

The Level of Maternal Methemoglobin during Pregnancy in an Air-Polluted Environment

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The objective of this prospective study was to determine if a correlation could be established between the ground-level concentrations of sulfur dioxide and methemoglobin concentrations in pregnant women when a coal-powered thermoelectric power plant was in operation ("dirty" period) and when it was closed ("clean" period). The location of the power plant, Plomin 1, in Labin, Croatia, was taken into consideration. Blood and urine samples of each pregnant woman in the study were tested three times in the clean period ($n = 138$) and three times in the dirty period ($n = 122$), with 1 month between each test. I observed a correlation between the increase in mean values of methemoglobin and the ground-level concentration of SO_2 on corresponding dates during the dirty period ($r = 0.72$, $p < 0.01$). In the clean period, the negative mean value of methemoglobin was significant ($r = -0.60$, $p \leq 0.05$), whereas in the dirty period, the positive mean value of methemoglobin was significant ($r = 0.73$, $p \leq 0.01$). The increase of maternal methemoglobin could be a useful biomarker to determine when the health of pregnant women is threatened by toxic substances in the environment. **Key words:** biomarker, environmental toxicants, maternal methemoglobinemia, precursor, pregnancy, toxic substances. *Environ Health Perspect* 111:1902–1905 (2003). doi:10.1289/ehp.6055 available via <http://dx.doi.org/> [Online 13 November 2003]

In recent years, researchers have focused on explaining the role of oxygen, free radicals, and oxidative stress during embryogenesis and placental stages, and development of pathologic pregnancy, especially preeclampsia and fetal intrauterine growth restriction (IUGR). Because I found no evidence that methemoglobin levels had been tested during human pregnancy in an air-polluted environment, I reevaluated research performed in this laboratory in the past. My objective was to identify a biomarker for methemoglobin to be used as a precursor and proof of the presence of oxidants before clinically manifested symptoms occur, even in early pregnancy. The statistical analysis was incomplete, thus requiring additional research, which was delayed because of the Croatian War for independence that lasted from 1991 to 1995.

Methemoglobinemia is a condition in which hemoglobin is oxidized to the ferric form and is unable to transport oxygen to tissues, therefore causing hypoxia. The physiologic level of methemoglobin is 1% in peripheral blood, and it may increase because of a variety of genetic, dietary, idiopathic, toxic, and other factors. Methemoglobinemia primarily occurs when erythrocytes are affected by xenobiotics and pharmaceutical compounds with toxicologic properties, such as volatile organic compounds, oxidants, nitrogen oxides, peroxydinitrites, phenacetin, and sulfonamides.

Materials and Methods

To determine the toxic substances in the environment and the level of air pollution, I chose to study the population living near Plomin 1, a coal-powered thermoelectric

power plant in Labin, Croatia. The plant, with a 110-m-tall chimney, is the single major air polluter within a 40-km radius of the target population. Every hour of operation, the plant emits about 8.5 tons (18,080 mg/m^3 , or 6,900.8 ppm) of sulfur dioxide in addition to nitrogen oxides (NO_x), carbon dioxide, carbon monoxide, total suspended particles, iron, titanium, vanadium, chromium, nickel, copper, zinc, selenium, lead, and other products of coal combustion. The coal from this area has a high sulfur content (9–11%) and a high level of radioactivity (the activity of ^{238}U is 300 Bq/kg, which is 10–15 times higher than the average for other types of coal in the world). In the approximately 700,000 tons of crude waste from coal combustion surrounding the plant, the concentration of radionuclides was 5–10 times higher than that in the unburned coal (Saric 1996). Because the plant was closed from 19 February 1989 to 6 September 1989, I was able to carry out research during two separate periods: the "clean" period from April to July 1989 and the "dirty" period from December 1989 to March 1990. In the dirty period, the daily ground-level concentrations of SO_2 were monitored at three different locations.

Air quality data and samples. Daily minimum, maximum, and average air temperatures; quantity and type of precipitation; wind direction and strength; and relevant data on weather conditions were provided by the Labin meteorologic station. Air quality (SO_2 , fumes, and particulates) was analyzed by an acidimetric method based on the British standards recommended by the World Health Organization (WHO 1976). Briefly, air samples were collected in a weak solution

of hydrogen peroxide; after particulates were removed by filtration, the quantity of the absorbed SO_2 in the H_2O_2 solution was determined by titration. The Regional Institute of Health Care (Pula, Croatia) performed the air quality measurements.

Subjects. Pregnant women were selected for the research target group from patients of the Primary Health Center, which is responsible for the health of about 25,500 residents, of which about 6,180 women of reproductive age had a permanently assigned obstetrician or gynecologist. Subjects were informed about the purpose of the research and gave written consent. Out of 273 women who were pregnant at the time of the study, 260 women were considered representative based on the criteria that they were pregnant and came to the center for regular monthly checkups during the clean and dirty periods. Patients received care from the Primary Health Center, the Obstetric-Gynecological Clinic in Rijeka, or the regional Obstetric-Gynecological Hospital in Pula.

Blood and urine samples from women in the study were tested three times in the clean period ($n = 138$) and three times in the dirty period ($n = 122$), with 1 month between each test. All 260 of the pregnant women in the study lived in Labin and the surrounding area.

The pregnant women were divided into six groups on the basis of the location of their places of residence within zones defined by concentric circles around the Plomin 1 plant. Most of the pregnant women lived in the zones 3.5–7.5 km (71.63%) and 7.5–12.5 km from the plant (21.63%). The town of Labin, with 12,000 inhabitants, is also in these zones.

The Obstetric-Gynecological Clinic and the Obstetric-Gynecological Hospital, where women from the area surrounding Plomin 1 were treated, provided data on reproductive loss for the clean and dirty periods.

Blood samples. According to the planned prospective study, 2 cm^3 of each blood sample

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I thank Aleksandra Frkovic, M. Vukelic, Anton Frkovic, I. Svel, S. Bulesic, S. Mladinov, M. Ljubacev, and E. Tirelli.

This study was supported in part by PP Plomin Ltd. The author declares a competing financial interest because the Croatian electricity company, PP Plomin Ltd., the management company for Plomin 2, provided funding for the study.

Received 14 October 2002; accepted 30 October 2003.

(heparinized) was tested. Hemoglobin and methemoglobin were measured by spectrophotometric method in the toxicology laboratory at the Department of Occupational Medicine (Rijeka, Croatia). Briefly, the erythrocytes were lysed with Triton X-100 and spectrophotometry was performed using the cyanohematin method. The maximal absorption of methemoglobin is 630–633 nm.

Samples (10 mL buffer, 0.1 mL blood from exposed or nonexposed pregnant women, or one drop of Triton X-100) were placed into test tubes, mixed well by turning the tubes five to eight times, and then left for 5 min. The mixture was then poured into another test tube, and the photometry was performed toward water at 633 nm (measurement A1). Afterward, one drop of neutral sodium cyanide was added to the mixture, which was stirred, left for 5 min, and measured again at 633 nm (measurement A2).

$$\text{Methemoglobin (g/L)} = (A1 - A2) \times F$$

F is determined by adding 0.1 mL potassium ferricyanide solution to 10.0 mL buffer and 0.1 mL normal blood. The mixture was then stirred and left for 2–3 min so that all of the hemoglobin could oxidize into methemoglobin. The absorption was measured at 633 nm (measurement A3). A drop of neutral sodium cyanide was then added to the mixture, and after 5 min, the absorption was measured again at 633 nm (measurement A4). The difference ($A3 - A4$) corresponds to the absorption of total hemoglobin in the form of methemoglobin. I also used the cyanohematin method to determine the difference in absorption between hemoglobin and methemoglobin.

$$F = \text{g/L hemoglobin} \div A3 - A4$$

$$\text{Percentage methemoglobin in total hemoglobin} = (\text{g/L methemoglobin} \div \text{g/L hemoglobin}) \times 100$$

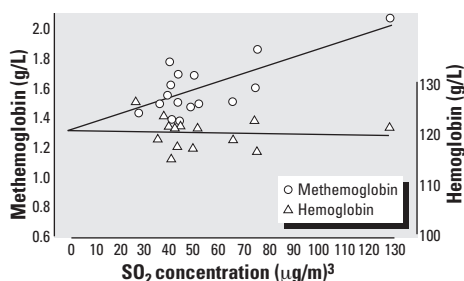


Figure 1. Correlation between ground-level concentration of SO_2 ($\mu\text{g}/\text{m}^3$) and the average value of methemoglobin and hemoglobin in blood samples of pregnant women during the dirty period when Plomin 1 was in operation (December 1989 to March 1990). For methemoglobin, $y = 1.286 + 0.0054x$; $r = 0.72$, $p < 0.01$. For hemoglobin, $y = 120.6 - 0.013x$, $r = -0.10$, $p > 0.1$.

The mean of two blood samples was used to determine F .

The standards used to relate absorbance values to hemoglobin and methemoglobin concentrations were 120–160 g/L and 0.0–2.5 g/L, respectively.

Results

I found a statistically significant positive correlation between the mean concentration of methemoglobin and the daily ground-level concentration of SO_2 in the dirty period on the days that blood samples were taken ($r = 0.72$, $p < 0.01$) (Figure 1). Linear correlation (Figure 2) shows that, in the clean period, the downward trend of methemoglobin is statistically significant ($r = -0.60$, $p \leq 0.05$). In contrast, in the dirty period, the upward trend of methemoglobin is significant ($r = 0.73$, $p \leq 0.01$).

The monthly value of SO_2 during the dirty period ranged from $34.1 \mu\text{g}/\text{m}^3$ (0.013 ppm) to $252.9 \mu\text{g}/\text{m}^3$ (0.10 ppm), varying with weather conditions. The ground-level concentration of SO_2 was proportionally higher on sunny and predominantly sunny days with no wind. To provide a weekly weather pattern, the following data were recorded: daily temperatures; wind from the southeast or northeast, which influences the ground-level concentrations of SO_2 ; and meteorologic conditions (the ground-level concentration of SO_2 is lower on rainy and cloudy days).

The frequencies of miscarriages and stillbirths were significantly different between the clean and the dirty periods ($p < 0.05$). The stillbirths occurred only during the second half of pregnancy, and only mothers of stillborn babies (and the mother of a baby who died on the second day) had methemoglobin values

$> 1.5 \text{ g/L}$ (1% of hemoglobin) (Table 1). In one case of stillbirth and the only case of spontaneous abortion after ≥ 20 weeks of exposure to SO_2 , methemoglobin was not detected in the mothers' blood. The adverse outcome of these cases can be explained by the influence of other intrinsic and extrinsic causes, but not by the influence of environmental toxicants (Table 1). Cases of increased methemoglobin values with confirmed symptoms of preeclampsia are in accordance with the assumption that environmental toxicants influence the course and outcome of pregnancy. I found no differences in methemoglobin levels or pregnancy loss by zones around the power plant. These data lead to the supposition that methemoglobinemia is related to the adverse outcomes and linked with IUGR, preeclampsia, complicated pregnancy, and a high percentage of perinatal mortality and morbidity.

In blood samples from pregnant women, the mean incidence of methemoglobin concentrations $> 1.5 \text{ g/L}$ fell from 22.7% to 13.4% in the clean period, whereas in the dirty period it increased from 10.8% to 32.8% (Figure 3).

The level of sulfates in urine samples significantly decreased during the clean period ($p < 0.05$), whereas it increased in the dirty period, but not statistically significantly ($p > 0.05$).

Discussion

Methemoglobinemia during pregnancy is often unrecognized and underemphasized by obstetricians. Methemoglobinemia is a precursor to preeclampsia and eclampsia and has the same symptoms: headache, breathing difficulties, dyspnea, skin discoloration, cyanosis, weakness, confusion, palpitations, chest pains,

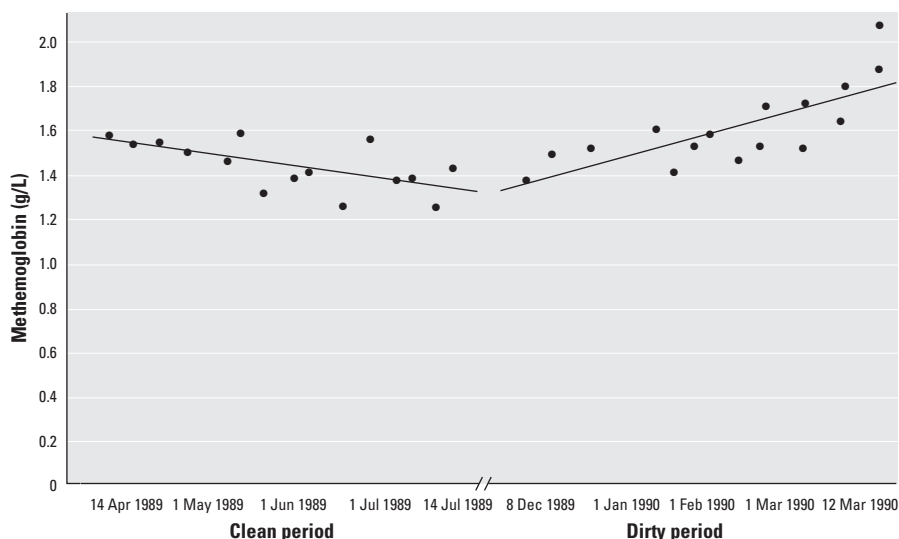


Figure 2. Trend of the methemoglobin concentrations in blood samples from pregnant women during the clean and dirty periods (samples without methemoglobin were omitted). Values shown are means, which are lower during the clean period than during the dirty period ($t = 4.74$, $p \leq 0.001$). For the clean period, $y = 1.569 - 0.070x$; $r = -0.60$; $p \leq 0.05$. For the dirty period, $y = 1.304 + 0.135x$; $r = 0.73$; $p \leq 0.01$.

altered mental status, and delirium leading to coma.

As methemoglobin levels increase, patients demonstrate evidence of cellular hypoxia; death occurs when methemoglobinemia during pregnancy approaches 70%. Tabacova et al. (1997) confirmed that methemoglobinemia is connected with complications during pregnancy. In their study, Tabacova et al. (1997) measured methemoglobin as a biomarker of individual exposure; the most common complications were anemia (67%), threatened abortion/premature labor (33%), and signs of preeclampsia (23%). Methemoglobin was elevated significantly, compared with normal pregnancies. These results suggest that maternal exposure to environmental oxidants can increase the risk of pregnancy complications through stimulation of the formation of cell-damaging lipid peroxides and from a decrease in maternal antioxidant reserves (Gladen et al. 1999; Little and Gladen 1999; Tabacova et al. 1997).

In the present study, the level of methemoglobin was monitored during pregnancy in an air-polluted area. The claim that the methemoglobin levels during pregnancy can indicate the adverse outcomes was based on the observation that, out of 10 cases of reproductive loss during the dirty period, three pregnant women had methemoglobin values > 1.5 g/L. Also, during the same period, a statistically significant linear increase of methemoglobin was established in the pregnant women tested.

When inhaled NO_x enters the mother's blood circulation directly through the alveolar-capillary membrane, oxidant electron loss changes hemoglobin to its pathologic reversible form—methemoglobin. Hemoglobin oxidation causes not only the rise in methemoglobin concentration but also the inhibition

of enzyme and nonenzyme antioxidants, causing methemoglobinemia and adverse effects of hypoxemia and hypoxia on particularly sensitive target organs.

Additional effects of methemoglobinemia include *a*) hypoxia and misbalanced production of reactive oxygen species, normal by-products of cellular metabolism that can cause problems when present in excessive amounts; *b*) increased exogenous toxic oxidants; *c*) oxidative stress to cellular membranes; *d*) cellular damage to DNA, proteins, lipids, and carbohydrates; and *e*) tissue damage in target organs. These effects occur during early placentation, when "local" placental vascular endothelium dysfunctions manifest clinically in nonsymptomatic fetal IUGR in the first half of pregnancy.

Therefore, the continuous monitoring of methemoglobin concentrations in the maternal bloodstream would be the first reliable early indicator of adverse effects of free radicals, oxidants, and oxidative stress.

Several articles have been published on the impact of toxic substances, metabolic oxygen free radicals, and oxidative stress on the placenta; other articles have described how placental circulation, nutrition, and oxygen concentration are vital to the fetus (Burton et al. 2001; Ferre 2001; Jaffe et al. 1997; Walsh et al. 2000). Increased fetal–maternal transfusion appears to result from the disintegration of the fetal–maternal barrier (Evain-Brion 2000; Morikawa et al. 1997; Pertl and Bianchi 1999; Wang and Walsh 1998).

It is well known that NO_x species from coal combustion are hemoglobin oxidants; however, the mechanism of toxicity of inhaled SO₂ is not fully understood. Low levels of SO₂, when inhaled, are immediately neutralized to form sulfite or bisulfite, which is metabolized and eventually excreted as sulfate.

The effect of SO₂ on the antioxidant status and lipid peroxidation of red blood cells and their reactions to ingested, inhaled, and parenterally administered sulfite have been described (Etlik et al. 1997; Jenkins et al. 2000). Inhalation of SO₂ causes bronchoconstriction (Lazarus et al. 1997). An increase in plasma endothelin levels has been observed during severe preeclampsia; because endothelin is a strong vascular constrictor, this suggests a correlation between plasma endothelin levels and the disease process (Dekker et al. 1991). However, the effect of placental transfer of toxicants during pregnancy and its impact on fetal hemoglobin—an immature form that is more readily oxidized than is adult hemoglobin—has not yet been fully clarified.

Conclusion

This prospective study of methemoglobin in pregnancy showed a positive correlation between the level of methemoglobin and exposure to SO₂, NO_x, and other coal combustion products.

Plomin 1 is a coal-powered thermoelectric power plant 3.5 m above sea level, and the

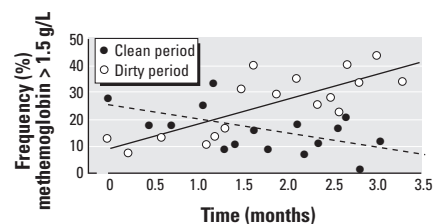


Figure 3. Incidence of methemoglobin > 1.5 g/L in blood samples of pregnant women during the clean and dirty periods. For the clean period, $y = 24.7 - 5.14x$, $r = -0.59$. For the dirty period, $y = 9.0 + 9.11x$, $r = 0.78$.

Table 1. Reproductive loss in clean and dirty periods.^a

Case no.	Maternal methemoglobin level	Pregnancy outcome	Date ^b	Gestational week	Residence location ^c	Weeks of SO ₂ exposure ^d
Clean period (4 April–13 July 1989)						
1	Negative	Twins, ^e one macerated, one death	12 August 1989	11	SW	0
2	Negative	Spontaneous abortion	22 June 1989	15	WSW	0
3	Negative	Spontaneous abortion	25 May 1989	17	SW	1
4	Negative	Premature birth ^f	28 June 1989	27	SW	8
Dirty period (7 December 1989–15 March 1990)						
1	Negative	Spontaneous abortion	22 December 1989	14	SW	14
2	Negative	Spontaneous abortion	31 December 1989	8	SW	8
3	Negative	Spontaneous abortion	30 January 1990	13	SW	13
4	> 1.5 g/L	Stillbirth	1 February 1990	40	SW	22
5	Negative	Spontaneous abortion	7 February 1990	20	SW	20
6	Negative	Spontaneous abortion	11 February 1990	15	N	15
7	Negative	Missed abortion	15 February 1990	13	SW	13
8	> 1.5 g/L	Stillbirth	23 February 1990	39	SW	25
9	Negative	Stillbirth	9 May 1990	41	SSW	35
10	> 1.5 g/L	Premature birth ^g	28 May 1990	28	SW	28

Abbreviations: N, north; SW, southwest; WSW, west southwest.

^aThe "clean" period was from April to July 1989; the "dirty" period was from December 1989 to March 1990. The plant was closed 19 February–6 September 1989. ^bDate of stillbirth, spontaneous abortion, or premature birth. ^cLocation of pregnant woman's residence, which is important to the population density and unfavorable weather conditions (winds) in relation to the source of air pollution and distance from the plant. ^dWeeks each pregnant woman was exposed during the "dirty" period. ^eThe twins were aborted at the same time; the next day the second twin briefly showed signs of life. ^fThe child died 3 days later. ^gThe child died 2 days later.

town of Labin is on a 200–300-m-high plateau 10 km to the southwest. The power plant is in an unsuitable location because northeast winds (40% of all winds) blow pollution directly from the plant to Labin. Instability of the atmosphere, temperature inversions, the chimney height, and fumes could explain the ground-level concentrations of toxic products of coal combustion in the research area.

The role of NO_x as an oxidant is well known, but the role of SO₂ and its metabolites on human antioxidants is not clear and requires further epidemiologic and laboratory research. Methemoglobin, which is a result of exposure to toxic substances in the environment and which may lead to hypoxia and hypoxemia in pregnant women, has an important influence on maternal health and placental and fetal development.

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