Nitrate in drinking water and risk of death from colon cancer in Taiwan

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Abstract

The relationship between nitrate levels in drinking water and colon cancer has been inconclusive. A matched case-control and a nitrate ecology study were used to investigate the association between colon cancer mortality and nitrate exposure from Taiwan’s drinking water. All colon cancer deaths of Taiwan residents from 1999 through 2003 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 sex, year-of-birth, and year-of-death. Each matched control was selected randomly from the set of possible controls for each case. Data on nitrate–nitrogen (NO$_3$–N) level of drinking water throughout Taiwan have been collected from Taiwan Water Supply Corporation (TWSC). The municipality of residence for cases and controls was assumed to be the source of the subject’s nitrate exposure via drinking water. The adjusted odds ratios for colon cancer death for those with high NO$_3$–N levels in their drinking water, as compared to the lowest tertile, were 0.98 (0.84–1.14) and 0.98 (0.83–1.16), respectively. The results of the present study show that there was no statistically significant association between NO$_3$–N in drinking water at levels in this study and risk of death from colon cancer.

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Keywords: Nitrate; Drinking water; Colon cancer; Mortality

1. Introduction

Nitrate in drinking water comes from numerous natural and man-made sources, including waste waters and agricultural and urban runoff. Nitrogen fertilizer is the largest contributor to anthropogenic nitrogen worldwide and has been implicated as an even more important source of drinking water nitrate in rural areas (Fields, 2004). The U.S. Environmental Protection Agency (EPA) has established a maximum contaminant level (MCL) in drinking water of 10 mg/L as nitrate–N to protect infants from methemoglobinemia (Ward et al., 2005). However, the effectiveness of this regulatory limit for preventing other health risks such as cancer has not been adequately studied (De Roos et al., 2003).

Nitrate can be endogenously reduced to nitrite, which can then undergo nitrosation reactions in the stomach with amines and amides to form a variety of $N$-nitroso compounds (NOC) (Walker, 1990). Most of these compounds are potent animal carcinogens (Tricker and Preussmann, 1991). Several studies support a direct relationship between nitrate intake and endogenous formation of NOCs. High nitrate levels in drinking water have been associated with increased excreted $N$-nitrosoproline levels in urine (Mirvish et al., 1992; Moller et al., 1989), and nitrate administered via drinking water has been shown to be directly related to 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 demonstrate a contribution of drinking water to overall nitrosation and suggest that nitrate intake can be used as a surrogate for exposure to target tissues to NOCs (De Roos et al., 2003).

Nitrosation can also occur in the large intestine (Bruning-Fann and Kaneene, 1993). NOCs are potent animal carcinogens, inducing tumors at multiple organ sites including the colon...
Table 1: Characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total subjects</td>
<td>2234</td>
<td>2234</td>
</tr>
<tr>
<td>Enrollment municipality</td>
<td>252</td>
<td>252</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1310 (58.6)</td>
<td>1310 (58.6)</td>
</tr>
<tr>
<td>Female</td>
<td>924 (41.4)</td>
<td>924 (41.4)</td>
</tr>
<tr>
<td>Mean age in years (SD) a</td>
<td>61.8±5.5</td>
<td>61.8±5.5</td>
</tr>
<tr>
<td>Mean NO\textsubscript{3}–N concentration (SD)</td>
<td>0.43±0.44</td>
<td>0.44±0.44</td>
</tr>
<tr>
<td>Mean calcium concentration (mg/L)(SD)</td>
<td>34.5±18.9</td>
<td>36.4±19.1</td>
</tr>
<tr>
<td>Drinking water served by waterworks (%)</td>
<td>91.8±16.4</td>
<td>91.4±16.0</td>
</tr>
<tr>
<td>Urbanization level of residence (%) b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metropolitan</td>
<td>831 (37.2)</td>
<td>756 (33.8)</td>
</tr>
<tr>
<td>City</td>
<td>516 (23.1)</td>
<td>514 (23.0)</td>
</tr>
<tr>
<td>Town</td>
<td>584 (26.1)</td>
<td>592 (26.5)</td>
</tr>
<tr>
<td>Rural</td>
<td>303 (13.6)</td>
<td>372 (16.7)</td>
</tr>
</tbody>
</table>

a SD: Standard deviation.  
b The urbanization level of each municipality was based on the urban–rural classification scheme of Tzeng and Wu (1986).

2.2. Subject selection

Data on all deaths of Taiwan residents from 1999 through 2003 was obtained from the Bureau of Vital Statistics of the Taiwan Provincial Department of Health which is in charge of the death registration system in Taiwan. For each death, detailed demographic information including sex, year-of-birth, year-of-death, cause of death, place of death (municipality), and residential district (municipality) were recorded on computer tapes. The case group consisted of all eligible colon cancer deaths occurring in people between 50 and 69 years of age (International Classification of Disease, ninth revisions [ICD-9], code 153). In all, 2234 colon cancer deaths with complete records satisfied this criterion.

The control group consisted of all other deaths excluding those deaths which were associated with gastrointestinal disease. The deaths excluded were those caused by malignant neoplasms of stomach (ICD-9 code 151), malignant neoplasm of small intestine, including duodenum (ICD-9 code 152), malignant neoplasm of colon (ICD-9 code 153), malignant neoplasm of rectum, rectosigmoid junction and anus (ICD-9 code 154), gastric ulcer (ICD-9 code 531), duodenal ulcer (ICD-9 code 532), peptic ulcer, site unspecified (ICD-9 code 533), gastrojejunal ulcer (ICD-9 code 534), and gastrointestinal hemorrhage (ICD codes 578). Subjects who died from bladder (Morales Suarez-Varela et al., 1993; Weyer et al., 2001), lung (Hoffmann et al., 1994), esophagus (Yang, 1980; Wu et al., 1993, Cantor, 1997), head and neck (Andre et al., 1995; Herity et al., 1981) cancers, and non-Hodgkin lymphoma (NHL) (Cantor, 1997; Gulis et al., 2002; Ward et al., 1996) were also excluded from the control group because of previously reported associations with nitrate or NOC exposures. A total of 78,325 deaths satisfied this criterion. Control subjects were pair matched to the cases by sex, year-of-birth, and year-of-death. A case had an average of 35 appropriate controls who satisfied the criteria for matching. We used a random sampling method to select one control from the set of possible controls for each case. The most frequent causes of death among the controls were diabetes mellitus (12.3%), liver cancer (11.0%), chronic liver disease and cirrhosis (6.9%), intracerebral hemorrhage (4.0%), and acute myocardial infarction (3.9%).

2.3. Nitrate–nitrogen (NO\textsubscript{3}–N) levels

Information on the levels of NO\textsubscript{3}–N in each municipality’s treated drinking water supply was obtained from the Taiwan Water Supply Corporation (TWS/CORC, 1991), to which each waterworks is required to submit drinking water quality data including the levels of nitrate. Four finished water samples, one for each season, were collected from each waterworks. The samples were analyzed by the waterworks laboratory office using standard method (cadmium reduction method). Since the laboratory office examines nitrate levels on a routine basis using standard method, it was thought that the problem of analytical variability was minimal. Among the 322 municipalities, 70 were excluded as they had more than one supply of drinking water and the exact population served by each could not be determined. Their details are given in an earlier publication (Yang, 1998; Yang et al., 2000). The final complete data comprised NO\textsubscript{3}–N data from 252 municipalities. Data collected were the annual mean levels of NO\textsubscript{3}–N for the year 1990. The municipalities of residence for all cases and controls were identified from the death certificate and were assumed to

Table 2: Odds ratios (ORs) and 95% confidence intervals (CIs) for colon cancer death by nitrate levels in drinking water, 1999–2003

<table>
<thead>
<tr>
<th>Nitrate, mg/L (median)</th>
<th>≤0.22 (0.00)</th>
<th>0.23–0.45 (0.38)</th>
<th>0.48–2.86 (0.74)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>775</td>
<td>758</td>
<td>701</td>
</tr>
<tr>
<td>No. of controls</td>
<td>746</td>
<td>749</td>
<td>739</td>
</tr>
<tr>
<td>Crude odds ratio a</td>
<td>1.0</td>
<td>0.98 (0.85–1.12)</td>
<td>0.92 (0.79–1.06)</td>
</tr>
<tr>
<td>Adjusted odds ratio b</td>
<td>1.0</td>
<td>0.98 (0.84–1.14)</td>
<td>0.98 (0.83–1.16)</td>
</tr>
</tbody>
</table>

\(X^2\) for trend=1.52, \(p=0.22\).  
a Odds ratio adjusted for age and sex.  
b Adjusted for age, sex, calcium levels in drinking water, and urbanization level of residence.
be the source of the subjects’ nitrate exposure via drinking water. The levels of NO$_3$–N of each municipality were used as an indicator of exposure to NO$_3$–N for an individual residing in that municipality.

2.4. Statistics

In the analysis, the subjects were divided into tertiles according to the levels of NO$_3$–N and calcium in their drinking water. Conditional logistic regression was used to estimate the relative risk in relation to the NO$_3$–N levels in drinking water. Calcium levels in drinking water was included in the multiple regression analysis because our previous study reported a protective effect of calcium intake via drinking water against colon cancer (Yang et al., 1997). Odds ratio (ORs) and their 95% confidence intervals (CIs) were calculated using the group with the lowest exposure as the reference group (Breslow and Day, 1981). Values of $p<0.05$ were considered statistically significant.

3. Results

Of the 252 studied municipalities, 80 (31.7%) had a NO$_3$–N levels ≤0.22 mg/L; 81 (32.1%) had a NO$_3$–N levels of 0.23–0.45 mg/L; and 91 (36.1%) had a NO$_3$–N levels ≥0.48 mg/L.

A total of 2234 colon cancer cases with complete records were collected for the period of 1999–2003. Of the 2234 cases, 1310 were males and 924 were females. The mean NO$_3$–N concentration in the drinking water of the colon cancer cases was 0.43 mg/L (SD=0.44). Controls had a mean NO$_3$–N exposure of 0.44 mg/L (SD=0.44). Both cases and controls had a mean age of 61.8. Cases lived in municipalities in which 91.8% of the population was served by a waterworks. For controls this number was 91.4%. Cases had a slightly higher rate (37.2%) of living in metropolitan municipalities than the controls (33.8%) (Table 1).

Table 2 shows the number of cases and controls and ORs for colon cancer in relation to NO$_3$–N levels in drinking water. The ORs for death from colon cancer were lower for the two groups with high levels of NO$_3$–N in their drinking water but they were not able to reach statistical significance. Adjustments for possible confounders only slightly altered the ORs. The adjusted ORs (95% CI) were 0.98 (0.84–1.14) for the group with water NO$_3$–N levels between 0.23 and 0.45 mg/L and 0.98 (0.83–1.16) for the group with NO$_3$–N levels of 0.48 mg/L or more. There was no difference between the groups with different levels of NO$_3$–N in drinking water.

4. Discussion

This study uses a death certificate-based case-control study to examine the relationship between colon cancer mortality and NO$_3$–N levels in drinking water in Taiwan. The results of the present study show that there is no statistically significant association between NO$_3$–N levels in drinking water and risk of death from colon cancer. Our finding is consistent with previous reports (Geleperin et al., 1976; Thouez et al., 1981; Jensen, 1982; Morales Suarez-Varela et al., 1995; Weyer et al., 2001; De Roos et al., 2003) and is in contrast to ecologic data from Slovakia (Gulis et al., 2002). Occupational studies of nitrogen-based fertilizers workers have also found no association with colon cancer (Fandrem et al., 1993; Fraser et al., 1989; Hagmar et al., 1991), although these estimates were based on small cases and lacked precision.

Despite their inherent limitations (Morgenstern, 1982), studies of the ecological correlation between mortality and environmental exposures have been used widely to generate or discredit epidemiological hypotheses. Before any conclusion based on such a mortality analysis is made, however, the completeness and accuracy of the death registration system should be evaluated. Since it is mandatory to register death certificates at local household registration offices, the death registration in Taiwan is very 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 colon cancer, have been reported to be one of the most unequivocally classified causes of death in Taiwan (Chen and Wang, 1990). Because of its fatal outcome, it is believed that all colon cancer cases exposed to either high or low levels of NO$_3$–N in drinking water all currently access to medical care regardless of geographical location.

Of greater concern is whether the relative levels of NO$_3$–N in the period around 1990 correspond to the relative levels in periods 20–30 years earlier. This is important since it is likely that exposure to causal factors would precede cancer mortality by at least 20 years (the latency period for carcinogen exposure). The historical levels of NO$_3$–N are not available for the study areas. However, we believe that the correlation between the levels of 1990 and levels in the previous levels of 20–30 years might be high since the use of nitrogen fertilizers have not changed over time in study areas.

Migration from a municipality of high NO$_3$–N exposure to one of low NO$_3$–N exposure or vice versa could have introduced misclassification bias and bias in the ORs estimate (Gladen and Rogan, 1979; Polissar, 1980). Mobility is age dependent, and diseases usually occur with a higher incidence among older groups and near the location of the environmental “cause” (Polissar, 1980). However, neighboring water sources tend to have similar chemical composition, and hence even if an individual moved, the change in exposure to NO$_3$–N in drinking water would probably not be significant provided that the old and new residence were relatively close to one another, which also reduces the uncertainty created by the fact that some residents consume water at their workplaces or elsewhere. Also, all subjects used for the present study were at least 50 years old, it is generally assumed that the elderly are more likely to remain in the same residence for a significant portion of their life span. Furthermore, urbanization levels were included as a control variable in the analysis. Since it is conceivable that municipalities with similar urbanization levels may have similar migration rates, this probably minimized the migration problem in our study.

Since the measure of effect in this study is mortality rather than incidence, migration during the interval between cancer diagnosis and death must also be considered. During this period, cancer diagnosis may influence a decision to migrate and possibly introduce bias. Data are not available for the differences in survival rate of colon cancer patients between high and low areas. If there was a trend toward migration to more urban or lower nitrate exposure areas because of proximity to medical care, for example, a spurious association between nitrate exposure and colon cancer death would have existed.
Formation of NOC (Coss et al., 2004). Dietary intakes of red and therefore, high intakes may not result in high rates of formation (Mirvish et al., 1992; Moller et al., 1989). The principal cause increased exposure to NOC through endogenous nitrosation (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 the dietary nitrate sources from vegetables and meat for individual study subjects in this study. However, there is no reason to believe that there would be any correlation between the sources of dietary nitrate and the levels of NO$_3^-N$ in drinking water. Furthermore, a study has indicated that when the concentration of waterborne nitrate is high, drinking water contributes substantially to total nitrate intake (Chilvers et al., 1984), and the potential for nitrite and NOC 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 increased risk of colon cancer.

There are a number of major risk factors for colon cancer that should be taken into account when investigating the possibility of an additional factor (drinking water nitrate exposure). On the basis of scientific knowledge from epidemiologic studies, the most important risk factor for colon cancer is physical activity, meat and fat consumption (Schottenfeld and Fraumeni, 1996). There is unfortunately no information available on this variable 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 nitrate in drinking water. It is also unlikely that there would be a direct relationship between other risk factors and the level of NO$_3^-N$ in drinking water.

We determined the exposure to NO$_3^-N$ by linking each study subject’s residence to their individual water source. However, we were unable to calculate the exact NO$_3^-N$ intake from water for individual subjects, because the amount of water consumed at home or at other places could not be determined. The data on individual exposure were thus still characterized by a lack of precision.

The mean level of NO$_3^-N$ in the drinking water of the control group was 0.44 mg/L in this study. This value is low compared with those found in previous studies. In De Roos et al. (2003) study, 55% of the control participants had average NO$_3^-N$ levels over 1 mg/L. In Weyer et al. (2001) study, the median of the NO$_3^-N$ levels from the municipal water supplies was 1.01 mg/L. In Gulis et al. (2002) study, 78.4% of the study population had an average nitrate level over 10 mg/L in their municipal water supply.

The NO$_3^-N$ concentration in drinking water in Taiwan is below the guideline value recommended by the World Health Organization (1984) of 10 mg/L. This guideline was not based on estimates of cancer risk. Also, 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. Forman (1989) notes that although environmental NO$_3^-N$ exposure probably plays 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 not associated with risk of death from colon cancer. Future studies should increase the precision of the estimation of the individual’s intake of nitrate, through both food and water, and control for confounding factor, especially personal risk factor such as physical activity, and meat and fat consumption.

Acknowledgments

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References
