ORIGINAL PAPER

The Influence of Smoking on the Variations in Carboxyhemoglobin and Methemoglobin During Urologic Surgery

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ABSTRACT

Introduction: Surgery is supposed to modulate the production of carbon monoxide by the reduction of heme oxygenase activity or transcriptional regulation of inducible heme oxygenase. On the other hand, the inhalation of tobacco smoke can substantially raise the level of carboxyhemoglobin in the blood. Furthermore, methemoglobin is maintained at a constant level. However, excessive production of methemoglobin relative to total methemoglobin reductase activity results in methemoglobin increase. **Aim:** The aim of our study was to investigate the perioperative variations of carboxyhemoglobin and methemoglobin during urologic surgeries, and at the same time to evaluate the changes in methemoglobin as a possible indicator of nitric oxide generation. Our second aim was to evaluate the effect of preoxygenation on the level of carboxyhemoglobin and methemoglobin and the influence of blood transfusion on their changes. **Material and methods:** The study included 30 patients scheduled for urologic surgery under general endotracheal anesthesia, aged 18–60 years without any history of respiratory disease, divided into two groups. The study group comprised patients who were smoking cigarettes or tobacco pipe, while the control group included non-smokers. In both groups carboxyhemoglobin (COHb) and methemoglobin (MetHb) levels were determined preoperatively, after preoxygenation, and postoperatively. **Results:** COHb levels were decreased postoperatively in both groups. The average values of COHb between the two groups were statistically significantly different (p=0.00). MetHb levels increased postoperatively in the group of smokers and decreased in the group of non-smokers. There were no statistically significant differences in the average postoperative MetHb levels between the two groups. **Conclusion:** Changes in carboxyhemoglobin and methemoglobin concentrations in arterial blood occur during urologic surgery, although these amplitudes are small when compared with carbon monoxide intoxication and methemoglobinemia. It is likely that organ perfusion and functions are affected by these monoxide gas mediators during urologic surgery.

Keywords: carboxyhemoglobin, methemoglobin, hemoglobin perioperative, urologic surgery.

1. INTRODUCTION

Carboxyhemoglobin (COHb) and methemoglobin (MetHb) are variants of normal hemoglobin, which is contained in red blood cells and is responsible for oxygen's transportation from lungs to tissue cells. Both molecules are formed from hemoglobin throughout different processes. Carboxyhemoglobin is formed when hemoglobin binds endogenous and exogenous inhaled carbon monoxide (CO). Endogenous CO is synthesized mainly by heme oxygenase, which catalyzes heme catabolism to CO, iron, and biliverdin (1, 2). The majority of CO is removed from the body via expiration (3).

CO has been reported to take part in a variety of pathological functions such as vasodilation, angiogenesis, vascular remodeling, and inflammatory response (1, 2). Heme oxygenase-1 (HO-1) is induced by heme, hemoglobin, hypoxia, oxy-radicals, cyclic adenosine monophosphate (AMP), heat shock, and cytokines via activation of the transcriptional process (4, 5). However, it still remains unknown whether surgically induced stress, sepsis, orchiectomy, splenectomy or hepatic resection in the clinical situation increases the concentration of HO-1 by induction of the enzyme or decreases it by reduction of the enzyme. It is also

unclear whether or not blood transfusion increases the CO concentration as a result of increased substrate load, such as hemoglobin (Hb), cytochrome P450 and other heme proteins. It is well known that CO tightly binds with Hb and forms COHb, while COHb is slowly converted to Hb in the presence of sufficient concentrations of oxygen (O_2) (6).

Major source of exogenous CO is tobacco smoke, thus the inhalation of tobacco smoke containing up to 5% carbon monoxide can substantially raise the level of COHb in the blood (7, 8). The reported blood COHb saturation is approximately 1% in nonsmokers and about 5.5% in smokers with a smoking history of almost 20 cigarettes per day (9, 10). The COHb level can thus identify those people at risk from any of the diseases associated with the inhalation of tobacco smoke even if carbon monoxide is not directly implicated in the pathogenesis of those diseases. Because it is difficult to metabolize CO in vivo, and CO binds with high affinity to hemoglobin, it is easy to measure the endogenous production of CO as COHb using spectrophotometry (11).

On the other hand, MetHb formation is the result of an oxidative process during which 1 or more of the 4 iron atoms in the hemoglobin molecule convert to ferric state and therefore are incapable of binding oxygen. Endogenous MetHb is produced when naturally produced nitric oxide (NO) interacts with hemoglobin, while MetHb is converted to hemoglobin enzymatically by MetHb reductase (12). Therefore, MetHb is maintained at a constant level. However, excessive production of MetHb relative to total MetHb reductase activity results in MetHb increase.

The aim of the study was to investigate the ranges of COHb and MetHb in smokers and non-smokers and the effect of pre-oxygenation with 100% of oxygen on the level of COHb and MetHb. We also investigated changes in COHb and MetHb during urologic surgery as a possible indicator of CO and NO generation. Furthermore, we evaluated the perioperative changes in COHb and MetHb during urologic surgery.

2. MATERIAL AND METHODS

2.1. SUBJECTS

This prospective clinical study was performed at the Clinic for Traumatology, Orthopedic Diseases, Anesthesia, Reanimation and Intensive Care in Skopje. The study was conducted after the approval by the Ethics Committee and the signed informed consent by every patient. We enrolled 30 consecutive patients scheduled for elective urologic surgery under general endotracheal anesthesia, aged 18–60 years, under physiological score for preoperative assessment of health – ASA (American Society of Anesthesiologists) 1 and 2. The study excluded all patients having surgery under local or regional anesthesia, patients with any history of respiratory disease, pregnant patients, and transplant patients. The patients were assigned into two groups: smokers (smoking ≥10 cigarettes or 30 grams of pipe tobacco per week) (13) and never-smokers (those who had never smoked cigarettes or pipe tobacco) (13).

2.2. STUDY DESIGN

All patients underwent standard preoperative protocol for nothing per mouth (for 6 hours); were normothermic and premedicated with oral Diazepam 5 mg, 90 min before surgery. The standardized anesthesia protocol was applied in all patients. After preoxygenation with 100% O_2/ 6L/min, for 3 minutes, the induction was started with midazolam 1-2 mg fentanil (2-10 µg/kg) and propofol (1-2 mg/kg). The intubation was facilitated with rocuronium bromide 0.6 mg/kg. Following induction of anesthesia all patients were manually ventilated for 2 min. After two minutes the patients were intubated and mechanically ventilated with inhaled fraction of a mixture of O_2 (50%) and air (50%). The anesthesia was maintained with continuous infusion of propofol 0.1 – 0.2 mg/kg/min, fentanil 1-2 mcg/kg and rocuronium bromide 0.3 mg/kg.

2.3. MEASUREMENTS

Arterial blood gas analysis was performed at three time points: T_0–before surgery under respiration with room air; T_1–after pre-oxygenation with 100% O2 for 3 minutes with 6L/min flow, and T_2–one hour after surgery in post-anesthesia recovery room under respiration with room air or oxygen inhalation with or without endotracheal intubation. Levels of COHb and MetHb were determined using a heparinized blood sample that was collected by puncture from peripheral artery. COHb, MetHb, total Hb, and partial pressure of carbon dioxide (PCO_2 mmHg) and oxygen (PO_2 mmHg) in arterial blood samples were performed by blood gas analyzer (SIEMENS RAPID Point 500 Systems). COHb and MetHb were analyzed spectrophotometrically using their specific absorption and reference wavelengths. The analyzer runs a zero calibration of the optical system against a colorless calibration fluid at least every 4 hours to guarantee accuracy. Other biochemical measurements and electrolyte levels were determined by standard laboratory methods.

2.4. STATISTICAL ANALYSIS

Continuous variables are reported as medians and ranges, and categorical variables are expressed as percentages. Statistical analysis was performed by analysis of variance and difference test. P value less than 0.5 was considered statistically significant. All analyses were performed with the SPSS statistical software.

3. RESULTS

In accordance with the inclusion criteria, the study enrolled 30 patients in both groups. Baseline demographic characteristics were similar in both groups with respect to sex, age, weight, height BMI and ASA. Baseline demographics and clinical characteristics of patients are shown in Table 1. There was a variety of diagnosis, and the most represented clinical diagnosis in I and II group was kidney cancer (26.6%). Table 2 illustrates the characteristics of the surgery. In both groups, smokers and nonsmokers, 30% of the interventions were laparoscopic. (Table 1, Table 2).

Table 3 shows the effect of smoking on the perioperative changes in arterial blood gas analysis. In the group

Parameters	Group Smokers n=15	Group Non-smokers n= 15	P
Sex M/F	11/4	10/5	NS
Age	50.2 ± 12.84	49.93 ± 13.99	NS
ASA I/II	0/15	2/13	NS
BMI (m²)	25.84 ± 3.5	25.92 ± 2.76	NS

Table 1.Demographic and clinical characteristics (Mean ± SD). Abbreviations: F−female; M − male; ASA- American Society of Anesthesiologist, BMI-Body Mass Index; NS-Not significant (p > 0.05)

Groups	Surgery Open/Laparo-scopic	Anesthe-sia time (min)	Operation time (min)	Blood transfu-sion (no/yes)	Trans-fusion unit
Smokers n=15	11/4	143.3 ± 62.38	174.66 ± 68.2	10/5	1
Non-smokers n=15	11/4	159.33 ± 64.65	196.66 ± 67.65	7/8	1.4

Table 2.Characteristics of the surgery (Mean ± SD)

	T_0	T_1	T_2
Group Smokers n=15			
COHb %	2.1 ± 1.02	1.92 ± 0.97	1.88 ± 0.63
MetHb %	0.18 ± 0.11	0.27 ± 0.14	0.3 ± 0.11
Total Hb g/L	137.73 ± 24.3	121.13 ± 30.18	115.4 ± 18.76
FO_2Hb %	93.7 ± 2.46	96.17 ± 2.34	92.5 ± 2.42
PCO_2 mmHg	38.07 ± 6.16	45.62 ± 10.31	44.54 ± 7.91
PO_2 mmHg	78.96 ± 10.81	166.67 ± 128.2	85.12 ± 19.57
Group Non-smokers n=15			
COHb %	0.47 ± 0.27	0.42 ± 0.34	0.4 ± 0.18
MetHb %	0.3 ± 0.16	0.38 ± 0.21	0.29 ± 0.12
Total Hb g/L	139.46 ± 22.9	131.2 ± 22.54	114.26 ± 18.22
FO_2Hb %	96.52 ± 1.66	98.08 ± 0.78	95.35 ± 2.37
PCO_2 mmHg	38.61 ± 5.07	39.3 ± 8.62	40.11 ± 6.35
PO_2 mmHg	83.94 ± 8.95	157.25 ± 56.85	93.21 ± 12.78

Table 3 .Perioperative changes in carboxyhemoglobin and methemoglobin in the Smoking and Non-smoking Group. Values are expressed as mean ± standard error of the mean. T0 − preoperative; T1−after preoxygenation; T2−one hour after surgery; COHb− carboxyhemoglobin; Hb−hemoglobin; MetHb−methemoglobin; FO2Hb − fractional oxyhemoglobin; PCO2−partial pressure of carbon dioxide; PO2−partial pressure of oxygen.

Group Non – Smokers n=15	T_0	T_1	T_2
Without blood Transfusion n= 10			
COHb %	0.62 ± 0.21	0.62 ± 0.21	0.3 ± 0.12
MetHb %	0.34 ± 0.18	0.46 ± 0.26	0.32 ± 0.14
Total Hb g/L	153.8 ±14.1	145.8 ± 14.7	125.4 ± 6.18
PCO_2 mmHg	41.4 ± 6.42	44 ± 8.74	44.2 ± 7.94
PO_2 mmHg	88.48 ± 10.17	141.66 ± 35	92.6 ± 12.7
With blood Transfusion n= 5			
COHb %	0.32 ± 0.16	0.2 ± 0.12	0.56 ± 0.21
MetHb %	0.26 ± 0.2	0.34 ± 0.25	0.28 ± 0.04
Total Hb g/L	118.8 ± 24.19	112 ± 24.44	93.6 ± 9.63
PCO_2 mmHg	38.12 ± 4.02	41.06 ± 5.26	39.24 ± 5.09
PO_2 mmHg	81.08 ± 9.77	140.4 ± 48.67	92.8 ± 7.69

Table 4.Effect of blood transfusion on the perioperative changes in carboxyhemoglobin and methemoglobin in the Non−Smoking Group. Values are expressed as mean ± standard error of the mean. T0 − preoperative; T1−after preoxygenation; T2−one hour after surgery; COHb−carboxyhemoglobin; Hb−hemoglobin; MetHb−methemoglobin; PCO2−partial pressure of carbon dioxide; PO2−partial pressure of oxygen.

| Group Smokers n=15	T_0	T_1	T_2
Without blood Transfusion n= 7			
COHb %	2.14 ± 0.91	1.87 ± 0.97	1.88 ± 0.56
MetHb %	0.15 ± 0.11	0.2 ± 0.1	0.28 ± 0.1
Total Hb g/L	151.85 ± 16.68	144.85 ± 18.91	130.57 ± 11.07
PCO_2 mmHg	39.65 ± 4.11	46.55 ± 9.41	44.45 ± 8.92
PO_2 mmHg	78.54 ± 11.16	195.04 ± 163.34	84.42 ± 15.38
With blood Transfusion n=8			
COHb %	2.07 ± 1.17	1.97 ± 1.04	1.87 ± 0.73
MetHb %	0.2 ± 0.11	0.33 ± 0.15	0.33 ± 0.1
Total Hb g/L	125.37 ± 23.83	100.37 ± 21.46	102.12 ±12.94
PCO_2 mmHg	36.68 ± 7.53	44.8 ± 11.62	44.62 ± 7.54
PO_2 mmHg	79.33 ± 11.25	141.85 ± 92.16	85.73 ± 23.71

Table 5.Effect of blood transfusion on the perioperative changes in carboxyhemoglobin and methemoglobin in the Smoking Group Values are expressed as mean ± standard error of the mean. T0 − preoperative; T1−after preoxygenation; T2−one hour after surgery; COHb− carboxyhemoglobin; Hb−hemoglobin; MetHb−methemoglobin; PCO2− partial pressure of carbon dioxide; PO2−partial pressure of oxygen.

of smokers, COHb was significantly higher at all three time points than in the group of nonsmokers, and decreased after preoxygenation as well as postoperatively due to mechanical ventilation. In both groups, MetHb increased after preoxygenation. Postoperatively there were differences regarding MetHb in both groups, in the control group the level of MethHb was lower than preoperative baseline level, but without significant difference (p>0.05) and significantly increased in the group of smokers (p<0.05) (Table 3).

Table 4 and Table 5 shows the effect of intraoperative blood transfusion on the perioperative changes in COHb and MetHb in nonsmokers (Table 4) and in smokers (Table 5). There was no significant difference in total Hb preoperatively and postoperatively between blood transfusion and no-transfusion groups, indicating that total Hb was adequately corrected by intraoperative blood

transfusion. In the blood transfusion group, COHb % was significantly higher compared to the non-transfusion group (1.36 ± 0.88 vs. 1.22 ± 0.92). In the blood transfusion group, MetHb was higher than that in the non-transfusion group (0.315 ± 0.08 vs. 0.3 ± 0.12). (Table 4, Table 5).

The level of COHb decreased postoperatively (2.1 ± 1.02 vs. 1.88 ± 0.63) in smokers and (0.47 ± 0.27 vs. 0.4 ± 0.18) in nonsmokers. The average values of COHb between the two groups were statistically significantly different. At baseline preoperative value T_0 was p=0.000, at the second time point T_1 after preoxygenation the p value was 0.000 and at the postoperative time point T_2, p=0.000. MetHb level increased postoperatively (from 0.18 ± 0.11 vs. 0.3 ± 0.11) in smokers and decreased (from 0.3 ± 0.16 vs. 0.29 ± 0.12) in nonsmokers. The average postoperative values of MetHb were as follows: at
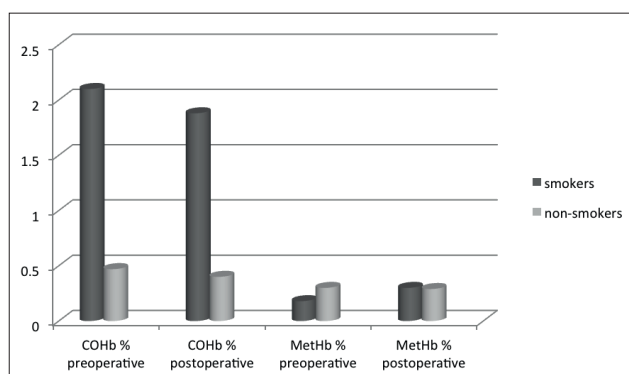
Graph 1. Change of the average values of CoHb and MetHb in the groups

baseline preoperative time point T_0, p=0.02, at the second time point after preoxygenation T_1, p=0.10 and at the last postoperative time point T_2, p=0.88. (Graph 1).

4. DISCUSSION

The presented study was conducted as a pilot study; it evaluated and tested in clinical practice the influence of smoking on the prognostic values of COHb and MetHb perioperatively. The analysis clearly indicated that smoking increases exogenous COHb and that the effect of smoking remains even after mechanical ventilation. Blood transfusion increases endogenous COHb, because hemoglobin is a major substrate for heme oxygenase. Although it has been reported that reaction of sevoflurane with soda lime, which has not been used for more than 2 weeks generates CO (14), the possibility was ruled out due to the intravenous anesthesia used in our study and due to high frequent use of the anesthetic circuit in our hospital.

A significant increase of CO was routinely observed by Levy et al. (15) during general anesthesia in infants and children when low flow anesthesia was used. In our study we used normal flow anesthesia in all patients.

Our study indicated that intubation anesthesia in smokers increases MetHb and that blood transfusion also increases MetHb. Although nitroglycerin and sodium nitroprusside have been reported to cause methemoglobinemia, which is recognized by cyanosis (16), nitroglycerin and sodium nitroprusside were never used for hypotensive anesthesia in the present series.

Contrary to the study of Takeda, were nitrous oxide (N_2O) has been used in all cases (17), but contamination of NO in N_2O gas was completely negligible. In our study N_2O was not used, therefore, the increase of MetHb postoperatively was ascribed to endogenous NO or some other autoxidizing mechanism which converts ferrous heme (Fe^{+2}) to ferric heme (Fe^{+3}). Increase of MetHb postoperatively after blood transfusion can be assigned to autoxidation of heme of the preserved red cell in the recipient.

The laparoscopic surgery creates a pneumoperitoneum with carbon dioxide (CO_2), but blood gas analysis does not indicate retention of CO_2 at several hours after extubation, nor is there a pathway which converts carbon dioxide to carbon monoxide. It has also been proposed that laparoscopic surgery with electric cautery is likely to generate CO due to incomplete combustion of the tissue, but comparison between laparoscopic (n=4) and open surgery (n=11) in our groups did not show any significant differences in COHb and MetHb. It has also been reported that the use of electric cautery in the laparoscopic surgery did not increase COHb (18).

Half-life of carboxyhemoglobin is 250 minutes in patient's breathing room air. This is reduced to 40 to 60 minutes with inhalation of 100% oxygen according to the review article of Dries (19). In our study preoxygenation with 100% oxygen for 3 minutes before anesthesia and surgery decreased the level of carboxyhemoglobin but without significance.

Locally generated CO is eliminated by Hb in circulating erythrocytes and is gradually released into the alveolar space of the lungs, where molecular oxygen is alternatively bound to the heme. Most endogenous generated CO is thus exhaled into the airway, and the alveolar oxygen tension determines the exchange rate between oxygen and CO (11). For this reasons, COHb in blood samples collected from patients could be altered by multiple factors such as surgical insults, hemoglobin concentrations, tissue oxygenation and pulmonary function (20). In this study, we chose patients who did not suffer from obvious respiratory or inflammatory disease, and we fixed the inspired oxygen fraction at 0.5 during the study except during preoxigenation. We tried to maintain Hb concentrations in normal ranges, so that Hb concentration at sampling time did not differ between groups and did not correlate with COHb concentration.

Carbon monoxide binds to hemoglobin to form COHb without oxygen-carrying capacity because of its 200 times stronger affinity, compared to oxygen. When COHb reaches to 10% of total hemoglobin, namely around 10 mg/mL, CO intoxication appears as working disability and headache. When the concentration exceeds 30%, consciousness is lost. Thus, CO is known as a toxic gas (21). Nevertheless, inducible and constitutive heme oxygenases that produce CO from

heme proteins are inherent in the human body, and the physiological role of CO as a toxic gas has yet to be elucidated completely. In the present study, the amplitude of COHb changes was not so large compared to CO intoxication. Because MetHb lacks oxygen carrying capability, methemoglobinemia is regarded as a causative factor of hypoxia. The body is equipped with methemoglobin reductase to eliminate MetHb from the blood (22). The amplitude of changes in MetHb during urologic surgery is also rather small compared to methemoglobinemia, which causes cyanosis. However, since the generation velocities of these two monoxides fluctuate during urologic surgery, these gas mediators are likely to affect organ function, although the present study did not investigate the effects.

Our study has limitations. A major limitation of this study is the relatively small sample size in both subgroups that makes statistical analysis difficult. Thus, our results should be interpreted with caution. Another limitation was that measurements were made without blinding of the researcher to the experimental group. The prelimi-

nary observations of our study require further validation in larger prospective populations in order to clarify the underlying mechanisms and further verify the prognostic ability of COHb and MetHb levels. This would be of particular importance since these indices are widely and readily available in everyday clinical practice.

- Conflict of interest: none declared.

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