Clinical Investigation of The Role of Tumor Necrosis Factor-α and Other Risk Factors In The Evolution of Breast Cancer in Kerbala City

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Abstract

Background :Breast cancer is the most common malignancy in females worldwide .It is a leading cause of death in women. The tumor markers CEA ,CA 19-9 and CA15-3 and inflammatory cytokine TNF-α were shown to contribute to breast cancer development and metastasis.

***Objective*:** In this study, we wished to determine whether there are associations between these factors along stages of breast cancer progression, and to identify the possible implications of these factors to disease course.

Materials and Methods: Forty female patients with Breast cancer with ages ranged between (29- 68) years were taken from (Al-Hussein Medical City/Kerbala).Control group consisted of 20 healthy people who were free from signs and symptoms of breast cancer who matched in age and gender with patients, and had no history for any cancer problem. TNF-α(TNF-α EASIA Kit, DIA source) was studied using the enzyme-linked immunosorbent assay (ELISA) method, Estimation of CEA,CA19-9 , CA15-3 by Enzyme-linked fluorescence assay (VIDAS. Biomerieux SA/France). T-test, ANOVA and Pearson correlation used to analyze results by using SPSS version 20. P-value ≤ 0.05 was considered significant.

Results: TNF-α ,CEA ,CA15-3 levels increased significantly ($p< 0.05$) in patients compared with control group. But the level of CA19-9 does not show significant increase when compared with control groups.

Conclusion :Significantly correlation of TNF-α, CEA and CA15-3 with breast cancer but there is no significant correlation withCA19-9. So no significant correlation after chemotherapy .

Keyword : Breast cancer, TNF-*a* **,**CEA ,CA19-9,CA15-3**.**

الخلاصة

خلفية الموضوع :يعد سرطان الثدي من أكثر أنواع السرطان شيوعا" في العالم .والذي قد يسبب الموت لدى النساء .هنالك بعض العلامات الخاصة بالأورام مثل المستضد السرطاني المضغي CEA ،البروتين CA 19-9، البروتين CA 15-3 وعامل تنخر الأورام نوع ألفا TNF-α والتي قد تساهم في تطور سرطان الثدي وانتشاره.

الأهداف: نأمل في هذه الدراسة أن نحدد فيما إذا كان هنالك ارتباط بين هذه العوامل ومراحل تطور المرض وكذلك التعرف على النتائج المحتملة لهذه العوامل

المواد وطرائق العمل: نفذت هذه الدراسة على أربعون(40) حالة نساء مصابات بسرطان الثدي بأعمار تتراوح ما بين 29–68 سنة . جمعت العينات من مدينة الحسين الطبية في محافظة كربلاء . كما شملت الدراسة على عشرون (20) أنثى أصحاء كمجموعة سيطرة وبأعمار تتراوح مابين(29–68) سنة.استخدمت تقنية الامتزاز المناعي المرتبط بالأنزيم لفحص عامل تنخر الأورام نوع ألفا(TNF-α) ، بينما تم قياس العلامات الدالة على السرطان ،المستضد السرطاني المضغي CEA ،البروتين CA19-9 والبروتينCA15-3 باستخدام تقنية الاختبار المتفلور المرتبط بالإنزيم ،حللت البيانات إحصائيا"باستخدام الرزمة الإحصائية SPSS – ANOVA- person correlation) (version 20 و تم مقارنة القيم بواسطة العينة المستقلة (T- test) ، إذا كان مستوى المعنوية اصغر أو يساوي 0,05 مع المقارنة بمجموعة السيطرة فتعتبر عالية المعنوية.

النتائج : أظهرت النتاج ارتفاع مستوى عامل تنخر الأورام نوع ألفا TNF-α،المستضد السرطاني المضغي CEA ، والبروتين CA15-3 بشكل معنوي عند المقارنة مع مجموعة السيطرة .أما مستوى البروتين CA19-9 فلم يظهر أي فرق معنوي عند المقارنة مع مجموعة السيطرة.

الاستنتاجات: هنالك علاقة معنوية بين عامل تنخر الأورام نوع ألفاTNF-α، المستضد السرطاني المضغي CEA والبروتين CA15-3مع سرطان الثدي .لكن لم تكن هنالك علاقة معنوية مع البروتين CA19-9مع سرطان الثدي . كذلك لم تكن هنالك علاقة معنوية لهذه العوامل مع المرض بعد إعطاء العلاج الكيماوي.

الكلمات المفتاحية: سرطان الثدي ، عامل تنخر الأورام نوع ألفا ، مستضد سرطاني مضغي CEA ،بروتين CA19-9، بروتين CA15-3 .

Introduction:

Breast carcinoma is the most common malignancy in females worldwide (**Siegel** *et al.,* **2014)**. It is a leading cause of death in women (**Sin ghai R** *et al.,* **2011**). Cytokines constitute a diverse group of proteins comprising hematopoietic growth factor, interferons, lymphokines and chemokines (**Romagnani**, *et al*.,**2004**). Tumor necrosis factor (TNF)-*α* is a key cytokine involved in inflammation, immunity, cellular homeostasis and tumor progression .It was first identified as an anti-tumor cytokine accompanied by serious toxicity involving in the innate and adaptive immune system**.** (**Balkwill, 2009**).

The association of inflammation and cancer has been well recognized in many types of cancer and inflammation has been regarded as the 'seventh hallmark of cancer' (**Mantovani** *et al*., **2008** , **Mantovani, 2009**). Accumulating evidence has shown that TNF-*α* is a key mediator of inflammation and cancer (**Sethi** *et al***., 2008** , **Balkwill, 2009**). TNFα is a special interest because of reports showing that under specific circumstances it may have cytotoxic and anti-tumor effects in several malignant diseases (**Bertazza ,2008**). Furthermore, the chronic expression of TNF-α in breast tumors has been demonstrated to be correlated with lymph node involvement, suggesting the role of TNF-α in enhancing tumor cell metastasis **(Leek , 1998)**. Moreover, it has been observed that breast cancer patients with elevated levels of TNF-α in the circulation have a poor prognosis. TNF-α thus constitutes a useful biomarker in cancers **(Ferrajoli** *et al*., **2002** and **Garcia-Tunon** *et al* **., 2006**). The most widely used serum markers in breast cancer are cancer antigen15-3 (CA15-3) and carcinoembryonic antigen (CEA) .Although CA 15-3 and CEA are not currently recommended as markers for breast cancer screening or therapeutic response monitoring according to the American Society of Clinical Oncology guidelines , CA 15-3 and CEA are the markers most widely used for surveillance purposes and monitoring of treatment response in clinical practice. (**Harris** *et al.,* **2007**). CA 15-3 is the most frequently used tumour marker in invasive breast cancer. Despite poor prognosis associated with an initially high value, scientific societies have not yet recommended its determination in the initial evaluation as regards the extent of disease(**Sturgeon** *et al .,* **2008**).

Patients and Methods

Selection of patients

During the period 1/June/2014 to 1/November/2014, forty female patients with breast cancer with ages ranged between (29-68) years were taken from (Al-Hussain Mediacl City/Kerbala. Control group consisted of 20 healthy people who were free from signs and symptoms of cancer who matched in age and gender with patients, and had no history for any breast problem.

Sample collection and assay procedure

Blood sample (5ml) was collected left at room temperature and then centrifuge for 15 min. at (3000 RPM). Serum was then separated and freezed until time of analysis. Estimation of CEA,CA19-9,CA15-3 9Vidas(Biomerieux SA/France), TNFα ELISA kit (Cusabio/China) in serum using commercially available and performed as recommended in leaflet with kit

Statistical Analysis : Results are expressed as mean ± standard error ,mean (SEM), student t-test, ANOVA and Pearson correlation used to analyze results by using SPSS version 20. P-value ≤ 0.05 was considered significant .

Results

A total of forty female patients with breast cancer are divided into two groups according to the age (29-45) yrs 13 (32.5% 13 out of 40 cases) ,and age (▪ 45)yrs 27(67.5% 27 out of 40 cases) .The distribution of patients according to pathological evaluation was as followings : Three stages (I,II,III) were 19 (47.5%),11(27.5%),10(25%) respectively table 1.

Table1: Identification of information of patients with breast cancer.

Variable		No.	Percentage(%)
Total number of patients		40	100 %
Age groups	29-45	13	32.5 %
	٠ 45	27	67.5%
Stage	I	19	47.5%
	II	11	27.5%
	III	10	25.0%
Drug	treated With Chemotherapy	15	37.5%
	No treated with Chemotherapy	25	62.5%

The distribution of cases and controls according to the presence of risk factors is depicted in (Table 2) . The cases had a higher age at marriage as compared to the controls; however, the difference was statistically significant. The mean duration of breast feeding in cases and controls was 14±5.78and 20±3.56months, respectively ($P < 0.023$). However, the cases had a significantly higher number of abortions (1.68±1.0) as compared to the controls (0.9±1.0). There was a statistically significant difference in the mean age of menopause of cases and controls with cases attaining menopause at a late age (50.64±4.21) as compared to the controls (47±1.46)years.

Table 2: Distribution of risk factors in control and patients with breast cancer .

Risk Factors	Mean ±SD (n=60)		Sig
	Breast cancer cases (40)	Controls (20)	
Age	47±10.31	47±10.7	0.67
age at a marriage (per year)	21.48±4.84	16.95±3.18	0.04
Age at menopause	50.64±4.21	47±1.46	0.046
Breast feeding duration (per months)	14±5.78	20±3.56	0.023
Number of abortion	1.68±1.0	0.9±1.0	0.033

Serum CEA ,CA 15-3, and TNF-α were estimated in 40 patients compare with 20 healthy control ,these parameters increased significantly (P٠ 0.05) in patients compare with control groups ,while no significant correlation in CA19-9 (table 3).

Table 3: Distribution of CEA,CA15-3,CA19-9,and TNF-α in control and patients with breast cancer .

	g	N	Mean ±Std.Devation	sig
CEA	Control	20	.733±.1798	0.00
	Patients	40	5.750±5.6599	
CA 15-3	Control	20	5.919048±3.7588055	0.00
	Patients	40	72.686000±19.2836384	
CA 19-9	Control	20	1.704762±1.3328451	0.19
	Patients	40	1.797500±1.1741855	
TNF-α	Control	20	80.966667±12.8720757	0.01
	Patients	40	190.200000±48.4690491	

While table 4 showed no significant differences in CEA ,CA 15-3,CA19-9and TNF-α when compare between the patients were treated with or without chemotherapy drugs as in(Table 4).

Table 4: Distribution of tumor markers in patients according to chemotherapy .

	chemotherapy	N	Mean Std. Deviation	*P* value
CEA	Non Chemotherapy	25	5.092±5.6454	0.763
	Chemotherapy	15	6.847±5.7045	
CA 15-3	Non Chemotherapy	25	74.564000±18.7945444	0.336
	Chemotherapy	15	69.556000±20.3359754	
CA 19-9	Non Chemotherapy	25	2.036000±1.1614933	0.709
	Chemotherapy	15	1.400000±1.1212238	
TNF-α	Non Chemotherapy	25	221.252000±32.7631414	0.716
	Chemotherapy	15	172.866667±35.1434340	

The correlation between the parameters in patients was shown in (table 6) the results revealed a strong correlation ($p<0.05$) between TNF-α and CEA. while there is no significantly correlation ($p<0.05$) found in the studying parameters in patients

Table 6: Correlation between parameters in patients with breast cancer

		CEA	CA 15-3	CA 19-9	TNF-α
CEA	Pearson Correlation	1	-.113-	.146	.363*
	Sig. (2-tailed)		.488	.369	.021
	N	40	40	40	40
CA 15-3	Pearson Correlation	-.113-	1	.184	-.116-
	Sig. (2-tailed)	.488		.255	.475
	N	40	40	40	40
CA 19-9	Pearson Correlation	.146	.184	1	.183
	Sig. (2-tailed)	.369	.255		.259
	N	40	40	40	40
TNF-α	Pearson Correlation	.363*	-.116-	.183	1
	Sig. (2-tailed)	.021	.475	.259	
	N	40	40	40	40

There were no significant correlation of tumor markers in patients according to the stages of disease and show increasing of CEA, CA15-3,CA19-9 and TNF-α values at stage 1(30.184±16),stage3(71.6±13),stage3(40.1±17) and stage 2(203.8±50).

Table 7: Stage level of CEA,CA15-3 ,CA19-9and TNF in patients with breast cancer.

	stage	N	Mean± Std. Deviation	*P* value
CEA	1	19	30.184±16.2973	0.154
	2	11	7.718±4.7159	
CA 15-3	1	19	71.426316±18.0827064	0.657
	2	11	71.276364±25.7928158	
CA 19-9	1	19	14.210526±18.0483577	0.379
	2	11	22.172727±9.8236542	
TNF-α	1	19	188.568421±54.3315343	0.657
	2	11	203.845455±50.0746316	
CEA	1	19	30.184±116.2973	0.159
	3	10	10.610±3.8530	
CA 15-3	1	19	71.426316±18.0827064	0.197
	3	10	71.610000±13.5733767	
CA 19-9	1	19	14.210526±18.0483577	0.720
	3	10	40.190000±17.4460725	
TNF-α	1	19	188.568421±54.3315343	0.439
	3	10	178.290000±33.1566802	
CEA	2	11	7.718±4.7159	0.108
	3	10	10.610±3.8530	
CA 15-3	2	11	71.276364±25.7928158	0.296
	3	10	71.610000±13.5733767	
CA 19-9	2	11	22.172727±9.8236542	0.147
	3	10	40.190000±17.4460725	
TNF-α	2	11	203.845455±50.0746316	0.109
	3	10	178.290000±33.1566802	

Discussion:

In the current study ,the age of cases and controls were between 29 to 68 years of age. **Ozmen** *et al* **., 2009** conducted a similar study with the cases and controls between 18 to 70 years of age. Also conducted a similar study with study population between 20 to 65 years age group. **Hussain** *et al***., 2010** .In the present study maximum numbers of subjects (67.5%) were observed above ٠ 45 years of age group. The average age of the cases was 47 years (SD 10.31) and that of controls was 47 years (SD 10.7), similar findings were noted in the study conducted by (**Meshram;** *et al***., 2009**)., the study reported most of the patients between 40 to 49 years of age with the average age of 48.4 years for cases. (**Abbasi** *et al* **., 2009**) reported average age of cancer cases 47.49 years in a similar study conducted Iran. The present study reported 32.5% of the cases with less than age 45 years.

Now due to globalization and adoption of western life styles, Iraqi women are marrying late, not having first child birth at an early age and also not using breast feeding for long time. These are the risk determinants of breast cancer as evident in many studies **Rao** *et al*., **2005, Grindel** *et al*., **2004**). Risk factors of breast cancer are manifold. There is difference between risk determinants and risk modulators whereas determinants cannot be influenced, risk factor modulator can be. On assessing the risk determinants it was found that 1/3 rd of women had delivered at age more than 30 years . These findings concurred with that of other studies. (**Alkhasawneh ,2007 and Yaren** *et al* ., **2008**).

The breast cancer cases attained a late age of menopause as compared to the controls. (**Marubini E; *etal* 1988**) Other studies have also reported increase in risk with late age at menopause.(**Toniolo PG; *et al* 1995**)

The present study revealed that the breast cancer cases had lower mean duration of breast feeding (14±5.78months) as compared to controls (20±3.56). Studies conducted in different countries have also reported similar findings (**Tryggvadóttir** *et al* .,**2001** ,**Chang-Claude** *et al*.**, 2000**)

The history of abortions was found to be significantly higher in breast cancer cases as compared to controls. Results of other studies are agreement and reported an increase in risk with induced abortion (**Lipworth L; *et al*1995**)

TNF-α is a major regulator of the inflammatory response through inducing the expression of other pro-inflammatory and chemotactic cytokines and adhesion factors. TNF-α is also produced by neoplastic cells or cells in the tumor microenvironment and can act as an endogenous tumor promoter. In normal breast tissue, TNF-α regulates cell proliferation through its pro-apoptotic effects, but in breast cancer, the inhibition of the apoptotic pathway and the enhancement of the survival and proliferation effects contribute to tumor cell proliferation (**Garcia-Tunon** *et al*.,**2006**).

Numerous studies have linked TNF-α to breast cancer progression .As a result, the mechanisms by which TNF-α promotes breast cancer have been recently explored using both *in vitro* and *in vivo* models. Similarly a prospective study was conducted on forty patients with invasive breast cancer and their serum concentration of TNF-α were found to be elevated (**Moore** *et al*.,**1999** , **Pikarsky** *et al* .,**2004**) studied the role of TNF-α polymorphism and its levels in breast cancer .Number of studies have explored the role of TNF-α in relation to an increased risk for cancer. Among them, most case-control studies have shown a higher cancer risk in people with elevated TNF-α levels.

Previous studies have shown that CA 15-3 is the most sensitive tumor marker in breast cancer disease [**Guadagni** *et al*.,**2001** , **Loprinzi** *et al*., **1986**). The current study confirms this finding. Recent literature suggests there is a modest advantage of performing tumor markers other than CA 15-3 in metastatic breast cancer. Our study found elevation of another tumor markers (CEA and/or CA19-9).

Although controversial, there are accumulating data that support our finding that elevated tumor markers are associated with a worse prognosis (**Sturgeon** *et al*., **2009**).

Conclusion : Significant correlation of TNF-α, CEA and CA15-3 with breast cancer. But there is no significant correlation for CA19-9 . So no significant correlation after chemotherapy

Reference:

Abbasi S, Azimi C, Othman F, Einollahi N, Dashti N, Nabatchian F.(2009). Risk factors for breast cancer in Iranian women: A case control study. Int J Cancer Res,5:1–11.

Alkhasawneh I.M. 2007. knowledge and practice of breast cancer screening among Jordanian nurses Oncol Nurs Forum. Nov,34(6):1211-7. 5

Balkwill F.(2009). Tumour necrosis factor and cancer. Nat Rev Cancer, 9: 361–371 .

Bertazza L, Mocellin S. 2008. Tumor necrosis factor (TNF) biology and cell death.*Front Biosci*, 13:2736-2743.

Ferrajoli A, Keating MJ, Manshouri T, Giles FJ, Dey A. 2002. The clinical significance of tumor necrosis factor-alpha plasma level in patients having chronic lymphocytic leukemia. Blood ,100: 1215–1219.

Garcia-Tunon I, Ricote M, Ruiz A, Fraile B, Paniagua R. 2006. Role of tumor necrosis factor-alpha and its receptors in human benign breast lesions and tumors (in situ and infiltrative). Cancer Sci ,97: 1044–1049.

Grindel CG, Brown L, Caplan L and Blumenthal D. 2004. The effect of breast cancer screening messages on knowledge, attitudes, perceived risk, and mammography screening of African American women in the rural South. Oncol Nurs Forum.Jul13;31,(4):801-8.

Guadagni F, Ferroni P, Carlini S. 2001. A re-evaluation of carcinoembryonic antigen (CEA) as a serum marker for breast cancer: a prospective longitudinal study.Clin Cancer Res ,7:2357-2362.

Harris L, Fritsche H, Mennel R. 2007. American Society of Clinical Oncology update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol. 2007;25:5287–5312.

Hussain GA, Rehab MM, Mohmmad ME and Mohmmad OH. 2010. Role of some risk factors in etiology of breast cancer in Sudan. Open Breast Cancer J,2:71–8.

Leek RD, Landers R, Fox SB, Ng F, Harris AL. 1998. Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Brit J Cancer, 77: 2246–2251.

Lipworth L, Katsouyanni K, Ekbom A, Michels KB, Trichopoulos D. 1995. Abortion and the risk of breast cancer: A case-control study in Greece. Int J Cancer,61:181-4.

Loprinzi CL, Tormey DC, Rasmussen P.1986. Prospective evaluation of carcinoembryonic antigen levels and alternating chemotherapeutic regimens in metastatic breast cancer. J Clin Oncol ,4:46-56.

Mantovani A, Allavena P, Sica A, Balkwill F. 2008. Cancer-related inflammation. Nature, 454: 436–444 .

Mantovani A. 2009. Cancer: Inflaming metastasis. Nature 457(7225): 36–37.

Marubini E, Decarli A, Costa A, Mazzoleni C, Andreoli C, Barbieri A. 1988. The relationship of dietary intake and serum levels of retinol and beta-carotene with breast cancer. Results of a case-control study. Cancer,61:173-80.

Meshram II, Hiwarkar PA, Kulkarni PN. 2009. Reproductive risk factors for breast cancer: A case control study. Online J Health Allied Sci,83:5.

Moore RJ, Owens DM, Stamp G, Arnott C, Burke F. 1999. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med ,5: 828-831.

Ozmen V, Ozcinar B, Karanlik H, Cabioglu N, Tukenmez M, Disci R. 2009. Breast cancer risk factors in Turkish women A university hospital based nested case control study. World J Surg Oncol,7:37.

Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S. 2004. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature ,431: 461-466.

Rao RS, Nair S, Nair NS and Kamath VG. 2005. Acceptability and effectiveness of a breast health awareness programme for rural women in India. Indian J Med Sci, 59:398-402

Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S. 2004. CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends Immunol*, 25(4):201-209.

Sethi G, Sung B and Aggarwal BB. 2008. TNF: a master switch for inflammation to cancer. Front Biosci ,13: 5094–5107 .

Siegel R, Ma J, Zou Z and Jemal A. 2014. Cancer statistics . *CA Cancer J Clin*, 64, 9-29.

Sin ghai R, Patil VW and Patil AV . 2011. Status of HER-2/neu receptors and Ki-67 in breast cancer of Indian women. *Int J App Basic Med Res*, **1**, 15-9.

Sturgeon CM, Duffy MJ, Stenman UH. 2008. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast and ovarian cancers. Clin Chem, 54(12): e11–e79.

Sturgeon CM, Lai LC and Duffy MJ. 2009. Serum tumour markers: how to order and interpret them. BMJ ,339:b3527.

Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE. 1995. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J Natl Cancer Inst,87:190-7.

Tryggvadóttir L, Tulinius H, Eyfjord JE, Sigurvinsson T. 2001. Breastfeeding and reduced risk of breast cancer in an Icelandic cohort study. Am J Epidemiol,154:37-42.

Yaren A, Ozkilinc G, Guler A . 2008. Awareness of breast and cervical cancer risk factors and screening behaviours among nurses in rural region of turkey. Eur J cancer care,17(3) 278-84.