

ADVERSE HEALTH EFFECTS ASSOCIATED WITH METHEMOGLOBINEMIA IN CHILDREN

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Abstract. In order to point out some health effects associated with methemoglobinemia in children, we investigated a battery of oxidative and antioxidant defence bioindicators (methemoglobin, reduced glutathione, lipid peroxides and glutathione peroxidase) in 336 children aged 0-3 years living in eight rural localities within three districts, confirmed as high risk territories for nitrate exposure in drinking water. The results showed significant increased lipid peroxides levels in the children exposed to nitrates vs. unexposed group, as a possible early reversible effect of exposure. The investigation also highlighted significant differences of the biochemical changes pattern in 0-3 year aged children at two levels (moderate and high) of nitrate concentrations in drinking water.

Key words: epidemiological study, bioindicators, methemoglobin, lipid peroxides, glutathione

Rezumat. În scopul de a semnala o serie de efecte asupra sănătății asociate cu methemoglobine-mia la copii, am investigat o baterie de bioindicatori oxidativi și de apărare antioxidantă (methemoglobina, glutatation redus, peroxizi lipidici și glutatation peroxidază) la un lot de 336 de copii 0-3 ani din opt localități rurale din trei județe, confirmate ca teritorii cu risc pentru expunerea la nitrați din apa de băut. Rezultatele au arătat niveluri semnificativ crescute ale peroxizilor lipidici la copiii expuși față de martori, ca un posibil efect precoce, reversibil al expunerii. Investigația a mai evidențiat diferențe semnificative între tabloul modificărilor biochimice la copiii 0-3 ani expuși la două niveluri diferite (moderat și înalt) de expunere la nitrați din apa de băut.

Cuvinte cheie: studiu epidemiologic, bioindicatori, methemoglobină, peroxizi lipidici, glutatation

BACKGROUND

Our previous experimental studies concerning the health effects of nitrates and/or nitrites exposure by drinking water outlined significant modifications of methemoglobin (MetHb) levels associated with other biochemical disorders (1):

- increased values of MetHb after a 7 days-subacute exposure of Wistar white rats to nitrites (30 mg NaNO₂/body weight/day) comparing to control; more increased values after a 90 days-exposure of rats to

nitrates (45 mg NaNO₃/kg body weight/day);

- decreased values of blood reduced glutathione (RG) levels after 7 days- and 90 days-exposures to nitrites;
- increased values of lipid peroxides (LP) levels and glutathione peroxidase (GPx) activity after 90-days exposure to nitrates and to nitrites;
- after a 7 days-recovery of white rats exposed for 7 days to nitrites, the experimental results have emphasized the reversibility of toxic effect on Hb (MetHb values have

normalized) but the RG, LP and GPx levels were still increased. The analysis of these experimental results and those ones obtained in five cross-sectional studies on 0-3 years children exposed to nitrites (urban area) and of nitrates (rural areas) in drinking water of Bacău district (2), showed the same pattern of MetHb values modifications. There is still an important notification to be made: if in both experimental and population studies, after cessation of nitrates/nitrites exposures, the MetHb values have normalized, the experimental data have indicated that this normalization was accompanied by high significant levels of LP and GPx comparing to unexposed group. Gupta and col. (3) have also found high cytochrome-b₅ reductase activity in children and adolescents consuming high levels nitrate-drinking water, as an adaptation effect to reduce MetHb. Based on our experimental findings and on other epidemiological data (3) we have been interested to find out the biochemical pattern in children

exposed to high nitrate level-drinking water. For this purpose, the battery of the already experimentally-investigated bioindicators was utilized.

STUDY DESIGN

For checking up our experimental results we have conducted, between 1999 and 2000, a cross-sectional study on 0-3 years aged children in rural areas of three districts of eastern Romania: Botoșani, Iași and Galați. The preliminary results found in Botoșani district were presented elsewhere (4). Recent studies have indicated the presence of high risk rural territories, with frequencies of inadequate drinking water samples up to 96% of the total water samples investigated (5).

The acute infant intoxications by nitrates are annually reported in the rural areas of Moldova. In 1996-2000 period, 979 cases were diagnosed, representing an annual incidence rate of 3.26 ‰ (with territorial variations between 1.50 and 7.00 ‰).

Table 1. Frequency of water samples with nitrate concentration over MAC* in rural territorial collectivities in Moldova (% of total samples analyzed)

District	No. of investigated localities	Period of study	No. of water samples	Frequency of samples over MAC (% of total samples analyzed)
Botoșani	120	1999	1468	94.70
Galați	1	2000	144	76.40
Iași	5	2000	159	79.20

*MAC – maximum allowable concentration

There is a growing trend in Moldova territory for the last five years, more increased in Iași, Botoșani, Galați and Bacău districts, recognized as high

risk areas. The territorial distribution of intoxication cases outlined the high risk areas within these districts.

ADVERSE HEALTH EFFECTS ASSOCIATED WITH METHEMOGLOBINEMIA

Eight localities within the three districts (Botoșani, Iași, Galați) confirmed as highest risk territories for nitrates exposure by drinking water were selected for epidemiological investigations on the chronic exposure effects in 0-3 years aged children.

366 children 0-3 y old (14.9% of total 0-3 y group) were investigated in these

localities for MetHb, RG, LP and GPx levels in blood samples. The sample of 366 children was divided in two groups – exposed and unexposed – according to the nitrate concentration in drinking water. Sex and age distribution of children are presented in table 2.

Table 2. Sex and age distribution of children

Groups of children	Age groups							
	0 – 1 year		1 – 2 years		2 – 3 years		Total	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
Exposed group	25	27	52	36	81	62	158	125
Unexposed group	9	8	17	9	17	23	43	40
Total	69		114		183		366	

MATERIAL AND METHODS

The heparinated blood samples of children were kept in refrigerator for two hours until the analyses.

The estimation of MetHb concentration (expressed as a percentage of hemoglobin) was performed using the modified method of Evelyn and Malloy (6). The MetHb values up to 3% as normal upper limit were considered (6).

The measurement of RG in whole blood was spectrophotometrically made using Ellman reagent, and expressed as $\mu\text{mol/g Hb}$ (7).

The remaining blood was processed for plasma preparation and kept at -20°C for maximum a week until the other bioindicators were measured.

The LP levels were measured by the thiobarbituric acid-method, expressed in $\mu\text{mol malon dialdehyde/L plasma}$

(8). The GPx activity was performed using hydrogen peroxide as substrate and Ellman reagent as colour reagent, being expressed in U/L plasma (9).

The data were subjected to statistical analysis using Excel software to establish average values (\bar{x}), standard deviations (σ), 95 % confidence intervals (CI) and t-Student test.

RESULTS

The comparison of bioindicators levels measured in the two groups of 0-3 years aged children indicated no significant differences for MetHb, RG and GPx values. Only the LP levels were significantly increased ($p < 0.001$) in the exposed group vs. unexposed (Table 3).

The data shown in Tables 4 and 5 indicated that the most children of

unexposed group (67 of a total of 83 children) had increased MetHb levels ($p < 0.001$) associated with increased PL and GPx values ($p < 0.001$ and $p < 0.01$, respectively) and decreased RG values

($p < 0.05$), until 49 children of the exposed group (of a total of 283 children) had MetHb levels under 3% (as normal upper limit).

Table 3. Levels of bioindicators in the group of children exposed to nitrates in drinking water vs. control group

Groups of children (n)		Met Hb, %	Reduced glutathione $\mu\text{mol/g Hb}$	Lipid peroxides $\mu\text{mol MDA/L}$	Glutathione peroxidase U/L	NO_3^- concentration in drinking water mg/L
Exposed group (283)	– $\bar{X} \pm \sigma$ (95 % CI)	4.69 ± 1.52 (4.51 – 4.87) NS	6.83 ± 1.09 (6.70 – 6.96) NS	6.83 ± 1.56 (6.65 – 7.01) $p < 0.001$	44.50 ± 6.71 (43.72 – 45.28) NS	207.1 ± 121.0 (193.0–221.2) $p < 0.001$
Unexposed group (83)	– $\bar{X} \pm \sigma$ (95 % CI)	4.64 ± 1.67 (4.28 – 5.00)	6.99 ± 1.30 (6.71 – 7.27)	5.79 ± 1.69 (5.43 – 6.15)	43.47 ± 7.54 (41.85 – 44.09)	22.0 ± 14.1 (19.0 – 25.0)

Table 4. Average values of bioindicators according to MetHb values in the unexposed group of children

MetHb values (n)	MetHb, %	Reduced glutathione, $\mu\text{mol/g Hb}$	Lipid peroxides, $\mu\text{mol MDA/L}$	Glutathione peroxidase, U/L
$\leq 3\%$ (16)	2.44 ± 0.49	7.69 ± 1.39	4.13 ± 1.79	37.39 ± 4.88
$> 3\%$ (67)	5.17 ± 1.39 $p < 0.001$	6.82 ± 1.23 $p < 0.05$	6.21 ± 1.39 $p < 0.001$	44.95 ± 7.35 $p < 0.01$

Table 5. Average values of bioindicators according to MetHb values in the group of children exposed to nitrates in drinking water

MetHb values (n)	MetHb, %	Reduced glutathione, $\mu\text{mol/g Hb}$	Lipid peroxides, $\mu\text{mol MDA/L}$	Glutathione peroxidase, U/L
$\leq 3\%$ (49)	2.40 ± 0.47	6.81 ± 1.42	7.13 ± 1.98	41.62 ± 7.05
$> 3\%$ (234)	5.17 ± 1.18 $p < 0.001$	6.83 ± 1.02 NS	6.76 ± 1.45 NS	45.13 ± 6.49 $p < 0.001$

These results suggested the division of exposed group into two subgroups:
- moderately-exposed group (46-150 mg nitrates/L in drinking water) consisted of 111 children (ME);

- highly-exposed group (over 150 mg nitrates/L) consisted of 172 children (HE).
The moderately-exposed children hadn't elevated MetHb levels comparing to

ADVERSE HEALTH EFFECTS ASSOCIATED WITH METHEMOGLOBINEMIA

control group, neither RG and GPx levels, but PL levels are significantly increased ($p < 0.001$) in these cases (Table 6). The biochemical significance of PL level increase in moderately-exposed children could be related to the already recognized oxidative effects of nitrates including lipid peroxidation, as an early damaging effect. Because this biochemical change was found in moderately-

exposed children without a significant increase of MetHb levels, it could be considered as an early, reversible effect, preceding the Hb oxidation. The highly-exposed children had elevated MetHb levels ($p < 0.05$) comparing to unexposed group, associated with significant changes of all bioindicators investigated in children (Table 6).

Table 6. The bioindicators levels in moderately-and highly-exposed children vs. unexposed group

Group of children (n)		NO ₃ conc. in drinking water, mg/L	MetHb, %	Reduced glutathione, $\mu\text{mol/g Hb}$	Lipid peroxides, $\mu\text{mol MDA/L}$	Glutathione peroxidase, U/L
ME group (111)* I	- $\bar{X} \pm \sigma$ (95 % CI) p(II vs.III) p(I vs.II)	101.4 \pm 28.3 (96.1-106.6) < 0.001 < 0.001	4.28 \pm 1.42 (4.02-4.54) NS < 0.001	7.08 \pm 1.05 (6.88-7.27) NS < 0.01	6.71 \pm 1.71 (6.39-7.03) < 0.001 NS	43.17 \pm 6.23 (42.00-44.34) NS < 0.01
HE group (172)** II	- $\bar{X} \pm \sigma$ (95 % CI) p(II vs.III)	277.2 \pm 94.9 (263.0-291.3) < 0.001	5.00 \pm 1.37 (4.80-5.20) < 0.05	6.68 \pm 1.05 (6.52-6.84) < 0.05	6.90 \pm 1.05 (6.74-7.06) < 0.001	45.46 \pm 6.50 (44.49-46.43) < 0.05
Unexposed group (83)*** III	- $\bar{X} \pm \sigma$ (95 % CI)	22.1 \pm 14.2 (19.0-25.1)	4.57 \pm 1.65 (4.22-4.92)	7.07 \pm 1.26 (6.80-7.34)	5.76 \pm 1.67 (5.39-6.12)	43.14 \pm 7.35 (41.56-44.72)

*ME moderately-exposed group (46 - 150 mg nitrates/L in drinking water)

**HE group – highly-exposed group (over 150 mg nitrates/L in drinking water)

*** Unexposed group (under 45 mg nitrates/L in drinking water – MAC)

The analysis of the two exposure levels (Table 6) indicated that all bioindicators but LP have significantly changed. This fact could be explained by GPx increased activity found in highly-exposed group of children vs.

those ones moderately-exposed. This enzyme catalyses the LP detoxication in organism and its induction (via MetHb- and GSH-reductases system) is a classical mechanism of antioxidant defense. These findings are similar to

other previous results obtained in a population - rural of Bacău district (10). In that study a significant increase ($p < 0.001$) of MetHb levels was found in 130 of 0-3 years aged children highly-exposed to nitrates vs. MetHb levels in 120 children moderately-exposed to nitrates in drinking water.

Other possible explanation of these results could be related to the inflammation of gastrointestinal system, frequently associated with infant nitrate intoxication.

Given the involvement of free radicals, especially nitric oxide, in the inflammation mechanism, further research are necessary to clarify the biochemical disorders associated with infant nitrite exposure.

CONCLUSIONS

1. The significantly-increased PL levels found in children exposed to nitrates vs. unexposed group could be an early, reversible effect of exposure, which probably precedes the increase of MetHb.
2. All the bioindicators investigated in the highly-exposed subgroup to nitrates emphasized significant changes vs. unexposed group, proving the relationship between the nitrate exposure by drinking water and the biochemical changes associated with the increase of MetHb levels.
3. The comparison of the two exposure levels-effects outlined the biochemical changes pattern in 0-3 year aged children and suggested possible preventive

measures according to the level of exposure to nitrates in drinking water to counteract these effects.

4. The results suggest that the methemoglobinemia, as main health effect of high nitrate concentration in drinking water, is only the top of the iceberg and further research are needed to clear up these aspects

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ADVERSE HEALTH EFFECTS ASSOCIATED WITH METHEMOGLOBINEMIA

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