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Association between genetic polymorphism of *XRCC6* T-991C and risk of varicocele

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Abstract

Background: The DNA non-homologous end-joining repair gene *XRCC6* (Ku70) plays an essential role in the DNA double-strand break (DSB) repairs. Defects in the DSB repair pathway results in genomic instability. Varicocele is characterized by high pressure and stasis in the veins of the testis. There is little knowledge about the molecular mechanisms underlying varicocele. One of the reasons for increased spermatozoa DNA damage is high concentrations of reactive oxygen species (ROS), which leads to DNA-DSBs. We assumed that a promoter T-991C (rs5751129) polymorphism in the *XRCC6* gene was associated with susceptibility to varicocele in infertile men. Therefore, 63 infertile varicocele men and 150 healthy controls were recruited in our study. The healthy controls had no history of varicocele, and they were matched with patients by age.

Results: Our results showed that infertile varicocele patients and control groups had significant differences in the distribution of their genotypic and allelic frequency ($p = 0.00$) in the *XRCC6* promoter T-991C polymorphism. Men who carried CC genotype had a 5.22-fold increased odds ratio of developing infertile varicocele compared to those who carried the wild-type TT genotype (95% CI 2.31–11.81, $P < 0.001$).

Conclusions: Our results suggested that the CC genotype and the C allele in the promoter region of *XRCC6* gene might play an important role in developing infertility in the varicocele men. Further research is needed to provide the effect of this polymorphism.

Keywords: X-ray repair cross-complementing group 6 gene (*XRCC6*), Varicocele, Polymorphism, DNA repair, Non-homologous end-joining, Genotype

Background

Varicocele is characterized by high pressure and stasis in the veins of the testis; it organizes the pampiniform vein plexus in the male spermatic cord [1]. It is mostly detected in young adults, which can harmfully affect the testicular function. The molecular mechanism underlying the pathogenesis of varicocele has not been elucidated yet.

The common feature of infertile varicocele patients is the increase in the sperm nuclear DNA fragments [2]. Saleh et al. [3] reported that sperm DNA damage in the infertile varicocele patients is significantly increased. The reasons for increased sperm DNA damage in infertile varicocele patients are high concentrations of reactive oxygen species (ROS) as well as a reduction in antioxidant defenses [4]. Many reports documented the relationship between the polymorphism of DNA repair genes and the amount of ROS production, which leads to the risk of male infertility diseases [5–8].

One of the most harmful effects of ROS on DNA is double-strand breaks (DSBs) which affect the loss of physical integrity and content of DNA [9]. The homologous recombination (HR) and non-homologous end-joining (NHEJ) are two main DBS repair sub-pathways. Some studies have indicated that single nucleotide polymorphisms (SNPs) in NHEJ genes might cause suboptimal DNA repair and, as a result, accumulation of DNA damage. The most important component of NHEJ

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pathway is *XRCC6* gene. It codes the Ku70 protein, a catalytic subunit of the NHEJ pathway, and plays an essential role in the maintenance of genome integrity [10]. A number of studies have evaluated the contribution of *XRCC6* gene polymorphism and cancers; however, no study has yet confirmed the association between the polymorphisms of *XRCC6* and the risk of varicocele [9–13]. Therefore, in this study, we assumed that polymorphisms of the gene in NHEJ pathway, like *XRCC6*, might also contribute to varicocele susceptibility. To test this hypothesis, we determined the genotypic frequency of polymorphism of the *XRCC6* gene at the promoter T-991C (rs5751129), the region in this case-control study.

Methods

Study population

A total of sixty-three infertile patients with varicocele were recruited from the Nemazi Hospital between 2017 and 2019 from the Fars province, south of Iran. The clinician approved the diagnosis of varicocele with infertility in all patients. All the married patients were adult infertile men with a primary diagnosis of unilateral or bilateral varicocele. All cases had grades 2 and 3 varicocele without other diseases that lead to infertility, e.g., sexually transmitted diseases (STDs) and diabetes. They had an infertility problem from 1.5 to 5.8 years. Patients lacking a defined grade or grade 1 were excluded.

Age-matched healthy married men with children (150 men) from the same place were collected. They were without varicocele and no infertility problem. The controls with a history of varicocele and/or known causes of infertility such as hormone abnormalities, cryptorchidism, infection problems, orchitis, obstruction of the vas deferens, drug abuse, diabetes mellitus, and abnormal karyotypes were excluded. All our patients and controls were chosen from Caucasian Persian Muslim people living in the Fars province. Both the patient and control group filled out the questionnaire and provided written informed consent to participate in the study. This study was conducted in accordance with the guidelines of the Declaration of Helsinki and its subsequent amendments.

Genotyping method

Genomic DNA was extracted from EDTA-whole blood samples, from patients and healthy controls, using a Genomic DNA miniprep kit (Bio Basic, Canada). The extracted DNA was stored at − 20 °C until the performance of PCR analysis. RFLP-PCR and designing of the primers were performed, as described previously [11]. Briefly, PCR conditions were 1 cycle at 94 °C for 5 min, 29 cycles of 94 °C for 30 s, 55.5 °C for 40 s, 72 °C for 45 s, and 72 °C for 10 min as a final extension step. Then, 5 μl of *XRCC6* T-991C products was mixed with 2 U of *DpnII*. Then, the mixture was incubated for 2 h at 37 °C. The wild-type C allele polymorphism had no *DpnII* cleavage site, with 301 bp size, whereas the mutant T allele was digested to 200 and 101 bp fragment size. The polymorphism was categorized as either T/T homozygote (undigested), C/C homozygote (digested), or C/T heterozygote. PCR and/or digestion products were electrophoresed on a 2.5% agarose gel containing ethidium bromide.

Statistical analyses

Hardy–Weinberg equilibrium was calculated for both patients and control groups. Pearson's chi-square or Fisher's exact test was used to analyze the test differences in the frequency of alleles and genotypes. The association between *XRCC6* polymorphism and varicocele risk was obtained by the odds ratios (OR) and 95% confidence intervals (CIs). All statistical analyses were done using SPSS software (version 16) for Windows (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as significant, which was two-sided for all tests.

Results

The demographic characteristics of infertile varicocele patients and controls were studied. The average age of the patients was 32.4 ± 2.3 years, and the average age of the control group was 31.9 ± 1.4 years. The difference was not statistically significant ($P = 0.58$), which indicated that the two groups of patients were equal in age. Hence, in terms of body mass index, there was no difference between the two groups ($P = 0.51$).

The genotype distribution of the *XRCC6* T-991C (rs5751129) gene polymorphism in sixty-three patients and one hundred fifty healthy men are presented in Table 1. The genotype frequency of *XRCC6* polymorphism in infertile patients ($\chi^2 = 2.17$, df = 1, $P = 0.14$) and controls ($\chi^2 = 1.69$, df = 1, $P = 0.20$) was in Hardy-Weinberg equilibrium. SNP analysis of *XRCC6* (rs5751129) polymorphism showed that the wild TT genotype was present in 14/63 patients (22.20%), while the variant genotypes TC and CC were present in 25/63 patients (39.7%) and 24/63 patients (38.1%), respectively. The genotype TC (OR = 1.96, 95% CI 0.93–4.11, $P = 0.075$) had no significant effect on the risk of varicocele in comparison with TT. The mutant homozygotes (CC) in patients had a significant 5.22-times higher risk than the controls (95% CI 2.31–11.81, $P < 0.001$) in comparison with TT (Table 1). In addition, the combined C allele carrier (TC + CC) of infertile varicocele patients was 2.82-times higher than that of the control group, and there was a significant difference between the two groups (95% CI 1.44–5.55, $P = 0.003$). The frequency of allele C in the varicocele group was significantly higher than the control group, and the difference was statistically significant (95% CI 1.22–3.46, $P < 0.001$) (Table 1).

Table 1 Association between polymorphism of *XRCC6* T-991C and risk of varicocele

XRCC 6 polymorphism	Cases (*n* = 63)	Controls (*n* = 150)	OR (95% CI)	*P* value
	N (%)	*N* (%)		
TT	14 (22.2)	67 (45.0)	1	
TC	25 (39.7)	61 (41.0)	1.96 (0.93–4.11)	0.075
CC	24 (38.1)	22 (14.0)	5.22 (2.31–11.81)	< 0.001
TT	14 (22)	67 (45)	1	
TC+CC	49 (78)	83 (55)	2.82 (1.44–5.55)	0.003
T	53 (42.1)	195 (65)	1	
C	73 (57.9)	105 (35)	2.98 (1.22–3.46)	< 0.001

Discussion

In this study, the association between the *XRCC6* gene polymorphism and the risk of varicocele in the Iranian population was investigated. The genotyping analyses revealed that individuals who carried the CC genotype had a higher risk of varicocele compared with those carrying the TT genotype of *XRCC6* T-991C. Previous reports have shown that there is a correlation between varicocele and body mass index, which has been observed to be more common in tall and fat men [14, 15]. However, in terms of body mass index, we did not find any correlation in all groups. To the best of our knowledge, this is the first report to investigate the susceptibility of the *XRCC6* T-991C polymorphism in infertile varicocele men.

It is known that the NHEJ pathway plays a principal mechanism for the removal of DNA DSBs damages. It has a main role in genetic stability and maintenance of normal spermatogenesis. In this pathway, *XRCC6* plays an important role, which may work with/without *XRCC5* as a heterodimer [10].

The results of our study showed that carriers of the CC genotype and the C allele of *XRCC6* were associated with a significantly increased risk of infertile men with varicocele. Our result may indicate that the mutant allele C of *XRCC6* T-991C in the promoter region is considered to be as a risk factor for varicocele susceptibility with infertility.

The *XRCC6* T-991C polymorphism, rs5751129, is located in the promoter region. It might influence the transcriptional activity of the gene [12, 16–18]. The genetic susceptibility of this polymorphism has been studied in a variety of diseases, including renal cell carcinoma [10], hepatocellular carcinoma [11], lung cancer [13], and male infertile patients [9]. Besides, previous results indicate that this SNP may affect the stability of Ku70 protein and its protein expression level, which may lead to alterations in DSB repair capacity [19, 20].

Therefore, the deficiency in *XRCC6* may lead to not only a lower DSB repair capacity, but also *hypersensitivity* to *ionizing radiation*, growth retardation, and severe combined immune deficiency [21]. On the other hand, *XRCC6* polymorphisms or small genomic variations might escape the cell cycle checkpoints and reduce DNA repair capacity, which might increase the fragmentation of sperm DNA in nuclear and trigger infertile varicocele patients [22, 23]. Therefore, the screening for specific DNA polymorphisms may have an application in infertile men in order to guide a targeted antioxidant treatment. It might bring a new insight into the management of men with infertility, especially infertile men with varicocele.

The present study had some limitations, which are suggested to be improved in future investigations. First, our population size was moderate, which may restrict the reliability and feasibility of analysis. Second, the lack of insufficient clinical and behavioral information, such as occupational exposure, smoking, and physical exercise habits limited our ability to analyze risk factors.

Conclusions

In summary, the present study is the first report to investigate the association between *XRCC6* promoter T-991C polymorphism and varicocele. Our findings suggest that the CC genotype is significantly associated with a higher susceptibility to varicocele. However, it is a preliminary study, so further large cohort studies adjusted for risk factors in varicocele and functional analysis are recommended to confirm the finding of our study.

Abbreviations

DSBs: Double-strand break; HR: Homologous recombination; NHEJ: Non-homologous end joining; RFLP: Restriction fragment length polymorphism; ROS: Reactive oxygen species; *XRCC6*: X-ray repair cross-complementing group 6

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Authors' contributions

M.R.N., M.J.M., B.G., and Z.B. conceived and planned the presented experiment. M.R.N. and Z.B. performed the experiments and analyzed the data. Z.B. supervised the research, designed experiments, and wrote the paper. The authors have read and approved the manuscript.

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Ethics approval and consent to participate
Parents of patients or legal guardians provided a written informed consent form for participation in the study. The Institutional Review Board and Human Ethics Committee of the Islamic Azad University of Kazerun approved this study (IR.IAU.KAU.REC.1398.189).

Consent for publication
Not applicable

Competing interests
Not applicable

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