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Risks of Urinary Infection and Exposure to Nitrites through Consumption of Water in SO-AVA

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Abstract

Our previous works had shown that drinking water in SO-Ava was contaminated with nitrates and their by-products. These nitrates by-products are extremely toxic for human being because of their capacity to transform hemoglobin in methemoglobin under the effect of Nitrosomonas and Nitrobacter bacteria especially with infants. It is in this context that the present study was conducted in order to examine the toxicity of these compounds on the inhabitants of Ahomey and Houédo to Sô-Ava, localities the most exposed to these xenobiotics through the drinking water. After urine and blood analyses on empty stomach, the results showed the presence of nitrites in urines in the case of 80 % of investigated followed by leucocyturia and by proteinuria. These symptoms added to the results of the blood parameters are proofs of an exposure with nitrates and an infection of enterobacteriological origin. The populations of these localities are thus exposed to the methemoglobin.

Keywords: Nitrates, bacteriological infections, methemoglobin, Sô –Ava

Introduction

Nitrates (NO3) and nitrites (NO2)[1] are ions naturally present in the environment. They are the result of a nitrification of ammonium ion (NH_4^+), present in the water and the ground, which is oxidized in nitrites by the Nitrosomonas bacteria, then in nitrates by the Nitrobacter bacteria[1]. Nitrates are very soluble in water[1]. The toxicity of nitrates results from their reduction from nitrites and from formation of methemoglobin on one hand and from their possible contribution for the endogenous synthesis of N-nitrose compound on the other hand[2].

During the acute poisoning, methemoglobinemia in infants is the only effect on the health which certainly proved the exposure with nitrates by the drinking water. Methemoglobinemia results from the reduction of nitrates to nitrites by microorganisms of the digestive system, followed by the oxidation by nitrites of ferrous iron (Fe^{2+}) of hemoglobin in ferric iron (Fe^{3+}), which engenders methemoglobin. Unlike hemoglobin, methemoglobin is incapable to bind to oxygen and thus reduce the transport of oxygen from lungs towards tissue[3]. The process of reduction of nitrates to nitrites is the object of a controversy. The formation of nitrites could sometimes result from a bacterial contamination of the water, having the effect of reducing nitrates to *vivo* nitrites[4,5].

Nitrites also have a toxic effect on reproduction and development following an exposure to very high doses of nitrites which also induced methemoglobinemia in mothers[6]; in particular on the development of the embryo and / or the fetus[2]. The combination of nitrites with amines can cause cancerous tumors of liver, kidney and lung[7,6].

Among a whole series of xenobiotic pollutants, it turns out that nitrates are top on the list considering their particular harmfulness on the environment and the public health. Nitrate ion (NO_3) is made up of nitrogen and oxygen; it results from the dissociation of nitric acid. Most of the nitrate salts (of ammonium, potassium, sodium) are very soluble in the water. In other conditions, in particular in acid medium, nitrites (NO_2^-) are naturally formed from nitrates. Nowadays, the largest part of nitrates absorbed daily, on average from 50 to 100 mg (from 200 to 400 mg for the vegetarians) resulting from vegetables, the rest from water (and other drinks), then from some meat and from other meat-based products (fishes). The aim of this work was to estimate the toxic effects of nitrates on certain hematologic parameters on people in the municipality of Sô-Ava.

Material and Methods

The qualitative and quantitative analyses of blood and urine (sampling method) are a very delicate operation because it requires a lot of care. It influences the analytical results and, consequently, the interpretation of the results. The survey was conducted during April and May, 2012, on a sample of 31 cases selected at random in the villages of Ahomey Gblon, Ahomey Lokpo and Houédo Gbadji, the localities where the populations are the most exposed to nitrates and their by-products, through the contaminated drinking water[8]. Sô-centre served as control. Blood and urine are the biological fluids analyzed. People selected according to a random sampling were all interviewed. Beforehand, ethical approval from the research ethics committee was obtained before the commencement of the investigation. Written informed consent for participation of the people was received from all participants. For urine collection, each of them received a clean plastic flask.

Venous Blood collection was carried out on empty stomach with the help of Vacutainer method (vacuum taking). Two tubes of 5 mL with anticoagulant and a dry tube of 5 mL were used by everyone. The anticoagulants used were: Ethylenediaminetetraacetic acid (EDTA) for blood count (NF), analysis of lead and cadmium then sodium fluoride for the analysis of glycemia and creatinine. The dry tube was used for the analysis of other biochemical analyses. These blood samples correctly identified (by an anonymous code) were centrifuged at 2000 tours for 15mn for serum separation and preservation at 4 °C was done. (refrigerator in the LAB laboratory of the campus). Urines are collected in flasks of 5 ml[9].

Urines were collected in flasks of 5 ml between 11 am and 5 pm according to the method recommended by WHO[9]. All the tubes of blood and flasks of urine and the survey papers regarding each person had the same anonymous code till the end of the study. The transport of samples was carried out in icebox at 4°C before being preserved at - 20 °C at laboratory.

Blood tests were conducted according to Elitech protocols (Clinical System) certified by European society of Standardization. Molecular Absorption Spectrophotometer of Screen Master make was used for analysis of glycemia, uremia, creatinemia, transaminases, uric acid, calcemia and cholesterols on one hand and automated hematology of RAYTO model RT-7200 brand was for blood counts (NF) in the laboratory (LAB / CAMPUS) of the Research for Health and Development Institute on the other hand.

Measurement of Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate-Pyruvate Transaminase (SGPT) on one hand and glycemia, cholesterols and uric acid on the other hand was carried out using enzymatic kinetic method at 340 nm and colorimetric method at 505 nm respectively[3]. For calcemia, colorimetric and complexometric method at 600 nm was used. Uremia was measured by kinetic- enzymatic method at 340 nm. Creatinemia was measured by colorimetric - kinetic method of Jaffé (505 nm).

All the manipulations were checked by serum multiparametric Elitrol 1 of Elitech brand.

Urinary calcium was measured with the same reactive as in the blood. The reference values used for the interpretation of each of the parameters were from other scientist'works[10, 11]. Urea and creatinine are waste of the metabolism which inform about the purifying function of the kidney, the main target of heavy metals. Any nephrotoxic substance (lesion of tubules) - even in the short term - may modify the blood concentrations of this two waste matter in the sense of an increase (they are reabsorbed in the damaged tubules and return to the blood, resulting in their increase). Tansaminases synthesized in liver increase to indicate the contamination of the body with nitrites.

These analyses were completed by the analyses of glycemia and NF in order to a possible problem with metabolism of glucose and anomalies of blood cells (red and white blood cells) owed to the exposure to identified xenobiotics[8]. These parameters were analyzed in the laboratory of biomedical analyses of the university of Abomey-Calavi.

Results and Discussion

Analysis of urine revealed the presence of nitrites in urines, presence of blood in 43 %, of leukocytes in 40 %, of glucose in 0 % and of proteins in 45 % of the investigated. These symptoms added to the results of the blood parameters are proofs of an exposure to nitrates and an entero-bacteriological infection. Results are grouped in table 1.

Discussion: The analysis of the results showed that a long-term exposure to by-products of nitrates through the consumption of water could cause renal damage, evidenced by the occurrence of leukocytes and some blood in urine to approximately 50 % of the studied population. Several works were dedicated to the study of the toxicity of nitrates and nitrites. Most of them aim at red blood cells as main target[11]. These compounds can have serious even deadly effects on the capacity of the hemoglobin to transport oxygen by transforming the ferric iron into ferrous iron[1]. This type of toxicity on red blood cells was demonstrated on mammals such as mouse[13,14]. Methemoglobinaemia (cyanosis) can happen in the following cases: ingestion of high quantities of nitrites (vegetables containing a lot of nitrates), coupled with lack of hygiene causing a bacteriological contamination; a proliferation of nitrato-reducing bacterial in a site where usually the very low bacterial density does not allow this reduction and when happens an accidental ingestion of rich nitrites products. The nitrites oxidizing effects on hemoglobin was confirmed by many works conducted on nitrates and nitrites and their effects on red blood cells of rat which have proved a strong methemoglobinemia.

Table-1
Biochemical and hematological values in Blood and urine

Villages	N°	URINE							SANG Blood						
		Glucose (mg/dL) Standard : 0	Proteins (mg/dL) Standard : 0	Nitrites (mg/dL) Standard : 0	(RBCc/µL) Standard :0	WBCc/µL) standard :0	Billirubins (mg/dL) Standard :0	pH Standard :5.0	Glycemia (g/L) Standard 0.70-1.10	Urinemia (g/L) Standard 0.10-0.40	Creatininemia (mg/L) Standard 8-14	Uricémia (mg/L) Standard 30-57	Total cholesterol (g/L) Standard 1.50-2.50	SGOT (UI/L) Standard < 46	SGPT (UI/L) Standard <49
A.Gblon	F	0	0	1.5	10	0	0	7.5	0.75	0.19	11	40	1.41	21	15
	F	0	10	1.0	0	0	0	7.5	0.80	0.17	10	35	1.36	29	30
	F	0	0	1.0	10	0	0	8.0	0.70	0.16	09	50	1.36	23	20
	F	0	30	1.5	250	50	0.5	6.5	0.60	0.17	10	37	1.37	13	15
	F	0	0	1.0	10	75	0	6.5	0.69	0.10	08	60	1.25	21	35
	M	0	0	1.0	0	0	0	6.0	0.90	0.18	09	50	1.43	13	25
	M	0	30	1.5	300	50	0	9.9	0.70	0.12	13	47	1.57	19	15
	M	0	0	0	10	0	0	9.0	0.68	0.25	13	56	2.25	14	20
A.Lokpo	M	0	0	0	50	0	0.5	8.0	0.80	0.16	13	33	2.31	18	21
	M	0	0	0.5	0	0	0	6.0	0.90	0.21	10	36	1.28	46	30
	F	0	30	0.5	250	75	0	6.0	0.85	0.24	13	42	2.10	16	19
	E	0	10	0.5	250	50	0	6.0	0.80	0.15	08	40	1.36	22	25
	E	0	0	1.5	0	0	0	7.5	0.60	0.14	08	39	1.67	24	20
	M	0	30	1.5	250	75	0	6.5	0.80	0.17	11	37	1.51	18	20
	F	0	0	0.5	0	0	0	6.5	0.70	0.20	10	44	1.53	20	18
HOuédo	F	0	0	1.5	0	0	0	7.0	0.65	0.21	12	51	1.67	14	07
	M	0	30	1.5	50	75	0.5	6.0	0.85	0.22	13	77	1.50	15	30
	M	0	0	0.5	0	75	0	8.0	0.74	0.12	11	60	1.35	10	15
	F	0	10	0.5	10	0	0	7.0	0.89	0.23	11	47	1.44	20	14
	M	0	10	1.5	0	0	0	6.5	0.60	0.19	09	23	1.19	15	10
	F	0	10	1.5	0	0	0.5	6.5	0.70	0.23	10	42	1.40	35	21
	F	0	0	1.5	10	0	0	7.5	0.65	0.16	10	47	2.73	30	11
	F	0	0	0	0	25	0	6.0	0.60	0.22	11	58	1.94	20	09
	F	0	200	0.5	0	75	0	9.0	0.70	0.28	11	30	1.90	15	18
	M	0	0	0	10	75	0	6.5	0.75	0.18	12	35	2.04	110	51
Avacentre (Control locality)	M	0	10	0.5	0	0	0	6.0	0.80	0.32	14	42	1.67	38	28
	M	0	0	1.5	0	0	0	6.0	0.73	0.17	11	80	2.02	44	34
	M	0	0	0.5	0	0	0	6.0	0.71	0.28	14	44	1.87	26	25
	M	0	10	0.5	0	0	0	6.0	0.69	0.19	12	56	1.45	20	12
	M	0	0	0.5	0	0	0	6.0	0.70	0.24	13	36	1.24	30	23
	M	0	0	0.5	0	25	0	6.0	0.68	0.24	08	25	1.30	15	06
m ± SD		0	13.55 ± 36.29	0.85 ± 0.55	47.4 ± 95.9	37.10 ± 21.28	0.06± 0.17	6.88 ± 1.06	0.73 ± 0.09	0.20 ± 0.05	10.90 ± 1.81	45.13 ± 12.99	1.63 ± 0.37	24.97 ± 18.15	20.71 ± 9.47

Our results indicated a high presence of nitrites in urine coupled presence of red blood cells and leukocytes. This pathological situation can be noticed easily. Indeed, nitrates ingested with food are partially reduced with saliva to nitrites by bacteria - nitrites which finally reach the digestive tract[15, 16]. These nitrites are then expelled through urine. Thus the presence of nitrites in urine is a proof of exposure to nitrates and a urinary infection of bacteriological origin. And this was evidenced by the presence of red blood cells, leukocytes and carbohydrate in urine. Indeed, in acidic gastric environment, nitrites can react with amines and amides and they can form N-nitroso compounds as nitrosamines, which are possibly carcinogenic[5]. The acute toxicity of sodium nitrate is rare. Nitrite on the other hand is toxic because it binds on hemoglobin to form methemoglobine and as a result the combined compound could no more perform its role of oxygen carrier.

Methemoglobine is reversibly reduced to hemoglobin due to the intervention of erythrocytic enzymes: the NADPH-metHb reductase and the NADH-metHb reductase with the cooperation of redox reactions of glutathione and ascorbic acid. Besides, nitrates cause an ionic imbalance and a need in ATP, which could be understandable by a rise of the rate of the blood glucose. Also, Nitrites are at the origin of the methemoglobinemia and of the hemolysis[17]. These two phenomena are connected and their origin is the absorption of the ammonium nitrate which once absorbed undergoes chemical modifications ending in the formation of nitrites. In that case of toxicity, red blood cells develop its own defense thanks to glutathione which represents one of the most important elements in red blood cells, as well as two other mechanisms of detoxification: NADPH-metHb reductase and NADH-metHb reductase which can regenerate the hemoglobin from the methemoglobin.

These biochemical processes require ATP supplied essentially by the erythrocytic glucose. So the damage of the membrane of red blood cells and the release of glucose would be at the cause of the toxicity of nitrate[18]. Fortunately, our results did not reveal such an increase. The obtained results did not reveal a significant increase of total cholesterol oncentration. Some works have reported an increase in serum cholesterol, explained by the reduction of the secretion of T3 and T4 thyroid hormones. Indeed, certain disorders of metabolism of lipids are connected to a thyroid dysfunction[19]. Also a hypothyroidism can trigger hyperlipidemia and hypercholesterolemia.

In all attacks by the xenobiotics, the hepatic metabolism of proteins is modified towards the production of defense systems and glyconeogenesis. The degradation of hepatic protein

compounds can explain the increase of urea and trigger the extreme values of serum creatinine in some investigated of Ahomey and Houédo. Proteins can be degraded in amino acids then in urea and creatinine.

These formed amino acids can be transformed under the effect of transaminases into carboxylic compounds such as pyruvic acid. This implies the intense enzymatic activity of the SGOT and the SGPT. Our results showed alarming increase of both types of transaminases. In this case it could be inferred that the intense enzymatic activity of SGOT and SGPT was connected to the hepatic-toxic effect due to nitrites, knowing that the liver is the main organ of the detoxification because it contains most of the enzymes of metabolism. This problem of the hepatic enzymatic system was indirectly highlighted by the increase of the body weight ratio for liver after the treatment with ammonium nitrate[13,17]. This result was in agreement with another report showing an increase of weight of the liver of rats treated with nitrates and nitrites.

Conclusion

The results recorded in the present study allowed us to confirm the exposure to nitrates by the inhabitants of the municipality of Sô-Ava through the consumption of water. It raises the issue of the sanitary safety of food. Indeed, in the long term, the permanent exposure of the population to nitrates by-products has caused changes at the renal level (occurrence of leukocytes and blood in urine), in spite of the stability of certain serum biochemical parameters. It would be desirable to complete this work by the analysis of the direct toxicity markers with the people exposed in order to know the serum glutathione and the methemoglobin.

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