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Yoga Up-regulates MTHFR Expression Levels; a Pilot Study: Yoga for MTHFR Disease in Children

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ABSTRACT

Introduction: Methylenetetrahydrofolatereductase (MTHFR) enzyme, an important regulator of folate-homocysteine metabolism pathway is encoded by MTHFR gene. Prevalent supraphysiological oxidative stress (OS) in either children or their parents with simultaneous presence of MTHFR deficiency may act hand in hand to make the consequences more worsen. Taking into account the ubiquity of MTHFR polymorphism and the possible ramification in several disorders including causation of male infertility, we conducted a pilot study for observing the impact of 21 days of supervised yoga-based lifestyle intervention (YBLI) on MTHFR gene expression.The study showed over fivefold up-regulation of MTHFR gene expression with accompanying decline in seminal oxidative stress ($p<0.05$) at the end of 21 days ininfertile men. It was also observed that infertile men possessed statistically significant raised polymorphic variant of MTHFR C677T ($p=0.03$) and A1298C ($p<0.01$). A holistic approach-YBLI may be endorsed in daily routine lifestyle by parents as well as adolescents especially the ones who have documented MTHFR deficiency to interrupt the OS and uplift antioxidant capacity of an individual.

Keywords: MTHFR; Yoga; Oxidative stress(OS); MTHFR Polymorphism; Folate-homocystine metabolism.

Introduction

Methylenetetrahydrofolatereductase (MTHFR) enzyme, an important regulator of folate-homocysteine metabolism pathway is encoded by MTHFR gene whose cytogenetic location is 1p36.22[1]. The transcription product of this gene is a 77kDa protein, MTHFR which catalyses the conversion of 5, 10, methylenetetrahydrofolate (obtained from dietary folate) to 5-methyl tetrahydrofolate, which further remethylates homocysteine to methionine by acting as a methyl donor in biological systems[2]. The two most widely studied common polymorphisms in the MTHFR gene are C677T and A1298C. In the first one there is substitution of cytosine to thymine at nucleotide position 677 which leads to alanine replaced by valine and in the second there is substitution of adenine to cytosine at nucleotide position 1298 which causes replacement of glutamate to alanine; both leading to decline in enzyme activity[3]. Earlier studies have revealed associations of MTHFR polymorphisms with a variety of conditions including poor pregnancy outcomes (e.g. premature birth), disorders of embryonic development like cleft lip/ cleft palate, neural tube defects, congenital heart defects(CHD), neurodevelopmental disorders like autism spectrum diseases(ASD) and attention deficit hyperactivity disease(ADHD), acute leukaemia, cardiovascular system diseases, occlusive vascular disease in children[4–9]. Atypical haemolytic uremic syndrome(HUS) has also been reported to be associated with this polymorphism recently[5]. A pilot study in Indonesia by Gamayani U et. al. could not reveal direct independent association of MTHFR C677T polymorphism with cerebral palsy in children10,yet another study claimed this polymorphism andresultant hyperhomcysteinemia to have causal relationship11. It has been strenuously highlighted that MTHFR polymorphisms with concomitant hyperhomocysteinemia may a have causal relationship with the occurrence of cerebral stroke[12].

An offspring's universal health course is influenced in the critical periconceptional period well before the commencement of actual life[13]. Also, pre-conceptional germline integrity in both the parents has a potential role in determining the future course of offspring's health. Therefore the pre-conceptional as well as periconceptional period can be point of intervention to improve outcomes[14]. Fate of the reproductive outcomes and future offspring health is to a large extent dependant on the integrity of sperm DNA and RNA. Very mild to severe unfavourable ramifications of damaged sperm DNA or RNAis observed in offspring as these accumulating damages have adverse effect on the embryonic development. One of the principal contributor for seminal oxidative DNA damage and dysregulated sperm epigenome is from ongoing or preceding oxidative DNA damage in male germcells which can be attributed to de novo increase in seminal oxidative stress (OS)[15,16]. Physiological level of unequivocal amount of reactive oxygen species (ROS) is prerequisite for normal spermatogenesis as well as spermiogenesis, capacitation and ultimate fertilization. But undesired and elevated amount of ROS may endanger

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the entire process of spermatogenesis resulting in qualitative and quantitative decline of semen. De novogerm line mutation of sperm and universal sperm epigenome hypomethylation may bring about diseased progeny if they pass on to the subsequent generations[17]. Recent researches have focussed a lot to elucidate the role of yin yang or balanced dualism nature of OS in male germ cells. ROS damages DNA by bringing about nucleotide modification, chromatin cross linking and DNA strand breaks[18]. The most commonly used biomarker of oxidative DNA damage in sperm is 8-hydroxy 2'-deoxyguanosine (8-OHdG)[19]. Impairment of DNA methyltransferases (DNMTs) function occurs with continued build-up of 8-OHdG stores with elevated ROS. Dysfunctional DNMTs culminates in global hypomethylation promoting genomic instability and increasing vulnerability for epimutations. Eventually dysregulated transcripts are generated which is followed by faulty foetal and embryonic development[20]. Undesired pregnancy outcome is frequently observed in the form of rise in neonatal and infant mortality rates. Rise in incidence of autosomal dominant disorders are also increasingly reported[21]. There are studies linking oxidative damage in germline cell especially sperms with surge in the propensity of occurrences of multifactorial inheritance disorders like type-II diabetes, high blood pressure, coronary vasculardisease, neurodevelopmental disorders like autismand seizure disorder, neuropsychiatric disorders like bipolar affective disease, schizophrenia etc. either in the childhood or later in life.

Yoga is an ancient holistic practice of union of mind and body. The art and science of yoga comprises of a group of physical, mental, and spiritual practices that originated in ancient India.Yoga and meditation practices lessen free radical levels and up-regulate antioxidant capacity. It has proven benefits seen in up regulating the anti-oxidant genes and reducing OS. It has proven health benefits including rejuvenation and increased life expectancy. OS is implicated directly or indirectly in several pathological conditions in human body e.g., cancer. Regular YBLI increases antioxidant components and reduces OS biomarkers. It also enhances plasma serotonin levels and reduces plasma adrenaline levels to further benefit psychological stress. OS, telomerase activity and oxidative DNA damage which are markers of ageing are reversed by practicing regular yoga. Its beneficial effect is seen in cardiovascular diseases, cancers, arthropathies, ocular disorders and immune dysfunctions as well as psychological disorders, such as anxiety or depression. It also stabilizes baseline functions through mechanism involving both afferent and efferent vagal activity. Domar et. al. reported that 55% infertile patients could achieve pregnancy within one year of practicing yoga and meditation. Overall effect of regular yoga is that it enhances the antioxidant defence status and also upregulates expression levels of various genes which maintain DNA integrity. So,OS and oxidative DNA damage are reduced which may reduce the incidence of male infertility.

Methods

This pilot study was undertaken for observing the impact of 21 days of supervised yoga-based lifestyle intervention (YBLI) on MTHFR gene expression in infertile men. Fifty (50) infertile male patients visiting the andrology clinic, department of urology at All India Institute of Medical Sciences, New Delhi were enrolled for the study (Fig 1). Subjects were recruited after obtaining informed consent and ethical clearance (Ref

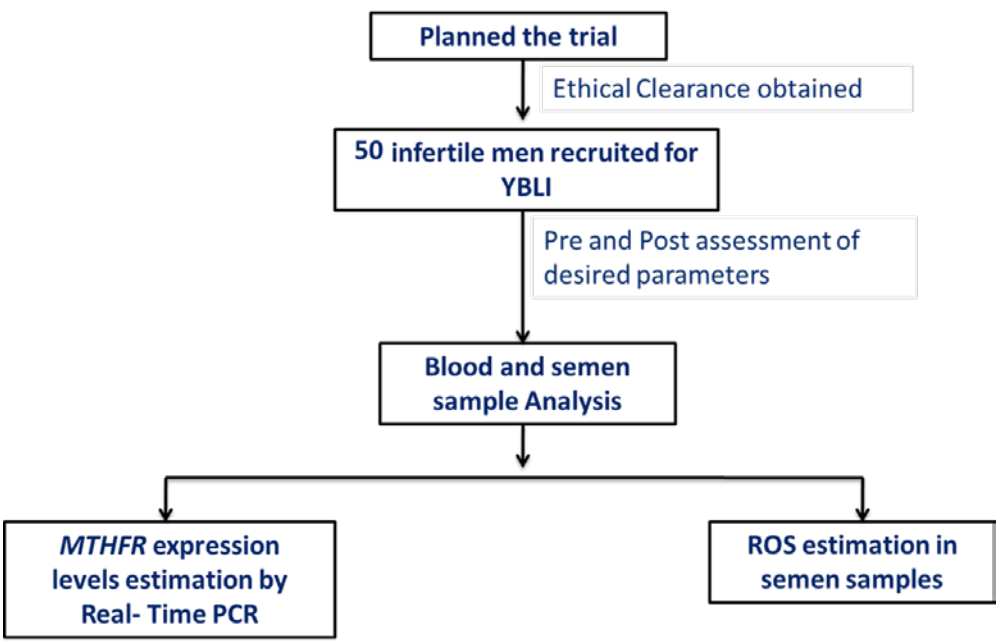


Figure 1. Methodology for the study.

no. IEC PG-352/18.07.2018, RT-37/30.08.2018 dated 5th September, 2018). Only primary male infertility cases were included for the study. The demographic profile and comprehensive clinical assessment were recorded on a structured proforma. Blood samples of the participants were taken at day 0 of yoga intervention (baseline) followed by undergoing a structured and pre-tested yoga program. The supervised yoga-based intervention included gentle physical movements and postures (asanas), regulated breath-ing techniques (pranayamas), meditation (dhayana), and lectures on yoga philosophy. The YBLI intervention was a regular outpatient-based program, practiced for two hours each day for the 21 days. The participants attended the Yoga based intervention program at the Department of Anatomy, AIIMS, New Delhi. The detail of a typical session is provided in table 1. Pre and post (Day 0 and Day 21) intervention MTHFR expression was measured by real time PCR employing commercially designed gene specific primers after reverse transcribing total RNA obtained from peripheral blood. Validation of the selected genes was done with the help of real-time PCR using gene specific primers whose sequences are provided in table 2. Bio-Rad CFX96 real-time system (Bio-Rad, California, USA) was used for quantitative analysis and relative quantification was done by $2^{-\Delta\Delta Ct}$ method after normalization of expressed mRNA using 2 internal housekeeping genes β-ACTIN and GAPDH.

Table 2. Primers used and their annealing temperature for qPCR amplification.

Genes	Primer sequence	Product length
MTHFR	F- CCATCGAAAAGCCAGGGTCCTCR-TGGGTTCCTACTGATGGAAGGTG	115 bp
β- ACTIN	F- CACTCTTCCAGCCTTCCTTCCTG R- CGATCCACACGGAGTACTTGCG	246 bp
GAPDH	F- CTTCAACAGCGACACCCACTCCTC R- CACCCTGTTGCTGTAGCCAAATTC	118 bp

Results

The mean age of the participants wasmean age 32.74 + 4.63 years. The study showed over fourfold up-regulation (4.21 axis fold change) of MTHFR gene expression with accompanying decline in seminal oxidative stress ($p<0.05$) at the end of 21 days (Table 3, Fig 2, Table 4, Fig 3).

Table 1. Elements of a typical supervised YBLI session.

Yoga practice acts			Duration
Preparation for the session			5 minutes
Initial prayer with practice of loosening exercises (yogic SukshmaVyayama)	Finger loosening, Wrist loosening, Elbow loosening, Shoulder loosening, Toe bending, Ankle bending, Knee cap tightening, patella movement, Knee bending & Hip rotation		10 minutes
Salutation to the Sun (Surya Namaskar)			5 minutes
Specific yogasana	Standing posture	Trikonasana Katichakrasana Tadasana Veerabhadrasana	45 minutes
	Sitting posture	Gomukhasana Paschim-utaanasana Shashaankasana Vakrasana	
	Prone posture	Ek-pada-shalabhasana Bhujangasana Poorna- shalabhasana	
	Supine posture	Uttanapadasana Setubhandhasana Matsyasana	
Pranayama	Breathing exercises involving-Nadishodhana, Bhramari, KapalBhati&Brahmamudra		20 minutes
Nada anusandhana (AUM- Aumkar recitation)			5 minutes
Dhyana (meditation)			10 minutes
Shanti mantra- the closing prayer			5 minutes
Lectures on yoga			20 minutes
Total duration			120 minutes

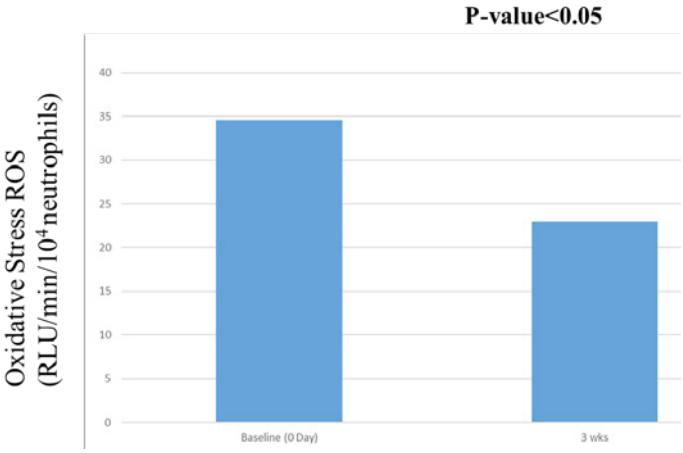


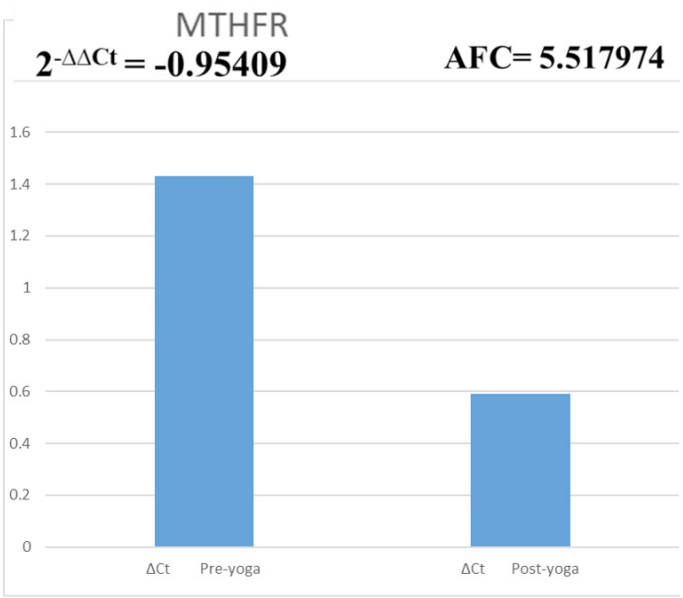
Figure 2. Change in Reactive oxygen species (ROS) in infertile men participating in YBLI (n=50). NOTE: Values of the Control Group not shown.

Table 3. Change in Reactive oxygen species (ROS) in infertile men participating in YBLI (n=50). NOTE: Values of the Control Group not shown.

Characteristics	Baseline	3 wks	p Value
Oxidative Stress ROS (RLU/min/104 neutrophils)	34.54 (4.94 to 167.19)	23.01 (0.87 to 119.51)	<0.05

Table 4. Relative expression (average ΔCt) of MTHFR with respect to β-ACTIN and GAPDH in blood samples of infertile men and AFC in the gene expression post-yoga with respect to pre-yoga. Values are expressed as mean (n=50). GOI, genes of interest; AFC, axisfold change.

GOI	ΔCt Pre-yoga	ΔCt Post-yoga	$2^{-\Delta\Delta Ct}$	AFC
MTHFR	1.432561	0.591345	-0.798219	4.213865



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Discussion

Supraphysiological levels of ROS can cause more oxidative damage to sperm due to limited antioxidant reserve of spermatozoa, inadequacy of base excision repair (BER) mechanisms etc. Therefore spermatozoadepends substantially on the oocyte cellular apparatus for oxidativedamage refurbishment[22]. But remedial measures undertaken in fertilized zygote is dependent on the extent of oxidative damage it has to tackle and also on oocyte age. Higher prevalence of childhood cancers, other paediatric disorders with linked or suspicious epigenetic component e.g., allergies, asthma, childhood obesity, childhood leukaemia etc. are believed to be encountered due to insufficient antioxidant capacity of spermatozoa. Similarly, fathers experiencing chronic oxidative stress have higher chance of procreating progeny with congenital anomaly. It is further augmented by increased paternal age, non-healthy lifestyle habitslike smoking, alcohol consumption, long term exposure to pollutants, chemical toxins, heavy metals etc [23,24]. Fathers of children with sporadic non-familial retinoblastoma exhibited high amount of 8-OHdG compared to fathers of normal children. Even children with diagnosed retinoblastoma were found to have higher 8-OHdG levels suggesting increased preponderance for somatic mutations of RB1 gene resulting in retinoblastoma[23].

Origin of yoga practice can be traced back thousands of years ago in India as a divine philosophical speciality area where yoga practitioners aimed at healing people from diseases or their sufferings. The standard yoga practice includes synchronized breathing exercises (pranayama), postural movement (asana), and meditation (dhyana) which foster overall wellness, lessen stress by unifying mind and body[25]. The recent decade has witnessed an overwhelming response in yoga application in clinical research to explore its numerous health benefits and the molecular mechanisms bringing about its positive advantageous effects, with a parallel increase of yoga practice among general public. Yoga revamps general as well as nervous system wellbeing through optimization of neurotransmitters, evolving neural synapses, and by harmonising circadian rhythm[26]. There is proven reduction of disease activity score in rheumatoid arthritis (RA)patients which is believed to be due to optimization of inflammatory mediators and immune modulators. Outstanding positive boosting effect on telomerase activity, decrease of ageing biomarkers and enhancement of neuroplasticity were observed in RA patient adopting yoga based lifestyle[27]. A significant decrease in OS and comprehensive amelioration of sperm DNA integrity was noted in fathers of retinoblastoma children who had undergone regular yoga practice for 6 months in previous studies from our laboratory[28,29]. In healththere is an equilibriumobserved

between oxidative stress and body's antioxidant ability. Disruption of this balanced state is considered a contributory factor for deviation from healthy state like ageing, inflammatory conditions, malignancy and numerous other chronic disorders[30]. There is proven fall of harmful oxidizing factors and uplift of one's anti-oxidant defence mechanism with regular practice of yoga. It is reflected in the form of gradual decline in oxidative damage biomarkers, e.g., ROS, 8-OHdG, DNA fragmentation index (DFI) etc. Yoga practice leads to all-inclusive boosting of antioxidant defence level along with upregulation of expression of genes responsible for maintaining DNA integrity thereby minimising oxidative stress induced DNA damage. These beneficial effects of yoga based lifestyle has been documented in erstwhile research from our laboratory[31,27,32]. It was also revealed more recently that mitochondrial integrity overhaul with increment of fertility prospects after embracing yoga based lifestyle in RA patients[33]. There is a consistent and increasing body of evidence stating that yoga and meditation-based lifestyle may enhance sperm health by mitigating seminal oxidative stress, oxidative DNA damage and by promoting sperm DNA integrity through decrease in OS induced damages and increasing the efficiency of DNA repair mechanisms. Overall health benefits is reflected in succeeding generations of offspring's as healthy genetic material is carried forward through a genetically healthy sperm with a reduced burden of genetic-epigenetic disorders. Irrefutable corroborations gathered from cotemporary research have been able to provide proof of efficacy yoga and meditation-based lifestyle in boosting genetic health of sperm genome and epigenome.

Conclusions

We are reporting here the first ever evidence of upregulation of MTHFR expression after practicing YBLI for a brief period of only 21 days with an accompanying significant reduction in seminal oxidative stress. MTHFR polymorphisms notably the widely studied C677T and A1298C are independently associated with a vast array of disorders in paediatric population. Prevalent supraphysiological OS in either children or their parents with simultaneous presence of MTHFR deficiency may act hand in hand to make the consequences more worsen. Embracing YBLImay ameliorate the oxidative nucleotide damage due to elevated OS; and also increase the efficiency of MTHFR enzyme to handle this undesired OS leading to epimutations of the sperm genome.Even though MTHFR level is not routinely ordered by clinicians, its polymorphism does have remarkable ramifications in a number of paediatric disorders. A holistic approach-YBLI evolved from a 3000 years old tradition and which is a simple intervention may be endorsed in daily routine lifestyle by parents as well as adolescents especially the ones who have documented MTHFR deficiency to interrupt the OS and uplift antioxidant capacity of an individual. However, it is emphasised that more concrete and large body of evidences in the form of well-designed randomized control trial in the specific target population is still required to recommend YBLI as a therapeutic modality to counter MTHFR deficiency.

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Mini Curriculum and Author's Contribution

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3. Rima Dada -MBBS, MD, PhD; Conceived the study, guided every stage of the protocol development, reviewed the final draft of the manuscript; final approval of manuscript.

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