“OVIDIUS” UNIVERSITY CONSTANTA
FACULTY OF MEDICINE

ABSTRACT OF DOCTORAL THESIS

Scientific coordinator
University professor ILEANA ION

Doctoral Candidate
University assistant Mertan (Severin) Beatrice Marcela

Constanta 2012

“OVIDIUS” UNIVERSITY CONSTANTA
FACULTY OF MEDICINE

Disturbances of hemoglobin metabolism by nitrogenous compounds pollutants in water and soil

Scientific coordinator
University professor ILEANA ION

Doctoral Candidate
University assistant Mertan (Severin) Beatrice Marcela

Constanta 2012

CONTENTS

INTRODUCTION

Water is an essential factor in ensuring and maintaining quality of life and public health. Because of multiple possibilities of disease by water consumption, it must meet a number of quality conditions to be considered potable: organoleptic, physical, chemical, microbiological.

Sanitary controls on drinking water quality revealed in many parts of the world a content of nitrates exceeding the maximum permitted by the World Health Organization.

For this reason serious manifestations are seen in artificially fed infants that make acute and often deadly so-called "blue baby disease" or methemoglobinemia.

In the same environmental conditions (polluted water) adults make chronic poisoning, which unfortunately are not notified in time by the patient or end up being attributed to other causes, and therefore are treated incorrectly.

Given these data, through this study I wanted to analyze some aspects of the health situation in relation to water quality in Constanta.

KEY WORDS: water, nitrates, health, methemoglobin, antioxidants

1. MOTIVATION OF DOCTORAL THEME

I chose this topic because exposure to nitrates from environmental factors, in particular the water is still a public health problem, both in Romania and in many countries (England, Ireland, France, Belgium, Germany, Italy , Spain, Israel, USA, Canada, China, Russia, and so on).

Importance of the theme:

- Nitrates are chemical substances with dangerous actions to the body, and their presence in water may not exceed the maximum permissible limit set by law.
- Drinking water with nitrates in high concentrations can cause nitrite poisoning (methemoglobinemia).
- In general population the category most affected by poor water quality is the artificially fed infants with powdered milk to which preparation is used such type of water.
- In our country there is a high incidence of methemoglobinemia, with significant mortality in children under 1 year.
- The disease can occur through consumption of plant foods that have been grown on land fertilized intense with nitrogen chemical fertilizers.
- However, continuous intake of nitrates from water and food lead to chronic poisoning of children and even adults.
- If for cases of acute nitrates poisoning are more data of reference to our country, for chronic intoxication they are few.
- At international level the results for chronic exposure are controversial.

2. OBJECTIVES OF DOCTORAL THESIS

The study aims to analyze the some favoring causes of toxic methemoglobinemia and the health effects caused by chronic exposure to elevated levels of nitrates and nitrites in the water in order to improve prevention of this condition difficult to predict.

I approached an interdisciplinary study linking data of Physiology with those of Hygiene to determine whether certain investigations are important for early detection and sizing risk to human health in the case of chronic exposure to nitrates and nitrites.

3. MATERIALS AND METHODS FOR STUDY

Study materials for water analysis

To analyze water quality in Constanta and identify areas that have exceeded the maximum permitted levels for nitrate and nitrite, we performed chemical and microbiological analyzes on water samples collected from Constanta county, both rural and urban areas.

The determinations were made in the laboratory of the Public Health Department in the period 2006-2011.

In order to estimate the consequences of chronic exposure to elevated levels of nitrates we processed the data having regarded the environment of origin, concentrations of indicators and types of water supply systems.

Study materials for analysis of morbidity

For study the morbidity we examined nitrites poisonings in Constanta County during 2006-2011, as well as chronic cases recorded by family doctors in localities selected for the study, in the same period.

To analyze cases of poisoning we used data taken from the Public Health Department, Constanta, from data sheets of reporting cases of infant methemoglobinemia caused by water consumption.

For the analysis of chronic disease we used data taken from chronic disease registry of family doctors, from patient record files and from records for reporting to the Health Insurance Constanta.

Study groups

The study included 180 persons selected from the countryside, from two localities in Constanta County. All persons are in the records family doctors from their localities.

People were divided into two groups as follows:

- **Test group** - made up of 90 persons from a village where the water supplied to the population in the centralized system of supply exceeds maximum allowable concentration for nitrate (locality with chronic exposure to nitrates - Corbu);

- **Control group** - consists of 90 persons from a village where levels of nitrates in water supplied to the population in a centralized system are within the limits set by law (Limanu).

Criteria for inclusion in the study

- Subject age between 18 - 38 years
- To live at least 5 years in area monitored in study
- To use drinking water from centralized system
- Subject apparently healthy
- Non-smoker.

Exclusion criteria from the study

- Daily consumption of bottled water
- Taking nutritional supplements in last 3 months
- Subject recorded with chronic illness.

The selection of the two localities included in the study was based on:

- concentration of nitrates in water provided to population by centralized system water supply
- number of inhabitants
- receptiveness and willingness of family doctors to be involved in the study .

Study Methods

For evaluation of water quality we did biochemical analyzes (nitrates, nitrites, ammonia, organic substances) and microbiological analyzes (Total number of germs enterococci, Escherichia coli).

For evaluation of study groups we collected blood samples for determination of: total antioxidant status, glutathione peroxidase, superoxide dismutase and methemoglobin.

4. RESULTS AND DISCUSSION

4.1. Analysis of water supply systems in Constanta County

In the first stage of research, we aimed to identify suppliers and water supply systems in Constanta County as well as establishing the drinking water quality in rural and urban areas to identify places at risk of exposure to nitrogen compounds pollutants (nitrates / nitrites).

4.2. Nitrites poisoning Constanta County

Having regard to the large number of villages that have registered constantly increased levels of nitrates in drinking water in 2006-2010, the next step of my research was to study the cases of nitrite poisoning in Constanta County.

We analyzed the cases according to the distribution by year, area of origin, gender, age, type of food, clinical form of the disease, the methemoglobin value, the presence of diseases associated and characteristics of water sources used in patient's diet.

4.3. Water analysis from the two villages selected for study

Analyzing the quality of drinking water supplied to the population in 2006-2010 we identified localities with normal levels of nitrates in water supplied by centralized system and localities which frequently recorded high concentrations of nitrates.
For continuation of study, we selected one village from each category.

Water analysis from village with chronic exposure to nitrates (test area)

In the period 2006-2012 were collected 51 water samples from centralized system of the village. In the chemical analysis of water we analyzed the following parameters: nitrates, nitrites, ammonia, organic substances.

37 of samples, representing 72.54% of total not complied with the legal standards for nitrates. Average of nitrates often exceeds the double of normal values. Maximum value (458 mg / l) is obtained in 2008 and is nine times higher than sanitary rules!

Of the total sample, 21 have registered inadequate values regarding the presence of coliform bacteria.

The existence of these bacteria in a drinking water represents a major epidemiological risk.

The water analysis for village with normal levels of nitrates (control area)

In the period 2006-2012 were collected 46 water samples from centralized system of the control village. The chemical analysis of water revealed that all samples are in the established sanitary rules for nitrate content.

For coliform bacteria in the period 2006-2012 there were 7 samples where do not fall under the rules set by the Ministry of Health.

Health significance of these changes is the same as in the previous case.

4.4. Setting the pattern of morbidity in the two localities selected in the study

To determine the pattern of morbidity in the two selected localities we analyzed the number of cases of chronic diseases registered by the family doctors in the period 2006-2011, during which we analyzed the water quality from centralized system.

4.4.1. The prevalence of ischemic cardiopathy

Studying the prevalence of ischemic cardiopathy in both villages (Figure 1) we found that the control village has not major changes over the 6 years of study, ranging from 1.19 ‰ in 2006 to 1.62 ‰ in 2010, maintained at the same level in 2011.

In village exposed to nitrates the values of prevalence are in a continuous increase from 1.04 ‰ in 2006 to 2.44 ‰ in 2011.

This trend may suggest that a reduced ability of blood to carry oxygen through the formation of methemoglobin together with other factors contribute to the occurrence of myocardial ischemia.

We have not found studies that establish a relationship between exposure to nitrates in environmental factors and prevalence of coronary heart disease.

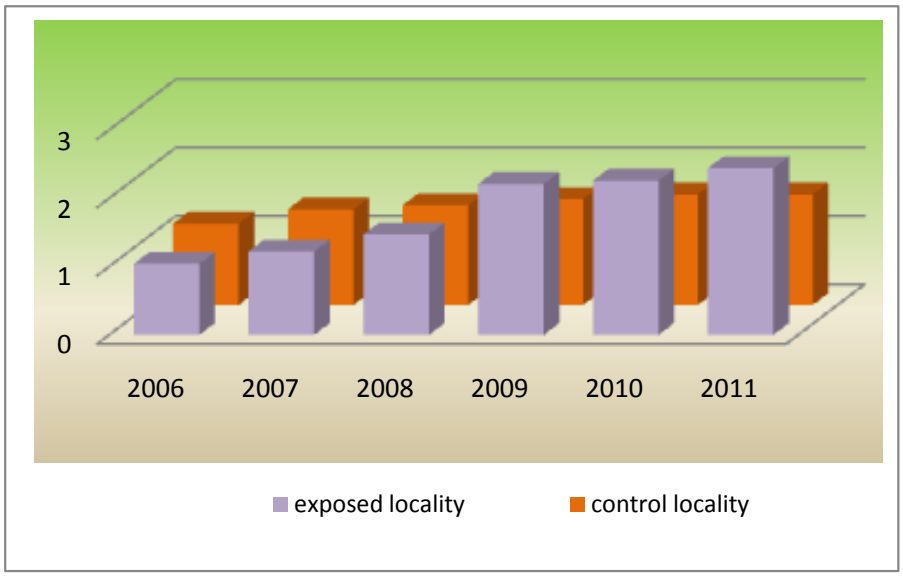

Figure 1 - Prevalence of ischemic cardiopathy

4.4.2. The prevalence of non-insulin-dependent diabetes (type 2)

Following prevalence of type 2 diabetes we noticed over the analyzed period that in the control village is an upward trend, from 16.2 ‰ in 2007 to 19.87 ‰ in 2010 followed by a stagnation of registration of new cases of disease (Fig. 2).

For locality exposed to nitrate trend of growth continues each year and is much higher than in the control area. If in 2006 the prevalence of diabetes had a value of 8.15 ‰ in 2011 it reaches 20.89 ‰. Thus we can say that in 2011 there are about two and a half times more cases than in 2006.

Increasing the number of cases is correlated with the increase of nitrate concentration from water supplied by centralized system.

No data was found in the literature about the prevalence of diabetes in areas exposed to nitrates, however according to studies on the pathogenesis of diabetes, oxidative stress is involved in the occurrence and development of diabetes cases and methemoglobin measurement is an important marker of stress oxidation in these patients [1, 2].

A study published in 2008 by specialists from the National Research Center in Cairo demonstrates that in patients with non-insulin-dependent diabetes methemoglobin concentration is three times higher than in healthy subjects [3].

In these circumstances it is clear that the health of persons in general and patients with diabetes in particular, will be profoundly affected by exposure to nitrate, which is a risk factor for oxidation of iron ferrous (Fe2 +) in the structure of hemoglobin and the formation of methemoglobin.

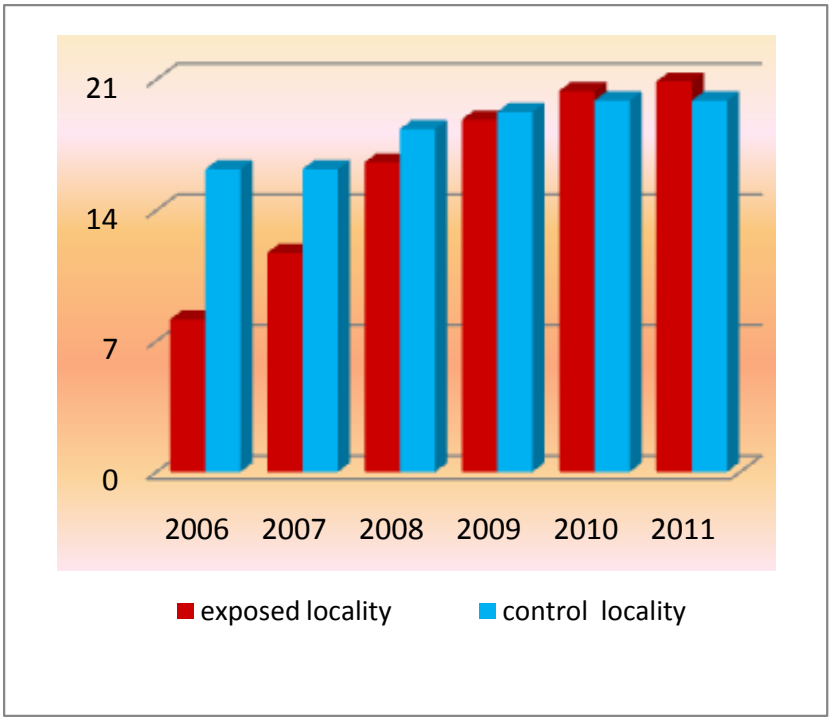

Figure 2 - Prevalence of diabetes type 2

4.4.3. The prevalence of chronic liver disease

Prevalence of chronic liver disease is higher in the chronic exposure to nitrates (Fig. 3) and is continuously increasing from 9.1 ‰ in 2006 to 17.58 ‰ in 2010. In 2011 a slight decrease in prevalence is determined by death of patients.

For locality which presents normal levels of nitrate in water from centralized system, the prevalence of chronic liver disease is extremely low, between 1.2 and 2.75 ‰.

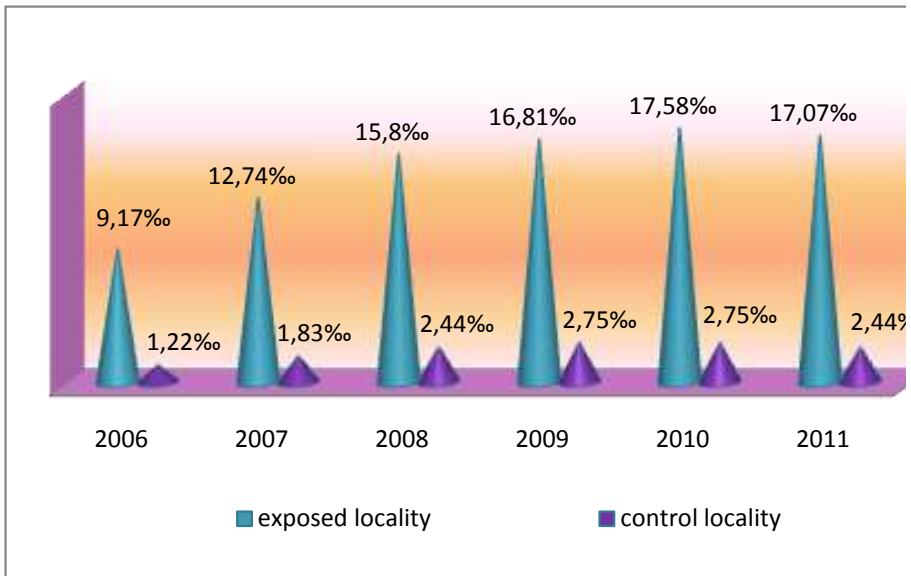

Figure 3 - Prevalence of chronic liver disease

There are no data about the hepatotoxicity of water with a high content of nitrates, but the big difference between the prevalence of chronic liver disease in the two localities can not be accidental.

Water with a high content of nitrates may also contain chemical structures that do not follow or escapes us and which affects the liver.

On the other hand, water with nitrates is a water which had at one time the organic load. Such water is very likely to contain parasites, and Giardia parasite is most commonly transmitted through water. It is set in the gut, but can reach the gall bladder. Therefore, biliary dysfunction in chronic giardiasis will lead to a chronic disturbance of liver function.

We can discuss about nutritional deficiencies or alcohol consumption, but knowing the two communities through movements that I made in those two localities I exclude that some people are chronic consumers of alcohol and others not.

As a result, the subject deserves to be investigated more thoroughly in the future, having regarded the serious consequences of chronic hepatitis: liver cirrhosis and liver cancer.

4.4.4. The prevalence of chronic lung disease

In case of chronic lung disease in the locality with high levels of nitrates in water, it is noted the same upward trend in prevalence from one year to another to 10.19 ‰ to 15.54 ‰

(Figure 4). In control locality chronic lung disease prevalence has increased slightly in the period 2006-2008, from 7, 33 to 8, 25 ‰, after which values remain relatively constant until 2011.

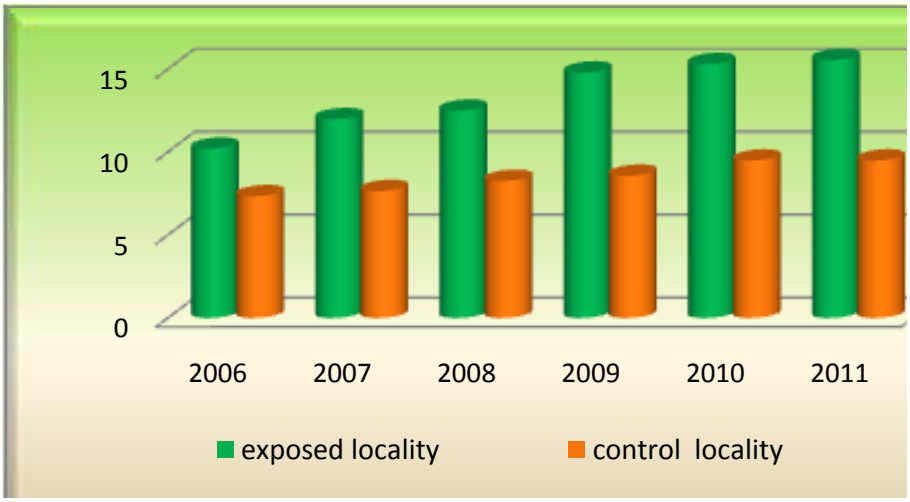

Figure 4 - Prevalence of chronic lung disease

Achieving of a high intake of nitrates from water and food increases the risk of forming a larger quantity of methemoglobin with impaired ability to transport oxygen from the lungs to the tissues, thus disturbing the respiratory function.

4.4.5. The prevalence of cancers

Analyzing the cases of cancers in records of family doctors, we found that in the locality exposed to high levels of nitrate in water the prevalence is constantly growing in 2006-2010. For control locality in the same period aren't major variations from one year to another (fig.5).

For 2011 in both villages there is a decrease in the prevalence of cancers, following the deaths among this group of patients.

As can be observed in the figure below for each year studied the prevalence of neoplasm has values over two to three times higher in locality exposed to nitrates compared with the control locality.

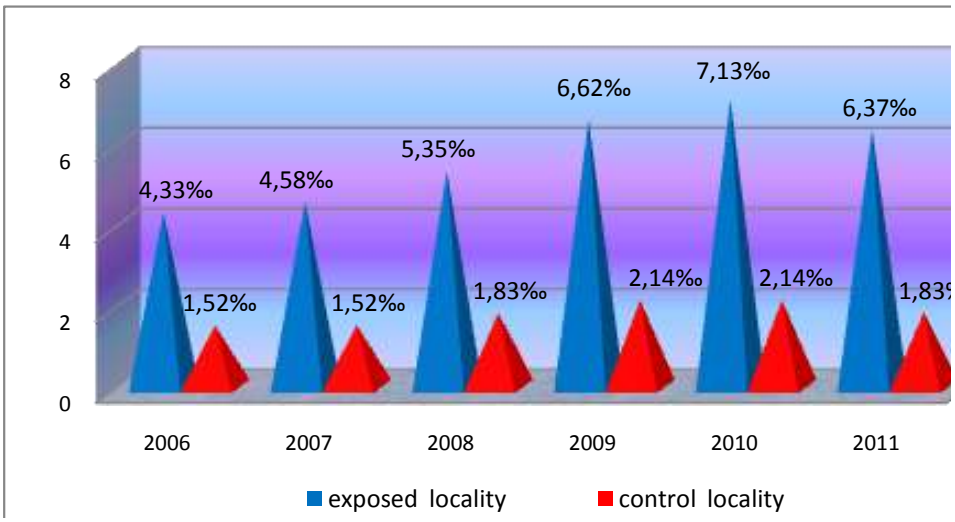

Figure 5 - Prevalence of cancers

The mechanism by which tumor cells are generated is based on the formation of nitrosamines, compounds which are generated by unification of nitrites with secondary amines, resulting from the cleavage of proteins. The formation of nitrosamines occurs predominantly at stomach due to acid environment that favors chemical reactions.

The specialists have different opinions about the role of nitrogen compounds from water in occurrence of cancer, and most studies are related to gastric cancer. Thus, the involvement of nitrates in water in the pathogenesis of gastric and colorectal cancer is supported by results of studies performed in America, Spain, China, Slovakia [4-8].

On the other hand the specialists from Canada say that there is no causal relationship between gastric cancer and nitrates in water [9]. Moreover, American Environmental Agency is taking steps to include nitrates and nitrites in the category of potential free carcinogenic substances.

Having regard to those data I believe that any study on this subject is useful for determining the exact role of nitrogen compounds in the development of tumors.

Both for coronary heart disease and diabetes, chronic pulmonary disease, chronic liver diseases and cancers we found more pronounced increase in prevalence in the area exposed to nitrates in 2006-2009, when the concentration of nitrates in water supplied through centralized system of the population was on growing.

4.4.6. The analysis of somatic-weight development and health status of infants

To determine the particularities related to this aspect in the two localities under study, we analyzed the following parameters: period of the pregnancy, sex, weight and length of the newborn and Apgar score.

After processing of data we can say that the exposure of pregnant women to nitrates from environmental factors disturb the somatic-weight development of the babies. I did not find studies about somatic-weight development of infants in case of chronic exposure of pregnant women to nitrogen compounds from water. There is only evidence of physical development of children with a history of methemoglobinemia.

4.5. Evaluation of study groups

4.5.1. Evaluation of methemoglobin

In the test group we identified 52 persons with high levels of methemoglobin and in the control group 18. We have introduced the data into a 2x2 contingency table. Based on data calculated OR value (5.47 for a confidence interval of 95%) indicates that the risk of elevated levels of methemoglobin is significantly higher in people who consume water rich in nitrates ($p<0, 000002$).

Determination of etiologic faction of attributable risk (FRA) shows that an increase in MetHb values above the normal limit of 1% can be attributed in the 81.71% of cases of chronic exposure to nitrates.

Analyzing the distribution of persons with high levels of methemoglobin by age we found that the most cases appear in group of 33-38 years. Therefore we can state that chronic exposure to nitrates increase the proportion of methemoglobin in blood.

Data are correlated with those in the literature which indicating an increase of methemoglobin not only in children but also in adults exposed to nitrates from environmental factors [10].

4.5.2. Evaluation of antioxidant defense markers

4.5.2.1. Evaluation of Total antioxidant status

By determining this parameter to the people under study we found an increase of values among people in test group compared with controls (Fig. 6).

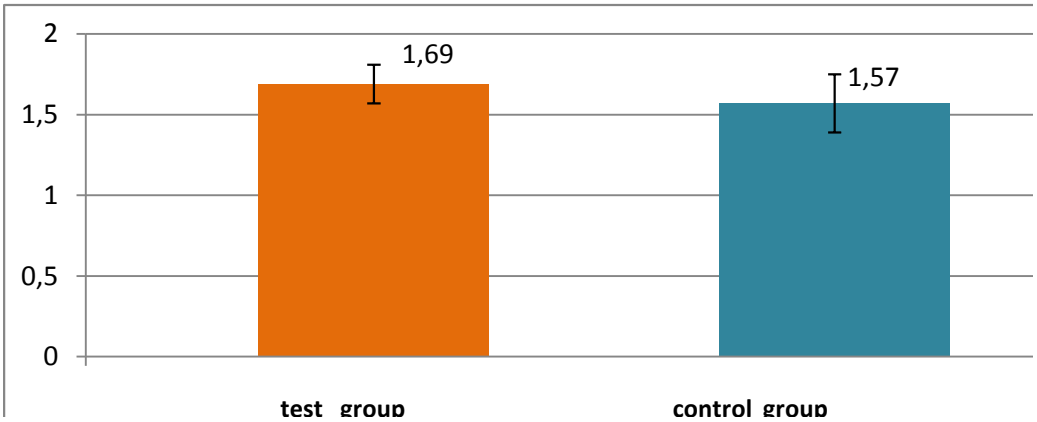

Figure 6 - The average values of TAS

Antioxidant defense of the body is done through the endogenous and exogenous antioxidants, of which the most important are superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione, catalase, vitamins A, E, C, selenium, coenzyme Q, uric acid, bilirubin, albumin, melatonin, succinate, porphyrins, arginine, citrulline, glycine, histidine, taurine, estrogen, etc. [11,12]. These antioxidants neutralize or remove reactive species of oxygen (ROS) and nitrogen.

By evaluating the total antioxidant status are obtained data about global antioxidant activity of the body and therefore we determined this parameter.

4.5.2.2. Evaluation of superoxide dismutase (SOD)

Superoxide dismutase is the body's first line of defense against oxidative stress.

In this study, the concentration of erythrocyte SOD showed a decrease of values to people from test group compared with controls (Fig. 7).

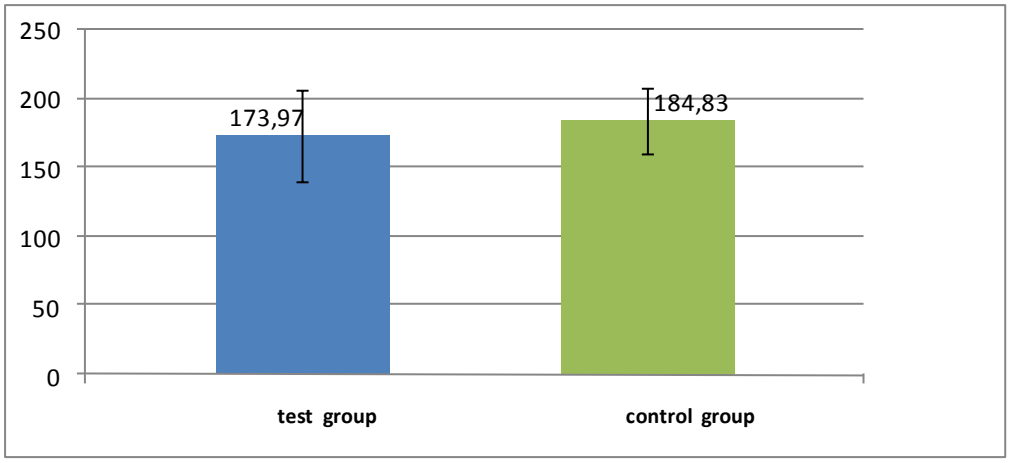

Figure 7 - The average values of SOD

Superoxide dismutase is the main "scavenger" of superoxide in the cells with predominantly aerobic metabolism.

The enzyme catalyzes the removal of two superoxide radicals which unite to form hydrogen peroxide. Thus SOD controls the superoxide anion level, preventing the initiation of forming reactions of peroxynitrite and hydroxyl radicals, which are harmful to the body [13, 14]. Intracellular concentration of enzyme affects the body's response to the cytotoxic effects of ROS.

Specialized studies show a correlation between the decreases in intracellular concentration of the enzyme and increase the cytotoxic effects of reactive oxygen species (ROS) [11, 13].

As a result, decreased SOD activity in the chronic exposure to nitrates favors the production of molecular lesions while generating changes in health

For the two markers of antioxidant defense (TAS and SOD) I found no data in the literature on changes that occur in the case of consumption of water with an increased levels of nitrates.

4.5.2.3. Evaluation of glutathione peroxidase (GPx)

Glutathione peroxidase also is part of protection mechanism against oxidative aggression. The average values of GPx for the two groups are shown in Figure 8.

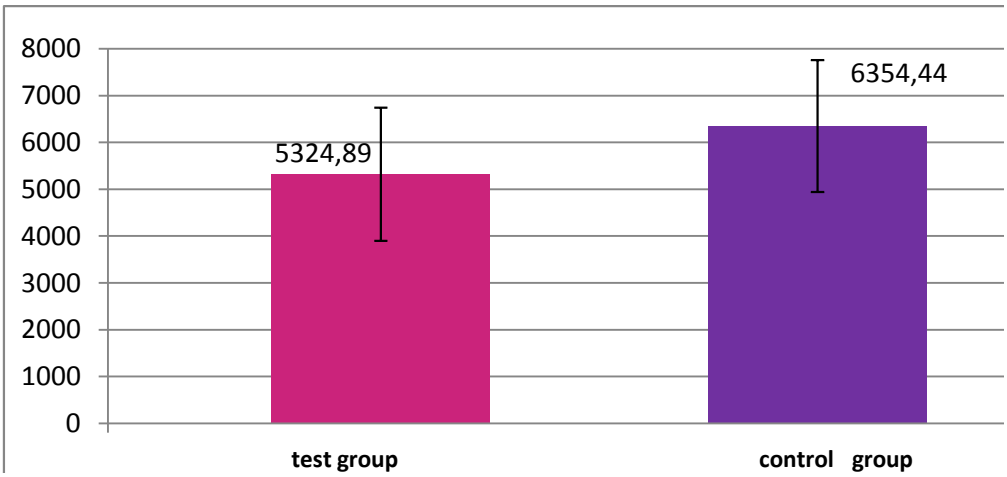

Figure 8 - The average values of GPx

4.6. Correlation between methemoglobin value and antioxidant defense markers

4.6.1. Correlation between the value of methemoglobin and total antioxidant status (TAS)

Analyzing the correlation between methemoglobin and TAS value (fig.9) we found that there is a positive linear connection between the two sets of data ($r = 0.44$, $p < 0.0001$). Square of the correlation coefficient indicates that 19.5% of the TAS variation is due to linear relationship with methemoglobin (MetHb).

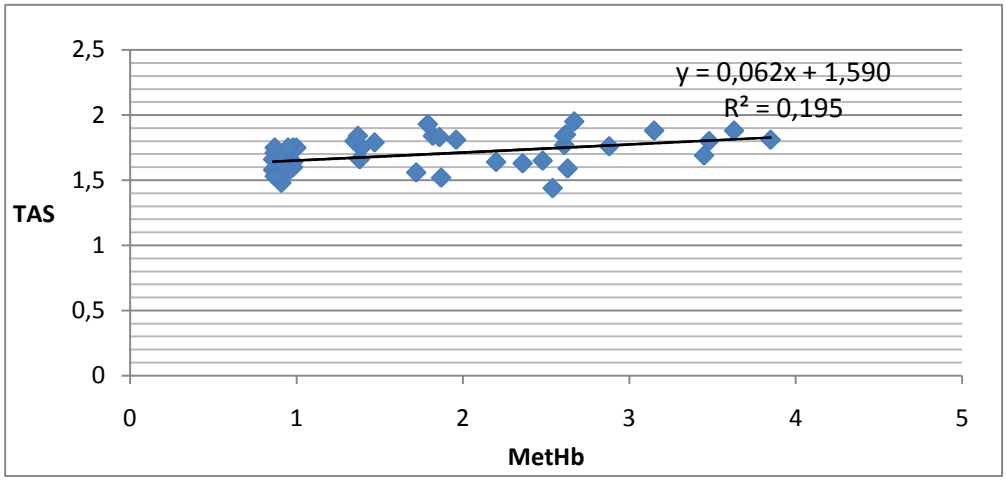

Figure 9 - Correlation between MetHb and TAS

4.6.2. Correlation between the value of methemoglobin and SOD

The correlation coefficient between the values of SOD and MetHb recorded in this study a value of - 0.30 ($p < 0.003$), suggesting that an increase in MetHb is accompanied from a decrease of the SOD (Fig. 10), in case of chronic exposure to nitrate.

Square of the correlation coefficient indicates that 9.4% of the variation of SOD is due to linear relationship with MetHb.

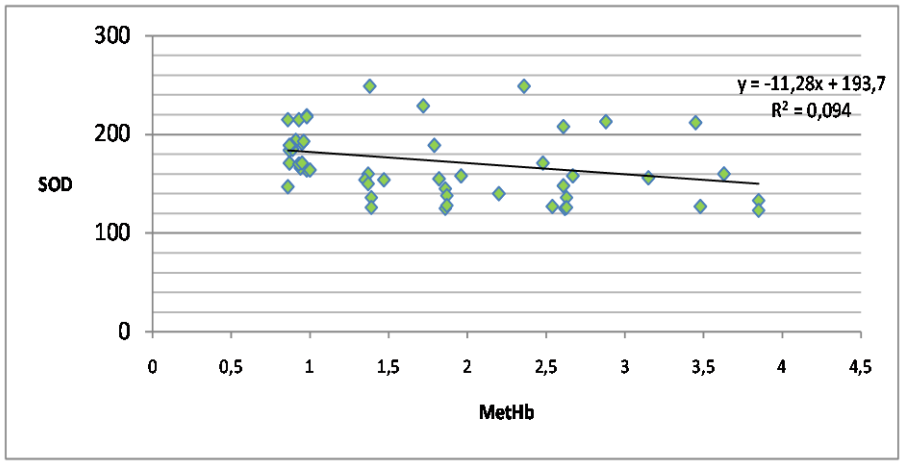

Figure 10 - Correlation between MetHb and SOD

4.6.3. Correlation between the value of methemoglobin and GPx

Glutathione peroxidase is also a protective mechanism against oxidative aggression. It occurs to reduce hydrogen peroxide and organic hydroperoxides [14-17].

In this study we found that GPx activity decreases in chronic exposure to nitrogen compounds.

Studying the correlation between MetHb and GPx we found a value of "r" of -0.12 ($p = 0.23$), indicating a weak correlation between the two sets of data values (fig.14).

Square of the correlation coefficient indicates that only 1.5% of the GPx variation is due to linear relationship with MetHb (fig.11).

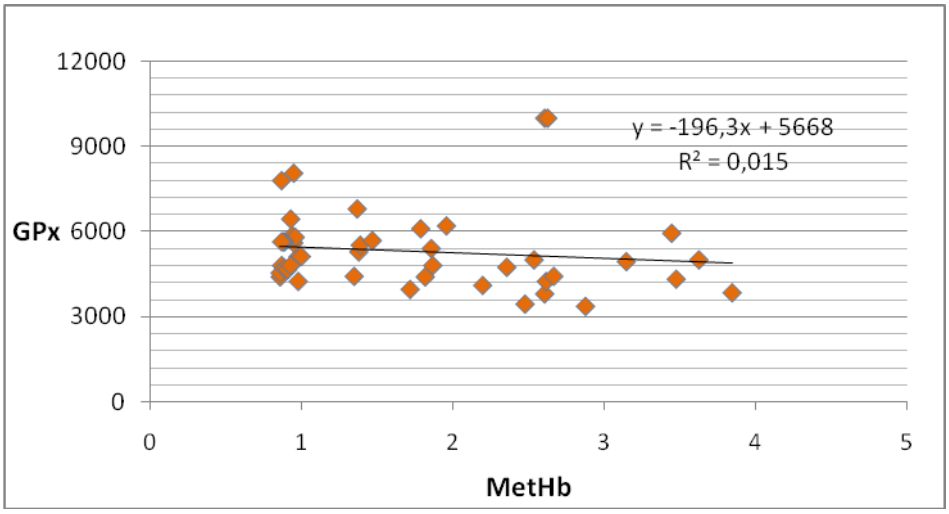

Figure 11 - Correlation between MetHb and GPx

Analyzing the correlation between MetHb value and the three antioxidant defense markers we found a significant relationship in terms of MetHb values and those of TAS and SOD.

For GPx correlation is weak. The data are consistent with those found in 2002 by Mancas and collaborators, in a group of childrenwhich shows that there is no significant correlation between MetHb level and GPx in patients exposed to nitrates compared with unexposed group [18]. No data was found regarding the exposure for adults.

5. CONCLUSIONS

- In rural areas are recorded most cases of deviation from sanitary rules for water quality;
- The individual supply system of rural areas the chemical and microbiological quality of water is more common inadequate than water supplied by central system;
- Also in rural centralized systems are unfortunately a chronic exposure to nitrates and relatively constant risk of epidemic waterborne;
- In urban areas chemical water quality parameters were within the sanitary norms in 90% of cases;
- Analysis of water samples on localities demonstrates the existence of areas whose population is constantly exposed to waterborne pollution;
- The research shows that they are subject to periodic organic discharges which are being overtaken in late stages, as nitrates;
- Due to inclement weather, year 2007 recorded the most frequent changes in quality parameters of water.

- In 2006-2010 in Constanta County there were 28 cases of nitrite poisoning.
- All patients were aged less than one year, and the diet of children was mixed type or artificial favoring the emergence of intoxication.
- The average value of methemoglobin registered on patients was 28.85 ± 23.31% of total hemoglobin.
- Over 90% of children had comorbidities of infectious cause that create optimal conditions for the conversion of nitrate to nitrite, in action of nitrate-reducing germs.
- Most cases of poisoning have occurred through the use of individual water supply systems and only one case by using centralized case.
- Cause of water pollution is the location near the water source of the manure storage areas, latrine, animal housing and the agricultural use of natural or artificial fertilizers generating nitrogen compounds.
- There were also two cases in which the levels of nitrates in water was within normal limits suggesting the involvement of vegetable consumption in the occurrence of methemoglobinemia (vegetable with high content of nitrates).

- Analyzing the water quality in the locality test, we found that from the total of 51 water samples collected in the period 2006-2012, 72.54% have exceeded the maximum limit for nitrates.
- For the chemical parameters aimed the recent pollution (organic substances, ammonia) we found no exceedances of the sanitary norms in any of the samples taken during this period.
- In case of nitrites (parameter indicating relatively recent pollution) only 3 samples were inadequate.
- It follows that the organic pollution of water is often caught in late stages, as nitrates.
- Regarding the evaluation of the microbiological quality of water in the locality test, we found that of the total samples, 21 recorded improper values for coliform bacteria which are a major epidemiological risk.
- The analysis of water in the unexposed locality found that all chemical parameters monitored are within the sanitary norms.
- Regarding the evaluation of microbiological quality of water we found also here the existence of samples (7 in total), which are not within the rules laid down by law, sanitary significance is the same as that described above.

- Studying the evolution of chronic diseases (ischemic cardiopathy ,type 2 diabetes, chronic lung disease, chronic liver disease, cancers) in the two localities studied we found a significant increase in the number of cases in area chronically exposed to nitrates.
- Exposure of pregnant women to nitrates from environmental factors disrupts the development of the babies: the average duration of pregnancy is lower as well as children's weight and length at birth.
- 61.83% of cases of newborns with low birth weight and 72.14% of cases of newborns with low birth length can be attributed to chronic exposure to nitrates.
- Apgar score had lower values for exposure to nitrates, and the highest grade is only 2% of cases, while in the control area it occurs in 36.6% of cases.

- By determining MetHb and analyzing statistical data based on the model of epidemiological investigation type case-control we found that the risk of developing of some elevated levels of MetHb is significantly higher in people who use water rich in nitrates (OR 5.47 to CI of 95%).
- Age group 33-38 years has the most elevated levels of MetHb.
- Given the fact that by boiling the nitrates existing in water and food concentrates and that over half of the test group used mainly food from their own households (as shown in the questionnaire), we can say that in this case, nutrition, along with water, is a risk factor for increased levels of methemoglobin.
- When determining total antioxidant status we found a significant increase among people in test group compared to control group. Based on data collected in this study we can say that this is a mechanism by which the body responds to oxidative stress caused by exposure to nitrates.
- Determination of SOD and GPx showed a decrease of the values in people in test group compared to control group.
- Data from this study suggest that in chronic exposure to nitrates enzymatic antioxidant defense mechanisms are overcome, enzymes displaying low values.
- Reduction of these antioxidant defense systems not determined compulsorily acute organ dysfunction, but certainly by the association with other etiological factors, the risk of developing chronic diseases increases, such as demonstrated by morbidity previously presented.
- Taking into account the results I think that this research is an important step in the detection and evaluation of disturbances caused by chronic exposure of adults to nitrogenous compounds from environmental factors and that it is important and interesting to extend the research for Giardia.

BIBLIOGRAPHY

1. Moussa S.A – Oxidative Stress in Diabetes Mellitus, Romanian J. BIOPHYS., Vol. 18, No. 3, 2008, Bucharest, p. 225–236;
2. Halliwell B, Cross Ce, Gutteridge Jmc. Free radicals, antioxidants and human disease: where are we now?. J Lab Clin Med 1992; 119**:** 598-620;
3. Hura C.- Conţinutul în nitraţi şi nitriţi în unele produse vegetale, Revista Jurnal de Medicină Preventivă, vol.3, nr.3-4, 1995, p.35;

4.Ward M.,Dekok T.,Levallois P.,Brender J.,Gulis G.,Nolan B.,Vanderslice J.- Workgroup report: drinking-water nitrate and health--recent findings and research needs- Environ Health Perspect. 2005 Nov ; 113 (11):1607-1614;

5.Morales-Suárez-Varela MM., Llopis-Gonzalez A., Tejerizo-Perez ML.- Impact of nitrates in drinking water on cancer mortality in Valencia, Spain- Eur J Epidemiol. 1995 Feb;11(1):15-21;

6. Chen K.,Yu W.,Ma X.,Yao K. and Jiang Q.- The association between drinking water source and colorectal cancer incidence in Jiashan County of China: a prospective cohort study- The European Journal of Public Health 2005, 15(6): 652-656;

7.Weyer PJ., Cerhan JR., Kross BC. et al.- Municipal drinking water nitrate level and cancer risk in older women: the Iowa Women's Health Study- Epidemiology 2001,12: 327–338;

8. Gulis G., Czompolyova M., Cerhan JR.-An ecologic study of nitrate in municipal drinking water and cancer incidence in Trnava District, Slovakia- Environ Res 2002; 88:182–187;
9. Alexa L, Gavăt V, Melinte C. - Curs de igienă - Litografia UMF Gr.T.Popa Iaşi 1994: 136-142, 193-199;
10. Gupta SK AK., Bassin., Gupta RC., Gupta AB., Seth JK., Gupta A.- Bulletin of the World Health Organization, 1999, 77(9), 749-752.
11. Tache S. – Capacitatea antioxidantă a organismului. In: Dejica D.(ed): Antioxidanţi şi terapie antioxidantă., Casa Cărţii de Stiinţa 2001, Cluj-Napoca, p. 71-101.
12. Adumitresi Cecilia, N. Ceamitru, Ileana Ion, Ninela Rădulescu, Carmen Ciufu, Cristina Farcaş, G. Badiu- Evaluation of Total Antioxidant Status in Guinea Pigs in Simulated Unitary Dives at 6 Absolute Atmospheres; Physiology, 2006; Vol.16, 2 (50): 41-43.

13. Scott M.D., Eaton J.W., Kuypers F.A., Chiu D.T.-Y. Lubin B.H. -Enhancement of Erythrocyte Superoxide Dismutase Activity: Effects on Cellular Oxidant Defense, Blood, Vol. 74, Nr. 7 (Nov. 15), 1989, 2542-2549.
14. Yamamoto Y, Takahashi K.-Glutathione peroxidase isolated from plasma reduces phospholipid hydroperoxides. Arch Biochem Biophis 1993 Sep; 305(2):541-5.
15. Takahashi K, Avissar N, Whitin J, Cohen H.- Purification and characterization of human plasma glutathione peroxidase: a selenoglycoprotein distinct from the known cellular enzyme. Arch Biochem Biophis 1987 Aug 1; 256(2): 677-86.
16. Condell R.A., Tappel A.L. - Evidence for suitability of glutathione peroxidase as protective enzyme: Studies of oxidative damage restoration and proteolysis. Arch Biochem. Biophys. 1983; 223: 407-416.
17. Thorpe, G. W.; Fong, C. S.; Alic, N.; Higgins, V. J.; Dawes, I. W.- Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes. Proc. Natl. Acad. Sci. U.S.A. 2004; 101: 6564–6569.
18. Mancaş G, Vasilov M, Albu G - Adverse Health Efects Associated with Methemoglobinemia in children -The journal of Preventive Medicine 2002; 10 (1):11-17;