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Molecular expression of inflammatory cytokines (interleukin IL-1B, IL-6, IL-10 and INF-ɤ among patients with breast cancer

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Summary

The total number of breast cancer patients involved in this study are (80) individual were taken from oncology center in Basrah province, the age of patients range from 27-76 yrs and (80) individuals considered as control group after they were checked and confirmed to be free from any chest or any other health problems. the maximum number of breast cancer females was found within the age groups 50-59 yrs (32.5%), fand the lowest rate in age group 20-29 (2.5%), with P =0.0163. PCR amplification results which was done on the extracted DNA have been confirmed by using gel electrophoresis which appear as compact DNA separated bands which were results from the accurate and specific binding between the target DNA template for IL-1β and its specific primer, only bands with expected molecular size 249bp were observed. The target DNA template for IL-6 and its specific primer, these bands viewed with specific size 408pb. the target DNA template for IL-10 and its specific primer, these bands viewed with specific size 295pb. The target DNA template for IFN-γ and its specific primer, these bands viewed with specific size 262 pb.

Keywords

breast cancer, PCR, IL-1B, IL-6, IL-10 and INF-ɤ

Breast cancer is the most prevalent cause of cancer death and the most common type of cancer in women, with a 9 percent lifetime diagnosis rate (Lacroix and Leclercq, 2005). According to the most recent Iraqi Cancer Registry, breast cancer is the most common type of malignancy in women, accounting for one-third of all reported female cancers.

Cytokines are macromolecules with biological features that imply they play a crucial role in infections, hematopoiesis, and homeostasis, indicating their multifunctional significance in governing immune responses as well as cancer by controlling tissue regeneration, cellular sprouting, and growth. ILs are secretory immunomodulatory proteins that belong to the cytokine superfamily and have complex immunological roles as cytokines. The primary function of ILs in the immune system is to mediate intercellular communication, which includes cell migration, proliferation, maturation, and adhesion, all of which are important in the inflammatory response (Dmitrieva, et al.,2016). Both acute and chronic inflammatory reactions require interleukins (Fasoulakis, et al.,2018). Kim, et al. (2011) discovered that standard RT-PCR for NB detection is quick, easy, inexpensive, and widely available.Using the Basic Local Alignment Search Tool, the sequences acquired were validated as NB by comparing them to those in the Gene Bank (National Center for Biotechnology Information) database.

The goal of this study was to employ conventional (PCR) to detect molecular expression of interleukins IL-1B, IL-6, IL-10, and INF- among females with breast cancer.

Materials and methods

Breast cancer Cases

Breast cancer cases in this study (case\control study) have been collected in Basra province particularly which attending Al-Basra Oncology Center, in period was extended from January to May 2021. All medical information for patients were recorded in questionnaire paper that included name, age, residency and marital status, breast Cancer females who are in the early stages, second stages, and the last stages after taking all the treatments. All information's were collected from patients after the accurate diagnosis by the oncologist which confirmed by other findings (Cystoscopy, biopsy, ultra sound and CT scan).

DNA extraction

EasyPure Blood Genomic DNA Kit isolates high-quality genomic DNA from 5-250 gl of fresh or frozen blood in a simple and convenient manner. To liberate DNA, whole blood is treated with binding/lysis buffer.

DNA amplification

Conventional PCR was used for the amplification of patients DNA samples to obtain the targeted genes by using their specific primers as in (Tables.1,2,3 and4) according to manufacture instruction of DNA extraction kit (EasyPure® Blood Genomic DNA Kit) where use in our study.

Table (1). the components of DNA extraction kit

Component	EE121-01 (50 rxns)	EE121-02 (200 rxns)
	E E121-11 (50 rxns)	EE121-12 (200 rxns)
Binding Buffer 3 (BB3)	30 ml	110 ml
Clean Buffer 3 (CB3)	6 ml	24 ml
Wash Buffer 3 (WB3)	12 ml	2*22 ml
Elution Buffer (EB)	25 ml	80 ml
RNase A (20 mg/ml)	500 µl (EE121-01)	2Ч1 ml (EE121-02)
	0 (EE121-11)	0 (EE121-12)
Proteinase K (20 mg/ml)	1 ml	4Ч1 ml
Genomic Spin Columns with Collection Tubes	50 each	200 each

Table (2) PCR master mix volume

component	volume
10 µM Forward Primer	0.5 µl
10 µM Reverse Primer	0.5 µl
Template DNA	3.5 µl
OneTaq Quick-Load 2X Master Mix with Standard Buffer	12.5 µl
Nuclease-free water	10 µl
total	27 µl

Table (3) Illustrate the primers sequences and product size

Genes	Primers sequence	Product size (bp)	Referance
IL-1B1	F: GTTGTCATCAGACTTTGACC	249	Conti,et al.,2000
	R: TTCAGTTCATATGGACCAGA		
IL-6	F: GCG ATG GAG TCA GAG GAA AC	408	Liaquat,et al.,2014
	R: ATC TTT GTT GGA GGG TGA GG		
IL-10	F: GTCAGTGTTCCTCCCAGT	295	Giordani,et al.,2003
	R: TTACCTATCCCTACTTCCTC		
INF-γ	F: TTCTTACAACACAAAATCAAATCT	262	Karakus,et al.,2011
	R: TCAACAAAGCTGATACTCCA'3		

Forward: (5´-3´)

Reverse: (5´-3´)

Gene	Temperature (°C)\ Time					Cycle No.
	Initial denaturation	Cycling temperature			Final extension	
		Denaturation	Annealing	Extension		
IL-1β	94єc \30sec	94єc \30 sec	45-68єc \ 15-60 sec	68єc\1 min	68єc \5 min	30
IL-6	94єc \30sec	94єc \30 sec	45-68єc \ 15-60 sec	68єc\1 min	68єc \5 min	30
IL-10	94єc \30sec	94єc \30 sec	45-68єc \ 15-60 sec	68єc\1 min	68єc \5 min	30
INF-γ	94єc \30sec	94єc \30 sec	45-68єc \ 15-60 sec	68єc\1 min	68єc \5 min	30

Results

Distribution of patients within various age groups.

Table (5) show the minimum number of breast cancer patients was found within the age groups 20-29 yrs (2.5%),followed by age group 30-39 yrs (12.5% ,above 40-49yrs (31.25%), the age group 50-59 yrs (32.5%) and >60 yrs (21.25%). P-value <0.0163.

Table (5): The number and percentages of patient with breast cancer and control group according to age groups.

Age Groups	Breast Cancer		Control		P-value
	No.	%	No.	%	
20 -29 year	2	2.5	2	2.5	0.0163
30 -39 year	10	12.5	8	10.00	
40 -49 year	25	31.25	23	28.75	
50-59 year	26	32.5	24	30.00	
>60 year	17	21.25	23	28.75	
Total	80	100%	80	100%	

P = 0.0163, xI = 20. 50, Df = 1

Molecular study

DNA extraction of whole blood from patients with breast cancer at various stages and control group then followed by DNA amplification through using conventional PCR technique and specific primers and DNA sequencing for each gene.

DNA extraction, very high quality and purity DNA have been extracted from whole blood of patients with breast cancer and control group by using DNA extraction kit (EasyPure® Blood Genomic DNA Kit USA) Figuer (1) show the The look of Genomic DNA extracted on an agarose gel electrophoresis stained with Ethidium Bromide.

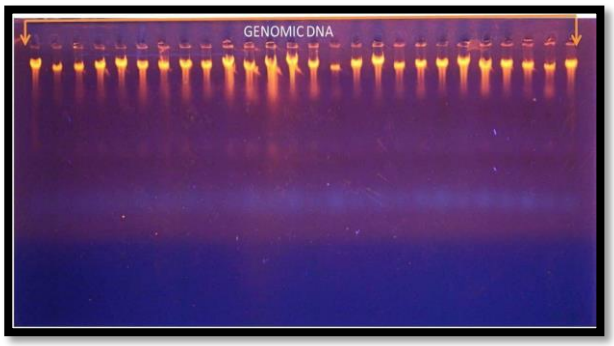

Figure (1): Ethidium Bromide-stained agarose gel electrophoresis appearance that displays Genomic DNA that was extract

DNA amplification

DNA extracted from whole blood samples have been amplified by using conventional PCR, then PCR product results confirmed by using 1% gel electrophoresis, in this analysis the DNA band that appear on the gel after successful attachment between extracted DNA template and the target specific primer for each one of interleukins (IL- 1β, IL-6, IL-10 and IFN-γ) that have been used in this study, only bands with expected molecular size 249bp, 408bp, 295bp ,and 262 bp respectively were observed for each interleukins. As shown in the figure (2, 3, 4, 5)

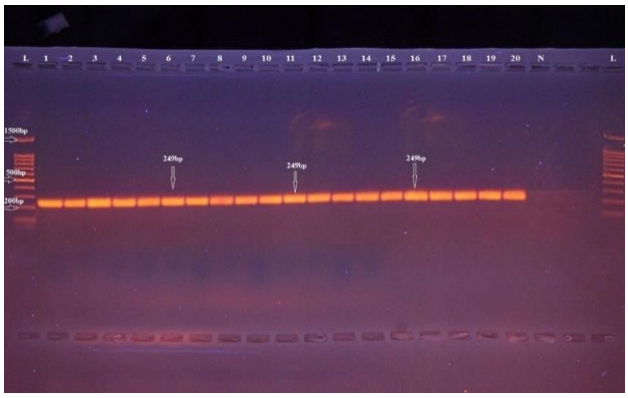

Figure (2): - Gel electrophoresis of the (IL1B1primer) PCR product, which shows 249bp Primer, L: ladder (1500-100) bp, Lanes (1-20) positive findings, and Lane N: negative control.

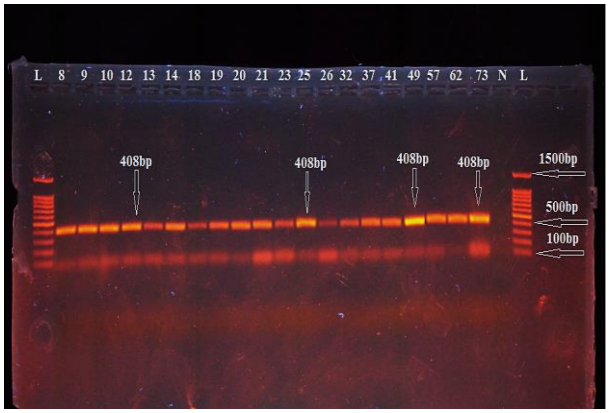

Figure (3): - Lane L: ladder (1500-100)bp, Lanes (1-20) represented positive results, and Lane N: negative control on gel electrophoresis for PCR product of (IL6 primer).

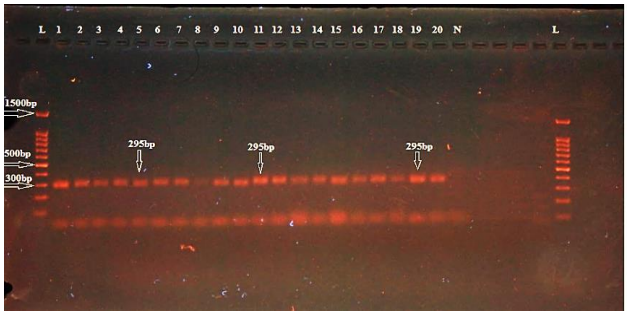

Figure (4): - Gel electrophoresis for PCR product of (IL10 primer) which show 295bp Primer, L: ladder (1500-100) bp, Lanes (1-20) represented positive results , N: negative control .

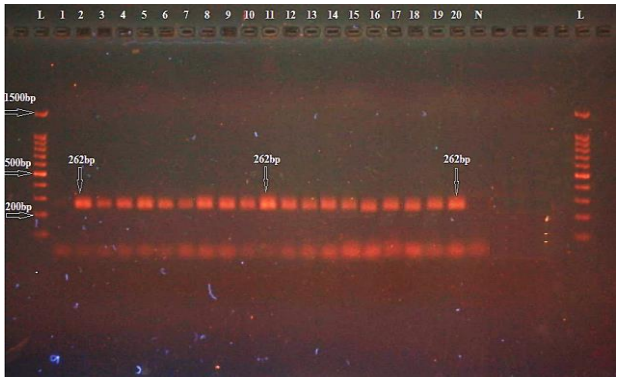

Figure (5): - Gel electrophoresis for PCR product of (INF-gamma primer) which show 262bp Primer, Lane L: ladder (1500-100)bp , Lanes (1-20) represented positive results, N: negative control .

Discussion

From total number of (80) patients with breast cancer, The majority of disease was in age group 50-59 yrs (32.5%), and the lowest infection was in age group 20-29 yrs (2.5%) with significant differences (P≤0.05). This results was similar with other studies (Andre, et al., 2013). One of the most important risk factors for breast cancer is age; the risk of developing breast cancer rises progressively as one gets older. Breast cancer is uncommon before the age of 25, and when it does develop, it is more aggressive. There is a significant increase after the age of 30, with more than 80% of instances in women identified after the age of 50, with the greatest rate of increase occurring before menopause, indicating a link with hormonal condition (Fourkala, 2011). Aging-related risk may be partly attributed to genetics (Hoeijmakers, 2009).

Interleukin-1 (IL-1 β) is a pro-inflammatory cytokine whose presence in primary tumors has been discovered as a potential biomarker for identifying breast cancer patients who are at an elevated risk of bone metastases. The quintessential proinflammatory cytokine is interleukin-1 β (IL-1 β) (Filipi, et al., 2015), and its expression in most cancers correlates with tumor invasiveness, metastasis, and angiogenesis (Naldini, et al.,2005). Several studies have shown how IL-1β may contribute to breast cancer development and metastasis among it multiple effects, (Filippi, et al.,2015).

IL-6, in particular, has been implicated in the start and maintenance of neuropathic pain due to its function in the inflammatory process following nerve injury (Lee, et al.,2004). However, the location of receptors on distinct cell types to which IL-6 can bind determines its function. This indicates that only IL-6R+ cells are capable of responding to IL-6 alone, whereas practically all cells respond to the IL-6/sIL-6R complex. Unlike other soluble cytokine receptors, such as those for TNF-alpha, sIL-6R prolongs the half-life of IL-6 and enhances its inflammatory effects by allowing gp130 + cells to respond to IL-6 (Ernst, et al.,2004).

Activated T cells, monocytes, B cells, and thymocytes are the main producers of IL-10, an important immunoregulatory cytokine. IL-10 can both stimulate and decrease the immune response as an immune response modulator (Mocellin, et al.,2005). IL-10 has been implicated in the development of cancer in a number of investigations, however the results have been mixed. Increased serum IL-10 levels, on the one hand, may aid cancer development by reducing MHC class I and II antigen expression and preventing tumor antigen presentation to CD8-cytotoxic T cells. The antiangiogenic properties of IL-10, on the other hand, are thought to protect and prevent tumors (Kong, et al.,2010).

IFN-γ is a cytokine with immunoregulatory and antiproliferative properties (Karakus,et al.,2011). Many tumor-derived cell lines, including breast cancer cell lines, are inhibited by IFN- (Ruiz, et al., 2000). Low levels of IFN- stimulate tumor growth, whereas high levels of IFN-g have anticancer activity (He, et al., 2005). The IFN- gene is found on chromosome 12 (12q24.1long)'s arm (Tegoshi, et al., 2002). A T>A polymorphism at the 874 position in the first intron of the IFN gene has been found, and this polymorphism overlaps with a putative NF-kB binding site, which could have functional implications for the transcription of the human IFN-g gene (Pravica,et al.,2000).

Using the Basic Local Alignment Search Tool, the sequences acquired were validated as NB by comparing them to those in the Gene Bank (National Center for Biotechnology Information) database, also some studies used PCR in diagnosis of breast cancer (Kim.,2007) , One of methods of PCR is multiplex PCR, which is a single step

method that employs one tube and permits the simultaneous detection of more than one organism in prostatitis (Zhou, et al., 2016). Using particular primers for conventional PCR, DNA was extracted from the blood of breast cancer patients, these patients, and the Control group. The molecular results of current study which performed by using conventional PCR to amplify IL-1β, IL-6, IL-10 and IFN-γ genes showed the percentage of the four genes which involved in this study among transitional cell breast cancer patients. There were no previous study isolated IL-1β, IL-6, IL-10 and IFN-γ from DNA samples of patients with breast cancer. Joshi et al., (2014) describe how the interleukin-4 receptor (IL-4R) is overexpressed in a variety of human malignancies and can act as a target for the IL-4 immunotoxin, which is made up of IL-4 and a mutant Pseudomonas exotoxin.

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