A Nested Case–Control Study of Methemoglobinemia Risk Factors in Children of Transylvania, Romania

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In this nested case–control study, we investigated the risk factors for methemoglobinemia (MHG) in 71 children in the Transylvania region of Romania. This study was unique in that the exposures for cases and controls were calculated as continuous values and were reported in milligrams per kilogram per day of nitrate/nitrite based on careful dietary reconstruction and environmental sampling. This procedure allowed us to compare point estimates of nitrate/nitrite exposure with other continuous, categoric, and ranked risk factors such as the presence or absence of diarrheal disease, reported severity of diarrheal disease, the use of vitamin supplements, the presence, absence, and/or duration of breast-feeding, and whether or not first-generation relatives experienced MHG. Analysis of these factors and exposure levels using both univariate and multivariate whole-model tests was performed to understand the relative significance of risk factors at varying levels of exposure to the development of MHG. Univariate and multifactorial analysis of risk factors for MHG underscored that, for this population, MHG is most strongly associated with nitrate/nitrite exposure through the dietary route (p = 0.0318), via feeding of formula and tea made with water containing high levels of nitrates, and that breast-feeding protects infants younger than 6 months of age (p = 0.0244). Our findings also raise questions about the role of diarrheal disease in the development of MHG, as likelihood ratios (likelihood 4.323, p = 0.0376) and multifactorial analysis indicated a significant role for diarrheal disease for some individuals.
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Many opportunities arise during the course of research including the opportunity to "nest" epidemiologic studies focusing on related, but separate, research questions (*1*). Such an opportunity occurred during the course of a recent pilot, retrospective cohort study examining the neuropsychologic impacts of nitrate exposure in children 5 years of age in the Transylvania region of Romania (*1,2*) (Figure 1). A joint Romanian/American research team developed an additional, nested case–control study examining risk factors for the development of methemoglobinemia (MHG).

Recently, questions have been raised about the role that diarrheal disease and infectious and inflammatory states play in the development of infantile MHG (*3,4*). The current study provided an opportunity to examine the risk factors for MHG in a relatively large group of infants.

The development of a detailed exposure assessment (milligrams per kilogram per day) for nitrate/nitrite intake, considering all dietary sources of nitrate/nitrite for each of the children in the study, allowed us to examine the relationship between nitrate/nitrite exposure as a risk factor for MHG and other risk factors such as diarrheal disease. Further, the use of a continuous exposure value made this analysis easier to accomplish through univariate, relational chi-square tests, the likelihood ratio and Pearson tests, and multivariate analyses—in this instance, the Wald chi-square statistic (*5*).

Ferrous iron associated with the hemoglobin molecule is sensitive to oxidative stress (*6–8*). A certain amount of auto-oxidative stress normally occurs and oxidizes ferrous iron (*9*). This leads to a normal methemoglobin level in the blood of about 1–2% (*10*). Ferric (Fe^{3+}) iron is reduced to the ferrous (Fe^{2+}) state again through reductase-mediated electron transfer involving electrons contributed by NADH (reduced diphosphopyridine nucleotide) and cytochrome b5 reductase (methemoglobin reductase), which reduces the methemoglobin (*8,10,11*).

Clinical MHG is defined as a condition of the blood in which > 2% of the hemoglobin is in the oxidized or methemoglobin state containing ferric iron molecules, although clinical symptoms are generally not present until the blood reaches 3–15% Fe^{3+}, depending on the individual (*12*). MHG can be induced by environmental contact with a number of substances including nitrates/nitrites in drinking water, inhalation of gaseous nitrites such as amyl nitrite and butyl/isobutyl nitrite, and ingestion or exposure to drugs, environmental toxins, and/or industrial by-products such as nitrobenzene, anilines, and copper sulfates (*11,13–15*). Dysfunctional and unstable hemoglobin due to genetic factors may also create a clinical MHG (*11,16–18*).

Infants 6 months of age and younger are at higher risk of MHG (*14,19–24*). In the newborn infant, the activity of the NADH–cytochrome b5 reductase is about 50–60% of the activity found in the adult (*8*). Likewise, the intestinal pH in infants is high enough to contribute to the growth of intestinal organisms that are particularly efficient at the conversion of ingested nitrate to nitrite (*25–27*).

Diarrheal disease has been explored recently as a cause of MHG at lower environmental exposures near the maximum contaminant limit (MCL) (*3,4,28*). The physiologic basis for this relationship is the activity of the macrophages involved in the inflammatory/defense response, which induce the production of a variety of free radicals including nitric oxide radical, NO· (*29–31*). It has been postulated that infants experiencing gastrointestinal irritation from infectious agents or allergic reactions to soy or cow's milk proteins have an elevated endogenous production of nitric oxide radical that leads to formation of excessive methemoglobin levels in the blood (*3,32–35*).

Several case histories reinforce this observation (*36,37*). Sager et al. (*38*) reported the case of an infant less than 6 months of age with renal disease, acidosis, and MHG. Other case histories have linked elevated methemoglobin levels to diarrheal disease caused by *Campylobacter* sp. and generalized septic shock in the infant (*39,40*). An early study conducted in Czechoslovakia (*41*) noted the presence of *Bacillus subtilis* organisms in the powdered formula of infants suffering from MHG. Although this is a common bacterial species, it is not necessarily known for its pathogenicity. A prospective study of infantile diarrheal cases in Arizona examined the methemoglobin levels of infants and found elevated levels of methemoglobin (*42*). In particular, these investigators found an association between

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the child's ranking in weight for age and the incidence of methemoglobin in the blood above the physiologic normal value of 1% (*42*). Data concerning children in the lower 10th percentile of weight for their age exhibited an analysis of variance (ANOVA) *f* factor of 0.039 for low weight and incidence of elevated methemoglobin level (*42*).

The association between endogenous production of nitric oxide radical, as mediated by macrophages, involved in the infectious and inflammatory response has been a topic of interest and toxicologic research for more than 10 years (*43–48*). Debate remains concerning the significance and amount of this endogenous nitric oxide production in terms of the organism's overall oxidative stress (*49,50*). However, a case history or a case series is descriptive in nature and is thus only able to raise questions rather than test associations. Testing associations between risk factors and disease is the role of the analytic study design, such as the case–control study.

Methods

The retrospective cohort study, in which this case–control study was nested, was designed to recruit 80 individuals: 30 previous MHG cases, along with their parents, into a high-exposure cohort (cohort I, cases); 25 exposed controls with no experience of MHG, along with their parents, into an exposure without infantile MHG cohort (cohort II, controls); and 25 more controls, also having no experience of MHG, along with their parents, into the no significant exposure and no infantile MHG cohort (cohort III, controls). The cohorts were to be individually matched on the basis of socioeconomic status, sex, and age. Restriction criteria for entering the study were a chronologic age of 5 years at the time of the study, Romanian speakers, the absence of significant previous neurologic sequella such as polio and head injury, the absence of a known chelated case of lead poisoning, no severe malnutrition and anemia, and a birth weight of ≥ 2,000 g; in the assumed high-exposure cohort or cases, the restriction criteria also included a clinically diagnosed case of infantile MHG. Some of these exclusion criteria were of greater significance to the cohort study than to this nested case–control study.

Due to difficulties in recruiting cases that met the age- and sex-matching restrictions on an individual basis and due to attrition when informed consent was given, 26 highly exposed cohorts (cohort I) who had experienced an episode of MHG, 22 exposed cohorts (cohort II) who had not experienced a case of MHG, and 23 low- or no-exposure cohorts, who also had not experienced a case of MHG (cohort III) were recruited and completed the study. This necessitated that the study subjects be frequency or category matched for the criteria of age, sex, and socioeconomic status. Table 1 presents an overview of the cohort characteristics. In terms of the nested case–control study, we referred to cohort I as infantile MHG cases, while cohorts II and III are the category-matched controls.

The overall sample was a convenience sample design in which cohorts were stratified according to known, previously established ranges of nitrate in drinking water in the regions in which the subjects lived. Cases and controls were recruited from the Sanitary Police records in the counties of Alba, Satu-Mare, Maramures, Salaj, Mures, and Bistrita-Nasud, Romania. Cases were located via records from the district physicians' offices coordinated through the Sanitary Police Headquarters for that county. Known cases of infantile MHG meeting the recruitment criteria (a clinically diagnosed case of MHG with positive ascorbic acid response) and not exhibiting the exclusion criteria (hereditary MHG, medication- or dye-induced MHG, early perinatal central nervous system damage, birth weight < 2,000 g, and not speaking Romanian as a first language) were admitted to the study. Controls were recruited by randomly selecting records from the district physicians' offices, coordinated through the Sanitary Police Headquarters for the respective county. Once again, the recruitment and exclusion criteria were applied, with the exception of the additional exclusion criteria that no known cases of infantile MHG were recruited as controls.

The data collection tool, a survey, was carefully translated into Romanian by Romanian/English-speaking scientists, reviewed by senior research personnel for both the Romanian and American research teams, and piloted in the field on four subjects. Subjects were then contacted, and full, informed consent of the study participant was obtained and an interview scheduled. Interviews were conducted in person with the primary caregiver serving as proxy for the child. Interviewees were identified by subject code, questionnaires were identically designed between cases and controls, and the interviewer was trained to carefully follow the script in every instance. Despite this, it is possible that the interviewer could determine the case and control status of the subject by their response to questionnaire items (recall bias is further addressed in the following section). The interview lasted 1 hr and was carried out in Romanian. After the interview, a yard and well inspection was performed, and environmental and biologic samples were taken as necessary (blood for lead levels, stool for parasites, water for nitrates). Data were obtained from these interviews and biologic sampling to reconstruct the level of exposure and examine risk factors relative to MHG.

Because this was a nested case–control study, part of a separate cohort study working with children who were 5 years of age at the time of the study, the primary caregiver was asked to recall the dietary habits of the child at both 2 and 6 months of life, as average age at the MHG incident for the cases was approximately 2 months of age (Table 1). This is a concern because recall bias is always an issue for case–control study designs. The literature dealing with survey design related to dietary recall indicates that, while dietary recall can be up to a 36% underestimate of the true dietary intake, certain design features aid in the accuracy of reconstructed dietary intake (*51,52*). These design features include structured questions carefully followed by the interviewer (*53*). Further, structured dietary questions provided an opportunity for the interviewee to respond to food category, amount, and frequency separately. The use of structured recall techniques contributed to increased recall accuracy (*53*).

We paid particular attention to the dietary habits and food and beverage choices listed in the feeding history portion of the questionnaire (*54–56*). We reviewed these questions with Romanian/English-speaking scientists with expertise in the area of nitrates and dietary exposures and Romanian dietary practices and habits. The survey instrument and dietary recall history was identical for

Figure 1. Map detailing the Transylvania region of Romania.

Table 1. Characteristics of cohorts.

Characteristic	Cohort I	Cohorts II/III
Age at time of study	≤ 5 years	≤ 5 years
Age at MHG incident	≤ 2 months	—
Sex (%)		
Male	40	47
Female	60	53
Total no.	26	45

cases and controls. Traditional foods and beverages commonly included in the diet of infants < 6 months of age were included in a food inventory/frequency format to assure that the widest possible choices were provided to Romanian caregivers being interviewed about their child's feedings (*54–56*).

A certain degree of recall bias on the part of cases is inevitable, although research indicates that the recall of particularly personal, salient, and highly relevant events, such as the recall by a parent of the first 6 months of their child's life, is often much clearer than other events (*51*,*53*). Another benefit of the exposure study design used here is that individual exposures are calculated for each study participant, and errors in classification based on actual versus calculated exposures will tend to distribute evenly in a nondifferential manner as they occur (*2*,*57*). To examine the possibility of recall bias functioning between cases and controls, data were spot checked by evaluating the amount of chi (tea) feeding reported between cases and controls. Tea is one of the most important sources of nitrate poisoning in this study group. Boiling the tea concentrates nitrate in the liquid, which is then bottle fed to infants. Our hypothesis was that, if significant recall bias were operating, cases would recall more clearly tea feedings for measures of both dose and frequency than would controls. This hypothesis was not supported, however; an ANOVA (SAS, Version 4; SAS Institute, Cary, NC, USA) run on cases and controls relative to the amount of reported tea feeding in milliliters per day (F ratio = 0.8912, p = 0.35004) revealed no significant difference between the groups.

Results

Family background and prenatal/pregnancy data indicated that both mother and father were, on average, in their late twenties to early thirties. The mother's occupation was overwhelmingly homemaker and the father's occupation was overwhelmingly laborer, with good agreement between cases and controls. This compares well with the rest of the Romanian population. Only small percentages of the mother's or father's relatives had experienced a case of MHG according to their recollection (Table 2). These findings suggest that socioeconomic status between the cases and controls were comparable, as were the ages of the parents. Children in both groups likely received comparable maternal attention, and close relatives did not present a pattern of intergenerational MHG.

Table 3 presents findings related to MHG, cyanosis, and diarrheal disease. Several areas of interest are apparent, including the association between MHG and diarrhea, which is marginally significant when analyzed using likelihood ratios and Pearson's chi-square tests of association (likelihood ratio = 4.323, p = 0.0376). This association is not as highly significant as the association between MHG and nitrate/nitrite exposure (likelihood ratio = 29.7, p = 0.0001).

Despite the cases' stronger association with nitrite/nitrate exposure than with diahrreal disease, the findings suggest that more cases than controls experienced recurring bouts of diarrheal disease, some of which were of slightly greater intensity in cases than in controls. However, cases overwhelmingly indicated that the diarrheal episodes were not associated with cyanosis. Cyanosis persisted for a few hours and in a small percentage (8%) for a few days. This may be related to use of ascorbic acid in Romania, rather than methylene blue, to reverse MHG, or it may reflect delay in obtaining emergency medical intervention. Only 20% of the cases (five individuals) reported other siblings experiencing a diagnosed case of MHG.

Another important area of concern is well-water sources, well depth, the level of well-water nitrate (parts per million), and boiling of liquids. Findings in this area indicate that controls had more diverse choices in regard to water source, and cases had significantly higher levels of nitrate in their potable water than did controls (Tables 4 and 5).

More than 90% of controls and 79% of cases reported boiling water for infant formula preparation. The duration of boiling for both cases and controls was adequate to kill most pathogenic organisms (*58*). Cases reported fewer choices in water sources than did controls, and well depth in both cases and controls was similar. Breast-feeding, the use of medications during breast-feeding, and the use of vitamins and mineral supplements for the infants were also examined. Select findings are presented in Table 6.

A high number of cases and controls breast-fed at least initially during their child's infancy. Significantly, however, the cases breast-fed for considerably shorter periods of time, although the frequency of breast-feeding was comparable between cases and controls. Further, infants in the control group were 3-fold more likely to be given vitamin and mineral preparations. The majority of these preparations were multivitamin supplements containing vitamins D and C. Twenty-five percent of the cases gave vitamin C exclusively after the MHG incident and 15% of controls gave vitamin C regularly. During breast-feeding, only a small percentage (12%) of cases reported the use of ampicillin.

Exposure values to nitrate are provided for both cases and controls in Table 7. Although exposure ranges and values are reported as nitrates, as a validation of the exposure assessment, a correlation between actual levels of nitrates in well water and the calculated nitrite exposure in milligrams per kilogram per day was run, giving a correlation of 0.71 (F ratio = 170.7, p < 0.0001; see the bivariate fit of calculated nitrite exposure by nitrate level in the well in Figure 2). The lower limits of the high-exposure category were determined by using the current MCL of 10 ppm nitrate-nitrogen (1 ppm nitrite). As Table 7 indicates, when the exposure assessment was performed on cases and controls, there was a small percentage of individuals who had experienced a particularly high nitrate/nitrite exposure who did not develop MHG and a small percentage of individuals who experienced a particularly low nitrate/nitrite exposure that did develop MHG. This led us to examine additional risk factors

Table 2. Characteristics of parents determined by prenatal and pregnancy survey questions.

Characteristic	Mother	Father
Age at interview (mean ± SE; n =71) (both cases and controls)	29 ± 0.58 years	32 ± 0.70 years
Occupation (n =71)	Homemaker > 90% (cases and controls)	Cases 92% laborer Controls 84% laborer
MHG	Cases 3.8% (1/26)	Cases 3.8% (1/26)
MHG reported in relatives	Controls 3.6% (3/45)	Controls 2.2% (1/45)

Table 3. Methemoglobinemia, diarrhea, and cyanosis reported in cases (n = 26) and controls (n = 45).

	Percent of cases (SE)	Percent of controls (SE)
Diarrhea	54 (0.10)	28 (0.07)
Severity of diarrhea		
Rare	64 (0.13)	86 (0.10)
Often	29 (0.12)	14 (0.09)
Very often	7 (0.07)	—
No cyanosis with diarrhea	92 (0.6)	100 (0)
Duration of MHG episode		
< 1 hr	4 (0.04)	—
Few hours	88 (0.06)	
Few days	8 (0.05)	
Cyanosis of siblings needing treatment	20 (0.08)	—

reported by these individuals that may have contributed to these findings such as breast-feeding presence or absence and the frequency of diarrheal disease. As Table 7 illustrates, nearly identical percentages of both cases and controls reported recurring bouts of diarrheal disease. However, the four cases that received doses of nitrate/nitrite below the MCL (in the medium exposure category) all reported recurring bouts of diarrheal disease, while the remaining individuals in the medium exposure category (11 individuals) did not report recurring bouts of diarrheal disease—a possible basis for the correlation between diarrheal disease found in the likelihood ratio test. To further confuse the issue, a full 48% of controls reported recurring diarrheal bouts without a case of MHG. Clearly, there is some pattern of relationship here between diarrhea and MHG, but the etiology and root cause remain vague.

Other biologic parameters of interest included the determination of blood lead, hemoglobin, hematocrit, and the presence or absence of parasites in stool samples. Table 8 presents descriptive statistics in regard to whole-blood parameters, blood lead, and biologic samples. Although the cases and controls are comparable in terms of hemoglobin and hematocrit levels, the mean blood lead level in the cases is about 1 µg/dL above the recommended limit of 10 µg/dL (statistically significant, $p = 0.0187$). It should also be noted that a majority of the lead burden was found in a few highly exposed individuals, and in the cases, 50% of the sample exhibited a level of ≤ 10 µg/dL, while 75% of the controls exhibited blood lead levels < 10 µg/dL.

No evidence was seen of a statistically disproportionate parasite burden between cases and controls using chi-square analysis ($\chi^2 = 3.55$, $p = 0.06$). However, only 19 (73%) cases and 28 (62%) controls provided a stool sample for analysis, and thus nonparticipation limits the certainty of the findings, and the dichotomous nature of the variables decrease the sensitivity of the test (*5*).

Finally, it should be noted that no significant findings were seen in regard to environmental parameters such as prenatal exposure to solvents. No individuals reported the use of herbal medications or home remedies. The mix of home heating methods was comparable between cases and controls (primarily wood-burning and gas stoves). No individuals reported headaches that resolved upon leaving the house, and neither cases nor controls reported the significant use of room deodorizers or incense.

Univariate and multivariate analyses. Given current questions surrounding the causes of infantile MHG at lower exposure ranges, univariate and multiple logistic analyses were run on known risk factors for MHG relative to the individual's exposure classification, history and intensity of diarrheal conditions, duration of breast-feeding, and use of vitamin supplements. This was performed on both the data as a whole, comparing cases to controls, and for the select group of high- and medium-exposure individuals that had reassorted as a result of the exposure assessment, which included both cases and some controls. The high-exposure cases and controls were analyzed separately from the medium and low exposure of the cases and controls in an effort to understand what differences may have accounted for the resistance of the high-exposure controls to developing MHG. Summaries of chi-square values, probabilities, and ANOVA on means are presented in Table 9 for all cases and controls and Table 10 for the high-exposure group cases and controls.

A cross-tab analysis (univariate) comparing only the gross presence or absence of diarrheal conditions with presence or absence of diarrhea indicated that a degree of relationship did exist between reporting diarrheal episodes and MHG (likelihood = 4.323, p =

Table 4. General characteristics of water for cases and controls ($n = 71$).

Characteristic	Percent	Mean (SE)
Well depth ≤ 10 m	84	—
Well covered	61	—
Depth to water surface (m)	—	3.8 (0.3)

Table 5. Water and water source characteristics by case and control status.

Characteristic	Cases ($n = 26$)	Controls ($n = 45$)
Water source before MHG incident (%)		
Well	96	59
Tapped or piped	4	39
Bottled	0	2
Boiled water used for formula (%)	79	93
Duration (min) of boiling [mean (SE)]	13 (1.1)	13 (1.1)
Well depth (m) [mean (SE)]	7.84 (0.56)	9.11 (1.18)
Nitrate level in well (ppm)		
Mean	253	28
Range	47.2–1,185	0–157

Table 6. Breast-feeding, medication use, and vitamin use.

	Cases ($n = 26$)	Controls ($n = 45$)
Breast-fed (%)	92	100
Median duration of breast-feeding (months)	0.75	4.0
Median frequency of breast-feeding	6 times/day	6 times/day
Infant given vitamins or minerals (%)	15	42
Mother took medications during lactation (%)	12	0
What type of medication did mother take?	Ampicillin	NA

NA, not applicable.

Table 7. Exposure levels to nitrate and reported diarrheal disease in cases and controls with distribution by high, medium, and low exposure categories.

		No. (%) individuals			
	Dietary exposure (mg/kg/day)	High (> 10 ppm)	Medium (1–10 ppm)	Low (< 0.5 ppm)	No. (%) reporting diarrheal disease
Cases					
Range	2.83–451.20	22 (84.6)	4 (15.4)	0 (0)	14 (51.85)
Mean nitrate	103.6				
Controls					
Range	0–182	13 (28.9)	11 (24.4)	21 (46.6)	13 (48.15)
Mean nitrate	11.2				

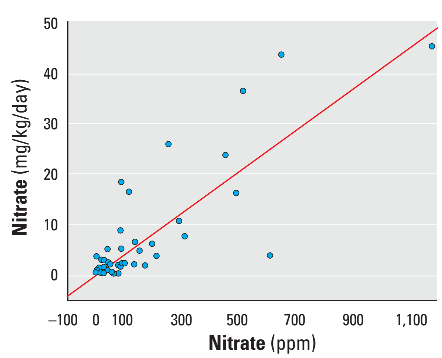

Figure 2. Bivariate fit of nitrite exposure by nitrate level in wells. The red line indicates linear fit.

0.0376). Cross-tabs performed independently for nitrate exposure category and MHG indicated a stronger relationship (likelihood = 29.7, $p < 0.0001$), and, as illustrated in Table 9, cases (92%) and controls (100%) overwhelmingly reported no cyanosis accompanying diarrheal episodes. Thus, these risk factors were compared along with a number of others using regression analysis (multivariate) to determine which factors were most predictive of positive MHG outcome.

For this sample as a whole, infant exposure to nitrate/nitrite was more highly correlated with infantile MHG than was the presence or absence of diarrheal conditions. This is further supported by the finding that intensity of diarrheal episodes (rarely, often, frequently) did not appear to be correlated with the presence or absence of MHG, as indicated in Table 9. Duration of breast-feeding, which is protective for MHG, was significantly associated with the presence or absence of MHG. The use of vitamins was also significantly associated with a negative MHG status under a multivariate analysis, perhaps as a result of vitamin C intake with vitamin supplements (*7,59*).

Table 10 indicates that the most significant difference between the highly exposed controls and the highly exposed cases is that the former group was breast-fed for a longer period of time ($p = 0.0398$), and they experienced less frequent diarrhea. Further, it is probable that other as yet unrecognized factors may interact with individual susceptibility as it relates to the ability to handle oxidative stresses.

Additional multivariate analyses were performed on variables exhibiting a close association with risk of infantile MHG. These tests involved multiple, stepwise logistic regression on mixed continuous and nominal variables. Table 11 summarizes the findings of multiple stepwise regression for positive and negative methemoglobin status by the variables exposure level, frequent bouts of diarrhea, duration of breast-feeding in months, and vitamin use.

Table 10 indicates that for this population the model predicts a strong association between nitrate/nitrite exposure, duration of breast-feeding, which is protective of nitrate/nitrite exposure, and infantile MHG. These factors were more strongly correlated with MHG outcome than was frequent diarrhea. Further, vitamin use remained an important factor but not strongly associated under a whole-model analysis.

Discussion

Univariate and mulifactorial analysis of risk factors for MHG from the case–control perspective underscore that, for this population, MHG is most strongly associated with nitrate/nitrite exposure through the dietary route ($p = 0.0318$), via feeding of formula and tea made with water with high levels of nitrates. Our results also show that breast-feeding protects infants younger than 6 months of age from diarrheal disease ($p = 0.0244$; Table 11). Although the presence or absence of diarrheal conditions was somewhat predictive of the presence or absence of infantile MHG, under the conditions of univariate analysis, when the data were analyzed multivariately from the perspective of intensity/frequency of diarrheal episodes, the association was no longer significantly predictive of infantile MHG ($p = 0.166$; Table 11). Further, use of vitamin supplements was mildly protective in this group, although sample size was small ($p = 0.0479$; Table 11).

The group of high-exposure individuals comprised both cases and some controls. These controls differed from the cases in two significant ways: They were less likely to have experienced severe diarrhea (likelihood ratio = 4.004, $p = 0.0454$), and they were more likely to have been breast-fed for more than 2 weeks (ANOVA, $p = 0.0398$; Table 10). Only the duration of breast-feeding and the reporting of frequent diarrheal conditions were significant. Although differences appear to exist in this small number of individuals ($n = 30$), the possibility that a case of subclinical MHG went undetected or that feeding history was incorrectly reported by the primary caregiver cannot be ruled out. Thus, a number of factors may be needed to explain the presence or absence of particular outcomes along the fringes of an exposure category (*60*). Without further research it would be difficult to assign these multiple factors primary or secondary status, although it should be noted that diarrheal conditions have been implicated in a number of MHG cases in the case-history literature (*61,62*). However, this ambiguous yet persistent association of MHG with diarrheal disease raises the possibility that confounding exposures or unrecognized intrinsic factors, or both, could be operating in some circumstances, influencing the relationship between diarrheal disease and methemoglobinemia. This issue deserves further investigation, and future work will focus on understanding the relationship between diarrheal disease and MHG and any contributing or confounding factors that are playing a role in this relationship.

Table 8. Biologic parameters for blood and stool samples.

Parameters	Cases (n = 26)	Controls (n = 45)
Blood lead level (µg/dL)	11.3 (1.0)	8.0 (0.46)
Hemoglobin[a] (g)	13.2 (0.22)	12.9 (0.17)
Hematocrit (v/v%)	36.5 (0.35)	36.7 (0.26)
Parasites[b]		
No. positive (%)	1/19 (5)	7/28 (25)
Species present (no.)	*Giardia*	*Giardia* (4)
		Ascaris (2)
		Enterobius (1)

Values shown are mean (SE) except where indicated.
[a]One-way ANOVA; $F = 0.0187$. [b]$\chi^2 = 0.55$; $p = 0.06$.

Table 9. Likelihood ratios and ANOVA relating MHG status to risk factors (cases vs. controls).

Test	Statistic	p-Value
+/– MHG by high, medium, or low exposure category	Likelihood = 29.7	< 0.0001
+/– MHG by +/– diarrhea	Likelihood = 4.323	0.0376
+/– MHG by intensity of diarrhea	Likelihood = 2.496	0.2871
+/– MHG by +/– vitamin use	Likelihood = 5.818	0.0159
+/– MHG by duration of breast feeding		
Positive	Mean = 2.2 months (ANOVA)	0.0093
Negative	Mean = 6.3 months (ANOVA)	

+/–, presence or absence.

Table 10. Likelihood ratios and ANOVA relating MHG status to risk factors for individuals in the high-exposure category (cases vs. controls).

Test	Statistic	p-Value
+/– MHG by intensity of diarrhea (n = 30)	Likelihood = 4.004	0.0454
+/– MHG by duration of breast feeding (n = 27)		
Positive (cases)	Mean = 0.96 months (ANOVA)	
Negative (controls)	Mean = 2.21 months (ANOVA)	0.0398

+/–, presence or absence.

Table 11. Multifactorial analysis of MHG risk factors.

Parameter	Estimate	Probability
Intercept	0.10256991	0.801195
Preexposure levels	–0.1977573	0.0318
Frequent diarrhea	0.86361735	0.1663
Duration of breast-feeding	0.32648915	0.0244
Vitamin use	–0.871533	0.0479

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