Glutathione S-transferase polymorphisms in varicocele patients: a meta-analysis

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ABSTRACT. The glutathione S-transferase (GST) family represents a major group of detoxification and antioxidant enzymes. Studies have shown that high oxidative stress levels are associated with varicocele. The objective of this study was to assess the relationship between GSTM1 and GSTT1 null polymorphisms and varicocele using a study group of 497 varicocele patients and 476 control subjects. A systematic literature search (for articles published up to September 2014) utilizing Google Scholar and PubMed was conducted. The chi-square-based Q test and I² index were used to evaluate data from retrieved studies. The possible publication bias was evaluated by Begg funnel plot and the Egger test. No statistically significant association was found between GSTM1 or GSTT1 null genotypes and varicocele in the overall data analysis. In a subgroup analysis, only the null GSTM1 genotype was observed at a significantly higher frequency in Caucasian varicocele patients. In the Chinese subgroup, no association...
was established between the \textit{GSTM1} and \textit{GSTT1} null genotypes and this condition. More attention should be drawn to oxidative stress-related pathological manifestations for Caucasian varicocele patients.

\textbf{Key words:} \textit{GSTM1}; \textit{GSTT1}; Meta-analysis; Polymorphism; Varicocele

\section*{INTRODUCTION}

Varicoceles (dilations of the pampiniform venous plexus) are found in approximately 15\% of the general adult male population, a figure that increases to 35\% for men presenting with primary infertility, and 81\% for those with secondary infertility (Gorelick and Goldstein, 1993). The exact pathophysiology of varicocele remains unknown, but the most widely accepted concept is that it leads to elevated testicular temperature, which has a deleterious effect on spermatogenesis (Goldstein and Eid, 1989). Several studies have also suggested that this condition is associated with increased oxidative stress (Saleh et al., 2003; Allamaneni et al., 2004; Mancini et al., 2004), and reactive oxygen species (ROS) are found at significantly increased levels in varicocele patients compared to control groups (Hendin et al., 1999).

In testis tissues, GSTA, GSTM, GSTT, and GSTP, which belong to the glutathione S-transferase gene family, act as important protective factors against oxidative stress (Strange et al., 2001). The homozygous deletion (null genotype) of the \textit{GSTM1} or \textit{GSTT1} gene results in the total absence of corresponding enzyme activity. Varicocele patients’ susceptibility to ROS may be due to one or more of these GST genetic polymorphisms. As GSTs are important for male reproduction and their malfunctioning is involved in impairment of spermatogenesis, deletion polymorphisms of \textit{GSTM1} or \textit{GSTT1} might be related to male infertility.

Only a small number of studies have focused on the association between GST genotypes and varicocele, and their results are contradictory. We thus carried out the current meta-analysis to assess the association between \textit{GSTM1} and \textit{GSTT1} gene polymorphisms and varicocele, in an attempt to evaluate possible pathophysiologies.

\section*{METHODS}

\textbf{Data collection, extraction, and study design}

We conducted a systematic literature search (for articles published up to September 2014) of Google Scholar and PubMed databases using the following keywords: "GST-M1", "GST-T1", and "varicocele". The primary reports retrieved were filtered using the following inclusion criteria: 1) studies must consist of a case-control study of \textit{GSTM1} or \textit{GSTT1} polymorphisms and varicocele; and 2) must provide detailed genotype data. Non-case-control studies, reviews, meta-analyses, and duplicate data were excluded. The following information was independently extracted from eligible studies by two investigators (xx and xx), and tabulated: first author and year of publication, country, ethnicity, frequency of each genotype in case and control groups, and genotyping method. All disagreements were resolved by consensus between authors. Subgroup analyses were performed based on ethnicity (Caucasian and Asian) according to the studies involved. A detailed data collection flow chart is shown in Figure 1, and the characteristics of each included study are given in Table 1.
Statistical methods

Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for each study under the null vs present genotype genetic model to explore associations between \textit{GSTM1} and \textit{GSTT1} and varicocele. Each eligible study was weighted by sample size. Heterogeneity among studies was evaluated by the chi-square-based \(Q\) test and \(I^2\) index. When no heterogeneity (\(I^2 < 50\%), P\ value >0.5\) was observed, the fixed-effects model (Mantel-Haenszel method; Mantel and Haenszel, 1959) was applied for OR estimation. Otherwise, the random-effects model was used. Begg’s funnel plot and the Egger test were used to evaluate possible publication bias. All analyses were performed using the Stata software (version 12; StataCorp, College Station, TX, USA).

RESULTS

Subject characteristics

Six eligible case-control studies incorporating 497 cases and 476 controls were included.
in our meta-analysis (Chenet al., 2002; Ichioka et al., 2009; Wu et al., 2009; Acar et al., 2012; Tanget al., 2012; Dehghani et al., 2014). The characteristics of these studies are shown in Table 1. For analysis of GSTM1 polymorphism, the case group comprised 434 patients and the control group 422 individuals; for GSTT1, 355 patients were included, along with 356 healthy controls. All polymorphisms were assessed by multiplex polymerase chain reaction genotyping. Five studies were conducted in Asian populations, while two involved Caucasian subjects. Data regarding null, denoted as GSTM1(-) and GSTT1(-) hereafter, and present genotypes, denoted as GSTM1(+) and GSTT1(+) hereafter, are shown in Table 2.

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<thead>
<tr>
<th>First author</th>
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<th>Controls</th>
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<th>Controls</th>
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Table 2. Genotype distributions in studies included in the meta-analysis.

Meta-analysis

Heterogeneity tests of the overall datasets returned I² values of 0% for both genes, with P values >0.05 (Table 3). Thus, no statistically significant heterogeneity was observed and we therefore used a fixed-effects model to assess associations within the data. In the overall analysis, no significant association was detected between genotype and varicocele for either GSTM1 (GSTM1(-) vs GSTM1(+); OR = 1.29, 95%CI = 0.98-1.70, P = 0.07) or GSTT1 (GSTT1(-) vs GSTT1(+); OR = 0.97, 95%CI = 0.71-1.33, P = 0.87; Figure 2A and B). Potential publication bias in the overall dataset was determined by generating Begg’s funnel plots and applying Egger’s linear regression test. The Begg’s funnel plot for both GSTM1 and GSTT1 was symmetric (Figure 3), and the Egger’s test P values were both greater than 0.05 (Table 3).

Figure 2. Results of the meta-analysis using the overall dataset. A. GSTM1 null vs GSTM1 present genotype. B. GSTT1 null vs GSTT1 present genotype. GST = glutathione S-transferase; OR = odds ratio; CI = confidence interval.
Heterogeneity tests of the subgroup datasets gave similar results to those above, with $I^2$ values of 0% and $P$ values >0.05 for both genes, indicating no statistically significant heterogeneity in either Asian or Caucasian subsets (Table 4). Therefore, the fixed-effects model was applied to estimate ORs. Forest plots of the two subgroup analyses are shown in Figure 4. We found that the $GSTM1(-)$ genotype was statistically strongly associated with varicocele in the Caucasian dataset (OR = 1.61, 95%CI = 1.02-2.50, $P$ = 0.03; Figure 4A), while in the Asian subgroup, no significant association was detected (OR = 1.13, 95%CI = 0.79-1.60, $P$ = 0.54; Figure 4B). However, analysis of the $GSTT1$ gene revealed no significant relationship in either the Caucasian (OR = 0.94, 95%CI = 0.58-1.54, $P$ = 0.81) or Asian datasets (OR = 1.00, 95%CI = 0.66-1.50, $P$ = 0.98; Figure 4C and D). Given the relatively small sample size involved, publication bias was not assessed for the subgroup analysis.

<table>
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<td>$GSTT1(-)$ vs $GSTT1(\cdot)$</td>
<td>Fixed</td>
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<td>0.97 0.71</td>
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Table 3. Results of the $GSTM1$ and $GSTT1$ meta-analysis.

OR = odds ratio. *$P$ value from the heterogeneity test. **$P$ value from the OR test.

Figure 3. Begg’s funnel plots relating to the association between $GSTM1$ and $GSTT1$ null polymorphisms and varicocele using the overall dataset. A. $GSTM1$ null vs $GSTM1$ present genotype. B. $GSTT1$ null vs $GSTT1$ present genotype. GST = glutathione S-transferase; SE = standard error; OR = odds ratio.

Figure 4. Forest plots from the subgroup analysis of A. $GSTM1$ in the Caucasian population, B. $GSTM1$ in the Asian population, C. $GSTT1$ in the Caucasian population, and D. $GSTT1$ in the Asian population. GST = glutathione S-transferase.
DISCUSSION

The glutathione S-transferases are a family of isoenzymes that play important roles in protection against oxidative stress. Under aerobic conditions, human spermatozoa generate ROS (Holland et al., 1982) as a normal physiological process (de Lamirande and Gagnon, 1993; Aitken and Fisher, 1994). However, in healthy men’s seminal plasma, excessive ROS are neutralized by antioxidants. When the balance between ROS production and antioxidant capacity is shifted, e.g. in pathological conditions where GST activity is reduced, surplus ROS might lead to sperm malfunction (Aitken and Clarkson, 1987; Alvarez et al., 1987; Gopalakrishnan and Shaha, 1998; Aydemir et al., 2007) and a high rate of DNA damage (Lopes et al., 1998).

Here, we presented an up-to-date meta-analysis including 497 cases and 476 controls, investigating the role of \textit{GSTM1} and \textit{GSTT1} null polymorphisms in varicocele patients, in an attempt to explore possible pathophysiology of this disease. Our results showed that in an overall population analysis, \textit{GSTM1} and \textit{GSTT1} null polymorphisms were not observed more frequently in varicocele patients than in control subjects. Similarly, Chen et al. (2002) also detected no difference between control and varicocele patient groups regarding the \textit{GSTM1} null genotype. Interestingly, only in the Caucasian subgroup analysis did we find a significantly higher frequency of the \textit{GSTM1} null genotype amongst varicocele patients, with an OR of 1.61 (P = 0.03). In contrast, no such association was discerned in the Asian subgroup. We failed to detect any statistically significant correlation concerning the \textit{GSTT1} null genotype in either Caucasian or Asian populations. As the sperm of varicocele patients with \textit{GSTM1} null genotypes are more vulnerable to oxidative damage (Chen et al., 2002), more attention should be paid to oxidative stress-related pathological manifestations for varicocele sufferers carrying such a null polymorphism. Although the impact of varicocele on male fertility remains unknown (Baazeem et al., 2011), reduced detoxification capacity during oxidative stress seems likely to be a contributory factor for those patients in Caucasian populations with a \textit{GSTM1} null genotype.

It should be noted that there are several limitations to this study. Firstly, only a small number of investigations have focused on the association between \textit{GSTM1} and \textit{GSTT1} null polymorphisms and varicocele. Although we included a comprehensive, up-to-date list of publications, it would be preferable to incorporate further data for a more extensive meta-analysis, particularly for ethnicity-based subgroup tests. In addition, other members of the GST family might compensate for the loss of \textit{GSTM1} and \textit{GSTT1} activity. Due to the limited eligible data, only null genotypes of \textit{GSTM1} and \textit{GSTT1} were assessed in the current meta-analysis. Future studies should include other genetic polymorphisms of varicocele patients.

In conclusion, we investigated \textit{GSTM1} and \textit{GSTT1} polymorphisms in varicocele patients...
and found that only amongst those from Caucasian populations was the GSTM1 null genotype observed at a significantly higher frequency. The performance of epidemiological studies is strongly recommended to validate the role of the GSTM1 gene in male infertility in Caucasian populations.

Conflicts of interest

The authors declare no conflict of interest.

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