

Association of *MUC1* 5640G>A and *PSCA* 5057C>T polymorphisms with the Risk of Gastric Cancer in Northern Iran

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Abstract

Background: Gastric cancer is the fourth most common cancer worldwide and the second leading cause of cancer-related death. Genome-Wide Association Studies (GWAS) have shown that genetic diversities *MUC1* (Mucin 1) and *PSCA* (Prostate Stem Cell Antigen) genes are involved in gastric cancer. The aim of this study was to determine the association between rs4072037G>A polymorphism in *MUC1* and rs2294008 C>T in *PSCA* gene with gastric cancer in northern Iran.

Methods: DNA was extracted from 99 formalin fixed paraffin-embedded (FFPE) tissue samples of gastric cancer and 96 blood samples from healthy individuals (sex matched) as controls. DNA was extracted followed by PCR using specific primers. Two desired polymorphisms, 5640G>A and 5057C>T for *MUC1* and *PSCA* genes were genotyped using PCR-RFLP method.

Results: The G allele at rs4072037 of *MUC1* gene was associated with a significant decreased gastric cancer risk (OR = 0.507, 95% CI: 0.322–0.799, $p = 0.003$). A significant decreased risk of gastric cancer was observed in people with either AG vs. AA, AG+AA vs. GG and AA+GG vs. AG genotypes of *MUC1* polymorphism (OR = 4.296, 95% CI: 1.190–15.517, $p = 0.026$), (OR = 3.726, 95% CI: 2.033–6.830, $p = 0.0001$) and (OR = 0.223, 95% CI: 0.120–0.413, $p = 0.0001$) respectively. Overall, no significant association was observed between the *PSCA* 5057C>T polymorphism and risk of gastric cancer in all genetic models.

Conclusion: Results indicated that the *MUC1* 5640G>A polymorphism may have protective effect for gastric cancer and could be considered a potential molecular marker in gastric cancer in the Northern Iran population.

Background

Gastric cancer is the fourth most common cancer worldwide and the second leading cause of cancer-related death (1). The prevalence of gastric cancer varies among different geographic populations around the world (2). More than 70% of deaths from stomach cancer occur in developing countries (3, 4). Northern and northwestern regions of Iran are at high risk for gastric cancer (2). In addition, the mortality rate from stomach cancer is the first cause of death due to cancer in both sexes in Iran (5, 6). From 2000 to 2005, the incidence rate was highest in northern Provinces, including Mazandaran, Golestan, and Ardabil (2). It is believed that different environmental risk factors like alcohol consumption, smoking habits, diet and infectious agents (*Helicobacter pylori*) are involved in the development of gastric cancer (7-9), but a genetic predisposition to stomach cancer is unknown yet. Previous studies suggested an association between *MUC1* and stomach (10), colon (11), lung (12), ovarian (13), breast (14), pancreas (15), and thyroid cancers (16). In addition, Genome-Wide Association Studies (GWAS) proposed genetic diversities like rs4072037G>A (5640G>A) in *MUC1* gene and rs2294008C>T (5057C>T) polymorphisms in *PSCA* (Prostate Stem Cell Antigen) gene as genetic risk factors in gastric cancer (17).

MUC1 (Mucin 1) gene is located on chromosome 1q21 and includes seven exons (18). This gene encodes a membrane-bound glycoprotein with the transmembrane domain, which is attached to the upper surface of the gastrointestinal epithelium. It plays important roles in protecting epithelial surfaces against external agents (19, 20). MUC1 protein in tumor cells or tumor-associated MUC1 (TA-MUC1) differs from MUC1 expressed in normal cells, with regard to its cellular distribution, biochemical features, and function. TA-MUC1 is hypoglycosylated and overexpressed in a variety of cancers, which plays a crucial role in progression of the disease (21). It is believed that hypoglycosylation may potentiate *MUC1* oncogenic signaling by decreasing its cell surface levels and increasing intracellular accumulation (21, 22).

PSCA gene is located on chromosome 8q24.3, which encodes a glycosylphosphatidyl inositol (GPI)-anchored protein with 114 amino acids. *PSCA* gene plays an important role in cell adhesion, proliferation, and survival (23, 24). *PSCA* is expressed in the epithelial cells of the bladder, stomach, kidney, skin, and esophagus. The function of *PSCA* in normal cells and carcinogenesis is not clearly known (25). Several reports indicated that *PSCA* is associated with various cancers, such as prostate (26), bladder (27), and pancreatic cancer (28). *PSCA* is up-regulated in the prostate, and pancreas cancers, while in the esophagus and stomach cancers it is down-regulated (29). 5057C>T is a missense single nucleotide polymorphism (SNP) in 5'-UTR region of *PSCA*. This polymorphism leads to changes in transcriptional activity in the upstream regions of *PSCA* gene (30). The C allele changes the initiation codon from ATG to the alternative start codon ACG (threonine instead of methionine) and leads to a truncated protein lacking nine amino acids from the N-terminus of *PSCA* protein. This alteration changes the protein folding and impairs intracellular processes (31).

The aim of this study is also evaluating the association between *MUC1* gene polymorphism, rs4072037G>A and *PSCA* gene polymorphism rs2294008C>T with gastric cancer in northern Iran.

Methods

The study population

Ninety nine formalin fixed paraffin-embedded (FFPE) tissue samples (64 males and 35 females, average age 67.5 ± 10.9) were collected from gastric cancer patients who referred to the pathology department of the Sari Imam Khomeini hospital from 2009 to 2016 (Table 1) (Mazandaran Province). Tumor characteristics of the patients are summarized in table 2. In addition, 96 blood samples were collected as control from healthy individuals (57 males and 39 females, average age 34.3 ± 7.0) who referred to Novin genetics laboratory of Sari for other reasons except gastric diseases (Table 1). The normal control individuals were not age-matched with patients, but they were selected randomly to find the allele/genotype frequency of the polymorphisms in the local population. Blood samples were collected in EDTA containing tubes and preserved at $-20\text{ }^{\circ}\text{C}$. This research was approved by the ethics committee in the Mazandaran University of Medical Sciences.

DNA extraction

DNA was extracted from paraffin-embedded tissues using “YTA Genomic DNA Extraction mini kit (Yekta Tajhiz Azma, Iran) and standard salting out methods from blood samples, respectively. The extracted DNA was maintained at -20 ° C until further study.

Genotyping using PCR-RFLP method

PCR (*Polymerase chain reaction*) was applied to amplify exon 2 of *MUC1* and 5'-UTR region of *PSCA* genes, including desired polymorphisms using specific primers (Table 3). Each reaction consists of 2µl template DNA, 11µl ready 2x PCR master mix RED (Ampliqon, Denmark), 11µl distilled water, 0.5µl of each primer at 25µM, in a total volume of 25µl. The PCR reactions were carried out under the following conditions: 94°C for 5 minutes, followed by 35 cycles 94°C for 60 seconds; 72°C for 60 seconds. The annealing temperature of 60 °C for 60 seconds was used for *MUC1* gene and 56 °C for 60 seconds used for *PSCA* gene. PCR products were visualized on 1% agarose gel containing SYBR Safe staining. Then, PCR products were subjected to restriction digestion using 6µl of the PCR products and five units of restriction enzyme. Briefly, 188 bp PCR fragment of the *MUC1* gene was digested using *A/III* enzyme; a homozygous GG allele remained uncut, homozygous AA genotype was digested into 114 and 74 bp fragments, and heterozygous GA produced three fragments, 118, 114 and 74 bp, respectively (Figure 1, A). Also, 139 bp PCR fragment of the *PSCA* gene was digested using a *N/III* restriction enzyme, genotype TT was digested to 2 fragments, 101 and 38 bp, genotype CC remained uncut and heterozygous CT showed three fragments, 139, 101 and 38 bp, respectively (Figure 1, B).

Statistical analysis

Chi-square test was used to evaluate differences in the distributions of demographic characteristics. The expected frequency of control genotypes was tested against the Hardy–Weinberg equilibrium. The odds ratio (OR) and 95% confidence interval (CI) were calculated using a logistic regression model. A *p*-value < 0.05 was considered statistically significant. Otherwise, the pooled ORs and 95% CIs without adjustments were calculated for the *MUC1* polymorphism rs4074037 as allele (G vs. A), homozygous genotype (GG vs. AA), heterozygous (AG vs. AA), dominant (AG+AA vs. GG), recessive (AA vs. AG+GG) and over-dominant model (AA+GG vs. AG). For *PSCA* polymorphism rs2294008, it was considered as allele (T vs. C), homozygous (TT vs. CC), heterozygous (CT vs. CC), dominant (CT+CC vs. TT), recessive (CC vs. CT+TT). The statistical analysis was performed using the IBM SPSS Statistics v21 software package (SPSS, Chicago, IL, USA).

Results

Demographic and clinical information of 99 patients with gastric cancer and 96 controls are shown in tables 1 and 2, respectively. Ninety nine patient samples were genotyped for *PSCA* gene (rs2294008) and 91 patient samples for *MUC1* gene (rs4072037) polymorphism. Genotype and allele frequencies of

rs4072037 and rs2294008 and their associations with the risk of gastric cancer are presented in tables 4 and 5, respectively.

The G allele frequency of rs4072037 is 64% in patients with gastric cancer compared with 78% in controls, respectively (Table 4). *MUC1* rs4072037 polymorphism is associated with significant decreased gastric cancer risk in four genetic models: G vs. A (OR = 0.507, 95% CI: 0.322–0.799, $p = 0.003$); heterozygous AG compared with AA (OR = 4.296, 95% CI: 1.190–15.517, $p = 0.026$); dominant model AG+AA vs. GG (OR = 3.726, 95% CI: 2.033–6.830, $p = 0.0001$); and over-dominant model AA+GG vs. G (OR = 0.223, 95% CI: 0.120–0.413, $p = 0.0001$) (Table 4).

In the current study, no significant association was observed between the *PSCA* 5057C>T polymorphism and risk of gastric cancer in all genetic models (T vs. C (OR = 1.050, 95% CI: 0.652 –1.693, $p = 0.840$); homozygous TT vs. CC (OR = 0.903, 95% CI: 0.327 –2.492, $p = 0.533$); heterozygous CT vs. CC (OR = 1.228, 95% CI: 0.644 –2.339, $p = 0.533$); dominant model CT+CC vs. TT (OR = 1.137, 95% CI: 0.420 –3.080, $p = 0.800$); and recessive model CC vs. CT+TT (OR = 0.904, 95% CI: 0.505 –1.620, $p = 0.735$)). As a result, There was no statistically significant differences between the genotype and allele frequency of *PSCA* (5057C>T) in cases and controls in this study (Table 5).

Furthermore, the result of interaction effects between *MUC1* (rs4072037) and *PSCA* (rs2294008) genotypes with gender is summarized in table 6.

Discussion

Different studies revealed a decreased incidences of gastric cancer globally, but it is still the most common cancer in the north and northwestern parts of Iran (2). Mazandaran, Golestan, and Ardabil Provinces are high-risk for gastric cancer (2, 32). Gastric cancer is a multi-factorial disease; a high dietary intake of salt, the high prevalence of *Helicobacter pylori* (*H. pylori*) infection, smoking and gastroesophageal reflux disease are the environmental factors in the pathogenesis of this disease in Iran (2, 9). Among genetic risk factors, *MUC1* (5640G>A) and *PSCA* (5057C>T) polymorphisms were reported in several studies earlier (33-36).

MUC1 is a large, transmembrane mucin glycoprotein expressed at the apical surface of most simple epithelia including stomach. Functions related to *MUC1* are generally associated with mucins such as lubrication, hydration of cell surfaces, protection from microorganisms and degradative enzymes (37). *MUC1* protein contains three different domains: short cytoplasmic, transmembrane and a large extracellular domain. The *MUC1* gene in human is comprised of 7 exons that can be alternatively spliced to form different transcripts (37). At least twelve splice variants of *MUC1* have been described (38). The SNP rs4072037G>A (5640G>A) located in the 5' side of the second exon determines the splicing acceptor site and produce two major *MUC1* transcripts. The "G" allele determines the variant type 2 or *MUC1*/B and contains the first 27 bp of the 2nd exon and have 9 amino acids longer than "A" allele, which create variant type 3 or *MUC1*/A in the N-terminal domain (39-41).

This additional sequence is potentially changing its intracellular trafficking or subsequent processing. The impact of the nine additional amino acids of MUC1/A on the signaling functions and intracellular localization was investigated by Imbert-Fernandez and colleagues (41). They showed that COS-7 cell line transfected by plasmid containing MUC1/A or MUC1/B had similar protein expression and plasma membrane localization. This study also showed MUC1/B and MUC1/A differs in their ability to modulate tumor necrosis α (TNF α)-induced transcription of IL-1 β and IL-8, inducing the basal TGF β expression; finally they show different inflammatory activities (41).

MUC1 could protect the gastric epithelial cells as physical barriers, so could inhibit *H. pylori* binding to gastric epithelial cells. The carriers of the short-short (SS) homozygous variant genotype, were at a high risk of *H. pylori* infection, compared to the carriers of the long-long (LL) and long-short (LS) genotypes of *MUC1* variable number of tandem repeats (VNTR) (42). Miao Li and colleagues, examined the relation between *H. pylori* infection and three polymorphisms (rs4072037 at 1q22, rs13361707 at 5p13, and rs2274223 at 10q23) involved in the inflammatory response to the increase of gastric cancer risk. They reported that people infected by *H. pylori*, carrying the genotypes AA rs4072037, CT/CC rs13361707 and AG/GG rs2274223 showed an increased risk of gastric cancer(43).

Different studies (Hanze Zhang et al., 2011, J Kupcinskis et al., 2014) have *shown* that the G allele locus on rs4072037 was *significantly associated* with a *decreased gastric cancer risk* (44, 45). Xinyang Liu et al., 2014 suggested the G allele at rs4072037 of the *MUC1* gene might have a protective rule in people from Asian countries against gastric cancer. Also, they reported that the association was more prevalent in Asian population than in Caucasians (46).

In this study, allelic comparison (G vs. A), genotype comparison (GA vs. AA; GG vs. AA), were done and dominant model (AG+AA vs. GG), recessive model (AA vs. AG+GG), over-dominant model (AA+GG vs. AG) was tested (47). We found that in this study, the rs4072037 AG genotype was significantly associated with the reduced risk of gastric cancer or it plays a protecting role [odds ratios (OR) = 4.296; 95% confidence interval (CI) = 1.190-15.517, $p = 0.026$ for AG vs. AA].

A study by Hye-Rim Song et al., 2014, with 3,245 GC cases and 700 controls suggests that the rs4072037 AG genotype was significantly associated with a reduced risk of gastric cancer [odds ratios (OR) = 0.78; 95 % confidence interval (CI) = 0.67–0.91 for AG vs. AA]. Their data indicated a significant association between the rs4072037 G allele and reduction in risk of gastric cancer (48). Another meta-analysis by Xi Gue et al., 2018, which included seventeen case–control studies (12551 cases and 13436 controls), suggests that the *MUC1* rs4072037 polymorphism might decrease the risk of gastric cancer in four different genetic combination (G vs. A; AG vs. AA; GG vs. AA; AG+GG vs. AA). They also performed analysis using ethnicity along with G allele and found the cancer risk was decreased among Asian population but not Caucasian (49).

Similar results (including 4,220 cases and 6,384 controls) have been obtained by Duan et al, 2014 through evaluating the association between *MUC1* (rs4072037) polymorphism and gastric cancer (50). Their study demonstrated that the *MUC1* (rs4072037) polymorphism is associated with risk of cancer in

all genetic models (G vs. A; GA vs. AA; GG vs. AA; AG+AA vs. GG; GG vs. AG+AA). They suggested *MUC1* (rs4072037) polymorphism had a slightly reduced gastric cancer risk among Asian population. Also, this polymorphism was significantly associated with a decreased cancer risk in all genetic models except for homozygous recessive (AA) model in Caucasian population (50).

In this study, *MUC1* rs4072037 polymorphism was significantly associated with a decreased risk of cancer in all genetic models except for homozygous GG vs. AA and recessive model AG+GG vs. AA (G vs. A: OR = 0.507, 95% CI: 0.322–0.799, $p = 0.003$; AG vs. AA: OR = 4.296, 95% CI: 1.190–15.517, $p = 0.026$; AG+AA vs. GG: OR = 3.726, 95%CI: 2.033–6.830, $p = 0.0001$; AA+GG vs. AG: (OR = 0.223, 95% CI: 0.120–0.413, $p = 0.0001$)). Data from this case-control study indicated that four genetic models G vs. A, heterozygous GA (GA vs. AA), dominant (AG+AA vs. GG), over-dominant model (AA+GG vs. AG), were significantly associated with the decreased risk of cancer. Therefore, *MUC1* rs4072037 polymorphism was associated with decreased risk of gastric cancer in northern Iran.

The Prostate Stem Cell Antigen (*PSCA*) is expressed in the gastric epithelium of the isthmus area. This area includes immature epithelial cells. *PSCA* expression increases in prostate cancer but it is reduced in gastric cancer and gastric intestinal metaplasia (51, 52). T allele carriers show lower transcriptional activity compared with the C allele carriers (5057C>T) in their gastric mucosa (31, 51). T allele carriers have nine amino acids more than C allele carriers (natural protein of 114 amino acid residues) (53). The association of *PSCA* (5057C>T) variant was described in Korea and Japan population for the first time and the T allele was reported as a risk factor for gastric cancer (31). A study in Japan and Korea showed that 5057T allele plays a more significant role in diffuse gastric cancer compared with the intestinal gastric cancer (31, 54). Such results were also similar in the population of Poland and the United States (34). Other studies in Caucasian and Chinese population also confirmed that this polymorphism is a risk factor for diffuse and intestinal gastric cancer (55).

In this study, *PSCA* rs2294008 polymorphism was not significantly associated with an increased risk of cancer in all genetic models (T vs. C (OR = 1.050, 95% CI: 0.652 –1.693, $p = 0.840$); homozygous TT vs. CC (OR = 0.903, 95% CI: 0.327 –2.492, $p = 0.533$); heterozygous CT vs. CC (OR = 1.228, 95% CI: 0.644 –2.339, $p = 0.533$); dominant model CT+CC vs. TT (OR = 1.137, 95% CI: 0.420 –3.080, $p = 0.800$); and recessive model CC vs. CT+TT (OR = 0.904, 95% CI: 0.505 –1.620, $p = 0.735$) (Table 5). Therefore, *PSCA* rs2294008 polymorphism was not associated with increased risk of gastric cancer in Northern Iran.

Conclusion

In conclusion, this study evaluated the effect of *MUC1* (rs4072037) and *PSCA* (rs2294008) polymorphisms on GC risk in the Northern Iran population. Data from this study revealed that the *MUC1* rs4072037 polymorphism was significantly associated with decreased risk of gastric cancer. However, the results could be important, especially for the interpretation of a genetic variant, which cause susceptibility to gastric cancer, and could be used as diagnostic markers in gastric cancer. In addition,

PSCA polymorphism did not show any relation to gastric cancer in our findings probably because of the type of gastric cancer or the small sample size.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of Mazandaran University of Medical Sciences and complied with the guidelines and principles of the Declaration. All participants signed written informed consent.

Consent for publication

All participants signed written informed consent for publication.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the author for correspondence upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Mohammad bagherHashemi-soteh conceived and designed the study.

Reza Alikhani performed the experiments.

Ali Taravati analyzed the data.

Mohammad bagherHashemi-soteh and Reza Aikhani wrote and edited the manuscript.

All authors read and approved the final manuscript.

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Abbreviations

GWAS: Genome-Wide Association Studies

MUC1: Mucin 1

PSCA: Prostate Stem Cell Antigen

FFPE: Formalin fixed paraffin-embedded

TA-MUC1: tumor-associated MUC1

GPI: Glycosylphosphatidyl inositol

SNP: Single nucleotide polymorphism

PCR: *Polymerase chain reaction*

RFLP: Restriction fragment length polymorphism

H. pylori: Helicobacter pylori

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Tables

Table 1: Demographic characteristics of the cases and controls.

Variables	Cases (n = 99)		Controls (n = 96)		P
Age (years) (mean ± SD)	67.5 ± 10.9		34.3 ± 7		0.000
Sex	n	%	n	%	
Male	64	52.9	57	47.1	0.448
Female	35	47.3	39	52.7	

Table 2. Tumor characteristics, including tumor site, tumor grade, lymphatic invasion, perineural invasion, tumor stage and tumor type.

Characteristic		Frequency (%)
Tumor site	Cardia	18 (33.3)
	Fundus	0 (0.0)
	Body	11 (20.4)
	Antrum	18 (33.3)
	overlapping	7 (13.0)
	Total	54(100)
Grade	I	13 (18.8)
	II	39 (56.5)
	III	17 (24.6)
	Total	69(100)
Lymphatic invasion	Present	27 (67.5)
	Absent	13 (32.5)
	Total	40(100)
Perineural invasion	Present	28 (54.9)
	Absent	23 (45.1)
	Total	51(100)
T	1	3 (6.1)
	2	19 (38.8)
	3	26 (53.1)
	4	1 (2.0)
	Total	49(100)
N	0	19 (36.6)
	1	18 (37.5)
	2	9 (118.8)
	3	2 (4.2)
	Total	48(100)
M	0	6 (66.7)
	1	3 (33.3)
	Total	9(100)
Stage	I	8 (19.0)
	II	16 (38.1)
	III	15 (35.7)
	IV	3 (7.1)
	Total	42(100)
Tumor type	Diffuse	14 (73.7)
	Intestinal	5 (26.3)
	Total	19(100)

Table 3. Primer sequences, PCR size and PCR-RFLP fragments using *AIWNI* and *NIaIII* restriction enzymes for *MUC1* 5640G>A (rs4072037) and *PSCA* 5057C>T (rs2294008) polymorphisms, respectively.

SNP	Primer sequence	PCR Size (bp)	Restriction enzyme	Digestion products (bp)		
				Wild-type	Mutant	Heterozygous
<i>MUC1</i> 5640G>A (rs4072037)	F: TAAAGACCCAACCCTATGACT R: AGAGTACGCTGCTGGTCATACTC	188	<i>AIWNI</i>	188	114,74	188,114,74
<i>PSCA</i> 5057C>T (rs2294008)	F: GAAACCCGCTGGTGTGACT R: GCCAAGCCTGCCATCAACAG	139	<i>NlaIII</i>	139	101,38	139,101,38

Table 4. Comparison of genotype and allele frequencies of *MUC1* gene polymorphisms between gastric cancer patients (n=91) and normal controls (n=96) using chi-square analysis.

SNP ^a	Genotype/Allele	Controls(n=96)	Cases(n=91)	OR ^b (95% CI ^c)	P-value
<i>MUC1</i> ^d 5640G>A (rs4072037)	AA	8 (8.4%)	4 (4.1%)	-	-
	AG	27 (28.1%)	58 (63.9%)	4.296 (1.190-15.517)	0.026
	GG	61 (63.5%)	29 (32%)	0.951 (0.265-3.417)	0.938
	AG+GG	88 (91.6%)	87 (95.9%)	0.506 (0.147-1.741)	0.280
	AG+AA	35 (36.5%)	62 (68%)	3.726 (2.033-6.830)	0.0001
	AA+GG	69 (71.9%)	33 (36.1%)	0.223 (0.120-0.413)	0.0001
	A	43 (22%)	66 (36%)	-	-
	G	149 (78%)	116 (64%)	0.507 (0.322 - 0.799)	0.003

a. Single nucleotide polymorphisms, b. odds ratio, c. confidence interval, d. Mucin 1

Table 5. Comparison of genotype and allele frequencies of *PSCA** gene polymorphisms between gastric cancer patients (n= 99) and normal controls (n= 96) using chi-square analysis.

SNP ^a	Genotype/Allele	Controls(n=96)	Cases(n=99)	OR ^b (95% CI ^c)	P-value
<i>PSCA</i> ^d 5057C>T (rs2294008)	CC	63 (65.6%)	62 (62.6%)	-	-
	CT	24 (25%)	29 (29.3%)	1.228 (0.644 - 2.339)	0.533
	TT	9 (9.4%)	8 (8.1%)	0.903 (0.372 -2.492)	0.844
	CT+TT	34 (34.4%)	37 (37.4%)	0.904(0.505-1.620)	0.735
	CT+CC	90 (90.6%)	91 (91.9%)	1.137(0.420-3.080)	0.800
	C	150 (78%)	153 (77%)	-	-
	T	42 (22%)	45 (23%)	1.050 (0.652 - 1.693)	0.840

a. Single nucleotide polymorphisms, b. odds ratio, c. confidence interval,* *Prostate stem cell antigen*

Table 6. Distribution of genotypes *MUC1* (rs4072037) and *PSCA* (rs2294008) polymorphisms according to Sex.

Sex	Group	<i>MUC1</i> 5640G>A (rs4072037)			p-value	<i>PSCA</i> 5057C>T (rs2294008)			p-value
		GG	GA	AA		CC	CT	TT	
Male	Case	18(30.5%)	40(67.8%)	1(1.7%)	0.0001	41(64.1%)	17(26.6%)	6(9.4%)	0.92
	Control	35(61.4%)	17(28.9%)	5(8.8%)		35(61.4%)	17(29.8%)	5(8.8%)	
Female	Case	11(34.4%)	18(56.3%)	3(9.4%)	0.06	21(60.0%)	12(34.3%)	2(5.7%)	0.25
	Control	24(61.5%)	12(30.8%)	3(7.7%)		28(71.8%)	7(17.9%)	4(10.3%)	

Figures

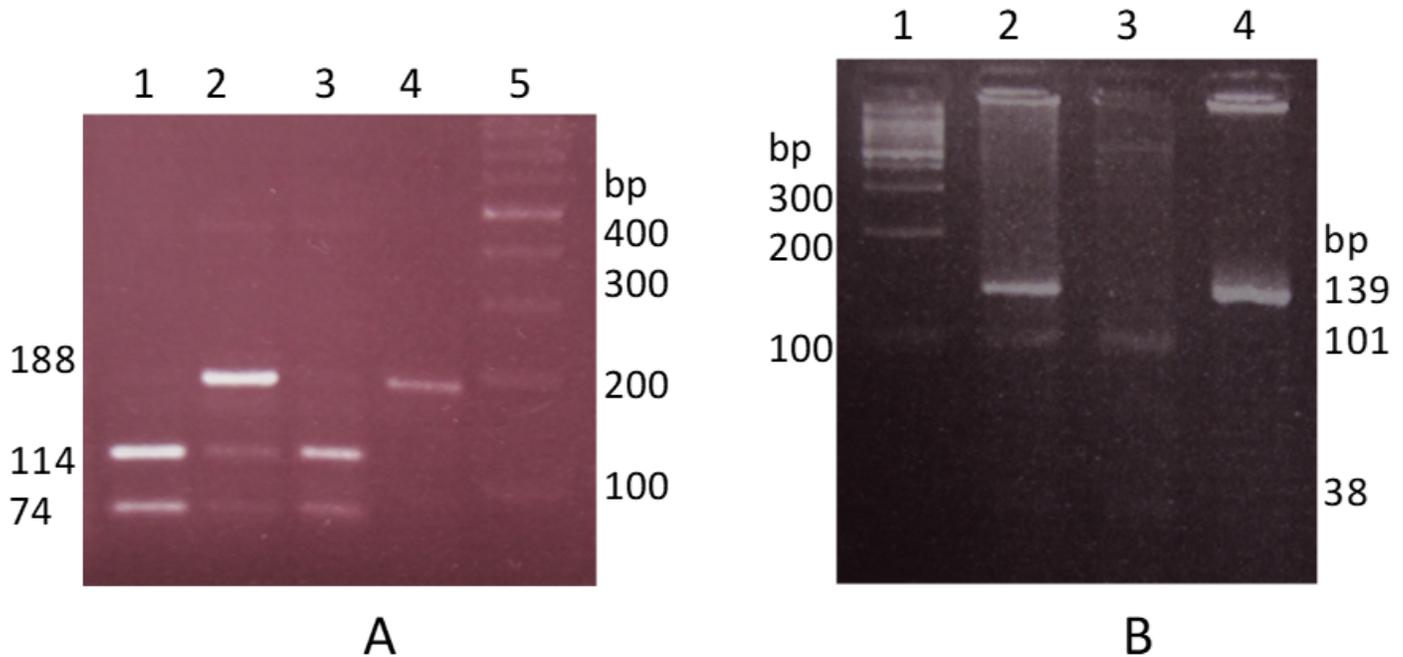


Figure 1

3% agarose gel electrophoresis of the restriction enzyme digestion (A) 188 bp PCR fragment of the MUC1 gene restriction enzyme digestion results using A1WNI enzyme; lane 1 and 3 shows homozygous AA, lane 2 heterozygous GA genotype and lane 4 shows homozygous GG genotype respectively, lane 5 shows 100 bp DNA marker (B) Restriction enzyme map of the 139 bp PCR fragment of the PSCA gene using NlaIII enzyme. Lane 1= 100 bp DNA marker, lane 2 heterozygous CT, lane 3 (101 and 39 bp) shows homozygous TT and lane 4, uncut PCR product shows homozygous CC genotype respectively.