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Original Article

Physiological variables and molecular study of *KLK2* and *KLK3* among patient with benign prostatic hyperplasia

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ABSTRACT

Prostatic hyperplasia is benign tumor occur in prostate. Benign prostatic hyperplasia is common disease in old men. The incidence of disease arises with increase in age. The patient with benign prostatic hyperplasia are estimated 20% of men in 40s old, and 90% in of men in 80s old, and main causes of prostatic hyperplasia are unknown but there is evidence referring to genetic and hormonal disorders that may cause the disease. This study includes 60 patients with prostatic hyperplasia with an average age of 64 years old and 30 samples as a control with same age group. The study obtained that there was significant association ($P \leq 0.05$) between PSA (KLK3) and prostatic hyperplasia. Result also mentions that there was significant decrease in testosterone level and significant increase in dihydrotestosterone level. The present study for *KLK2* and *KLK3* genes showed molecular variation in both genes, varied between polymorphism and allele polymorphism. PCR amplification of specific primers of *KLK2* gene showed polymorphisms ranged between 14%, 8%, 10%, and 6% in each KLK2a, KLK2b, KLK2c, and KLK2d primers respectively, while the allele polymorphism in KLK2c amplification with primer reaches 18% of patient. PCR amplification of specific primers of *KLK3* gene showed polymorphisms ranged between 10%, 6%, 2%, and 4% in each KLK3a, KLK3b, KLK3c, and KLK3d primer respectively, and allele variation was not detected in amplification product of *KLK3*.

1. Introduction

Benign prostatic hyperplasia is a common disease in older men,[1] ranging from 20%, 40%, 70%, 80%, and 90% in men between 40, 50, 60, 70, and 80 years old respectively.[2,3] Despite the availability of medical and surgical treatments for benign prostatic hyperplasia, the understanding of the processes involved in pathological benign growth in human prostate is not clear enough.[1]

At the level of the population, there are five categories that increase the risk of benign prostatic hyperplasia regardless of age, which are steroid hormones, sex hormones, genetics, lifestyle, and infections.[4]

Since the inflationary growth of the transitional zone of the prostate associated with prostate clinical hyperplasia may be the result of the abnormal expression of genes that respond to main androgens, this leads to an imbalance between cell division and apoptosis.[1]

Testosterone (TES) encourages the proliferation of prostate cells and increases the size of prostate with age at natural men and either man who have low levels of TES has prostate size smaller than normal, but when it is overcome by the shortfall in TES level, the size of the prostate increases, but only to normal within the same age group.[5] It has proven that the increase in testosterone above normal limit could result in high risk of prostate cancer rate.[6]

Kallikreins related peptides (KLKs) are a group of serine proteases found in various tissues and biological fluids.[7] KLKs divided into two main categories are Plasma KLKs and Tissue KLKs.[8] These two groups vary widely in molecular weight, substrate, immunological characteristics, gene structure, and the type of Alcanin release.[7] All KLK genes encode single-chain preproenzymes with lengths varying between 244 and 293 amino acid residues and ~40% protein identity among each other.[9]

Gene expression of kallikreins related peptides has been detected in many tissues and cell lines for many of the human organs, so the kallikreins related peptides involved in a wide range of physiological processes, including pressure regulating peel off the skin, semen fluid liquefaction, tissue regeneration, regulating inflammatory processes.[10]

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The human kallikreins related peptides have a great importance in the very specific expression for each of KLK2 and KLK3 in the prostate tissue, which qualifies them to be a biological marker for prostate diseases. Prostatic specific antigen has particular gained prominence in recent years as the most valuable tumor marker and is currently used on a large scale for the diagnosis, monitoring and examining cases of prostate cancer and enlarged prostate. The KLK2 is expressed in prostate mainly in addition to a number of other tissues in low rate such as breast, thyroid, and salivary glands.[11] This gene has several similarities with KLK3. This gene encodes a protein with amino acid sequence similar to the sequence of amino acids for PSA (KLK3) by 77%. As in the PSA (KLK3), all of the mRNA of the *KLK2* gene and protein KLK2 are expressed in epithelial cacuminal human prostate cells and secreted in the seminal fluid.[12] Later, it was shown that KLK2 cleaves to pro-PSA to generate active PSA, and this evidence strongly supports the physiological role for *KLK2* in regulating the activity of PSA (KLK3) These two particles perhaps work together in normal, benign and cancerous prostate tissue.[12]

The regulation of kallikreins related peptides includes *KLK2* and *KLK3* on the transcriptional and post-transcriptional level by steroid hormones, and each of *KLK2* and *KLK3* is under the influence of androgenic hormones, as a complex of androgen-receptor with androgenic response element which is located at the beginning of promoter to stimulate the expression of both gene elements. The studies showed that the concentration of each of the *KLK2* and *KLK3* decreases after treatment with anti-androgen during the process of sexual transformation.[13]

This study aimed to evaluate physiological parameter along with molecular screening of *KLK2* and *KLK3* in patient with prostatic hyperplasia in western parts of Iraq.

2. Materials and methods

The study samples were collected from patients for the period from 01/02/2016 until 05/15/2016, and the study included samples in two groups.

Patient group consists of 60 men diagnosed with a benign prostatic hyperplasia within the age group ranging between 45 and 80 years.

Control group composed of 30 samples, who were normal man and who were not diagnosed to have any symptoms or signs of prostatic hyperplasia.

The information was collected for each member of the sample through a personal interview and recorded in a questionnaire prepared in advance for this purpose.The concentration of each of Testosterone, DHT, and tPSA was determined by following steps of kits by ready-made analysis, according to the method of Enzyme-linked immune sorbent assay (ELISA) using ELISA Reader device.

Genomic DNA extracted from the blood of 7 control samples and 50 patient samples by Geneaid kit and the DNA were checked by gel electrophoresis. Then the extracted DNA is detected and the resulting PCR technique was done using Agarose gel and according to Maniatis et al.[14]

Primers were designed using NCBI www.ncbi.nlm.nih.gov, to detect possible mutations in patients compared with the natural sequences on NCBI.

A pair of specialized primers was used to amplify exons of *KLK2* and *KLK3*, 4 primers for each gene (Table 1), where the prefixes are equipped lyophilized as 10 Bekomul/μl.

Table 1
Primer and their size (refer this study).

Primer of KLK2 gens			
Primer	Sequence	Size	Annealing temperature
KLK2a	F: TCATCCAGTCTCGGATTGTG R: GAGGAGGAGAGGCATGAGG	1235	60
KLK2b	F: TCACTCCCTTCCTGGTTCTG R: GGGAGGCAGATGTCTGGTTA	997	59.5
KLK2c	F: TGAGGGAAAGGGAGAGGATGA R: CAAACCAGTCCCACAGGTGC	725	61
KLK2d	F: ATCAGGTGGTCTATGGGGCTA R: GCATTCACTATCCCCCGTCTT		60
KLK3a	F: CACTGTTTCTTTTTCTCTTTTGGA R: TAGGGATGACTCACCGAGCA	684	60.5
KLK3b	F: TCTGATCACCGAACTGACCA R: CCCACTGGGAGAAAACAACT	1345	59.5
KLK3c	F: GGGTCTTCCTTTGGCATGGG R: CCCCTTTGAGTTCAGTGTCCT	844	60
KLK3d	F: GGGTGGGATCCACACTGAGA R: CCTAGGCGGTTTCCTCATCTT	923	59

3. Results and discussion

The questionnaire was conducted according to Index of Symptoms of the American Association of Urology (AUASI). A total of 250 samples of people aged between 41 and 80 years, were asked questions outlined in the questionnaire, 60 patient samples were selected after they proved to be positive for benign prostatic hyperplasia and 30 control samples did not have any symptoms of hyperplasia.

Testosterone Hormone levels showed decrease ($P \leq 0.05$) in serum for people with benign prostatic hyperplasia (444.0) when compared with the control sample (614.2).

This result is consistent with the findings of the Wayne et al.,[15] who stated that the levels of testosterone hormone were low in men over the age of 50 years and those with hypertrophic prostatic hyperplasia when compared with healthy people of the same age group.

The present study showed significant differences ($P \leq 0.05$) between benign prostatic hyperplasia and control group in relation to DHT hormone level which was 70.21 and 57.17 in each group respectively.

The current study, agreed with the findings of Habeeb[16] and Pentti and Jean.[17], who found a significant increase in serum DHT levels in benign prostatic hyperplasia patients.

This variation in levels may happen as a result of higher activity enzyme 5-alpha reductase, leading to positive feedback and increase the production of dihydrotestosterone levels, as a result of the conversion of testosterone to dihydrotestosterone.[16]

It has been suggested that DHT may have a role in protecting cells from apoptosis by restricting androgen receptors within cells and thus increase cell division at the expense of apoptosis which leads to hyperplasia.[18]

The present study recorded a significant increase ($P \leq 0.05$) in the level of serum prostatic specific antigen in patients with benign prostatic hyperplasia (3.312) compared with control samples (0.9033).

These results agreed with the findings of Nadler et al.[19] and Irani et al.[20] as the cavity of the prostate gland contains high concentrations of PSA (KLK3) and there are a number of barriers between the cavity and capillaries of these barriers include all of the basement membrane, stroma prostate cells capillary endothelial, and increasing levels of total PSA in the blood in patients with benign prostatic hyperplasia as a result of damage to the barriers between the epithelial layer and the bloodstream,[21]; usually, there are a small amount of leaking of the prostatic specific antigen into bloodstream, while in hyperplasia patients there are a large amount of leaking.[22] This is probably the reason leading to the increase in PSA (KLK3) in men with benign prostatic hyperplasia.

Indeed, there is strong relation between values of PSA (KLK3) and DHT, so high value of DHT may lead to expression of PSA

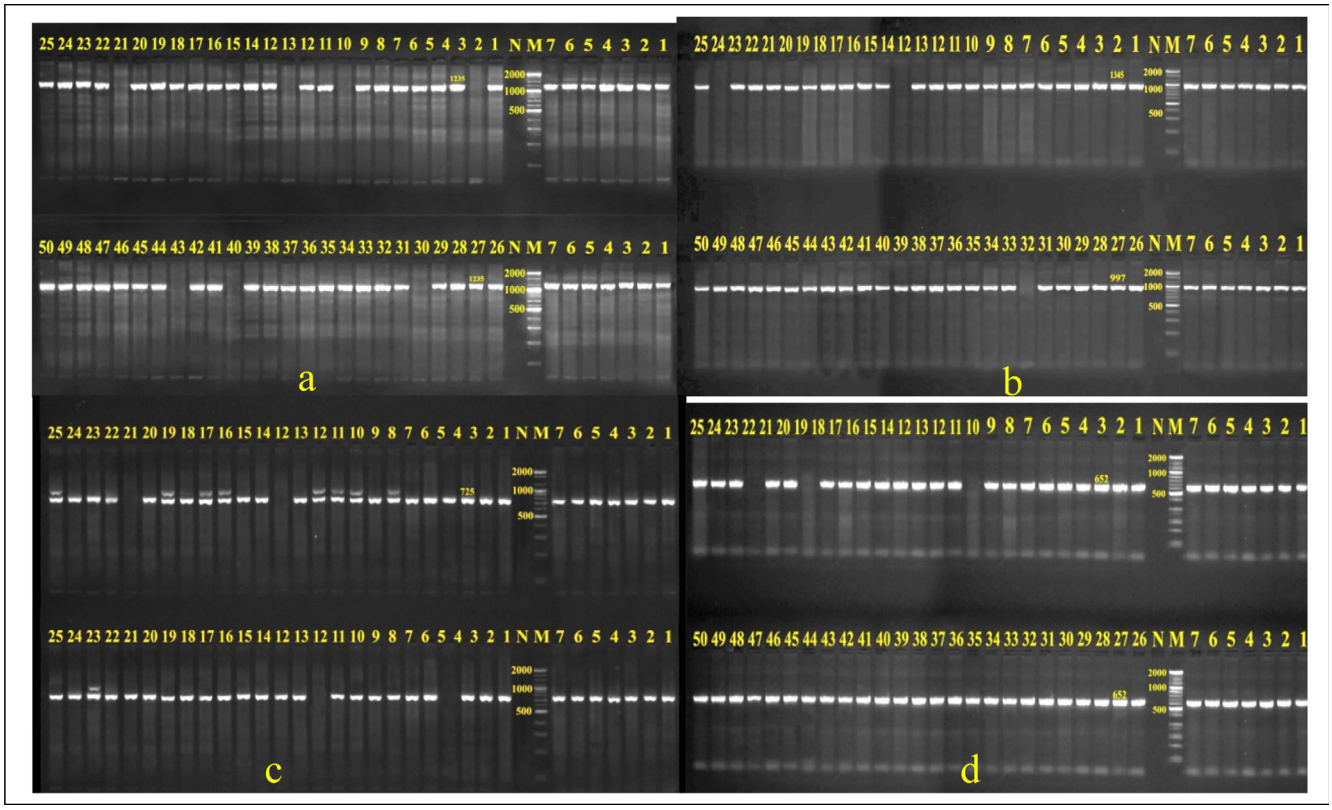

Fig. 1. Gel electrophoresis on Agarose gel in concentration 1.5% for a: KLK2a, b: KLK2b, c: KLK2c, and d: KLK2d.

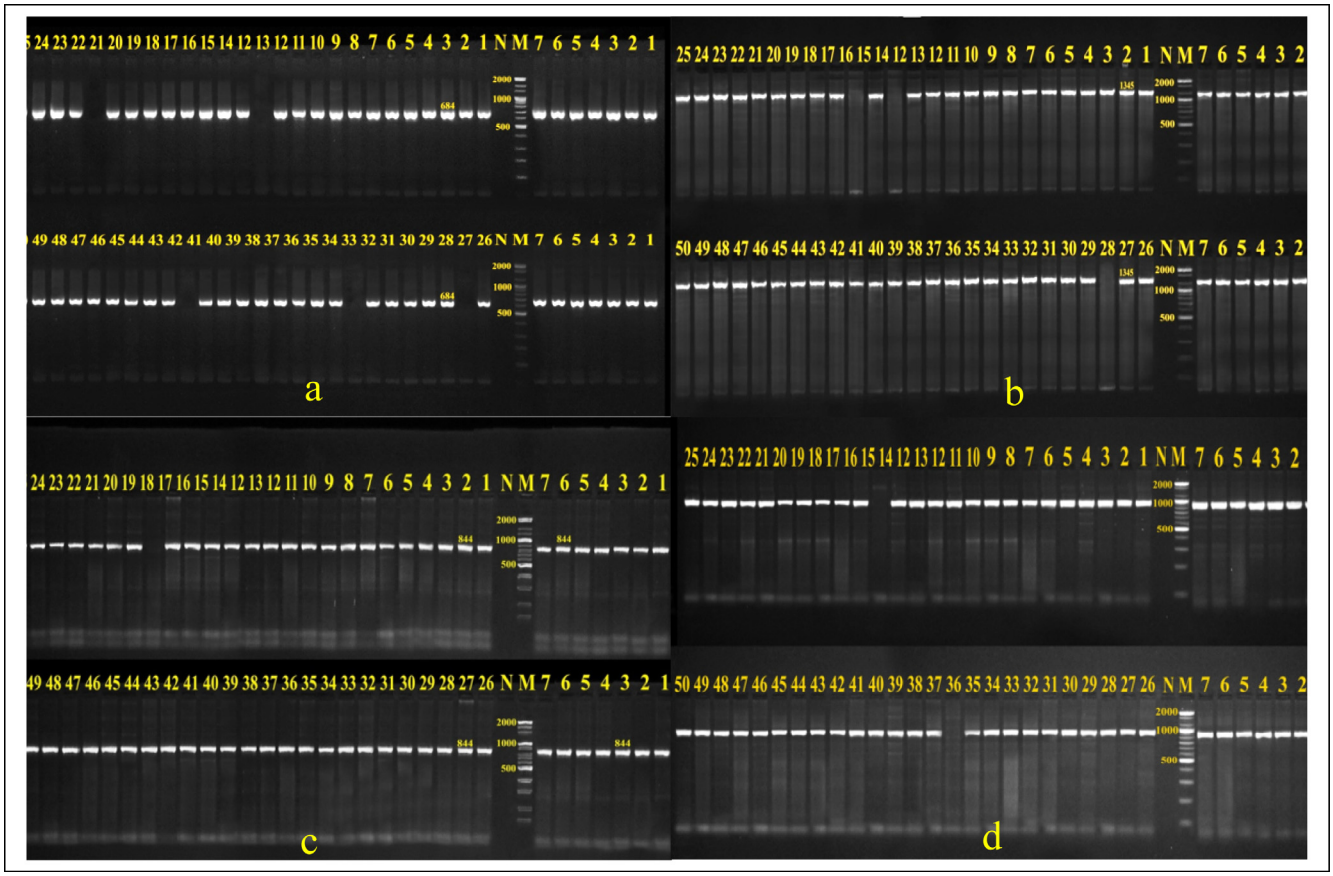

Fig. 2. Gel electrophoresis on Agarose gel in concentration 1.5% for a: KLK3a, b: KLK3b, c: KLK3c, and d: KLK3d.

(KLK3) and thus raise in blood. This may give the hormone importance in *KLK3* gene regulation.[13]

The results of polymerase chain reaction of *KLK2* gene showed 19 (38.00%) of the study sample suffering from various genetic disorders represented the absence of the expected appearance in four primers and it is distributed as follow, 14% for KLK2a, 8% for KLK2b, 10% for KLK2c, and 6% for KLK2d. The results of polymerase chain reaction of the primer KLK2c also showed allele polymorphism in 9 samples, while not appears among the control group any absence or variation in studied sites, as shown in Fig. 1.Results of the polymerase chain reaction of KLK3 gene showed 11 (22.00%), member of the study sample suffering from various genetic disorders represented the absence expected from bands in different primers and distributed as follows, 10% for KLK3a, 6% for KLK3b, 2% for KLK3c, and (4%) for the first KLK3d, and the rest of the samples only gave correct bands as expected as well as the control group did not show any lack of bands, as described in Fig.2.

The phenomenon of nucleotide polymorphism may regulate genes by stimulation or inhibition of them and may affect causing a particular disease and its progress, and genetic strategy proposed, such as the phenomenon of single nucleotide polymorphism as one of the causes of benign prostatic hyperplasia, where they were to determine the number of point mutations in several genes may have a positive effect on prostate growth and inflation.[23]

The genetic targets such as SNPs may evaluate as genetic signs promising to better understand genetic bases of various complex diseases, including benign tumor or cancer.[24]

Single nucleotide polymorphism represents approximately 90% of the variations. This means that the SNPs are most common variations in human genome.[25]

But these SNPs that occur in the prostate specific genes and their role in pathogenicity and disease progression for prostate hyperplasia are not entirely clear.[26] As functional SNPs are based on the location of polymorphisms, its regulation with protein production and maturation depends on the type of mutation which may lead to change in cellular processes and function of cells.[25]

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