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website: http://jsci.utq.edu.iq Email: utjsci@utq.edu.iq

Determine concentration of Immunoglobulin IgG,IgM,IgA and complement components C_3 and C_4 in serum of women effected with breast cancer

Syoof Khowman Alwan
Department of Biology - Science Collage - AL-Qadisyia University

Abstract

This study was carried out to investigate some immunological aspects in women effected with breast cancer compared with healthy control groups .The study included (20) patients with breast cancer and (18) healthy women, their age ranged between (21-74) years.The concentration of immunoglobulins (IgG, IgA, IgM) and complement component C_3 and C_4 were evaluated in two studied groups .

The concentration of immunoglobulins IgG was showed a significant increase pvalue in patients with breast cancer as compared with control group. No significant differences were observed in respect to the immunoglobulins levels of IgM and IgA between the studied groups .The concentration of complement component (C_3,C_4) were showed a significant increase pvalue in patients with breast cancer as compared with control group (healthy women).

Introduction

The breast is a modified, specialized, coetaneous, glandular structure. At puberty the breast develops under the influence of the hypothalamus, anterior pituitary and ovaries, also required insulin and thyroid hormones. The mature female breast has a distinctive protuberant, conical form with roughly round base (1).

The breast tumors are abnormal breast tissue, swelling hard mass, may be either benign or malignant. Breast tissue is never static; the breast constantly responding to changes hormonal, nutritional, genetic, psychological, and environmental stimuli such as radiation that cause continual cellular changes (2).

Recent insights into the molecular and cellular mechanisms underlying cancer development have revealed that immune cells functionally regulate epithelial cancer development and progression (3).

Leukocytes represent a diverse assortment of immune cells composed of both innate (myeloid) and adaptive (lymphoid) lineages. Innate immune cells, including macrophages, granulocytes, mast cells, dendritic cells (DCs),and natural killer (NK) cells , represent the first line of defense against pathogens and foreign agents . When tissue homeostasis is perturbed , tissue-resident macrophages and mast cells locally secrete soluble factors such as cytokines , chemokines , bioactive mediators , and matrix –remodeling proteins that recruite additional leukocytes from the circulation to the damaged tissue (that is inflammation) (4 , 5 , 6).

Recruited innate immune cells can directly eliminate pathogenic agents in situ. At the same time, DCs take up foreign antigens (including tumor antigens) and migrate to lymphoid organs, where they present their antigens to adaptive immune cells . Upon recognition of foreign antigen presented by DCs or other professional APCs. Presenting cells , adaptive immune cells , such as T lymphocytes or B lymphocytes , undergo clonal expansion in order to mountain " adaptive" response targeted against the foreign agent (7,8)

B-cell precursors mature within bone marrow ,where somatic recombination genes results in expression of a diverse array of B-cell receptors . Mature antigen committed B cells migrate to secondary lymphoid organs (lymph nodes or spleen , predominantly) . Upon antigen recognition by B-cell receptors , the B lymphocytes become activated and undergo clonal expansion , resulting in their enhanced capacity to recognize foreign antigens(9). Acute activation of B-lymphocyte responses (to foreign antigens or tissue damage) can also result in rapid induction of several soluble mediators , including diverse immunoglobulin sub types , B-cell-derived cytokines such as IL-6 and activation of complement cascades , which together trigger recruitment of innate immune cells from the circulation . In this manner, acutely activated B cells or chestrate phagocytic or cytotoxic destruction of immunoglobuline . complexed antigens (pathogens or damaged cells) by innate immune cells . Such acute B-cell responses are critical for protecting tissues from pathogens and nonself-antigens . Chronic activation of B cells can be deleterious , however , as evidenced by their association with several pathologic diseases states (rheumatoid arthritis and other autoimmune diseases) and some cancer types (10).

Immunoglubulines are effector molecules of the humoral limb of immunity and have become essential tools in life science research (11). The most outstanding break through in antibody research has been the treatment of cancer with monoclonal antibodies (MAb).

There are two major mechanisms by which antibodies may mediate tumor lysis , complement fixing antibodies bind to the

tumor cell membrance , activate complement and lyse the tumor cell. (12)

Another mechanism of tumor cell lysis by antibody is antibody dependent cell – mediated cytotoxicity (ADCC) , in this mechanisms antibodies (usual of IgG class) from and inter cellular bridge by binding to a specific determinant on the target cell and the fc receptor of effector cells such as macrophage , granulocytes and killer cells (13).

The complement system is an integral part of the immune system . It involves more than 30 soluble and cell-bound proteins that circulate in the plasma in an inactive form (13)

These proteins can be activated by three independent pathways , namely-classical , alternative and lectin pathway leading to generation of products that have important biologic activities including tumor cell destruction (14).

There are two major mechanisms by which complement components mediate tumor cell destruction :

1- The binding of complement fixing antibodies to tumor cell membrane , promote attachment of complement component that make pores in the membrane , resulting in cell distraction due to loss of osmotic and biochemical integrity (15) .
2- The complement components enhance the tumor cells killing by a process involving opsonization and subsequent phagocytosis by macrophages (16) .

Aim of The study

1-To evaluate the concentration of immunoglobulins (IgG,IgM,IgA) and concentration of complement component(C3,C4) in breast cancere women compared with healthy control group.

Materials and methods

20 females with breast cancer with age rang from 26 to 74 years were studies .

All these studied cases were diagnosed by clinicians and pathologists (confirmed histologically), and all these cases were selected for this study before surgery and all had received no treatment .A total of –18— healthy female volunteers who were age matching to the patient group , were selected as a healthy control group . Five milliliters of venous blood were with drawn from each patient (preoperatively) by vein – puncture and was collected separately in plane tube with no anti coagulant , left to clot at room temperature then centrifuged and serum was collected in two separated tubes and stored at (-80° C) until used . The concentration of Immunoglobulins (IgG,IgM,IgA) and complement component(C3,C4) were measured by single radial immunodiffusion (SRID) method , in which equal volumes of reference sera and test samples are added to wells in an agarose gel , containing mono-specific anti-sera .

The samples diffuse radically through this gel and the substance being assayed from a precipitin rings with the mono-specific anti-sera .Ring diameters are measured and a reference curve is constructed on graph paper . Unknown concentration are determined from the reference standard curve (17).

Results and discussion

Table (1) demonstrated that there were a significant pvalue changes in the levels of IgG in breast cancer of patients sera in comparison with healthy controls.

The results revealed that patients with breast cancer have a higher values in concentration of IgG wich reached(1578.9 ± 241.9 mg/dL) with significant differences ($p < 0.01$) compared to that of healthy control (1188.2 ± 297.7 mg/dL) .

While the mean serum levels of IgA and IgM in control patients were (240.1 ± 106.9 , 130.9 ± 35.4) mg/Dl and (262.6 ± 78.3 , 126.1 ± 36.8) mg/d L respectively in

malignant breast tumor patients , and ther were no significant differences between the control groups and the malignant group .

The results of study were in agreement with investigators (18, 19 , 20 , 21) who collectively cited that plasma levels of IgM and IgA from breast cancer patients were quite close to their concentration in healthy and benign control groups .

On other hand the IgG was found to be significantly increased in patients with cancer of the skin and lung , but decreased in patients with cancer of the prostate and breast . Serum IgM was reported to be elevated in patients with sarcoma , melanoma , brain tumors , but decreased in patients with carcinoma of the ovary . Serum IgA was found to be elevated in patients with cancer of epithelial secretory organs , such as skin , breast , head and neck , lung , gut, prostate , and uterine cervix whether these findings reflect specific changes of humoral arm of tumor –host interaction remains to be investigated (22).

The elevation in IgG levels cited in the present study and the other concordant studies can be attributed to increase antigenic stimulation , suggesting a humoral defensive reaction against increasing tumor load .

Table (4,5) demonstrate that the mean serum levels of C_3 and C_4 complement components of breast cancer patients were (207.1 ± 46.2 , 49.7 ± 18.9) mg / dL respectively. which were significantly higher than those in control group. (140.6 ± 20.1 , 31.9 ± 17.7) mg / dL respectively ($p<0.01$).

The results of the present study and those of (23) and (22) demonstrated that the stage of the neoplastic disease influence the level of complement activity , which can be attributed to the persistent presents of a tumor mass which serves as an antigenic stimulus for increasing antibody production that were complexed with antigenic , and the immune complexes may activate the complement system resulting in an increased the components production .

The results of this study were in agreement with (24) who is found a significant rise in sera of patients with benign lesions , whereas a significant rise in C_3 and C_4 was observed in all the cancer patients studied .

The complement activity increased significantly with the progression of the disease up to stage III and remained persistently elevated thereafter, patients who had a clinical cure had normal level of C_3 and C_4, whereas the values remained elevated in patients who were still undergoing treatment for residual lesions .

On other hand the (24) support a hypothesis that blocking complement inhibitor function on tumor cells will not only enhance monoclonal antibody – mediated immunotherapy but may also be effective at enhancing a normally ineffective humoral immune response in the absence of administered anti tumor antibody

Table (1) The concentration of IgG (mg/dL) in patients and control group

Study groups			
Values	Healthy control	Malignant breast tumor	P(ANoVA)
Minimum	840	1283	t= -4.426 P<0. 01
Maximum	1695	1938	
Mean	1188.2	1575.9	
SD	297.7	241.9	
SE	70.2	54.1	
N	18	20	

Table (2) The concentration of IgA (mg/dL) in patients and control group

Study groups			
Values	Healthy control	Malignant breast tumor	P(ANoVA)
Minimum	81	132	t= -0.747(NS) P<0. 01
Maximum	294	381	
Mean	240.1	262.6	
SD	106.9	78.3	
SE	25.2	17.5	
N	18	20	

Table (3) The concentration of IgM (mg/dL) in patients and control group

Study groups			
Values	Healthy controls	Malignant breast tumor	P(ANoVA)
Minimum	79	74	t= 0.412(NS) P<0. 01
Maximum	188	180	
Mean	130.9	126.1	
SD	35.4	36.8	
SE	8.3	8.3	
N	18	20	

Table (4) The concentration of C3(mg/dL) in patients and control group

Study groups			
Values	Healthy controls	Malignant breast tumor	P(ANoVA)
Minimum	102	142	t= 5.605 P<0. 01
Maximum	169	271	
Mean	140.6	207.1	
SD	20.1	46.2	
SE	4.9	10.3	
N	18	20	

Table (5) The concentration of C4(mg/dL) in patients and control group

Study groups			
Values	Healthy controls	Malignant breast tumor	P(ANoVA)
Minimum	19.2	19.5	T= -3.706 P<0. 01
Maximum	43.5	71.1	
Mean	31.9	49.7	
SD	7.7	18.9	
SE	1.8	4.2	
N	18	20	

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تحديد تراكيزالغلوبيولينات المناعية IgM,IgA,IgG ومكوني المتمم (C_3,C_4) في مصول نساء مصابات بسرطان الثدي

سيوف خومان علوان

قسم علوم الحياة - كلية العلوم - جامعة القادسية

الخلاصة

اجريت هذه الدراسة من اجل الكشف عن بعض المظاهر المناعية لدى نساء مصابات باورام الثدي مقارنة مع نسوه المجموعة الضابطة (النساء السليمات) .

تضمنت الدراسة (٢٠) مريضة مصابة بسرطان الثدي و (١٨) امرأة سليمة . تراوحت اعمارهن بين (٢١-٧٤) سنة .

حددت تراكيز الغلوبيولينات المناعية (غلوبيولين G و غلوبيولينA و غلوبيولين M ومكوني المتمم (C_3,C_4) كمياً بطريقة الانتشار المناعي المفرد (SRio) فاظهرت النمتائج ارتفاعاً معنوياً في مستوى الغلوبيولين G لدى المريضات بسرطان الثدي مقارنة بالمجموعة الضابطة و لم يلاحظ مثل ذلك الاختلاف في مستوى الغلوبيولينين A,M بين مجموعتي الدراسة .

اما مكوني المتمم (C_3,C_4) فقد ابديا ارتفاعاً معنوياً في مستواهما المصلي لدى المريضات المصابات بسرطان الثدي بالمقارنة مع نسوة المجموعة الضابطة (النسوة السليمات) .