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Evaluation of T-helper 22 and T-helper17 in patients with breast cancer

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Abstract

The interplay between Th-17 and Th-22 cells plays vital role in tumor immunity . Therefore, this study aimed to focus on these cells in patients with breast cancer. The results of this study showed that there were a highly significant increment in concentrations of IL-17A(139.5±17pg/ml) compared with control group which was (41.33±11.3pg/ml) and showed results were a highly significant elevation in concentration of IL-23P19 (192.73±22.3pg/ml) while control group was (121.41±14.7pg/ml). Also The results showed a highly significant increment in concentration of IL-22 (137.25±24.6pg/ml) compared to control group which was (77.67±13.7pg/ml) and also in TNF-α concentration was (200±23.7pg/ml) compared with control group which was (10.5±7.3pg/ml). Molecular findings recorded a significant elevation in the levels of AP-1 gene expression were Fos 18.76±7.8 in patients group and 9.05±1.12 control group, also the values of JunD gene 15.77±9.43 and 6.18±0.112, and lastly JunB 23.223±11.34 in comparison to control group7.33±3.76.Overall findings revealed considerable inflammatory response by Th-17 and Th-22 and remarkable AP-1 gene expression.

Keyword : Breast cancer, Th17 ,IL-17A , TNF-α and AP-1 Genes.

Introduction

Breast cancer is of the most common malignant tumors in humans, which is responsible for increasing numbers of morbidity and mortality every year in all over the world, this tumor type affect mainly women but also males can affected too [1,2,3]. In this disease the transformed cells of breast grow in uncontrollable manner the spread to the surrounding and sometimes distant tissues, resulting in the formation of the second type of cancer that led to death in women [4,5]. The etiology of the disease in complex including family history ,age, weight (obesity) and lifestyle such smoking, alcohol or the exposure to chemical or physical carcinogens. The disease globally constitute about 23% of all other invasive cancers [6,7] .

It is well known that healthy competent immune status play crucial role in the development of tumors, this controlling defense mainly occur via effector T cells such as Th-1,Th-17 and Th-22 and regulated by T-reg. on the molecular levels each cancer result from genetic mutation in such mutations led to losing the ability of proto-

oncogene to control the cell division resulting in formation oncogene. It was found that inflammation contribute the pathogenesis of breast cancer [8,9] .

Immune cells orchestrate their activities in human body by cytokines which are glycoproteins working as immune mediators that manage the inflammatory immune response and disease progression, these mediators affect directly on the immune and tumor cells, by up-regulation or activation of inflammation (pro-inflammatory cytokines) and down regulation or inhibition (anti-inflammatory cytokines), so cytokines can stimulate the development and growth or can inhibit the development of breast cancer[10,11].

During the cellular ontogeny of naïve CD4+ T-cells can develop to many types including effector cells such as Th-1, Th-2, Th-17, Th-22 and regulatory cells T-reg, this cells release and respond a complex network of cytokines, the ratio of each type of cells and the concentration of the cytokines are in a fine homeostasis, the stability or balance of their cytokines is very important for the regulation of the immune system [12,13,14,15].

Th-17 cells is a another subset of T helper cells changed the classical Th-1/ Th-2 example of T helper cell differentiation and Th17 cells are CD4+ T cells which are responsible for the production of interleukin-17A (IL-17A), Also the unique transcription factor are expressed with potent and effective biological activities[16].These cells also can secret TNF-α and IL-22 and CCL20, notably IL-17 could be secreted in the absence of IL-22 and vice –versa indicating differential molecular and cellular requirement expression of these cytokines [117,18,19,20].

Tumor necrosis factor is general inflammatory cytokine, its work as a key cytokine, this cytokine effectively manage the immune status of the inflammatory tumor microenvironment then can promote the migration and invasion of the tumor cells, in addition to this activities its appeared that TNF-α have many other effective roles but it's still unknown completely[21].

The activator protein-1(AP-1) transcription factor that’s found nearly in all eukaryotic cells affect the mitotic cellular behavior, such as proliferation, tumor cells growth, invasion, migration and the metastasis [22]. This transcription factor composed of three subunits homodimers Fos,JunD and JunB. Jun family include (JunD,JunC and JunB) homodimers, While fos (FosC,FosL1,FosL2 and FosB). The heterodimers of Fos/Jun crucial for activation than Jun/Jun homodimers during the activation of AP-1 due to response to extracellular stimulus, that’s leading to the binding of AP-1 to specific cognate sequences of DNA then controlling the cell proliferation[23]. Due to the critical role of AP-1 in the development of cancer, Ap-1 considered as one of the most important targets of the therapy of cancer [24]. This work was aimed focus on the interplay activities of Th-17,and Th-22 with expression levels of AP-1 gene.

Materials and Methods

Collection of Samples :

Blood sample were collected from 43 woman patient of breast cancer who attended to the conchology clinic department in Al-Diwaniya city hospital from the period between 1/4/2014 – 1/4/2015 their ages ranged from 31-63 years old , 17 control group were closed apparently healthy their ages ranged from 30-45 years old . Sera were isolated by centrifugation and preserved at-20C° until the day of the usage.

Cytokines assay:

Serum levels of IL-17A , IL-23P19 , IL-22 and TNF-α were measured using a commercial enzyme-linked immunosorbent assay(ELISA) kit (KOMA biotech / Korea) according to the manufacturer's instructions.

Primers design:

All the primers that used in this work which listed in (table -1) .These primers (Housekeeping gene, Fos gene , JunD, and JunB) had been designed by the NCBI-Gene Bank data base and also the use of the Primer 3 design online, the primers were used to assay the relative gene expression ,quantitation of the gene expression as qRT-PCR technique which use the SYBER Green DNA binding dye(Bioneer, Korea).

Table-1: The Primers, sequences, gene bank accession number, and references

Primer		Sequence	Reference
GAPDH	F	ATGGGAGTTGCTGTTGAAGTCA	[25]
	R	CCGAGGGCCCACTAAAGG	
Fos	F	CTCTGACTCGCTCAGCTCAC	This study
	R	CAGGAACCCTCTAGGGAAGA	
JunD	F	GACATGGACACGCAGGAG	This study
	R	CCGTGTTCTGACTCTTGAGG	
JunB	F	CCATCAACATGGAAGACCAA	This study
	R	TTGAGCGTCTTCACCTTGTC	

Quantitative Reverse Transcription Real-Time PCR (RT-qPCR):

Real-Time PCR technique (Quantitative Reveres Transcription) was used for measurement(quantitation) of the relative and comparative gene expression analysis. This assay was done depending on the technique of [26].The reaction conditions of Thermocycler protocol were shown in the following (table-2) .

Table -2: Thermocycler protocol

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	3 min	1
Denaturation	95 °C	20 sec	45
Annealing\Extension Detection(scan)	60 °C	30 sec	
Melting	60-95°C	0.5 sec	1

Statistical Analysis:

The results that obtained by this study were analyzed statistically by the using of statistical package SPSS specialized program (Statistical Package for Social Sciences) version 10.0 for windows. All the tested parameters were written as mean ± standard error (S.E.). All the differences between and among the tested parameters were listed in ANOVA (analysis of variance), the least statistical significant differences (LSD) also used to improve the valuable changes in data on the probability (*P*) value was ≤ 0.05 [27].

Results

Interleukin-17A(IL-17A) :

The results of this study showed that there was a highly significant increment in the concentration of IL-17A(139.5±17pg/ml) compared with a control group which was (41.33±11.3pg/ml). as shown in figure 1**.**

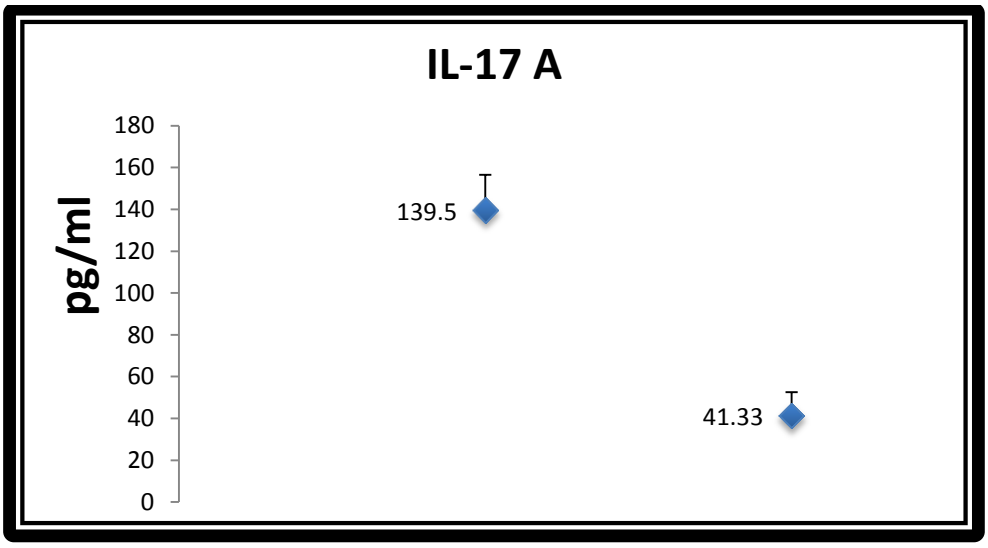

Figure 1: The levels of IL-17A in sera of breast cancer patients compared with control group.

Interleukin- 23P19 (IL-23P19) : The results of this study showed that there was a highly significant increment in the concentration of IL-23P19 (192.73±22.3pg/ml) compared with a control group which was (121.41±14.7pg/ml). as shown in figure 2.

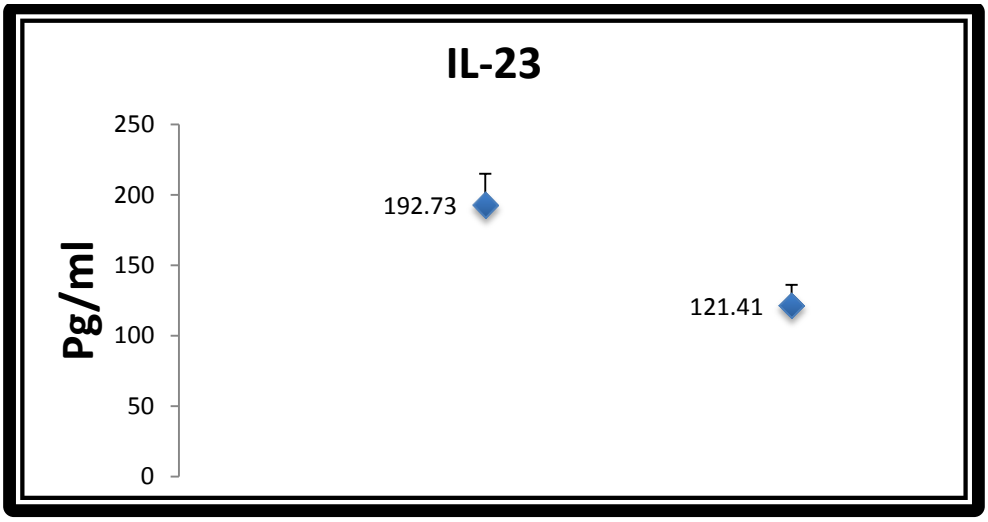

Figure 2 : The level of IL-23P19 in sera of breast cancer patients compared with control group.

Interleukin- IL-22 (IL-22) : The results of this study showed that there was a highly significant increment in the concentration of IL-22 (137.25±24.6pg/ml) compared with a control group which was (77.67±13.7pg/ml). as shown in figure-3.

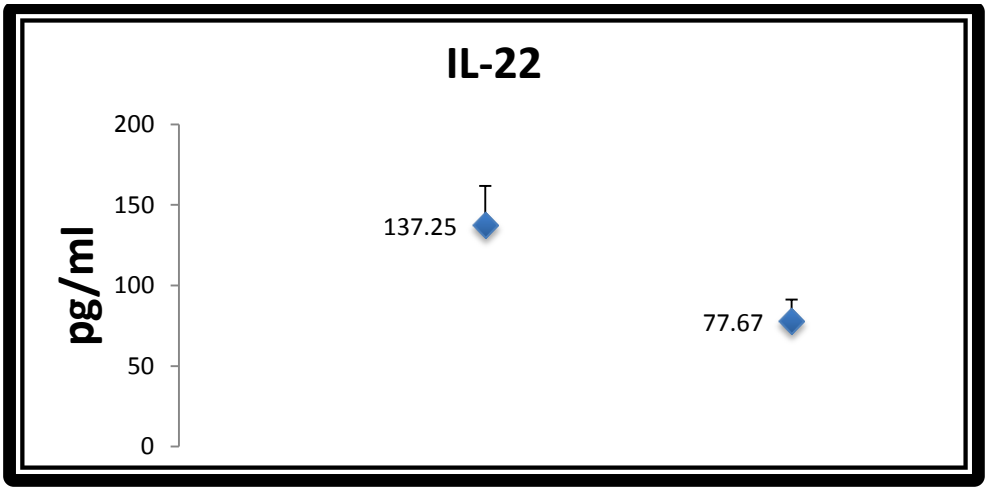

Figure 3 : The level of IL-22 in sera of breast cancer patients compared with normal group.

Tumor necrosis factor- alpha (TNF-α) :

The results of this study showed that there was a highly significant increment in concentration of TNF-α (200±23.7pg/ml) compared with a control group which was (10.5±7.3pg/ml). as shown in figure 4.

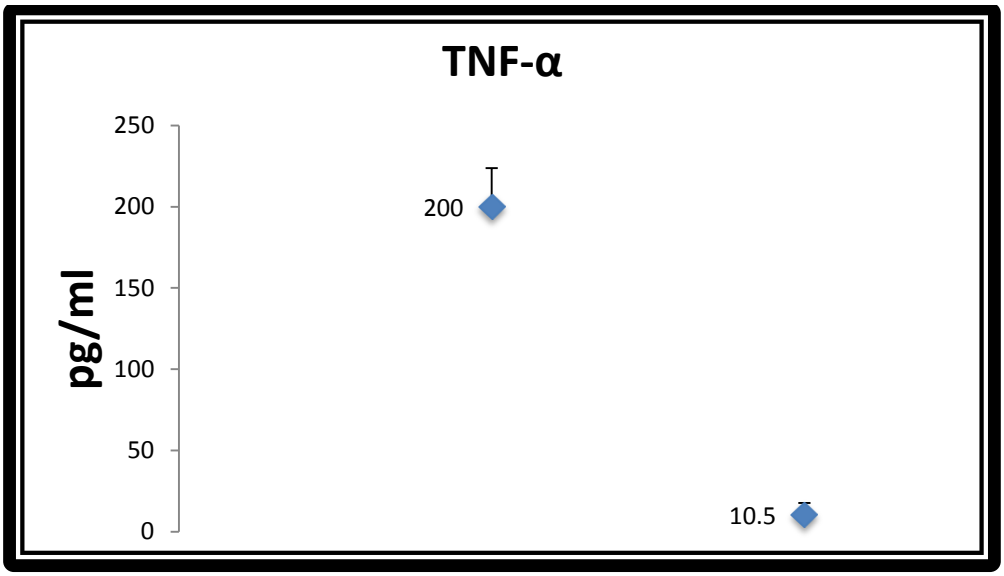

Figure 4 : The level of TNF-α in sera of breast cancer patients compared with control group.

AP1 Genes Expression:

Collected results indicated a remarkable increment in the levels of the gene expression by using the technique of real time-PCR, which were Fos 18.76±7.8 in patients group and 9.05±1.12 control group, also the values of JunD gene expression 15.77±9.43 and 6.18±0.112, and lastly JunB 23.223±11.34 in comparison to control group7.33±3.76. All these findings were statistically significant at the ($p \leq 0.05$) Table -3.

Table-3: AP1 genes expression in breast cancer group and control group.

Gene	Patients group	Control group
Fos	18.76±7.8	9.05±1.12
JunD	15.77±9.43	6.18±0.112
JunB	23.223±11.34	7.33±3.76

Discussion

This study showed a significant increase in the levels of IL-17A , IL-23 ,IL-22, And TNF- α in sera of patients with breast cancer in women. The differentiation of human naïve CD4 +T cells can be resultTh-22 or Th-17 cells by the coordination of the action of IL-6 ,IL-1β and IL-23[25].

Also the obtained data in this work reveled a significant increment in IL-23 of patients with breast cancer in comparison to control which may due to the fact, that IL-23 receptor (IL-23R) is crucial factor of Th-17 cell-mediated processes, which have potent role in the pathogenesis of the cancer[26]. Both Th-22 and Th-17 cells have complex role and controversial in tumor pathology and suggesting that have a fluctuating identity within the patient of cancer[27,28].Finding of the current study declared an increment in IL-17A of patients with breast cancer compared with control, this finding support the opinion that the role of effector cells and its activity in the orchestrating the events of the inflammatory response in which cells determine the required activity in cancer such as the switching on the cellular response[30].

IL-22 concentrations were also elevated in patients, IL-22 is secreted by Th-22 and by innate lymphoid cells [29,31]. This cytokine bind to class II receptor and its can modulate the intracellular events as a response to external signal driving the epithelial cells to proliferate and tumor-genesis of breast cells [28]. TNF-α as general inflammatory cytokine expected to be elevate as shown in the obtained results in the group of patients with breast cancer in comparison to control, TNF-α usually represent estrogen receptor positive (ER+) breast cancer, importantly, it responsible for maintaining cancer-associated fibroblasts in an undifferentiated status, resulting in the increased transcription and activity of key estrogen-producing enzymes such as aromatase. High levels of TNF-α are detected within the tumor microenvironment, and though infiltrating immune cells are thought to contribute a significant amount of TNF-α [31].

During the cellular signal transduction, The AP-1 transcription factor is a key component. AP-1 family of transcription factors consists of three subunits, this transcription factor have specific DNA sequences to bind during the cellular proliferation[24]. AP-1 has been appeared to regulate target genes by binding to consensus DNA-regulatory elements, known as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) response elements (TREs) [23,24,25].
The obtained data reflect the fact of the medical importance of the AP1 transcription factor during the breast cancer patients which may explain the role of cellular immune mechanism against the tumor, where cell-mediated cytotoxicity occur. AP-1 activity in drug resistant human breast cancer increased usually [22]. Also in another study , it was noticed that the transcription factor AP-1 contributes to the EpCAM dependent breast cancer invasionand also regulated by RNA binding protein RBPMS1which repress the AP-1 [36,37].

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