http://pmjournal.ir Original Article
Summer 2019, Volume 4, Issue 14 (8-10)

MTHFR Gene Expression and Promoter Methylation in Oligozoospermia Infertile Men

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Check for updates

DOI: 10.21859/pmj01023

Submitted: 2019/04/13
Accepted:2019/05/27

Keywords:
MTHFR Gene
Promoter Methylation
Gene Expression
Oligozoospermia

Abstract

Methylenetetrahydrofolate reductase (MTHFR) has a critical role in spermatogenesis process and altered expression level of this gene may leads to male infertility. In the present study, the assocaitions of gene expression and promoter methylation of MTHFR with risk of oligozoospermia was assessed. Sperm DNA and RNA were extracted from 20 oligozoospermia men and 20 controls consisting of healthy fertile men. Following the modification of DNAs by sodium bisulfite treatment, the methylation status of the MTHFR gene promoter was quantified by methyl specific PCR. MTHFR expression evaluated by Quantitative PCR, or real-time PCR, (qPCR). Our data revealed a significant association of CpG island promoter methylation and MTHFR expression with risk of oligospermia (P = 0.031). The prevalence of methylation in promoter region of MTHFR can be a useful molecular biomarker to predict the predisposition of male infertility.

INTRODUCTION

Infertility is a major health problem for 1 to 2% of couples worldwide. About 50% of infertility cases are related to men. Environmental, genetic and epigenetic factors are involved in male infertility. About 1 to 5% of infertility cases are caused by genetic factors such as chromosomal or monogenic abnormalities, mitochondrial mutations, Y chromosome micro-deletions and autosomal chromosomes, defects in DNA repair mechanisms, Y chromosome-related syndromes, and some polymorphisms. In addition to genetic factors, epigenetic factors also affect male infertility [1]. DNA methylation is the most common epigenetic factor involved in infertility. DNA methylation occurs by the addition of a methyl group at the fifth position of the cytosine ring in the CpG dinucleotide. Sperm epigenetics is an emerging interest in reproductive medicine. Variations in the sperm epigenome by DNA methylation may contribute to male fertility [2]. Looking into DNA methylation of specific genes could therefore prove a valuable diagnostic marker in clinical andrology. One of the key enzymes involved in spermatogenesis processes is methylene tetrahydrofolate reductase (MTHFR) [3]. The MTHFR gene produces an enzyme involved in the processing of folate and regulation of homocysteine in the body. Folate is a critical nutrient involved in methylation, DNA synthesis, and amino acid metabolism [4]. Change in methylation patterns can affect several cellular processes including gene expression. The the MTHFR gene has been found to be overexpressed in the testicles than in other organs in the adult male mouse. Moreover, inactivation of the MTHFR gene, that occurs due to deficiency in folate, results in hyperhomocysteinemia and infertility in male mice. This condition supports the role of MTHFR in spermatogenesis [1]. The MTHFR gene is located on the long arm of chromosome 1 (1p36.3) and has 12 exons. The promoter of this gene exhibited two isoforms of CpG island, Island A contains 62% CG and Island B consisted of 75% CG [5]. Any changes in the MTHFR gene sequence can modify the spermatogenesis leading to the transmission of infertility to the carriers [6]. One of the factors involved in the expression of this gene is promoter methylation of this gene. In the present study, the expression level of MTHFR gene was assessed in men with oligoazoospermia and then evaluated the promoter methylation rate of this gene in comparison with healthy fertile individuals.

METHODS

The statistical population of this study included 20 men with oligoazoospermia and 20 healthy fertile individuals. Men, who had spouses having confirmed normal gynaecological assessment, have had an infertility history of at least 2 years. Sperm samples were collected according to the World Health Organization [7] guidelines. Semen samples were collected after 2–5

days of sexual abstinence, then subjected to somatic cell lysis for 20 min on ice and afterward washed with sperm wash buffer (SWB) to eliminate white blood cell contamination. Sperm DNA extraction was applied to isolate DNA from samples according to the manufacturer's protocol (BioBasic® One-4-All Genomic DNA Miniprep Kit) and for RNA isolation used for Trizol® reagent (Invitrogen).

To investigate the methylation status of the promoter, the DNA was first bisulfiteated by Kit protocol (EpiTect Bisulfite Conversion, Qiagen), during which the non-methylated cytosines were converted to thymine and the methylated cytosines remained unchanged. Then, the PRC reaction was performed with methylated and non-methylated primers (Table 1). Furthermore, the results were analyzed by agarose gel electrophoresis.

For gene expression evaluation, cDNA synthesis was first performed by Biofact cDNA synthesis kit (Biofact, Southern Korea), Real-time PCR was then carried out using SYBR Master Mix (Ampliqon, Denmark). Primers were used for amplification of MTHFR (forward primer 5'- GAGCGGCATGAGAGACTCC-3' reverse 5'-CCGGTCAAACCTTGAGATGAG-3') and b-actin (forward primer5'-AACAAGAGGCCACACAAATAGG-3' reverse 5'-CAGATGTACAGGAATAGCCTCCG-3') as reference gene.

Table 1: Methylation-Specific PCR Primer Sequence

Primer	Sequence
Methylated	F- TAGATTTAGGTACGTGAAGTAGGGTAGAC
	R- GAAAAACTAATAAAAAACCGACGAA
Non-methylated	F- TTTAGGTATGTGAAGTAGGGTAGATGT
	R- CAAAAAACTAATAAAAAACCAACAAA

RESULTS

The percentage of MTHFR promoter methylation in patients with oligoazoospermia infertility was significantly higher than that in the healthy control group (P = 0.021). Overall, 53% (11/20) of oligoazoospermia infertile men exhibited hypermethylation in their spermatozoa as compared to (5%; 2/20) the fertile controls. The frequency of hypermethylation in MTHFR promoter region was significantly higher in idiopathic infertile men than in fertile controls (P = 0.001).

The relative expression was found to be significantly different between oligoazoospermia and control fertile individual (P = 0.002).

Our findings showed a significant relationship between promoter methylation of MTHFR gene and decreased expression of mRNA (P=0.031). On the other hand, a significant relationship of promoter methylation with gene expression reduction and oligozoospermia was revealed (P = 0.001).

DISCUSSION

Male infertility is due to low sperm production, abnormal sperm function or blockages, leading to prevention of the sperm delivery [8]. Illnesses, injuries, chronic health problems, lifestyle choices and other factors can play a role in male infertility [9]. Current evidence suggests that male infertility maycontribute to epigenetic alterations. One of the most epigenetic alteration has been described to be change in methylation pattern. Houshdaran et al. (2007) suggested a broad epigenetic defect associated with abnormal semen parameters [10]. Their results indicated that sperm abnormalities may be associated with a broad epigenetic defect of DNA methylation in numerous sequences of multiple types. Hypermethylation of CpG islands is usually linked to gene silencing and epigenetic changes are potentially reversible [11]. Genetic variations in the folate-related enzyme genes have been revealed to play a significant role in male infertility. Our study aimed to evaluate promoter methylation pattern in MTHFR gene and its effect on expression. We observed hypermethylation of the promoter in 53% of oligozoospermia samples, as well as a decrease in the expression of this gene that was significantly associated with hypermethylation. Wu et al. (2010) focused on DNA methylation in poor quality human spermatozoa in the same region of the same gene. They reported a statistically significant elevation in the frequencies of MTHFR hypermethylation in idiopathic infertile men with oligozoospermia [12]. Since epigenetic changes are reversible, they can therefore be classified as drug targets. Comprehensive studies with larger sample size are required to investigate the effect of methylation inhibitors on such changes.

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