Influence of Tumor Necrosis Factor – Alpha -308 gene Polymorphism and its Adverse Effects of Seminal Plasma concentration on sperms characteristics of infertile men

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Abstract and objective: Tumor Necrosis Factor – Alpha -308 gene Polymorphism have been related to different disease conditions due to increased TNF-α. However, its relation with Seminal fluid analysis is not studied in Iraq. Objective: this study aimed to investigate the association of TNF-α-308 gene Polymorphism and TNF-α seminal plasma concentration with of abnormal seminal fluid analysis among infertile men. Methods: Prospective correlational study including 85 infertile men collected during October 2019 to March 2020. From each participant blood and semen samples were collected according to the standard method. The blood used for DNA extraction and TNF-α-308 genotyping using allele specific PCR. Seminal plasma was separated and used for TNF-α measurement using sandwich ELISA method. Results: the results found that mutant allele was frequent among infertile men 28%. TNF-α seminal plasma concentration was significantly higher in men carrier for variant allele58.92±21.03 pg/ml in comparison with infertile carrying wild type allele27.1±13.34 pg/ml. Seminal plasma TNF-α was inversely correlated with sperm motility (progressive and none progressive (r=-0.350 and -0.282 respectively. Conclusion: The mutant allele A might negatively affect sperm motility and morphology by increasing seminal plasma TNF-α level.

Key words: Tumor necrosis factor alpha, gene polymorphism and infertility

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Background:
Tumor necrosis factor alpha is a pleotropic cytokine acting through two transmembrane receptors as a powerful pro-inflammatory cytokine (Musco and van Staden, 2010). Its released by macrophage in have inflammatory actions in both innate and adaptive responses (Agbanoma et al., 2012). One of the single nucleotide polymorphism that have been studied in wide disease models is rs1800629 -308G/A of TNF-α gene where the allele G substituted to A and associated with higher level of production(Louis et al., 1998).
The genetic polymorphism have been reported that TNF-α linked to higher risk for infertility among males (Mostafa and Taymour, 2016). However, TNF-α has been linked to male infertility in a lot of literatures. Its seminal plasma level have been linked with lower sperm motility (Agbanoma et al., 2012; Qian et al., 2011) or its might induce apoptosis via linking with TNF-alpha Receptor-1 and conserved death domain and activation of caspase 8 that affect permeability of sperms followed by activation of executioner caspase 3 leading to cell death (Zalata, Mokhtar, et al., 2013). The pro apoptotic activity of TNF-α might be related to reactive oxygen species that cause membrane peroxidation of sperm plasma membrane and sperm DNA fragmentation and lower acrosome reaction (Eggert-Kruse et al., 2007).

So, his study aimed to determine the TNF-α G308A gene polymorphism among Iraqi infertile men, and determination of its impact on seminal plasma TNF-α protein level and semen characteristics.

Material and Methods:

Study design and settings:

This cross-sectional study involved 85 infertile Iraqi men attending to routine Seminal fluid analysis in private laboratory in Baghdad during the period of October 2019 to March 2020. From each participant blood and semen samples were collected after verbal approval for sample intake. A detailed for history, duration of infertility, drug intake, previous surgery, varicocele or trauma and type of infertility. All types of male infertility were included and only patients taking immunosuppressive or anti-inflammatory drugs were excluded from this study.

Genomic DNA Extraction and Tumor Necrosis Factor - α -308 genotyping:

Blood samples were collected in EDTA-K2 collection tubes and stored in -20°C. Genomic DNA was extracted using Blood/Cell DNA Mini Kit (cat. No. GB100) Geneaid®. Allele specific polymerase chain reaction was performed for genotyping of TNF-α -308 as described previously (Richardson et al., 2001).

Measurement of seminal plasma Tumor Necrosis Factor - α:

After liquefaction of semen sample, equal volume of phosphate buffered saline was added for each sample and stored -20°C until assay. Human Tumor Necrosis Factor Alpha (TNFA) ELISA Kit (abx050218) purchased from Abbexa® UK used for quantitative measurements of seminal plasma TTNF-α according to manufacturer instructions.

Statistical analysis:

All statistical analysis done by using GraphPad Prism 7.0 San Diego, CA. independent sample t-test used for comparison of numerical data. While, Chi-square or Fisher exact test used to estimate the association between categorical data. P value less or equal to 0.05 considered as significant. Heat map graph used for illustration of correlation of coefficient among variables.

Results:

A total of eighty-five infertile men were included in the study, the mean age of them was 34.78 years. 30 (35.29%) were primary infertility. None of infertile subjects was taking antibiotics. The genotyping results of -308 gene polymorphism was 59 (69.41%) was homozygous wild type, 15 (17.65%) was heterozygous genotype and 11 (12.94%) were homozygous mutant genotype. The allele frequency of mutant allele A was 21.76% among our selected infertile men (Table 1).
Table 1: Genotype and allele frequency of TNF-α G308A gene polymorphism.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>59</td>
<td>69.41%</td>
</tr>
<tr>
<td>GA/AG</td>
<td>15</td>
<td>17.65%</td>
</tr>
<tr>
<td>AA</td>
<td>11</td>
<td>12.94%</td>
</tr>
<tr>
<td>Allele G</td>
<td>133</td>
<td>78.23</td>
</tr>
<tr>
<td>Allele A</td>
<td>37</td>
<td>21.76</td>
</tr>
</tbody>
</table>

According to the allelic variation in TNF-α G308A, the results in table 2 showed that infertile men carrying mutant allele did not show difference in the mean of sperm concentration (p=0.187), none progressive motility (p=0.098), round cell count (p=0.253) and the state of leukocysperma (p=0.473). A significant lower percentage of progressive motile sperms and none progressive motile sperm was found among mutant allele carriers (21.75) in comparison with wild type allele carriers (25.31), similarly, normal sperm morphology was lower among mutant allele carriers (20.31) when compared with wild type allele carriers (32.91).

Figure 1: Agarose gel electrophoresis of allele specific primer PCR of G308A TNF-alpha gene in 2% agarose gel. Lane 1: molecular marker 100bp. Lane 2 and 3: represent homozygous mutant. Lane (4 and 5), (6 and 7) and (8 and 9) represent homozygous wild type.
Interestingly, the mutant allele carrier have higher percentage of immotile sperm (mean=44.49%) compared to wild type allele carriers (mean=36.91) also, seminal plasma TNF-α concentration (mean=58.92) in mutant carriers and wild type carriers have (mean=27.1).

Table 2: Comparison between laboratory results according to allelic variation of TNF-α G308A.

<table>
<thead>
<tr>
<th></th>
<th>Mutant allele</th>
<th>Wild type allele</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration</td>
<td>27.79±13.29</td>
<td>23.4±18.9</td>
<td>0.187 NS</td>
</tr>
<tr>
<td>Progressive motile sperm</td>
<td>21.75±5.9</td>
<td>25.31±6.59</td>
<td>0.003 *</td>
</tr>
<tr>
<td>None progressive motile sperm</td>
<td>33.2±12.2</td>
<td>37.23±13.29</td>
<td>0.098 NS</td>
</tr>
<tr>
<td>Immotile sperm</td>
<td>44.49±15.3</td>
<td>36.21±12.9</td>
<td>0.001 **</td>
</tr>
<tr>
<td>Normal Sperm Morphology</td>
<td>20.31±5.94</td>
<td>32.91±8.93</td>
<td>0.001 **</td>
</tr>
<tr>
<td>Round cells</td>
<td>11.58±7.9</td>
<td>13.29±8.02</td>
<td>0.253 NS</td>
</tr>
<tr>
<td>TNF-alpha (pg/ml)</td>
<td>58.92±21.03</td>
<td>27.1±13.34</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>Leukocytospermic</td>
<td>8 (21.62%)</td>
<td>22 (16.54%)</td>
<td></td>
</tr>
<tr>
<td>None leukocytospermic</td>
<td>29 (78.38%)</td>
<td>111 (83.45%)</td>
<td>0.473 NS</td>
</tr>
</tbody>
</table>

NS: none statistical significance p>0.05.

*: statistical significance (p≤0.05).

**: high statistical significance (p≤0.001).

Seminal plasma TNF-α is adversely affect sperm motility:

The results of correlation of coefficient (Figure 2) showed that seminal plasma TNF-α was inversely correlated with progressive motility (r=-0.350) and none progressive motile sperms (r=-0.282) and increased proportionally with increased percentage of immotile sperms (r=0.442).
Discussion:

Polymorphism of TNF-α has been linked to infertility in a lot of literatures. Here in this study it’s the first time to investigate the frequency of TNF-α G308A gene polymorphism among Iraqi infertile men and their relation with seminal plasma concentration of TNF-α and seminal fluid parameters.

We reported that GA/AA genotypes was presented in 30.59% of Iraqi infertile men, this frequency was comparable with French study by Tronchon, et al., in 2008 who found 33.3% of infertile men carrying these genotypes (Tronchon et al., 2008). Similarly, Egyptian study by Zalata, et al., who reported 33.6% (Zalata, Atwa, et al., 2013). Chinese study by Li, et al., reported higher frequency of these genotypes (47.6%) (Li et al., 2010). However, despite this difference the impact of variant allele is well documented in these studies. The impact of this polymorphism is explained by amino acid change on promoter region at -308 when Guanin replaced by adinin, will affect the transcriptional activity of TNF-α gene and resulting in higher level of TNF-α protein that mediate pathological activities in different diseases (Qidwai and Khan, 2011).

The current results highlighted elevated seminal plasma TNF-α level among infertile men carrying mutant allele in comparison with wild type allele carriers. This results in agreement with Li, et al., who found similar findings (Li et al., 2010). Furthermore, the pathological effect of TNF-α on infertility is widely investigated by several reports. The certain amount of seminal plasma TNF-α can inhibit the sperm activity and acrosomal reaction (Bian et al., 2007), also, it can interfere with sperm energy and function of mitochondria that ultimately affects sperm motility (Bian et al., 2004). Furthermore, it can affect nitrous oxide synthesis which is essential for sperm motility (Wu et al., 2004).

Our results recorded that higher seminal plasma TNF-α was not associated with leukocyte number in those patients. This result in contrary with Liu et al. because of any cases with severe urogenital infection was
excluded in order to avoid its interference with our hypothesis (effect of genetic variation of TNF-α on its production). The raised seminal plasma TNF-α was ignored, its well known that infection is responsible for increased seminal plasma pro-inflammatory cytokines that have negative impact on sperms (Pergialiotis et al., 2018).

Our results highlighted the influence of mutant allele on increased level of TNF-α in certain infertile patients. However, we support the idea of using infliximab for improving the semen characteristics via neutralizing secreted TNF-α and preventing its harmful effect on spermatozoa (Said et al., 2005).

In conclusion, TNF-α G308A variant allele might influence increased seminal plasma TNF-α that negatively affect characteristics of spermatozoa of infertile men.

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Reference:


