European Science Methodical Journal
ISSN (E): 2938-3641
Volume 2, Issue 5, May - 2024

EVALUATING THE ROLE OF GENETIC POLYMORPHISMS AND GENE EXPRESSION OF THE KLK2 GENE WITH THE RISK OF BENIGN PROSTATIC HYPERPLASIA IN IRAQI PATIENTS

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Abstract:

Benign prostatic hyperplasia (BPH) is a non-malignant tumor of the prostate that enlarges prostate tissue and reduces the flow of urine from the bladder. Testosterone plays a role in the disease as low testosterone with age leads to hyperplasia of the prostate The present study aimed to evaluate the role of genetic polymorphism and gene expression in the KLK2 gene at the variant locus for rs198972, rs198977, a gene located on chromosome 19 that mainly encodes for enzymes involved in BPH by increasing gene expression for rs198972 and by Fisher's probability (P = 0. 569) between patients and controls. 569) between patients and healthy controls. Homozygous CC and C allele genotypes were considered a causative factor with an odds ratio of 1.43 and 1.43, respectively. The TT and T allele genotypes have a Fisher probability value of P = 0.427, so the TT and T allele genotypes are a protective factor for the disease according to the odds values of 0.45 and 0.70, respectively. In front of heterozygosity and rs198977, the CC and C genotypes have a Fisher probability (P = 0.778) between patients and healthy people. The homozygous CC and C genotype is a protective factor for the disease with a value of Odd ratio of 0.84 and 0.90, respectively. The TT and T allele genotypes had a Fisher's exact probability value of P = 0.666, so the TT and T allele genotypes were considered as an etiologic factor according to the likelihood values of 1.00 and 1.11, respectively. The results of the current study show higher levels of KLK2 gene expression in BPH patients compared to healthy people, as the real time PCR results showed a difference in the CT values between the study groups, and the CT values in patients were lower than the control group, and the folding level in patients (14.35±5.21) was higher than healthy people (1.00±0.00) with statistically significant differences (P<0.001***).

Keywords: KLK2 gene, SNP, Translocation, Odd ratio, Prostatic.

Introduction

There are several factors that affect the prostate: sex hormones, age, genetic history and inflammation in the prostate (Parsons et al., 2010) Some androgenic hormones may play an important role in this, as testosterone is converted into dihydrotestosterone and the effect of this compound on the prostate leads to its enlargement (Yu Tong and Ren-yuan Zhou, 2020). Benign prostatic hyperplasia is caused by the abnormal expression of certain genes that do not respond to androgens, leading to disruption of cell division and apoptosis (Letteria et al., 2014) Some evidence suggests that genetics may be involved in prostate enlargement (Ayush et al., 2015). A study of men under the age of 64 who underwent surgery for BPH found a four-fold increase in the likelihood of having an enlarged prostate in the presence of a second-degree relative and a six-fold increase in the likelihood of having a first-degree relative with BPH. Researchers estimate that about 50% of men under the age of 60 who undergo surgery for prostate enlargement have inherited the disease (Parsons et al., 2010) One study estimated that genetic factors may contribute as much as 72% of the risk of prostate enlargement among the elderly (Rohrmann et al., 2005) Kallikreins are a large family of genes that possess great similarities in terms of their structure and the proteins they encode This family consists of 15 genes, and extends 280 kilobases along chromosome 19. KLK2 are classic kallikreins and one of the first kallikreins to be discovered. KLK2 genes are characterized by a high similarity between them, as they both have a transcriptional orientation from centromere to telomere, unlike other genes, and have a major role through expression in the prostate and a major role in diseases affecting the prostate, including BPH and prostate cancer (Hannu et al., 2008) kallikrein are a subset of serine proteases that are proteins capable of breaking peptide bonds in proteins that differ greatly in molecular weight, substrate, gene structure, and immunological properties (Guzel et al., 2014). They are found in different tissues and biological fluids (Kornberg et al., 2018). They are divided into two main categories: plasma kallikrein and tissue kallikrein (Wang et al., 2017) .

Tissue Kallikrein is part of a large, polygenic family of 15 genes with significant similarities at the level of genes and proteins as well as its tertiary structure. The term tissue kallikrein is used to describe a group of genes that share highly similar sequences and are located on the same chromosome (Bonk et al., 2020). kallikrein share several characteristics including the sequence and organization of exons and neutrons, the number and length of the exon region, the location of the methionine in the code, and the termination codon A gene of the kallikrein family located on chromosome 19 in region q13. 4 It is 5790 base pairs in length and consists of five exons and four introns. The first exon is 75 base pairs in size and contains a 29-base 5' untranslated region followed by a methionine start codon; the second, third, and fourth exons are 160, 287, and 137 base pairs in size The fifth exon has a size of 830 bases and the stop codon is located at a distance of 156 bases from the beginning of the exon and this exon contains an untranslated region of 674 bases and the transcriptional direction of this gene is from centromere to telomere (Capogrosso et al., 2021). The KLK2 gene is mainly expressed in the prostate and a few other tissues, such as the thyroid, breast, and salivary glands (Kričković et al., 2020). The KLK2 gene has several similarities to KLK3. This gene encodes a protein with

an amino acid sequence that is 77% similar to the amino acid sequence of PSA. As in PSA, both KLK2 mRNA and hk2 protein are expressed in human prostate columnar epithelial cells and secreted into semen (Roumeguère et al., 2017) In vitro studies have shown that hk2 converts PSA into an active enzymatic protein and can cleave semenogelins and fibronectin. Furthermore, hk2 can activate urokinase plasminogen activator, inactivate plasminogen activator inhibitor-1 (PAI-), and deactivate plasminogen activator inhibitor-1 (PAI-1). 1) in addition to its ability to bind to IGFBPs (Kričković et al., 2020) .

Materials and Methods

The research investigation was carried out at the University of Diyala in Iraq, in the Molecular Genetics Lab of the Faculty of Education for Pure Science The current study was conducted on a group of patients and healthy people visiting Baquba Teaching Hospital / Consulting Clinics, as blood samples were taken from healthy people and patients with BPH from October 2022 until February 2023, and the number of study samples amounted to 80 samples divided into 50 men with BPH and 30 healthy men. DNA was extracted using the System gDNA Miniprep Blood ReliaPr extraction kit, which was supplied by Bioneers in South Korea. To amplify the *KLK2* gene at the location of variants rs198972 , mixture for the polymerase chain reaction 1.5 µl forward primer 5"-CCACACTGTATCACCCCTGG-"3 , 1.5 microliters of the reverse primer and 5"-CCTTTCCCCTCCAGATGTTGG-3" ,and rs rs198977 forward primer 5"TCACTGAACTGACCATACCCA-3" and reverse primer 5"-CGGCCTTATCTAGGCTTCCC-3", 3 µl DNA, 5 µl master mix, and 14 µl free nuclease water. For every sample, the reaction product had a total volume of 25 microliters. The reaction mixture for the samples of healthy people and diabetes patients was then added to the polymerase chain reaction device. The following reaction conditions were programmed into the apparatus: five minutes at 94°C for initial denaturation, thirty seconds at 94°C for denaturation, thirty seconds at 63°C for primer annealing, five minutes at 72°C for extension, and five minutes at 72°C for final extension. This was carried out due to a total of 35 cycles involving primer annealing, denaturation, and extension. After the data from the polymerase chain reaction were collected, the samples were electrophoresed for 1.5 hours at 90 volts on a 1% agarose gel. The amplification product was shipped to Macrogen Company in South Korea, where it enabled Sanger nucleotide sequencing of the *KLK2* gene. The Hardy-Weinberg equation was used to ascertain which genotype was a causative factor and which genotype was a protective factor based on the analysis of the nucleotide sequencing data using the Genius application.

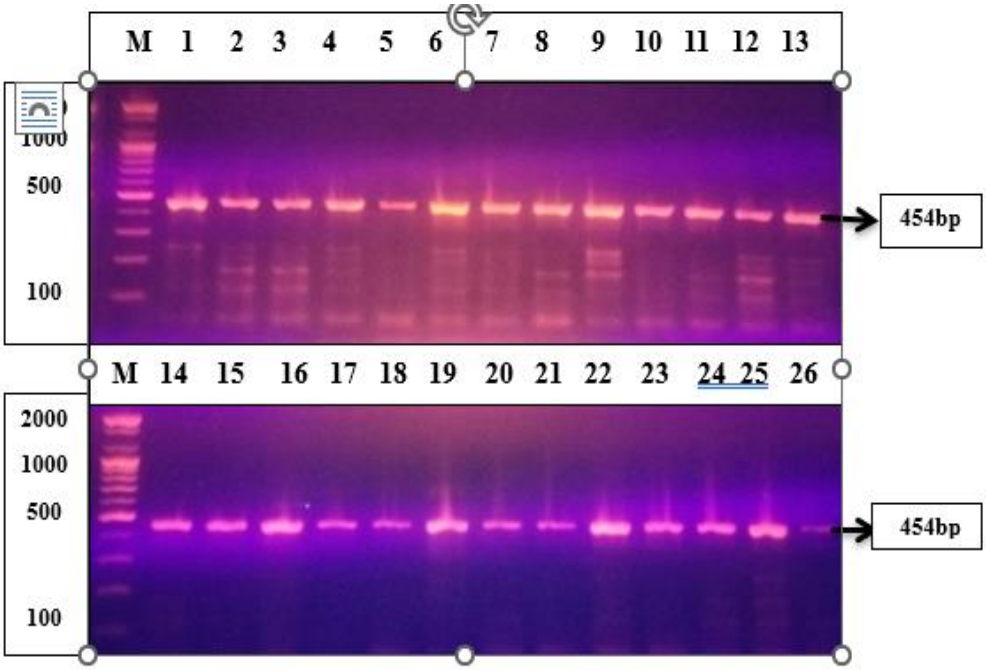
Quantitative real-time polymerase chain reaction

KLK2 genes' relative expression level was measured by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR). Beta-actin was used as the reference gene. All primers were designed by Third Author according to the NCBI-primer blast. The sequences of the designed primers are as KLK2 gene foreword 5"-TCTTCAGTGTGTGAGCCTCCA-3" and Reverse5"- ACAAGTGGACCCCCAGAATCA -3"and B-Actin gene Forward 5"-CCCATCACCATCTTCCAGGAGGG-3" and Reverse 5"-

CATGCCAGTGAGCTTCCCGTTCA-3". Beta-actin with an amplicon size of 90 bp and annealing temperature 60°C. KLK2: F with an amplicon size of 196 bp and annealing temperature 60°C. real-time PCR reactions were performed in 20µl reaction volume containing(0.5µl) of each primer (100pmol/µl),10 µl NEB luna universal qPCR Master Mix, 3 µl cDNA and 6 µl Water RNase-Free. Standard cycling protocol was applied to perform Real-time PCR. Amplification condition included: 10 min at 95°C for Initial Denaturation, 15 s at 95°C for denaturation, 60 s for annealing at the specific temperature for each gene, followed by 15 s at 72°C for extension, with forty cycle. Gene expression assessment was performed based on the 2−ΔΔCT method.[15] .

Results

KLK2 gene amplification from DNA in BPH patients If the amplification results show that the molecular weight of the resulting bands for all patient and healthy samples is 454bp at the location of rs6070 and rs198977 heterozygotes.



Amplification product of the KLK2 gene fragment of the coding segment comprising the two variants in rs6070, rs198972 and rs198977 for BPH patients and healthy people of Diyala governorate community, gel electrophoresed on 1.5% agarose gel for 1.5 hours at 90 V voltage, stained with ethidium bromide dye and visualized under UV light, Numbers 1-13 represent patient samples and 14-26 represent healthy people samples.

The result KLK2 gene amplification results are shown in Figure 1. for both BPH patients and healthy people. In each sample of patients and healthy subjects, if the amplification results show that the rs198972 and rs198977 variations have a molecular weight of 454 bp at the location of the resulting bands.

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ISSN (E): 2938-3641
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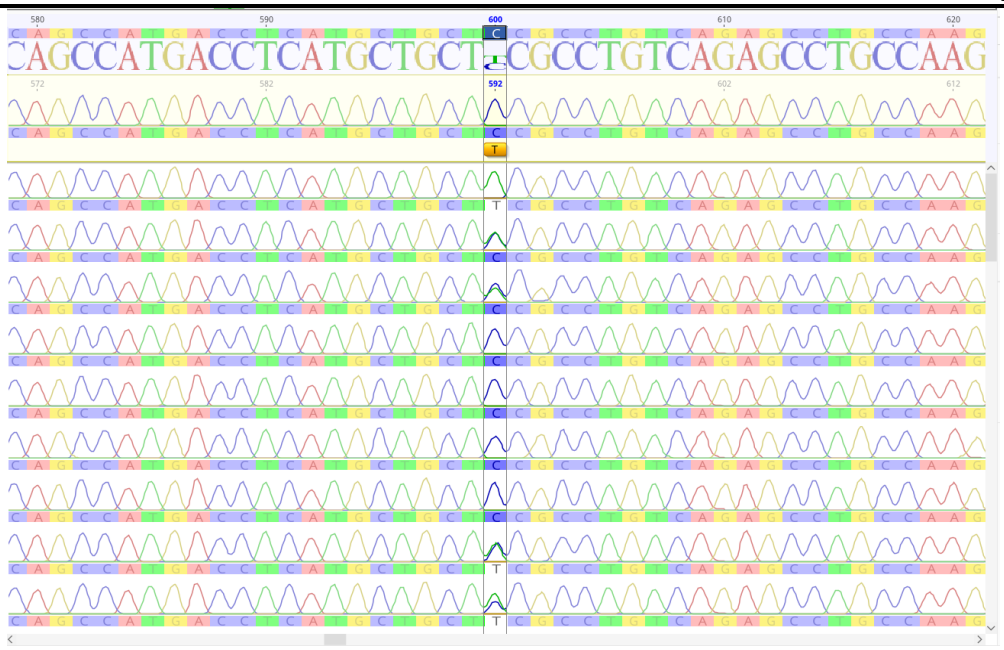

Figure 2: The position of the rs198972 C/A/T variant and the kind of mutation are displayed by comparing the alignment of the nitrogenous bases of a portion of the *KLK2*gene between samples from BPH**, healthy controls, and the GenBank sample (NCBI, 2023).**

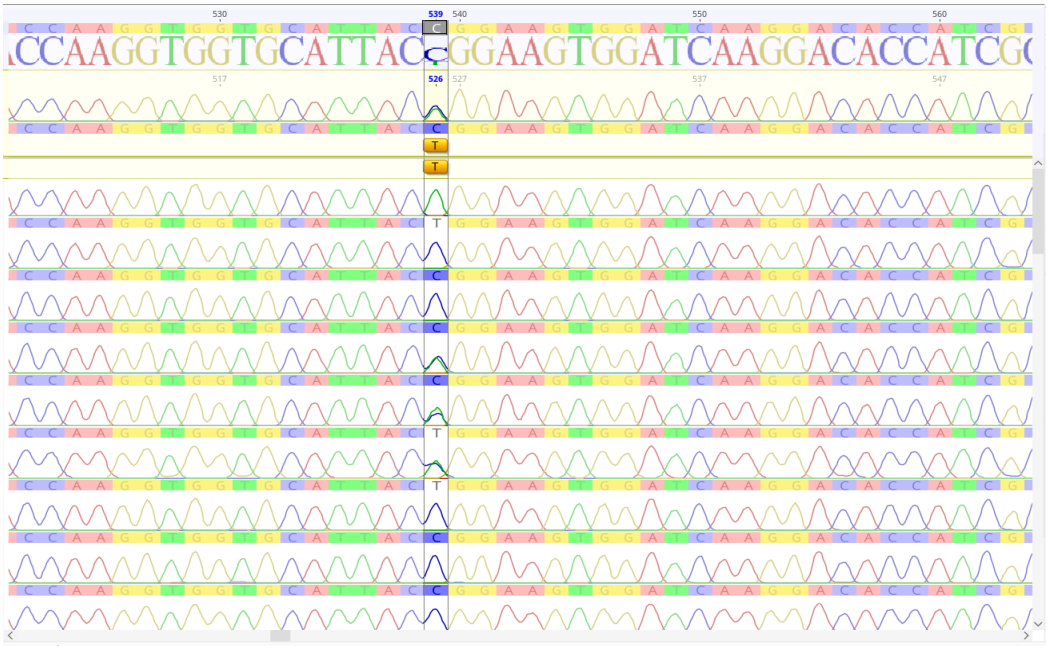

Figure 3: The position of the 198977C/T variant and the kind of mutation are displayed by comparing the alignment of the nitrogenous bases of a portion of the *KLK2* gene between samples from BPH**, healthy controls, and the GenBank sample (NCBI, 2023).**

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Table 1. Expected frequencies of genotype and alleles of the coding region 198972 C/A/T for*KLK2*by using Hardy-Weinberg equilibrium

Hardy P-values	Allele frequencies		Genotype// rs198972 C/A/T				Groupe
0.5403 NS	T	C	TT	CT	CC	No.	Observed	24 Patients
	16	32	2	12	10		
	33.33	66.67	8.33	50	41.66	%	
	Not diagnosed		2.67	10.67	10.67	No.	Expected
			11.11	44.44	44.44	%	
0.8887 NS	20	28	4	12	8	No.	Observed	24 Control
	41.67	58.33	16.66	50	33.33	%	
	Not diagnosed		4.17	11.67	8.17	No.	Expected
			17.36	48.61	34.03	%	

*(P≤0.05), **(P≤0.01), NS: Non-Significant

Table 2. Genotype distribution and allele frequency of KLK2 rs198972 C/A/T SNPs

Genotype// rs198972 C/A/T	Patients No. (%)	Control No. (%)	Fisher's/P-value	O.R. (C.I.)
CC	10 (41.66%)	8 (33.33%)	0.569 NS	1.43 (0.38 - 5.46)
CT	12 (50%)	12 (50%)	0.773 NS	1.00 (0.31 - 3.18)
TT	2 (8.33%)	4 (16.66%)	0.427 NS	0.45 (0.05 - 2.89)
Total	24 (100%)	24 (100%)		
Allele	*Frequency*			
C	32 (66.67%)	28 (58.33%)	O.R. (C.I.) = 1.43 (0.62 - 3.32)	
T	16 (33.33%)	20 (41.67%)	O.R. (C.I.) = 0.70 (0.30 - 1.62)	
*(P≤0.05), NS: Non-Significant.				

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Table 3. Expected frequencies of genotype and alleles of the coding region rs198977 C/T for *KLK2* by using Hardy-Weinberg equilibrium

Hardy P-values	Allele frequencies		Genotype// rs198977 C/T				Groupe
0.2001 NS	T	C	TT	CT	CC	No.	Observed
	13	35	3	7	14		
	27.08	72.92	12.5	29.16	58.88	%	
	Not diagnosed		1.76	9.48	12.76	No.	Expected
			7.34	39.5	53.17	%	
0.1025 NS	12	36	3	6	15	No.	Observed
	25	75	12.5	25	62.5	%	
	Not diagnosed		1.5	9	13.5	No.	Expected
			6.25	37.5	56.25	%	

Patients 24

Control 24

*(P≤0.05), **(P≤0.01), NS: Non-Significant.

Table 4. Genotype distribution and allele frequency of *KLK2* rs198977 C/T SNPs

Genotype// rs11549920 A/C/G	Patients No. (%)	Control No. (%)	Fisher's/P-value	O.R. (C.I.)
CC	14 (58.88%)	15 (62.5%)	0.778 NS	0.84 (0.26 - 2.75)
CT	7 (29.16%)	6 (25%)	0.759 NS	1.24 (0.33 - 4.65)
TT	3 (12.5%)	3 (12.5%)	0.666 NS	1.00 (0.12 - 8.35)
Total	24 (100%)	24 (100%)		
Allele	*Frequency*			
C	35 (72.92%)	36 (75%)	O.R. (C.I.) = 0.90 (0.35 - 2.27)	
T	13 (27.08%)	12 (25%)	O.R. (C.I.) = 1.11 (0.44 - 2.82)	
*(P≤0.05), NS: Non-Significant.				

Discussion

The results shown in Table 1 showed that the number of patients with CC homozygosity was (10) and C allele was (32). The number of patients carrying the CC and C genotypes was significantly higher in the BPH patient group according to the mentioned ratios (41.66 and 66.67, respectively) compared to the control group (healthy people), which recorded 33.33 and 58.33 according to the Fisher probability (P = 0.569) between patients and healthy people. Homozygous CC and C allele genotypes were considered as an etiologic factor with an odds

ratio of 1.00 and 1.43, respectively. The results shown in the same table also showed that the observed number of patients for the TT genotype is 2 and the T allele is 16, which recorded a significant decrease in the BPH patient group, as the ratios reached 8.33 and 33.33, respectively, compared to the control group, as the ratios reached 16.66 and 41.67, and according to the Fisher probability, the probability value reached P = 0.427, so this TT genotype and T allele is a protective factor for the disease according to the odds values, which reached 0.45 and 0.70, respectively While the CT genotype varied in patients with prostatic hyperplasia and healthy people, as the number of sightings for both patients and healthy people amounted to 12 if the proportions reached 50 for both patients and healthy people, while the C allele showed a significant increase, as the number of sighting values reached 32 in patients compared to the control, as the sighting values amounted to 28, and the proportion was 66. 67 for patients compared to the control group with a ratio of 58.33 and according to Fisher's probability of 0.773 P=, so the CT genotype and the C allele are an etiologic factor according to the odds values of 1.00 and 1.43, respectively. The results in Table (2) showed that the distribution of the three genotypes CC, CT, TT and allelic frequency of the KLK2 gene at the The CT and T allele genotypes were significantly elevated in the patients, with values of (7) 29.16 and (13) 27.08, respectively, compared to the control group, with values of (6) 25.0 and (12) 25.0, respectively. Therefore, the CT genotype and the T allele are the causative factor of the disease according to the likelihood values of 1.24 and 1.11, respectively, and the differences in the likelihood values of the genotypes and their alleles are due to the small sample size .The results in Table (1) showed that the distribution of the three genotypes TT, CT, CC and the allelic frequency of the KLK2 gene at the heterozygous site /C/T rs198977 in the study population according to Hardy-Weinberg law is balanced, as there are statistically significant differences between the observed and expected values of the three genotypes and alleles, as the Hardy-Weinberg probability value was 0.1025 NS and 0.2001 NS in patients and healthy people, respectively. In the same regard, a study by Bonk et al in 2020 of 284 people with chronic myeloid leukemia with 280 healthy people, in which the KLK2 gene was studied for two sites (rs2664155 and rs198977) and the results of the genetic polymorphism revealed that rs198977 was clearly associated with the disease and the study showed that people with the rs198977.TT genotype have an increased risk of developing hyperleukemia and the study showed that it also has a role in doubling the risk and response to chemotherapy. Another 2014 study by Shang et al. showed that the KLK2 gene at several loci, including rs198977, plays an important role in promoting tumor cell proliferation and can prevent apoptosis of cancer cells through the bax and bcl2 pathway. The protein encoded by this gene is a highly active trypsin-like serine protease that spontaneously cleaves from arginine residues This protein is mainly expressed in prostate tissue and is responsible for the cleavage of prostate-specific antigen into its enzymatically active form. It is highly expressed in prostate tumors and may be a precursor to prostate malignancy(Catalona et al;2011).

Conclusions

Benign prostatic hyperplasia is associated with hormonal changes and genetic polymorphisms of the KLK2 gene at rs198972 and rs198977. The results of rs198972 showed that the CC and TC genotypes and their C allele are considered a causative factor, while the TT genotypes and their T allele are considered a protective factor, while the results of rs198977 showed that the CC genotype and its C allele are considered a protective factor. TT genotype and its T allele is a protective factor for the disease while the rs198977 results showed that CC genotype and its C allele is a protective factor for the disease while the results showed that TT and TTC genotype and their T allele is a causative factor for the disease .

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