Periodontal Health Status And Assessment of Vitamin D Levels in The Serum of Women with Breast Cancer

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Abstract
Background:*Periodontal diseases are a group of inflammatory diseases that affect the periodontium of the tooth. It results from interactions between microorganisms and the host's immune response.Breast cancer is the malignant tumor of breast tissue that forms from uncontrolled growth of abnormal breast cells. Vitamin D exhibits physiological and pharmacological effects in human body; it is present in normal breast tissue and has antiproliferative effects on the differentiation of breast cancer cells.*
Aims of the study: *1.To determine the serum levels of Vitamin D in women with breast cancer having periodontal diseases (gingivitis and chronic periodontitis) in comparison to women without breast cancer.*
2. To correlate between serum levels of Vitamin D with clinical periodontal parameters (plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL)) in women with breast cancer in comparison to women without breast cancer.
Subjects, Materials and Methods:*80 participants, females only were recruited in this study with age ranged from (25-65) years old, they were divided into four groups: the first group (BC +CP) group which included participants with breast cancer and chronic periodontitis (n=20), the second group (BC+ G) group included participants with breast cancer and gingivitis (n=20), the third group (CP) group which included participants with chronic periodontitis only (n=20) and the fourth group (G) group which included participants with gingivitis only (n=20). Other than breast cancer, all subjects were systemically healthy females. Periodontal health status was determined by clinical periodontal examination of plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL). Blood samples were collected from each participant, serum levels of Vitamin D were determined by enzyme -linked immune-sorbent assay (ELISA).*
Results:*The results of this study showed that the median values of PLI and GI were slightly increased in BC+G group in comparison to other groups with non-significant differences.Thepercentages of BOP showed highly significant differences among the groups at P <0.01. The median values of PPD showed slight increase in BC+CP group than CP group with non-significant differences. Median values of CAL were increased in CP group than BC+CP group with non-significant differences.Vitamin D serum levels showed highly significant differences among the groups at P <0.01, the G group had the higher median of Vitamin D level among the groups. Also by using Spearman's rank Correlation Coefficient, serum levels of Vitamin D were correlated negatively with all clinical periodontal parameters.*
Conclusion: *The present study provides evidence of association between periodontal diseases and breast cancer. Vitamin D serum levels could be used as biomarker for diagnosis and prediction of progression of both periodontal diseases and breast cancer.*
Keywords: *periodontal diseases, breast cancer, Vitamin D.*

I. Introduction

Periodontal diseases are inflammatory diseases that affect the periodontium of the tooth [1].This inflammation is affected by the innate and adaptive immune response of the host to the plaque biofilm that contain many microorganisms [2].The presence of these virulent microorganisms is responsible for the initiation and progression of periodontal diseases, host response to pathogenic infection is also very important in disease progression [3]. Periodontal diseases are classified into gingivitis and periodontitis. Gingivitis can be defined as the inflammation of the gingiva; it is reversible with good oral hygiene practice by the patient [4]. While periodontitis is the more destructive form of periodontal diseases in which the inflammation extends to the alveolar bone that support the teeth and results in periodontal pocket formation, periodontium destruction, attachment loss,

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Alveolar bone resorption, and these symptoms will lead to tooth mobility and finally tooth loss [5]. Cancer also known as a malignanttumor or malignant neoplasm, is a group of many diseases can be defined as abnormal cell growth in one part of the body with the possibility of metastasis to other parts of the body [6,7].

Breast cancer is the malignant tumor of breast tissue that forms from abnormal growth of breast cells, it is the most common and spreading malignancy in women worldwide and it is the biggest threats of women health and the most lethal cancer in women [8]. The normal breast is arranged in three layers which are epithelium, connective and fat tissues, so breast tumors are the tumors of these tissues and could be benign or malignant. Most of the malignant breast tumors originate from the epithelial tissue of the breast [9].Recently periodontal diseases are associated with increased prevalence of breast cancer [10]. There are many possible explanations for the link between periodontal disease and breast cancer. First possibility is that systemic inflammation with periodontal disease may affect the breast tissues. Second possible explanation is that bacteria from the oral cavity can enter the circulatory system and affect breast tissues [11]. Vitamin D is steroid hormone .It is essential for consumption of calcium from the intestines and re-absorption of calcium from kidneys and bone formation to maintain adequate calcium concentrations for calcium homeostasis, bone mineralization and immunity. It is potentially influencing risk of periodontal diseases through three mechanisms: maintenance of oral bone health, anti-inflammatory activity and anti-microbial activity [12, 13]. There is a strong association between low Vitamin D serum levels and an increased risk of breast cancer and it is believed that Vitamin D decreases fatality rates for patients with breast cancer. Vitamin D metabolites increase communication between cells by activating a protein that avoids aggressive cell division. As long as Vitamin D receptors are present, tumor growth is prevented and pause expanding its blood supply .Vitamin D receptors are not lost until a tumor is very advanced. These are the reasons for better survival in patients whose vitamin D blood levels are high. So, 25-hydroxy Vitamin D could be a biomarker for severity of cancer, rather than a factor that causes longer survival [14].

II.   Materials And Methods

The subjects who participated in this present study were consisted of (80) females with the age range from (25 to 65) years old. The participants recruited for the study were attendants of Oncology teaching hospital / Medical city, Baghdad, also attendants to the Iraqi blood bank / Medical city, Baghdad for blood donation. Samples collection was started from October 2015 to January 2016. All participants were informed about the aims of the study by a written informed consent and it was assigned by all participants.
The participants were divided into four groups:
1. First group (BC+CP); consisted of twenty patients with newly diagnosed breast cancer and chronic periodontitis, patients with chronic periodontitis should have at least 4 sites with periodontal pockets with depth of ≥4 mm with clinical attachment loss (CAL) of 1-2 mm or greater [15].
2. Second group (BC+G); consisted of twenty patients with newly diagnosed breast cancer and gingivitis.
3. Third group (CP); consisted of twenty patients with chronic periodontitis only with no history of any systemic diseases.
4. Fourth group (G); consisted of twenty patients with gingivitis only with no history of any systemic diseases.
Samples of 3 mls of blood were collected from each patient, and then full examination of clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) was done by;
1. Assessment of soft deposits by Plaque index by (Sillness and Löe, 1964)[16].
2. Assessment of Gingival Inflammation by Gingival index by (Löe and Sillness, 1963), (Löe, 1967)[17, 18].
3. Assessment of Bleeding on probing according to(Newbrun, 1996) [19].
4. Assessment of Probing Pocket Depth by (Langet al, 1999) [15].
5. Assessment of Clinical attachment level. then the collected blood samples were placed in serum separating gel tubes and allow samples to clot for 30 minutes then perform centrifugation for 15 minutes at 1000 x g (3500 Rpm) then serum samples were separated and placed in plain tubes and stored at deep freezer (-64 °C) in plastic containers for later analysis by Enzyme Linked Immunosorbent Assay (ELISA) kit for quantitative determination of serum levels of Vitamin D. The laboratory tests were done in the Teaching Laboratories of Baghdad Medical City. Samples storing was done in the Iraqi blood bank / Medical city.

Statistical analysis was done using mean, median, Min, Max, SD, SE, percentages, Kruskal-Wallis H test, Mann-Whitney U test, Chi-square test, Shapiro-Wilk test, Spearman's rank correlation coefficient test (r) and scatter plots.

III.   Results

The present study showed that the median values of PLI and GI were elevated in BC+G group in comparison to other groups with non-significant differences among the groups, the median values of PLI for BC+CP, BC+G, CP and G groups were (1.27, 1.41, 1.19, 1.27) respectively and The median values of GI for the BC+CP, BC+G, CP and G groups were (1.25, 1.45, 1.23, 1.32) respectively (Table 1). The percentages of non-bleeding and bleeding sites in the group BC+CP were (71.08% and 28.92%), in group BC+G were (52.61% and 47.39%) ,in group CP were (66.88% and 33.12%) ,and in group G were (59.20% and 40.80%) respectively. By using Chi-square test, the results of BOP revealed highly significant differences among the groups (Table 2).The

intergroup comparison of BOP parameter among the groups was showed in (Table 3). The median value for PPD of group BC+CP was (4.51), it was slightly higher than group CP which was (4.45) with non-significant differences between the groups. The median value of CAL for BC+CP group was (3.34) and for group CP was (5.29). The median value of CAL in CP group was higher than BC+CP group (Table 4). The median value of Vitamin D of the BC+CP, BC+G, CP and G groups were (8.38, 8.16, 8.53, 9.41) respectively. Group G showed the higher level of Vitamin D among the groups followed by group CP. There were highly significant differences found among the groups determined by using Kruskal-Wallis H test (Table 5). There were highly significant differences between BC+CP and G groups also there were highly significant differences between BC+G and G groups that were determined by using Mann-Whitney U test(Table 6). By using Spearman's rank Correlation Coefficient, serum levels of Vitamin D were correlated negatively with all clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) (Table 7). In figures (1, 2, 3, and 4) the scatter plots showed the inverse correlations between Vitamin D, PPD and CAL in BC+CP and CP groups.

Table 1: Analytic statistics of median values of PLI, GI parameters for the groups with comparison of significance

Groups	PLI				GI			
	Median	X^2	p-value	Sig.	Median	X^2	p-value	Sig.
BC+CP	1.27				1.25			
BC+G	1.41	4.276	0.233	NS	1.45	3.731	0.292	NS
CP	1.19				1.23			
G	1.27				1.32			

Table 2: The distribution of the numbers and percentages of sites according to the presence or absence of BOP with comparison of significance among the groups.

Groups		Score 0	Score 1	Total	X^2	d.f.	p-value	Sig.
BC+CP	No.	1288	524	1812				
	%	71.08	28.92	100	163.861	3	0.000	HS
BC+G	No.	1048	944	1992				
	%	52.61	47.39	100				
CP	No.	1240	614	1854				
	%	66.88	33.12	100				
G	No.	1236	852	2088				
	%	59.20	40.80	100				

Table 3: the intergroup comparison of BOP parameter among the groups.

Groups	X^2	d.f.	p-value	Sig
BC+CP vs. BC+G	136.61	1	0.000	HS
BC+CP vs. CP	7.549	1	0.006	HS
BC+CP vs. G	60.026	1	0.000	HS
BC+G vs. CP	81.162	1	0.000	HS
BC+G vs. G	17.938	1	0.000	HS
CP vs. G	24.842	1	0.000	HS

Table 4: Analytic statistics of median values of PPD and CAL parameters for BC+CP and CP groups with comparison of significance.

Groups	PPD				CAL			
	Median	X^2	p-value	Sig.	Median	X^2	p-value	Sig.
BC+CP	4.51	179	0.570	NS	3.34	150.5	0.180	NS
CP	4.45				5.29			

Table 5: Analytic statistics of median values of Vitamin D parameter for the groups with comparison of significance.

Groups	Vitamin D			
	Median	X^2	p-value	Sig.
BC+CP	8.38			
BC+G	8.16	19.987	0.000	HS
CP	8.53			
G	9.41			

Table 6: The intergroup comparison of Vitamin D parameter among groups.

Groups	BC+CP vs. BC+G	BC+CP vs. CP	BC+CP vs. G	BC+G vs. CP	BC+G vs. G	CP vs. G
Mann-Whitney U test	194.5	157.5	23.5	166	72	129.5
p-value	0.882	0.250	0.000	0.357	0.001	0.056

Sig.	NS	NS	HS	NS	HS	NS

Table 7: Spearman's Correlation Coefficient (r) between serum levels of Vitamin D and periodontal parameters for each group.

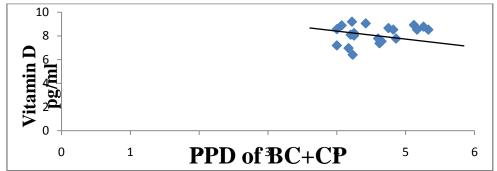| Periodontal parameters	Correlations	Vitamin D (BC+CP)	Vitamin D (BC+G)	Vitamin D (CP)	Vitamin D (G)
PI	r	-0.088	-0.016	-0.226	-0.484
	p-value	0.712	0.947	0.337	0.030
	Sig.	NS	NS	NS	S
GI	r	-0.051	-0.012	-0.199	-0.356
	p-value	0.830	0.958	0.399	0.123
	Sig.	NS	NS	NS	NS
BOP	r	-0.024	-0.043	-0.233	-0.335
	p-value	0.919	0.856	0.324	0.148
	Sig.	NS	NS	NS	NS
PPD	r	-0.206		-0.281	
	p-value	0.384		0.230	
	Sig.	NS		NS	
CAL	r	-0.021		-0.141	
	p-value	0.930		0.555	
	Sig.	NS		NS	

Figure 1: Scatter plot of the correlation between PPD and Vitamin D concentration in BC+CP group.
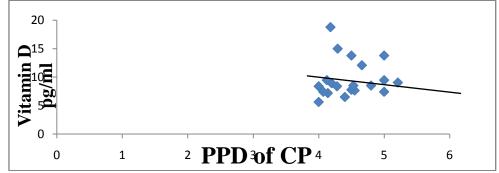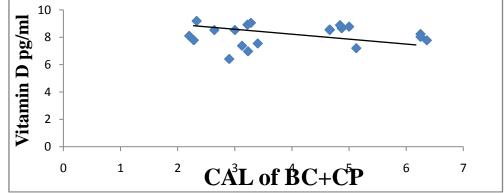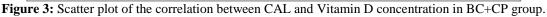

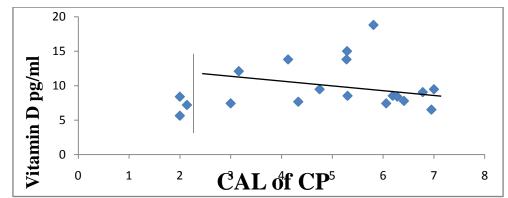**Figure 2:** Scatter plot of the correlation between PPD and Vitamin D concentration in CP group.

Figure 3: Scatter plot of the correlation between CAL and Vitamin D concentration in BC+CP group.

Figure 4: Scatter plot of the correlation between CAL and Vitamin D concentration in CP group.

IV.   Discussion

Periodontal diseases may be associated with breast cancer. This may be due to that periodontal inflammation may affect the breast tissues. Another possible explanation is that bacteria from the oral cavity can enter the circulatory system and also affect the breast tissues [20]. Both of these diseases are multi-factorial diseases and lead to release of many inflammatory mediators from the damaged tissues into saliva and blood. These mediators may be used for diagnosis and prediction of progression of both periodontal diseases and breast cancer. PLI, GI median values and BOP percentages were higher in BC+G group than other groups. Dental plaque is the major etiological factor in periodontal diseases. This bacterial aggregation leads to formation of microbial bio-film and bacterial invasion which result in destruction of the periodontium thus enhance plaque accumulation on the teeth surfaces [5]. Another possible explanation of such results that breast cancer patients neglected their oral hygiene follow up, this may be due to depressed state of mind which keeps them out from regular brushing of teeth and usage of cleaning aids. The median values of PPD were slightly higher in BC+ CP group than in CP group but the median values of CAL were higher in CP group than in BC+CP group. The development of chronic periodontitis is commonly preceded by several instances of gingivitis; PPD would tend to underestimate disease prevalence because gingival recession may reduce PPD. Second, gingival inflammation may be associated with fast progression in the PPD and PPD is an indicator of current illness status, CAL parameter would tend to overestimate periodontitis prevalence since attachment loss can be due to non-inflammatory causes and CAL is an indicator of cumulative tissue destruction, including past periodontal diseases [21]. The association between low levels of vitamin D and increased risk of breast cancer can be attributed to Vitamin D role in cancer prevention through functions as anti-proliferation, pro-differentiation and cell cycle stabilization. Vitamin D had been proven to have important anticancer, immune modulatory and innate immune effects.

It plays an essential role in oral homeostasis and its dysfunction or deficiency may lead to periodontal diseases [22]. Evidence suggested that vitamin D is involved in the development of breast cancer and there is an inverse association between vitamin D intake and breast cancer. Vitamin D is expressed in normal and malignant breast cells and has anticarcinogenic effects on mammary gland [23]. The results of the present study showed negative non-significant correlations between Vitamin D and the clinical periodontal parameters (PLI, GI, BOP, PPD and CAL) for the groups. The cause of non-significant results may be due to reduced number of subject's distribution in each group. The results of this study coincided with other previous studies that there were a decrease concentrations of Vitamin D with the presence of periodontitis and breast cancer.

In conclusion, Vitamin D can be used as a marker of periodontitis and breast cancer so it may contribute in identification of higher risk individuals as well as lead to new therapeutic approach.

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الحالة الصحية للثة وتحديد مستويات فيتامين (د) في مصل دم النساء المصابات بسرطان الثدي

الخلاصة

الخلفية:

أمراض اللثة هي الأمراض الالتهابية التي تؤثر على دواعم السن.ان هذه الالتهابات تنتج من التفاعل بين الكائنات الحية الدقيقة مع الاستجابة المناعية للمضيف. ان سرطان الثدي هو ورم سرطاني لأنسجة الثدي التي تتكون من النمو الغير مسيطر عليه لخلايا الثدي الغير طبيعة. ان فيتامين (د) يعرض خواص فسلجية وعلاجية في جسم الانسان ويتواجد بشكل طبيعي في انسجة الثدي. فيتامين د لديه تاثيرات ضد تكاثر وتمايز الخلايا السرطانية في الثدي.

أهداف الدراسة:

1. تحديد مستوى فيتامين (د)في مصل دم النساء المصابات بسرطان الثدي مع امراض اللثة (التهاب اللثة البسيط والتهاب دواعم السن المزمن) بالمقارنة مع نساء غير مصابات بسرطان الثدي.
2. الربط بين مستوى فيتامين (د)مع المؤشرات السريرية لتقييم الحالة الصحية لانسجه ماحول الاسنان (مؤشر الصفيحهالجرثوميه,مؤشر التهاب اللثه ,مؤشر النزف عند التسبير ,مؤشر عمق الجيوب واخيرا مؤشر فقدان الانسجه الرابطه) في النساء المصابات بسرطان الثدي بالمقارنة مع نساء غير مصابات بسرطان الثدي.

المرضى, المواد وطرق العمل:

(80) مشاركة من النساء فقط ادرجوا في هذه الدراسة ,تتراوح اعمارهن بين (25-65) سنة مقسمين الى اربعة مجاميع. المجموعه الاولى تضم مريضات مصابات بسرطان الثدي مع التهاب دواعم السن المزمن (عددهن 20). المجموعه الثانية تضم مريضات مصابات بسرطان الثدي مع التهاب اللثة (عددهن 20). المجموعة الثالثة تضم مريضات مصابات بالتهاب دواعم السن المزمن فقط (عددهن 20). والمجموعة الرابعة تضم مريضات مصابات بالتهاب اللثة فقط (عددهن 20). تم فحص الحاله الصحية لانسجة ماحول الاسنان سريريا بالمؤشرات التاليه:مؤشر الصفيحهالجرثوميه,مؤشر التهاب اللثه ,مؤشر النزف عند التسبير ,مؤشر عمق الجيوب واخيرا مؤشر فقدان الانسجه الرابطه. عينات الدم قد جمعت من كل مشاركة وتم تحديد مستوىفيتامين (د)بواسطه نظام مقايسة الانزيم المرتبط الممتز المناعي.

النتائج:

اظهرت نتائج هذه الدراسة ان القيم الوسطية للصفيحة الجرثومية و مؤشر التهاب اللثه كانت مرتفعة نسبيا في المجموعة الثانية بالمقارنة مع المجاميع الاخرى.كما اظهرت نتائج مؤشر النزف عند التسبير فروق ذات دلالة إحصائية عالية بين المجاميع. وجود ارتفاع بسيط في مؤشر عمق الجيوب في المجموعة الاولى عن المجموعة الثالثة. اظهر مؤشر فقدان الانسجه الرابطه ارتفاع في المجموعة الثالثة عن المجموعة الاولى. واظهرت نتائج فيتامين (د) عن وجود فروق ذات دلالة إحصائية عالية بين المجاميع وان المجموعة الرابعة كان لديها اعلى مستوى لفيتامين (د) بين المجاميع. وباستخدام معامل سبيرمان للارتباط تم تقييم العلاقات واظهرت النتائج وجود علاقات سلبية بين مستوى فيتامين (د)مع مؤشرات انسجه ماحول الاسنان السريرية.

الاستنتاج:

اظهرت نتائج هذه الدراسة وجود ارتباط بين امراض اللثة المزمنة وسرطان الثدي وان مستوياتفيتامين (د) في مصل الدم يمكن استخدامهاكدلالات بايولوجية لتشخيص والتنبؤ بتطور كل من امراض اللثة المزمنة و سرطان الثدي.

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