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CLINICAL STUDY

Homocysteine levels and bad obstetric outcome among female operating room personnel occupationally exposed to nitrous oxide

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Abstract: It is known that nitrous oxide (N_2O) inactivates vitamin B_{12} and causes hyperhomocysteinemia. The personnel working at the operating theatres are repeatedly exposed to N_2O in the ambient air. This prompted us to investigate the biochemical indices of vitamin B_{12} metabolic status among female personnel working under various levels of N_2O exposure. In this study, the homocysteine and folic acid levels were assessed and bad obstetric outcome was questioned. Sixty operating theatre female personnel were examined. Vitamin B_{12} and folic acid, total homocysteine level, anticardiolipin IgM, IgG, antiphospholipid IgM, IgG levels were measured in serum. A questionnaire inquiring about obstetric history was given. The serum concentration of folic acid was 10 ± 3.3 nmol liter^{-1}. The vitamin B_{12} level was 332 ± 134 pmol liter^{-1}, the serum concentration of homocysteine was 9.1 ± 2.4 nmol liter^{-1} and all were within normal ranges. There was no difference regarding homocysteine, folic acid, vitamin B_{12} levels and the obstetric history between the subjects who had abortus history and the subjects who had not abortus history. Exposure to N_2O in healthcare workers was not associated with alterations of homocysteine, folic acid status and bad obstetric outcome *(Tab. 4, Ref. 18)*. Text in PDF *www.elis.sk.*
Key words: operating rooms, drug contamination, nitrous oxide, pregnancy outcome.

Although several efforts have been made to minimize the exposure to waste anaesthetic gases by improving the working environment, contamination of operating theatre air is still unavoidable, whenever anaesthetic agents were given by inhalation (1).

Several independent groups suggested that there is a health risk associated with chronic occupational exposure to waste anaesthetic gases. These studies mainly focused on spontaneous foetal abortions leading to the assumption that genetic damage of foetus occurs during pregnancy (2–5). Nitrous oxide is an inhalation anaesthetic and analgesic, which is widely used in operating theatres. Several epidemiological studies have shown associations between occupational exposure to this agent and adverse pregnancy outcomes (6). Nitrous oxide acts by oxidizing vitamine B_{12} from the active cobalamin (cobI) to the inactive cobalamine (cobIII) (7).

Healthcare workers who are active in operating theatres are repeatedly exposed to N_2O in the ambient air (8). Although deleterious effects of N_2O were reported in patients undergoing anaesthesia, little effort has been directed towards evaluation of the influence of N_2O on vitamin B_{12} metabolism in operating personnel.

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Interest in the prognostic value of circulating homocysteine concentration continues to grow. There is already a large body of evidence indicating that elevated plasma total homocysteine (tHyc) (Hyperhomocysteinemia) may be causally related to risk of coronary, cerebral, peripheral arterial diseases, pregnancy disorders and recurrent pregnancy loss (7, 9–13). Preeclampsia, prematurity, very low birth weight, stillbirths, neural tube defects, and clubfoot were significantly associated with high tHyc levels (11–14).

Homocysteine is a thiol containing amino acid, which is not used in protein synthesis but which is, instead, involved at junction of two major metabolic pathways in human physiology. First, it can be condensed with the amino acid serine in an irreversible sulphuration reaction catalysed by enzyme cystathionine beta synthase in the presence of pyridoxine (vitamin B_6) to form cystathionine, which is then reduced to form cysteine, which can be excreted. Alternatively, homocysteine can be reversibly methylated to reform the essential amino acid methionine, taking methyl groups from the folic acid pathway in a reaction catalyzed by methionine synthase in the presence of cobalamine (vitamine B_{12}), thus allowing the further methylation processes vital to ongoing cellular activity on living organism. It will be seen that, in the absence of or with reduced concentrations of vitamin B_6, of vitamin B_{12} and of folic acid, homocysteine concentrations will rise. Similarly, if the enzymes controlling the metabolism of homocysteine are inactivated or made less efficient, once again homocysteine concentration will increase (7–10).

In this study, we aimed to investigate the correlation between high tHyc levels and bad pregnancy outcome among the female

operating theatre personnel actively working in the operating theatre.

Methods

After the institutional ethics committee approval and informed consent from each subject, our study was planned to take place in 60 nurses working in 25 different operating theatres for general anaesthesia and recovery rooms. The operating theatres, in which we conducted the study, are the main operating theatres rooms for extended operations. Isoflurane, sevoflurane, desflurane and nitrous oxide were used for anaesthesia. On an average, N_2O was used for 10 hours in a day for anaesthesia management. There was an air-change system without air intake in the operating theatres and recovery rooms. Anaesthesiology equipment was a closed breathing systems, and waste anaesthetic gas systems were passive scavenging systems.

In total, 60 female personnel of anaesthesia or surgery working in operating theatres or recovery area were included into the study. Our inclusion criteria were that the personnel worked a minimum of 6 hours daily for at least 3 years in the operating theatre or recovery room. All were asymptomatic with an unremarkable medical history and a normal physical examination. Blood samples were collected at the end of the daily shift. The serum was send to the

Table 1: Follow up Form

Name surname: **Age:**		**Medical record no:**	
Gravida:	Parity:	IUeX (after 20 week):_____	Abortus
D/C:	Living child:	PPex:_____	First 10 week ___ 10–20 week____ Not known:_____

Past medical and genetic history

Deep venous thrombosis (DVT) ☐ Pulmonary thromboembolism ☐
Cardiological disease ☐ Surgery ☐
DVT history in family ☐ Diabetus mellitus
Previeous pregnancy PIH (pregnancy induced hypertension)/preeclampsia ☐
Myocardial Infarction (MI) history before 40 years ☐
Preview pregnancy gestational diabetes mellitus (DM) ☐ Hypertension ☐
Pulmonary thromboemboli history ☐ Type I DM ☐ Type II DM ☐
Bronchial Asthma ☐ Goitre ☐ Allergy☐ Autoimmune disease ☐
Smoking ☐ Thromboembolic events ☐ Alcohol ☐ Child with a anomalia ☐Previous pregnancy preterm labour ☐

Patient's drugs:_____Other:_____

General questions

Status in operating room: doctor nurse
Part: anesthesia other
Working hours:
How many years have you been working in the operating room?
How many years have you been married?
How many years have you desired to become pregnant?
Mensturation profile
Partner: Sperm analysis Normal Abnormal
Known disease:

Pregnancy complication

Preterm labour ☐ Oligohydramnios ☐ Polihydramnios ☐
Postterm pregnancy ☐ Rh-Rh isoimmunisation ☐
Early pregnancy loss Ablatio placenta ☐
First 10 week ☐ Still birth ☐
10-20 week ☐ Gestational diabetus mellitus ☐
20< ☐ Multifetal pregnancy ☐
Fetal growth retardation PIH-preeclampsia ☐
SGA (small for gestational age) ☐ Macrosomia ☐
IUGR (Intrauterine growth retardation)☐ < 3 th percentil < 10th percentil
Hyperemesis gravidarum ☐
Advanced maternal age ☐

In which part of the pregnancy did you continue to work at the hospital?

1.trimester ☐ 2.trimester ☐ 3.trimester ☐

Birth

Type of birth Gestational week_____Sex E ☐ K ☐ BW_____ LH ☐
Type of birth Gestational week _____Sex E ☐ K ☐ BW _____ LH ☐

Complication related to the fetus

Mortality ☐ Retinopathy ☐
Intraventricular hemorhage ☐ Necrotizing enterokolitis ☐

Biochemistry Laboratory of the University Hospital for determination of the following parameters by electrochemiluminescence immunoassay (ECLIA) procedures: Homocysteine (normal range 5.5–17 nmol liter^{-1}), vitamin B_{12} (normal range 160–800 pmol liter^{-1}), folic acid (normal range 3–17 pmol liter^{-1}), anticardiolipin IgM, IgG and antiphospholipid IgM and IgG.

A questionnaire inquiring about reproductive history; employment, health problems, medications and lifestyle habits was given to each subject (Tab. 1). We excluded personnel with conditions that affect the determination of their oxidative stress status, such as those taking any chronic medications for autoimmune diseases, liver or pulmonary diseases, acute or chronic inflammation, vitamin supplements, antioxidants and those who smoked or consume alcohol on a regular basis. All the operating theatre female personnel were included into the study as no one was meeting the exclusion criteria.

Statistical analysis

An exploratory statistics was performed using the Statistical Package for the Social Sciences (SPSS version 11.5). The results were expressed as the mean ± standard deviation or as the median (25–75 % confidence intervals) or as frequencies (%). Kolmogorov–Smirnov test was used to test for normal distribution of the numeric variables. The subjects who had abortus history were compared to the subjects without abortus history regarding categorical variables with Chi-Square test and numerical variables such as age, serum folic acid, vitamin B_{12}, homocyteine levels with t-test and anticardiolipin IgM, IgG, antiphospholipid IgM and IgG with the Mann-Whitney U tests, respectively. p values < 0.05 were considered significant.

Results

The characteristics of the group is seen in the Table 2. The operating theatre personnel had either 8 hours (35 (58 %) subjects) or 9 hours (25 (42 %) subjects) working shifts.

Detailed obstetric history of the operating theatre personnel was shown on Table 3. Five (19 %) subjects out of 26 had early (during the first 10 weeks of gestation) pregnancy loss. One subject (1.7 %) had two pregnancy losses between 10 and 20 weeks of gestation. Two (8 %) subjects had preterm deliveries. One woman (1.7 %) had premature rupture of membranes. Five (21 %) women had intrauterine growth retardation. Two women (8 %) were observed to be in advanced maternal age during their pregnancies. There was only one (1.7 %) multifoetal pregnancy. The first deliveries were vaginal in 12 (50 %) and caesarean section in the other 12 subjects. The first deliveries of the subjects occurred at 38 ± 2.5 weeks of gestation. The mean birth weight of the first-born newborns were 3071 ± 636 grams and seven out of 24 were male. The second deliveries were vaginal in six (60 %) and caesarean section in the other four subjects. The second deliveries of the subjects occurred at 38.4 ± 1.3 weeks of gestation and the mean birth weight of the newborns were 3286 ± 414 grams (eight out of 10 newborns were male). One subject reported macrosomia of the newborn. None of the subjects reported stillbirths or postpartum neonatal deaths. Complications in the newborn such as intraventricular hemorrhage, retinopathy or necrotizing enterocolitis were not reported. One subject had pulmonary thromboembolism during her last pregnancy. Two (3.3 %) women had children with anomalies.

Table 2. Demographic characteristics of the study subjects.

Age (years)	31±6
Employment duration (years)	6.8±7
Married	34 (56.7%)
Occupation: physician/nurse/OR technician/nurse anaesthetists	15/29/14/2

Table 3. The obstetric history of the operating theatre room personnel. Values are frequency (%).

Ever wanted children	11 (18%)
Number of gravida	
0	5
1	5
2	13
3	5
7	2
Number of parity	
0	3
1	15
2	9
Number of dilatation and curettage	
0	18
1	6
2	2
Number of live births	
0	3
1	14
2	9
Number of intrauterine exitus	
0	24
1	2
Number of abortus	
In the first 10 gestational weeks	
0	21
1	1
2	2
4	1
5	1
10-20 weeks of gestation	
2	1

Table 4: Laboratory results

	Subjects who had abortus (n=5)	Subjects who had not abortus (n=55)	P
Homocysteine (nmol liter^{-1})	10.69±3.98	7.95±1.42	0.09
Folic acid (nmol liter^{-1})	9.59±3.75	12.05±3.98	0.26
Vitamin B12 (pmol liter^{-1})	369.14±33.23	371.07±157.46	0.42
Anticardiolipin IgM, n(%)	1 (% 1.8)	1 (% 20)	**0.101**
Anticardiolipin IgG, n(%)	1 (% 1.8)	0 (% 0)	0.917
Antiphospholipid IgM, n(%)	1 (% 1.8)	0 (% 0)	0.917
Antiphospholipid IgG, n(%)	1 (% 1.8)	0 (% 0)	0.917

Values are mean±standard deviation (homocysteine, folic acid, vitamin B12) and number (%)

None of the study subjects had cardiac disease, diabetes, alcohol consumption or deep vein thrombosis history. Three subjects had allergies and 44 subjects were non-smokers. One woman had asthma, five had goitre disease who did not need any medication.

The mean ± SD homocysteine level was 9.1 ± 2.4 nmol liter^{-1} (normal range 5.5–17 nmol liter^{-1}), the mean ± SD serum concentration of folic acid was 10 ± 3.3 nmol litre^{-1} (normal range 3–17 pmol liter^{-1}), the mean ± SD vitamin B_{12} levels were 332 ± 134 pmol liter^{-1} (normal range 160–800 pmol liter^{-1}), and all were within normal limits. There was no difference regarding homocysteine, folic acid, vitamin B_{12} levels and the obstetric history between the subjects who had abortus and the subjects who had not abortus ($p > 0.05$) (Tab. 4).

The serum anticardiolipin IgM and IgG were positive only in two (3.3 %) and one (1.7 %) subjects respectively. Antiphospholipid IgM was positive in one (1.7 %) and antiphospholipid IgG was positive in another (1.7 %) subject (Tab. 4).

Discussion

Nitrous oxide is widely used in the operating theatres for anaesthesia managements and the staff who work in these theatres are occupationally exposed to this agent. According to the recent well–documented reports, it is well known that N_2O causes Hyperhomocysteinemia (tHcy) which is a risk factor for bad pregnancy outcome (2, 10, 12, 15, 16). We hypothesized that occupational exposure to N_2O causes tHcy and this is a risk factor for recurrent early pregnancy loss. We wanted to examine the tHyc, vitamin B_{12}-folic acid levels of our female operating personnel and correlation with bad obstetric outcomes.

There are several reports of hyperhomocystenemia associated with pregnancy disorders and recurrent pregnancy loss (11–14). Krajewski and colleagues (15) reported impaired vitamin B_{12} metabolic status and significantly increased tHcy levels in female nurses with occupational exposure to N_2O. In this study, we examined the relation of tHcy levels with bad obstetrical outcome among the female operating theatre personnel. There isn't a control group because that design was planned to be the second stage in case of significant increases in tHcy levels in our exposed group. Fortunately, all measurements were in normal ranges according to our university laboratory ranges and we have not any significant rate of abortions. Krajewski et al (13) reported lower vitamin B_{12} and higher tHcy levels in their exposed group.

Currently, several randomized, placebo-controlled trials are ongoing and the results should make possible a meaningful assessment of whether lowering homocysteine concentration does indeed reduce the risk of cardiovascular diseases. Vollset et al (11) report that hyperhomocysteinemia may also be an important biological marker for, and possibly even a cause of or a contributor to complications and adverse outcomes of pregnancy. When comparing quartiles of homocysteine concentration measured in 5883 women aged 40–42 years, between 1992–1993 with outcomes and complications of 14492 pregnancies that occurred between 1967 and 1996 in the same women, authors noted an increased risk of preeclampsia, premature delivery, very low birth weight, neural tube defects, and clubfoot. Rowland et al (17) reported that occupational exposure to high levels of N_20 may adversely affect women's fertility. We did not detect any relation between bad obstetric history and working in the operating theatre probably due to advanced scavenging systems used in our operating theatres.

Despite advanced scavenging systems, the level of pollution in operating theatres depends on multiple factors such as the type of anaesthesiology equipment (closed/open system), the anaesthesiology techniques (high/low flow rate, use of face masks or laryngeal mask airways, use of uncuffed tracheal tubes), and the methods of anaesthetic induction (18). Low-flow anaesthesia should be used whenever possible to decrease the waste anaesthetic gas level in the operating theatres. The sole use of intravenous drugs such as propofol instead of volatile agents would eliminate occupational exposure but may result in environmental pollution by toxic metabolites. In our operating theatres, we have laminar air condition system preventing the circulation of exhausted air into operating theatre (> 15 air changes h^{-1}).

We studied the effect of exposure to waste anaesthetic gases on pregnancy outcome by studying the homocysteine, folic acid, vitamin B_{12}, anticardiolipin and antiphospholipid levels in female personnel working in operating theatre. In conclusion, tHcy, vitamin B_{12} and folic acid levels were within normal range in our operating theatre female staff. There was no difference between female personnel who had abortus and who had not abortus, in respect to blood levels of homocysteine and bad obstetric outcome.

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