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Nitrate contamination of ground water and Methemoglobinemia in Gaza Strip

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الملخص

تلوث المياه الجوفية بالنترات وعلاقتها بالميتهيموجلوبنيميا في قطاع غزة

إن تلوث المياه الجوفية بالنترات أصبح مشكلة جدية للصحة العامة في قطاع غزة، ولذلك جاءت هذه الدراسة من أجل قياس وتقييم تركيز النترات في المياه الجوفية ، وعليه فلقد أجريت الدراسة الميدانية وأخذت العديد من عينات المياه من الآبار الجوفية الخاصة بمياه الشرب وتم فحصها وتحليلها من حيث تركيز النترات فيها وفق طريقة التحليل الضوئي بجهاز التحليل الطيفي. ولقد وجدت الدراسة بأن المياه الجوفية في قطاع غزة تم تلوثها بشكل كبير مع معدل تركيز للنترات يساوى 129.7/ملجرام/لتر لجميع أبار مياه الشرب التي تم دراستها .

من ناحية أخرى أجريت دراسة مرض الميتهيموجلوبينيميا كأحد التأثيرات الصحية الأساسية الناتجة عن تلوث مياه الشرب بالنترات حيث تم إجراء فحص الميتهيموجلوبينيميا على 640 طفل تم اختيارهم عشوائيا من الأطفال الذين دخلوا مستشفيات الأطفال وتم اخذ عينات الدم من هؤلاء الأطفال خلال عام 1998 حيث اجري فحص تركيز الميتهيموجلوبين حسب طريقة التحليل الضوئي الواردة في كتاب باربرا براون.1992 .

كانت النتائج العملية تشير إلى أن معدل انتشار الميتهيموجلوبينيميا في قطاع غزة عالي بشكل ملحوظ، وان معدل الحالات غير الطبيعية مع اختلاف النسب الذين يعانون من مرض فقر الدم (الميتهيموجلوبينيميا) يساوى 72-75% من إجمالي الأطفال الذين تم فحصهم في هذه الدراسة.

ABSTRACT

The nitrate contamination of ground water is a serious threat to public health in Gaza Strip. This study was performed in order to evaluate the concentration of nitrate in ground water of Gaza Strip. Afield study was performed and various samples were taken from drinking water wells and analysed for nitrate concentration according to ultraviolet

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spretrophotometric method . It was found that the ground water of Gaza Strip has been greatly polluted by nitrates with average nitrate concentration of 129.7 mg/L for all study of drinking wells of Gaza Strip. In other hand , methemoglobinemia in the Gaza Strip was investigated as the health effect of nitrate exposure. Sex hundred forty (640) child blood samples during 1998 were randomly selected from paediatric hospitals in Gaza Strip and analysed for methemoglobin concentration according to spectrophotmetric method described in Barbara Brown 1992. The experimental results indicate that the prevalence of metemoglobinemia in Gaza Strip is very high and the average abnormal cases with varied percentage suffering from metemoglobinemia is equal to 72-75 % of the total investigated children .

Introduction :

Palestine is located in the Middle East surrounded by Lebanon and Syria to the North , Jordan to the West , Egypt to the South and Mediterranean Sea to the West . Gaza Strip is a part of Palestine about 360 KM^2 , located on the south eastern coast of Mediterranean Sea, bordered on the south by Sinai desert, on the east by Nagab desert. On this narrow strip about 1,003,490 of Palestinian people live as surveyed in 1998, they occupy about 70 % of the total area of Gaza Strip which considered as a high populated area with 1.936 person/Km^2 and in some refugee camps reach to 50-80.000 person/Km^2 . The annual total precipitation ranges from 200-400 mm with average of about 300 mm . The soil type in most area of Gaza Strip is quaternary soil with clayey material increasing towards the eastern border. Ground water is the most reliable gift in Gaza Strip because it represents the only significant source of fresh water for domestic, industrial and irrigation purposes. The ever increasing demand of Palestinian community for water consumption has generated a serious problem can be summarised as follows:

1.Unavailability of sufficient water resources mainly due to rapidly increase of population with uncontrolled water consumption and decrease of the annual replenishment rate of ground water with rainfall.
2.Unavailability of drinking water with appropriate qualities for most cities of Gaza Strip due to the ground water deterioration with anthropognic activity making most drinking water wells contaminated by virus pollutant turning water unhealthy for drinking.

One of the most important pollutants responsible for poor quality of water in Gaza Strip is the high concentration of nitrate which make more than 80

% of total drinking wells unsuitable for drinking according to WHO standards of45 mg/L for nitrate concentration for all area of Gaza Strip in October 1998 is 129.7 mg/L as NO3.

Background:

Nitrogen is an essential element in living matter but it can cause environmental and health problems when nitrogen as fertiliser, waste or plant residue reach to land, it undergoes many chemical, physical and biological reactions forming many components, the nitrate NO3 from can reach ground water and could become harmful and toxic to infants.

Major sources of nitrate pollution in Gaza Strip:

Anthropogenic source which increased the amount of nitrate to harmful level is the waste materials especially from sites used for disposal of human sewage, industrial wastes , septic tanks which represent a major local source of nitrate contamination of ground water especially in Khanyounis area of Gaza Strip . The other main source results from agriculture where Gaza farmers still consider nitrogen fertiliser the cheap and best growing fertiliser. Another source is from using soil cleaners such as methyl bromide widely used in Gaza Strip. Another potential source of nitrate leaching to the ground water is the application of animal and poultry manure on land as natural fertiliser.

In Gaza Strip the farmers usually apply an excess of manure to the crop to ensure that enough nitrogen available for growing. Anyhow, these nitrates formed from the above different sources get from the field into our water supply. It is mainly due to two reasons:

1. Applying too much nitrogen to the soil, result in excess nitrogen remaining in the environment and much of it leached into ground water.
2. The over-irrigation of agriculture area causes the leaching of these nitrates, passing the plants root zone and enters the groundwater in short time, especially the soil type in Gaza Strip is sandy and gravel soil.

The WHO drinking water standards do not allow more than 10 mg/L nitrate nitrogen in public water supply. Some international health authority has recognised the health risks for infants of nitrate exposure below the current WHO standards. The European community has established health guidelines for nitrate of 5-6 ppm in Germany and South Africa, the enforceable standards are 4.4 ppm and USA the level is 10 ppm. These levels were adopted after the clear evidence occur in two international studies (Simon, et.al 1962, Sattel Maher 1964) showing that methoglobinemia occurred at concentration below 10 ppm. Unfortunately the Palestinian health authority did not yet established any guidelines for water quality. The nitrate contamination of all water systems in Gaza Strip scoring relatively high levels already exceeding the WHO guidelines more than 8 times in which most of drinking wells contains nitrate concentration exceeding the WHO standard with the average concentration for all drinking wells of 129.7 ppm. Anyhow , the nitrate concentration higher than 10 mg/L may cause nitrate poisoning of infants during the first four-six months of life. The pH of an infant gastric juice is high (less acidic) between 5-7. So the nitrate reducing bacteria in infant digestive system converts nitrate to more toxic nitrite which could be absorbed and reaches the blood, forming methemoglobia which cannot carry oxygen and symptoms of oxygen deficiency occur with shortness of breath, heart attacks, increase susceptibility to illness, and if more than half of haemoglobin is converted to methemoglobin, death may occur. This condition is called as methemoglobinemia (blue baby syndrome). In another hand, other important factor may expose infants for high risk of methemoglobinemia, in which their blood contain special type of haemoglobin called Foetus-Haemoglobin (F-Hb), usually found during the first months after birth, considered more susceptible to nitrate exposure, in addition the enzyme system which convert methemoglobin to haemoglobin is not very active in early life of infants (G.F Craun et, al, 1991). Anyhow as child develops the acidity of the stomach becomes stronger, and the nitrate reducing bacteria are killed, so the nitrate is mot a cause of methemoglobinemia for older children and adults.

In this study, investigation and laboratory analysis of water of the drinking wells in Gaza Strip was performed to determined the concentration of nitrate and their possible human health risks, of causing methemoglobinemia.

Methods:

first: Determination of nitrate concentration in drinking wells of Gaza Strip.

This work has been designed and implemented to evaluate the current nitrate concentration in drinking ground water wells in Gaza Strip. The study area was divided into five major zoon areas. Water samples were taken from almost all drinking wells present in each area and analysed for nitrate concentration according to spectrophotometric method described in (standard method for analysis of water and wastewater 16th edition, 1985).

Apparatus:

1. Ultraviolet spectrophotometer: for using at 220 nm with matched silica cells of 1 cm light path.

2. Membrane filter. 45 nm pore diameter membrane filter for turbid sample.

Reagents:

1. Stock nitrate solution:

0.7218 g of dry potassium nitrate (KNO_3) was dissolved in distal water and diluted to 1000 ml then 2 ml of chloroform ($CHCl_3$) was added.
So 1 ml = 100 mg NO_3-N

2. Standard nitrate solution:

50 ml of stock nitrate solution were diluted to 500 ml with distal water.
So 1 ml of this solution =10 mg NO_3-N.

3. Hydrochloric acid solution, HCL (1 N) .

Procedure:

1. 1ml of HCl solution were added to 50 ml clear sample and mixed .

2. Preparation of calibration curve :
The NO_3-calibration standard in the rage of 0 to 7 mg NO_3-N/l were prepared by diluting to 50 ml the following volumes of the standard solution : 0, 1, 2, 4, 7...35 ml.
The NO_3 standard was treated in the same manner as samples. Then the calibration curve was drawn.

3. Measurement:

- spectrophotometer was adjusted to wave length at 220 nm.
- spectrophotometer set at zero absorbance by reading the absorbance of distal water (blank) .
- reading for the unknown water sample was recorded at the absorbency.
- the reading was calibrated with the nitrate calibration curve and the concentration of nitrate was calculated.

Results and comments:

The experimental results of the water wells in first area of Gaza Strip, the northern area including Beithanon . Beit Lahia, and Jabalia are tabulated in table No1 and the other area in tables 2,3,4 and 5. The average concentration of nitrate in drinking wells of Gaza Strip was given in table No 6 and suitability of these drinking wells for drinking according to WHO standard of nitrate concentration is given in table No 7.

Table No1. Nitrate concentration in drinking water wells in northern area of Gaza Strip. 1998

No	Well name and code	Location	Conc. NO3/ppm
1.	C-127	Beit Hanon	70
2.	Abu Gazala	Beit Hanon	43
3.	H-A-135	Beit Hanon	50
4.	BL-A-185	Beit Lahia	104
5.	BL-B-67	Beit Lahia	82
6.	BL-A-180	Beit Lahia	60
7.	JB-E118	Jabalia	195
8.	JB-E90	Jabalia	174
9.	JB-D60	Jabalia	226
10.	JB-Murad	Jabalia	105
11.	JB-E92	Jabalia	280
12	JB-AL Torak	Jabalia	174

Table 2. Nitrate concentration in drinking water wells in Gaza city. 1998

No	Well name and code	Location	Conc. NO3/ppm
1.	Rodwan 1A-162	Sheikh Rodwan	130
2.	Rodwan 7B-162	Sheikh Rodwan	165
3.	Rodwan 9E-157	Sheikh Rodwan	98
4.	Rodwan 10D-68	Sheikh Rodwan	63
5.	Rodwan 13G-162	Sheikh Rodwan	122
6.	Sofa 2A-25	Al shavaraIIIa3apa	140
7.	Ghosin GR-75	Al Remal	128
8.	Rodwan 1GR-64	Al Remal	84
9.	Ejlin 1	Shiekh Ejlin	102
10.	Ejlin 2	Shiekh Ejlin	60

Table 3. Nitrate concentration in drinking water wells in Med Zoon area (Deralbalah, Magazy, Buraje) 1998.

No	Well name and code	Location	Conc. NO3/ppm
1.	D1-J146	Der AlBalah	60
2.	Al Sahel 3	Der AlBalah	85
3.	Al Sahel 5	Der AlBalah	60
4.	Hosni Basheir	Der AlBalah	80
5.	Besaid 542	Magazy	42
6.	Abu Ganem	Magazy	50
7.	Baker Moslum	Buraj	93

Table 4. Nitrate concentration in drinking water wells in Khanyounis and eastern villages 1998.

No	Well name and code	Location	Conc. NO3/ppm
1.	Temwin K-41	Khan younis	300
2.	UN camp K-L86a	Khan younis Camp	50
3.	Al Sada K-L87	Khan younis	311
4.	Al Ahrash K-L127	Khan younis	346
5.	Amel K-L159	Al Amal	380
6.	Al Tebaha T44	Al Qurara	87
7.	Al-N9	Absan Kibera	300
8.	F2-N22	Absan Kibera	120
9.	S-M2A	Bani Sohila	240
10.	S-M2B	Bani Sohila	120

Table 5. Nitrate concentration in drinking wells of Rafah 1998.

No	Well name and code	Location	Conc. NO3/ppm
1.	R-P 15	Rafah city	147
2.	R-P124	Tel Saultan	120
3.	R-L138	Al Hododi	45
4.	R-CND	Canda camp	60
5.	R-P10	UN camp	210

Table 6. Average concentration of nitrate in drinking wells of Gaza Strip 1998.

No	Area	Total well No	Average nitrate conc. No3/ppm
1.	Northern area	12	130.2
2.	Gaza are	10	109.2
3.	Medzoon area	7	67.4
4.	Khan younis and eastern villages	10	225.4
5.	Rafah area	5	116.4

Table 7. Suitability of drinking water wells with nitrate in Gaza strip 1998.

No	Area	Total well No	No of wells suitable	No of wells unsuitable	Percentage % of unsuitable
1.	Northern area	12	2	10	83.3%
2.	Gaza area	10	non	10	100%
3.	Medzoon area	7	2	5	71.4%
4.	Khan Yonis and eastern villages	10	1	9	90%
5.	Rafah area	5	1	4	80%
6.	average of wells unsuitable				84.8%

The results of the above tables show clearly that the ground water of almost all drinking wells in Gaza Strip are observed to be contaminated with nitrate with average concentration of 129.7 mg/l and no well could be considered suitable for drinking according to WHO standards of 45 mg/l of nitrate. From table No 7 it is clear that more than 84 % of the total drinking wells in Gaza Strip are unsuitable for drinking due to nitrate contamination, in some areas like Khan Yonis and eastern villages exceeding the Who standards more than 5 times of the normal value of 45 ppm for nitrate.

So consuming this type of water with high level nitrate for drinking has a significant relation in causing methemoglobinemia in large number of Gazians infants below six months age, as concluded from laboratory work performed to investigate the incidence of methemoglobinemia in Gaza Strip.

Methemoglobinemia in Gaza Strip:

Methemoglobinemia is the most immediate life-threatening effect of nitrate exposure. The excessive ingestion of nitrate in infancy put them at high risk of developing methemoglobinemia which is characterised with cyanosis, bluish coloration of the skin around baby ᾽s eyes and mouth, due to lack of oxygen and accumulation of carbon dioxide (CO2) in the blood. These symptom ate directly proportional with the concentration of methemoglobin which is an abnormal haemoglobin containing iron molecule in ferric (+3) state rather than normal ferrous (2+) state. When methemoglobin forms approximately 10-30 % of total haemoglobin, only cyanosis occur and when the level is increased to 50 %, other serious symptoms may occur such as dyspnea (difficult in breath), tachycardia (rapid heart beat), dizziness, fatigue and headache, if the concentration level of methemoglobin increases to 50-70 % severe lethargy and brain damage, loss of consciousness, convulsion and death may occur (Charlotte.M.Fant et. 1993). The normal level of methemoglobin in adults and children is less than 2 % of the total haemoglobin.

Acquired methemoglobinemia results when infants are fed with well water contaminated with high level of nitrates. Unfortunately, the health records in Gaza Strip have no information on the impact of the deteriorating quality of water mainly due to nitrate contamination at health, also here in no information of infant methemoglobinemia due to nitrate exposure from well water used to dilute powdered milk or infant formula. This study designed to investigate the relation between the quality of drinking well water with high level of nitrate concentration that used to dilute powdered milk and infant formula and methemoglobin concentration among infants below six months in Gaza Strip. This target population was chosen for the following reasons:

1. Infants at this age are more susceptible to nitrate poisoning
2. Infants after this age are usually given some food supplement that may contain vitamin C, which is usually given to treat methemoglobinemia.
3. Infants at this age usually take powdered milk or infant formula as their source of food, which restricts the source of nitrite exposure to water used to dilute the milk or infant formula as their source of food .

Determination of methemoglobin concentration in infants below six months of age in Gaza Strip.

Selection of samples:

Gaza Strip was divided into two major zoon area according to presence of paediatric hospitals, the cases were randomly selected from both hospitals were those infants below six months of age who came for routine check-up or were already hospitalised for any other reason other than methemoglobinemia, for each patient the age and residence were recorded.

Procedure:

Three hundred twenty blood samples were selected from Gaza city:

Paediatric hospital (Al Nasser) and three hundred twenty other blood samples were randomly selected from Khanyonis general hospital, paediatric department. All blood samples were analysed. For methemoglobin concentration according to method described in Barbara A. Brown "Haematology principle and procedure@. 1992.

Apparatus: Spectrophotometer, Micropipetes.

Reagents:

1.Preparation of phosphate buffer, 0.067 m, pH 6.6

Solution 1: Potassium dihydrogen phosphate 0.067 m
Solution2: Sodium dihydrogen phosphate 0.067 m
So 63 ml of solution 1 with 37 ml of solution 2 to form phosphate buffer, 0.067 m, pH 6.6
2. Preparation of phosphate buffer 0.017, pH 6.6
100 ml of phosphate buffer, 0.067 m, pH 6.6 mixed with 300 ml of distal water, a few drops of saponin oil were added.
3. Preparation of sodium cyanide 10% w/v
10 gram of sodium cyanide mixed with 1 ml of 12% acetic acid

Note: this reagent was usually prepared immediately and used within 1 hour.

Determination of calculation factors:

Calibration factor for the spectrophotometer used in this test was determined as follows:-

1. determination of the haemoglobin concentration in g/dl in whole blood sample was performed .

2. 5 µL of 0.017 phosphate buffer was placed in each of two test tubes, then 50 ml (microliter) of whole blood specimen was mixed with one tube , the second tube remains as Blank.
3. 20 µL 20% of potassium ferricyanide were added to both test tubes.
4. Blank is used to set the optical density at zero, then first reading of the spectrophotometer at 630 nm and recording the absorbance D1.
5. 20 µL of neutralised sodium cyanide were added to both tubes and stood for 2 minutes, then second reading of the spectrophotometer at 630 nm, and recording the absorbance D2 then the calibration factor calculated as follows: $F = \frac{Hgb(g/dl)}{D1 - D2}$

Note: Two spectrophotometers were used

One for the northern area of Gaza Strip samples with calibration factor equal to 0.64 and the other spectrophotometer was used for the southern area samples with calibration factor equal to 0.66.

Determination of methemoglobin in children specimen:

- 5 µL of 0.017 m phosphate buffer was placed in each of two test tubes (one as test and the other as Blank).
- 50 µL of whole blood from each sample were added and mixed in the test tube.
- Blank was used to set the optical density at zero.
- the first reading of the test at wave length 630nm for each sample was taken and recording the absorbance of the test (D1).
- 20 µL of neutralised sodium cyanide were added and mixed to each of two tubes (the test and the blank) and stood for 2 minutes, then Blank is used to calibrate the spectrophotometer are zero, then the second reading for the test was taken at the same wave length 630nm and recording the absorbance (D2).
- the percentage amount of methemoglobin in each sample was calculated as follows: methemoglobin (g/dl)=D1-D2 x factor.

Results and comments:

from the data obtained it was clear that the methemoglobinemia in Gaza Strip is widely present in infants below six months of age and it has very significant relation with the nitrate concentration in drinking water used for the preparation of child formula. The conclusion results were tabulated in the following tables (8,9) as the percentage of methemoglobin concentration and the total number of effected patients and the locality were recorded.

Methemoglobinemia in Gaza Strip 1998:

Table 8. Methemoglobinemia at southern area of Gaza Strip

Percentage % of methemo-globin	Total	45	41-45	36-40	30-35	26-30	21-25	16-20	11-15	6-10	0-5
No of patients	320	3	2	3	4	6	10	12	34	94	152
Percentaege of disease%	100	0.9	0.6	0.9	1.25	1.9	3.1	3.7	10.6	29.4	47.5

Table 9. Methemoglobinemia at Northern area of Gaza Strip

percentage of methemo globin %	Total	> 45	41-45	36-40	30-35	26-30	21-25	16-20	11-15	6-10	0-5
No of patients	320	6	6	6	10	18	50	62	74	67	21
Percentage of disease %	100	1.7	1.7	1.7	3.3	5.8	15.8	19.2	23.3	20.8	6.7

It is clear from the above tables (8,9) that the prevalence of methemoglobinemia in Gaza Strip is very high. If we consider that the normal percentage of methemoglobin concentration from 0-10 % of the total haemoglobin, the prevalence of the abnormal cases suffering from methemoglobinemia in the southern area of Gaza Strip is equal to 23.1 % , while the prevalence of the disease in northern area of Gaza Strip is above 72.5 % . Anyhow, the symptoms of methemoglobinemia depend on the percentage of methemoglobin concentration, so when methemoglobin concentration ranges from 10-30% of the total haemoglobin, only cyanosis occurs and as the level increases the complicated symptoms of methemoglobinemia occur and death may occur when level of methemoglobin increases above 70% (Randall, Grubs, et. al. 1998). It is important to say that long term suffering with 30% of methemoglobin could not show any physical symptoms of the disease but unfortunately those infants in future will be with low mental development and intellectual activities retardation due to long exposure to inadequate amount of oxygen.

Recommendation:

- **Treatment of methemoglobinemia**

Infants with cyanosis and methemoglobin level below 30% is simply treated once occur. The infants should be avoided from drinking water contaminated with nitrate for a few days. The body will replenish the haemoglobin by itself in a few days (Johnson et. al./ 1987). But in patient is severely cyanotic with methemoglobin concentration from 30-70%, methyl blue should be administrated intravenously in a dosage of 1-2 mg /kg of body weight, in case of failure of methyl blue to improve methemoglobinemia, ascorbic acid of 500 mg orally may be helpful, if the patient is severely ill, an exchange of blood transfusion or administration of hyperbaric oxygen may be required (Charlotte et. al. 1993)

- **Prevention:**

many steps should be taken to prevent child methemoglobinemia includes residents of rural areas should routinely test their drinking wells especially if pregnant women or infants consume well water and if found contaminated with nitrate, other water source should be used like other safe drinking wells, bottled water, water purification apparatus which can clean water from nitrate, such as ion exchange, reverse osmosis (Lukens, et. al. 1987)

Breast feeding is considered the bet solution to avoid infants methemoglobinemia, and for those women who are not breast feeders, the use of bottled water in infant formula preparation offers the safest solution.

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