

Association between the Asp327Asn Polymorphism of Sex Hormone-Binding Globulin Gene and Prostate Cancer

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Abstract

Background: By binding SHBG hormone to sex hormones, in addition to carrying them in the blood, they regulate the amount of tissue availability. The genetic changes in the structure of globulin affect its binding to hormones, so in this study the effects of single nucleotide change in exon 8 or rs 6259 in the incidence of prostate cancer was evaluated.

Materials and Methods: The study population included 120 patients with prostate cancer and 120 control subjects. After collecting blood samples, DNA was extracted by salting out procedure in order to determine the genotype of individuals by RFLP-PCR method. According to Hardy-Weinberg equilibrium genotypes the allele frequencies were calculated and a relationship between this variation and prostate cancer were evaluated using SPSS V.23. Also, the relationship between variations was investigated. P-values smaller than or equal to 0.05 were considered significant.

Results: Results indicated that homozygous mutant genotype AA 2.58 (p value= 0.007, OR: 2.58, CI95%: 1.52-4.38) and heterozygous AG increase the risk of prostate cancer in carriers by 1.18 times (p-value =0.5, OR: 1.18, CI95%: 0.38-3.61). But homozygous of wild genotype GG have a protective role against prostate cancer (p-value =0.005, OR: 0.385, CI95%: 0.23-0.65).

Conclusion: Asn allele is one of the main factors in prostate cancer, therefore, it could be used as the non-invasive and suitable marker for early detection in susceptible individuals.

Keywords: Prostate cancer; Sex hormone-binding globulin; PCR-RFLP

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Introduction

Prostate cancer is caused by malignancy and irregular proliferation of prostate cells. In most cases, it has slow growth and in some circumstances has fast growth (1). Evidence suggests that both environmental and genetic factors are involved in prostate cancer but complete understandings of the causes of prostate cancer are still unknown (2). The main risk factors for this cancer include smoking, poor diet, obesity, older age, family history, and genetic factors. Prostate cancer before 45 years of age is very unusual but its incidence increases by age (3).

Men who have first-degree relatives with prostate cancer are twice more at risk compared with men without a family history (4). Also, people with two first-degree relatives with prostate cancer are five-fold at risk of developing the disease (5).

Several studies have found a possible link between levels of androgens, such as testosterone and dihydrotestosterone circulating (DHT) and risk of prostate cancer in different ethnic groups (6). Androgens, which are male sex hormones are produced by the testes and adrenal glands and play a

crucial role in male reproduction and sexual behavior (7). In addition to androgens which are essential for development, operation and maintenance of prostate, estrogen and female sex hormones also have roles in the male reproductive system through specific receptors of estrogen (8).

Sex hormone-binding globulin (SHBG) is a plasma glycoprotein that binds to active androgens and estrogens and carries them in the blood. The affinity of this protein for its ligand is about four to five times to albumin (9). Plasma SHBG is secreted by the liver (10), but SHBG gene mRNA have also been identified in a variety of hormone-responsive tissues including prostate (11), endometrium (12), fallopian tubes (13, 15), specifically in granulosa cells-lutein (14), and placenta (16). The local production of SHBG represents the regulation of access to steroids and activating signals, by autocrine and paracrine processes (10). SHBG gene is located on the short arm of chromosome 17p12-13 (17) with 8 exons, 7 introns, and 3.2kb in length (18). The mature protein

is a plasma homodimer which is made up of two identical or nearly equal monomers. Each monomer includes O-linked glycosylation site in the tyrosine amino acid 7 (Thy7) and two N-linked glycosylation sites at Asn351 and Asn367 positions (19).

Three polymorphisms have been reported in the protein-coding region of SHBG which leads to changes in its amino acid sequence. Genetic variants have different effects on protein. One of the changes is point mutations in exon 8 at the N- glycosylated site in which aspartic acid in position 327 is replaced by asparagine. Initial reports suggested that changes in glycosylation have no effect on steroid binding properties, but today it is recognized that in wild type, protein of carbohydrates is essential for correct attachment of glycoprotein to its receptor (20). The aim of this study was to investigate the relationship between polymorphism Asp 327 Asn (D327N) in exon 8 SHBG gene and risk of prostate cancer in Iranian population.

Table 1. Biochemical variables and clinical indicators in patient and control groups.

Variable	Range	Mean± Std Error Difference		P value
		Case	Control	
Age (years)	40-50	18 (15%)	59 (49.17%)	0.000
	50-60	30 (25%)	42 (35%)	
	60 ≥	72 (60%)	19 (15.83%)	
	Mean ± Std Error Difference	67.88 ± 0.77	41.03 ± 1.53	
BMI (kg/m ²)	25 ≥	59 (49.17%)	62 (51.67%)	0.398
	25 >	61 (50.83%)	58 (48.33%)	
	Mean ± Std Error Difference	26.17 ± 2.42	25.08 ± 2.05	
PSA total (nmol/L)	Mean ± Std Error Difference	32.56 ± 1.27	15.72 ± 2.06	0.000
PSA Free(nmol/L)	Mean ± Std Error Difference	7.86 ± 0.17	1.92 ± 2.16	0.000
Smoker	Never	37	36	0.922
	Former	49	52	
	Current	34	32	
Stage of cancer	Stage I	71 (42.5%)		
	Stage II	42 (35%)		
	Stage III	15 (12.50%)		
	Stage IV	12 (10%)		

Materials and Methods

Study population

A case/control study was designed in which 120 Iranian men with primary prostate adenocarcinoma (patient

group) attending Imam Khomeini Hospital (Research Center of The genitourinary) were recruited. They were selected based on physical examination, Digital Rectal Exam (DRE), serum levels of PSA, prostatic

needle biopsy, histopathology report, and radical prostatectomy surgery. The same number of individuals were selected as control group who had no particular disease and also did not use any specific drug and had no acquired or genetic background of cancer, especially prostate cancer.

Genotyping

About 3 to 5 ml blood were taken from all subjects that were mixed with EDTA, then frozen at -20°C . DNA of all samples were extracted by salting out method and a spectrophotometer was used to determine the concentration of it. The polymorphism D327N forward primer with 5'-AGAAGAAGATATGGGGCAGTG-3' sequences and reverse primer with 5'-GGCCTGGTCCACATCC-3' sequence was used in order to amplify the fragment with 209bp length (20).

For every PCR reaction, 10 ml of water and 10 ml of the commercial master mix 2X amplicon (including MgCl₂ 1.5mM, buffer, Taq enzyme, dNTP and other materials needed for reaction), 1 microliter of each primer and 100 ng (1 μ l) from genomic DNA of each patient were added and after mixing were placed in thermocycler (Eppendorf Master cycler gradient, Germany). Programs included 95°C for 5 minutes, 95°C for 60 seconds, 62°C for 30 seconds, 75°C for 30 seconds at 37 cycles and 72°C for 5 minutes in one cycle for amplification of 209 bp of SHBG gene. The amplified products were electrophoresed on agarose gel 1.5% with DNA Safe beside 100bp size marker (Roche) at a constant voltage of 95 volts for 15 to 20 min, till bands were seen.

Hinf I restriction enzyme was added to the amplified products containing 209bp band and incubated for 16 to 18 hours at 37°C . Then product of digestion enzyme was poured onto the 3% agarose gel. The amplified fragment contained two incision sites. First and second incision sites were located after 75 and 144 nucleotides from 5' end of the string. The first incision site in all the samples had been cut, but the second incision site which was a location of D327N polymorphism of SHBG gene, had cut just for unchanged samples for this position. In the presence of nucleotide G in the desired position, three bands namely 75bp, 69bp 64bp had appeared which indicated GG genotype. But if it changes to A only two bands namely 133 bp and 75bp were seen, indicating that the genotype was AA. Heterozygous individuals possessed 133bp, 75bp, 69bp, and 64bp bands.

Ethics Statement

This research was done in compliance with all norms and standards of the ethics committee (Islamic azad university of Tehran Medical Sciences Branch) and

also by receiving written consent of the patients.

Statistical Analysis

Using genotype counts, their percentages and frequency were determined in cancer and control groups. The allele frequency in both groups was achieved according to Hardy-Weinberg equilibrium. The relationship between genotype and risk of prostate cancer in two groups were studied in SPSS ver. 23 applying Chi-square test.

Results

The body mass index (BMI) in 121 subjects was ≤ 25 and in 119 it was more than 25. BMI in the two groups were not significantly different ($p = 0.398$). According to the pathology reports, 71 (42.5%) patients were in Stage I, 42 in stage II (35%), 15 in stage III (12.5%), and 12 patients were in stage IV (10%).

After ensuring the amplification of 209bp segment, the samples were exposed to Hinf I enzyme. Results of some digestion of the samples are shown in Fig. 1. To ensure the accuracy of genotypes determined, a number of samples were randomly selected and sequenced.

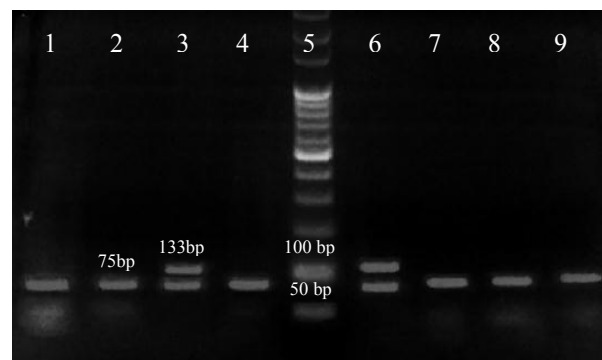


Figure 1. Results of the enzymatic digestion for some cancer and control samples: wells 1, 2, 4, 7, 8 & 9 is GG genotype, wells 3 & 6 is AA genotype, well 5 is marker 100bp

Results

The results of the genotypes count are presented in Table 2. According to Hardy-Weinberg equilibrium, the frequency of allele A in the cancer group was 0.55 versus 0.33 in the control group. G allele frequency in the cancer group and control group were 0.45 and 0.67, respectively.

Statistically, a significant relationship was found between the two groups ($P=0.007$). By calculating the risk of prostate cancer, we found that individuals with AA genotype were 2.58 times, and people with AG / GA heterozygous genotype were 1.18 times more likely to develop prostate cancer. But GG homozygous genotype had a protective role against prostate cancer (odds Ratio = 0.385).

Table 2. Statistical analysis of genotypes

Genotype	Tumor group	Control group	P Value	Odds ratio	CI 95%
AA	63 (52.5%)	36 (30%)	0.007	2.58	1.52-4.38
GG	50 (41.6%)	78 (65%)	0.005	0.385	0.23-0.65
AG/GA	7 (5.8%)	6 (5%)	0.5	1.18	0.38-3.61

Discussion

Prostate cancer is the main cause of cancer death in Iran (21). However, some studies have shown that unlike America and Europe, prostate cancer is not common in Iran (22). Accurate statistics are not available in Iran but in 2007 the incidence of this cancer in Iran was about 1.5 per 100,000 people in a year which was much lower compared to Western countries. In western countries, this rate is extremely high, from 50 to 500 cases per 100,000 people in a year. However, the number of males with prostate cancer is increasing compared to previous years, so it is highly important to prevent and control it for the health of the society (22). Prostate cancer is dependent on testosterone and androgen metabolism, so it seems reasonable to assume that genetic changes in active genes which are involved in hormones pathways can affect the risk of developing the disease (8). Since plasma glycoprotein SHBG is considered as a carrier protein for the active androgen and estrogen, genetic changes affect its plasma distribution and can change the access of target tissues cells (23).

The D327N polymorphism is a functional polymorphism that causes adding Glycosylation to N-Linked into the C-terminal of laminin G-like globular domain protein (24). In animal models it was identified that additional carbohydrate chains in the protein SHBG (which codes by Asn 327 allele) reduced its metabolic clearance and increased the half-life of the protein. For this reason, an increase in SHBG serum levels for carriers of these alleles can be seen (25).

Testosterone controls how the prostate gland grows and develops. For patients with advanced or metastatic prostate cancer, hormone therapy with anti-androgen compounds might be the last best hope (26). Also, some found a strong relationship between SHBG gene polymorphism and risk of developing prostate cancer (27). But, there is no study about the detection of Rs 6259 polymorphism of SHBG in Iranian men and prostate cancer. We decided to evaluate the relationship between polymorphism of exon 8 SHBG and prostate cancer in order to diagnose the disease in early stages.

The rs6259 polymorphism in exon 8 SHBG occurs due to change of G nucleotide to A nucleotides. After

analyzing the genotypes in two groups AA homozygous genotypes were found in 63 people (52.5%) in cancer group and in 36 (30%) of the control group (p-value=0.007). Also GG homozygous genotype included 50 people (41.6%) in cancer group and 75 (65%) in control group (p-value=0.005). Genotype AG / GA in cancer group and controls included 7 (5.8%) and 6 (5%) individuals, respectively (p-value=0.5). The frequency of A mutant allele in the cancer group was more than the control group while G allele was more frequent in the control group. The results of the present study were in line with those of Berndt & et al in which polymorphisms in associated genes with hormones and risk of prostate cancer were investigated. rs 6259 Exon 8 was one of the polymorphisms examined among 14 polymorphisms. The study showed an association between rs 6259 Exon 8 and risk of developing prostate cancer, and it was found that genetic variation of SHBG could influence the susceptibility to prostate cancer (27).

A study was done to understand the molecular mechanism of hormone resistance and its association with disease progression in patients with prostate cancer and AR and SHBG gene polymorphisms were evaluated. Results showed that polymorphism 5790 G> A (rs 6259) can be proposed as a good marker for patients with hormone resistance (28).

In another study, serum concentrations of total testosterone, estradiol, and ratio of testosterone to estradiol and also ratio of binding globulin SHBG to sex hormone which are associated with prostate cancer were evaluated in 631 healthy volunteers and the results led to the hypothesis that prostate tissue biology depends on the duration of exposure to sex steroids and is effective on people's lives (29).

The association between breast cancer and rs 6259 polymorphism was investigated by Cui Y (30), Piccioni (31), Dunning et al. (32). Similar to the current results, they found that this polymorphism might be involved in causing cancer. This polymorphism was also found to be associated with risk of type 2 diabetes and increase in SHBG serum levels by 10% (33).

The present study suggests that Rs 6259 polymorphism could be associated with the susceptibility of Prostate cancer. The odds ratio of the

AA mutant genotype was calculated as 2.58 (p value= 0.007, OR: 2.58, CI95%: 1.52-4.38), showing the significant effect of this genotype in causing the disease. It seems that SHBG gene polymorphisms like genetic changes could influence the development of prostate cancer. Inconsistent results may be due to differences in genetic studies in different populations or other changes such as different techniques applied for polymorphism identification. Further studies with larger sample sizes are recommended in order to assess the accuracy of these findings.

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Authors' contributions

TZ developed the study concept, designed the protocol, and wrote the manuscript. HM analyzed the data. NMR & ZhH introduced patients for the research.

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Conflict of interest

The authors have no conflicts of interest to declare.

Role of sponsor

The funding organizations are public institutions and had no role in the design and conduct of the study.

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