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Association between the concentration of nitrate and phosphate ions in drinking water and the occurrence of goiter in Nandi Hills, Kenya

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Abstract
Nitrates and phosphates are among the major inorganic pollutants in the environment, primarily contributed by nitrogenous and phosphate fertilizers, organic manures, human and animal wastes, and industrial effluents through biochemical activities of microorganisms. Beside other known detrimental effects, research findings suggest that increased nitrate and/or phosphate intake affects the functioning of the thyroid gland in humans. In Kenya, goiter remains endemic despite iodization of table salt in households. Notably, the area around Nandi hills in Nandi County has recorded high incidences of goiter in the recent past. This indicates that factors such as inorganic ion content of water (among others) may have etiological role in endemic goiter cases in the region. This study sought to determine the etiological factors leading to observed high prevalence of goiter. Both nitrate and phosphate levels in stream and borehole water were found to be within the WHO acceptable limits. Pearson Correlation results reported an insignificant positive association ($P>0.05$; $r>0$) between nitrate-phosphate-iodine ions and occurrence of goiter in the study. The study therefore concluded that concentrations of nitrate-phosphate ions and iodine levels in water and table salt respectively are not the main cause of goiter in the study area. This calls for additional studies to be carried out on other possible causes of goiter in the study area.

Keywords: Goiter, drinking water, nitrate ions, phosphate ions

Introduction

Globally, the Total Goiter Prevalence (TGP) in the general population is estimated to be 15.8%, varying between 4.7% in America to 28.3% in Africa (Andersson *et al.*, 2010) [1] Worldwide, over 90.54% (approximately 200 million people) cases of goiter are caused by iodine deficiency (De Benoist *et al.*, 2004) [6].

In Africa, goiter is endemic in many countries notably Congo, Uganda, Kenya and Sudan. The prevalence is as high as 81% in some parts of these countries (Elnour *et al.*, 2000) [9]. It is the chief consequence of iodine deficiency, resulting from either low iodine intake or ingestion of goitrogens. Iodine deficiency also occurs in lowland regions far from the oceans, such as central Africa (Zimmermann & Andersson, 2012) [25]. It is characterized by a swelling of the larynx resulting from enlargement of the thyroid gland (thyromegaly) normally associated with a thyroid gland that is not functioning properly (Knudsen *et al.*, 2002) [16]. Goiter is caused by deficiency of iodine in the human body. Selenium deficiency is also considered a contributing factor (Aydin *et al.*, 2002) [3]. In countries that use iodized salt, Hashimoto's thyroiditis is the most common cause.

Lately, it has been also suggested that increased nitrate and/or phosphate intake affects the function of the thyroid gland, as was observed in a study in pigs by competitive inhibition of iodide transport leading to decreased thyroid hormone secretion, followed by an increase in thyroid-stimulating hormone (Zoeller, 2007; Eskiocak *et al.*, 2005) [26, 10]. This has been theorized to happen through the inhibition of steroidogenesis without involving the guanylatecyclase-cyclic guanosine monophosphate pathway (Zaki *et al.*, 2004) [23]. This has been hypothesized to be a major cause of goiter in humans though no conclusive studies have been conducted.

In Kenya, Goiter remains endemic despite iodization of salt. Notably, the area around Nandi hills in Nandi County has recorded high incidences of goiter in the recent past. According to the Kapsabet District Hospital and Moi Teaching and Referral Hospital (MTRH) records goiter accounts for 50 - 72% of the diseases in the Nandi Hills area (Ali *et al.*, 2014). This is despite the fact that the table salt used in most of the households in Kenya is iodized.

This suggests that other etiological factors such as inorganic content of water may have a role in the causation of endemic goiter cases in the region. In addition, tea farming is the main economic activity in the study area. In Nandi Hills tea plantations, about 5,000 hectares of land are under mature tea and fertilizer is applied at a rate of 140 Kg /ha/year, with N: P: K being the commonly used fertilizer. These inorganic ions can be discharged to water bodies through leaching and surface run-off. Against this background, the present study sought to determine the association between inorganic ions and goiter occurrence in the study area. This was achieved through the determination of the concentration of nitrates and phosphates in the drinking water commonly used in this area and urine samples.

Material and Methods

Study Area

The research was conducted in Nandi Hills ward, Nandi County. The area is an urban settlement in Nandi County, Kenya. It is located in a highland area of lush green rolling hills at the edge of the Great Rift Valley in the southwestern part of Kenya, approximately 303 kilometres, by road, northwest of Nairobi. The coordinates of Nandi Hills, are: 0°06'01.0"N, 35°10'35.0"E (Latitude: 0.100278; Longitude: 35.176389). Nandi Hills lies at an elevation of approximately 2,047 metres (6,716 ft), above sea level (Globalfeed, 2015) and has a cool and wet climate with two rain seasons during the equinoxes. Temperatures vary between 18°C and 24°C which coupled with the rich volcanic soils make the area ideal for growing tea (KIG, 2014). Tea production in the Nandi Hills area uses high concentration of nitrogenous fertilizer. Nitrates can be discharged to water bodies through leaching and surface run-off and ends up in surface and ground water sources causing pollution (Maghanga *et al.*, 2012) [17].

Sampling and sample collection

A total of 50 households were randomly sampled to obtain water and urine samples. Fifty questionnaires were self-administered to the respondents. The urine and water samples were analyzed to determine the concentration of nitrates and phosphates.

Collection of water samples

A total of 50 water samples were collected from both surface water (stream) and ground water (boreholes) sources using plastic bottles. Out of the 50 samples collected, 40 of them were from boreholes while 10 were from streams. The samples collected were kept in a cooler containing ice and transported to the laboratory within a period of 24 hours. This was done to inhibit metabolic processes of microbes and biodegradation reactions that could significantly change the levels of nitrates and phosphates.

Collection of Urine Samples

From the 50 households, 50 samples of first morning urine were obtained in labelled sample cups. This was achieved by first explaining to the respondents the purpose of the study. Also, the researcher was accompanied by public health officer who assisted in urine sample collection. The sample cups were kept in cooler containing ice at 4^0 C and transported to the laboratory within a period of 24 hours

Analysis of Nitrate Concentration in water and urine

Analysis of nitrates was done using cadmium reduction method according to (Doane & Horwath, 2003) [8]. In this case, the sample was filtered using a filter paper, and passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) was determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically using UV-Vis spectrometer. An absorbance measurement was made at 220 nm and corrected by subtracting a second measurement at 275 nm. This was done to compensate for the presence of organics.

Analysis of Phosphates Concentration in water and urine

Spectrophotometric method as described by (Doane & Horwath, 2003) [8] was employed in the analysis. The method involves the formation of molybdophosphoric acid, which is reduced to the intensely colored complex, molybdenum blue. This analytical method is usually extremely sensitive and is reliable down to concentration of 0.1mg of phosphorus per liter of water (Aspila *et al.*, 1976) [2].

Ethical Consideration

Prior to urine sample collection, the participants were asked not to give their names thus maintaining their privacy and anonymity. The researcher also obtained all the required permits from Ministry of Education, National Commission for Science, Technology and Innovation (NACOSTI) and Ministry of Public Health and Sanitation before commencing the research.

Data Analysis

Data was analyzed descriptively using Statistical Package for Social Science (SPSS), version 20. Pearson correlation test was carried out to determine the association between the concentration of phosphate ions, nitrate ions and prevalence of goiter. Findings were presented using graphs, tables and charts.

Results and Discussion

Demographic information

Gender and age formed the demographic variables in the present study. From 50 respondents in the study, majority of them were female (64%), while males were 36%.

Results in Figure 1 revealed that majority of the respondents (38%) were between 31-40 years. Respondents between the age of 41-50, 18-30 and 51-60yrs were 34, 20 and 8% respectively.

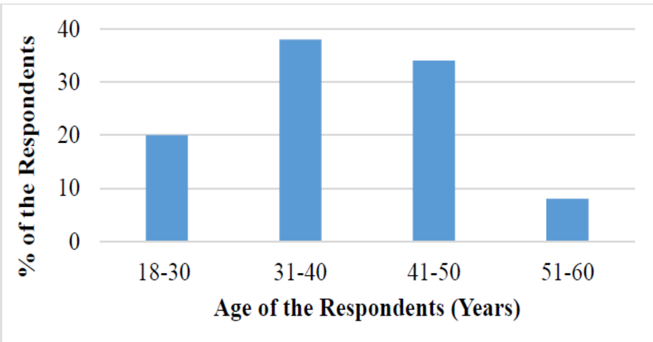

Fig 1: Age of the Respondents

From Figure 2, 42%, 34%, 14% and 10% of the respondents used borehole, stream, rain and piped water for their general purposes respectively.

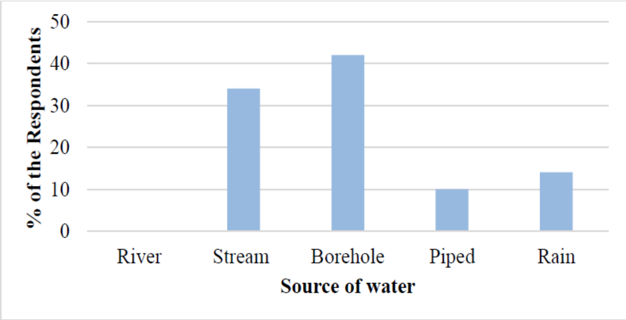

Fig 2: Source of water for general purposes in the study area

Figure 3 below clearly indicates that concentrations of phosphate ions in both borehole and stream water were within WHO acceptable limits (0.3 mg/l). Borehole water recorded phosphate ions that was slightly below the WHO acceptable limits (0.29 mg/l). On the other hand, stream water recorded a mean value of 0.302 mg/l which was found to be just within the WHO guidelines.

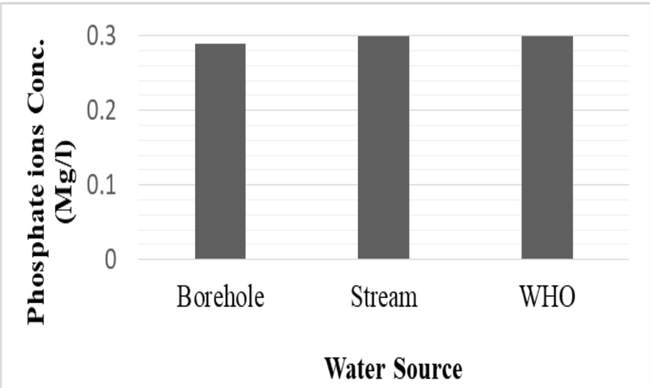

Fig 3: Concentration of Phosphate ions in drinking and WHO acceptable limits

Figure 4 indicates that the concentrations nitrate ions in both borehole and stream were within WHO acceptable limits (10 mg/l). About 1.69 and 2.91mg/l of nitrate levels in borehole and stream water were recorded respectively.

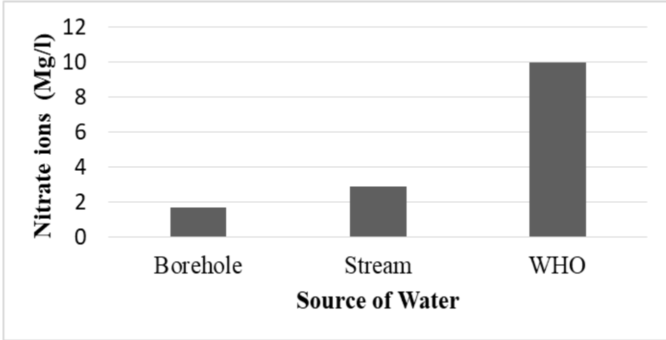

Fig 4: Concentration of Nitrate in drinking and WHO acceptable limits

About 54% of the respondents reported that at least one of their family members had suffered goiter while the remaining 46% had not suffered as shown in Figure 5.

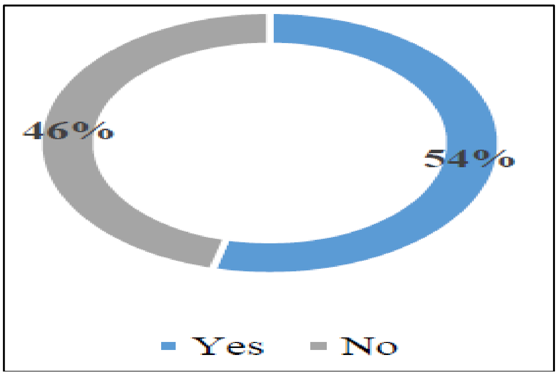

Fig 5: Occurrence of Goiter among respondents in the study area

From Table 1, the concentration of nitrate ions and phosphate ions in drinking water recorded an insignificant negative relationship (p=0.936; r=-0.012). On the other hand, the concentration of nitrate ions and phosphate ions in drinking water showed positive associations with prevalence of goiter. However, the associations were found to be insignificant (p>0.05).

Table 1: Relationship between nitrate- phosphate ions and prevalence of goiter in drinking water

		Conc. of NO_3^-	Conc. Of PO_4^{3-}	Occurence of Goiter
Conc. of NO_3^-	Pearson Corr (r)	1	-.012	.179
	Sig. (2-tailed) (P)		.936	.218
Conc. of PO_4^{3-}	Pearson Corr. (r)	-.012	1	.201
	Sig. (2-tailed) (P)	.936		.166

Correlation results in Table 2 showed that nitrate concentration had a significant positive association with prevalence of goiter (r=0.734; P<0.05). However, the concentration phosphate ions in urine recorded an insignificant positive relationship with occurrence of goiter (r=0.227; P>0.05).

Table 2: Relationship between nitrate- phosphate ions and prevalence of goiter in Urine samples

Variables		Conc. Of PO_4^{3-}	Conc. of NO_3^-	Occurence of goiter
Conc. of PO_4^{3-}	Pearson Corr. (r)	1	0.240	0.227
	Sig. (2-tailed) (P)		0.136	0.159
Conc. of NO_3^-	Pearson Corr.(r)	0.240	1	0.734
	Sig. (2-tailed) (P)	0.136		.000**

** denotes significance at P<0.05

Findings from the present study recorded an insignificant positive relationship between nitrate-phosphate- iodine ions and goiter occurrence. Several studies on the association between inorganic components in drinking and goiter occurrence have focused on nitrate, fluoride, iodine and manganese ions (Van Maanen *et al.*, 1994; Fernando *et al.*, 2009) [21, 12]. However, less has been done on phosphate ions. The results obtained in the present study are in contrast with several studies that have been carried out. Ward *et al.* (2010) [22] recorded a significant association between nitrate intake from public drinking water supplies and incidence of thyroid cancer. Furthermore, studies by Van Maanen *et al.* (1994) [21];

Tajtakova *et al.* (2006) reported that populations with sufficient iodine intake provided some evidence that nitrate ingestion via drinking water was associated with subclinical hypothyroidism and hypertrophy of the thyroid. Earlier on, Hettche (1956); van Maanen *et al.* (1994 described an association between high nitrate concentrations in drinking-water and goitre incidence. A dose–response relationship between inorganic ions in drinking water and goiter occurrence was demonstrated by Höring *et al.* (1991). Morris (2010) investigating on the relationship between water chemistry and goiter development in two species of bamboo shark, *Chiloscyllium*spp, reported a positive associated between the two variables.

A number of subsequent studies in Slovakia, Bulgaria, Germany and the USA have reported a correlation between various measures of nitrate intake and effects on thyroid function, but all suffer from methodological and data problems that preclude definitive conclusions (Tajtakova *et al.*, 2006; Gatseva & Argirova, 2008a, b; Radikova *et al.*, 2008; Ward *et al.*, 2010) [22]. Dhanwal (2011) [7] reported a significant effect of increased phosphorous on thyroid hormones on tissue phosphate metabolism and renal phosphate handling. The effect led to increased filtered load of phosphorous causing hyperthyroidism. The positive relationship between inorganic ions in drinking water and goiter incidence is explained by Zoeller (2007) [26]. The researcher attributed it to competitive inhibition of iodide transport leading to decreased thyroid hormone secretion, followed by an increase in thyroid-stimulating hormone.

On the other hand, insignificant relationship between inorganic ions in water and goiter occurrence in the present study could be attributed to some reasons. Firstly, the concentrations of both phosphate and nitrate in borehole and stream water were within the recommended limits by WHO. Secondly, it could be due to the season in which the water samples were collected. Finally, most of the studies that have reported positive association were done on laboratory animals while the present study used humans, hence the difference. Even though, results of the present study are in agreement with other studies. A clinical study in the Netherlands, did not find any relationship between nitrate intake and thyroid structure or function (Blount *et al.*, 2009) [4]. Furthermore, Fernando *et al.* (2009) [12]. The researchers reported an insignificant positive relationship between inorganic ions in drinking water and goiter occurrence in Sri Lanka. In addition, other studies (Crow *et al.*, 1998; Zaki *et al.*, 2004; Eskiocak *et al.*, 2005) [5, 24, 11] have demonstrated insignificant relationship between inorganic components of water and goiter occurrence. However, these studies reported that chronic exposure ($\geq$ 5 years) to nitrate ions and phosphate ions could result in the development of diffuse colloid goiter

Conclusion

The relationship between nitrate-phosphate-iodine ions and goiter occurrence in water was insignificant, concluding that the occurrence of goiter in the study area is not dependent on the ions tested in the study. Hence, there could be other factors that contribute to the prevalence of goiter in the study.

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Conflict of Interest

The authors declare no conflict of interest.

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