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Methylene Tetrahydrofolate Reductase C677T Gene Polymorphism In Male Infertility

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Background: Methylenetetrahydrofolate reductase is an important enzyme which plays major role in folate metabolism and DNA methylation. The objective of this study was to analyse the MTHFR C677T polymorphism in infertile men.

Study Design: Blood samples were collected from 58 infertile men and 55 control subjects were taken which are fertile. C677T mutation analysis was done by Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) Method.

Results: The present study reveals that 23 Homozygous CC, 28 heterozygous CT, 7 TT were found in infertile men, whereas in control, the allele variations were 38 Homozygous CC, 17 heterozygous CT, but no Homozygous TT was found.

Conclusion: As a result, Genotypes and allele frequencies found in infertile patients and controls but mutant allele was found only in infertile men. Homozygous TT was observed only in azoospermia infertile men whereas heterozygous CT was found in both oligozoospermia and azoospermia subjects. The study has to be extended with large number of samples.

Keywords: Male infertility, MTHFR C677T, Folate, Homocysteine, Azoospermia

Introduction

Folic acid metabolism is significant for integrity and stability of the genome as it plays a major role in error free DNA synthesis, DNA methylation pattern and repair (1). Therefore, deficiency in folate intake or polymorphism(s) in the enzymes of folate pathway may result in aberrant DNA synthesis and methylation. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme of methionine and folate metabolism, crucial help for DNA synthesis and methylation. MTHFR reduces 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate which gives methyl group to homocysteine to form methionine. Methionine is ultimately converted to S-adenosyl methionine which acts as a 'methyl' donor for DNA methylation (2). Also, methylenetetra hydrofolate involved in DNA synthesis by changing uracil to thymine (3). In adult testis

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of a mouse than other organs the activity of MTHFR is high, representing its role in spermatogenesis (4,5). Recent research has identified epigenetic regulation of several genes playing important role in spermatogenesis and fertility (6). Therefore, alterations in the MTHFR gene could change the process of spermatogenesis and predispose carriers to infertility (7, 8).

The length of MTHFR gene in human is 20 kb (20,336 bp) and located at 1p36.3 (OMIM 607093), having 11 exons (9). There are more than 40 polymorphisms have been identified in MTHFR, but the most severe and clinically significant common variants are C677T in exon 4 and A1298C in exon 7 but most common are C677T of MTHFR gene has been identified to affect activity of this enzyme (10-15). C677T variant replaces alanine with valine (A222V, rs1801133) and it is the most common as well as severe form of MTHFR (16-21). This substitution results in reduced MTHFR specific activity and increased thermolability (22).

Homozygous T677T variant has, 30% of remaining activity and heterozygous C677T variant has 70% of residual activity when compared to C677C variant (23). This substitution also results in enhanced level of homocysteine and low plasma folate level. The frequency of C677T polymorphism differs with environmental and ethnic place (24). It has been recommended that low level of folate related to MTHFR polymorphism would be the reason for infertility due to alteration the synthesis of DNA and RNA molecules (4, 15).

Concentration of sperm can be increased based on folic acid and zinc sulfate supplementation, this additionally suggests the importance of this pathway in spermatogenesis (14). This is also supported by study in which stimulation of low methylation by 5-aza deoxy cytidine, which inhibits the differentiation of spermatozoa to spermatocytes in murine model (25). Hence analysis of C677T polymorphism is important in infertile men to elucidate the association between allele variants and male infertility.

Methodology

Genomic DNA was extracted from the peripheral blood of patients and control samples using phenol-chloroform-isoamyl method (26, 27). Isolated DNA was dissolved in 1x (working) TE buffer with the help of thermomixer. The quantification and quality check of DNA was performed by subjecting the DNA to spectrophotometry (Nanodrop method). The nucleic acid sample was analysed at 260 nm and 280 nm by using Nanodrop Spectrophotometer (Thermo Scientific, Germany).

Pure DNA is A260/A280 ≥1.8 to 2. Value of <1.8 indicates protein and phenol contamination. Value of >2 indicates the possibility of RNA contami-nation. Further, the integrity of the genomic DNA was assessed by running it on 0.8% agarose gel. PCR was carried out in a total reaction volume of 20 µl each in thin walled tubes consisting of DNA 2µl, ADW (autoclaved distilled water) 7.6µl, forward primer (59CATC CCTATTGGCAGGTTACCC39) 0.2µl, reverse (59 GGGAAGAACTCAGCGAAC TCAG39) primer (28,29) 0.2µl, mastermix 10µl. PCR cycling was carried out in Roch gradient PCR.PCR amplification conditions included denaturation at 95°C for ten minutes and then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds and polymerization at 72°C for 40 sec, and a final stage of polymerization at 72°C for 7 minutes.

After amplification, 0.5µl of Hinf I as a restriction enzyme added to 0.5µl of amplified product and kept for 4 hours. Immediately after 4 hours vial was transferred to water bath at 80°C and kept for 20minutes. Finally the

product was runned in 3% agarose gel electrophoresis. The gel image was viewed using UV transilluminater.

Results

23 Homozygous CC, 28 heterozygous CT, 7 TT were found in infertile men, whereas in control, the allele variations were 38 Homozygous CC, 17 heterozygous CT, but no Homozygous TT was found in control samples. Mutant allele variation of homozygous TT was found in 7 infertile samples which belong to azoospermia and oligozoospermia category and 28 heterozygous CT was found in which majority belongs to oligozoospermia as well as azoospermia subjects but low number allele frequencies were observed in other categories of infertile subjects such as asthenozoospermia, oligoasthenoteratozoospermia.

Figure-1. Gel electrophoresis showing RFLP. C1 and C2 are con-trols; MI1 to MI10 are Male infertile samples; M-50bp molecular marker.

These results were also supported by meta-analysis in the previously carried out studies. The observations of our study get interesting particularly from the two previous studies shown on Indian population that have reported contradictionary outcomes for what they have been reported in a meta-analysis study by Zhu and colleagues. Their results revealed that association between *MTHFR C677T* polymorphism and male infertility.

Additionally, MTHFR C677T was linked with a considerable high risk of azoospermia in each genetic models. Meanwhile, no significantly increased risks of oligoasthenotertozoospermia (OAT) were found in most of the genetic models (30). Another meta analysis study involving 26 studies on the association of MTHFR C677T polymorphism with male infertility has concluded that MTHFR C677T polymorphism has a strong association with increased risk of male infertility (31). Furthermore, a study involved 360 of non obstructive infertile men and reported the association of *MTHFR C677T* with azoospermia (32).

Conclusion

Genotypes and allele frequencies were found to be higher comparatively with the normal subjects. Since, *MTHFR* gene plays a major role in regulation of spermatogenesis, allele variants can disturb the process resulting in male infertility. However, a large number of samples, well-designed and high quality epidemiological studies will be required to confirm the association between *MTHFR C677T* polymorphism and male infertility.

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