The risk of cancer as a result of elevated levels of nitrate in drinking water and vegetables in Central India

Pinky Taneja, Pawan Labhasetwar, Pranav Nagarnaik and Jeroen H. J. Ensink

ABSTRACT

The objective of the present study was to determine the effect of nitrates on the incidence of gastrointestinal (GI) cancer development. Nitrate converted to nitrite under reducing conditions of gut results in the formation of N-nitrosamines which are linked to an increased gastric cancer risk. A population of 234 individuals with 78 cases of GI cancer and 156 controls residing at urban and rural settings in Nagpur and Bhandara districts of India were studied for 2 years using a case-control study. A detailed survey of 16 predictor variables using Formhub software was carried out. Nitrate concentrations in vegetables and primary drinking water supplies were measured. The logistic regression model showed that nitrate was statistically significant in predicting increasing risk of cancer when potential confounders were kept at base level (P value of 0.001 nitrate in drinking water; 0.003 for nitrate in vegetable) at $P < 0.01$. Exposure to nitrate in drinking water at >45 mg/L level of nitrate was associated with a higher risk of GI cancers. Analysis suggests that nitrate concentration in drinking water was found statistically significant in predicting cancer risk with an odds ratio of 1.20.

Key words | cancer risk, drinking water, gastrointestinal cancer, nitrate, vegetables

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INTRODUCTION

India continues to be challenged in terms of key health outcomes including those related to access to water, sanitation, and hygiene. The majority of urban and rural India is fed by groundwater, which serves as the major source of drinking water with 85% of drinking water supplies met by groundwater in India (Ministry of Water Resources 2009). Although groundwater is, in general, considered a good drinking water source as it is less prone to fecal contamination than surface water source, groundwater can be contaminated by agriculture and industrial processes. The use of animal manure and chemical fertilizers, especially in areas with extensive and intensive agriculture could lead to the contamination of groundwater by the leaching of nitrogenous fertilizers from soils. In urban areas, nitrates may leach from septic systems, municipal wastewater drains and sewer-pipes, refuse dumps and, finally, contribute to the contamination of drinking water sources.

Adverse human health effects due to high nitrate content in drinking water are currently of concern. The oxidation of normal hemoglobin (Hb) to methemoglobin (metHb) by nitrates is highly toxic to humans causing fatal conditions like cyanosis and asphyxia at sufficiently high concentrations (World Health Organization 2011). Nitrates have also been reported to be a potential confounder in cancer incidence and act as an inhibitor in iodine uptake by the thyroid gland. Based on the best available evidence, the WHO and Bureau of Indian Standards (BIS) have set up the guideline value and acceptable/desirable level of nitrate in drinking water as 50 mg/L (World Health

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Organization 2011) and 45 mg/L (Bureau of Indian Standards BIS: 10500:2012) as NO_3^-, respectively.

Nitrates can be ingested through food as well as from drinking water. Plants take up nitrates from contaminated soils and water leading to accumulation of a higher concentration of nitrates in leafy vegetables. Seventy to eighty percent of the consumption of nitrates is estimated to be from plant sources (Hord *et al.* 2009). Due to the presence of vitamin C and vitamin E in vegetables as inhibitors, there is a significant reduction in the possibility of formation of nitroso compounds. Also, it has been reported that processing of vegetables, including washing, peeling, boiling, and cooking can cause loss of nitrates (EFSA Panel on Contaminants in the Food Chain 2008). Nitrate is also used as a preservative in processed foods to prevent bacterial growth and as a color enhancing agent in meat products.

Nitrates react with secondary and tertiary amines through a series of reactions to form nitrosamines which are proven carcinogens. Research in the area of carcinogenicity due to high nitrate ingestion is still ongoing. Higher concentrations of nitrates in drinking water have been positively associated with the increased risk of several types of cancers. Nitrates having the potential to convert to dinitrogen trioxide under acidic conditions of the stomach have been possibly related to cancer risk due to the formation of N-nitroso compounds. Nitrate once ingested is converted to nitrite in the presence of commensal bacteria in the saliva. Nitrite then forms nitrous acid which yields dinitrogen trioxide, finally producing nitrosamine by reaction with secondary and tertiary amines in the gastrointestinal (GI) tract (Ward *et al.* 2005; National Toxicology Program 2011). These N-nitrosamines are proven carcinogens and their exposure results from the consumption of nitrosating agents as nitrates, tobacco and cigarette smoke (Hebels *et al.* 2011; Stepanov *et al.* 2014).

A case-control study in Iowa linked average nitrate concentrations above 5 mg/L (NO_3-N) with colon and rectum cancer risk for a 10-year exposure. Public water supplies data on nitrate concentration in water for Iowa towns retrieved from the Center for Health Effects of Environmental Contaminants were related to the incidence of colon and rectum cancer with an odds ratio (OR) of 1.2 and 1.1, respectively, depicting a significant risk of cancer due to nitrates (De Roos *et al.* 2003). An average nitrate concentration of 88 mg/L was reported to be positively associated with stomach cancer mortality in an ecological study to determine the levels of nitrate involved with the development of gastric cancers (Sandor *et al.* 2001). A similar study in Slovakia on high nitrates in drinking water and cancer incidence linked nitrate concentrations of up to 50 mg/L with non-Hodgkin lymphoma and colon cancer (Gulis *et al.* 2002). Drinking water supplies of 258 municipalities in Valencia, Spain, reported higher nitrate concentrations (>50 mg/L) to be associated with gastric cancer risk (Zaki *et al.* 2004).

Tobacco consumption, radiation exposure, alcohol, hormones, age, diet, genetic history of cancer and some viruses and bacteria are some of the leading causes of the disease. Studies on tea and coffee consumption have also linked them with the incidence of cancer (Inoue *et al.* 1998; Ren *et al.* 2010; Chen & Long 2014; Green *et al.* 2014). The risk of cancer was also found to be associated with chronic consumption of alcohol (Bagnardi *et al.* 2001; Seitz & Becker 2007). Several factors leading to cancer development could thus be present and nitrate is one of them to explore.

The epidemiological studies conducted so far show heterogeneous results in the association of cancer with nitrates. This study aims at determining the association of drinking water nitrates with gastric cancer risk. After reviewing the status of nitrate contamination of drinking water in Nagpur, a case-control study design was adopted to assess health risk due to nitrates. Considering the geographical differences and population characteristics, the study investigates gastric cancer risk due to nitrates in drinking water in Nagpur and Bhandara districts, India.

METHODOLOGY

This study intends to determine the nitrate contamination and associated cancer risk in and around Nagpur. A gastric cancer exposed population was identified with the help of three cancer hospitals in Nagpur. The MIDAS multispeciality hospital, Government Medical College, and Mandhania hospital were enrolled for the study. The data on gastric cancer patients from 2000 to 2014 collected from the hospitals were used to identify the sampling locations by plotting the locations on Google Earth and identifying dense areas

having at least more than one patient within a similar area. The 78 gastric cancer cases admitted during 2000–2014 were considered as the subject group of the study. A control group of 156 individuals was identified, with non-cancer patients residing at similar locations as cases and utilizing drinking water and vegetables from the same source as that of cases. The surveys were administered electronically using the Open Data Kit platform. Patients' experience was recorded on the basis of 16 predictor variables along with the collection of drinking water and vegetable samples to identify nitrate contamination. The questionnaire consisted of questions about demographic information, residential history, working pattern, sources of drinking water, dietary habits, vegetable procurement, tea/coffee consumption, smoking history, alcohol consumption, tobacco consumption, history of genetic diseases in the family, and other water-related diseases. The results were then analyzed to achieve a conclusion on the hypothesis about the association of nitrate and cancer incidence.

Prior informed consent was obtained from the human subjects under study.

Survey design

The following exposure variables, risk variables, and confounders were considered for the study (Table 1).

Table 1 | Different sections of survey including exposure variables, risk variables, and confounders

Variables	Category
Exposure	Drinking water nitrate consumption
	Vegetable nitrate consumption
Risk factors	Time of stay
	Working pattern
	Drinking water source
	Water quality
	Food habit
	Procurement of vegetables
Confounders	Tea/coffee consumption
	Smoking history
	Alcohol consumption
	Tobacco consumption

Exposure

The case-control study under consideration was based on two of the exposure variables of nitrate. Drinking water and vegetables were considered as the main exposure variables by which the population had the chance to be exposed to nitrate.

Drinking water: Persons can be exposed to a high level of nitrates present in the water they are consuming on a daily basis for drinking purpose if it exceeds the permissible limit of nitrate.

Vegetables: Individuals also have the probability to be exposed to nitrates from vegetables if high nitrate-accumulating vegetables are consumed on a daily basis and the daily intake of nitrate exceeds the acceptable intake of nitrate in vegetables as prescribed by the Joint FAO/WHO Expert Committee on Food Additives.

Risk factors

Risk variables which had the possibility of increasing cancer incidence were then identified and are shown below.

Time of stay: The residential history of the person living in a particular area was recorded in years by stratifying the individuals in two categories: the ones who were living for less than 10 years in a particular area and the ones who were living for more than 10 years in the particular area (<10 yr/ >10 yr). This question was based on an assumption that the respondents living in the particular area for more than 10 years had a higher exposure to the similar environmental conditions for a longer period of time.

Working pattern: The working pattern was divided into three categories: farming, employed, or unemployed. This factor was based on subsequent possible risk to nitrates to the individuals practicing farming due to the application of nitrogenous fertilizers in the field in comparison to the other two categories.

Drinking water (DW) source: The following categories were considered for the drinking water source: communal tube well; borehole, hand pump; communal hand dug well; communal tap; water treatment system; river, stream, spring; purchased water, water delivery tanker; personal hand dug well; personal tube well, borehole, hand pump; personal tap.

The respondents were asked to choose one of the categories of drinking water source to determine the source information. There could be a possible risk of nitrates to the individuals drinking water from the communal tube well and hand dug well as nitrates could leach directly from the agricultural fields to the surrounding area, thereby contaminating groundwater.

Water quality: This was divided into the following categories: drinking water rating; drinking water smell; drinking water color; and drinking water purification technique.

Some questions were then asked of the respondents to describe the water quality to which they were exposed. The drinking water rating was categorized into the three categories of good, acceptable, or poor, according to the perception of the individual consuming the water.

Further, the respondents were asked to rate the smell of the water as having foul smell/no smell. The color of the water as being yellowish/muddy which is distinguishable in comparison to clear water was also taken into consideration. Water purification techniques, if utilized in households, including reverse osmosis (RO) membrane/filtration/boiling/alum were also recorded.

Food habit: Consumption of some green vegetables in a vegetarian diet has been found to have accumulated more nitrates, for which, vegetable samples were collected and analyzed for the presence of nitrate. The respondents were asked to report if they were following a non-vegetarian/vegetarian diet and how frequently. They were also asked about frequent consumption of nitrate-rich vegetables such as lettuce, spinach, beetroot, cabbage, and turnip.

Procuring vegetables: Respondents were asked to report whether the vegetables consumed by them were brought from the market/local vendor/mall or from the agricultural fields to identify the source of vegetables.

Confounders

Tea/coffee consumption: Respondents were asked to report the consumption of tea/coffee at subsequent intervals, as once a day/twice a day/three times a day/more than three times a day/never.

Smoking: The habit of cigarette smoking by individuals was recorded on a scale of daily, weekly, rarely, to get an insight into the practice of smoking that can enhance cancer risk if practiced periodically. A pack of cigarettes consumed daily was considered to be the highest frequency of smoking that can lead to increasing the risk of cancer. Alcohol consumption and tobacco consumption was also rated on a scale of daily, weekly, and rarely to gain information about the habits of individuals that increase the risk to cancer.

Genetic diseases: The individuals were asked to report the genetic history of diseases in their family. This was categorized into cancer, thyroid, and other. The individuals having a familial genetic history of cancer and thyroid could possess a greater risk of cancer development.

Multiple diseases: Within this category the individuals having a history of water-related diseases in their family were listed. Common diseases considered for the survey were cholera, diarrhea, typhoid, jaundice, cirrhosis, peptic ulcer, gastric acidity, upset stomach, dyspepsia, vomiting or kidney/bladder stone.

Study area

Three major hospitals in Nagpur were identified and 60, 68, and 74 patients affected by GI cancer including rectum, colon, ovary, stomach, pancreas, and gall bladder from MIDAS multispeciality hospital, Mandhania hospital, and Government Medical College, respectively, were selected. The gastric cancer patients enrolled during 2000–2014 were selected for the study. The residence locations of patients were recorded and immediately converted to GPS positioning for analysis to safeguard the identity of respondents. These patients were identified to be living in 42 rural and 36 urban areas of Nagpur and Bhandara districts (Figure 1). Visits were made to the households of the patients and the study was undertaken. People consuming water and vegetables from the same source as cancer patients in the same area were considered as controls.

Sample collection

Drinking water and vegetable samples were collected from the sources of patients and controls. Each sample was collected in triplicate to verify the nitrate concentration.

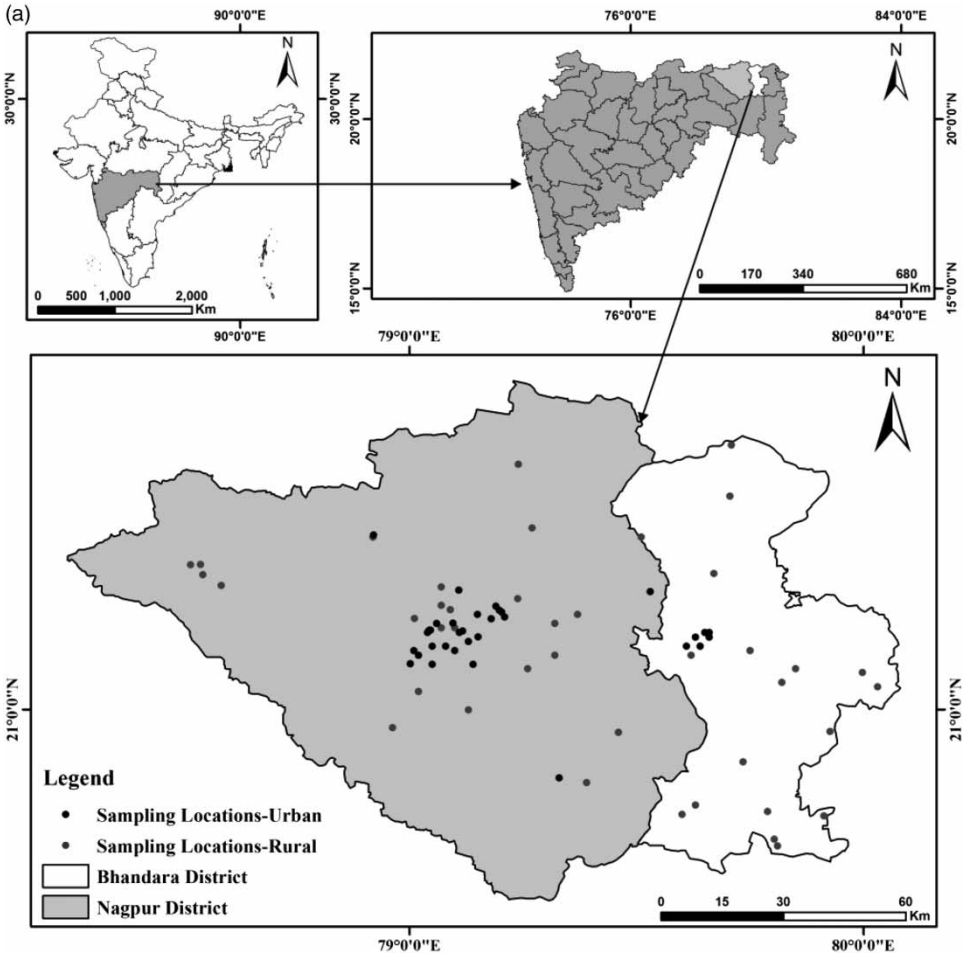

Figure 1 | (a) Study area in Maharashtra, India. (b) Rural study areas in Nagpur and Bhandara districts. (c) Urban study areas in Nagpur and Bhandara districts. (*Continued.*)

Drinking water samples were collected in I L pre-cleaned plastic sampling bottles and transported to the laboratory for analysis of physico-chemical parameters including pH, EC, turbidity, and nitrates. Nitrate was measured in mg/L using a spectrophotometer (APHA 2012).

Vegetable samples were collected in zip lock bags and transported to the laboratory within 4 hours of sampling. Vegetables were thoroughly washed with distilled water, chopped to an approximately uniform size and dried. Nitrate concentrations in vegetable samples were analyzed using the AOAC method (AOAC 1994).

In addition to the above analyses, a detailed survey was conducted in each household and data were collected electronically using formhub software developed by the University of Columbia, USA. Along with general demographic information, the duration of residence in the location, as well as personal perception information such as water quality ratings, were obtained. The primary drinking water source for each patient/relative as well as the area from where they procure vegetables was surveyed. Alcohol/tobacco consumption, tea/coffee consumption on a daily, weekly, or monthly basis was recorded. Genetic history of cancer and other prevalent waterborne diseases in the family were also recorded.

Data analysis

Logistic regression was utilized to estimate the association and model the relation between nitrate concentration and GI cancer development. All the 16 parameters (Table 2) were regressed against the cancer incidence to estimate the risk. OR at 95% confidence interval (CI) was also

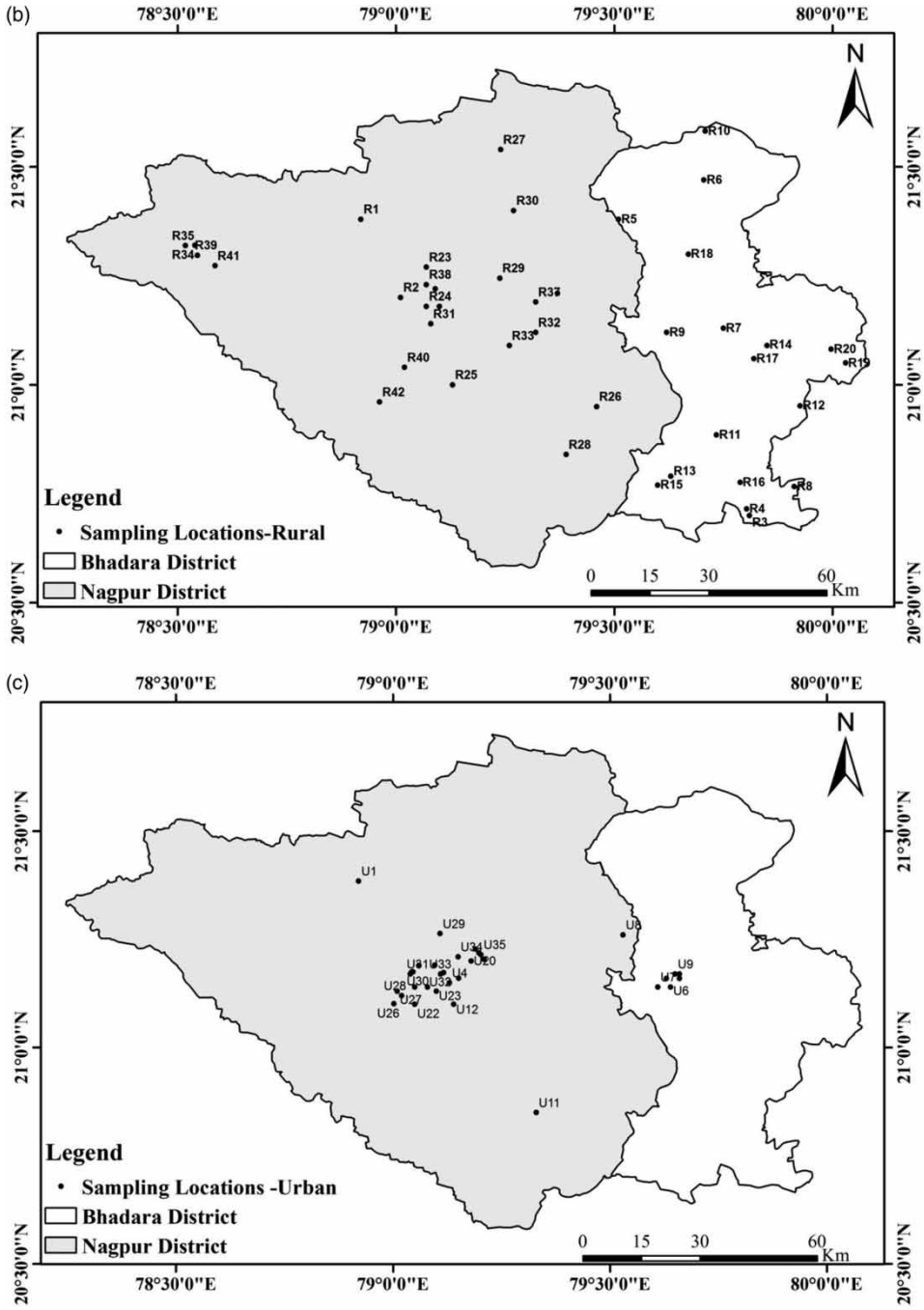

Figure 1 | Continued.

calculated using R Stat software (R-project). Values of $P < 0.01$ were considered statistically significant. The nitrate concentration from drinking water was categorized into two categories: low (below $\leq$45 mg/L) and high (above >45 mg/L). After determining significant factors, crude OR and adjusted OR were calculated for the association of nitrate and GI cancers. Mean daily estimated intake of nitrate from vegetables was calculated using the formula $EDI = F \times R$ where EDI is estimated daily intake of nitrate; F is average daily vegetable consumption (mg/kg body

Table 2 | Characteristics of study population

Parameter	Cases ($n = 78$)	Control ($n = 156$)	Chi-square value	Significance
Age (year)			4.5	$P > 0.05$ non-significant
10–40	24 (30.8%)	66 (42.3%)		
40–70	50 (64.1%)	82 (52.6%)		
70–100	9 (11.5%)	10 (6.4%)		
Gender			2.5	$P > 0.05$ non-significant
Male	47 (60.2%)	110 (70.5%)		
Female	31 (39.7%)	46 (29.5%)		
Marital status			1.4	$P > 0.05$ non-significant
Unmarried	4 (5.1%)	15 (9.6%)		
Married	74 (94.9%)	141 (90.4%)		
Time of stay (year)			5.0	$P < 0.05$ significant
>10	71 (91%)	124 (79.5%)		
<10	7 (9%)	32 (20.5%)		
Working pattern				
Farming	9 (11.5%)	14 (9%)		
Drinking water source			86.3	$P < 0.05$ significant
Groundwater	51 (65.3%)	98 (62.8%)		
Piped water	29 (37.1%)	58 (37.2%)		
Water quality			6.3	$P < 0.05$ significant
Good	20 (25.6%)	55 (35.3%)		
Acceptable	25 (33%)	76 (48.7%)		
Poor	20 (25.6%)	25 (16%)		
Food habit			1.7	$P > 0.05$ non-significant
Vegetarian	34 (43.6%)	82 (52.6%)		
Non-vegetarian	44 (56.4%)	74 (47.4%)		
Procuring vegetables			20.2	$P < 0.05$ significant
Agricultural area	26 (33.3%)	15 (9.6%)		
Marketplace	52 (66.6%)	141 (90.4%)		
Tea/coffee consumption	70 (89.7%)	139 (89.1%)	209.5	$P < 0.05$ significant
Smoking	13 (16.6%)	12 (7.7%)	32.5	$P < 0.05$ significant
Alcohol consumption	11 (14.1%)	14 (9%)	29.2	$P < 0.05$ significant
Tobacco consumption	23 (29.4%)	27 (17.3%)	60.5	$P < 0.05$ significant
Genetic diseases			6.1	$P < 0.05$ significant
Cancer	13 (16.6%)	5 (3.2%)		
Diabetes	2 (2.6%)	7 (4.5%)		
Thyroid	5 (6.4%)	5 (3.2%)		
Multiple disease	2 (2.6%)	1 (0.64%)	4.5	$P > 0.05$ non-significant

weight) – 0.5·kg person^{-1}·day^{-1}; and R is concentration of nitrate in vegetable (mg/kg).

Similarly, mean daily estimated intake of nitrate from drinking water was calculated as EDI = Average daily water consumption (2 L person^{-1}·day^{-1}) × Concentration of nitrate in water (mg/L).

RESULTS

Data analysis indicates that there is a significant difference in mean nitrate concentrations of drinking water supplies in the cancer cases and controls. The consumption of nitrate through drinking water is higher in the case of individuals consuming drinking water containing nitrate greater than 45 mg/L, as shown by the box plot in Figure 2.

Table 2 presents the different characteristics of the population with mean nitrate intake. Out of 234 people, 78 were cancer cases and 156 individuals served as control. Both the control and cancer cases residing within 1 km distance had similar exposure to environmental factors. Of the 78 cancer cases, 31 were females and 46 were males. Seventy-one cases and 124 controls had been residing at the same place for a period of more than 10 years. The major drinking water consumption was from groundwater sources, as 51 cases and 98 controls were using groundwater as the source of primary drinking water (Table 1). Also, 13 cancer cases reported the presence of cancer in their family, linking the disease to genetic factors. Around 41 cases were identified to be consuming nitrates in drinking water above the acceptable level (BIS nitrate >45 mg/L) while only 24 controls were exposed to nitrate concentration above the limit (Table 3). The estimated daily intake of nitrate from water and vegetables was higher in the cases in comparison to the controls. The cases were reported to have a consumption of nitrate in drinking water above 45 mg/L as compared to the controls (Figure 3(a) and 3(b)). The higher prevalence of diseases (categorized as multiple diseases) in cases showed a higher probability of health risk (Figure 4). The individuals registered as cases were reported to be significantly affected with water-related diseases, reconfirming that the cases were prone to higher risk of contamination (Figure 5). Table 4 presents the conditional logistic regression model of the characteristics associated with the population. Results indicate that age, tobacco consumption, and nitrates in the water as well as vegetables were the significant factors at $P < 0.05$.

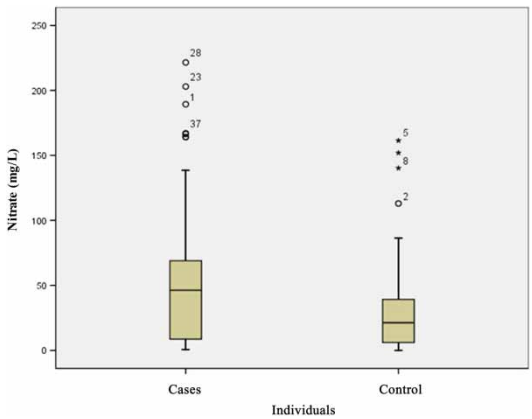

Figure 2 | Mean nitrate concentration in cancerous and non-cancerous individuals.

Table 3 | Nitrate concentrations

Nitrate in drinking water and vegetables	Cases ($n = 78$)	Control ($n = 156$)
Mean estimated daily intake of nitrate in drinking water (mg/L) ± S.D.		
≥ 45	116.9 ± 8.3	175.73 ± 4.2
< 45	20.4 ± 6.4	51.5 ± 2.9
Mean estimated daily intake of nitrate from vegetables (mg/day) ± S.D.	69.59 ± 4.9	35.73 ± 1.6

At 95% CI, adjusted OR of nitrate in drinking water was 1.20 and nitrate in vegetable 1.02 (Table 5). The adjusted OR for nitrate in drinking water illustrated that this confounding factor has a significant potential to increase the risk of cancer, while the adjusted OR for nitrate in vegetables shows that there was no association between total nitrate intake and gastric cancer. On this basis, nitrate in drinking water was further stratified at levels ≤45 mg/L and >45 mg/L.

Table 6 shows the adjusted association of nitrate with GI cancers. After adjustment for age, gender, and tobacco consumption, nitrate concentration ≤45 mg/L is shown to have no association with GI cancer at this stratification level, while at >45 mg/L level the association was statistically significant.

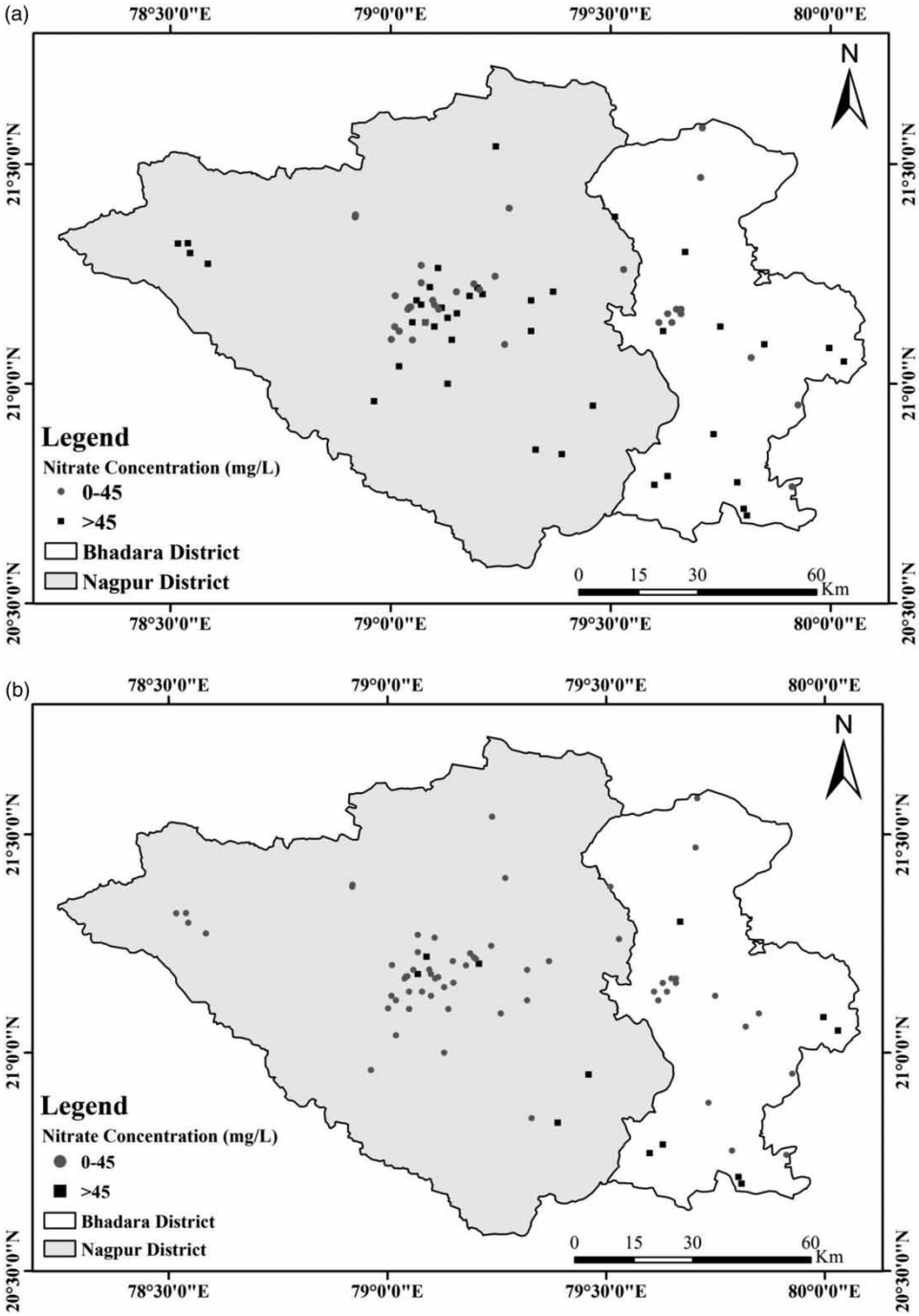

Figure 3 | (a) Nitrate concentration in cases within the range 0–45 mg/L and above 45 mg/L. (b) Nitrate concentration in controls within the range 0–45 mg/L and above 45 mg/L.

DISCUSSION

According to the WHO (2015), the third most common cause of cancer-related deaths is attributed to gastric cancers. Some of the compounds found in cured meats and drinking water as nitrates and nitrites convert themselves into carcinogenic nitrosamines causing gastric cancers. There are several risk factors for gastric cancer occurrence in

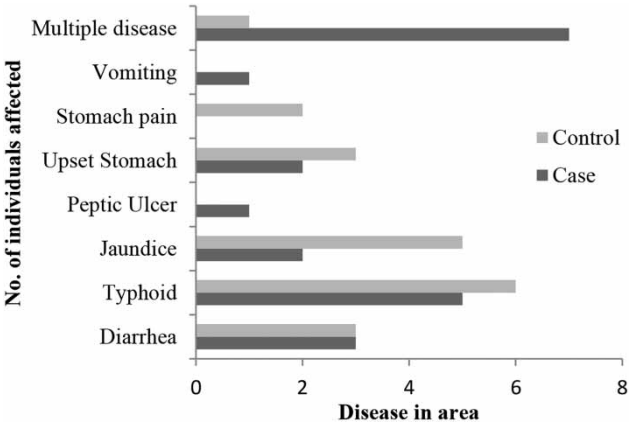

Figure 4 | Graphical representation of diseases prevalent in the area.

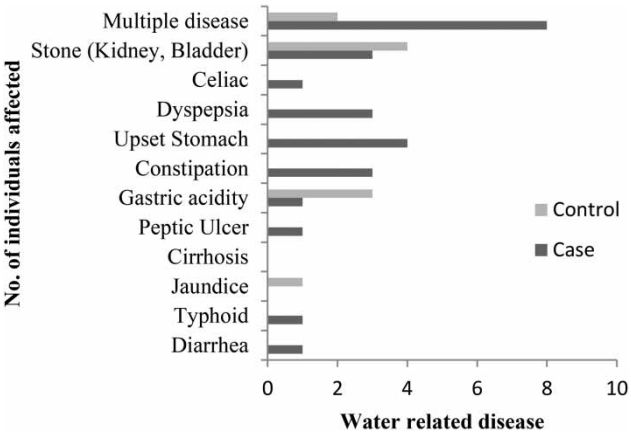

Figure 5 | Representation of water-related diseases in the family.

Table 4 | Logit results

Coefficients	Pr (>|z|)	Significance
Age	0.00575	**
Tobacco consumption	0.01900	*
Nitrate	0.00176	**
Nitrate in vegetables	0.00360	**

Significance codes: **0.001; *0.01.

Table 5 | OR for significant factors

Characteristic	Adjusted OR (95% CI)[a]	Crude OR
Nitrate in drinking water	1.20 (1.04–1.34)	1.1
Nitrate in vegetables	1.02 (1.00–1.04)	1.00

[a]Adjusted for age, gender, and tobacco consumption.

Table 6 | Association of nitrate with GI cancers

Nitrate in drinking water	Cases	Control	Adjusted OR[a]
≤45 mg/L	41 (52.6%)	24 (15.4%)	1.00 (0.98–1.01)
>45 mg/L	37 (47.4%)	132 (84.6%)	1.10 (0.99–1.15)

[a]Adjusted for age, gender. and tobacco consumption.

humans including tobacco consumption, alcohol consumption, cigarette smoking, tea/coffee consumption, vegetables rich in nitrate, cured meat products, a genetic history of cancer, and high concentration of nitrates in drinking water.

We have used a population interview-based case-control study to determine the relationship between nitrates and gastric cancer incidence. This study examined the effect of nitrates in GI cancer cases with an assumption that they were exposed to similar environmental conditions for more than 10 years as 91% of the population reported to be residing at the same place for ≥10 years. The mean nitrate concentration to which the cancer cases were exposed was higher in comparison to the controls, as shown in Figure 2. Our findings supported the hypothesis of the study that nitrate concentration in drinking water plays a significant role in determining the increased risk of GI cancer. The logistic regression model shows the statistical significance of nitrates in drinking water and vegetables with $P = 0.001$ and 0.003, respectively, at $P < 0.01$. Thus, nitrate with GI cancer risk should be considered as a significant factor while determining cancer risk in areas with higher nitrates in drinking water. A study in Baranya county, Hungary showed the significant positive association of high nitrates in drinking water with an increase in the probability of gastric cancer mortality (Sandor *et al.* 2001). The groups exposed to an average nitrate concentration of 88 mg/L (standardized mortality ratio, SMR 0.96; 95% CI 0.79–1.13) and above were at high risk of mortality. Gastric cancer mortality linked with a high intake of drinking water nitrate of NO_3-N concentration greater than 0.45 mg/L (OR 1.2; 95% CI 0.93–1.11) has also been reported in Taiwan (Yang *et al.* 1998). Also, in China, nitrate intake from drinking water was found to be positively associated with gastric cancer as mean nitrate levels were high, up to 109.6 mg/L, significantly impacting gastric mucosal changes (Xu *et al.* 1992). Our data suggest a similar association with GI cancers.

There were several strengths found in the study. The study relied upon data directly from cancer affected cases rather than mortality data which is of less importance due to the inherent causes of several related factors which could relate to the cause of death and mislead the study. We have considered 16 different risk variables to identify the major causes of the disease occurrence, which, to our knowledge, has not yet been carried out by any recent research study. Adding to this, the study has taken into consideration the incidence data collected from the residential locality at the time of diagnosis of the disease. The field data collection on nitrate concentration of drinking water and vegetables along with source identification proved the validity of the research.

Further, adjusted OR determined at 95% CI confers that nitrate in drinking water is the potential confounder in increasing the risk of cancer as has been reported by several other studies. This is confirmed with an adjusted OR of 1.20 for nitrate in drinking water (Table 6). However, the adjusted OR of 1.02 for nitrate in vegetables depicts nitrate exposure from vegetables is not associated with GI cancers. In the absence of drinking water nitrates, vegetables play a major role in nitrate intake for humans. However, vegetables also contain vitamin C and vitamin E which can act as potential inhibitors of endogenous nitrosation. This can hinder the formation of nitrosating compounds due to nitrates from dietary vegetables (Zeegers *et al.* 2006). The endogenous formation of N-nitroso compounds from nitrate/nitrite and secondary and tertiary amines in the absence of vitamin E and selenium in the diet can be a cause of human cancer, as established by Chow & Hong (2002). Nitrite treatment in the presence of low selenium and vitamin E intake leading to the formation of peroxynitrite ONOO- has been found to cause animal mortality. It has been already proven that processed meat can cause colon cancer due to higher N-nitroso compounds in comparison to fresh meat (Ward *et al.* 2005). The dietary intake of nitrate was found to be positively associated with adult glioma and thyroid cancer risk in a metanalysis study (Xie *et al.* 2016).

Nitrosating compounds as nitrate thus proves to possess the potential to cause a risk of cancer. Further, the segregation of data at 45 mg/L levels according to the BIS permissible limit for nitrates in drinking water, is reported to have (adjusted OR of 1.00 and 1.10 for values below and above 45 mg/L) a distinct significance to the association between nitrate and GI cancer. This implies that the Indian, as well as international standards for nitrate, should include the risk of cancers due to prolonged exposure to nitrates as a major factor while prescribing permissible limits.

Considering data collection based on a living population as subjects, the study was programmed for 2 years with a small sample size. The limitations of the study could involve the lack of previous data on nitrate in drinking water. However, this has been resolved by the reference to counter data collection from monitoring agencies such as the Central Groundwater Board and there was found to be no significant difference in nitrate concentrations. The study did not include data on calcium and magnesium, which have often been correlated as having a cumulative effect with nitrates on cancer risk in recent research.

The epidemiological evidence to support the link between drinking water nitrate and GI cancers is mixed. A study in Wisconsin for the association between nitrate levels in drinking water and gastric cancer mortality conferred no link of increasing gastric cancer risk and nitrate levels (Radmacher *et al.* 1992). However, further insights into the cause of the disease and its association with nitrate levels while minimizing the random classification errors is essential. In summary, high nitrates appear to be associated with a risk of GI cancers, although the association may differ because of the adjustment for different dietary habits and confounders among diverse populations.

In view of the above presented study, the effect of other parameters in modifying the effect of nitrate on the risk of cancer would be worth exploring. Studies over a larger population could also present further evidence of the risk towards cancer.

CONCLUSION

The findings of this research illustrate the significant determination of nitrates in drinking water and vegetables and the consequent gastric cancer risk. Our findings clearly suggest that prolonged exposure to nitrates in drinking water above 45 mg/L could be a significant confounder in gastric cancer incidence. The drinking water nitrate

monitoring studies and exposure data collected through the survey support that along with factors such as high frequency of tobacco consumption and alcohol consumption, the nitrate consumption and residential longevity positively affect the occurrence of GI cancers. Although some of the vegetables have been reported to contain high nitrate levels, the presence of antioxidants limits the formation of nitrosamines and their potential carcinogenic risk. Thus, the nitrate-rich drinking water sources should be periodically monitored. A further understanding of different sources of nitrate affecting human health is essential to help minimize cancer risk.

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