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Effect of Nitrate on Oral Health Status among General Population Residing in High and Low-Level Nitrate Blocks in Erode District, Tamilnadu, India - A Cross Sectional Study

Gousalya V[1], Prabu D[2], Raj Mohan M[3], Dinesh Dhamodhar[3], Bharathwaj VV[4], Sindhu R[4], Sathiyapriya S[4], Elakiya[1]

[1]Post graduate student, SRM Dental College and Hospital, Department of Public Health Dentistry, Ramapuram, Chennai, India
[2]Professor and Head, SRM Dental College and Hospital, Department of Public Health Dentistry, Ramapuram, Chennai, India
[3]Reader, SRM Dental College and Hospital, Department of Public Health Dentistry, Ramapuram, Chennai, India
[4]Senior Lecturer, SRM Dental College and Hospital, Department of Public Health Dentistry, Ramapuram, Chennai, India

Corresponding Author: Dr. Prabu D

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ABSTRACT

Background: Nitrate levels in our water resources have risen in many parts of the world, owing primarily to the use of inorganic fertilizers and animal manure in agricultural areas. When nitrate is consumed under conditions that promote the formation of N-nitroso compounds, the risk of certain cancers and congenital disabilities may increase**.**
Aim: The study aims to evaluate the nitrate levels in groundwater and their effect on oral health status among the general population of Erode district, Tamil Nadu, India.
Methodology: A cross-sectional study was conducted among the general population of index age groups 5 yrs, 12 yrs, 15 yrs, 35-44 yrs and 65-74 yrs. A total of 540 subjects participated in the study. The Oral Health Assessment form, WHO 2013 was used for assessing dental caries, enamel fluorosis and oral mucosal lesions. Clinical examination was performed using Type III clinixal examination. Data were processed and analyzed using SPSS version 26. A Chi-square test was employed to analyze the difference between the high and low fluoride nitrate blocks.
Results: There was no statistically significant difference between the high and low nitrate blocks, with a p-value of 0.062. Statistically significant differences were found between the index age groups for the prevalence of oral mucosal lesions (p-value <0.001)
Conclusion: This study's contradictory finding emphasizes the importance of drinking water and Central Ground Water Board Ministry of Water Resources should plan and organize a team for the upgradation of the water quality in India. Such lacunae might be due to the unqualified manpower, meticulous ground work can be done efficiently by Public Health dentist alone but they are not recruited for the Central, State and District level Officials. Hence the Public Health Dentist should be recruited as a District Oral Health Officer for formulating a meticulous plan to solve the community dental problem.

Keywords: nitrate, oral mucosal lesions, water resources, n-nitroso compounds, candidiasis, ANUG

INTRODUCTION

Water is the most important compound for human survival. Daily water consumption from various sources exposes an individual to various elements present in water, which can be beneficial or harmful. The continuous growth in the global population over the last few decades, along with unplanned development and irresponsible human interference, has put freshwater surface resources under severe strain. Over-exploitation of groundwater resources leads anthropogenic load to groundwater. In recent decades the incidence of cancer has been emerging rapidly in Tamil Nadu. In erode district the prevalence of lung cancer was 3%, prostate cancer was 3%, kidney cancer was 2%, nasal and paranasal sinus cancer was 4%, stomach cancer was 2%, mouth cancer was 3.1%. To find out the reason related to dentistry we came across various elements present in the drinking water, the element which was above the permissible limit is nitrate which has the carcinogenic properties of causing cancer. Researches have been conducted in various geological, geomorphic, and climatic settings to determine how the geochemistry of groundwater influences its quality and found that high levels of salinity, nitrate, phosphorus, fluoride, arsenic. threaten groundwater quality in many countries such as India, USA, China, Canada, Spain, Pakistan, Iran and Namibia. [1] In 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as probably carcinogenic to humans, and after reviewing the studies, the international scientists established the association between glyphosate and non-Hodgkin lymphoma [22]

Nitrate is a compound that occurs naturally and also has many human-made sources. Consuming too much nitrate can be harmful. Groundwater pollution with anthropogenic nitrate (NO3)– pollutants has been identified as an emerging concern in India. Nitrate contamination is anthropogenic groundwater pollution that can come from both point (such as sewage systems, dairy lagoons, wastewater discharge precipitation, and animal wastes) and non-point sources (such as fertilisers, pesticides, manure application, atmospheric deposition, industrial emissions and so on).[2][3] Increased NO3 – pollution in drinking water causes health problems such as methemoglobinemia in new-borns, thyroid dysfunction, and hypertension.[4][5][6] Nitrogen fertiliser is the most important anthropogenic source of nitrogen in the environment, and its use has expanded dramatically since the introduction of the Haber–Bosch process in the 1920s. After 1980, most synthetic fertiliser applications on agricultural land began.[7] Nitrate concentrations in our water resources have expanded as nearly half of all applied nitrogen flows from agricultural fields to pollute surface and groundwater.[8] Nitrate from drinking water is easily absorbed in the upper gastrointestinal system and transported throughout the human body. It is actively transferred from blood into saliva when it reaches the salivary glands, and levels can be up to 20 times greater than in plasma.[9][10] Around 6–7% of total nitrate in the oral cavity may be converted to nitrite, primarily by nitrate-reducing bacteria.[11] When nitrate is secreted, it enters the gastrointestinal tract, along with the nitrite produced in the oral cavity. Nitrite can be protonated to nitrous acid (HNO_2) in the stomach, resulting in dinitrogen trioxide (N_2O_3), nitric oxide (NO), and nitrogen dioxide (NO_2). It has become obvious that NO is implicated in a wide spectrum of NO-mediated physiological functions since the discovery of endogenous NO production. These include the management of blood pressure and blood flow through vasodilation, the preservation of blood vessel tone, the prevention of platelet adhesion and aggregation, the modulation of mitochondrial activity, and a variety of other functions.

On the other hand, nitrous acid (HNO_2) and other nitrate and nitrite generated

metabolites are strong nitrosating agents that are known to stimulate the development of N- nitroso compounds (NOC), which are thought to be the primary agents in many of the nitrate-associated deleterious health effects. NOC is made up of N-nitrosamines and N-nitrosamides and can be generated when nitrosating substances interact with N-nitrosatable amino acids, which are also derived from food. The nitrosation process is affected by the reaction processes involved, the concentration of the chemicals involved, the pH of the reaction environment, and other modifying variables such as the presence of N-nitrosation catalysts or inhibitors. [12] To preserve the health of the most vulnerable group, bottle-fed new-borns, the recommended value for nitrate in drinking water is 50 mg/L as nitrate ion. As a result, it protects other populations, such as older children and adults. This recommendation number is based on epidemiological research that found no detrimental health consequences (methemoglobinemia and thyroid impacts) below 50 mg/L in drinking water. [13] The World Health Organization (WHO) established an acceptable daily intake (ADI) for nitrate of 3.7 mg/kg body weight per day, equivalent to 222 mg nitrate per day for a 60-kg adult, and nitrite of 0.07 mg/kg body weight per day, equivalent to 4.2 mg nitrite per day for a 60-kg adult, based on risk of methemoglobinemia. [14]

Ingested nitrate and nitrite that may produce NOCs are probably carcinogenic to humans, according to the International Agency for Research on Cancer (IARC) classification, they are classified under group 2A. Ecological research identified an association between nitrate and both colorectal cancer and all digestive tract cancers together.[15] There is also a positive association between nitrate and bladder cancer, brain cancer. More research is needed to determine the impact of nitrate from private wells, which may be a significant supply of water in rural regions and may have a greater degree of pollution than municipally provided public water.[16] Nitrate is a precursor in the synthesis of N-nitroso compounds (NOC) by endogenous nitrosation; the majority of NOC are carcinogens and teratogens. As a result, exposure to NOC produced because of nitrate consumption from drinking water and food sources may result in cancer, birth abnormalities, or other serious health effects.[12] Early diagnosis of oral cancer has emerged as a priority to the public health objective whereby oral health professionals play leading role. [23]

According to Central Ground Waterboard Ministry of water resources (CGWB), Government of India, 2010 Erode district has nitrate content above the permissible limit in water. [29] According to the development of an atlas of cancer in India (1985-2002) oral cancer prevalence of Erode district in the female is 5.2% and in male is 7.5%. [30] The former data indicates that the nitrate has the potential to cause cancer, especially oral cancer. With this evidence the current study focussed on identifying the nitrate levels in drinking water of Erode district and to find its association with oral mucosal lesions.

MATERIALS & METHODS

A descriptive cross-sectional study was conducted among the general population residing in high and low nitrate blocks in Erode district, Tamil Nadu, India. This study was conducted for 15 months, from July 2021 to September 2022. The nature and purpose of the study were explained to the SRM Dental College Ramapuram Institutional Review Board, and ethical approval was obtained (IRB NO: SRMDC/IRB/2020/MDS/No.701). Sample size calculation was done using G Power software (3.1). The power (1-β) of the study was kept at 95% and α error at 5%.[6] A total of 540 samples were obtained for examining participants for the high and low nitrate blocks. Erode district has 14 blocks. [7] Out of 14 blocks 4 high nitrate and 4 low nitrate blocks were taken. After dividing into high and low blocks for nitrate using multistage random sampling, 2 blocks were selected for each category, i.e., high nitrate

and low nitrate blocks, 135 participants were examined in each block. High nitrate blocks of Erode district were selected based on the CGWB, Government of India, 2010 [4] and the low Nitrate blocks were taken from the Groundwater yearbook of Tamil Nadu and U.T. of Puducherry, 2018.[5]. 25 water samples from each block were collected. Hence a total of 100 water sample was collected, measuring approximately 200ml in a clean, dry polythene container from 4 blocks [Table 1]. All the containers were rinsed with distilled water before the water sample collection, and they were immediately labelled with the location name. The spectrophotometric method was used to analyze the nitrate level. The inclusion criteria employed in this study were 1) participants who were residents of the block from birth, 2) who have given informed consent, 3) participants who were in the index age groups, 4) participants who used one source of drinking water from birth to 10 years of life and 5) those who have at least 50% of their permanent teeth (except third molars) erupted. Exclusion criteria for this study were 1) participants who had moved from another location or who were not permanent residents of the particular block of examination, 2) those who had a history of drinking water from multiple sources 3) participants who drink purified water and packaged mineral water. Written informed consent was obtained from the participants after explaining to them the aim of the study and methods used in the vernacular language. Intra-examiner reliability was evaluated using Kappa statistics, in which the outcome was satisfied (>0.81). Oral examination was done for 540 participants in the index age group (5yrs, 12 yrs, 15 yrs, 35-44 yrs, 65-74 yrs). The oral health status of the participants was assessed using WHO proforma 2013 for adults and children. Oral mucosal lesions were evaluated for all the participants in the study.

Table 1: High and Low nitrate block selection plan

Total no. of Blocks in Erode district [7]	**High nitrate blocks, according to the CGWB [4]**	**Low nitrate blocks, according to the GWYB of Tamil Nadu and U.T. of Puducherry 2018[5]**	**High nitrate blocks, according to the CGWB which were selected in the current study [4]**	**Low nitrate blocks, according to the GWYB of Tamil Nadu and U.T. of Puducherry 2018 which was selected in the current study [5]**
Ammapet Andiyur Bhavani Bhavanisagar Chennimalai Erode Gobichettipalayam Kodumudi Modakurichi Nambiyur Perundurai Sathyamangalam Talavadi T.K. Palayam	Ammapet Gobichettipalayam Andiyur Bhavani Sathyamangalam Erode	Bhavanisagar Nambiyur Modakurichi Perundurai Bhavani Chennimalai	Gobichettipalayam 135 population 25 water samples	Bhavanisagar 135 population 25 water samples
			Sathyamangalam 135 population 25 water samples	Nambiyur 135 population 25 water samples

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS software (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019). Descriptive statistics determined the frequency, percentage, mean and standard deviation for the prevalence of variables. A Chi-square test was applied for the association between high and low nitrate blocks, gender differences, and prevalence of oral mucosal lesions in the nitrate blocks.

RESULT

Table 2 Water Quality Analysis Of High Nitrate Blocks In The Drinking Water According To The Cgwb And Low Nitrate Blocks According To The Gwyb Of Tamilnadu And The Mean Value Of The Nitrate Levels Obtained From This Study Analysis For Both High And Low Nitrate Blocks.

High nitrate blocks	**Nitrate levels based on CGWB (mg/dl)**	**Nitrate levels obtained from the current study analysis Mean (mg/dl)**
Gobichettipalayam	124	20.12
Sathyamangalam	112	26.64
Low nitrate blocks	Nitrate levels based on the GWYB of Tamil Nadu (mg/dl)	Nitrate levels obtained from the current study analysis Mean (mg/dl)
Bhavanisagar	8	29.16
Nambiyur	6	22

Table 2 represents the presence of nitrate in the drinking water according to the CGWB and the nitrate levels obtained from the present study analysis of High nitrate blocks, i.e., Gobichettipalayam (124) and Sathyamangalam (112). There is a decline in the nitrate levels of our study analysis compared to the CGWB. For low nitrate blocks there is an increase in the nitrate level in the present study, but the value lies within the optimal range.

TABLE 3: COMPARISON OF PREVALENCE OF ORAL MUCOSAL LESIONS IN HIGH AND LOW NITRATE BLOCKS

	No abnormal condition %(n)	**Malignant tumour %(n)**	**Ulceration %(n)**	**Acute necrotizing ulcerative gingivitis %(n)**	**Candidiasis %(n)**	**Abscess %(n)**	**P value**
High nitrate blocks							0.062
Gobichettipalayam	81.5 (110)	5.2(7)	10.4 (14)	0(0)	2.2(3)	0.7(1)	
Sathyamangalam	77.0(104)	2.2(3)	10.4 (14)	1.5(2)	2.2 (3)	6.7(9)	
Low nitrate blocks							
Bhavanisagar	78.5(106)	1.5(2)	11.9(16)	2.2(3)	3(4)	3(4)	
Nambiyur	77 (104)	1.5(2)	8.1(11)	0(0)	5.9(8)	7.4(10)	

Table 3 describes the prevalence of oral mucosal lesions in the high nitrate and low nitrate blocks. There was no statistically significant difference between the high and low nitrate blocks, with a p-value of 0.062.

TABLE 4: PREVALENCE OF ORAL MUCOSAL LESIONS ACCORDING TO THE INDEX AGE GROUPS OF THE STUDY PARTICIPANTS

AGE	**Malignant tumour %(n)**	**Ulceration %(n)**	**Acute necrotizing ulcerative gingivitis %(n)**	**Candidiasis %(n)**	**Abscess %(n)**	**P value**
5 YEARS	0 (0)	12(13)	0 (0)	5.6(6)	2.8 (3)	< 0.001*
12 YEARS	0 (0)	13.9 (15)	0 (0)	2.6(3)	0 (0)	
15 YEARS	0 (0)	12(13)	0 (0)	5.6(6)	0.9(1)	
35-44 YEARS	74.1(80)	8.3(9)	8.3(9)	0 (0)	0.9(1)	
65-74 YEARS	74.1(80)	4.6(5)	4.6(5)	4.6(5)	1.9(2)	

Table 4 represents the frequency and percentage of oral mucosal lesions among the index age groups 5 years, 12 years, 15 years, 35-44 years and 65-74 years. Statistically significant differences were found between the index age groups for the prevalence of oral mucosal lesions (p-value <0.001)

TABLE 5 PREVALENCE OF ORAL MUCOSAL LESIONS BETWEEN MALES AND FEMALES.

GENDER	**Malignant tumour n (%)**	**Ulceration n (%)**	**Acute necrotizing ulcerative gingivitis n (%)**	**Candidiasis n (%)**	**Abscess n (%)**	**P value**
MALE	5 (0.9)	27 (5)	2 (0.4)	11 (2)	13 (2.4)	0.789
FEMALE	9 (1.7)	28 (5.2)	3 (0.6)	7 (1.3)	11 (2)	

Table 5 represents the frequency and percentage of oral mucosal lesions between

males and females, and no statistically significant differences were found between them.

DISCUSSION

This cross-sectional study evaluated the nitrate levels in drinking water and its effect on oral health among the general population residing in high and low nitrate blocks classified according to the CGWB. On the water quality analysis of high nitrate blocks according to CGWB in the current study there is a contrary decrease in the nitrate levels in drinking water. Similarly in the low nitrate blocks (classified according to groundwater yearbook of TamilNadu), there is a contrary increase in the nitrate levels obtained from the current study results in the drinking water. This groundwork study was initiated to compare the oral health status based on the nitrate level data obtained from the CGWB and the GWYB of Tamil Nadu. However, the current study results vary from the CGWB, and GWYB of Tamil Nadu, the values of the nitrate level in our study were within the permissible limit. Hence this study suggests the CGWB and groundwater yearbook of Tamil Nadu to update the data to the current year not only for nitrate but also including other parameters in drinking water. The prevalence of malignant tumour from the current study results in the high nitrate block according to the CGWB Gobichettipalayam was 5.2% and Sathyamangalam was 2.2%. The prevalence of malignant tumour from the current study results in low nitrate block according to the CGYB Bhavanisagar and Nambiyur was 1.5%. The reason states that the nitrate which is a precursor of N- nitroso compounds is carcinogenic in humans causing DNA damage. [19] The prevalence of ulceration from the current study results in the high nitrate block according to the CGWB Gobichettipalayam and Sathyamangalam is 10.4% respectively. The prevalence of ulceration from the current study results in low nitrate block according to the CGYB Bhavanisagar was 11.9 % and Nambiyur was 8.1%. With the results in the prevalence of ulceration after excluding all the potential confounding variable there is a possibility for other unavoidable factors such as aphthous ulcers might have been involved. The prevalence of candidiasis from the current study results in the high nitrate block according to the CGWB Gobichettipalayam and Sathyamangalam was 2.2%. The prevalence of candidiasis from the current study results in low nitrate block according to the CGYB Bhavanisagar was 3% and Nambiyur was 5.9 %. The reason for this might be due to nitrite and NO which is reduced from nitrate may inhibit the microorganisms which includes Candida albicans [20]. The prevalence of abscess from the current study results in the high nitrate block according to the CGWB Gobichettipalayam was 0.7% and Sathyamangalam was 6.7%. The prevalence of abscess from the current study results in low nitrate block according to the CGYB Bhavanisagar was 3% and Nambiyur was 7.4%. The prevalence of acute necrotizing ulcerative gingivitis from the current study results in the high nitrate block according to the CGWB Gobichettipalayam was 5.2% and Sathyamangalam was 2.2%. The prevalence of acute necrotizing ulcerative gingivitis from the current study results in low nitrate block according to the CGYB Bhavanisagar was 1.5% and Nambiyur was 1.5%. In this scenario nitrite which is reduced from the nitrate plays a potential role as a biomarker in the gingivitis and periodontitis.[21] This study serves as a pioneering study because of lack of literature about the interaction of nitrate in drinking water and the oral mucosal lesions. Owing to the lack of literature further analytical studies are need to be conducted in the future.

According to various studies nitrate had a direct relationship with the cancer. The aim of the current study was to find the correlations between nitrate in drinking water and cancer. In Tamil Nadu Cancer Registry Project, there is an insufficient data on the block-wise prevalence of cancer,

because the Tamil Nadu Cancer Registry Project has only the district wise data. This is the reason why the data becomes elusive, that we could not correlate the current study findings with the results of the TNCRP since in the current study we have taken certain specific sampling sites i.e., blocks of the Erode district. The in-depth data regarding blockwise prevalence of cancer was not available, hence we recommend TNCRP for the future research on the blockwise prevalence of cancer by eliminating all the possible confounding factors for the welfare of the public health.

CONCLUSION

This study's contradictory finding emphasises the importance of drinking water and Central Ground Water Board Ministry of Water Resources should plan and organize a team for the upgradation of the water quality in India. Such lacunae might be due to the unqualified manpower, meticulous ground work can be done efficiently by Public Health dentist alone but they are not recruited for the Central, State and District level Officials. Hence the Public Health Dentist should be recruited as a District Oral Health Officer for formulating a meticulous plan to solve the community dental problem. National Program on Prevention and control of dental diseases should look on to the endemic areas for the prevention of dental diseases which should be assessed, implemented and monitored by the public health Dentist who should be recruited as oral health program officers in both central and state governments. Oral health policy should be formulated for the prevention of oral cancer. Public health awareness should be given by the public health dentist to the people about the harmful and beneficial effects of Nitrate and its effect on oral health.

Declaration by Authors

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