baghdad. Iraq. The sociodemographic characteristics and medical history data gathered by direct interview with the patients that includes; age, history of contraceptive pills, family history of breast cancer, body mass index(BMI). The Forty-five (45) patients with breast cancer that included in this study were divided to two groups depending on status of expression of estrogen receptor as it was reported in their medical records. The first group) ER+ve) was consist of 26 patients while the second group (ER-ve) was consist of 19 patients .

The saliva collected by having the subjects spit for some minutes into a sterile plastic cup, the volume of the collected saliva measured in milliliters, and the calculation of the salivary flow rate based on a collection time (milliliter per minutes). The samples centrifuged and analyzed for the assessment of the level of SIgA using enzyme linked immunesorbent assay (ELISA) according to the manufacture protocol by using kit supplied by immunotech- Company-France.

Estimation of SIgA

1- Standard constituted to 1000 pg. / ml with standard dilution buffer. From original standard a serial diluent of the standard was prepared
2- One hundred ml of sample was added per well, then 100 ml of diluent were added for each well. The plate incubated for 4 hours, then the content of well discarded and washed by washing solution.

3- Conjugate reconstituted (200 ml) added into each well then incubated at 25 C° and washed three times.
4- Substrate (200 ml) added into each well, followed by incubation for 15 minutes at 25 C° with continuous shaking in the dark.
5- Stopping solution H_2SO_4, (50 ML) added into each well and mixed gently.
6- Absorbance measured by spectrophotometer at 450 nm within two hours.

The flow rate of saliva (SFR) ml/min was estimated by dividing the total collected saliva volume (ml)by collecting time (min) that was measured by sample collection: SFR ml/min = saliva sample volume (ml)/ collection time (min). Salivary pH measured with a digital pH-meter (Hanna Instruments, USA) 30 to 60 minutes after saliva samples collected, and pH considered as a quantative variable.

Statistical analysis: SPSS version 23 used for data entry and analysis, mean and standers deviation (SD) were used to represent the numerical data. Independent student T- Test and Pearson correlation used to confirm significance. The level of significance was set at ≤0.05.

Results

The mean age of (45) patients included in this study was 52± 9.4SD year, 37.8% of patients was in age group of (41-50) years old, 88.9% was in post menopause status, 57.8% had had no history of contraceptive, 71.1% with family history of breast cancer, 62.2% with no history of breast-feeding and 48.9% with BMI (25-30) as displaced in table 1.

Table-1: Discrptive statistic of studied group.

Study variable		No.	%
Age category/years	20-30	4	8.9%
	31-40	4	8.9%
	41-50	17	37.8%
	51-60	14	31.1%
	>60	6	13.3%
Menopause statues	Pre-menopause	5	11.1%
	Post-menopause	40	88.9%
History of contraception	Yes	19	42.2%
	No	26	57.8%
Family history	Yes	32	71.1%
	No	13	28.9%
Breast feeding	Yes	17	37.8%
	No	28	62.2%
Past medical history	No- comorbid illness	28	62.2%
	DM	8	17.8%
	HT	9	20.0%
BMI	BMI less 18	0	0.0%
	BMI (18-25)	16	35.6%
	BMI (25-30)	22	48.9%

	BMI MORE 30	7	15.6%

The finding of current study demonstrated there was statistical significant difference in mean value of SIgA between ER +ve and ER-ve patients, where ER+ve patients reported low level of SIgA (136.8) incomparison to ER-ve patients (304.5) as seen in table.2

Table.2- The mean value of SalivaryIgA of studied group

	Study groups	No.	Mean	Std. Deviation	P-value
SIgA	ER+ve	26	136.8	40.1	0.01
	ER-ve	19	304.5	156.4	

The finding of current study demonstrated a significant statistical difference in mean value of salivary flow rate between ER+ve and ER-ve patient groups, where ER+ve patients reported low level of SFR (0.7) ml/min. in comparison to ER-ve patients (1.4) *ml*/min. as seen in table.3.

Table.3- The mean value of SFR of studied groups.

	Study groups	No.	Mean	Std. Deviation	P-value
SFR	ER+ve	26	0.7	0.04	0.001
	ER-ve	19	1.4	0.07	

Our data indicated that the mean value of PH was a significantly differed between ER+ve and ER-ve patients, where ER+ve patients reported low level of PH (5.8) in comparison to ER-ve patients (6.1) as seen in table.4.

Table-4: The mean value of PH of studied groups.

	Study groups	No.	Mean	Std. Deviation	P-value
PH	ER+ve	26	5.8	0.2	0.01
	ER-ve	19	6.1	0.3	

The finding of current study showed there was a significant positive direct correlation between SIgA and SFR (r =0.7, p =0.001), and negative correlation between SIgA and PH (r = -0.3, p=0.04) and SFR and PH (r = - 0.4, p=0.001) as seen in table.5.

Table-5: The correlation between parameters under study.

		SFR	SIgA	PH
SFR	**R**	1	0.7	-0.4
	p-value		0.001	0.001
SIgA	**R**	0.7	1	-0.3
	p-value	0.001		0.04
PH	**R**	0.4	0.3	1
	p-value	0.001	0.04	

R= Person correlation

Discussion

The cancers of breast classified into hormone receptor–positive and negative groups to guide patient treatment. The responsive breast cancers are ER and/or PR positive [17]. A number of literatures proposed that oral tissues are sensitive to changes in female sex steroid hormones. Some disorders of the oral cavity show a predilection for females and samples from these lesions appear to be ER-positive, supporting a role for estrogen in disease etiology and such finding of these studies in line with our finding of low level of protective factor (SIgA) in ER+ve breast cancer patient. [18]

The decrease in estrogen levels during menopause thought to affect the oral epithelial maturation process, leading to thin, atrophic epithelium prone to inflammatory changes rather than infection due to impairment of local protective factors such as SIgA in addition to decreased salivary flow rate in senior people in general due to aging process. Secretory immunoglobulin A (SIgA) is the dominant immunoglobulin in external secretions that bathe mucosal surfaces (respiratory, intestinal, and reproductive), where it acts as a key component of the immune system's "first line of defense" against microbial invasion [19]. The decreased SIgA secretion of oral surfaces surly will increase the incidence of oral infection, then the ER+ve patients with breast cancer possibly more liable for oral infection than negative one where the level SIgA was low according to our finding. The exact mechanism of association salivary IgA level and expression of estrogen receptor yet not clearly identified. The authors [20,21] satiated that the important saliva proteins play important roles not only in protecting the integrity of oral tissues, but also in providing clues for local and systemic diseases, such as breast cancer (systemic inflammation). However, the saliva proteins can affected by some physiological and pathological factors, such as psychological and hormonal status, ages, physical exercises, oral hygiene, drugs, and smoking [14]. A study conducted by Welinder and his co-workers demonstrates high frequency of IgA1 positive cells in primary breast tumors. IgA1 found to be present in both the cytoplasm and plasma membrane of 35 out of 36 individual breast cancer tumors [22].

Our data indicated that the SFR and PH of saliva were significantly lower in patients of ER+ve expression in comparison to ER –ve patients. Actually, we did not find a solid scientific base for explanation of this relation and more studies recommended for identifying the exact factor, which stand behind this association

Few studies [23, 24] reported an increase of overexpression of tumor markers c-erbB-2 (erb) and cancer antigen 15-3 (CA15-3) in the saliva of women with breast cancer when compared with patients who had benign lesions and healthy subjects.

We expect an association between expression of sex hormone in-patient with breast cancer and constituent of saliva and such possibility in need for further research to identify the specific relation.

Conclusion

The Salivary IgA, Salivary Flow Rate and PH were lower in ER+ expression breast cancer.

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