دور الحركيات الخلوية الالتهابية
IL-10 و IL-6 و IL-4 لمرضى سرطان المثانة

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الملخص

خلفية وهدف البحث: الهدف من هذه الدراسة هو تحديد التعبير الجزيئي المناعي للإنترلوكينات الالتهابية IL–4 و IL–6 و IL–10
مواد وطرق البحث: استخدام ELISA وتقنيات PCR التقليدية والتسلسل، لإعطاء الاعتراف بأدوار هذه الإنترلوكينات في مرضى سرطان المثانة في محافظة البصرة. شملت دراسة الحالات والشواهد 85 من مرضى سرطان المثانة المؤكدين و 80 فردًا كمجموعة ضابطة. تم جمع البيانات حول العمر والجنس والتدخين وشرب الكحول والتاريخ العائلي والمهنة والإقامة والنتائج السريرية لجميع المرضى المصابين بسرطان الظهارة البولية.
نتائج البحث: كشفت الدراسة الحالية عن الوزن الجزيئي لـIL–4) ، (IL–10 & IL–6والتي كانت على النحو التالي (180 قاعدة نايتروجينية، 600 قاعدة نايتروجينية و150 قاعدة نايتروجينية) على التوالي مع نسبة تعبير موجب ل (90.%) IL–4 (95٪) IL–6 و(% 60) IL–10
الاستنتاج : نستنتج أن الإنترلوكينات الالتهابية IL–4 و IL–6 وIL–10 مفيدة للتشخيص المبكر لسرطان المثانة

الكلمات المفتاحية: الإنترلوكينات، سرطان المثانة، تفاعل البوليميراز المتسلسل

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The role of inflammatory interleukins (IL-4, IL-6 & IL-10) of bladder cancer Patients

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Abstract

The aim of this study was to determine Immunomolecular expression of inflammatory interleukins (IL-4, IL-6 & IL-10) by using ELISA, conventional PCR and Sequencing technologies, to acknowledge the roles of these interleukins in patients with bladder cancer in Basrah province. a controlled case study included 85 confirmed bladder cancer patients and 80 individuals as a control group. Data about age, gender, smoking, alcohol drinking, family history, occupation, residency and clinical findings for all patients with urothelial carcinoma were collected. The current study detects the molecular weight of (IL-4, IL-6 & IL-10) which were as follow (180bp, 600bp & 150bp) respectively with positive expression percentage for IL-4 (90%), IL-6 (95%) and IL-10 (60%).

Conclusion: We conclude that inflammatory interleukins (IL-4, IL-6 & IL-10) are usefull for early diagnosis of bladder cancer

Key words: interleukins, bladder cancer, PCR

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Introduction

Urothelial carcinoma is a disease in which the lining of the bladder lose their ability to regulate their growth and begin to divide uncontrollably, this abnormal growth can form a tumor, This abnormality may be caused by secondary chronic inflammation of lower urinary tract, stones, smoking and exposure to various chemicals, exposure to carcinogens products and compounds, and secondary schistosomiasis (Cohen *et al.*, 2000). Bladder cancer is a heterogeneous either, low-grade, superficial papillary lesion or high-grade, invasive tumor that usually has metastasized at the time of presentation (Ferri, 2003).

Transitional cell carcinoma accounts for almost 5% of all human cancers and represents 95% of all urothelial tumors (Ashoor, 2007). It is the second most common tumor of the genitourinary tract, it's also the second most common cause of death from these cancers (Williams *et al.*, 2001). It is the most common malignant tumor in the Western countries and the fifth most common cancer among males with an incidence of 29.8 per 100.000 males per year (Ashoor, 2007).in addition, it is the most common malignant tumor in the Middle East and Africa where Schistosomiasis is a prevelant problem (Kadhim, 2009). In Iraq it's the third most common malignant tumor with incidence 6.6% in both males and females reported by Iraqi cancer registry (ICR), It's the second most common tumor in males (10.3%) and the eighth in females (3%)(ICR, 2000).

Genomic testing examines the DNA of a tumor to find alterations that may be driving the growth of cancer. By identifying the mutations (changes) that occur in a cancer cell's genome, doctors may better understand what caused the tumor and tailor treatment based on these findings. (CTCA, 2021) In this era of the Human Genome Project, quantitation of gene expression in tumor or host cells is of paramount importance for investigating the gene patterns responsible for cancer development, progression and response or resistance to treatment. Quantitative realtime PCR (qrt-PCR) technology has recently reached a level of sensitivity, accuracy and practical ease that supports its use as a routine bioinstrumentation for gene level measurement. Several applications have already been implemented in the field of cancer research, and others are being validated, showing that this molecular biology tool can provide both researchers and clinicians with precious information concerning the behavior of tumors (Mocellin, *et al.*, 2003). The nested RT-PCR assay was used to analyze the CK20 transcript in the peripheral blood, bone marrow, lymph nodes, the tumor and normal biopsies of bladder from patients with invasive TCC of the bladder (Ribal, *et al.*, 2006).

Using multiplex-PCR assays for the detection of circulating tumor cells in peripheral blood and urine samples of patients with bladder cancer (Leotsakos, *et al.*, 2014).

Materials and methods

Sampling

A Case-control study was conducted between October 2020 to July 2021 which carried for patients with bladder carcinoma according to minimum sampling size equation that depend on the disease ratio, the total number of bladder cancer patients involved in this study are (85) individual were taken from Basrah oncology center in Basrah province, the age of patients range from 30->60 years and (80) individual considered as control group after they were checked and confirmed to be free from any urological or any other clinical problems. during collection process data about each patient were reported in questionnaire paper for each one, which included age,

gender, family history, smoking, alcohol drinking, occupation, residency and clinical findings. Five ml of venous blood was taken from 165 participant (85 for patients and 80 for control), 2ml kept in EDTA tube for molecular study (nucleic acid extraction) which kept at -20 for preservation prior use. All controls that involved in this study (80 individual) were checked to be sure that they were free from any urological disease, tumor, allergies and other infectious disease.

Conventional PCR Technique

DNA extraction: very high quality and purity DNA have been extracted from whole blood of patients with bladder cancer and control group by using DNA extraction kit (ZYMO RESEARCH, Quick-DNA™ Miniprep kit) which give very high quality and quantity of DNA and every sample was extracted separately and then run on gel electrophoresis for result confirmation.

primers of the markers that used in the study: Table (1) Illustrate the primers sequences and product size

Table (1) show Interleukins primer.

Genes	Primers sequence	M.W	Referance
IL-4	F: CCCCCACCAGTGGCTACC	182bp	(Mohan, *et al.*,2009)
	R: CCAGGAATGAGGTCTTGGAA		
IL-6	F: TAGCCGCCCCACACAGACAG	600bp	(Nakanishi, *et al.*,2004)
	R: GGCTGGCATTTGTGGTTGGG		
IL-10	F: TGAGGTGGTGTACTACCATA	149bp	(Nishimura, *et al.*,1992)
	R: GATCATGCCATTGCACTCTA		

Preparation of agarose gel: 1% of agarose gel was Prepared by mixing 1 gram of agarose powder with 100ml of already prepared TBE buffer in Pyrex conical flask, then dissolved the mixture very well in microwave oven for about 4 min at medium temperature until it start boiling with no thread appearance throughout agarose liquid, allow the agarose to cool until 50 C° then ethidium bromide was added to the gel (5µl of the stain per 100ml of agarose gel), after that the gel poured into the mold and let it at room temperature to solidify and be ready to use.

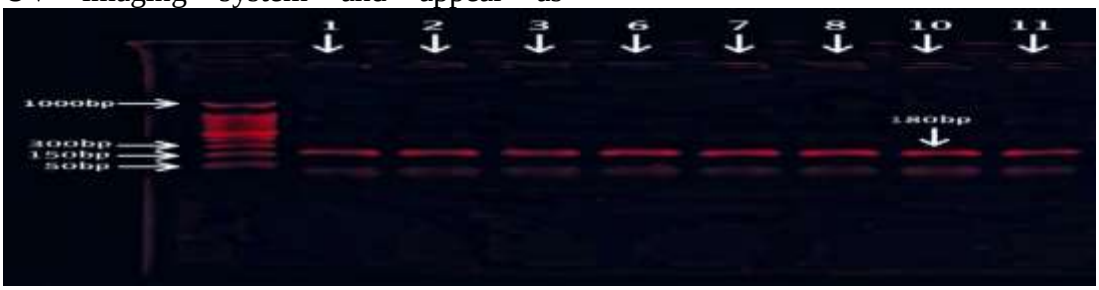
Statistical analysis Statistical analysis was carried out by using SPSS VER.23 two way T test (student's T-test) and chi square to find out the statistical differences between all variables. probability less than 0.05 is significant ($P<0.05$).

Results

DNA amplification: DNA extracted from whole blood samples have been amplified by using conventional PCR, then PCR product results showed 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 that have been used in this study(IL-4,IL-6 and IL-10), the bands appeared under UV imaging system as orange compact bands due to using ethidium bromide stain as indicator DNA stain, gel electrophoresis DNA band have been estimated by using (100-1500bp) & (50-1000bp) DNA ladder as DNA band size indicator, the results shown amplified DNA for each interleukin.

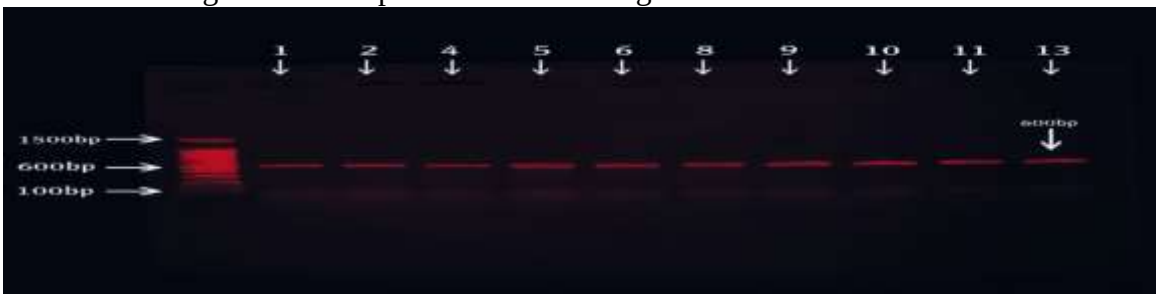
Interleukin 4: PCR amplification results which was done on the extracted DNA have been confirmed by using gel

electrophoresis which appear as compact DNA separated bands which were results from the accurate and specific binding between the target DNA template and its specific primer, these bands viewed under UV imaging system and appear as illuminating orange bands due to ethidium bromide stain, only bands with expected molecular size 180bp (IL-4 specific primer) were observed. As shown in the figure below.



Figure(2) show positive PCR amplification result for IL-4 which have 180bp M.W in compression with ladder size, lane(1,2,3,6,7,8,10) for patients and (11) for control were positive samples on 1% agarose gel on (70 voltage for 45 min)

Interleukin 6: 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-6 and its specific primer, these bands viewed under UV imaging system and appear as illuminating orange bands due to ethidium bromide stain, only bands with expected molecular size 600bp (IL-6 specific primer) were observed. As shown in the figure below.



Figure(3) show positive PCR amplification result for IL-6 which have 600bp M.W in compression with ladder size, lane(1,2,4,5.6,8,9,10 ,13) for patients and (11) for control were positive samples on 1% agarose gel on (70 voltage for 45 min)

Interleukin 10: 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-6 and its specific primer, these bands viewed under UV imaging system and appear as illuminating orange bands due to ethidium bromide stain, only bands with expected molecular size 150bp (IL-10 specific primer) were observed. As shown in the figure below.

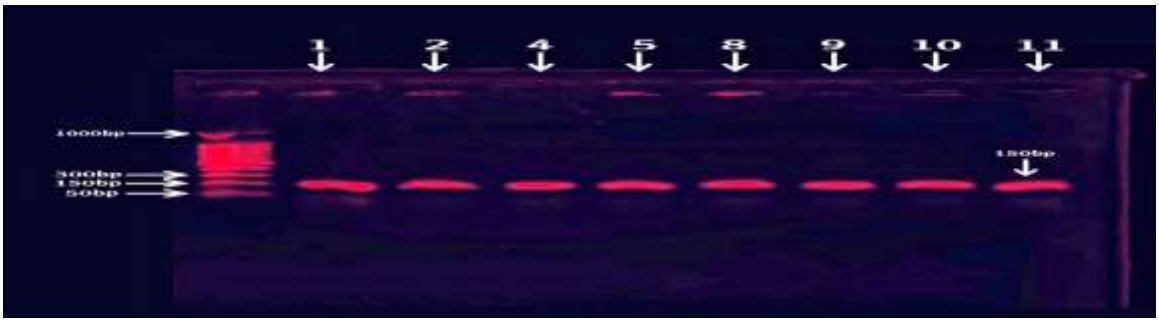


Figure (4) Show positive PCR amplification result for IL-10 which have 150bp M.W in compression with ladder size, lane(1,2,4,5.,8,9,10) for patients and (11) for control were positive samples on 1% agarose gel on (70 voltage for 45 min).

Presence of IL-4, IL-6 and IL-10 genes in patients and control groups: Table below show the number and percentage of the three genes which involved in this study among patients with bladder cancer and control, as we see IL-6 gene show the highest percent which was 95% as a total followed by IL-4 was (90%), and IL-10 gene show the lowest percent was 60%.

Table (2): Show IL-4, IL-6 and IL-10 genes number and percentage.

Gene	Males				Female				Total		
	Total No	%	Positive No	%	Total No	%	Positive No	%	Total No	Total Positive No	%
IL-4	16	80	15	93.75	4	20	3	75	20	18	90
IL-6	16	80	15	93.75	4	20	4	100	20	19	95
IL-10	16	80	9	56.25	4	20	3	75	20	12	60

Table (3) Show IL-4, IL-6 and IL-10 genes number and percentage for patients and control group.

Gene	Patients				Control			
	Total No	%	Positive No	%	Total No	%	Positive No	%
IL-4	20	100	18	90	1	100	1	100
IL-6	20	100	19	95	1	100	1	100
IL-10	20	100	12	60	1	100	1	100

Discussion

The molecular result of current study which performed by using conventional PCR to amplify IL-4, IL-6 and IL-10 genes showed that the percentage of the three genes which involved and express in this study among transitional cell carcinoma patients for both gender, as we see IL-6 gene show the highest percentage which is 95% as a total for both male and females, and IL-10 gene show the lowest percentage which is 60%. There were no previous study isolated IL-4, IL-6 and IL-10 from DNA samples in patients with urothelial carcinoma.

(Joshi *et al.*, 2014) explain that interleukin-4 receptor α (IL-4Rα) is overexpressed on a various human cancers and can serve as target for IL-4 immunotoxin constitute of IL-4 and a mutated *Pseudomonas* exotoxin. but, its expression and correlation with grade and clinical stage of bladder cancer has not been studied. (Chen, *et al.*, 2013) Study data suggest that IL-6 was overexpressed in the urothelial carcinoma

specimens compared with non-malignant tissues at both mRNA and protein levels.

(Hedrich & Bream, 2010) explained that human IL-10 expression patterns appear to be under genetic influence resulting in differential expression and disease susceptibility.

(Abdulwahhab, *et al.*, 2021) this study contributes to a growing body of work on gene expression signatures in bladder cancer and urine samples collection from Bladder cancer early detection center in Mosul, during the study period patients N: 101 and controls N: 19 .The final study is to establish a concept of stratification of patients with bladder cancer. For this, they have developed a multi-parameter test for the quantification of the selected biomarkers.

(Cao, *et al.*, 2018) Long Non-Coding RNA Expression Profiles for the Characterization of Different Bladder Cancer Grade, Study findings revealed the significant role of lncRNAs in the development process of bladder cancer.

(Lipunova, *et al.*, 2019) the study suggested that all UBC prognostic outcomes may have different biological origins with limited overlap. Further validation of these observations is essential to target a phenotype that could best predict patient outcome and advance current management practices.

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