Human catalase gene polymorphism (CAT C-262T) and risk of male infertility

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Catalase—idiopathic male infertility—oxidative stress—polymorphism

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Summary
Infertility is the failure of a couple to engender after endeavouring at least one full year of unprotected intercourse. It has been reported that reactive oxygen species contributed to pathogenesis of various disease. To inactivate ROS cells biosynthesise several antioxidant enzymes, one of them is catalase which contributes H2O2 to H2O and O2. This study set out to delineate the association of catalase C-262T polymorphism with idiopathic male infertility. The study included 195 men with idiopathic infertility and 190 healthy volunteers. Genomic DNA was extracted from peripheral blood leucocytes. Genotype and allele frequencies were determined in patients and controls using allele-specific PCR (AS-PCR). The prevalence of genotype frequencies of the CAT CC/CT/TT was 31.79%, 65.12% and 3.07%, respectively, in infertile subjects, as against 24.73%, 55.26% and 20%, respectively, in healthy volunteers. Statistical analysis has emerged significant difference from the comparison of either genotype ($P < 0.05$). Taking into accounts of results, the catalase C-262T polymorphism indicates that CAT-262T/T genotype confers less susceptibility to male infertility. Further studies with larger numbers of patients are required for further evaluation and confirmation of our finding.

Introduction
Infertility is a privileged medical condition because of a couple in which more than one person is involved. The World Health Organization (WHO) defines infertility as a couple’s inability to conceive after 1 year of regular intercourse in the absence of contraception. About 15% of couples will not attain gestation within 12 months of trying a baby (Shiva et al., 2011). Among infertile couples, the male factor is roughly responsible for half of the cases (Poongothai et al., 2009). Male infertility of unknown origin is called idiopathic male infertility due to an unexplained reduction in semen quality (Hamada et al., 2011). The significant impact of reactive oxygen species (ROS) has been documented in various diseases such as infertility. However, many questions, considering physiologic role of ROS remain unanswered. Studies have demonstrated that antioxidants have substantial impact in andrology. It has been reported that the male germ cells at different stages of development have potential to turn out ROS (Agarwal et al., 1994a,b). Semen ROS are produced by spermatozoa and semen leukocytes (Novotný et al., 2003). Although the low levels of ROS are demanding for sperm capacitation and hyperactivation, human spermatozoon is susceptible to ROS at higher levels (Kefer et al., 2009). Human spermatozoa are supersensitive to ROS due to the paucity of antioxidant storage. The most pernicious effects of ROS on spermatozoa include DNA damage and lipid peroxidation of sperm membrane (Sikka, 2001). The eternal exclusion of H2O2 is needed for the aerobic organisms. Aggressive H2O2 has been hypothesised to play a substantial role on sperm motility due to the decrease in membrane fluidity which is momentous for sperm–oocyte fusion (Makker et al., 2009). As H2O2 is the only substrate for catalase, here upon, we evaluated the association of functional polymorphism in this gene (C-262T) which alters the level of catalase in blood and influences the promoter activity in Iranian men (Forsberg et al., 2001). Catalase (EC 1.11.1.6) is a ubiquitous antioxidant enzyme which is intensively perused, because of the easement of isolation from tissues such as liver. It is dumbbell-shaped antioxidant which is mostly located in peroxisome, and it is predominantly in erythrocyte, hepatocyte and kidney.
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whereas the connective tissue has a minimum level of catalase (Quan et al., 1986). Catalase is assigned to chromosome 11p13, comprising 13 exons (Konings et al., 2007). It is a heme containing enzyme with high performance of converting \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \) and \( \text{O}_2 \) in an energy-efficient way. Human catalase includes four stable polypeptide chains, each comprising 526 amino acid residues (Korneluk et al., 1984). The relation between (C-262T; rs1001179) polymorphism and catalase activity has been studied in multiple studies such as Alzheimer (Goulas et al., 2002), asbestosis (Franko et al., 2008) and vitiligo (Gavalas et al., 2006). Forsberg et al. (2001) documented substantially higher catalase levels in homozygous of TT genotype in comparison with the CC genotype. It has been suggested that genetic variations in antioxidant genes may contribute to oxidative sperm DNA damage and male infertility (Ji et al., 2012). To the best of our knowledge, the association between idiopathic male infertility and C-262T polymorphism has not been studied. Therefore, we assessed the distribution of this functional polymorphism in Iranian men with the idiopathic infertility.

### Materials and methods

#### Participants

Cases were 195 infertile patients who had been unable to conceive within a reasonable period of the time (at least 2 years). The research was approved by the local institution review board. Semen samples were obtained after at least 72 h of abstinence by masturbation. Second examination was performed to further elucidation of the aetiology. Each idiopathic infertile man sustained serum determination was performed to further elucidation of the aetiology. The laboratory personnel were unaware of case-control status. Genomic DNA was extracted from peripheral blood leukocytes, by standard procedure using the Gpp Solution DNA extraction kit (genpajoohan, Iran). DNA concentrations were quantified by gel electrophoresis. Purified genomic DNA was maintained at a temperature of \(-20^\circ\text{C}\) until molecular analysis.

#### Laboratory assessment

Blood (1 ml) was withdrawn into EDTA-K3 coated Venojects. The samples were stored at 4 °C and centrifuged at 578 g for at least 10 min within the next 24 h. The laboratory personnel were unaware of case-control status. Genomic DNA was extracted from peripheral blood leukocytes, by standard procedure using the Gpp Solution DNA extraction kit (genpajoohan, Iran). DNA concentrations were quantified by gel electrophoresis. Purified genomic DNA was maintained at a temperature of \(-20^\circ\text{C}\) until molecular analysis.

#### Genotyping

The variant alleles of CAT C-262T were determined with allele-specific PCR (AS-PCR) assay, using primers were listed in Table 1 (Khodayari et al., 2013). Primers were synthesised by MWG-Biotech (Ebersberg, Germany). Genomic DNA (30 ng) was added to a PCR mixture, comprising 2 pmol of each primer, 0.1 mM dNTP, 10× PCR buffer (10 mM Tris–HCl, 50 mM KCl and 0.1% Triton X-100), 1.5 mM MgCl\(_2\) and 0.5 unit of Taq polymerase (gene Fanavaran, Iran) in a total volume of 25 μL. The amplification was performed as follows: initial denaturation at 95 °C for 5 min, amplification for 34 cycles at 95 °C for 45 s, 56 °C for 45 s and 72 °C for 45 s. The final cycle included extension for 5 min at 72 °C to ensure full extension of the product. The PCR products were electrophoresed in 2% agarose gels and were visualised under UV illumination as shown in Fig. 1.

#### Statistical analysis

The statistical analysis was performed by Med Calc chi-square test (version 12.1.4.0; Mariakerke, Belgium) to make genotype and allele comparisons. A value of \( P < 0.05 \) was considered statistically significant.

#### Results

The study included 190 controls and 195 patients with idiopathic male infertility. The patients ranged in age from 25 to 42 years old, and the healthy controls ranged in age from 29 to 40 years old. In this study, data from semen analyses obtained from infertile men. The main

### Table 1 Primer used in amplification

<table>
<thead>
<tr>
<th>Primer designation</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward (C allele)</td>
<td>5′-GCCCTGGTTCTGGCTATC-3′</td>
</tr>
<tr>
<td>Forward (T allele)</td>
<td>5′-GCCCTGGTTCTGGCTATT-3′</td>
</tr>
<tr>
<td>Common reverse</td>
<td>5′-GGTTTGCTGTGCAAGACACT-3′</td>
</tr>
</tbody>
</table>

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clinical characteristics of the infertile men are summarised in Table 2.

Both alleles C and T have the same PCR products in size (340 bp). The allele and genotype frequencies of the C-262T in the promoter region of the catalase gene of the control and patient groups are shown in Table 3. In comparison of allele frequencies between the control and patient groups, there was a significant difference in the distribution of the allele ($P = 0.0008$). Also, significant difference was found between genotype frequencies of the control and patient groups determined by the expected value of the $x^2$ test. ($P = 0.0001$). Genotype frequencies of the CAT CC/CT/TT were 31.79%, 65.12% and 3.07%, respectively, in infertile subjects; whereas in healthy volunteers were 24.73%, 55.26% and 20% respectively.

### Table 2 Clinical characteristics of the infertile patients

<table>
<thead>
<tr>
<th>Semen variable</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligospermia(^{a})</td>
<td>24 (12.30%)</td>
</tr>
<tr>
<td>Asthenospermia(^{b})</td>
<td>32 (16.41%)</td>
</tr>
<tr>
<td>Teratospermia(^{c})</td>
<td>46 (23.58%)</td>
</tr>
<tr>
<td>Oligoasthenospermia</td>
<td>40 (20.51%)</td>
</tr>
<tr>
<td>Normospermia</td>
<td>53 (27.17%)</td>
</tr>
</tbody>
</table>

According to the (WHO) criteria: \(^{a}\)less than 20 million spermatozoa per ml; \(^{b}\)decreased motility of spermatozoa; and \(^{c}\)more than 70% abnormal spermatozoa.

### Table 3 Genotype and allele distributions of catalase (C-262T) polymorphism

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Patients</th>
<th>OR (95% CI)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>47 (24.73%)</td>
<td>62 (31.79%)</td>
<td>0.11</td>
<td>0.0001</td>
</tr>
<tr>
<td>CT</td>
<td>105 (55.26%)</td>
<td>127 (65.12%)</td>
<td>(0.04–0.30)</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>38 (20%)</td>
<td>6 (3.07%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls</th>
<th>Patients</th>
<th>OR (95% CI)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>199 (52.36%)</td>
<td>251 (64.35%)</td>
<td>0.60</td>
<td>0.0008</td>
</tr>
<tr>
<td>T</td>
<td>181 (47.63%)</td>
<td>139 (35.64%)</td>
<td>(0.45–0.81)</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

Nowadays, infertility is a serious problem. Hitherto, nobody has been able to fully explain the cause of the idiopathic male infertility. Modulating of male infertility remains disappointing due to the paucity of our knowledge about prognosis, aetiology and treatment. According to the WHO, about 80 million couples suffer from this problem annually. Given the prevalence of male infertility and the cost of treatment, it is crucial to evaluate the causes and risk factors. Male infertility is a polygenic multifactorial syndrome which accounts for 10–15% couples (Ferlin et al., 2006). Polymorphisms and antioxidant genes mutation are the most genetic modifications that have been studied in recent years in connection with numerous diseases. Genetic polymorphism as a risk factor makes a great contribution to male infertility by resonancing spermatogenic defect. The promoter sequences are the areas that have potential impact on gene expression (Hoogendoorn et al., 2003). Numerous SNPs in gene regulatory regions have been coupled with the alteration in enzyme levels and human diseases. However, the regulation of the human catalase gene is still unclear (Forsberg et al., 2001).

Several SNP have been accounted, most of which are in association with acatalasemia (Goth & Eaton, 2000; Goth et al., 2001). Blood pressure has also been reported in association with CAT gene (Jiang et al., 2001).

In this study, we evaluated a functional polymorphism of the single nucleotide C-262T in the catalase gene, and we also observed that CAT C-262T polymorphism was mutated in a different proportion in cases and controls. The most obvious finding to emerge from this study is that the TT genotype was significantly higher in the healthy control group. In Foresberg et al. study, TT genotype indicates a higher catalase level in comparison with CT and CC genotypes. The second major finding was a higher percentage of heterozygous (CT) genotype in both infertile patients and healthy controls. In contrast to our findings, Frako et al. reported that CC and CT genotypes have a less risk of asbestosis compared to TT genotype (Franko et al., 2008). Different association was noted in Choi et al. study, they declared that TT genotypes were significantly associated with increased risk of prostate cancer (Choi et al., 2007). There are some other studies which CAT genotypes were not in connection with rheumatoid arthritis (El-Sohemy et al., 2006), Alzheimer’s disease (Goulas et al., 2002) and recurrent depressive disorder (Galecki et al., 2009). In summary, we...
observed the consistency of the association with respect to idiopathic male infertility. It may indicate the importance of antioxidant gene polymorphism on idiopathic male infertility. While the randomised multicenter trials with greater sample size are still needed to clarify our results.

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References


