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Investigating Perchlorate Concentration in Drinking Water and its Relationship with the Frequency of Hypothyroidism Among Infants in Damavand City

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A B S T R A C T

Background: The newfound pollutant perchlorate, with global distribution, long-term stability in the environment, and solubility in water, is the most crucial health threat of the present era by causing changes in the level of thyroid hormones. Perchlorate enters the body by drinking water or contaminated food with perchlorate. The United States Environmental Protection Agency has provided a guideline of 15 µg/L in drinking water. This study aims to determine the relationship between the concentration of perchlorate in drinking water and the frequency of hypothyroidism in newborns.
Methods: 238 samples were collected from the water supply network of the study area. The samples were analyzed according to the standard water and wastewater tests and ion chromatography methods.
Results: The results showed that the quality characteristics of drinking water in the water supply systems of the studied area are within the normal range and in accordance with the standard. Drinking water is classified as hard water in Damavand city. Perchlorate concentration of drinking water was detected to be 0.002 - 0.009 mg/liter.
Conclusion: The concentration of perchlorate in the drinking water of the study area is according to the guidelines of the U.S. Environmental Protection Agency and does not threaten consumers' health.

1. Introduction

Humans are constantly exposed to various environmental pollutants [1]. Perchlorate (CLO_4^-) is one of the types of environmental pollutants with global distribution [2-4]. CLO_4^- is an emerging pollutant that is regarded as a considerable threat of human health [3,5]. It is an anion that is highly soluble in water and environmentally stable [6,7]. CLO_4^- consists of a tetrahedral array of oxygen atoms around a central chlorine atom [2]. CLO_4^- is colorless and odorless, and as a strong oxidizing agent, it can react explosively at high temperatures [8]. It is found in two forms: natural (atmospheric origin, dry, semi-arid deserts) [9,10] and synthetic (man-made in the production and storage of solid fuel for missiles and rockets, weapons, lights, making car airbags, making matches, battery and rubber, paint,

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combustion and explosion to dispose of materials containing CLO_4-, some fertilizers) [2,5,6,11-14]. Especially as a pollutant of drinking water, the dissolution of ammonium, potassium, magnesium, lithium, or sodium salts in groundwater and surface water has become an alarming concern for public health [2-4,6,15]. It potentially impacts human health, aquatic and terrestrial animals [2-4]. CLO_4-enters the body through drinking water or eating food contaminated with perchlorate [8]. According to a study by the U.S. Centers for Disease Control (CDC), almost any amount of CLO_4- exposure was associated with a significant change in thyroid hormone levels [14,16]. Consequently, exposure to CLO_4- prevents iodine absorption and disrupts the thyroid gland function of women in producing thyroid hormone, especially those who do not receive enough iodine, leading to metabolism disruption [2, 6,13,17-19]. Long-term reduction of thyroid hormone in the fetal period and newborns can cause developmental defects in the nervous system. Further, it can cause adult metabolic problems [17, 20]. As a fattening chemical, CLO_4- causes nonalcoholic fatty liver disease in humans [21]. CLO_4- has a biological half-life of approximately eight h and is excreted unchanged in the urine [22]. After CLO_4- was observed in drinking water sources throughout the southwestern United States, the U.S. Environmental Protection Agency (EPA) added CLO_4- as a high-priority contaminant to its drinking water candidate contaminant list in 1998 [2,23,24]. Regarding its health effects, EPA set an interim health advisory level of CLO_4- at 15 µg/L in 2009. It decided to regulate CLO_4- under the Safe Drinking Water Act in 2011 [24]. Recommended levels for CLO_4- in several U.S. states range from 1-18 µg/L [9]. Several studies have been conducted to determine the amount and extent of CLO_4- contamination in surface and underground water, drinking water, soil, and other foodstuffs such as milk, etc, in other countries. Their main purpose was to evaluate the amount of CLO_4- exposure in existing media in the environment and reducing the risks caused by their entry into the environment. Therefore, CLO_4- can have special importance in human health and the environment. The high concentration of CLO_4- in drinking water is one of the major factors in increasing the incidence of hypothyroidism among infants in epidemiology. Other factors include iodine disorders such as iodine deficiency and high iodine exposure, using betadine with high concentrations in gynecology and obstetrics departments for disinfection, excessive use of iodine-containing drugs during pregnancy, consanguineous marriages, autoimmune diseases thyroid, and selenium deficiency [25]. This study aimed to investigate the relationship between the concentration of CLO_4- in drinking water and the frequency of hypothyroidism in newborns identified in Damavand city.

2. Materials and Methods

2.1 Type of study

The present research is an experimental-laboratory study based on the ethical standards with obtaining necessary permits.

2.2 Area of Study

Damavand city is located at 35′ and 52^0 east longitude and 43′ ‛and 35^0 north latitude and 70 km from Tehran, with an average altitude of 2000 m above sea level. The rainfall regime of the region is a function of the Mediterranean regime. The average recorded temperature is 9.3 °C, and the annual rainfall in Damavand region is 350.3 mm, which is in the form of rain and snow. Damavand climate is classified as semi-arid in De Martonne classification and cold dry regions in Amberege′ s classification. In the classification published by the country′ s water engineering standard and the statistical yearbook of 1971, Damavand is placed in the cold semi-desert region [26]. The subordinate cities of Damavand include Damavand, Roodehen, Abali, Kilan, Absard. Agriculture, animal husbandry, various industries, and factories in the field of food and other products are active in this city.

2.3 Sampling

A sampling of the water supply systems of the studied area was done instantaneously during the spring and summer of 2022. The studied area is covered by five municipalities (Damavand, Roodehen, Kilan, Absard and Abali). Piped water (distribution network) is supplied from underground water (well water). A sampling of 66 water supply systems (14 urban and 52 rural) was done based on availability and accessibility. Urban and rural tap water samples were randomly collected from homes, restaurants, and water tanks (pumps or taps directly connected to groundwater sources without water treatment). In this study, tap water refers to water that has been distributed through general treatment (simple chlorination) in the public plumbing system. Under standard conditions [27], water samples were collected in a polyethylene container to measure the qualitative characteristics, transferred to the water and wastewater chemistry laboratory, and kept in a refrigerator at a temperature of 4 °C until the tests were performed in order to prevent their quality from changing. After flushing the water for at least 5 min and three times from the study area, we collected 198 samples to measure qualitative water characteristics (pH, dissolved oxygen, temperature, residual free chlorine, nitrite, nitrate, electrical conductivity, total hardness, total alkalinity, chlorides, sulfate, and turbidity). 40 samples equivalent to 60% of the water supply systems of the study area were collected to measure CLO_4- ion concentration of the water after the water flush for at least 5 min, once and without repeating. The criterion for choosing water supply systems was the proximity of the wells to the industrial, agricultural, and residential areas of the city and the village and population distribution. Therefore, 238 samples were collected to measure water qualitative characteristics and perchlorate ion concentration. To measure the thyroid hormone based on the TSH test, the researchers of the present study took the samples from the heels of all newborns in the city by a laboratory sampler according to the approved method and sent them to the medical diagnosis reference laboratory of Shahid Beheshti

University of Medical Sciences for analysis by ELISA method. The characteristics of the hormone-measuring device are mentioned in the preparation and sample analysis section. The statistical information of the results of implementing the screening program for newborns in the city during 2016 to the spring of 2022 was obtained from the managing the prevention and fight against diseases in the region.

2.4 Preparation and Sample Analysis

The primary characteristics of the water sample were measured according to standard methods for water and wastewater tests [27]. To determine CLO_4- ion concentration in drinking water, the widely used method 332 was used [9]. Method 332 is an extension of Standard Methods 314.0, 314.1, 314.2, and 331.0 by the USEPA to analyze perchlorate in raw and treated drinking water. This method can be used to detect and quantify low levels of CLO_4-with a detection limit in the ng/L range. Method 332 uses I.C. and electrospray mass spectrometry (ESI-MS) techniques [9,28,29]. To measure the concentration of CLO_4- in drinking water, we filtered the sample with a 0.22 μm needle filter and injected it into a liquid chromatography-double mass spectrometer without a column. The mobile phase was 65% methanol and 35% water containing 1% formic acid. The limit of quantification (LOQ) for this method was 20 ng/L, and the limit of detection (LOD) was 6.06 ng/L. The m/z ratio for the Quantifier and the Qualifier were 98.2 > 82.2, 100.2 >, and 84.2, respectively. A pH-meter (model 3305 from JENWAY company), turbidity meter (model 2100Q from HACH company), EC-meter (model CONDUCTIVITY/TDS METER LOVLAND COLO USA from HACH company), spectrophotometer (model DR 5000 from HACH company), Laser thermometer (model MS6520B of MASTECH company), CLO_4- of I.C. Alliance (model ACQUITY TQD LC MS MS of Waters company) were used to measure P.H. ; turbidity; electrical conductivity; nitrite, nitrate, sulfate; and temperature, respectively. All compounds and chemicals used were manufactured by Merck, Germany. The Alliance I.C. system consists of an isocratic pump, a Waters conductivity detector, an autosampler attached to the device, an analysis column, a Waters separation module and chromatograph management software. The ELISA Reader device (Stat Fax 3200 from Awareness USA Company) was used to measure thyroid hormone based on the TSH test.

3. Results and Discussion

This study was conducted in order to evaluate the concentration of CLO_4- as an emerging pollutant in drinking water and its relationship with the frequency of hypothyroidism among infants in Damavand city. The authors of the present study measured the qualitative characteristics of drinking water and the concentration of CLO_4- in drinking water during two seasons of the year. Moreover, they analyzed the types of fertilizers and poisons consumed and their distribution during a three-year period to evaluate the relationship between drinking water contamination and CLO_4- with regard to the frequency distribution of hypothyroidism among babies born during a 6-year period. The results of examining the qualitative characteristics of drinking water in Damavand city′ s water supply systems are presented in Table 1. According to Table 1, the qualitative characteristics of drinking water in the study area are within the normal range and in accordance with the Iranian national standard No. 1053, fifth edition [30], the international standards of the European Union, the World Health Organization, and EPA [31]. Based on the results of average alkalinity and total hardness measured in Table 1, it can be concluded that carbonate hardness is equal to total alkalinity, and according to pH, most of the hardness of the water in this city is of carbonate type, which can be removed by conventional methods. According to the classification of water, the city′ s drinking water is classified as hard water. Table 2 illustrares CLO_4- concentration of drinking water in Damavand city′ s water supply systems. The results showed that CLO_4- was detected with different concentrations in all collected samples. According to Table 2, the concentration of CLO_4- in the water supply systems of Damavand city is between 0.002 to 0.009 mg/L with an average of 0.00663 ± 0.0018 mg/L. In addition, the results indicate that the measured concentration of CLO_4- is within the standard range of 15 µg/L [24,32], which is assigned as the temporary health recommended level by EPA for CLO_4-. The results of this study are in line with the results of the study by Kosaka et al. (2007) on the occurrence of CLO_4- in urban drinking water sources conducted in a catchment area contaminated with CLO_4- from industrial sources in Japan. CLO_4- in 19 samples out of 27 was 1 µg/L, of which 13 samples had concentrations in the range of 10 µg/L. CLO_4- concentrations in water samples taken from the middle and lower Tone River catchment were generally 10 - 20 µg/L [18]. Similarly, Iannece et al. (2013) found 44 cases out of 62 tested drinking water samples, with different concentrations from 5-75 ng/L in Italy. CLO_4- was found to be similar in magnitude to values reported in drinking water from the USA and not of immediate health concern [33]. In contrast, a study by Giahi et al. (2015) reported different results of CLO_4- concentration in water and surface soil of war zones in Khorramshahr due to the occurrence of wars in the region and the pollution caused by the residual pollutants of weapons, the excessive use of fertilizers and agricultural poisons, the entry of industrial effluents into the surface water flowing in the region, and the occurrence of dust and the transfer of pollutants [8]. Significantly, the possible reasons for the reduction of CLO_4- concentration in drinking water can be found in environmental factors such as pH, dissolved oxygen, nitrate, temperature, salinity, and molybdenum ion [32], favorable environmental conditions, appropriate rainfall, and the absence of excessive use of fertilizers and pesticides. Agriculture and the scientific use of fertilizers and pesticides were searched under the supervision of the experts of the Jihad Agricultural Department of the city. Tables 3 to 6 show the types of consumed fertilizers and poisons and their distribution in Damavand city compared to the whole country during a three-year period [34,35]. Indiscriminate and inappropriate use of fertilizers, especially nitrates and chemical poisons, in fields and gardens for soil fertility and pest control is another possible source of $CLO_{4}-$. In practice, some of these materials always permeate through the soil, which either causes groundwater pollution through leakage; or during rains through surface runoff. They pollute surface water in addition to soil pollution. Regarding the possible role of fertilizers and chemical poisons in the environment, pollution of $CLO_{4}-$ is due to two reasons: agricultural runoff containing $CLO_{4}-$ can lead to the pollution of natural waterways that are a source of drinking water and edible plants that provide an alternative route of exposure by absorbing soluble compounds containing $CLO_{4}-$salts [8]. According to the studies, the total agricultural land of Damavand city is 17000 hectares (9% of the agricultural land of Tehran province). Based on Tables 3 to 6, Damavand city accounted for an average of 0.028% of chemical fertilizer consumption and 0.005% of chemical poison consumption in the country between 2019 - 2021 [34,35]. Agricultural experts believe that the decrease in the quality of fertilizers and agricultural pesticides and the decrease in rainfall are the reasons for the increase in the consumption of fertilizers and pesticides in Damavand in 2021 compared to the years before 2019. As presented in Table 1, nitrate concentration was measured at the lowest value of 1.7 mg/L and the highest value of 52 mg/L, with an overall average of 20.05 ± 1.86 mg/L. Furthermore, Table 3 illustrates that about 75% of the fertilizers consumed in 2019 and 96% of the fertilizers consumed in 2020 were of nitrogen type, which is a serious threat in terms of increasing nitrate concentration in water sources if using agricultural fertilizers is not managed correctly. The nitrate concentration, two units higher than the permissible limit, indicates the increase in the consumption of nitrogen-type agricultural fertilizers and the entering natural waterways as a source of drinking water due to the decrease in rainfall. shows the frequency distribution of hypothyroid disease in the screening program of infants born during a 6-year period was observed in the study area except for Abali city. Congenital hypothyroidism screening studies started in 1972 in North America and gradually spread to other countries. Since 2006, the screening program has been carried out all over Iran based on blood samples from the heels of 3 to 5-day-old babies. Prevalence is influenced by various factors and varies in different areas. The prevalence in the world is estimated to be one in 3000-4000 births. The implementation of this program in Iran also shows that the prevalence of hypothyroidism in newborns is increasing, reaching from 2.2 in 2011 and to 2.7 in 2014 in 1000 births [36]. Table 7 also explains out of 13157 screened babies (51.75% boys and 48.25% girls), 63 babies with a prevalence rate of 19 babies out of 4000 births were identified with hypothyroidism. The prevalence of hypothyroidism in New Zealand is 1 out of 3192, and in Iran, it is 2 out of 1000 live births [37]. The conducted studies reported the prevalence of hypothyroidism among infants in Qazvin as 2.2 per thousand (1 of 446 infants), in Isfahan 3.4 per thousand (1 of 294 infants), in East Azerbaijan 1.5 per thousand (1 of 666 infants), and in Central Province 2.3 in one thousand (1 of 307 infants). In the U.S. , the prevalence of hypothyroidism in infants has increased from 1 in 3000 to 1 in 2000. The prevalence in the member countries of the Council of Europe varies from 1 of 1300 to 1 of 13000. This amount has been reported in Turkey as 0.4 per thousand (1 of 2326 newborns) [36]. According to Table 7, the screening results in this study showed that the prevalence of hypothyroidism in newborns in the study area is higher than in other studies. The prevalence rate in the country should be analyzed for increasing factors. Based on Tables 2 and 7, the results of this research are consistent with the results of a study conducted by Javidi et al. (2015) in which no significant relationship was observed between $CLO_{4}-$ concentration in water and thyroid function TSH level in infants [38,39].

Table 1: Qualitative characteristics of drinking water of Damavand city water supply systems

Parameter	Measured Values		
	Minimum	Maximum	Mean ± SD
Temperature (°C)	9	21	16.4 ± 2.8
Chlorine Residual (mg/L)	0	1.2	0.3±0.04
pH	7	8	7.51±0.022
Turbidity (NTU)	0.17	1.88	0.63±0.049
Electric Conductivity (ms/cm)	126	2850	657.3±45.45
Chloride (mg/L)	1.4	82	24.55 ± 2.58
Nitrite (mg/L)	0.025	0.075	0.03 ± 0.001
Nitrate (mg/L)	1.7	52	20.05±1.86
Sulfate (mg/L)	2.2	192	32.57±3.69
Total Hardness (mg/L $CaCO_3$)	80	818	288.7 ± 146.7
Total Alkalinity (mg/L $CaCO_3$)	80	272	172.6 ± 58.7
Dissolved Oxygen (mg/L)	5.4	8.1	7.19±0.059

Table 2: Perchlorate concentration in Damavand city drinking water

Parameter	Measured Values		
	Minimum	Maximum	Mean ± SD
Perchlorate (mg/L)	0.002	0.009	0.00663 ± 0.0018

Table 3: Type of fertilizers consumed in Damavand city

Type of Fertilizer		
Nitrogen*	Phosphate	Potassium

*75% of the fertilizers consumed in 2019 and 96% of the fertilizers consumed in 2020 were nitrogen type.

Table 4: Types of poisons consumed in Damavand city

Type of Poison	
Insecticide	Types of Granules (Diazinon, Reagent Granules, Emulsifying oil)

Table 5: The amount of distribution of fertilizers used in Damavand city

Consumption rate	year		
	2019	2020	2021
Whole country (thousand tons)	2153	1991	1873
Tehran Province (thousand tons)	40	37	36
Damavand city (thousand tons)	0.510	0.530	0.650
Annual consumption percentage of the city compared to the whole country	0.023	0.027	0.034

Table 6: The amount of distribution of poisons consumed in Damavand city

Consumption rate	year		
	2019	2020	2021
Whole country (thousand tons)	277	194	215
Tehran Province (thousand tons)	0.155	0.420	0.450
Damavand city (thousand tons)	0.013	0.010	0.012
Annual consumption percentage of the city compared to the whole country	0.0046	0.0051	0.0055

Table 7: Frequency distribution of hypothyroid disease among infants born in Damavand city from spring 2016 to spring 2022

Year	Screened newborns	baby boy	baby girl	Hypothy roidism cases	Distribution of the disease frequency by urban area
2016	2378	1258	1120	11	Damavand(5), Absard(5), Roodehen(1)
2017	1253	649	604	17	Damavand(7), Absard(8), Roodehen(2)
2018	2371	1218	1153	14	Damavand(7), Absard(4), Roodehen(2), Kilan(1)
2019	2233	1138	1095	5	Damavand(2), Absard(1), Roodehen(1), Kilan(1)
2020	2662	1364	1298	9	Damavand(3), Roodehen(6)
2021	1710	889	821	6	Damavand (1), Roodehen (5)
2022	550	292	258	1	Roodehen (1)

4. Conclusion

Considering the frequency of hypothyroidism in babies born during a 6-year period (with a prevalence rate of 19 babies per 4000 births) and the particular importance of CLO_4^- as an environmental pollutant with an impact on public health by preventing iodine absorption and dysfunction of the thyroid gland of women in the production of thyroid hormone (especially people who do not receive enough iodine) and disorders in metabolism, monitoring of drinking water in the region in terms of CLO_4^- concentration was studied. This study showed that the qualitative characteristics of drinking water in the studied area are normal and agree with the standard. Also, the concentration of CLO_4^- in the drinking water samples is in line with the guidelines provided by the EPA for CLO_4^- that is not a threat to consumers′ health. However, if fertilizers and poisons are not properly managed, human activities will be considered as possible and potential sources of CLO_4^- release in water in the future. Therefore, it can be stated that the concentration of CLO_4^- in the drinking water of the studied area is not considered an important factor in the frequency of hypothyroidism in newborns. The frequency of the disease is related to other factors that increase the incidence of the disease, such as the iodine status of the area. And also, other cases of environmental pollutants and genetic background should be examined.

Authors' Contributions

Hassan Jalilvand: investigating; sample collecting; data collecting; data analysis; writing the original manuscript. Mohammad Rafiee, Ahmad Reza Yazdanbakhsh, Hossein Hatami: supervising; managing the research project; revising the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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