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IL-18 Gene Polymorphisms Impacts on Its Serum Levels in Prostate Cancer Iraqi Patients

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Abstract

Prostate cancer is one of the most common types of cancer in men. A total of 110 Iraqi Arab individuals were included in this study; 60 individuals of them had prostate cancer with increased levels of TPSA (patients group); their age range 52-90 years. They were referred for diagnosis and treatment to the National Al-Amal Hospital for oncology in Baghdad during the period from July 2017 to October 2017. While the other 50 apparently healthy subjects were the control group, their age range similar to patients group. Sera and blood samples were collected from all patients and controls than used to assess for the level of IL-18 and DNA extraction, respectively. The polymorphisms were analyzed using polymerase chain reaction-single specific primer (PCR-SSP), at the position -137 G/C (rs187238) in the promoter of *IL18* gene. The genetic polymorphism of the *IL18* gene promoter -137G/C (rs187238) was determined and presented with three genotypes (GG, GC, and CC) in prostate cancer patients and controls. Testing for Hardy-Weinberg (H-W) equilibrium revealed that Prostate cancer patients showed insignificant variation in the distribution of IL-18 -137 genotypes ($P > 0.05$). While the control samples showed significant variation ($p \leq 0.05$) between the observed and expected. Comparing patients with controls indicated that IL-18-137 alleles or genotypes showed no association with the risk of prostate cancer development in Iraqi Arab population or protection against them. Serum level of IL-18 was highly significant ($P \leq 0.001$) increased in patients compared to control. The IL-18 serum levels differences in GG and GC genotypes was significant ($p < 0.05$) between patients and control. While there were no significant differences between the three IL-18 -137 genotypes inpatient or in controls.

Keywords: Human prostate cancer, IL-18 gene polymorphisms, Odds ratio, ELISA, Iraq.

تأثير تعدد الإشكال الوراثي للحركي الخلوي - 18 على مستواه في مصل دم مرضى سرطان البروستات
العراقيين

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الخلاصة

سرطان البروستاتا هو واحد من أكثر أنواع السرطان شيوعاً لدى الرجال. شملت هذه الدراسة 110 شخص عراقي عربي، 60 شخصاً منهم مصابون بسرطان البروستاتا مع ارتفاع في مستوى الكلي ل PSA (مجموعة المرضى)؛ تراوحت أعمارهم ما بين 52-90 سنة والذين تمت إحالتهم للتشخيص والعلاج إلى مستشفى الأمل الوطني لمعالجة الأورام في بغداد خلال الفترة من تموز 2017 إلى تشرين الأول 2017. بينما الخمسين الآخرون فهم من الأصحاء ضاهرياً (مجموعة السيطرة)، كانت أعمارهم تتشابه مع اعمار مجموعة المرضى. جمعت عينات دم وموصول من كل المرضى والأصحاء ومن ثم استخدمت لاستخلاص الدنا و لقياس مستوى الحركي الخلوي-18 على التوالي. تم تحليل تعدد الأشكال الوراثي باستخدام طريقة تفاعل انزيم البلمرة المتسلسل- للبريمر المفرد المتخصص في موقع -137G/C (rs187238) لبادئ حفاز الحركي الخلوي -18. وضهرت النتائج بثلاثة أنماط جينية (GG و GC و CC) في مرضى سرطان البروستاتا والسيطرة. كشف اختبار توازن هاردي- واينبرغ (H-W) أن مرضى سرطان البروستاتا أظهروا تبايناً غير معنوي ($P > 0.05$) في توزيع الأشكال الوراثية للحركي الخلوي -18 في الموقع-137. في حين أظهرت عينات السيطرة تبايناً معنوياً ($p \leq 0.05$) بينهما. كما أشارت المقارنة بين المرضى والأصحاء إلى أن الأنماط الوراثية والليلايتها للحركي الخلوي -18 في الموقع -137 لم تظهر أي ارتباط مع خطر الإصابة بسرطان البروستاتا لدى الأشخاص العرب العراقيين أو الحماية ضده.

وأظهر المستوى المصلي للحركي الخلوي -18 زيادة معنوية ($P \leq 0.001$) في مرضى سرطان البروستاتا مقارنة مع السيطرة. وايضا أظهر المستوى المصلي للحركي الخلوي -18 اختلافات معنوية ($p < 0.05$) في الأشكال الوراثية GG و GC بين المرض والأصحاء بينما لم تظهر اختلافات معنوية بين الأشكال الوراثية لمجموعة المرضى او مجموعة الأصحاء.

Introduction

Cancer is a major public health problem worldwide. Prostate cancer (PCa) is one of the most common types of cancer in men, occurs in the prostate (a small walnut-shaped gland in men) which produces the seminal fluid, nourishes and transports sperm. Prostate cancer disease incidences have risen significantly in developing and Asian countries including Iraq. Rendering to the data published by World health organization (WHO) in 2017, PCa mortality in Iraq reached 0.25%. Moreover, the death rate is 7.02 per 100,000 of the population; which makes Iraq ranked no. 152 [1]. Nevertheless, PCa etiological factors are exceedingly complicated, and possibly correlated with quite a few factors, comprising smoking, surrounding environment, nutritional habits, hormonal status, age, and race. Yet, the precise etiology and pathogenesis still unpredictable. Of considerable interest, around 42% of the PCa risk is attributed to heritable factors such as inflammation and genetic factors may have an essential role in the etiology of prostate cancer [2]. Several studies have proposed genes such as *HPC1*, *CAPB*, *BRCA1*, and *BRCA2*, as susceptibility genes for PCa. Chronic inflammation has been associated with increased risk in PCa. The initiation, maintenance, and pathology of the inflammatory response depend upon pro- and anti-inflammatory signals. Single-nucleotide polymorphisms (SNPs) in cytokine genes have been associated with increased inflammation, increased cytokine production and possibly increased PCa risk [3]. Among the cytokines involved in inflammation, Interleukin-18 (IL-18), is a cytokine with a proinflammatory biofunction and produced by activated macrophages, epithelial cells, osteoblasts, keratinocytes, and also cancer cells. The role of IL-18 still remains incompletely understood. IL-18 plays a central role in the inflammation and immune response; because IL-18 immune activating effects have also antineoplastic characteristics, it was enticing to propose IL-18 as a new adjuvant treatment against cancer. A quantity of IL-18 SNPs gene have been recognized, investigated and the DNA sequence variations in the promoter of *IL18* gene may up to altered IL-18 creation and/or activity, and so this can modify susceptibility of an individual's to PCa [4].

Little is known about the pattern of cancer in Iraq; no study has been performed for the detection of *IL18* gene polymorphisms in Iraqi prostate cancer patients. So the present study is designed to determine the *IL 18* gene polymorphism defined by polymerase chain reaction at -137 G/C position in Iraqi prostate cancer patients.

Materials and methods

Study subjects

A total of 110 individuals were included in this study, they were referred for diagnosis and treatment to the National Al-Amal Hospital for oncology in Baghdad during the period from July 2017 to October 2017. 60 individuals of them had prostate cancer and had an increased level of total prostate specific antigen (TPSA) (patients group); their age range 52-90 years. To eliminate the influence of other diseases, we excluded patients with infectious diseases and diabetes mellitus. The diagnosis was done by the consultant medical staff in this Hospital. While the others 50 apparently healthy Iraqi Arab subjects were the control group, their age range similar with patients group.

Assessment of Interleukin-18 serum levels

Serum specimens were obtained from all study subjects and assayed for the level of IL-18 using Human IL-18 Enzyme linked immunosorbant assay (ELISA) kit (MyBiosorce, USA) following the manufacturer's instructions.

DNA extraction

Patients and controls blood samples were collected in vacuum tubes containing 5% EDTA. DNA was extracted by the Wizard[®] Genomic DNA Purification Kit (Promega/USA) upon kit instructions.

Detection of *IL18* -137 G/C

The polymorphisms were analyzed using polymerase chain reaction-single specific primer (PCR-SSP), at the position -137 G/C (rs187238) in the promoter of *IL18* gene according to Giedraitis *et al.*, [5] with minor modifications. Reactions were carried out in a Bio-Rad PCR thermal cycler/ USA. For the -137 genotyping, PCR reaction was performed with a final volume of 20 μ l consisting of 5 μ l of 10 ng DNA template, 1 μ l of each of the reverse primer and sequence-specific forward primers, and 12 μ l of Nuclease - free water in Accupower[®] PCR PreMix tube. Regarding IL18 -137, a common reverse primer 5'-AGGAGGGCAAATGCACTGG-3' and two sequence-specific forward primers 5'-CCCCAACTTTTACGGAAGAAAAG-3' and 5'-CCCCAACTTTTACGGAAGAAAAC-3' were used. Reaction conditions consisted of initial denaturation at 94 °C for 2 min followed by 5 cycles of denaturation at 94 °C for 20 sec., and annealing at 68 °C for 1 min. then 25 cycles of denaturation at 94 °C for 20 sec., annealing at 62 °C for 40 sec. and extension at 72 °C at 40sec. lastly final extension at 72°C at 5 min. PCR products (261 bp) were visualized by 2% agarose gel electrophoresis stained by ethidium bromide.

Statistical analysis

All statistical analyses were performed using statistical package for social science (SPSS) program version 17 for windows (SPSS INC., Chicago IL, USA). Results were expressed as mean \pm SD. Comparisons between two groups were performed using T test for categorical data. P value of <0.05 were considered to indicate statistical significance.

Genotypes of *IL18* -137 were presented as frequencies percentage, and significant differences between their distributions in prostate cancer patients and controls were assessed by two-tailed Fisher's exact probability (P). Also, odds ratio (OR), the etiological fraction (EF) and a preventive fraction (PF) were calculated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists.

Allele frequencies of *IL18* -137 and -607 genes were estimated by direct gene counting methods, however, a significant deviation from H-W equilibrium was calculated using H-W calculator for two alleles. Pearson's Chi-square test was employed for detecting significant differences between the expected and observed frequencies.

Results and discussion

Serum levels of IL-18

A highly significant increase ($P \leq 0.001$) in IL-18 level was noticed in patient group (429.6 ± 221.6 pg/ml) compared to control (298.1 ± 145.2 pg/ml) as shown in Figure-1. The present result revealed that IL-18 was up-regulated in PCa patients, an observation that suggests their involvement in the pathogenesis of it.

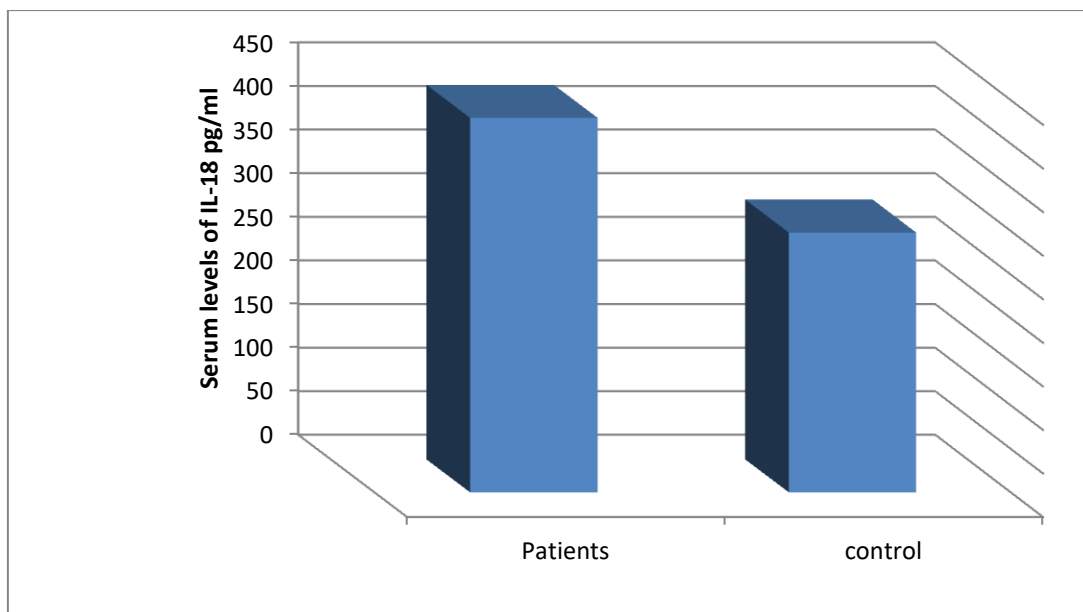


Figure 1- Serum level of IL-18 in prostate cancer patients in comparison to control group ($P \leq 0.001$)

One of the essential factors of immune response homeostasis is the cytokine network. However, any alteration in this network may cause an abnormal immune response. Furthermore, this network might be affected by various pathological and infectious events. Henceforth, investigators have concentrated on genes controlling the cytokine expression; mainly on gene polymorphisms that could have an influence on the expression level and consequently the whole immune response [6].

The present results are consistent with those obtained by Nong *et al.* [7] who found that the serum levels of IL-18 were significantly higher in PCa patients compared to controls ($P < 0.05$). Authors noticed that the serum levels of IL-18 in PCa patients at stage T1 was significantly lower ($P < 0.01$) than its level in patients presented with other stages of PCa. Also, they reported that the patients with IL-18 levels more than 316 pg /ml were at lower survival rate compared to the patients with lower levels. Besides, present findings are consistent with those obtained by Lissoni *et al.* [8] when they assayed IL-18 level in early and advanced cancer patients. Authors reported insignificant differences in IL-18 levels between controls and non-metastatic patients. Correspondingly, metastatic patients revealed significantly higher IL-18 mean values in both study groups.

IL-18 gene promoter - 137G/C

Interestingly, H-W equilibrium testing demonstrating that PCa presented insignificant variation in respect to the distribution of IL-18 -137 genotypes ($P > 0.05$), Furthermore, there were insignificant differences between the observed and expected frequencies of GG, GC and CC genotypes in patient samples. Nonetheless, the observed and expected genotype frequencies were in a good agreement with H-W equilibrium. Whereas control samples revealed significant variation ($P \leq 0.05$) between the observed and expected genotype frequencies and it did not agree with H-W equilibrium, as shown in Table-1.

Table 1-Observed and expected numbers and frequencies percentage and Hardy-Weinberg (H-W) equilibrium of IL-18 -137 genotypes and alleles in prostate cancer patients and controls

Studied groups			IL-18 137 genotypes or allele					H-W P ≤
			GG	GC	CC	G	C	
Prostate cancer patients	Observed	No.	21	25	5	67	35	Not significant
		%	41	49	10	65.7	34.3	
	Expected	No.	22	22.99	6	Not estimated		
		%	43	45	12	Not estimated		

Control	Observed	No.	26	23	0	75	23	Significant P ≤ 0.05
		%	53	47	0	77	23	
	Expected	No.	28.7	17.6	2.7	Not estimated		
		%	58.5	36	5.5			

Comparing patients with controls, IL-18 -137 GG genotype was insignificantly decreased in prostate cancer patients (41 vs. 53%; P = 0.316), with OR value of 0.62 and the EF of such difference was 0.2. IL-18 -137 GC genotype was insignificantly increased in prostate cancer patients (49 vs. 47%; P = 0.844), with OR value of 1.09 and the EF of such difference was 0.039. However, in terms of allele frequencies, the G alleles were insignificantly increased (65.7 vs. 77%, P= 0.119) in patients than controls, while allele C was insignificantly decreased (34.3 vs. 23.4%, P=0.119) in patients compared to controls, as shown in Table-2.

Table 2- Statistical assessment of associations between IL-18 -137 genotypes or allele and prostate cancer.

IL18-137 Genotypes or Allele	Patients (N:51)		Controls (N:49)		OR	Etiological or Preventive Fraction	Fishers Exact Probability	95%CI
	N	%	N	%				
Genotypes								
GG	21	41	26	53	0.62	0.20	0.316	0.28 to 1.36
GC	25	49	23	47	1.09	0.039	0.844	0.50 to 2.36
CC	5	10	0	0	-	-	-	-
Alleles	(102)		(98)					
G	67	65.7	75	77	0.59	0.31	0.119	0.32 to 1.09
C	35	34.3	23	23	1.70	0.14	0.119	0.92 to 3.16

OR= odds ratio; 95%CI = 95% confidence interval.

The results are indicated that IL-18-137 alleles and genotypes showed no association with the risk of prostate cancer development in Iraqi Arab population or protection against it, and the recorded OR, EF and PF values are in favor of such conclusion. In Han Chinese population, the genotype distribution of the controls was in agreement with HW equilibrium (P > 0.05). This is not compatible with the results of the present study. However, it's compatible in the association analyses of allele and genotype with prostate cancer susceptibility which revealed no changes between the genotype distribution in the cases and controls (P = 0.191 and 0.499 for -137G/C). And no significant association among the allele frequencies (P = 0.135 for -137G/C) [9]. Also Nong *et al.*, [10] found that the IL-18 -137 gene polymorphisms did not influence susceptibility to prostate cancer in the analyzed group of patients and control (IL-18-137 P = 0.715) In Chinese population.

Saalanti *et al.* [11], in a study performed in north India, reported compatibility between IL-18 genotypes (137 G/C) distribution between control and patient groups under H-W equilibrium. This is similar to the results of this study in the case of patients but not with the control. On the other hand, the present results disagreed with these results; given that both genetic variants (GC and CC) of -137 were noticed to be significantly correlated with increased risk of developing prostate cancer once compared with the wild homozygous GG (OR = 1.71; 95% CI: 1.20–2.46; P = 0.003 and OR = 3.35; 95% CI: 2.03–5.53; P < 0.0001 respectively).

One of the investigated loci IL18-137 was in agreement with H-W equilibrium in PCa patients whereas other IL-18 -137 in control disagreed with it. Such deviation from H-W equilibrium was determined as a major source of conflicting results being noticed in different ethnicities, given that the factors influencing the equilibrium are generally ethnicity – based [12]. In addition to different described factors, still the most important is a mutation that present new alleles into populations. Another important factor is the selection that takes place when genotypes experienced various

reproductive success. There are other several factors have a similar impacts such as migration, the movement of new individuals into or out of the population, and genetic drift, the random change in allele frequencies due to chance, and influential effect of these factors are related to the ethnicity of the population under investigation [13]. Also, clinical heterogeneity could enlighten such discrepancy. Owing to potential variety in patient population like the severity of the disease, age, and time of onset may lead to wide fluctuation in results. Nong *et al.* [10] found that IL-18 gene polymorphisms may participate in disease onset and severity. IL-18-137 GG genotype was associated with higher tumor grade ($P = 0.018$) and stage ($P = 0.007$). Polymorphism variants in the IL-18-137 may be associated with a worse prognosis for prostate cancer.

Deviations from HW equilibrium might correlate to the inconsistency. Testing for deviations from HW equilibrium is an essential quality control step in population genetic studies. In the current study with Iraqi Arab population, frequencies of genotype for IL-18 -137 C/G polymorphisms in controls slightly deviated ($P < 0.05$) from HW equilibrium, which may rise the chances of obtaining false-positive results because the sample size in patients and control may not permit a firm conclusion. Therefore conclusions of the present study must be interpreted with caution.

Giedraitis *et al.* [5] stated that the substitution of G into C at -137 position eliminates a histone 4 transcriptional factor-1 nuclear factor binding site. Nonetheless, at position -167 C is substituted into A, leads to disruption of the binding site of cyclic adenosine monophosphate. Same authors declared that cloning and gene expression data analysis has clarified that -137 and -167 SNPs of IL-18 promoter region being responsible for the alterations in the transcription factor binding and thus influencing the activity of IL-18 gene [5].

Single-nucleotide polymorphisms are thought to be the principal source of inconsistency among humans, particularly when they affect gene expression and/or function in accordance with their location in the DNA sequence. Consequently, an enormous number of SNPs regarding cytokine loci were reported and studied in wide range diseases such as cancer, autoimmune diseases and infections [14].

In males, prostate cancer is the foremost common disease and features such high heterogeneity that its pathogenesis and progression differ widely in different patients. It is assumed that genetic variants are the central risk factors that correlated with prostate cancer. There is now considerable knowledge that in a multiethnic population, many genes and chromosome segments are significant associated with prostate cancer risks, such as *PCA3* [15], *CYP17* [16], *TP53* [17], 8q24 [18], and 9q22 [19].

IL-18 plays an essential role in inflammation and immune response and is in general acknowledged as a key defense cytokine against infectious agents. Duo to immune stimulating effects of IL-18 have anti-neoplastic properties also, it was tempting to propose IL-18 as a new adjuvant therapy against cancer [20].

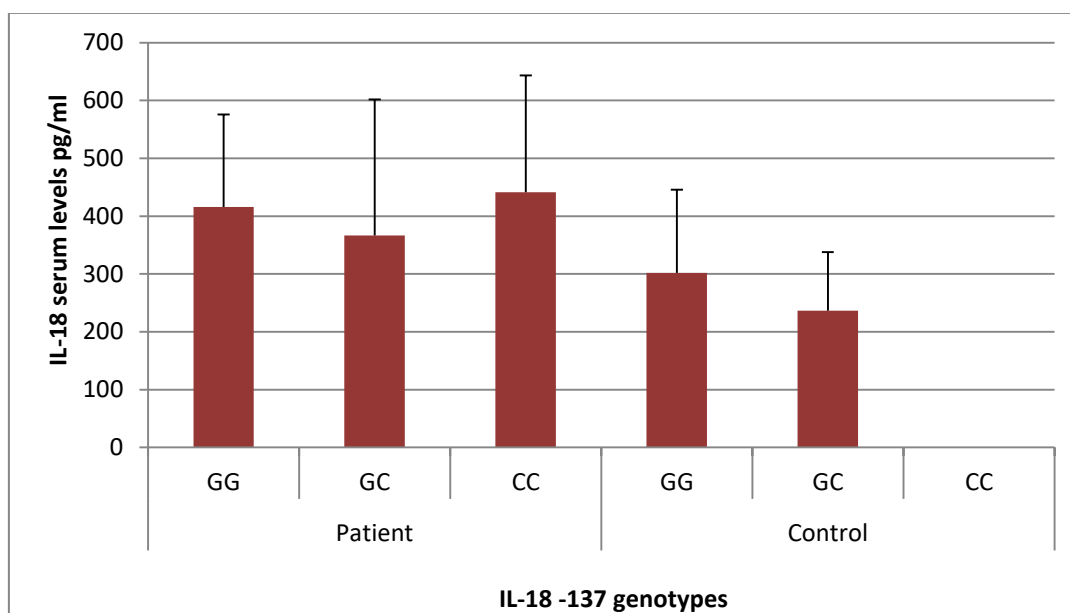
IL-18- 137G/C genotype impact on IL-18 serum levels

For advance understanding of cytokines gene polymorphisms role in prostate cancer patients, the impact of IL-18- 137 gene polymorphism on serum level of IL-18 was evaluated in patients and control, because Jin *et al.*, [21] reported that it has been well documented that cytokines gene polymorphism have a functional importance and might be associated with high or low production of the corresponding cytokines.

The impact of *IL18-137* gene polymorphism on IL-18 serum level was determined in patients and control (Table-3 and Figure -3). In patients group, CC genotype showed the highest level (441.1 ± 202 pg/ml) followed by genotype GG with 415 ± 159.9 pg/ml and GC with 366.4 ± 235.4 pg/ml. however, in controls, the GG genotype showed highest level (301.8 ± 143.9 pg/ml) followed by GC genotype with 236.3 ± 101.5 pg/ml, and the IL-18 serum levels differences in GG and GC genotypes was significant ($p < 0.05$) between patients and control. There were no significant differences between the three IL-18 -137 genotypes inpatient or in controls.

Table 3- Serum level of IL-18 in prostate cancer patients and controls distributed by IL-18 - 137 genotypes

	IL-18 -137 genotypes					
	Patients			Controls		
	GG	GC	CC	GG	GC	CC
Serum levels of IL-18 pg/ml	415.9±159.9*	366.4±235.4**	441.1 ± 202	301.8± 143.9*	236.3±101.5**	0
P value	*, ** Significant differences (P<0.05) in IL-18 serum levels between GG and GC genotypes in patients and control					

**Figure 2-** Serum level of IL-18 in prostate cancer patients and controls distributed by IL-18 -137 genotypes.

The current study differs, partly, from Dwivedi *et al.* [11] results in regard to patients group while it goes with control group. Dwivedi and his co-workers observed a tendency for IL-18 protein levels as GG > GC > CC in both of patient and control groups concerning -137 G/C. Conversely, this trend was statistically significant in patients group (P = 0.0074). Additionally, pairwise comparison between genotypes demonstrated that patients with GG wild genotypes developed an increase in IL-18 level. Such finding was statistically significant concerning patients group against CC mutant genotype (GG Vs CC: P < 0.05, P < 0.01), respectively.

Cytokine gene polymorphisms may change the structure and biological function of a certain cytokine coded by the defective gene leading to either increase or decrease of cytokine production. The inheritance of SNP may render people more susceptible or resistant to certain diseases, therefore affecting cytokine secretion levels, and eventually may influence the progression of tumor [22].

Conclusion

These results and irrespective of their different observed associations may highlight the role of *IL18* gene polymorphism in conferring susceptibility or resistant against the progression of prostate cancer.

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