Original Article

A Role for Heterozygosity of NF-κB1 rs28362491 Polymorphism in Patients with Idiopathic Oligospermia

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Abstract

Background: Nuclear factor-kappa B (NF-kB) activation and its inhibition by NF-kB inhibitor (IkB) have been functionally linked to germ cell apoptosis, which may affect human infertility. We hypothesized a possible relationship between the *NF-κB1*-94ins/del ATTG (rs28362491) and *NF-κBIA* 3'UTR A→G (rs696) polymorphism, which are common polymorphisms and the susceptibility to oligospermia in the context of the sperm apoptosis.

Methods: In order to evaluate this association, we studied the polymorphisms and sperm apoptosis rates of 114 men with idiopathic oligospermia, as well as 130 normospermic men, using PCR-RFLP and TUNEL staining methods, respectively.

Results: Univariate analysis revealed that heterozygous ID genotype at the *NF-κB1* -94ins/del ATTG polymorphism is associated with an approximately 2.4-fold reduced risk of oligospermia ($P = 0.006$, 95% confidence intervall = 1.34 – 4.13). However, the genotype and allele frequencies of *NF-κBIA* 3'UTR A→G polymorphism, and the genotype frequencies of all possible rs28362491/rs696 genotype combinations did not show any significant differences between oligospermic and normospermic men. Furthermore, neither polymorphism appeared to affect sperm apoptosis, although the sperm apoptosis index was detected to be significantly higher in the oligospermic patients compared with those in the controls ($P < 0.05$).

Conclusion: Our findings suggested that the heterozygosity of rs28362491 in the NF-κB1 gene may have a protective effect against oligospermia and could modify the susceptibility of oligospermia in a group with idiopathic male infertility in a Turkish population.

Keywords: *NF-κB1* and *NF-κBIA* polymorphisms, male infertility, oligospermic, idiopathic, apoptosis

Cite this article as: Tek B, Elçin P, Tunçdemir M, Onaran I, Özkara H, Kanıgür Sultuybek G. A Role for Heterozygosity of NF-κB1 rs28362491 Polymorphism in Patients with Idiopathic Oligospermia. *Arch Iran Med*. 2016; 19(4): 275 – 281.

Introduction

Spermatogenesis involves a highly orchestrated multiple-step and events in which male germ cells proliferate and differentiate into functional highly specialized male gametes called spermatozoa. Although there are actually thousands of genes expressed in the testes, several enzymes and proteins that regulate spermatogenesis are produced by testis-specific gene expression with the highly ordered process during this process.[1] Testis-specific gene abnormalities may induce sperm abnormalities such as oligospermia (low sperm count of, 20×10^6/mL) (WHO, 2010) and azoospermia (absence of sperm in semen). There are also a plethora of non-genetic factors that might potentially affect the male reproductive function. Some evidences from animal and gene expression studies of oligospermia suggest that several molecular pathways may be interrupted in postmeiotic spermatozoa. One of the interrupted pathways is cell apoptosis which is an extensive process in the mammalian testis needed for maintaining normal spermatogenesis by limiting the testicular germ cell population.[2] Male infertility has been shown to be associated with a modified apoptotic process.[3–5] A strong association has been shown between abnormal semen parameters and the abortive apoptosis in ejaculated sperm and testicular biopsies.[6–8] Depending on the results of very few report on apoptosis in ejaculated spermatozoa, it has been suggested that the observed apoptotic alterations in subfertile patients are likely to have originated from an abortive apoptotic mechanism which is triggered during spermatogenesis.[9–11]

During spermatogenesis, apoptotic cell death is regulated by highly controlled transcription of a number of genes. Nuclear factor-kappa B (NF-kB), a transcription factor, has a function in modulation of apoptosis as well as cell proliferation, adhesion, invasion, and angiogenesis in many cell types. Growing evidence suggests that NF-kB may also regulate male germ cell apoptosis. Human and animal studies suggest the involvement of NF-kB in spermatogenesis.[12,13] NF-κB p50 and p65 subunits have been identified in the nuclei of Sertoli cells and spermatocytes where they may have a role in the regulation of stage-specific gene expression during the process of spermatogenesis.[13] Pentikäinen *et al.*[12] have also shown that NF-kB proteins in Sertoli cell exert pro-apoptotic effects on germ cells during testicular stress. Furthermore, NF-kB is known to prevent apoptosis by inducing anti-apoptotic proteins.[14] The five members constitute the NF-κB family and a critical component among them is a heterodimer of the p50/p105 and p65/RelA subunits encoded by *NF-kB1* (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1) and *RelA* genes, respectively. In the absence of stimuli, NF-*k*B is inactivated by noncovalent interaction with inhibitory proteins in the cytoplasm.

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Accepted for publication: 22 January 2016

Inhibitor of kappa B alpha (IkBa) (encoded by the *NF-kBIA* gene) receiving external signals is rapidly phosphorylated and degraded. Thus, NF-kB translocates into the nucleus to start expression in target genes. It has been shown that impaired NF-kB signaling and defects in its regulation may be involved in the pathogenesis of various diseases as well as inflammation-related disorders.[15,16] There is a relationship between excessive apoptotic activity and increased NF-kB activity in human seminiferous tubules.[12,17] Furthermore, it has been reported that inhibition of NF-κB activity by a pharmacological inhibitor provides significant protection to the loss of spermatids and resulting infertility in rats.[18]

On the other hand, several nucleotide variants in the *NF-kB1* and *NF-kBIA* genes have been reported.[19–23] The results of studies examining the relationship between these polymorphisms and the susceptibility to many human diseases (including autoimmune, inflammatory diseases and cancers) have suggested that NF-kB1 plays an important role in complicated pathogenic regulation of apoptosis. Furthermore, polymorphic variations in promoter regions of the *NF-kB1* gene and in the 3'-untranslated region (3'-UTR) of *NF-kBIA* have been associated with a number of pathological conditions including apoptotic process. One of these is a functional polymorphism (-94 insertion/deletion ATTG, rs28362491) in the promoter region of *NF-kB1* gene. Deletion (D) of the ATTG sequence causes the loss of binding to nuclear proteins with reduced promoter activity than Insertion (I) variant.[20] The other is the 3'UTR A→G polymorphism (rs696) in *NF-kBIA* gene, which affects the expression, structure and function of Iκ Bα protein.[24] It has been also postulated that allelic differences in the *NF-kBIA* 3′UTR may alter IkBα expression and influence complex formation with NF-kB and thus, the regulation of cell growth and apoptosis.[15,22] Although several single nucleotide polymorphisms in apoptosis-related genes have been reported as a potential risk factor for impaired sperm production.[25–27] there is no data available on the prevalence of *NF-kB1*-94ins/del ATTG and *NF-kBIA* 3'UTR variants in patients with impaired spermatogenesis.

Considering the critical function of NF-kB in germ cell apoptosis, these two polymorphisms in *NF-kB1* and *NF-kBIA* gene would be attractive candidate factors for searching impaired spermatogenesis, opening the question whether individual variations in the expression of these genes might account for different susceptibilities to male infertility with spermatogenesis impairment in the context of the sperm apoptosis. Therefore, the present study carried out genotyping analyses for *NF-kB1* -94 ins/del ATTG or *NF-kBIA* 3'UTR A→G polymorphisms in a hospital-based case–control study of male infertility with idiopathic oligospermia in a Turkish population, including 114 infertile men with idiopathic oligospermia and 130 fertile normospermic men as controls. In addition, we evaluated the effects of these two polymorphisms on the apoptotic alterations in ejaculated sperm.

Materials and Methods

Subjects and sample collection

The study population consisted of men admitted to the Andrology Clinic of Urology Department, Cerrahpaşa Medical Faculty of Istanbul University for infertility evaluation between April 2010-February 2014. Out of 2803 admittances, 114 men who were unsuccessful to achieve pregnancy with their partners after one year of intercourse without contraception, were evaluated for infertility and diagnosed with idiopathic oligospermia. They underwent andrological examination and at least two semen samples for semen analysis were analyzed for each case. Patients with history of epididymitis, orchitis, mono-or bilateral cryptorchidism, and-varicocelectomy, as well as men under treatment with spermatogenesis-impairing medication were excluded from the study.

The control group consisted of 130 normospermic men recruited from the same department where they were admitted for other urological complaints other than infertility and they fathered in the last 2 years. The subjects were age-matched volunteers from similar ethnic background who lived in Istanbul. The characteristics of the study and control groups are summarized in Table 1.

Semen analyses were performed according to the World Health Organization Laboratory Manual for the examination and processing of human semen (5th 2010). Men were asked to abstain from sexual activity for 2–5 days before analysis and semen was collected in a private room in the Andrology Clinic near the laboratory. The samples were obtained into a clear container by masturbation. The average values of the 2 sample were calculated. In all patients, blood samples were drawn for the measurement of serum follicle stimulating hormone (FSH) and total testosterone (T). Idiopathic infertility was defined as men without any abnormal finding (without finding of varicocele, chryptorchidism) on andrological examination and hormone measurements (FSH and T) except abnormal sperm concentration (less than 15.10^6 /mL).

The Institutional Ethics Committee approved the study protocol in accordance with the Helsinki declaration and all participants (study and control group) gave informed consent (approval reference no. 83045809/14878).

Polymorphism Analysis

DNA was isolated with high salt DNA extraction method from peripheral blood samples obtained for routine clinical work-up and all the samples were isolated at -80°C until used. Concentration and purity of DNA was measured in Nanodrop® and determined by 260/280 nm optic density (OD) ratio. The polymorphisms of *NF-kB1* and *NF-kBIA* genes were determined by Polymerase Chain Reaction-Restriction Fragment Length polymorphism (PCR-RFLP) method according to our previous paper.[28] 285 and 424 base pair PCR fragment of the *NF-kB1* and *NF-kBIA* genes were amplified in a 25 µL reaction volume containing 100 ng genomic DNA, 200 pmol of each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 U Taq Polymerase (Sigma, St. Louis, MO, USA) and 2 mM $MgCl_2$ (Fermentas, Lithuania). PCR conditions were as follows: a denaturing step of 95°C (1 min), then 35 cycles of 95°C (30 s), 61°C (30 s), 72°C (1 min) and final incubation at 72°C (5 min). The products were digested overnight with 5U of PflMI (Van91I) and HaeIII (BsuRI) (Fermentas, Lithuania) at 37°C and were run on an ethidium bromide-stained 3% agarose gel for 45 minutes at 90 V and were directly detected under UV light. The primers used to amplify the polymorphic sites of the polymorphisms tested were F:5'-TGGGCACAAGTCGTTTATGA-3' and R:5'-CTGGAGCCGGTAGGGAAG-3' for rs28362491, and F: 5'-GGCTGAAAGAACATGGACTTG-3' and R: 5'- GTACACCATTTACAGGGAGGG-3' for rs696.

In situ DNA end labeling method (TUNEL)

Sperm DNA fragmentation related to the presence of apoptosis was evaluated by the TUNEL assay using an ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Peroksidase Kit (S7101-

Table 1. Characteristics of the study population.

	Oligospermic (n = 114)	Normospermic (n = 130)	P-Value
Age (years)	33.0 (29.0 – 37.0)	36.0 (32.0 – 39.0)	0.078
Sperm concentration ($\times 10^6$/mL)	3.0 (2.0 – 8.0)	30.0 (15.0 – 50.0)	<0.0001
Sperm motility (%)	20.0 (5.0 – 42.5)	50.0 (26.3 – 57.0)	0.0011
Sperm morphology (%)	1.0 (1.0 – 5.0)	5.0 (5.0 – 9.0)	<0.0001
Right testicular volume (mL)	15.0 (7.5 – 18.0)	18.0 (6.0 – 23.3)	0.412
Left testicular volume (mL)	15.0 (7.5 – 18.0)	18.0 (6.8 – 19.3)	0.494

Data are shown as median and interquartile range (IQR, 25th-75th percentile)

KIT, Chemicon), as described by the manufacturer and our previous work.[29] Briefly, sperms were washed and resuspended in fixed 10% neutral buffered formalin for 20 min at room temperature. After being rinsed with PBS, samples were incubated with proteinase K (20 mg/mL). Samples were subjected to 3% H_2O_2 for endogenous peroxidase inhibition, and were incubated with 1X equilibration buffer at room temperature for 30 min. The digoxigenin-labeled dNTP tail was incubated for 1 h with terminal deoxynucleotidyl transferase (Tdt) at 37°C, and samples were washed in stop/wash buffer for 10 min at room temperature. Samples were incubated with antidigoxigenin-peroxidase antibody at room temperature for 30 min, and were stained with diaminobenzidine used for peroxidase substrate. Staining was evaluated using a light microscope after counterstaining with methyl green. For staining specificity, a negative control slide without the addition of Tdt enzyme and a positive control slide with Tdt enzyme treatment were included.

Morphometric analysis of the positive cells in slides stained by TUNEL method was performed under high power magnification (×40) in a blinded fashion. At least 200 cells were randomly analyzed per slide from 10 different fields. Average cell per unit area number was found by taking the average of the results.

Statistical analysis

Statistical analysis was performed using the software Prism version 5.0 (GraphPad Software, Inc., USA). Independent t-test was used to examine the mean difference on clinical data of study participants between the groups, which have a normal distribution. The differences between the three genotype groups in percentage of TUNEL positive sperm were analyzed by unpaired one-way ANOVA. Hardy-Weinberg equilibrium was determined for compatibility between patient and control groups using χ^2-tests. The associations of rs28362491 and rs696 genotypes, and the susceptibility to idiopathic oligospermia were analyzed by calculating the odds ratios (OR) and their 95% confidence intervals (95% CI) using the χ^2-test, accepting the homozygous common genotypes as the reference category for each polymorphism. We applied Bonferroni correction to the significant P-values (less than 0.05).

Results

The distribution of genotypes of *NF-κB1* -94ins/delATTG genotypes in normospermic and idiopathic oligospermic men was found to be significantly different (Table 2). The frequency of wild genotype was observed to be 45.6% in oligospermic men as against 29.2% in fertile men. The statistical analysis of the data using χ^2-test showed that the I/D genotype of the -94ins/delATTG polymorphism was associated with a significantly decreased risk of oligospermia when compared to the I/I genotype (OR = 2.36, 95% CI: 1.34–4.13, P = 0.002). In addition, the difference remained statistically significant even after Bonferroni correction (P = 0.006). However, the I allele frequency in idiopathic oligospermic cases was not significantly different from that in the control group (P = 0.126).

We also tested the association of 3'UTR A→G (rs696) polymorphism in *NF-κBIA* gene with the susceptibility to male infertility with idiopathic oligospermia. Table 2 shows that there is no statistical difference between oligospermic and normosepermic men when compared for allele frequency and genotype distribution of the *NF-κBIA* 3' UTR A→ G polymorphism (P > 0.05).

Observed genotype distributions for both polymorphisms in control group were in general agreement with Hardy-Weinberg equilibrium expectations (*NF-κB1*, P = 0.351; *NF-κBIA*, P = 0.559).

To analyze the association of genotypic combinations with the risk of oligospermia, we grouped the NF-κB1 and NF-κBIA polymorphic genotype variants into nine subgroups, II/AA, II/AG, II/GG, DI/AA, DI/AG, DI/GG, DD/AA, DD/AG and DD/GG as shown in Table 3. The frequency of wild-type homozygous genotype combination (II/AA genotype) did not differ significantly between oligospermic and normospermic men when compared to other combinations (P = 0.924–0.254**)**.

The *NF-κB1* and *NF-κBIA* polymorphisms with respect to percentage of TUNEL positive sperm were determined in the two study groups. The percentage of TUNEL-positive sperm was higher in the oligospermic group (64.48 ± 15.79 %) than in the control group (36.21 ± 11.47%) (P < 0.0001). When oligospermic and normospermic individuals were divided according to presence of ATTG allele in the context of the NF-κB1 -94 ins/del ATTG polymorphism**,** one-way ANOVA test illustrated that there was no significant difference in sperm apoptosis rate among the genotypes of this polymorphism in oligospermic patients (three-group ANOVA, P = 0.058), as shown in Figure 1A. In the TUNEL staining method, cell nuclei were evaluated as brown TUNEL (+) (Figure 2). In addition, no significant association was found between this polymorphism and percentage of TUNEL-positive sperm in normospermic men (P = 0.106). Furthermore, the results of association between *NF-kBIA* 3' UTR A→G polymorphism and sperm apoptosis were consistent with *NF-kB1* -94 ins/del ATTG polymorphism results (Figure 1B).

Table 2. Distribution of NF-κB1 and NF-κBIA genotypes and allele in oligospermic and normospermic men.

Genotypes / alleles	Oligospermic *n* (%)	Normospermic *n* (%)	O.R (95% CI)	*P** Value
NF-κB1 rs28362491				
II	52 (45.6)	38 (29.2)	Reference	
ID	43 (37.7)	74 (57)	2.36 (1.34 – 4.13)	0.002 (0.006)
DD	19 (16.7)	18 (13.8)	1.30 (0.60 – 2.80)	0.507
I allele	147 (64.5)	150 (57.7)	Reference	
D allele	81 (35.5)	110 (42.3)	1.33 (0.92 – 1.92)	0.126
NF-κBIA rs696				
AA	19 (16.7)	21 (16)	Reference	
AG	67 (58.8)	74 (57)	0.99 (0.49 – 2.02)	0.998
GG	28 (24.5)	35 (27)	1.13 (0.51 – 2.50)	0.762
A allele	105 (46)	116 (44.6)	Reference	
G allele	123 (54)	144 (55.4)	1.06 (0.74 – 1.51)	0.750

O.R = Odds ratio; CI = confidence interval. **P* value in paranthesis were corrected by Bonferroni correction.

Table 3. Odds ratio for different genotypic combinations of NF-κB1 and NF-κBIA polymorphism.

Genotype combinations	No. of subjects (oligospermics / normospermics)	OR	95% CI	*P*-Value
II+AA	9/7	Reference (1.00)		
II+AG	33/23	0.896	0.298 – 2.753	0.928
III+GG	10/8	1.029	0.265 – 3.995	0.760
DI+AA	7/14	0.927	0.311 – 2.759	0.289
DI+AG	25/39	2.006	0.662 – 6.076	0.336
DI+GG	11/21	2.455	0.719 – 8.383	0.254
DD+AA	3/0	0.181	0.008 – 4.077	0.263
DD+AG	9/12	1.714	0.461 – 6.371	0.634
DD+GG	7/6	1.102	0.253 – 4.801	0.805

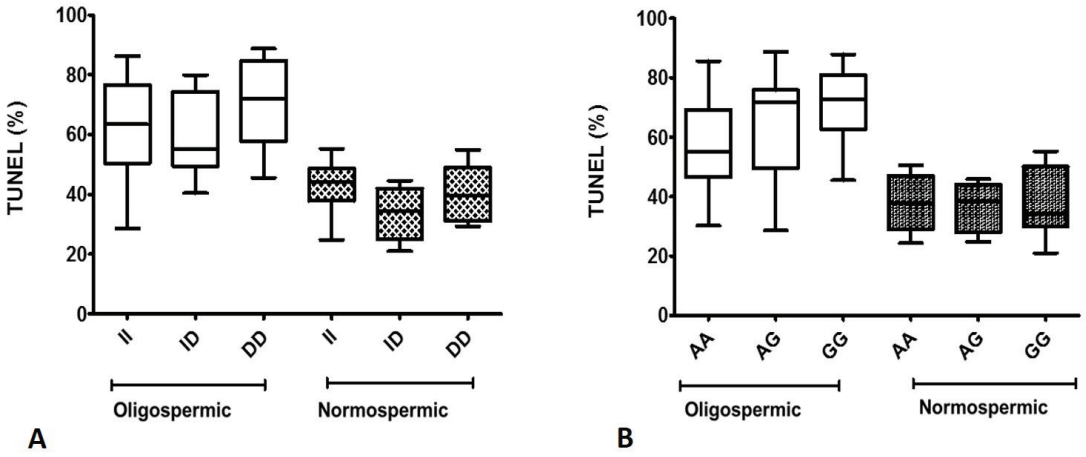

Figure 1. Box-and-whisker plots for sperm apoptosis measured by TUNEL in study subjects divided into three groups according to *NF-κB1* -94ins/delATTG (**A**) and *NF-κBIA* 3'UTR A→G (**B**) genotypes.

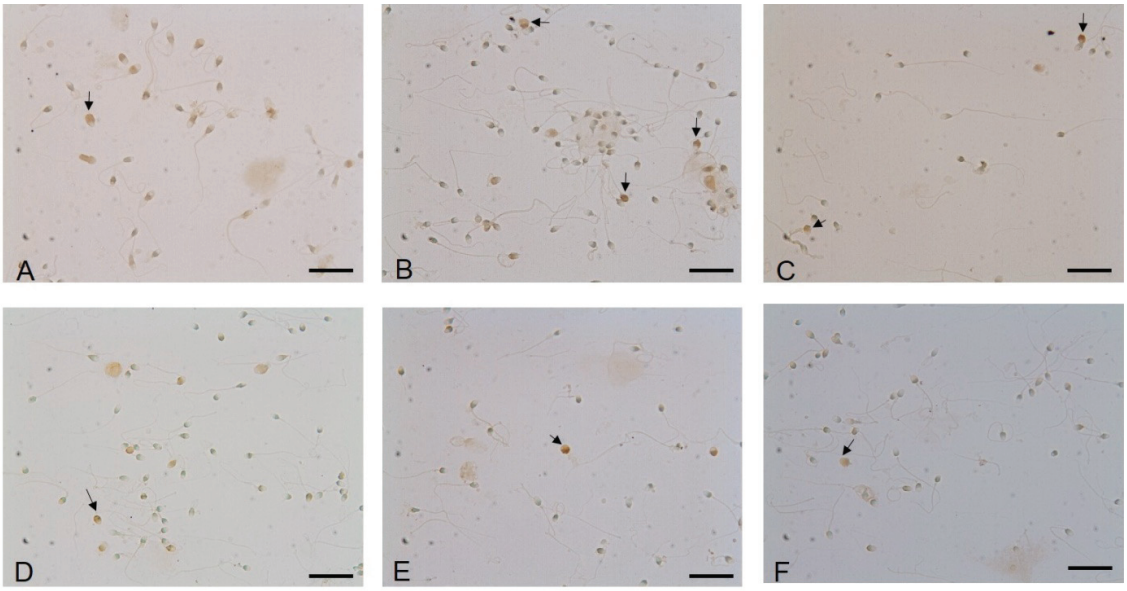

Figure 2. Representative figures of TUNEL staining for subjects according to *NF-κB1* -94 ins/delATTG genotypes. **A)** Oligospermic i/i, **B)** Oligospermic d/i, **C)** Oligospermic d/d, **D)** Normospermic i/i, **E)** Normospermic d/i, **F)** Normospermic d/d. Apoptotic sperms (arrows, dark brown nuclei). Healthy sperms (green nuclei). (Counterstain: Methyl green, Bar: 20 μm)

Discussion

Aberrant expression of NF-κB proteins, which is accepted as being one of multiple signaling pathways related to apoptosis, was found in chronic inflammatory diseases and several malignancies.[15,17,19] It has been suggested that an imbalance of the DNA binding activity of NF-κB may be part of the pathophysiological mechanisms in apoptosis-related diseases.[30] In addition, a few studies suggest that NF-κB proteins play a critical role in human spermatogenesis by pro-apoptotic effects on germ cells.[12,31] Pentikäinen *et al*.[12] showed that an increased amount of NF-κB activity is associated with an excessive amount of apoptotic activity in human seminiferous tubules. Furthermore, there are also some data suggesting that genetic variations of NF-κB proteins, including the NF-κB1 -94 ins/del ATTG and NF-κBIA 3'UTR A→G polymorphisms are associated with the development of several diseases.[20,23] Therefore, all these observations convinced us to examine the association between the mentioned NF-κB polymorphisms and idiopathic male infertility in a Turkish population in the context of sperm apoptosis. As far as we know, there is no data available in the English literature to report the association with defective spermatogenesis.

Although there are studies demonstrating that NF-κB has both anti- and pro-apoptotic effects within cells,[32] our expectation was that the I allele frequency regarding the NF-κB1 -94 ins/del ATTG polymorphism would be higher in oligospermic cases, as previous studies have suggested that D allele may result in decreased NF--B1 message and Sertoli cell NF-κB proteins exert pro-apoptotic effects on germ cells.[12,20] Although our univariate analysis showed that there was no significant difference in I allele frequency between oligospermic patients and normospermic men, the frequency of the heterozygous ID genotype was more common (2.4 fold) in normospermic men. Thus, it might be speculated that the heterozygous variant of the NF-κB1 -94 ins/del ATTG polymorphism has a protective effect on oligospermia. Positive heterosis occurs when heterozygous offspring exhibit superiority of a biological trait compared to their homozygous parents, as a result of an increase in the value of a quantitative trait. It may be present in a large number of gene association studies and it has been also reported for NF-κB1 -94 ins/del ATTG polymorphism.[33,34] The biological meaning of the protective effect of the heterozygote genotype of rs28362491 toward oligospermia is not easily explained by the existing observations on NF-κB. It has been demonstrated that the modulation of apoptotic response was more affected by heterozygous variants of some polymorphisms in various genes than those from homozygote variants[35] and NF-κB has a role in numerous stress responses including apoptosis within male testicular cells.[36] Thus, it may be simply speculated that a dosage-dependent gene regulatory hierarchy composed by the heterozygote genotype of the NF-κB may affect expression of genes involved in germ cell apoptosis, perhaps by altered apoptotic response. Therefore, this effect may confer a protective effect against oligospermia. However, this hypothesis requires further investigation.

As previously mentioned, several diseases with apoptosis pathogenesis are hypothesized to be caused by an imbalance between NF-κB and Iκ B. In addition, it has been suggested that a few polymorphisms of NF-κBIA may also play a role in this imbalance.[15] Among the NF-κBIA polymorphisms, the single nucleotide polymorphism in the 3'UTR (G/A) of the NF-κBIA gene is important because it may affect mRNA stability and translational efficacy.[20] In some case control studies, it was observed that there was association between this polymorphism and a few conditions associated with increased apoptosis.[37] In our study, we did not find any association between the *NF-κBIA* rs696 polymorphism and susceptibility to oligospermia. Moreover, there was no evidence for a combined effect of rs28362491 and rs696 on susceptibility to oligospermia.

In the second step, we concentrated on whether these polymorphisms are related to susceptibility to sperm apoptosis in our study population. Our expectation was that variants of the NF-κB1 and NF-κBIA genes would be associated with altered sperm apoptosis. In this study, we used terminal UTP nicked-end labeling (TU-

NEL) staining, which is one of the most commonly used methods to label apoptotic cells, to detect the apoptosis of sperm. However, we did not see any significant difference between the genotype groups of rs28362491 and rs696, and the levels of the assessed apoptosis marker in oligospermic infertile males, suggesting that these polymorphisms are not associated with increased apoptosis in oligospermia. This might be because apoptotic cell death during spermatogenesis is not related with NF-κB activation, or alternatively that the two polymorphisms studied do not have a critical effect on impaired spermatogenesis.

Alternatively, rather than predisposing to DNA fragmentation in mature spermatozoa, the mentioned NF-κB polymorphisms may have an important effect in an earlier stage of spermatogenesis or during embryonic development.

The results from our study, which is the first study in the literature to investigate the rs28362491 and rs696 genetic variants as a possible risk factor for susceptibility to oligospermia, have some limitations. One of the major limitations is the small sample size, which may have influenced the statistical power of our analyses, and the fact that it only considered Turkish patients, as racial differences may be important. Second, it should be kept in mind that there are other polymorphic sites of *NF-κB1* and *NF-κBIA* gene, and other variations or mutations of genes including, but not limited to, AZFb, AZFc, MTHFR, DNTM, DNMT3L and Pygo2 that may play a role in oligospermia. Furthermore, several studies have found an effect between sperm apoptosis and genetic variants of cell death pathway genes such as p53, FAS and caspase-8, but presently we do not know that these and other polymorphisms in the apoptosis-associated genes might be related with the susceptibility to oligospermia for our cases.[38,39] Thirdly, we have not yet evaluated the expression and activity of p50 (NF-κB1), which regulates important cellular events such as apoptosis and cell death independent of the NF-κB complex, in the samples from the cases. Therefore, further investigation seems warranted on the combined effects of these genetic variations on oligospermic phenotype. Third, the present study did not investigate whether NF-κB activation may be affected by rs28362491 and rs696 genotypes in the context of infertile patients with oligospermia.

In conclusion, we were not able to demonstrate an association between increased apoptosis in oligospermia and the rs28362491 polymorphism in the *NF-κB1* gene, or the rs696 variant in the *NF-κBIA* gene. However, this study demonstrates that the heterozygosity of rs28362491 in the *NF-κB1* gene may have a protective effect by modifying susceptibility to oligospermia.

Acknowledgment

We would like to thank all of the subjects who participated in this study. This work was partly supported by the Research Fund of Istanbul University (project number: 32981). The authors have no conflict of interest to declare.

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