

## Early detection of Breast Cancer by Tumor Marker CA15.3, CA27.29 and relationship with P53 and Vitamin D

الكشف المبكر لسرطان الثدي بواسطة الدالات الورمية CA15.3, CA27.29 وعلاقتها مع بروتين P53 وفيتامين D

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### Abstract

Breast cancer is the second most frequent cancer of women. Tumor markers are substances that can be found in the body when cancer is present. The classic tumor marker is a protein that can be found in the blood in increased levels when the cancer is present. The present study have been investigated the levels of cancer antigen CA15.3, CA27.29, P53 and Vitamin D in women with breast cancer, aged were 30-75 years old, they divided into four groups, each group composed of 50 women included breast cancer patients aged 30-59 year, breast cancer patients aged 60-75 years, milking nodules groups 30-60 year and control groups. This study showed significant  $P < 0.05$  increasing in the level of CA15.3, CA27.29 and P53 concentration in milking nodules and both breast cancer groups, also showed significant  $P < 0.05$  decreasing in vitamin D concentration in milking group and breast cancer group and significant difference in serum Ca concentration.

Key words: Breast Cancer, CA15.3, CA27.29, P53, Vitamin D

### المستخلص

سرطان الثدي من الأمراض الشائعة عند النساء ، الدالات الورمية هي المواد التي تتواجد في الجسم عند حدوث السرطان وتعتبر مواد بروتينية تتواجد في دم الشخص المصاب بالسرطان اجري هذا البحث لتقييم التغير في تركيز العامل السرطاني CA15.3 والعامل السرطاني CA27.29 وبروتين P53 وفيتامين D لمرضى سرطان الثدي في نساء العراق بواسطة فحص 200 امراه 30-75 سنة سليمة ومريضة مقسمة الى أربعة مجاميع كل مجموعة تتألف من 50 امرأة وتشمل مجموعة مرضى سرطان الثدي بعمر 30-59 ومجموعة مرضى سرطان الثدي بعمر 60-75 سنة ومجموعة العقد اللبئية بعمر 30-60 سنة ومجموعة الأصحاء السيطرة . من خلال نتائج هذه الدراسة لوحظ ارتفاعا معنويا  $P < 0.05$  لتركيز العامل السرطاني CA15.3 والعامل السرطاني CA27.29 وبروتين P53 وانخفاضا معنويا عند  $p < 0.05$  في تركيز فيتامين D في مجموعة الغدد اللبئية ومجموعتي سرطان الثدي كما لوحظ تغيرا معنويا في عنصر الكالسيوم .

الكلمات المفتاحية : سرطان الثدي ، P53 ، CA15.3, CA27.29 ، فيتامين D

### Introduction

Cancers represent a heterogeneous group of diseases characterized by uncontrolled growth and spread of abnormal cells in the body. The disruptive behaviours of cancer cells reflect dynamic changes in their genomes and in gene expression that result in disruption of normal regulatory signaling circuits [1]. Breast Cancer is the most commonly diagnosed cancer and the second leading cause of cancer related mortality. Projections for 2004 show an expected 215,990 new cases of invasive breast cancer, representing 32% of all new cancer diagnoses, and 40,110 breast cancer related deaths, and accounting for 15% of cancer related mortality. Breast cancer incidence has increased over the past 30 years in the U.S. [2]. This trend is thought to reflect increased diagnosis due to mammographic screening, and perhaps also to secular trends in the Prevalence of obesity and hormone replacement therapy (HRT) use by postmenopausal women. Over the same period, breast cancer mortality rates have declined, reflecting earlier breast cancer detection and treatment, and improvements in breast cancer therapies [3]. The risk of breast cancer increases with age. There is a rapid rise in breast cancer incidence with age up, and then the rate of increase slows dramatically. Breast cancer rates vary widely among different countries. The countries cited above have incidence rates six times higher than countries in Asia and Africa [4,5]. CA15-3 also known as MUCI, episialin, polymorphic epithelial or epithelial membrane antigen is mainly used to watch patients with breast cancer. Elevated blood levels are found in less than 10% of patients with early disease and in about 70% of patients with advanced disease. Levels usually drop if

treatment is working, but they may go up in the first few weeks after treatment is started. This rise is caused when dying cancer cells spill their contents into the bloodstream [6]. CA15.3 and CA27.29 may also contribute to cancer progression by modulating the immune response cancer cell expressing high levels of cancer antigen. The cell membrane has been shown to inhibit the function of cytotoxic T lymphocyte and lymphokine activating killer cell, this blockage may occur as result of elevating in cancer antigen 15.3 and masking cell membrane antigen involved in immune process [7,8] and also the elevated cancer antigen 15.3 concentration could be helpful for preoperative diagnosis of axillary lymph node metastasis in patients with Breast Cancer [9]. Research indicates that high concentration of CA15.3 and CEA were associated with poor prognosis [10]. Another research showed that high concentration of CA15.3, CA27.29 and CEA between aspiration fluid taken from cancerous breast tissue and that taken from breast tissue distance from the primary tumor, other tissue markers thyroglobulin and calcitonin [11]. Mutation of P53 is a common event in breast cancer [12]. This alteration can result in cellular accumulation of P53 and may also found in serum P53 antibodies (P53-Abs), [13] to clarify prognostic significance of these antibodies, Activation of P53 in response to cellular or genotoxic stress induces several responses, including DNA repair, senescence, differentiation, cell cycle arrest and apoptosis [14]. Inactivation of the P53 tumor suppressor gene is the most common genetic alteration in human cancers. This alteration is usually caused by missense point mutations of the gene. Sometimes, inactivation of the P53 protein may occur through complex formation with cellular proteins [15]. Alterations in P53 have prognostic value in colon, breast, and gastric cancers and so their recognition may be important for clinicians [16,17]. The role of vitamin D in relation to breast cancer risk [18]. Specifically, the main actions of the biologically active form of vitamin D, 1, 25(OH)<sub>2</sub>D, are mediated via the vitamin D receptor. The vitamin D receptor (VDR) is present in normal breast tissue [19]. 1,25(OH)<sub>2</sub>D has antiproliferative effects on and promotes the differentiation of breast cancer cells [20]. The aim of the present work were to investigate the levels of cancer antigen 15.3, CA27.29, protein P53 and vitamin D in the series patients with breast cancer.

#### Material & Methods

1. Samples two hundred healthy female and patients aged 30-75 years divided into four groups each groups formed 50 female (Control, Milking Nodule, Breast Cancer aged 30 -59 and breast cancer aged 60 - 75 group).
2. Determination of carcinogenic agent 15.3 by used vidas CA15.3 kit, this assay test uses two monoclonal antibodies (115D8 and DF3) which react with a circulating CA15.3 antigen expressed in human breast cancer cell. This assay principle combines a 2 step enzyme immunoassay sandwich method with final fluorescent detection (ELFA) [21].
3. Determination of P53 in serum. ELISA was used for the *in vitro* quantitative. The assay will recognize both endogenous and recombinant P53. The P53 Kit is a solid phase sandwich Enzyme Linked Immune Sorbent Assay (ELISA). According to manufactures institution [22].
4. Measurement of vitamin D by HPLC, blood samples were collected from women to measure total serum vitamin D participants were not required to fast for blood collection. Blood was collected via venipuncture, all tubes were protected from light, allowed to clot, centrifuged at 3000 rpm for 10 min, and then stored at -8 C until thawed for analyses by high performance liquid chromatography (HPLC). The method used has been described in detail. Briefly, the method utilizes a reversed- phase HPLC technique that shows a clear resolution of Vit D. The mobile phase is an acetonitrile extract of serum by solid phase extraction C18/ODS 4.6 ×25, wave (265nm), HPLC was prefor- med using a Shimadzu LC- 2010AHT system with Shima -dzu LC-2010 pump (Japan) [23].
5. Determination of free, calcium in blood. Sera were determined by utilizing atomic absorption spectrophotometer method. Two sets of standard solutions in the ranges (0.1 to 1 µg/ml) for Ca, was prepared. The sera were precipitated by using equal volume of 1.2N (TCA) after centrifugation, diluted (1: 10) for element measurements by the Atomic Absorption Spectrophotometer.

#### Statistical Analysis of Data [24]

Other data were analyzed by SPSS11.0 software and reported as mean ± standard deviation using one-way ANOVA. Student's T-Test was used for comparison between groups. P values of 0.05 or less are considered statistically significant.

## Results

Total of 200 healthy and patient women were including in this study with a determined the mean of Vitamin D  $16.4 \pm 12.65$  ng/mL in healthy group,  $15.41 \pm 12.25$  ng/mL in milking nodules group,  $9.4 \pm 3.52$  ng/mL in breast cancer aged (30 – 59 ) and  $7.65 \pm 3.66$  ng/mL in breast cancer aged 60-75 by HPLC technique showed decreased in vitamin D concentration in breast cancer group Fig (1,2,3,4,5 ), Table (1). Significant increased were seen on levels of CA 15.3 in patients with Breast Cancer and Milking Nodules groups as compared with the control group showed highly increased in levels of breast cancer concentration. Table (2). Significant increased were seen on levels of CA 27.29 in patients with Breast Cancer and Milking Nodules groups as compared with the control group showed highly increased in levels of breast cancer concentration. Table (3), significant increased were seen on levels of protein P53 in patients with Breast Cancer and Milking Nodules groups as compared with the control group showed highly increased in levels of breast cancer concentration. Table (4). the result showed significantly decreased of vitamin D in patients with breast cancer and milking nodules groups as compared with the control group. Table (5). the result showed significantly increased of Ca concentration in patients with breast cancer and decreased in milking nodules groups as compared with the control group.

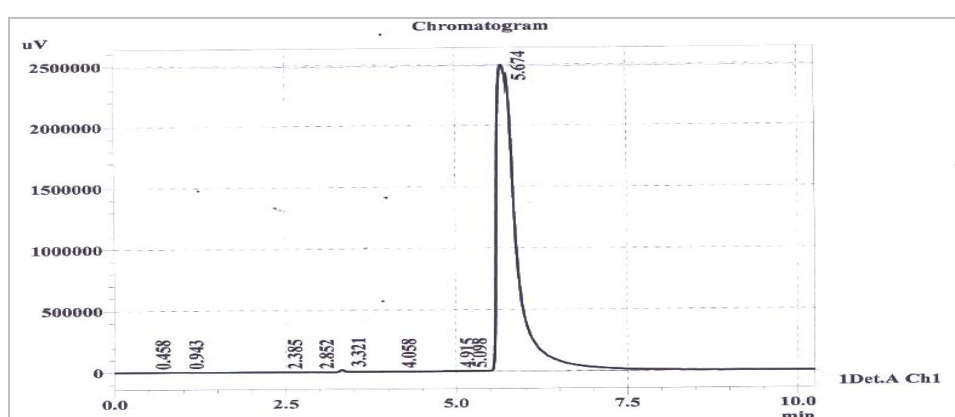


Fig (1): Vitamin D Standard HPLC Technique

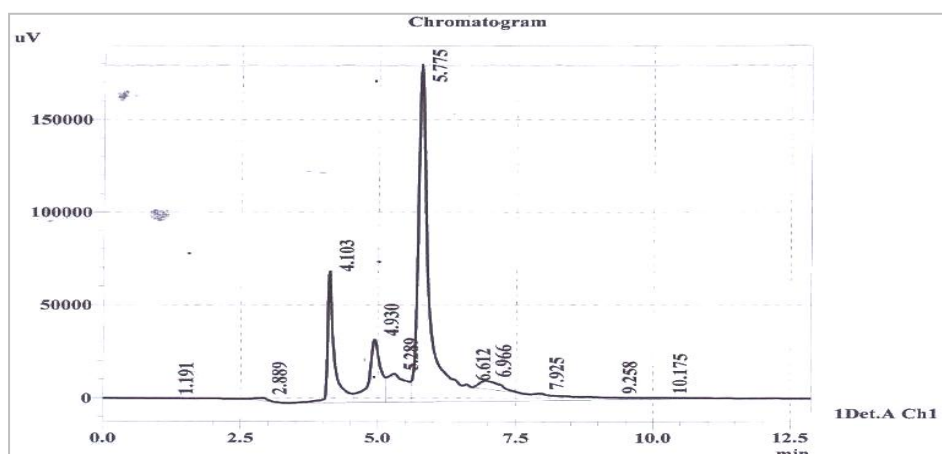


Fig (2): Chromatogram of Vitamin D in healthy group

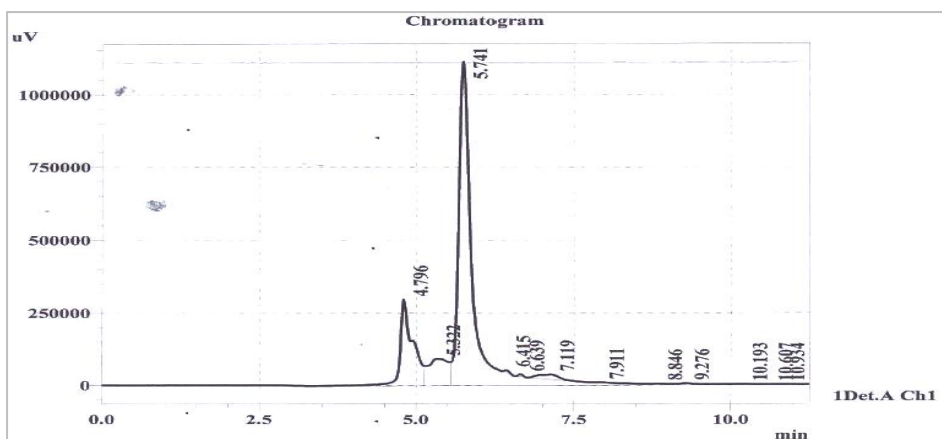


Fig (3): Chromatogram of Vitamin D in milking nodules patients

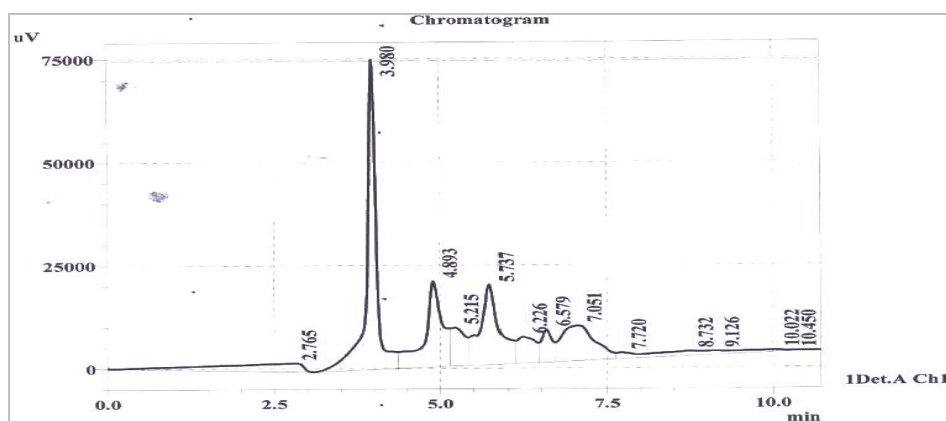


Fig (4): Chromatogram of Vitamin D in Breast cancer (age 30-59) patients

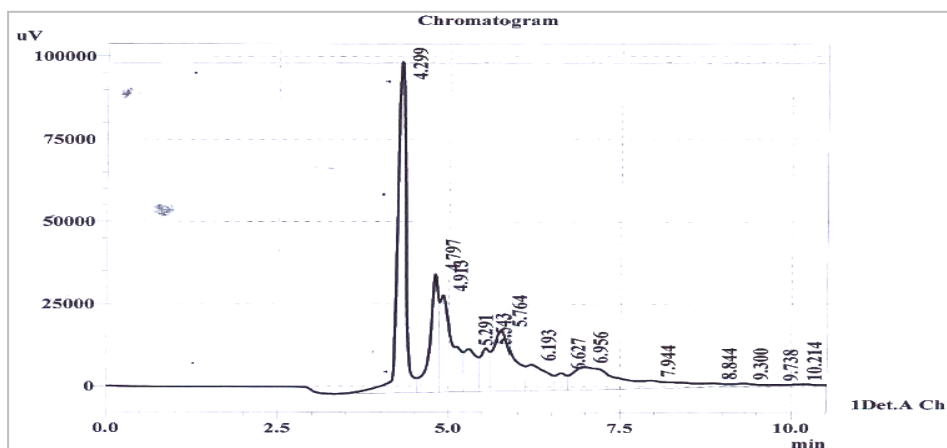


Fig (5): Chromatogram of Vitamin D in Breast Cancer (age 60-75) patients

Table (1): The level of CA15.3 in Milking Nodule and Breast Cancer Patient, M±SD.

	NO.	Mean±SD U/ml	P – value
Control group	50	8.176±2.055	
G1	50	20.362±4.324	P<0.05
G2	50	126.964±11.413	P<0.05
G3	50	156.916±12.096	P<0.05

Table (2): The level of CA27.29 in Milking Nodule and Breast Cancer Patient, M±SD.

	NO.	Mean±SD U/ml	P – value
Control group	50	10.23±2.66	-
G1	50	28.22±3.660	P<0.05
G2	50	194.85±15.71	P<0.05
G3	50	213.5±21.51	P<0.05

Table (3): The level of protein P53 in Milking Nodule and Breast Cancer Patient, M±SD.

	NO.	Mean±SD ng/ml	P – value
Control group	50	-	
G1	50	0.281±0.310	P<0.05
G2	50	1.251±0.852	P<0.05
G3	50	1.719±0.866	P<0.05

Table (4): The level of Vitamin D in Milking Nodule and Breast Cancer Patient, M±SD.

	NO.	Mean±SD ng/mL	P – value
Control group	50	16.4 ±12.65	
G1	50	15.41±12.25	P<0.05
G2	50	9.4±3.52	P<0.05
G3	50	7.65.±3.66	P<0.05

Table (5): The level of Ca in Milking Nodule and Breast Cancer Patient, M±SD

	NO.	Mean±SD ppm	P – value
Control group	50	43.787 ±6.641	
G1	50	29.682±7.660	P<0.05
G2	50	53.120±5.71	P<0.05
G3	50	54.213±6.10	P<0.05

## Discussion

CA15.3 is the most thoroughly investigated serum tumor marker in breast cancer. Some of the key decision in the current management of primary breast cancer involves the determination of prognosis [25]. The most commonly used prognostic factor in breast cancer are the levels of CA15.3. Concentration and changed Ca, in milking nodule and breast cancer patient. The role of tumor marker in the management of breast cancer patient is limited to patients with advanced disease since these are seldom abnormal in early disease or with local recurrence [26]. Thus marker analysis is appropriate in previously diagnosed patients at high risk for tumor recurrence to detect early disease dissemination and in patients with metastasis disease to evaluate therapeutic response [27]. In this study, slightly increased in levels of cancer antigen 15.3 concentration in women suffer from milking nodule in the breast, this result may be indicating a chance of disease prognosis or recurrence. Many studies suggests that there is a slight increase in CA15.3 of few patient this indicates recurrence of the disease. In the present study showed elevated in CA15.3 in many of patient, this antigen levels correlated positively and significantly with both tumor size and axillary lymph nod involvement. The poor prognostic value of high initial concentration of antigen in invasive breast cancer is well demonstrated [25]. In this study CA15.3 concentration is elevated in many of patient group this refer to metastases take place, thus a high initial CA15.3 concentration led us to assess the possible spread metastases that would otherwise remain undetected and had contributed to the high percentage of patient with metastases. The determination of CA15.3 in primary locoregional breast cancer is indicative of tumor size and nodal involvement and these marker are useful as prognostic indicator by both univariate and multivariate analysis, positivity of CA15.3 appear useful in identification of nod negative patient who have a high risk recurrence [21,27]. Studies were undertaken Cancer antigen 15.3 (CA 15.3) has been described as a useful marker in patients monitoring for breast malignancy. The CA15.3 was marke-dly elevated in patients with breast

carcinoma. Rising trend in serum CA15.3 will indicate appearance of early recurrence [26]. In the present research showed the difference in the concentration of protein P53 in milking nodules and breast cancer patients, this result of changed in physiological of mammary cells this difference take place result of present content malignant cell in blood stream[28]. This alteration can result in cellular accumulation of P53 and may also found in serum P53 antibodies (P53-Abs)[29]. In this study it found decreasing in Ca in milking nodule and increasing in Ca in Breast Cancer groups and decreasing in vitamin D in third group. The Epidemiological studies have suggested that there is an inverse association between serum vitamin D levels and risk of breast cancer, though these results vary by study design [30]. There is evidence of an association between high levels of vitamin D and decreased risk of breast cancer as has been concluded by The International Agency for Research in Cancer (IARC) [31]. nevertheless, there is not sufficient evidence to conclude that a causal effect exists, Several mechanistic studies have identified specific targets for vitamin D in cancer prevention including such functions as ant proliferation pro-differentiation [32] and cell cycle stabilization, the concentration of calcium may contribute to mammary carcinogenesis due to the roles of these elements in regulating cell proliferation, differentiation, and apoptosis [33], increased of calcium concentration may be effect of cancer cell in bloodstream conc. be divided into two cases with and without bony metastases, bony lytic lesion are frequently seen in patients with hematological malignancies [34] in present work showed increased in protein P53 this take place result of effect the cancer cell in bloodstream. P53 remains the most commonly mutated gene in many common human cancers, with mutations (principally, but not exclusively, missense) estimated to occur in 50% of all cancers. Mutant proteins are almost always defective for sequence specific DNA binding, and thus for transactivation of genes upregulated by the wild-type protein [29]. Interestingly, the proportion of missense mutations in P53 is higher than that seen in other tumor suppressor genes, suggesting that expression of P53 mutants may confer selective advantage over and above loss of wild type function [34].

### Conclusion

This study also suggests that there are elevated of cancer antigen CA15.3, CA 27.29 and P53 as well as variation in serum levels vitamin D and calcium are directly related to tumor burden and are independent prognostic factor for breast cancer. therefore both tumor marker and trace element could be considered for clinical use such as predicting patient outcome and determination adjuvant treatment for better outcome.

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