Allele frequency of promoter region -1082A>G interleukin10 gene and risk of prostate tumors in Iraqi patients

S H N Al-Ruba’i¹, M Sh Ali² and N S Ahmed³

¹Mustansiriyah University, College of Science, Department of Chemistry, Baghdad-Iraq
²University of Technology, Applied science Department, a Branch of Chemistry, Baghdad- Iraq
³Biotechnology Research Centre, Molecular, and Biotechnology Laboratory, Al-Nahrain University, Baghdad- Iraq

*Corresponding: salwahnaser@gmail.com

Abstract. Number of studies has demonstrated the relationship of interleukin 10 gene polymorphism with risk of prostate cancer. This research aimed to evaluate the effect of single nucleotide polymorphism in the promoter region rs1800896 of IL-10 -1082A >G on the incidence of benign prostate hyperplasia and prostate cancer in Iraqi patients. In this study, we studied IL-10 gene polymorphism in two groups of patients, thirty of whom have benign prostate hyperplasia and thirty have prostate cancer, as well as in thirty healthy subjects who were the control group. Relevant primers were used for the amplification by the polymerase chain reaction of the promoter region IL-10 rs1800896. Restriction fragment length polymorphism has been used to determine the frequencies of the alleles associated with each group of subjects studied. The amplified products of PCR were sequenced using the forward primer. The result of restriction fragment length polymorphism showed that AG, AA alleles were not found and GG allele was detected in all of the controls and patients, leading to a conclusion that AA, GG homozygotes and AG heterozygote alleles were not associated with both benign prostate hyperplasia and prostate cancer.

1. Introduction

Prostate cancer (PCa) represents one of the most malignant diseases globally; the occurrence of this disease depends on ethnicity, family history and age [1]. Several studies revealed that one of the reasons that play a role in prostate cancer development is inflammation [2]. Some studies found that about 20% of patients with chronic inflammation after 5 years follow up they developed prostate cancer in comparison with patients with no chronic inflammation change [3]. IL-10 is a significant immunosuppressive and anti-inflammatory cytokine. IL-10 has several immune regulator roles, which are located in the main immune management link. The cytokines family of IL-10 consists of nine individuals: IL-29, IL-28A, IL-28B, IL-26, IL-24, IL-22, IL-20, IL-19 and IL-10 [4, 5]. IL-10 is one of many interleukins that play an important role in cancer [6, 7]. There are five exons in the IL-10 gene of human, their position on the chromosome 1, at 1q31-1q32 [8]. A significant number of polymorphisms
(essentially single nucleotide polymorphisms (SNPs)) were found in the promoter area of the IL-10 gene [9-11]. Persuasive evidence has been obtained that some of those polymorphisms are associated with IL-10 differential expression [12-14]. We aimed in this study to determine whether single nucleotide polymorphism in the promoter region rs1800896 of IL-10 -1082A > G is associated with prostate tumors.

2. Methods

2.1 Study subjects
A total of 60 male patients, 30 of whom were identified with PCa and 30 with benign prostate hyperplasia (BPH), the range of age was (45-86 years) for PCa and (46-91 years) for BPH patients, who attended Medical City/ Ghazi Al-Hariri Hospital/ Baghdad, Iraq and 30 apparent healthy members (41-86 years) who participated as controls, from March 2018 to January 2019, were involved in this study.

2.2 Exclusion criteria
Subjects were removed from the study if they met one or more of the following criteria: patients with chemotherapy or radiotherapy, patients with prostate lift, patients with other tumors, patients with another form of inflammation, and diabetes patients.

2.3 Collection of samples
Three milliliters from the blood have been taken from 30 PCa patients, and 30 BPH patients. Also, 3 milliliters of blood were taken from the control group. The samples were added to tubes with EDTA to avoid blood coagulation. The samples were held at -20 °C until they were assayed.

2.4 DNA extraction
Genetic DNA was extracted from frozen samples after getting them to room temperature from the patients and the controls using Extraction kit type gSYNC™ DNA produced by (Geneaid company) according to the manufacturer instructions provided with the kit.

2.5 Polymerase chain reaction (PCR)
PCR was carried out by using pair of primers that were obtained from prior literature [15] forward 5’-GTC AGT GTT CCT CCC AGT-3’ and reverse 5’-TTA CCT ATC CCT ACT TCC TC-3’, in a 25µL final volume, the mixture of PCR consisted of 1.5µl of genomic DNA, 5µl Taq PCR Pre Mix, 10 picomols/µL (1µL) of every primer and 16.5 µL of Distilled water. After several attempts to detect the optimal temperature for annealing, traditional PCR was used to detect IL-10 gene using the described primers. For this gene, the best conditions for the reaction of PCR are: heating the samples at 95 °C for 3 min (primary denaturation) followed by 35 cycles of 95 °C for 45 s (denaturation), 54 °C for 45 s (annealing), and 72 °C for 45 s (extension), with a final extension of 72 °C. In a 1.5% agarose gel colored with red safe, PCR products were visualized.

2.6 Restriction fragment length polymorphism (RFLP)
The PCR – RFLP according to Wang et al. (2014) method was used to determine rs1800896 SNP polymorphism in the promoter region -1082A > G [16]. For identification of rs1800896 genotypes in the PCR products, Earl the restriction enzyme was used under the following conditions: the restriction fragment length polymorphism assay was carried out in a 10µL reaction volume containing 5µL PCR product and 0.5µL from Earl and 4.5µL buffer, the mixture was then mixed and vortexed very well for several seconds and then incubated at 37 °C for 15 minutes in the incubator. Afterwards, 5 µL from the mixture of the reaction was run on agarose gel (2.5%) stained with 1.5 µL of red stain pigment and 5 µL DNA ladder. Agarose gel electrophoresis was performed at 70 Ampere and 90 volts for 2 hours. The DNA was detected by observing the staining gel under vision gel documentation device (Scie-Plas/UK). Sequencing of the amplified product of the IL-10 gene was done by (Macrogen company/Korea).

Homology search was carried out using the Basic local alignment search tool (BLAST) available online
at the national center biotechnology information (NCBI). The findings have been compared with references sequences of the gene provided from the gene bank of NCBI as a control.

2.7 Statistical analysis
Statistical Package for the Social Sciences (SPSS) program (V. 25) has been used to analyze the data of genetic parameters and Chi-square test was used to compare percentages.

3. Results and discussion
Genomic DNA was obtained from patients and controls from whole blood samples. Gel electrophoresis was used to verify the integrity of the DNA that was extracted. In PCa patients, BPH and controls, standard PCR was used to amplify the IL-10 gene. IL-10 gene was detected as a 370 bp fragment in the aforementioned patient groups and controls. To detect IL-10-1082 alleles, RFLP-PCR technique was used, the 370 bp products of PCR were digested with EarI endonuclease, a single band (370 bp) was obtained in case of homozygous GG, other bands for AA and GA alleles were not detected in any of the controls and patients. (Fig. 1) shows the RFLP segments of the IL-10 gene.

![Figure 1](image)

**Figure 1.** Genotyping of IL-10 gene by RFLP-PCR using the endonuclease EarI on 2.5% agarose gel imaged by UV after coloring with red safe pigment. M represents a DNA ladder (100bp), lanes (1-5) represent controls, lanes (6-10) represent BPH patients and lanes (11-15) represent PCa patients. By using 70V for 2 hours in electrophoresis, one band at 370 bp detected -1082 GG, another bands two for AA and three for GA alleles were not found.

Table 1 shows the genotyping, distribution of alleles and frequencies through patients and controls.

| Genotype | Healthy | BPH | PCa |
|----------|---------|-----|-----|
| GG       | 30 (100)| 30 (100)| 30 (100) |
| AA       | 0 (0)   | 0 (0)   | 0 (0)    |
| GA       | 0 (0)   | 0 (0)   | 0 (0)    |
| Total    | 30 (100)| 30 (100)| 30 (100) |
From table 1, it can be seen that AA homozygote allele is not found in both benign prostate hyperplasia and prostate cancer patients and controls. The GA heterozygote allele also was not detected in the controls and both BPH and PCa patients, whereas GG homozygote allele was detected in all of the controls and patients.

To confirm the electrophoresis results of PCR products for the IL-10 gene, fifteen samples of amplified products for IL-10 gene from tumor and benign patients, as well as from controls, were sequenced to detect SNPs inside these sequences. These sequences have been matched with an IL-10 reference sequence at NCBI Gene Bank. All polymorphisms of these sequences were summarized in Table 2.

Several studies have investigated the relationship between PCa risk and the polymorphism of IL-10 -1082. McCarron et al. found that the genotype of IL-10 -1082 AA is increased significantly in patients relative to control group 31.6 against 20.6%; P= 0.01 [17]. Another study on Indian patients, by Bandil et al. showed that the genotypic distributions of GG, GA, and AA of -1082 A/G region were 23, 43, and 34% for cancer; 13, 49 and 38% for BPH; and 10, 30, and 60% for controls respectively. Variate G allele either in heterozygous AG or in homozygous GG condition revealed a strong relation with PCa [18], also in other cancers, some studies have investigated the relation with IL-10 -1082 polymorphism [19, 20], whereas other studies [21-23] found that the relation between IL-10 -1082 polymorphism and PCa risk was not significant.

4. Conclusion

To conclude, the findings showed that these prostate pathologies are not correlated with homozygotes GG and AA alleles and heterozygote GA allele in IL-10 rs1800896.

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