ASSOCIATION BETWEEN NITRATE IN DRINKING WATER AND RISK OF BLADDER CANCER; SYSTEMATIC REVIEW AND META-ANALYSIS UPDATE TO MAY 2016

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Received on 27-07-2016  
Accepted on 04-09-2016

Abstract

Nitrate is a water-soluble compound which can endanger the health of human and other living creatures at high concentrations. Although bladder cancer caused by nitrates is biological plausibility, but the number of studies on the relationship between nitrate exposure and the risk of bladder cancer is limited. Therefore, in this study, it has been tried to reevaluate the relationship between nitrate exposure and the risk of bladder cancer through systematic review and meta-analysis. The databases of ISI, Pubmed, Scopus, Irandoc and SID were used to find the studies in Iran and the world. Generally, 6 studies including 1 ecological study, 3 case-control studies and 2 cohort studies were entered at the meta-analysis stage. At the stage of meta-analysis, the heterogeneity of the studies with high scores was $I^2=52\%$, $P<0.001$, so, Random effect model was used for them and the heterogeneity of the studies with low scores was $I^2=0\%$, $P=0.53$, so, Fixed effect model was used for them. For the studies with high scores, REM=0.93 ($P$ value=0.22) and for studies with low scores, REM=1.77 ($P$ value<0.001). In high quality studies, the risk of bladder cancer was reduced by 7% significantly and in low quality studies, it was reduced by 77% significantly. The relative mean risk of 6 studies was REM=1.03 ($P$ value=0.58). The results of the systemic review and meta-analysis showed that the drinking-water nitrate has no effects on the increase or reduction in the risk of bladder cancer and performing further studies on this issue is needed.
1. Introduction

Nitrate is a natural compound that is essential for all living creatures. Nitrate enters the body through eating vegetables, meat and drinking water. Water pollution by organic substances, municipal and industrial waste, animal and chemical manures or municipal and industrial sewage can cause the entrance of nitrate in surface and underground sources [1,2]. Since nitrate has high solubility in water, large amounts of it can enter water resources [3,4]. To prevent Methemoglobinemia in children, WHO and EPA announced the maximum permissible drinking-water nitrate as much as 45 mg/l in nitrate [5,6]. Studies on animal showed that N-nitroso compounds (NOCs) are a potential carcinogen [7,8]. International agency for research on cancer considered the nitrate as probable human carcinogen [9]. NOCs can be produced with the chemical composition of nitrate and amine and amid in the stomach and bladder (Figure 1) [10,11].

![Figure 1. Formation of NOC in the stomach and bladder caused by the conversion of NO₃ to NO₂.](image)

Nitrate in the human body is caused by eating food (vegetables) and drinking water. Since vegetables contain vitamin C and E, they prevent the conversion of nitrite to nitrate [12,13]. Therefore, the largest share of nitrate is related to the nitrate in drinking water. Previous epidemiological studies have been done on stomach cancer [14,15], non-Hodgkin's lymphoma [16,17], Prostate neoplasm and esophagus cancer. The results showed that the nitrate increases the risk of the cancers [15,18]. Approximately, 70% of ingested nitrate is excreted through the urine. Therefore, the production of NOCs can also occur in the bladder [19]. Although bladder cancer caused by nitrate is biological plausibility [20,21], but, the number of studies on the relationship between nitrate exposure and the risk of bladder cancer is...
limited [19,22]. The factors of the bladder cancer risk are male gender, smoking, exposure to polycyclic aromatic hydrocarbons (PAHs) and arsenic in drinking water [23]. The studies on this field have discussable results. Some studies have noted a relationship between nitrate and bladder cancer [22,24] and some others have rejected [20,25]. Although a meta-analysis study has been done in 2012 by Wang et al but it has been done more than three years ago [26] and no new studies have been done on it up to now. Therefore, in this study, it has been tried to reevaluate the relationship between the drinking-water nitrate and the risk of bladder cancer through a systematic review and meta-analysis.

2. Materials and Methods

This research is a systematic and meta-analysis study on the relationship between drinking-water nitrate and the risk of bladder cancer. The databases of ISI Web of Science, Pubmed, Scopus, Irandoc and SID were used to find the studies in Iran and the world.

1.2. The selection and the quality evaluation criteria of the studies

Firstly, a list of all studies’ titles and abstracts included in the mentioned databases was provided by three researchers (Ya.F, Ha.K, Ya.Z) to prevent the bias of researchers. The related titles were investigated independently and, then the studies published between the dates 1995 and 2016 were searched. The search was done for two weeks from 11.03.2016 to 25.03.2016, then the related studies were evaluated initially by blinding method and entered in the research process independently.

Similar studies were excluded from the study. In this research, the inclusion criterion of different studies is that the drinking-water nitrate and the risk of bladder cancer were noted. The studies were not of the earlier studies or the studies were performed on the field of clinical decision-making or unrelated investigations to the bladder cancer were excluded from the study. In the second stage, the abstracts of the selected studies were investigated with the use of checklist STROBE which is a standard checklist.

This checklist includes 43 various sections and investigates various aspects of methodology, including sampling methods, measurements, statistical analysis and objectives of the study [27]. In this checklist, the minimum score is 40 and the maximum one is 45.

Finally, the top studies which have gained the minimum score (40) based on checklist, were entered in the research and their data was extracted for meta-analysis. Funnel Plot and Egger’s test were used to determine the Bias Publication [28].
2.2. Data extraction

In this study, 6 articles (3 case-control studies, 2 cohort studies and 1 ecological study) were investigated that their methodologies were almost the same and all of them were performed in 1993-2015.

The important required data for data analysis including research method, research type, study time, the score of each study in the NOS (Newcastle – Ottawa Scale) system, the ratio of bladder cancer, gender, sample size and confidence level were collected. According to NOS qualitative evaluation criteria which includes Selection, Comparability and Exposure, case-control and cohort studies were scored. In the NOS system, the score range is from 0 to 9. In this study, the studies were classified to low score (<7) and high score (≥7).

3.2. Statistical data analysis

The meta-analysis was done by Comprehensive Meta-Analysis V. 2.2.064 software. Higgins I² was used to calculate the heterogeneity of the studies. At meta-analysis stage, Random effect was used for the studies where I² was greater than 50% and fixed effect was used for the studies in which I² was smaller than 50%. In this study, the significance level was P-value<0.05.

3. Results

1.3. Identification of relevant studies

As shown in Figure1, the relevant studies were identified. Totally, 176 studies were found by researching in the databases of SID, Irandoc, Scopus, Embase, ISI web of science and PubMed. According to Title and abstract and some other reasons, 80 articles were excluded at the stage of Eligibility. Of 96 remaining studies, 90 studies were excluded because of the reasons including hazard risk, reported correlation, Letter, comments, or correspondence. Finally, 6 studies were remained (3 case-control studies, 2 cohort studies and 1 ecological study) and entered in the stage of meta-analysis (Figure2).

![Flow diagram for identification of relevant case-control and cohort studies.](image-url)
2.3. Characteristics of the studies

The general characteristics of the studies including first author, the year of publication, country, type of study, relative risk, significance interval, matched variables and results are shown in Table 1. The scope of the publication years of the studies was from 1993 to 2015.

Table 1. General characteristics of the 2 case-control, 1 ecological and 2 cohort articles included in the final analysis.

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Study design</th>
<th>Location</th>
<th>Follow-up time (years)</th>
<th>Cases-Control</th>
<th>CI Low</th>
<th>CI High</th>
<th>Variables adjusted</th>
<th>Outcome</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morales et al</td>
<td>1993</td>
<td>Ecological</td>
<td>Spain</td>
<td>-</td>
<td>-</td>
<td>1.76</td>
<td>1.28</td>
<td>2.42 Age</td>
<td>Revealed relative risks of over 1 in Valencia province in men and women, associated with the consumption of water containing a mean concentration of &gt; 50 mg/L.</td>
<td>[29]</td>
</tr>
<tr>
<td>Weyer et al</td>
<td>2001</td>
<td>Cohort</td>
<td>USA</td>
<td>12</td>
<td>Cases: 48438; Controls: 48011;</td>
<td>1.40</td>
<td>0.69</td>
<td>2.83 Age, education, smoking, physical activity, BMI, waistto-hip ratio, total energy, intakes of vitamin C/E, dietary nitrate, fruit, vegetables, water source</td>
<td>The positive association for bladder cancer is consistent with some previous data; the associations for ovarian, uterine, and rectal cancer were unexpected.</td>
<td>[21]</td>
</tr>
<tr>
<td>Ward et al</td>
<td>2003</td>
<td>Case-control</td>
<td>USA</td>
<td>-</td>
<td>Cases: 808; Controls: 1259</td>
<td>0.84</td>
<td>0.67</td>
<td>1.05 Age, education, cigarette smoking, years with chlorinated surface water and study period</td>
<td>Long-Term exposure to nitrate in drinking water at levels in this study (90th percentile 5.5 mg/liter nitrate-nitrogen) is not associated with risk of bladder cancer</td>
<td>[25]</td>
</tr>
</tbody>
</table>
Totally, 105959 persons (51179 cases and 54870 controls) participated in these 6 studies. A mean age of them was 48.3 years old. According to Newcastle–Ottawa scale (NOS), the case-control studies by Ward and Espejo (Table 2) and the cohort study by Zeegers had high scores (Table 3).

Table 2. Methodological Quality of Studies Included in the Final Analysis Based on the Newcastle-Ottawa Scale for Assessing the Quality of Case-Control Studies.

| Zeegers et al | 2006 | Cohort | Netherlands | 9.3 | Cases: 889; Controls: 4441; | 1.01 0.87 1.18 | Age, sex, smoking status, exposure from food | Not Support an association between nitrate exposure and bladder cancer rise | [20] |
| Chiu et al | 2007 | Case-control | Taiwan, China | - | Cases: 513; Controls: 513 | 1.76 0.28 2.42 | Age, gender, urbanization level of residence | There Was a significant positive relationship between the levels of nitrate in drinking water and risk of death from bladder cancer | [24] |
| Espejo-Herrera et al | 2015 | Case-control | Spain | - | Cases: 531; Controls: 556 | 0.65 0.41 1.03 | Age, sex and area of residence, smoking status, NSAIDs use, night-time urinary frequency, time working in farm/agriculture activities, tap water and vitamin C daily intake, and urinary infections (ever). | Bladder cancer risk was inconsistently associated with chronic exposure to drinking water nitrate at levels below the current regulatory limit | [30] |

Table-2. Methodological Quality of Studies Included in the Final Analysis Based on the Newcastle-Ottawa Scale for Assessing the Quality of Case-Control Studies.
Table-3. Methodological quality of studies included in the final analysis based on the Newcastle-Ottawa scale for assessing the quality of cohort.

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Selection (Score)</th>
<th>Comparability (Score)</th>
<th>Exposure (Score)</th>
<th>Total Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weyer et al</td>
<td>2001</td>
<td>1 1 1 0</td>
<td>1</td>
<td>0 1 1</td>
<td>6</td>
</tr>
<tr>
<td>Zeegers et al</td>
<td>2006</td>
<td>1 1 1 1</td>
<td>2</td>
<td>0 0 1</td>
<td>7</td>
</tr>
</tbody>
</table>

The studies by Ward et al (2005) and Cantor et al were excluded from meta-analysis because they are review studies [15,18]. The ecological study by Kuzma et al was excluded from meta-analysis because relative risks were not estimated in it. The study by Morales et al (1995) was also excluded from meta-analysis because they used the RR data collected in 1993 [22,29].

Also, some ecological studies were excluded from the study because the authors calculated the standardized incidence ratios [19,31,32].

3.3. Meta-analysis of the studies

The highest and lowest relative risk of bladder cancer was related to the studies by Chiu et al (RR = 1.96) and Espejo et al (RR=0.65), respectively. At the stage of meta-analysis, the heterogeneity of the studies with high scores was
\(\hat{\tau}^2=52\%, P<0.001\), so, Random effect model was used for them and the heterogeneity of the studies with low scores was \(\hat{\tau}^2=0\%, P=0.53\), so, Fixed effect model was used for them. For the studies with high scores, REM=0.93 (P value=0.22) and for studies with low scores, REM=1.77 (P value<0.001). Given the quality of the studies, the results were inconsistent, in high quality studies, the risk of bladder cancer was reduced by 7% significantly and in low quality studies, it was reduced by 77% significantly. The relative mean risk of 6 studies was REM=1.03 (P value=0.58) that showed the drinking-water nitrate doesn’t increase the risk of bladder cancer significantly (P value>0.05) (Figure 3). Among the case-control and cohort studies, the studies by Chiu et al and Weyer et al had the low score, respectively. Also, the ecological study by Morales et al had the low score (Table 2 and 3).

![Figure 3. Forest plot of meta-analysis on nitrate in drinking water and risk of bladder cancer.](image)

4. Discussion

In this study, the studies with low and high score published up to May 2016 were used to investigate the relationship between the drinking-water nitrate and the risk of bladder cancer. The results of the meta-analysis on 2 cohort studies, 3 case-control studies and one ecological studies showed that there is no significant relationship between the drinking-water nitrate and the risk of bladder cancer.

As shown in figure3, the relative risk in the study by Ward et al is much lower than the one in other studies. Unlike the meta-analysis study by Wang et al [26], excluding the study by Ward et al, increased the relative risk of bladder cancer by 7% (from 1.03 to 1.1) insignificantly, however, some properties of the study by Ward et al cannot be missed. The properties of the study by Ward et al: since only 70% of the population used the water including the measured nitrate, risk evaluation was not correct; the sample was not an appropriate representative of the total population because the sample was selected in a period of 50 years but other population was a period of 20 years.
They consumed the water sources of the region; the response rates of cases (85%) and controls (82%) were low. These reasons can be the cause of the low relative risk in the study by Ward et al[25]. On the other hand, in the study by Ward et al, the concentration of the drinking-water nitrate was greater compared to other studies but the relative risk of bladder cancer was lower. Since the studies group in his study included farmers, so, due to the lower consumption of cigarettes in this group, the relative risk of bladder cancer was lower [33]. On the other hand, the concentration of THM in drinking water used with this group is lower. Only 8% of farmers were faced with high concentrations of THM (> 28 mg/l) [25]. Also, in some places of urban regions, the concentration of nitrate was higher than the rural regions due to the pollutant sources (for example municipal sewage), so, the risk of bladder cancer can increase in urban regions [34].

Considering intervening factors in any study increases the accuracy in estimating the risk. Intervening factors in the relationship between the drinking-water nitrate and the risk of bladder cancers are age, gender, education, smoking, vitamin C and E, the consumption of meat, stomach ulcers, occupational exposure and the nitrate in food [10,35]. The most important intervening factors in this type of diseases are age and gender [36,37].

As was said, the bladder cancer caused by nitrate is biological plausibility but the study by Mirvish et al showed that taking vitamin C, E and polyflonprevents the production of NOC and subsequently, increased the risk of bladder cancer [38]. Studies Ward (2003), Ward (2006) and Ward (2007), Dubrow et al and Michaud et al showed that vitamin C can significantly reduce the risk of bladder cancer associated with nitrate or NOC [25, 39-42]. The cohort study by Freedman et al showed that the relative risk in those who were smokers in the past (RR = 2.22) and those who are smokers (RR = 4.06) was much higher than non-smokers [43].

On occupational exposure, the study by Samanic et al showed that the incidence of bladder cancer among hotel staff, repairers and machinery operators is more than one among the control groups [44].

Other intervening factors related to the water pollutants and cancer were considered in the studies by Bove et al [45], Doyle et al [46], Young et al [47] and Hrudey et al[48].

One of the problems of this study was the different concentrations of nitrate in different places due to different amount of water, different pollutant sources and etc. [49]. In this study, the concentrations of nitrate were different in the studies, the failure point for the groups with intermediate and high exposure was 0.48 mg/l and 7.7 mg/l, respectively, it was 3.09 mg/L for cohort studies and it was 2.48 mg/l for case-control study and 50mg/L for
ecological study. So, comparing the studies with each other was difficult in meta-analysis. For example in the study by Chiu et al the failure point of the group with high exposure was greater than 0.47 mg/l [24] and for the study by Zeegers et al, it was greater than 7.7 mg/l [20]. The difference in the concentration of nitrate was the main cause of high heterogeneity ($I^2 = 78\%$, P-value <0.001). Another reason of high heterogeneity was the different types of the studies. In this study, three case-control studies, 2 cohort studies and 1 ecological study were used. Cohort and case-control studies are done in the person but the ecological studies are done in the population or group. Also, ecological and case-control studies cannot provide enough evidences to show the relationship. So, the ecological study by Morales et al was considered as the study with low score.

Since many journals seldom publish the studies in which there is no relationship between the exposure and disease, there is a publication error. Also, there is a publication error in this study but the reverse Begg’s graph and Egger’s test showed that there is no overall publication error (Figure4).

![Funnel plot of the studies included in the meta-analysis.](image)

The average concentration of nitrate in water is 0.2 mg/l. Agricultural activities, industrial and urban sewage discharge have contaminated water supplies and increased the concentration of nitrate. So, WHO and EPA provided some guidelines and standards to control Methemoglobinemia in children.

Since no significant relationship was observed between the drinking-water nitrate and bladder cancer, Bogovski et al and Bryan et al studies showed that the nitrate is indirectly carcinogenic [50,51]. The nitrate becomes a carcinogenic compound for the animals by converting to NOC (Figure1) [7].

So, the carcinogenic risks of nitrate should not be ignored. Finally, it must be said that due to a few number of the studies, it cannot be concluded accurately on the relationship between the drinking-water nitrate and the risk of
bladder cancer. So, it is recommended to perform more studies, especially prospective studies on this issue to obtain more accurate conclusions.

5. Conclusions

Given the high and low score studies, the risk of bladder cancer was significantly decreased by 7% and significantly increased by 77%. Totally, it was increased insignificantly. The systematic review and meta-analysis showed that the drinking-water nitrate has no impact on the increase or decrease of bladder cancer risk (P-value>0.05). In this study, the conclusion was based on 6 studies performed up to 2016 and it is necessary to conduct more cohort and case-control studies with considering more intervening factors to obtain more accurate conclusions.

6. Acknowledgments

School Of Public Health, Semnan University of Medical Sciences was the financer of this review research (Code:A-10-268-8).

7. References


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