REVIEW

TNF-α –308 polymorphisms and male infertility risk: A meta-analysis and systematic review

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ABSTRACT

This study aimed to conduct a systematic review and meta-analysis of prospective studies discussing TNF-α –308 polymorphism and male infertility. This study was conformed to Preferred Reported Items for Systematic Reviews and Meta-Analyses guidelines. PubMed, Embase and Scopus databases were searched to identify relevant studies by two independent reviewers. Hazard ratios were pooled using fixed-effect or random-effects models when appropriate. Q-test was performed to evaluate study heterogeneity and publication bias appraised using funnel plots. The search yielded five studies (three of Caucasians ethnicity and 2 of Asian ethnicity) comprising 2939 men (2262 infertile men and 677 fertile controls). Most of the studied cases were carried out on TNF-α promoter region at positions –308 G/A (four studies) where
Polymorphism
Meta-analysis
Semen
Sperms

\(-308\) C/T was dealt with in one study. Overall, significant associations between TNF-\(\alpha\) – 308 gene polymorphisms and idiopathic male infertility risk were observed (fixed effect: OR = 0.472, 95% CI: 0.378–0.589; \(P = 0.001\); random effect: OR = 0.407, 95% CI: 0.211–0.785; \(P = 0.007\)) with robust findings according to sensitivity analyses. Funnel plot inspections did not give evidences of publication bias. A stratified analysis performed for ethnic groups revealed significant association in both Caucasian and Asian populations. It is concluded that there are evidences of associations between TNF-\(\alpha\) – 308 gene polymorphisms and male infertility risk.

Meta-analysis refers to methods that focus on combining and contrasting results from different studies to identify patterns among study results, sources of disagreement or additional relationships coming in this context [1,2]. Male infertility results from interaction of multi-factorial causes as, endocrinal environmental and/or genetic factors [3–6]. Many genetic studies were carried out to inspect the contribution of encoded genes whereas various studies exposed the association between different genotypes and the disease vulnerability [7–10].

Seminal plasma contains several cytokines that are normally present in the male genital tract. Tumor necrosis factor (TNF)-\(\alpha\) is one of the known cytokines regulatory peptides that is produced and secreted by leukocytes being implicated as growth and differentiation factors. In the testes, TNF-\(\alpha\) receptors are present in the Sertoli cells and Leydig cells, allowing TNF-\(\alpha\) to modulate their functions [11]. In men with unexplained infertility, seminal plasma levels of TNF-\(\alpha\) were demonstrated to correlate negatively with progressive sperm motility [12]. In their study, Said et al. [13] demonstrated that exposing sperms to high concentrations of TNF-\(\alpha\) results in a significant loss of its functional as well as genomic integrity.

Genetic factors as single nucleotide polymorphisms were revealed to affect TNF-\(\alpha\) level, where different polymorphisms in the TNF gene cluster were linked with its modified production. Several single nucleotide polymorphisms in TNF-\(\alpha\) promoter region were investigated at positions –1031 T/C, –863 C/A, –857 C/T, –575 G/A, –308 G/A, –244 G/A, and –238 G/A [14]. Tronchon et al. [15] associated –308 TNF-\(\alpha\) A allele with increased expression/production of TNF-\(\alpha\). They pointed that the frequency of –308 allele was significantly higher in fertile men with testicular failure or with altered sperm motility compared with patients with normal sperm parameters (19.4%). Lazaros et al. [16] pointed to the nonsignificant relationship between TNF-\(\alpha\) 857C/T polymorphisms and semen quality. Zalata et al. [14] associated the single nucleotide polymorphism in the TNF-\(\alpha\) (–308) gene with significantly increased seminal caspase-9 and significant decrease in sperm count, sperm motility, normal sperm morphology, acrosin activity and seminal \(\alpha\)-glucosidase. Lately, Shukla et al. [17] designated an association between TNF-\(\alpha\) G -308A genotype substitutions with fertile men supported by allele and genotype meta-analysis and thus established it as a risk factor.

This study aimed to assess the relationship of TNF-\(\alpha\) polymorphism with male infertility by conducting a meta-analysis of the available case-control studies.

Material and methods

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guiding principle where included studies were retrieved from many electronic databases including PubMed, Scopus and Embase. Combination of search terms was used as (“TNF-alpha” or “TNF-\(\alpha\)”), (“male infertility”) and (“polymorphism” or “allele” or “variant” or “mutation” or “gene” or “genotype”). No restrictions were executed on the search in terms of language till June 2015.

Selection criteria

The two investigators (TM, MT) independently assessed the literature eligibility where the discrepancies were resolved by consensus. Articles were considered for inclusion in the systematic review if:

a. Reported data from an original, peer-reviewed study (not conference abstracts, case reports, posters, editorials, review articles or letters to editors).

b. Case-control design.

c. Reported about the TNF-\(\alpha\) –308 genotype distribution in cases and corresponding controls.
d. Evaluated TNF-α −308 genotype polymorphism and male infertility risk.
e. All procedures carried out were in accordance with the ethical standards of the responsible committee on humans and with the Helsinki Declaration of 1975, as revised in 2008 and obtained informed consent from all subjects for being incorporated in these studies.

This information was extracted from the studies: first author’s name, publication year, country, ethnicity, and genotype frequencies (cases/controls).

**Statistical analysis**

Comprehensive meta-analysis program (Englewood, NJ, USA) was used for all statistical analyses. Combined odds ratio (OR) with its consequent 95% confidence interval (CI), was used to calculate the strength of the relationship between TNF-α polymorphisms and male infertility risk. Heterogeneity assumption was examined using Q-test [18]. Random-effects model (Der Simonian-Laird method) were used to analyze the pooled model (Der Simonian-Laird method) and fixed-effects model (Mantel–Haenszel method) which controls for each study was examined by [20]. In addition, Hardy–Weinberg equilibrium (HWE) among controls for each study was examined by χ² test.

**Results**

A total of six eligible citations were identified during this search that met our inclusion criteria involving 2939 men in the pooled analyses (2262 cases and 677 controls). These studies were published between 2008 and 2014 (Fig. 1, flowchart). In the articles eligible for the meta-analysis, three were conducted in Caucasian populations, and two studies involved Asian populations. All articles were in English except one in Chinese with English abstract [21]. The controls of all studies mainly came from healthy population from hospital-based populations being matched for sex and age. All included articles used blood samples for genotyping assay.

Applied exclusion criteria included the following: age > 50 years, vasectomy, azoospermia, hypogonadism, varicocele, diabetes, hypertension, chemotherapy, malignancy, tuberculosis, HIV, leukocytospermia, smoking, alcohol or drug abuse, abnormal karyotype, Y chromosome deletions and chronic genital infections.

To assess genetic polymorphism, polymerase chain reaction-restriction fragment length polymorphism was used in every study. Semen samples of cases were carried out according to WHO guidelines 1999 except one paper [14] that used WHO 2010 guidelines. Most of the studied cases were carried out on TNF-α promoter region at positions −308 G/A (3 studies) (GG is the wild and AA is the mutant) where −308 C/T (CC is the wild and TT is the mutant) was dealt with in one study. The characteristics of all case–control studies incorporated for the tested polymorphism were demonstrated (Table 1).

Kurz et al. [22] cases comprised azoospermic, oligozoospermic and teratozoospermia/asthenozoospermia men, Tronchon et al. [15] cases comprised azoospermia and oligozoospermia men, Li et al. [21] cases comprised oligozoospermia, Zalata et al. [14] cases comprised asthenozoospermia, asthenoteratozoospermia and OAT, and Shukla et al. [17] cases comprised oligozoospermic and asthenozoospermic men.

Overall, significant associations between TNF-α gene polymorphisms and male infertility risk were observed (fixed effect: OR = 0.472, 95% CI: 0.378–0.589; P = 0.001; random effect: OR = 0.407, 95% CI: 0.211–0.785; P = 0.007) with strong results according to the sensitivity analyses (Table 2, Fig. 2). Funnel plot assessment did not reveal evidences of publication bias. In the funnel plot, the results of the small studies are shown to be more widely distributed than those of the large one (Fig. 3). Evaluation of heterogeneity was analyzed according to the P-value for heterogeneity indicating a significant heterogeneity (Q-value = 31.969, P = 0.001). Among controls, all studies were in HWE except one [16].

Stratification based on ethnicity demonstrated significant association between TNF-α gene polymorphisms and male infertility risk in Caucasian population (fixed effect: OR = 0.698, 95% CI: 0.526–0.928; P = 0.013; random effect: OR = 0.657, 95% CI: 0.360–1.199; P = 0.171) as well as in Asian population (fixed effect: OR = 0.260, 95% CI: 0.182–0.370; P = 0.001; random effect: OR = 0.194, 95% CI: 0.069–0.541; P = 0.002) (Table 3).

**Discussion**

Genetic factors may impact TNF-α levels where several polymorphisms in the TNF-α gene cluster have been associated with the modified TNF-α production [23]. Since the original identification of the TNF-α polymorphisms, several studies investigated the genetic effect of these polymorphisms on the susceptibility to different human diseases such as prostate cancer, Crohn’s disease, pre-eclampsia and colo-rectal cancer [24,25]. In view of that, the association between TNF-α gene polymorphisms and susceptibility to male infertility was also raised and reported in different populations.

In this meta-analysis, there was a significant association between TNF-α gene polymorphisms and male infertility risk.

Tronchon et al. [15] pointed out, in French population, an increased frequency of the −308 TNF-α A allele in oligozoospermic and asthenozoospermic men compared with normozoospermic men. In oligozoospermic men exhibiting TNF-α A allele, compared with those with G allele, an altered hormonal balance was detected with increased inhibin B hormone levels and subsequent reduced FSH plasma hormone levels, leading to an FSH/inhibin B ratio roughly half as high [15].

Zalata et al. [14] associated, in Egyptian population, that the TNF-α GG genotype was more frequent in fertile men than asthenozoospermic, asthenoteratozoospermic or oligoasthenoteratozoospermic men compared with TNF-α AA genotype. Existence of A allele was significantly greater among fertile patients than fertile controls. Men with the TNF-α AA genotype revealed a significant decrease in the sperm concentration, sperm motility, normal sperm morphology, acrosin activity, and seminal α-glucosidase and demonstrated a
significant increase in seminal plasma caspase-9 apoptotic factor compared with those with the TNF-α GG genotype [14].

In Asians, Li et al. [21] in their study on Chinese population, observed that asthenozoospermic and oligoasthenozoospermic men exhibited significant differences from infertile men with normal sperms in the frequency of GA/AA at position −308 in the promoter region of TNF-α gene. They demonstrated negative correlation between the GA + AA type of the TNF-α −308 allele and progressive sperm motility. They pointed that seminal plasma TNF-α level was significantly elevated in asthenozoospermic and oligoasthenozoospermic men compared with infertile men with normal sperms being significantly higher in the GA + AA than in the GG type of the TNF-α −308 allele.

Shukla et al. [17] observed, in Indian population, that the substitution levels from G to A in the TNF-α gene were significantly higher in the infertile subjects compared with the healthy fertile controls. Apoptosis and necrosis levels were also higher in oligozoospermic and asthenozoospermic infertile subjects being associated with increased levels of reactive oxygen species.

However, Kurz et al. [22] demonstrated, in Austrian population, that allele frequencies of TNF-α −308 C → T, −863 C → A polymorphisms were nonsignificantly different between non-normozoospermic and normozoospermic men and that the mutant alleles were not over-represented in oligoasthenoteratozoospermic or asthenozoospermic men. Also, in their study, Lazaros et al. [15] did not associate between TNFα −857C → T polymorphism and semen quality in the Greek population.

Overall, Grataroli et al. [26] noted that TNF-α had a key role in adapting apoptosis throughout binding with type 1 TNF-α receptor, activating a number of transduction pathways leading to the enrollment of adaptor proteins, through interactions between conserved death domains. These adaptor proteins activate caspase-8, that increases mitochondria permeability conversion for releasing cytochrome C, followed by configuration of a high-molecular weight complex (apoptotic protease activating factor-1, cytochrome C, and caspase-9) that activates caspase-3, followed by cell death [27]. In their study, Perdichizzi et al. [28] associated TNF-α with increased sperm DNA damage and phosphatidylserine externalization explaining reduced fertility. Further, the pro-apoptotic effect
### Table 1  Characteristics of the included studies on TNF-polymorphisms and male infertility risk.

| Study | Country | Ethnicity | TNF-alpha | Cases/controls | Genotyping | Genotype data (cases) | Genotype data (controls) | Hardy–Weinberg equilibrium |
|-------|---------|-----------|-----------|---------------|------------|-----------------------|--------------------------|-----------------------------|
| 1     | Kurz et al. [22] | Austria | Caucasians | −308 CT | 446/128 | CC, CT/TT | 324/446 (72.6%) | 123/446 (23.4%) | 91/128 (71.1%) | Yes |
| 2     | Tronchon et al. [15] | France | Caucasians | −308 GA | 581/103 | GG, GA/AA | 425/581 (66.7%) | 156/581 (33.3%) | 83/103 (80.6%) | Yes |
| 3     | Li et al. [21] | China | Asians | −308 GA | 187/62 | GG, GA/AA | 98/187 (52.4%) | 89/187 (47.6%) | 57/62 (91.9%) | Yes |
| 4     | Zalata et al. [14] | Egypt | Caucasians | −308 GA | 268/124 | GG, GA/AA | 178/268 (66.4%) | 90/268 (33.6%) | 104/124 (83.9%) | Yes |
| 5     | Shukla et al. [17] | India | Asians | −308 GA | 1040/260 | GG, GA/AA | 508/780 (65.1%) | 172/780 (34.9%) | 224/260 (86.2%) | Yes |

### Table 2  Meta-analysis results of the included studies on TNF-polymorphisms and male infertility risk.

| Study | TNF-alpha | Odds ratio | Lower limit | Upper limit | Z-value | P-value | Log odds ratio | SE | Weight (fixed) Relative weight | Weight (Random) Relative weight | Hedges’s g | SE |
|-------|-----------|------------|-------------|-------------|---------|---------|---------------|----|-----------------------------|-------------------------------|------------|----|
| 1     | Kurz et al. [22] | −308 CT | 1.080 | 0.699 | 1.669 | 0.346 | 0.729 | 0.077 | 0.222 | 25.98 | 21.42 | 0.042 | 0.122 |
| 2     | Tronchon et al. [15] | −308 GA | 0.656 | 0.390 | 1.106 | −1.582 | 0.114 | −0.421 | 0.266 | 18.08 | 20.57 | −0.232 | 0.147 |
| 3     | Li et al. [21] | −308 GA | 0.97 | 0.037 | 0.252 | −4.781 | 0.001 | −2.337 | 0.489 | 5.38 | 15.74 | −1.258 | 0.269 |
| 4     | Zalata et al. [14] | −308 GA | 0.380 | 0.221 | 0.654 | −3.499 | 0.001 | −0.967 | 0.276 | 16.80 | 20.37 | −0.532 | 0.152 |
| 5     | Shukla et al. [17] | −308 GA | 0.300 | 0.205 | 0.40 | −6.183 | 0.001 | −1.203 | 0.195 | 33.77 | 21.89 | −0.663 | 0.197 |
| Fixed effect | | 0.472 | 0.378 | 0.589 | −6.631 | 0.001 | −0.750 | 0.113 | | | −0.413 | 0.062 |
| Random effect | | 0.407 | 0.211 | 0.785 | −2.681 | 0.007 | −0.898 | 0.335 | | | −0.494 | 0.184 |
Fig. 2   Forest plot for the association between TNF-α polymorphisms and male infertility [group A (cases) and group B (controls)]. The involvement of each study to the meta-analysis (its weight) is denoted by the area of a box, the center of which represents the size of the estimated OR. The overall OR is shown in the middle of a diamond where the left and right extremes represent the corresponding CI.

Table 3   Subgroup meta-analysis results of the included studies on TNF-polymorphisms and male infertility risk according to ethnicity.

| Study name         | Odds ratio | Lower limit | Upper limit | Z-value | P-value | Log odds ratio | SE | Weight (fixed) relative weight | Weight (random) relative weight | Hedges’s g | SE |
|--------------------|------------|-------------|-------------|---------|---------|----------------|----|-----------------------------|--------------------------------|------------|----|
| **Caucasians**     |            |             |             |         |         |                |    |                            |                                |            |    |
| Kurz et al. [22]   | 1.080      | 0.699       | 1.669       | 0.346   | 0.729   | 0.077          | 0.222 | 42.70                       | 35.30                          | 0.042      | 0.122|
| Tronchon et al. [15]| 0.656      | 0.390       | 1.106       | -1.582  | 0.114   | -0.421         | 0.266 | 29.73                       | 32.66                          | -0.232     | 0.147|
| Zalata et al. [14] | 0.380      | 0.221       | 0.654       | -3.499  | 0.001   | -0.967         | 0.276 | 27.57                       | 32.04                          | -0.532     | 0.152|
| Fixed effect       | 0.698      | 0.526       | 0.928       | -2.474  | 0.013   | 0.013          | 0.276 | 27.57                       | 32.04                          | -0.198     | 0.080|
| Random effect      | 0.657      | 0.558       | 1.281       | -1.370  | 0.171   | 0.171          | 0.276 | 27.57                       | 32.04                          | -0.221     | 0.169|
| **Asians**         |            |             |             |         |         |                |    |                            |                                |            |    |
| Li et al. [21]     | 1.104      | -1.774      | 0.719       | -4.630  | 0.001   | -2.267         | 0.490 | 13.64                       | 41.08                          | -1.246     | 0.269|
| Shukla et al. [17] | 0.300      | 0.205       | 0.40        | -6.183  | 0.001   | -1.203         | 0.195 | 86.63                       | 58.92                          | -0.663     | 0.107|
| Fixed effect       | 0.260      | 0.182       | 0.370       | -4.830  | 0.001   | -0.743         | 0.195 | 86.63                       | 58.92                          | 1.00       | 0.287|
| Random effect      | 0.194      | 0.069       | 0.541       | -2.015  | 0.002   | -0.902         | 0.195 | 86.63                       | 58.92                          | 1.00       | 0.287|
of TNF-α is demonstrated to be mediated by reactive oxygen species manufacture causing peroxidative damage to sperm plasma membrane and sperm DNA fragmentation correlated with impaired sperm competence [29–33].

Points of limitation in this meta-analysis are the limited available studies and the dealing with only two ethnic races: Caucasians and Asians where other ethnic decent studies were absent e.g., Africans and African–Americans. Moreover, the meta-analysis was limited to available English publications and the possibility of unpublished reports was not yet identified. Also, several models of different alleles were not demonstrated in detail since some of the available papers did not declare these information and fine points clearly.

Conclusions

The current meta-analysis provides evidence of associations between TNF-α gene polymorphisms and male infertility risk. Further studies are warranted to validate associations of TNF-α polymorphisms and male infertility as $-363 \text{ C}/\text{A}$ and $-857 \text{ G}/\text{A}$.

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Conflict of Interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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