Original Research Article

Study of methionine synthase (MTR) gene polymorphisms in personnel exposed to trace quantities of anesthetic gases in operation theatres

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Abstract

Background: Nitrous oxide irreversibly oxidizes the cobalt atom of vitamin B12, inactivating it which is a co-factor for methionine synthase. Methionine is an essential aminoacid that serves as a methyl donor (via its activated from s-adenosyl methionine) in hundreds of biological reactions.

Aim: The aim of the study was to evaluate Methionine Synthase (MTR) gene polymorphism in persons who are exposed to trace quantities of anaesthetic gases in operation theatres.

Materials and methods: 87 cases of physical status American society of anaesthesiologists (ASA) I and II exposed to anaesthetic gases in operation theaters at Gandhi Hospital and Osmania General Hospital, Hyderabad were selected for study. Also 150 controls who were not exposed to anesthetic gases were selected at random to compare with the data generated on the subjects exposed to these gases.

Results: This study entitled study of methionine "synthase (MTR) gene polymorphisms in personnel exposed to trace quantities of anaesthetic gases in operation theatres" was conducted on 87 exposed and 150 non exposed subjects. The objective was to evaluate the effect of Nitrous Oxide on Methionine Synthase (MTR) gene polymorphisms. No statistically significant difference was observed in the distribution of genotypes between the two groups.

Conclusion: The result of the study suggest that large studies would be required to provide statistical power and recommends that examination should be done on large populations to assure at better conclusions.

Key words

Methionine synthase (MTR), Gene polymorphisms, Anesthetic gases, Operation theatres.

Introduction

Nitrous oxide irreversibly oxidizes the cobalt atom of vitamin B12, inactivating it which is a co-factor for methionine synthase. Methionine is an essential aminoacid that serves as a methyl donor (via its activated from s-adenosyl methionine) in hundreds of biological reactions. The end product of methylation [1] is catalyzed by the vit B12 dependent enzyme methionine synthase. The present study is to evaluate Methionine Synthase (MTR) gene polymorphism in persons who are exposed to trace quantities of anesthetic gases in operation theatres.

Materials and methods

87 cases of physical status American society of anaesthesiologists (ASA) I and II exposed to anaesthetic gases in operation theaters at Gandhi Hospital and Osmania General Hospital, Hyderabad were selected for study. Also 150 controls who were not exposed to anesthetic gases were selected at random to compare with the data generated on the subjects exposed to these gases.

From all the cases and controls detailed information pertaining to various epidemiological parameters such as sex, age, weight, height, BMI, duration of OT exposure was collected using a specific proforma. 5 ml of blood was collected and DNA from all the patients and controls was isolated from leukocytes using rapid non-enzymatic method (Lahiri and Nurnberger 1991). Genotyping for the methionine synthase C.2756-G(D919G) mutation was done as follows. PCR amplification was carried out using an exonic forward primer (5′-TGT TCC CAG CTG TTA GAT GAA AAT C-3′) and an intronic reverse primer (5′-GAT CCA AAG CCT TTT ACA CTC CTC-3′) to amplify a 211-bp fragment spanning the polymorphism. The polymorphism introduces an Hae III site in the rarer G allele, resulting in fragments of 80 and 131 bp. PCR products were digested with Hae III for 2 hour, followed by electrophoresis on 3% agarose gels and visualisation by ethidium bromide staining. Methionine synthase genotypes were obtained.

Results

The demographic data of the subjects exposed to anesthesia gases in operation theatres and controls was as per **Table - 1**. The frequency of males was (34.5%) and females was (65.5%) in the exposed group while in nonexposed group the frequencies were 60% among males and 40% females. About 58.6% of the subjects had 1-10 years, 28.7% had 11-20 years, 11.5% had 21-30 years while only 1.2% had longer exposure of 31-40 years to nitrous oxide. The mean BMI recorded for the subjects exposed to anaesthesia gases and controls was 24.0± 0.25 and 24.14±0.39 respectively. Genotype frequencies of c.2756A>G polymorphism of MTR gene was as per **Table – 2**.

Table – 1: Baseline characteristics of cases (exposed) and controls (non-exposed) studied.

	CASES (exposed)	**CONTROL (non-exposed)**
Total	87	150
M	30 (34.5%)	90 (60.0%)
F	57 (65.5%)	60 (40.0%)
BMI	24.0± 0.25	24.14± 0.39
Age range	23-64 yrs	19-58 yrs
Duration of exposure to nitrous oxide (in yrs)		
1-10	51(58.6%)	--
11-20	25 (28.7%)	--
21-30	10 (11.5%)	--
31-40	1(1.2%)	--
BMI	24.0± 0.25	24.14± 0.39
Age range	23-64 yrs	19-58 yrs

The c.2756A>G polymorphism of *MTR* gene was genotyped using HaeIII enzyme. The allele frequencies were consistent with HWE in

subjects exposed to anaesthesia gases but not in controls **(**AN=0.13; CT=0.059; **Table-3).** The deviation from HWE may be attributed due to genetic drift, migration, selection, etc.

The distribution of different genotypes between the two groups (exposed and non-exposed) revealed high frequency of AA genotype (51%) in the exposed group as compared to control group (44%) while that of heterozygotes was more in the control group as compared to exposed group (CT-51% and AN-46%). However, no statistically significant difference was observed in the distribution of genotypes between the two groups in general as well as when both the sexes were compared (**Table – 4, 5**). The results were that no statistically significant difference was observed in the distribution of genotypes between the two groups in general as well as when both the sexes were compared.

Table – 2: Genotype frequencies of c.2756A>G polymorphism of MTR gene.

	CASES		**CONTROLS**	
Genotype	**N**	**Frequency**	**N**	**Frequency**
A/A	39	51%	55	44%
A/G	35	46%	64	51%
G/G	2	3%	7	6%

Table – 3: Allele frequencies of c.2756A>G polymorphism of MTR gene.

	CASES		**CONTROLS**	
Allele	**N**	**Frequency**	**N**	**Frequency**
A	113	74%	174	69%
G	39	26%	78	31%

HWE for cases: p-value-0.13; Controls-0.059

Table – 4: Risk of c.2756A>G genotypes under different models.

Model	**Genotype**	**Cases**	**Controls**	**OR (95% CI)**	**P-value**
Codominant	A/A	39 (51.3%)	55 (43.6%)	1.00	0.57
	A/G	35 (46%)	64 (50.8%)	1.17 (0.64-2.12)	
	G/G	2 (2.6%)	7 (5.6%)	2.26 (0.43-11.86)	
Dominant	A/A	39 (51.3%)	55 (43.6%)	1.00	0.5
	A/G-G/G	37 (48.7%)	71 (56.4%)	1.22 (0.68-2.21)	
Recessive	A/A-A/G	74 (97.4%)	119 (94.4%)	1.00	0.35
	G/G	2 (2.6%)	7 (5.6%)	2.09 (0.41-10.69)	
Overdominant	A/A-G/G	41 (54%)	62 (49.2%)	1.00	0.76
	A/G	35 (46%)	64 (50.8%)	1.09 (0.61-1.97)	
Log-additive	---	---	---	1.27 (0.76-2.12)	0.36

Discussion

For operating personnel exposed to high concentrations of nitrous oxide (i.e., in unscavenged operating rooms), there may be an increased incidence of abortions and birth defects [2, 3]. Such findings are particularly applicable to dental operatories [4, 5]. Two occupational studies have examined the capacity of nitrous oxide to produce abortions and teratogenic changes. In one it was found that a small subset

exposed to the highest levels of nitrous oxide had a delay in the time to conception [6]. In another, it was concluded that frequent, high occupational exposure to nitrous oxide may have a negative influence on the ability of women to become pregnant [7]. These untoward findings may be explained by the discovery that nitrous oxide can inactivate methionine synthase. Deacon R. Lamb M, Perry J. Chanarin I, Minty B. Halsey MJ, Nunn JF [8] studied the effect of nitrous oxide on methionine synthase and deoxyuridine utilization in rats. Exposure of rats to a 50% N2O/oxygen mixture led to a rapid loss of methionine synthase activity in both liver and brain.

Table – 5: Sex wise Genotypic distribution of c.2756A>G polymorphism among cases and controls.

F	Cases		Controls	OR (95% CI)	P.VALUE
	A/A	27 (52.9%)	28 (51.8%)	1	
	A/G	23 (45.09%)	23(42.5%)	0.96 (0.44-2.11)	0.93 0.68
	G/G	1 (1.90%)	3(5%)	2.89 (0.28-29.56)	
	A/A	12(48%)	27(37.5%)	1	
	A/G	12(48%)	41(56.9%)	1.52 (0.60-3.87)	0.38
	G/G	1(4%)	4(5%)	1.78 (0.18-17.63)	1

There was impaired conversion of deoxyuridine to deoxythymidine by bone marrow cells and this defect followed loss of methionine synthase activity. There was no homocystinuria. Withdrawal of N2O was followed by a relatively slow recovery of methionine synthase activity over four days. The inactivation of vitamin B12 by N2O promises to be a valuable tool in the study of vitamin B12 metabolism. Koblin DID, Watson JE, Deady JE, Stokstad ELK, Eger E, II [9] measured enzyme inactivation as a function of nitrous oxide concentration and exposure time. Mice exposed to 0.8 atm nitrous oxide exhibited more than a 50 per cent decrease in liver methionine synthetase activity within 30 min, and activity dropped to 5-25 per cent of the original value after a 4-hour exposure. Although 4-hour exposures to low nitrous oxide partial pressures (less than 0.05 atm) did not significantly alter methionine synthetase activity, higher concentrations of nitrous oxide caused a progressive inhibition over this time period. Continuous exposure to trace levels of nitrous oxide (approximately 1100 ppm) for eight to 22 days produced a small but significant reduction in liver and brain methionine synthetase activity. Methionine synthetase activity returned to control levels two to four days following inactivation. Other anesthetics (xenon, halothane, isoflurane, enflurane) did not produce inactivation. Koblin DID, Waskell L, Watson JE, Stokstad ELK, Eger El, II [10] studied the activity of methionine synthetase was measured in liver biopsies .Methionine synthetase activity (+/- SE) averaged 219 +/- 28 nmol of methionine per hour per gran of liver in patients given nitrous oxide, and 414 +/- 29 in control patients. Inactivation of methionine synthetase progressively increased as the product of the concentration of nitrous oxide and the exposure time increased and concluded that inactivation of methionine synthetase may play a role in the development of the pathologic effects seen in patients and medical personnel after exposure to nitrous oxide. Royston BID, Nunn JF, Weinbren HK, Royston D, Cormack RS [11] studied the

rate of inactivation of hepatic methionine synthase by nitrous oxide in 22 patients undergoing laparotomy during general anesthesia, including 70% nitrous oxide. Mean half-time of inactivation was 46 min. Metabolic consequences of nitrous oxide are, thus, critically dependent on the duration of anesthesia, and are unlikely to be significant during exposures of less than 40 min.

Conclusion

The result of the study suggest that large studies would be required to provide statistical power and recommends that examination should be done on large populations to assure at better conclusions.

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