



ASSOCIATION OF PROSTATE SPECIFIC ANTIGEN CONCENTRATION WITH (KLK3) RS2735839 IN INFERTILE PATIENTS

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Abstract

Male infertility has become a worldwide reproductive health problem. The aim of the present study was detection and genotyping for *KLK3* rs2735839 G/A Polymorphism and its association with tPSA concentrations, Find the correlation between infertile patients and tPSA concentrations. The (75) seminal fluid and blood samples include (50) samples from infertile men and (25) seminal fluid and blood samples from healthy men, these were collected from the Outpatient men infertility department of the Kamal AL, Sameraiae for fertilization and infertility Hospital. the result of genotype rs2735839 G/A polymorphism showed A/A frequency (24%) in infertile patients and (8%) in control, A/G frequency in infertile patients (34%) and control group (24%) and G/G frequency (42%) in infertile patients and (68%) in control group. The results of the show concentrations of tPSA in control (0.4148+0.1232) while concentrations in patients (0.2984+0.0915). It was concluded that the PSA levels were related with infertile men and that *KLK3* rs2735839 (G) allele was significantly associated with higher seminal PSA levels and (A) allele was significantly associated with lower seminal PSA levels in men. And decreased of PSA concentration in infertile patients causes a decrease of sperm motility.

Key words: Prostate Specific Antigen, *KLK3*, infertility.

Introduction

Infertility (clinical definition) a disease of reproductive system defined by is the inability to achieve a clinical pregnancy after one full year or more of regular, unprotected sexual intercourse (Sharma, 2017). The World Health Organization (WHO) labeled infertility as a worldwide public health issue which does not give attention it deserves. Reports suggest that infertility in 1990 and 2010 is similar in the developed world as 1.9% of child-seeking women aged 20-44 years experienced primary infertility and 10.5% secondary infertility, but Infertility prevalence was highest in developing countries including the Middle East (Al-Turki, 2015). Prostate specific antigen (PSA), also called human kallikrein 3 (hk3) or gamma-seminoprotein, is a serine protease produced primarily by the prostate gland. It is synthesized as a 261 amino acids pre-propolypeptide, including a 17-amino acids signal and a 7-amino acids activation peptide, which are cleaved by human glandular kallikrein 2 (hk2) during processing and secretion. Mature PSA is a glycoprotein of ~28 kDa comprised of 237 amino acids and a carbohydrate chain (Zhou *et al.*, 2018). PSA is secreted

at concentrations of 0.5-2g/l into seminal fluid (Naz *et al.*, 2017). PSA is one of the most abundant proteins in the secretion of the normal human prostate epithelium, has been suggested to be produced as a zymogen of the enzymatically active protein (Christensson *et al.*, 1990). It's main function is to dissolve the coagulum formed after ejaculation by semenogelin, allowing sperm to swim freely in the female genital tract (Brugh *et al.*, 2003). PSA in seminal fluid is also important for the breakdown of cervical mucus, allowing the entry of sperm into the uterus (Turek, 2005). PSA is present in small quantities in serum of men with a normal prostate but is elevated during several prostate abnormalities, including prostate cancer (Gunasekaran and Pandiyan, 2017); Restrepo and Cardona-Maya, (2013). One single nucleotide polymorphism (SNP) in the *KLK3* gene, *KLK3* rs2735839 G/A polymorphism, was reported to influence serum PSA levels (Nobata *et al.*, 2012). Considering that this *KLK3* SNP is located in the 3'-UTR (untranslated region) of *KLK3* gene, speculation of the effect of this polymorphism on serum PSA levels seems biologically plausible.

Materials and Methods

Materials

Human Total Prostate Specific Antigen (tPSA) ELISA kit were purchased from Blue Gene, All other chemicals and reagents that use in extraction DNA and genotyping were from Tonk Bio and Promega.

Patient population

This study was carried out during the period from October, 2018 to May, 2019. The samples were collected from the outpatient men infertility department of the Kamal AL-Samaraiae for fertilization and infertility Hospital. Patients have selected according to clinical and laboratory examination and divided into two groups: Group I 50 patients were examined and diagnosed as related with infertility. Group II 25 apparently healthy individuals (Control) with normal seminal fluid parameters.

Semen analysis

Semen samples from infertile patient and controls were collected by masturbation and ejaculation into sterile glass cups after 3-7 days of abstinence and to get the best sample should avoid ejaculation for 24 to 48 hours before the test. After sperm fluid liquefaction at 37°C for 30 min and the semen analysis, including sperm concentration, motility and Sperm morphology and leukocyte, white blood cell, round cell and epithelial cell counts were assessed with the use of pre-stained slides by using light microscope (World Health Organization, 2010).

Enzyme-linked immunosorbent assay (ELISA)

Total Prostate Specific Antigen ELISA kit applies the quantitative sandwich enzyme immunoassay technique. The micro titer plate has been pre-coated with a monoclonal antibody specific for TPSA by BlueGene Biotech.

Genomic DNA isolation

The genomic DNA isolated from the whole fresh blood collected in EDTA anticoagulant tubes for molecular studies has been applied using genomic DNA purification

Table 1: Genotypes and allele frequency for the *KLK3* rs2735839 G/A polymorphism in infertile patient and control.

Allele frequency(%)		Genotype % GG GC CC			Groups
G	A	GG	AG	AA	
40(80)	10(20)	17(68)	6(24)	2(8)	Control
59(59)	41(41)	21(42)	17(34)	12(24)	Patient
6.551		0.842	10.522	14.286	Chi square
0.010		0.359	0.001	0.00013	P-value
Sign.		Non.sign.	Sign.	Sign.	Significance
OR95%CI		P-value	Patient	Control	Genotypes
0.2754 (0.0565 to 1.3422)		0.1105	12	2	AA
0.6130 (0.2064 to 1.8205)		0.3782	17	6	AG
3.9464 (1.4609 to 10.6611)		0.0068	21	17	GG

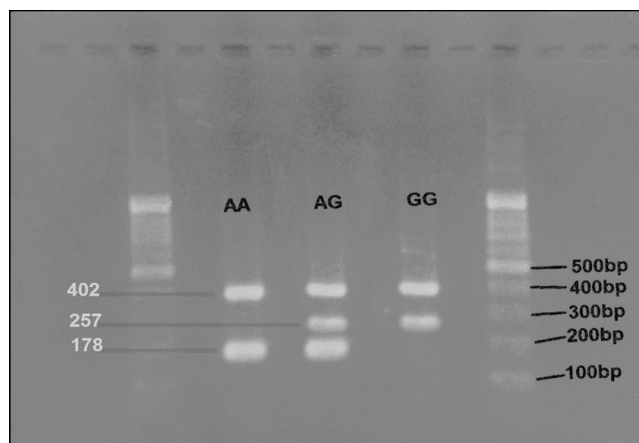


Fig. 1: 1.5% agarose gel showing the different genotypes for the *KLK3* rs2735839 G/A polymorphism.

kits (Tonk bio). After genomic DNA extraction, agarose gel electrophoresis has been adopted to confirm the presence and integrity of the extracted DNA (Sambrook, 1989).

Genotyping of polymorphisms

The genotyping of the *KLK3* polymorphism was conducted by the polymerase chain reaction with the confronting two-pair primers (PCR-CTPP) method. The primers used were F1: 5 CAC TGT TAG CAT GAA TCA TCT GG, R1: 5 GCC CCA TGG TCC ACT C, F2: 5 GGT TCT GTC TTG TGG CC A and R2: 5 CAG ACA TCT TCA TAA CCT CAG GG for the *KLK3* rs2735839 A/G polymorphism. The underlining shows the bases of the SNP. The thermal cycler conditions were 95°C 5 min denaturing followed by 35 cycles of 95°C 1 min, 63°C 1 min and 72°C 1 min and 72°C 10 min for final extension (Nobata *et al.*, 2012). Each genotype is distinguished as follows: A/A genotype (178- and 402-bp bands), G/A genotype (178-, 257- and 402-bp bands) and G/G genotype (257- and 402-bp bands). The representative gel for the genotyping of *KLK3* rs2735839 A/G polymorphism.

Statistical Analysis

The Statistical Analysis System- SAS, (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference -LSD test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Correlation coefficient between variables in this study.

Results

This study is designed for genotyping for *KLK3* rs2735839 G/A Polymorphism in blood by using

Table 2: Genotypes for the *KLK3* rs2735839 G/A polymorphism and total prostate specific antigen concentration.

Parameter	tPSA
Genotype	Mean \pm SD
AA	C0.18214 \pm 0.02806
GA	B0.28522 \pm 0.03356
GG	A0.43158 \pm 0.07554
P-value	0.00014
LSD	0.0627

Table 3: The compression between patients and control in concentrations of tPSA.

Parameter	tPSA
Groups	(mg/ml)
Control	A0.4148 \pm 0.1232
Infertile	B0.2984 \pm 0.0915
P-value	0.00031
LSD	0.0486

Polymerase chain reaction with confronting two-pair primers (PCR-CTPP). In addition measuring PSA levels in seminal fluid by using the enzyme-linked immunosorbent assay (ELISA) and finding the Correlation between PSA levels and *KLK3* rs2735839 G/A Polymorphism in infertile men and seminal fluid parameters.

The product of PCR reaction observed after horizontal gel electrophoresis by UV light (Fig. 1) the genotyping of *KLK3* rs2735839 A/G polymorphism is shown as following: A/A genotype (178-bp and 402-bp bands), G/A genotype (178-bp, 257-bp and 402-bp bands) and G/G genotype (257-bp and 402-bp bands).

The result of genotype rs2735839 G/A polymorphism in the table 1, showed A/A frequency 12(24%) in infertile patients and control g 2(8%), A/G frequency showed in infertile patients 17(34%) and control 6(24%) and G/G frequency 21(42%) in infertile patients and 17(68%) in control. the frequency of G allele is (59%) in patient and (80%) in control and allele frequency of A is (41%) in patient and (20%) in control.

In this study were measuring tPSA and the result show concentrations of tPSA in A/A (0.18214+0.02806) while concentrations in A/G (0.28522+0.03356) and G/G were (0.43158+0.07554) showed in table 2.

And table 3, showed the concentrations of tPSA in

Table 4: Compare the seminal fluid parameters between patients and control .

Parameter	Count	Rapid progressive	Progressive	Non- progressive	Immotile	Abnormality
Groups	(million/ml)	Motility A (%)	Motility B (%)	Motility C (%)	Motility D (%)	(%)
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Control	A39.20 \pm 13.82	A27.40 \pm 8.55	A34.20 \pm 8.98	A18.60 \pm 8.84	A20.20 \pm 9.18	A25.60 \pm 6.66
Infertile	B25.88 \pm 13.06	B10.40 \pm 16.20	B18.80 \pm 16.16	A18.00 \pm 9.24	B52.80 \pm 27.01	B55.60 \pm 15.96
P-value	0.00012	0.00015	0.00032	0.090	0.00036	0.00063
LSD	5.667	5.548	6.261	Non.Sign.	9.702	6.418

control were (0.4148+0.1232) while concentrations in infertile patients were (0.3072+0.0929).

The result obtained from seminal analysis of samples for all individuals (patients and control) showed that sperm counts in control group were (39.20 \pm 13.82) while sperm counts in patients were (25.88 \pm 13.06) and abnormal morphology of sperm in control were (25.60 \pm 6.66) while abnormal morphology of sperm in patients were (55.60 \pm 15.96) and motility of sperms showed that rapid progressive motility of sperm were (27.40 \pm 8.55), progressive motility of sperm (34.20 \pm 8.98) while non-progressive motility of sperm (18.60 \pm 8.84) and immotile sperm (20.20 \pm 9.18) in control. while in patients showed rapid progressive motility of sperm (10.40 \pm 16.20), progressive motility of sperm (18.80 \pm 16.16) while non-progressive motility of sperm (18.00 \pm 9.24) and immotile sperm (52.80+27.01) that showed in table 4.

Discussion

In approximately 50% of infertility cases are due to male factor that can be caused by abnormal characteristics in several parameters, including sperm concentration, motility and morphology, semen volume and motile sperm count. Prostate specific antigen (PSA) is a serine protease with chymotrypsin-like enzymatic activity have a physiological function is degradation of the gel-forming proteins semenogelins I and II in semen after ejaculation. (Jarai *et al.*, 2011). This leads to liquefaction of the seminal clot and the release of motile sperm, thus enabling the spermatozoa to travel through the female reproductive tract (Mattsson *et al.*, 2014). and many previous studies revealed that PSA concentration affected the sperm quality. This study is designed for genotyping for *KLK3* rs2735839 G/A Polymorphism by using Polymerase chain reaction with confronting two-pair primers (PCR-CTPP). In addition measuring PSA levels in seminal fluid and finding the Correlation between PSA levels and *KLK3* rs2735839 G/A Polymorphism in infertile men and seminal fluid parameters. The result of genotype rs2735839 G/A polymorphism showed A/A frequency 12(24%) in infertile patients have significant difference compared with control 2(8%), A/G frequency showed significant difference in

infertile patients 17(34%) compared to control 6(24%) and G/G frequency 21(42%) in infertile patients and 17(68%) in control showed non-significant difference. the frequency of G allele is (59%) in patient and (80%) in control, showed significant difference compared allele frequency of A is (41%) in patient and (20%) in control.

The results of the concentration of tPSA according to genotype showed that in A/A (0.18214+0.02806) while concentrations in A/G (0.28522+0.03356) and G/G were (0.43158+0.07554) and the statistical analysis of the result appeared that there is significant difference at (P<0.01) between A/A, A/G and G/G. While the tPSA concentration results in (patients and Control) showed concentrations of tPSA in control were (0.4148+0.1232) and concentrations in patients were (0.2984+0.0915) the result appeared a decrease of tPSA concentration in the patients compared with control. And the statistical analysis of results there is a significant difference at (P<0.01) between control and patients in concentrations of tPSA and G allele associated with increasing PSA concentrations and A allele associated with decreased PSA concentrations. The present data, which is similar tend to previous studies results in the world. And modulates PSA levels (Nowinski *et al.*, 2018). The study applied on Iranian men show Patients with AG and GG genotypes had a higher total serum level of prostate specific antigen (PSA) compared to those with AA genotype (Motamedi *et al.*, 2019). another study observed that tPSA concentration were lower in subjects carrying one or more A allele at the rs2735839 (Parikh *et al.*, 2011). This may indicate that one or more of this allele directly cause a reduction in tPSA concentration, possibly through regulatory effect on transcription of the gene, through altered protein stability or reduced detection of serum tPSA and study record that rs2735839 shows a much stronger association with PSA concentrations (Eeles *et al.*, 2008). Other study mentioned the major allele G of rs2735839 is associated with elevated PSA level compared with the minor allele A (He *et al.*, 2014).

Statistical analysis of the result obtained from seminal analysis of samples for all individuals (patients and control) shows that there is a relationship between the PSA concentrations and decrease in quality of semen parameters especially on sperm count, sperm motility and sperm morphology, the result found that had significant effect at level (P<0.01) on counts, progressive motility of sperm and sperm morphology. Which corresponds to the other studies (Schieferstein, 1999; Srettabunjong *et al.*, 2015; Sävbblom *et al.*, 2014; Lilja *et al.*, 1987).

Conclusion

In conclusion, the present study revealed that the

KLK3 rs2735839 G allele have positive correlation with increase PSA levels and A allele responsible to decrease PSA levels and the PSA levels were related to infertile men by modulating the sperm concentration, motility and morphology and that showed statistically significant differences between patients and controls that could be useful information for diagnosing male infertility.

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