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Association of Nitrate Levels in Drinking Water and Infantile Methemoglobinemia

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ABSTRACT

Over the past two centuries, nitrate production and consumption have significantly increased, especially in the field of agriculture. There is a higher possibility of observing nitrate concentrations above the maximum contaminant level in private water systems and shallow wells. The consumption of nitrate-contaminated drinking water can lead to entering of large nitrate quantities into the body. According to epidemiological studies, exposure to elevated levels of nitrate in drinking water causes methemoglobinemia in children. In this regard, even the guidelines related to the level of nitrate in drinking water provided by the US.EPA and World Health Organization were promulgated to protect infants from developing the acute condition of methemoglobinemia. Complex interactions between ingested nitrate and hemoglobin in the body might have adverse health effects, including reduced oxygen transferrin capability and methemoglobinemia known as blue baby syndrome. This study aimed to briefly review the epidemiological studies related to the source of nitrate in drinking water and adverse health effects on infants, such as methemoglobinemia.

1. Introduction

Nitrate is a chemical compound consisting of one nitrogen and three oxygen atoms, which is chemically bound to an anionic compound with a negative charge that could be neutralized in the environment through combining with cations [1]. Nitrate and nitrite are predominantly soluble in the environment. These substances are naturally produced by the oxidation of nitrogenous compounds as a result of the microbiological activities in water and soil, as well as electrical drainage (e.g., lightning) at lower concentrations [2].

Nitrogen has a natural cycle in the environment [3]. In this cycle, bacteria convert nitrogen into nitrate, and nitrate is absorbed by plants. The animals that feed on these plants use nitrates to produce protein chains. The nitrate found in animal tissues returns to the environment through excreting feces and degrading the microbial tissues of dead animals. Microorganisms initially convert nitrate into the ammonium ion, followed by nitrite. This reaction occurs in the environment, as well as the digestive system of humans and animals. After the bacterial conversion of nitrate into nitrite in the environment, the nitrogen cycle is completed by the conversion of nitrite into nitrogen [4].

In the past two centuries, human activities have essentially altered the global nitrogen cycle and increased the level of nitrate in most of the areas across the world [5]. The most common sources of nitrate in drinking water include agricultural activities, wastewater disposal, nitrogenous waste human products, motor vehicles, and industrial activities [6-8].

Nitrite can also be formed chemically in distribution pipes by *Nitrosomonas* bacteria during the stagnation of nitrate-containing, low-oxygen drinking water in galvanized steel pipes or in the case of chloramination use as a residual disinfectant while the process is not controlled properly [6]. The recommended values for nitrate and nitrite are based on their short-term effects since these compounds are considered to have protective effects in the long run. According to the guidelines of the World Health Organization (WHO), the recommended level of nitrate in drinking water is 50 mg/L in the form of the nitrate ion, while it is 3 mg/L for nitrite in the form of the nitrite ion [1].

The present study aimed to review the experimental and epidemiological findings on the nitrate levels in drinking water and assess their effects on methemoglobinemia

in infants. Furthermore, preventive measures were proposed based on the epidemiological data in this regard. Since drinking water sources may play a key role in exposure, we also discussed the sources of nitrate in drinking water and common treatment methods.

2. Nitrate in Drinking Water

In the past century, global population growth has led to the need for more food sources, and modern methods of food production have been adopted in the agricultural sector. One of the main influential factors in the increased level of nitrate in the environment is the application of this compound as a fertilizer in the agriculture [9]. Use of chemical fertilizers is considered the most important source of human nitrogen production worldwide [10, 11]. Accordingly, the increased production and consumption of fertilizers in agriculture and their penetration into the soil and water is a prominent contributing factor to the contamination of water resources with nitrogenous compounds.

Discharge of urban and industrial wastewater and leachate from landfills into the environment is another source of nitrogenous compounds, which could contaminate water sources with nitrate, especially in the areas where sewage collection systems are not available and absorbent wells are used for the disposal of domestic wastewater [12, 13].

Nitrate and nitrite are soluble in the aquatic environment. These compounds are easily discharged into the environment during rainfall or waste disposal (especially wastewater disposal) and move into surface water and groundwater through leakage [4]. Nitrate concentration in surface water is normally lower than 18 mg/L; however, agricultural runoff, waste dump runoff or contamination with human or animal wastes may increase the concentration to hazardous levels.

Nitrate concentration often fluctuates seasonally and may increase when rivers are fed by nitrate-rich aquifers. In some areas, nitrate concentration in the drinking water derived from surface water is higher than 10 mg/L due to the runoff and discharge of sewage effluent and industrial wastes. As mentioned earlier, nitrite could also be formed chemically in distribution pipes by *Nitrosomonas* bacteria during the stagnation of nitrate-containing, low-oxygen drinking water in galvanized steel pipes or in the case of chloramination use as a residual disinfectant while the process is not controlled properly [11].

The recommended value for nitrate in drinking water is 50 mg/L as the nitrate

ion (equivalent to 11 mg/L as nitrate-nitrogen), which exerts protective effects on the health of bottle-fed infants as the most sensitive subpopulation. Therefore, the standard level of nitrate in drinking water is also beneficial for the health of children and adults. Epidemiological studies have estimated the guideline value of 50 mg/L for nitrate in drinking water based on the absence of adverse health effects on methemoglobinemia and thyroid [14]. If the nitrate levels in drinking water exceed 50 mg/L, drinking water may become a major source of total nitrate intake, particularly in bottle-fed infants.

In bottle-fed infants, daily intake of nitrate from the formula containing drinking water with 50 mg/L of nitrate has been determined at 8.3-8.5 milligrams per a kilogram of the body weight. The WHO guideline value of 50 ppm and US maximum contaminant level (MCL) of 45 ppm for nitrate in drinking water have been established to protect infants against methemoglobinemia, which is commonly known as the 'blue baby syndrome' [1].

Nitrate effectively penetrates into soil and remains in groundwater for decades [15]. Groundwater is the main source for 50% of drinking water supplies [16]. The risk of exposure to nitrate-rich groundwater has been reported to be comparatively higher in the areas where drinking water is collected from shallow wells (less than 100 feet below the land surface) and those with drained soil and high nitrogen inputs.

Agricultural activities are the largest nonpoint sources of groundwater nitrate contamination due to the common use of nitrogen fertilizers for crops and the trend toward concentrated animal farming [17]. In rural areas, domestic water wells are often shallow and close to the sources of nitrate contamination, whereas public supply wells are usually in deep groundwater aquifers where contamination is less likely.

3. Nitrate Toxicity

Nowadays, nitrate concentration in aquatic environments, especially in drinking water, is a major health concern. Nitrate contamination of drinking water is on the rise, which may give rise to adverse health outcomes. Nitrosamines are formed as a result of nitrate reduction, which may also be involved in gastrointestinal cancer [18]. Evidence also suggests that nitrate may contribute to the development of thyroid tumors [19, 20] and increase the size of the thyroid gland since nitrate can interfere with the metabolism of iodine (I), thereby leading to goiters [21]. In the reports in this regard, several recommendations and standards have emphasized on the necessity of compiling nitrate regulations

for humans. For instance, in the United States, the Food and Drug Administration (FDA) recommends that the additive nitrate and nitrite in meat should not exceed 200 mg/kg of nitrate [22].

In this regard, WHO has estimated the disease burden associated with excess nitrate concentration. Health implications of nitrate exposure in drinking water were first reported in the scientific literature in 1945 after cyanosis diagnosis in the infants fed with the prepared formula from well water [23]. Since 1945, most of the studies on the health effects of nitrates in drinking water have focused on infants as the most vulnerable population to the exposure [23-26]. On the other hand, the health-protective value of nitrate has remained a public health issue, with the opinions varying on the exact levels of the compound.

In the evaluation of nitrate concentrations, nitrite levels should also be considered since these compounds are closely related in the nitrogen cycle in the environment and body. Moreover, nitrite plays a pivotal role in toxicity induction after formation from nitrate. Recent reports have associated the nitrate levels in drinking water with other health effects than methemoglobinemia, especially at higher levels than 50 ppm. This toxicological review aimed to provide an update on the health effects of nitrate, focusing on methemoglobinemia, reproductive and developmental outcomes, potential carcinogenicity, and endocrine/thyroid effects [27].

Nitrate has been implicated in methemoglobinemia, as well as a number of other currently inconclusive health outcomes, including its involvement in cancer via the bacterial production of N-nitrous compounds, hypertension, high rate of neonatal mortality, birth defects in the central nervous system, diabetes, spontaneous abortion, respiratory tract infections, and immune system changes [28-30]. However, the main health concern is about the high concentration of nitrate in drinking water and its short-term and long-term, which may lead to teratogenic effects, methemoglobinemia, and cancer in humans [10].

4. Nitrate Toxicity and Infantile Methemoglobinemia

The primary risk of nitrate in drinking water is associated with its conversion into nitrite in the gastrointestinal tract. Methemoglobinemia occurs when nitrite oxidizes the hemoglobin iron of the red blood cells and ultimately cannot carry oxygen [31]. Therefore, methemoglobinemia is caused in the presence of methemoglobin in the blood.

In the absence of oxygen, cells may die or bruise the skin. Infants aged more than one year have the ability for the rapid conversion of methemoglobin into hemoglobin. Despite the high levels of nitrate and nitrite, the methemoglobin content of the red blood cells remains low. Infants are particularly susceptible to developing methemoglobinemia for several reasons, including their increased capacity to convert nitrate into nitrite and low levels of the cytochrome b5 reductase enzyme, which converts MetHb into hemoglobin.

The enzymatic system of the infants aged less than six months is not able to reduce methemoglobin to hemoglobin due to the lack of development [32] resulting from the reactions of several oxidizing agents with the normal hemoglobin content of the blood. Unlike the oxygen in hemoglobin, methemoglobin is so firmly bound that the methemoglobin cannot function as an oxygen carrier through alternate oxygenation and deoxygenation. In such case, anoxemia may occur and lead to severe consequences and even death [33]. Although methemoglobinemia may result from congenital heart diseases or the ingestion, inhalation, absorption, and medicinal administration of several drugs or chemicals, ingestion of drinking water with excess nitrate has been reported to be the major cause [34].

Infants with methemoglobinemia may seem healthy, while they manifest intermittent signs of blueness around the mouth, hands, and feet. Moreover, they may occasionally have trouble breathing, diarrhea, and vomiting. In some cases, infants with methemoglobinemia have a peculiar lavender color although they only show slight distress. The blood samples of these infants appear chocolate-brown and do not turn pink with air exposure. High level of methemoglobin in infants is associated with marked lethargy, excessive salivation, and loss of consciousness. In addition, convulsions and death could occur at extreme methemoglobin levels [35, 36].

Infants are particularly susceptible to developing methemoglobinemia for several reasons, including low stomach acid, promoted bacterial growth and conversion of nitrate into nitrite (which binds to hemoglobin to cause methemoglobinemia), increased capacity to convert nitrate into nitrite, and low levels of the cytochrome b5 reductase enzyme (which converts methemoglobinemia into hemoglobin) [30]. Furthermore, the amount and activity of the NADH-dependent methemoglobin reductase enzyme, which reduces

methemoglobin, are deficient in infants until six months of age [32, 37, 38]. Other particularly susceptible populations to methemoglobinemia include fetus and the individuals that are genetically deficient in the NADH-dependent methemoglobin reductase enzyme.

5. Epidemiological and Clinical Studies

Complex cofactor relationships do not currently allow the establishment of a quantitative exposure-response relationship for the exposure of humans to nitrates in food or water sources and the subsequent development of methemoglobinemia. In general, the factors make it difficult to estimate the number of the cases with methemoglobinemia are that on the one hand, methemoglobinemia is not an evident disease, and on the other hand, the definition of methemoglobinemia varies in the literature.

Studies that have examined the relationship between the nitrate levels in drinking water and prevalence of infantile methemoglobinemia, proposing conflicting results [39]. The few experimental studies in this regard are largely negative; however, most of these studies have evaluated the low levels of nitrate in drinking water in small sample sizes of neonates. According to the findings,

cofactors such as diarrhea and respiratory diseases have been reported to increase the risk of methemoglobinemia [40, 41]. An epidemiological study conducted in South Africa showed the increased risk of methemoglobinemia in the infants fed with water containing more than 20 mg/L of nitrate-N; nevertheless, clinical methemoglobinemia was rare. Another finding denoted the protective effects of vitamin C intake against methemoglobinemia [42].

In an original study by Walton (1951), acute cases of clinical infantile methemoglobinemia were reported, which were associated with the ingestion of nitrate in drinking water exceeding 45 mg/L [43]. On the other hand, a literature review indicated no incidence of methemoglobinemia in the bottle-fed infants aged less than six months who ingested nitrate concentrations of less than 45 mg/L in drinking water [44]. The majority of the studies focusing on the association of infantile methemoglobinemia and ingestion of nitrate in drinking water reported correlations with the nitrate concentrations exceeding 100 mg/L [41, 44, 45]. However, most of the studies in this regard have failed to consider the bacterial contamination of the drinking water, which may cause intestinal inflammation

in infants and increase the endogenous conversion of nitrate into nitrite, thereby leading to methemoglobinemia [37].

In 1985, WHO reported that more than 1,300 cases of methemoglobinemia (mortality in 21 cases) occurred in Hungary over a five-year period. Until the late 1980s, methemoglobinemia was considered a significant health issue in Hungary [46]. Despite the reports on high nitrate concentrations (>50 mg/L) in drinking water across the world [47], they are rarely paralleled with the reports of methemoglobinemia. Many of the actual cases predate the early 1990s, proposing that the apparent decline in the incidence of methemoglobinemia may be suggestive of an infectious etiology [48].

According to L'hirondel, in the cases where methemoglobinemia has been associated with the infant formula made with drinking water or carrot soup preparations containing elevated nitrate levels, it is possible that bacterial growth inside the bottle or stored products have been the main cause of the exogenous conversion of nitrate into nitrite [49].

6. Treatment and Reduction of Health Hazards

In the case of resource management and control of nitrate levels in drinking water, there are two main options for when nitrate

water resources are higher than the standard levels. One of the solutions involves replacement with other water sources, and the other demands the application of purification methods for nitrate removal before determining the alternative sources of water [14]. Therefore, the costs required for the provision of refining equipment or alternative sources should be predicted. If nitrate contamination is caused by livestock or human wastewater storage, it is crucial to provide sufficient water supplies by digging wells in another locations or creating deeper wells in an underground table [50].

If a water source with higher nitrate content than the standard limit is in shallow wells, deeper layers may not be contaminated. Therefore, deeper water tables of clay layers or impervious layers to prevent the penetration of contamination into the lower aquifers should be protected properly. Moreover, water well must be refurbished in order to prevent the penetration of the contamination influenced by cement slurry. New wells for human water sources should meet the standards and be far from all the sources of pollution [51].

Nitrate-contaminated surface water or groundwater should be disinfected to eliminate pathogens and convert nitrite

into nitrate so as to reduce the risk of infantile methemoglobinemia. Blending could also be employed if an additional source of low-nitrate water is available although it is rather unlikely. Alternatively, bottled water could be consumed if nitrate or nitrite concentrations are high for pregnant women and newborns [14].

A number of measures must be taken in the case of elevated nitrate concentrations or when the inspection of well water indicates sources of nitrate contamination, particularly where there are indications of poor microbiological quality. For instance, water should be boiled or disinfected by appropriate methods before consumption. In the continuous or extended boiling of water to ensure microbiological safety in terms of nitrate concentration, the WHO guidelines for drinking-water quality (i.e., heat exposure only until the water reaches a rolling boil) must be observed. If available, alternative supplies (e.g., bottled water) are recommended for bottle-fed infants, while ensuring that they are microbiologically safe. In addition, steps should then be taken to protect water wells

and ascertain that nitrate and microbiological contamination sources are removed from the vicinity of the well [52].

7. Conclusion

According to several epidemiological studies, nitrate concentrations of more than 45 mg/L (as NO_3) in water supplies could cause infantile methemoglobinemia, particularly in the infants aged less than six months who are mainly fed with powdered milk formula made up of tap water containing high concentrations of nitrates. Water examination is the only effective approach to determining nitrate concentrations and distinguishing the acceptable or unacceptable standards based on the results. The key solution to prevent nitrate contamination in drinking water is selecting and improving a suitable location for agricultural activities. Proper management could reduce the risk of nitrate contamination in the areas where fertilizers and animal waste are present so as to maintain and safeguard water supplies. If nitrate levels in drinking water are higher than the standard limit, other sources of water should be selected or water treatment should be performed.

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