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NITRATES IN DRINKING WATER AND THE RISK OF DEATH FROM CHILDHOOD BRAIN TUMORS IN TAIWAN

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The objective of this study was to (1) examine the relationship between nitrate (NO_3-N) levels in public water supplies and risk of death from childhood brain tumors (CBT) and (2) determine whether calcium (Ca) and magnesium (Mg) levels in drinking water might modify the effects of NO_3-N on development of CBT. A matched cancer case-control study was used to investigate the relationship between the risk of death attributed to CBT and exposure to NO_3-N in drinking water in Taiwan. All CBT deaths of Taiwan residents from 1999 through 2008 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health. Controls were deaths from other causes and were pair-matched to the cases by gender, year of birth, and year of death. Information on the levels of nitrate-nitrogen (NO_3-N), Ca, and Mg in drinking water were collected from Taiwan Water Supply Corporation. The municipality of residence for CBT cases and controls was presumed to be the source of the subject's NO_3-N, Ca, and Mg exposure via drinking water. Relative to individuals whose NO_3-N exposure level was $\leq$0.31 ppm, and the adjusted odds ration (OR) (95% confidence interval [CI]) for CBT occurrence was 1.4 (1.07–1.84) for individuals who resided in municipalities served by drinking water with a NO_3-N exposure >0.31 ppm. No significant effect modification was observed by Ca and Mg intake via drinking water. Data suggest that exposure to NO_3-N in drinking water is associated with a higher risk of CBT development in Taiwan.

The U.S. Environmental Protection Agency established a maximal contaminant level (MCL) in drinking water of 10 mg/L as nitrate-N to protect infants from developing methemoglobinemia (Ward et al., 2005). However, the effectiveness of this regulatory threshold limit for preventing other adverse health risks such as cancer has not been adequately studied (De Roos et al., 2003).

Nitrates may act as procarcinogens, interacting with amines and amides in the stomach to form a variety of *N*-nitroso compounds (NOC) (nitrosation), most of which are potent animal carcinogens (Tricker and

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Preussmann 1991) following reduction of dietary nitrate to nitrite in saliva (Walker 1990). The generated nitrite is postulated to produce methemoglobinema and potentially carcinogenic processes. There is evidence of a dose-response increase in urinary nitrate secretion (Kleinjans et al. 1991), and elevated salivary nitrate and nitrite levels among those individuals exposed to higher levels of drinking water nitrates, relative to those subjects with low amounts of these compounds (Van Maanen et al. 1996).

Several studies support a direct relationship between nitrate intake and endogenous formation of NOC. High nitrate levels in drinking water are associated with increased excretion of *N*-nitrosoproline in urine (Mirvish et al. 1992; Moller et al. 1989). Nitrate administered via drinking water was shown to be directly correlated with concentration of total NOC in feces (Rowland et al. 1991). In addition, populations with high rates of esophageal, gastric, and nasopharyngeal cancer excrete high levels of *N*-nitrosoproline (Kamiyama et al. 1987; Lu et al. 1986; Yi et al. 1993). These results indicate a contribution of drinking-water nitrates toward nitrosation and suggest that nitrate intake may be used as a surrogate biomarker for exposure of target tissues to NOC (De Roos et al. 2003).

Brain tumors are the most common solid tumors occurring in children. The etiology of childhood brain tumors (CBT) is largely unknown. The postulation that exposure to NOC and their precursors is related to the risk of CBT is supported by observations in animal studies where Rice et al. (1989) demonstrated that *N*-alkylnitrosoureas induced brain tumors in the offspring of pregnant rodents and monkeys. Most epidemiological studies that examined the role of maternal consumption of cured meats during pregnancy found a significant positive association between maternal intake of cured meats and the risk of development of CBT (Dietrich et al. 2005; Preston-Martin et al. 1996; Kuijten et al. 1990; Sarasua and Savitz 1994; McCredie et al. 1994). Cured meats are a major dietary source of preformed NOC and their precursors (Dietrich et al. 2005). In contrast, no significant association with cured meats consumption was noted in some other studies (Cordier et al. 1994; Bunin et al. 1993; 1994; Lubin et al. 2000).

Given the widespread exposure of populations to nitrate, there are surprisingly few epidemiologic studies concerning the possible association of nitrates in drinking water with development of CBT. Mueller et al. (2001) determined nitrate levels in water supplies using dipstick measurements, often several years after women's pregnancies, and reported no significant correlation between CBT occurrence with levels of nitrate and nitrite. However, an increased risk of CBT occurrence was observed in western Washington State, one of the three study centers, among offspring of women who used private wells as their drinking-water source during pregnancy. In a SEARCH International Childhood Brain Tumor study (Mueller et al., 2004), risk of CBT development did not increase with rising nitrate levels measured in tap water. However, the risk of astrocytoma was associated with increasing nitrite levels measured in tap water of residences of pregnant women.

Several epidemiologic studies reported no significant association or inverse association between dietary nitrate intake and human cancers (Ward et al. 1996; 2003; Forman 1987), which may be attributed to antioxidants and nitrosation inhibitors in nitrate-containing foods (Bartsch et al. 1988). Antioxidants that inhibit endogenous nitrosation include vitamin C and alpha-tocopherol (Ward et al. 2005). Our previous studies showed that the association between NO_3-N exposure and risk of colorectal cancers was influenced by water hardness (calcium [Ca] and magnesium [Mg]) (Chiu et al. 2010; Chang et al. 2010). No apparent previous studies explored whether Ca and Mg levels in drinking water might modify the association between NO_3-N exposure and development of CBT. If a significant effect modification by Ca and Mg levels in drinking water exists, the true magnitude of the association between NO_3-N exposure and CBT occurrence may be obscured. Furthermore, knowledge of the modifying factors may help in

initiation of public policy, risk assessment, and setting threshold standards.

The objective of this study was thus to (1) examine the relationship between NO_3-N levels in public water supplies and the risk of death attributed to CBT and (2) determine whether Ca and Mg levels in drinking water might modify the effects of NO_3-N on development of CBT.

MATERIALS AND METHODS

Study Area

Taiwan is divided into 361 administrative districts, which will be referred to herein as municipalities. These are the units that will be subjected to statistical analysis. Excluded from the analysis were 30 aboriginal townships and 9 islets which had different life-styles and living environments (the diets of subjects in these municipalities are generally rich in fiber, antioxidants, and nitrosation inhibitors, which may yield beneficial properties and act in a way against brain carcinogenesis occurrence). This elimination of unsuitable municipalities yielded 322 municipalities.

Socioeconomic Factors

Each municipality in Taiwan was assigned to a degree-of-urbanization category from 1 to 8 based on the urban–rural classification of Tzeng and Wu (1986), which takes into account variables such as population density, age, economic activity and family income, educational level, environment, and health service-related facilities. A municipality with the highest urbanization score, such as the Taipei metropolitan area, was classified in category 1, whereas mountainous areas with the lowest score were assigned to category 8. The urbanization index used in this study serves as a proxy for a large number of explanatory variables such as socioeconomic status and differential exposures to environmental conditions, which contributed to the etiology of mortality. For the analyses, the urban–rural classification was further divided into four levels: I, metropolitan (categories 1 and 2); II, city (categories 3 and 4); III, town (categories 5 and 6); and IV, rural (categories 7 and 8).

Subject Selection

Data on all deaths of Taiwan residents from 1999 through 2008 were obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health, which is responsible for the death registration system in Taiwan. For each death, detailed demographic information including gender, year of birth, year of death, cause of death, place of death (municipality), and residential district (municipality) were recorded. The cancer case group consisted of all eligible malignant tumors of the brain (International Classification of Disease, 9th rev. [ICD-9], code 191) or cranial nerves (code 192) occurring in individuals between 0 and 19 yr of age. In all, 457 deaths due to CBT with complete records satisfied this criterion.

Controls were drawn from all other deaths excluding deaths due to neoplasms. Control subjects were pair-matched to the cases by gender, year of birth, and year of death. Each matched control was selected randomly from the set of possible controls for each case.

The most frequent causes of death among the controls were motor vehicle traffic accidents of unspecified nature (17.1%), accidental drowning and submersion (11.2%), ill-defined and unknown causes of morbidity and mortality (7.9%), congenital cardiac anomalies (3.1%), paralytic syndromes (2.8%), and accidents caused by unspecified fire (2.8%).

Nitrate-Nitrogen (NO_3-N), Ca, and Mg Levels in Drinking Water

Information on the levels of NO_3-N, Ca, and Mg in each municipality's treated drinking-water supply was obtained from the Taiwan Water Supply Corporation (TWSC) (TWSC/ROC, 1991), to which each waterworks is required to submit drinking-water quality data including the levels of NO_3-N, Ca, and Mg. Four treated water samples, one for each season, were collected from each waterworks. The samples were analyzed by

the waterworks laboratory office using standard methods (cadmium reduction method for NO_3-N and spectrophotometric method for Ca and Mg, respectively). Since the laboratory office examines NO_3-N, Ca, and Mg levels on a routine basis using standard methods, it was presumed that analytical variability was minimal. Among the 322 municipalities, 70 were excluded as these had more than one supply of drinking water and the exact population served by each could not be determined with details provided in earlier publications (Yang et al. 1998; Yang 1998). The final complete data comprised NO_3-N, Ca, and Mg data from 252 municipalities. Calcium and Mg concentrations remain reasonably constant for long periods of time and are a quite stable characteristic of a municipality's water supply (Bell and Doege 1984). Data collected were the annual mean levels of NO_3-N, Ca, and Mg for the year 1990. The municipalities of residence for all cancer cases and controls were identified from death certificates and it was presumed that municipal drinking water was the source of the subjects' NO_3-N, Ca, and Mg exposure via drinking water. The levels of NO_3-N, Ca, and Mg of each municipality were used as indicators of exposure to NO_3-N, Ca, and Mg for an individual residing in that municipality.

Statistics

In the analysis, the subjects were categorized into one of the two NO_3-N exposure categories: low (less than or equal to median among controls; $\leq$0.31 ppm) and high (greater than median among controls; $>$0.31 ppm). Conditional logistic regression was used to estimate the association between NO_3-N levels present in drinking water and risk of death attributed to CBT. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated using the low-exposure group as the referent group (Breslow and Day 1980). The association between drinking-water NO_3-N and risk of CBT occurrence was stratified by Ca and Mg levels in drinking water. The analyses were performed using the SAS software (version 8.2; SAS Institute, Inc., Cary, NC). All statistical tests were two-sided and values of $p < .05$ were considered statistically significant.

RESULTS

In total, 457 CBT cases with complete records were collected for the period 1999–2008. Of the 457 cases, 267 were males and 190 females (Table 1). The mean NO_3-N concentration in the drinking water of the CBT cases was 0.46 mg/L (SD = 0.47). Controls had a mean NO_3-N exposure of 0.43 mg/L (SD = 0.47). CBT cases lived in municipalities in which 91.6% of the population was served by waterworks. For controls this number was 88.9%. CBT cases had a higher rate (44.4%) of residing in metropolitan municipalities than controls (28.4%). CBT cases had a numerically lower rate (46%) of living in municipalities served by drinking water with high levels (>34.8 mg/L) of Ca than controls (47.7%). CBT cases had a similar rate (41.8%)

TABLE 1. Characteristics of the Study Population

Parameters	CBT cases (n = 457)	Controls (n = 457)
Enrollment municipality	252	252
Gender		
Male	267 (58.4%)	267 (58.4%)
Female	190 (41.6%)	190 (41.6%)
Age (yr)		
0–9	260 (56.9%)	260 (56.9%)
10–19	197 (43.1%)	197 (43.1%)
Mean NO_3-N concentration (mg/L) (SD)	0.46 ± 0.47	0.43 ± 0.47
Drinking water served by waterworks (%)	91.6 ± 18.7	88.9 ± 18.8
Urbanization level of residence (%)[a]		
Metropolitan	203 (44.4%)	130 (28.4%)
City	108 (23.6%)	105 (23.0%)
town	104 (22.8%)	139 (30.4%)
rural	42 (9.2%)	83 (18.2%)
Ca levels (mg/L)		
Less than or equal to median (34.8)	247 (54.0%)	239 (52.3%)
Greater than median	210 (46.0%)	218 (47.7%)
Mg levels (mg/L)		
Less than or equal to median_(9.3)	266 (58.2%)	264 (57.8%)
Greater than median	191 (41.8%)	193 (42.2%)

[a]The urbanization level of each municipality was based on the urban–rural classification scheme of Tzeng and Wu (1986).

TABLE 2. Odds Ratios (OR) and 95% Confidence Intervals (CI) for Childhood Brain Tumors (CBT) Death in Relation to Nitrate Levels in Drinking Water, 1999–2008

NO_3-N levels (mg/L)	CBT cases	Controls	OR (95% CI)[a]
≤0.31	190 (41.6%)	229 (50.1%)	1.00
>0.31	267 (58.4%)	228 (49.9%)	1.40 (1.07–1.84)

[a]Adjusted for age, gender, and urbanization level of residence.

of residing in municipalities served by drinking water with high levels (>9.3 mg/L) of Mg compared to controls (42.2%) (Table 1).

Table 2 shows the distribution of CBT cases and controls and OR with respect to the levels of NO_3-N in drinking water. Relative to individuals whose NO_3-N levels were ≤0.31 mg/L, the adjusted OR (95% CI) for CBT was 1.4 (1.07–1.84) for subjects who resided in municipalities served by drinking water with a NO_3-N levels >0.31 mg/L.

The association between NO_3-N levels in drinking water and risk of CBT among those with high (greater than median) and low (less than or equal to median) Ca intake via drinking water is shown in Table 3. There was no evidence of a significant interaction between drinking water NO_3-N levels and low Ca intake via drinking water.

The association between NO_3-N levels in drinking water and CBT risk was evaluated among those with high (great than median) and low (less than or equal to median) Mg intake via drinking water (Table 4). There was no evidence of a significant interaction between drinking-water NO_3-N levels and low Mg intake via drinking water.

TABLE 3. Odds Ratios for Childhood Brain Tumors (CBT) by Levels of Nitrate and Ca in Drinking Water

	NO_3-N levels (mg/L)					
	≤0.31			>0.31		
Ca levels (mg/L)	CBT cases	Controls	OR[a] (95% CI)	CBT cases	Controls	OR[a] (95% CI)
>34.8	58	86	1.00	152	132	1.30 (0.84–1.99)
≤34.8	132	143	0.93 (0.60–1.45)	115	96	1.41 (0.90–2.19)

[a]Adjusted for age, gender, and urbanization level of residence.

TABLE 4. Odds Ratios for Childhood Brain Tumors (CBT) by Levels of Nitrate and Mg in Drinking Water

	NO_3-N levels (mg/L)					
	≤0.31			>0.31		
Mg levels (mg/L)	CBT cases	Controls	OR[a] (95% CI)	CBT cases	Controls	OR[a] (95% CI)
>9.3	77	61	1.00	130	116	1.24 (079–1.95)
≤9.3	129	152	0.83 (0.53–1.29)	137	112	1.21 (0.77–1.9)

[a]Adjusted for age, gender, and urbanization level of residence.

DISCUSSION

Our findings are in contrast to results reported in previous epidemiologic studies (Mueller et al. 2001; 2004). The basis for these inconsistencies is not known. All these studies, including our investigation, have common problems concerning exposure assessment methods. Instead of using a real individual exposure to NO_3-N, our data estimated past NO_3-N levels by linking each study subject's residence to the subject's individual water source while not taking into account residential histories. Data on individual exposure were thus characterized by a lack of precision. A more basic reason for the inconsistent results may be that there is no causality between NO_3-N in drinking water and development of CBT. It is also possible that high level of NO_3-N is a proxy for some other contaminants, such as pesticides or metals, in the water for which no information is available (Mueller et al., 2001).

The biological mechanisms underlying NOC exposure and correlation to increase risk of CBT remain unknown. It is possible that, due to a decreased capacity for DNA repair and a high rate of neural cell division, the fetal brain is particularly susceptible to potential carcinogenic effects following exposures to NOC (Rice and Ward 1982; Berleur and Cordier 1995; Mueller et al. 2001). Furthermore,

an increased hypoxanthine-guanine phosphoribosyltransferase (HPRT) variant frequency in peripheral lymphocytes of subjects exposed to high levels of drinking-water NO_3-N was noted, suggesting the occurrence of genotoxic effects (Van Maanen et al. 1996).

Although there appears to be a consistent association between intake of high drinking-water NO_3-N and endogenous nitrosation capacity, intake of dietary NO_3-N is not likely to increase nitrosation due to the presence of nitrosation inhibitors in vegetables (Bartsch et al. 1988). Antioxidants that inhibit endogenous nitrosation include vitamin C and alpha-tocopherol, which reduce nitrite to NO (Bartsch et al., 1988). Previous findings suggested that Ca and Mg may act like vitamin C and alpha-tocopherol, which inhibited endogenous nitrosation produced by intake of NO_3-N in drinking water, and therefore individuals who had low levels of Ca or Mg intake via drinking water may be at increased risk for exposure to NOC and colorectal cancer (Chiu et al. 2010; Chang et al. 2010). It was further postulated that the effect of NO_3-N in drinking water on risk of CBT development might be modified by intake of Ca and Mg in drinking water. To our knowledge, this is the first to study the effect modification by Ca and Mg intake in drinking water in the correlation between NO_3-N exposure and CBT occurrence.

The completeness and accuracy of the death registration system needed to be evaluated before any conclusion based on mortality analysis is reached. In Taiwan, it is mandatory to register death certificates at local household registration offices, and the death registration in Taiwan is complete. Although causes of death may be misdiagnosed and/or misclassified, the problem has been minimized through the improvement in the verification and classification of causes of death in Taiwan since 1972. Furthermore, malignant neoplasms, including CBT, were reported to be one of the most unequivocally classified causes of death in Taiwan (Chen and Wang 1990). Because of a fatal outcome, it is believed that all CBT cases from exposure to high or low levels of NO_3-N in drinking water had access to medical care regardless of geographical location in recent years.

Inherent in this study design were the assumptions that CBT cases and controls were exposed to NO_3-N in drinking water attributed to the "usual place of residence" recorded on the death certificate and that individuals spent most of their daily life in this residential municipality. The probability that these assumptions were true among the subjects aged 0-19 yr was based on the fact that the majority of these study subjects (1) spent more time at home, (2) were studying in their usual residential municipality, and (3) were less likely to have resided in other locations.

Migration from a municipality of high NO_3-N exposure to one of low exposure or vice versa may have introduced misclassification bias and bias in OR estimates (Gladen and Rogan 1979; Polissar 1980). Our study population was stable in terms of mobility compared with populations in most industrialized countries, probably because of cultural factors and age distribution (Yu et al. 2006). Further, any misclassification of exposure is most likely to be nondifferential, which would reduce the estimated magnitude of association rather than introduce a positive bias in the estimation.

Since the measure of effect in this study is mortality rather than incidence, migration during the interval between CBT diagnosis and death must also be considered. During this period, CBT diagnosis may have influenced parental decision to migrate and may have possibly introduced bias. If there was a trend toward migration to more urban areas or municipalities with higher levels of NO_3-N in drinking water because of proximity to medical care, a spurious association between NO_3-N exposure from drinking water and death due to CBT would have resulted. Two aspects of this study presumably minimize this possibility. First, migration precipitated by a CBT diagnosis would have been less likely for the parents of the subjects, because for this cohort of decedents, the parental occupational status would weigh against a move requiring a job change. Second, urbanization level was included as a control variable in the analysis.

Intake of NO_3-N from drinking water and dietary sources may result in increased exposure to NOC through endogenous nitrosation (Mirvish et al. 1992; Moller et al. 1989). The principal dietary NO_3-N sources are vegetables. Vegetables also contain vitamin C and other nitrosation inhibitors (Bartsch et al. 1988), and therefore, high intakes may not necessarily result in high rates of formation of NOC (Coss et al. 2004). Dietary intakes of red and processed meat are of particular importance in the formation of fecal NOC (Bingham 1999; Bingham et al. 2002). There is unfortunately no information available for assessing dietary NO_3-N sources from vegetables and meat for individual study subjects in this investigation. However, there is no reason to believe that there would be any correlation between the sources of dietary NO_3-N and the levels of NO_3-N in drinking water. Furthermore, Chilvers et al. (1984) indicated that when the concentration of waterborne NO_3-N is high, drinking water contributes substantially to total NO_3-N intake and the potential for nitrite and NOC formation may be increased. It is thus proposed that individuals with higher daily NO_3-N intake from drinking water and lower intakes of nitrosation inhibitors may be at an elevated risk of CBT development.

There are other risk factors, not included in this study, that may play a role in the etiology of CBT, such as ionizing radiation, parental smoking, and certain heredity and genetic factors (Little 1999). There is unfortunately no information available on these variables for an individual study subject and thus it could not be adjusted for directly in the analysis. However, there is no reason to believe that there would be any correlation between these confounders and the levels of NO_3-N in drinking water. It is also unlikely that there would be a direct relationship between other risk factors and the levels of NO_3-N in drinking water.

The nitrate concentration in drinking water in Taiwan is below the guideline value 10 mg/L recommended by the World Health Organization (1984). This guideline was not based on estimates of cancer risk. In addition, there is no scientific evidence to justify firm conclusions about the safety of any concentration of NO_3-N in water with regard to cancer risk occurrence. Formam (1989) noted that although environmental NO_3-N exposure may play a role in the development of cancer, it does not show a rate-limiting effect.

In summary, our data suggest that exposure to NO_3-N in drinking water at levels in this study is associated with higher risk of death attributed to CBT. Future studies need to increase the precision of the estimation of the individual's intake of NO_3-N, through both food and water, and control for confounding factors such as radiation.

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