

# Relation of nitrate contamination of groundwater with methaemoglobin level among infants in Gaza

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علاقة تلوث المياه الجوفية بحمض النتريك مع معدلات المتهيموغلوبين بين الأطفال في غزة  
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**الخلاصة:** أُجريت دراسة وصفية، مستعرضة وتحليلية عام 2002، في ثلاث مناطق من قطاع غزة، في فلسطين، لتحديد العوامل المترافقة مع المستويات المرتفعة للمتهيموغلوبين بين الرضع، وعلاقتها بنسبة تركيز حمض النتريك في آبار مياه الشرب. ومثلت مصادر مياه الشرب السبب الأكثر احتمالاً وراء ارتفاع معدلات المتهيموغلوبين. فمن بين 338 طفلاً جاؤوا لتلقي التطعيم، تركزت المعدلات المرتفعة للمتهيموغلوبين في أولئك الذين يتغذون بالأغذية التكميلية، ويتناولون المياه التي سبق غليها، وتتراوح أعمارهم بين ثلاثة وستة أشهر. وبلغ أعلى متوسط لمعدلات المتهيموغلوبين في خان يونس حيث سُجّلت أعلى نسبة تلوث لمياه الشرب بحمض النتريك. وقد ركزت نتيجة الدراسة على أهمية اقتصار التغذية على الإرضاع من الثدي بالنسبة للرضع أقل من ستة أشهر، مع ضرورة اختيار مصدر مناسب للمياه لهم.

**ABSTRACT** A descriptive, cross-sectional and analytical study was carried out in 3 areas of the Gaza Strip, Palestine, in 2002, to determine the factors associated with high methaemoglobin (Met-Hb) levels in infants and the relationship with nitrate concentration in drinking water wells. Drinking water sources were likely to be the main factor for high levels of Met-Hb. Out of 338 infants attending for vaccination, having supplemental feeding, use of boiled water and age 3–6 months were associated with high Met-Hb levels. The highest mean Met-Hb level was in Khan-Younis, where the highest mean nitrate concentration was recorded in drinking water. The results emphasize the importance of exclusive breastfeeding for infants < 6 months old, and the choice of a suitable source of water for these infants.

## Rapport entre la contamination des eaux souterraines par les nitrates et la méthémoglobinémie chez le nourrisson à Gaza

**RÉSUMÉ** Une étude descriptive, transversale et analytique a été menée en 2002 dans 3 régions de la Bande de Gaza en Palestine dans le but de déterminer les facteurs associés à l'élévation de la méthémoglobinémie (MetHb) chez le nourrisson et son rapport éventuel avec la concentration en nitrates des puits d'eau potable. Il était en effet probable que les sources d'eau potable soient le principal facteur responsable de l'augmentation des taux de MetHb. Chez 338 nourrissons consultant pour une vaccination et bénéficiant d'une supplémentation nutritionnelle, l'utilisation d'eau bouillie et l'âge de 3 à 6 mois ont été associés à une hyperméthémoglobinémie. C'est à Khan-Younis qu'ont été enregistrés à la fois le taux moyen de MetHb le plus élevé et la plus forte concentration moyenne de nitrates dans l'eau potable. Les résultats soulignent l'importance de l'allaitement maternel exclusif pour les nourrissons de moins de 6 mois et du choix d'une source d'eau adéquate pour ces enfants.

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## Introduction

Contamination of drinking water supplies with nitrates is an issue of concern for public health and environmental health practitioners, mainly because it can be a health threat to infants less than 6 months old when contaminated drinking water is used to prepare formula milk [1]. Short-term exposure to high levels of nitrates in drinking water can cause methaemoglobinaemia, known as blue baby syndrome, especially in infants up to 6 months of age [2,3].

Groundwater is the only source of drinking water in the Gaza Strip region of Palestine, and water supplies from the wells in Gaza City, Jabalia and Khan-Younis have a high level of nitrates, above the international recommended level. In these areas, there is high population density; thus much of the Gaza population is exposed to high nitrates. Gaza Strip faces a serious problem of drinking water availability if current pumping rates continue or increase with the growth of the population and economy [4].

The most common causes of groundwater pollution in the Gaza Strip are over-pumping from wells for domestic and agricultural use leading to diminishing groundwater levels and hence seawater intrusion [5], the continuous seepage of wastewater [5] and the agricultural chemicals including fertilizers which are applied to cropland to raise agricultural production, and which then permeate the cropland groundwater [6]. The underlying problem is the high population in the Gaza Strip.

The general objective of this study was to evaluate the relationship between nitrate concentrations in the drinking water sources and the level of methaemoglobin (Met-Hb) in infants' blood in 3 areas of the Gaza Strip: Jabalia, Gaza City and Khan-Younis and to examine the relationship between

demographic, socioeconomic, nutrition and environmental status and Met-Hb levels among infants.

## Methods

A cross-sectional design was adopted for this study which was conducted over 6 months between the beginning of June 2002 and the end of November 2002.

### Sample

The study group was all 338 infants who visited one of the 12 Ministry of Health (MOH) primary health care centres for vaccination during the study period; these are the main health centres in Jabalia, Gaza City and Khan-Younis. The distribution by area was follows: 43 from Jabalia (12.7%), 199 from Gaza City (58.9%) and 96 from Khan-Younis (28.4%). The sample size calculation was made using *Epi-info*, version 6 in order to determine a representative number of subjects.

### Data collection

The mothers of the study infants were interviewed face-to-face to collect the following information: mother's age, mother's educational level, mother's occupation, father's occupation, family's source of drinking water, type of feeding for the infant (exclusive breastfeeding, complementary foods, formula milk) and if formula milk was used, how it was prepared.

Venous blood samples were collected from infants into EDTA tubes by a well-trained laboratory technician and tested for haemoglobin (Hb) and Met-Hb. The samples were promptly sent to the laboratory to be tested for complete blood count (CBC) using a haematology analyser cell (Dyne

1700) and Met-Hb assay using a manual spectrophotometric procedure.

The nitrate concentration in the drinking water wells of the study areas during the study period were taken from the records of the Public Health Laboratory of the MOH which tests wells twice annually in coordination with the municipality.

### Analysis

The data was entered into a computer and *SPSS*, version 8, and *Epi-Info* were used for data cleaning and analysis. Data analysis was carried out by preparing frequency tables for all the study variables, defining and recoding certain variables and cross-tabulation and advanced statistical analysis. Statistical relationships between the variables and methaemoglobin level were assessed using the chi-squared test. *P*-values < 0.05 were considered statistically significant.

### Ethical procedures

The necessary approval to conduct the study was obtained from the Helsinki Committee in the Gaza Strip. Patient confidentiality was maintained at all times during the study. Approval of the director general of the MOH was obtained in order to carry out this study following the permission of general director of primary health care. The idea and theme of the study were fully explained to the mothers of included infants. Both verbal and written methods were used in a clearly detailed performance. Assurance and confidentiality were satisfactorily attained.

## Results

### Characteristics of study group

Of the 338 infants, 53.3% were males and 46.7% were females (Table 1). The mean age was 4.5 [standard deviation (SD) 2.6]

months; 35.8% were aged 1–3 months, 53.3% > 3–6 months and 10.9% > 6 months. The mean weight of the study infants was 6.3 (SD 1.5) kg.

The mean Hb level of the study group was 10.6 (SD 1.2) g/dL; 58.0% of the infants were anaemic (Hb < 11 g/dL) (Table 2).

Approximately a quarter of the infants (24.0%) were exclusively breastfed, 13.6% were fed only by formula and 62.4% were fed by formula in addition to breastfeeding (Table 2). Three-quarters of the infants (76.0%) were supplemented by foods other than milk, and 24.0% were not. The mean age of beginning supplementation was 2.5 (SD 2.0) months. The study showed that 68.3% of infants were given boiled water.

The main source of drinking water for the families of each study infant was as follows: 59.5% of families used tap water, 20.4% used treated water, 11.2% used home-filtered water and 8.9% used reservoir water and water of private wells (Table 3). The source of family drinking water varied significantly in the different study areas ( $\chi^2 = 68.21$ ,  $P < 0.0001$ ), with tap water being more common in Jabalia (79.1% of infants' families), than Gaza City (52.8%) or Khan-Younis (64.6%).

### Met-Hb levels

There was a significant statistical association between infant's age and Met-Hb level in blood ( $\chi^2 = 47.55$ ,  $P < 0.0001$ ) (Table 1). The proportion of infants with Met-Hb level > 5.0% at 1–3 months of age was 17.7%, at > 3–6 months of age was 65.9% and > 6 months of age was 16.5%. The percentage of males with raised Met-Hb (54.9%) was not significantly different from females (45.1%).

There was no significant relationship between Met-Hb level and any of the socio-economic variables: mother's age, mother's

Table 1 Methaemoglobin levels (Met-Hb) of the study infants by mother's age, educational level, and occupation, father's occupation and family size

| Variable                     | Met-Hb level  |       |             |       | Total (n = 338) |       | P-value  |
|------------------------------|---------------|-------|-------------|-------|-----------------|-------|----------|
|                              | ≤ 5% (normal) |       | > 5% (high) |       | No.             | %     |          |
|                              | No.           | %     | No.         | %     |                 |       |          |
| <i>Infant's age (months)</i> |               |       |             |       |                 |       |          |
| 1-3                          | 92            | 52.9  | 29          | 17.7  | 121             | 35.8  | < 0.0001 |
| > 3-6                        | 72            | 41.4  | 108         | 65.9  | 180             | 53.3  |          |
| > 6                          | 10            | 5.7   | 27          | 16.5  | 37              | 10.9  |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |
| <i>Infant's sex</i>          |               |       |             |       |                 |       |          |
| Male                         | 90            | 51.7  | 90          | 54.9  | 180             | 53.3  | 0.72     |
| Female                       | 84            | 48.3  | 74          | 45.1  | 158             | 46.7  |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |
| <i>Mother's age (years)</i>  |               |       |             |       |                 |       |          |
| ≤ 18                         | 14            | 8.0   | 8           | 4.9   | 22              | 6.5   | 0.24     |
| > 18                         | 160           | 92.0  | 156         | 95.0  | 316             | 93.5  |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |
| <i>Mother's education</i>    |               |       |             |       |                 |       |          |
| Preparatory                  | 84            | 48.2  | 74          | 45.1  | 158             | 46.8  | 0.95     |
| Secondary                    | 78            | 44.8  | 78          | 47.6  | 156             | 46.2  |          |
| Higher                       | 12            | 6.9   | 12          | 7.3   | 24              | 7.0   |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |
| <i>Mother employed</i>       |               |       |             |       |                 |       |          |
| Yes                          | 5             | 2.9   | 7           | 4.3   | 12              | 3.6   | 0.49     |
| No                           | 169           | 97.1  | 157         | 95.7  | 326             | 96.4  |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |
| <i>Father employed</i>       |               |       |             |       |                 |       |          |
| Yes                          | 131           | 75.3  | 119         | 72.6  | 250             | 74.0  | 0.57     |
| No                           | 43            | 24.7  | 45          | 27.4  | 88              | 26.0  |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |
| <i>Family size</i>           |               |       |             |       |                 |       |          |
| ≤ 3                          | 16            | 9.2   | 13          | 7.9   | 29              | 8.6   | 0.78     |
| 4-6                          | 55            | 31.6  | 48          | 29.3  | 103             | 30.5  |          |
| 7+                           | 103           | 59.2  | 103         | 62.8  | 206             | 60.9  |          |
| Total                        | 174           | 100.0 | 164         | 100.0 | 338             | 100.0 |          |

n = total number of respondents.

educational level, mother's employment, father's employment or family size.

Concerning the type of milk feeding of infants, there was significant negative association between Met-Hb level in blood and exclusive breastfeeding ( $\chi^2 = 97.21$ ,

$P < 0.0001$ ) (Table 2). This result is also supported by ANOVA test showing that the mean Met-Hb level of infants fed by formula was the higher (8%), compared with infants fed exclusively by breastmilk (3%) ( $F = 69.33$ ,  $P < 0.0001$ ). There was

Table 2 Methaemoglobin levels (Met-Hb) of the study infants by type of feeding, water use, presence of diarrhoea, weight and anaemia

| Variable                    | Met-Hb level  |              |             |              | Total      |              | P-value  |
|-----------------------------|---------------|--------------|-------------|--------------|------------|--------------|----------|
|                             | ≤ 5% (normal) |              | > 5% (high) |              | No.        | %            |          |
|                             | No.           | %            | No.         | %            |            |              |          |
| <i>Type of feeding</i>      |               |              |             |              |            |              |          |
| Breastfeeding               | 77            | 44.3         | 4           | 2.4          | 81         | 24.0         | < 0.0001 |
| Formula milk                | 5             | 2.9          | 41          | 25.0         | 46         | 13.6         |          |
| Mixed                       | 92            | 52.9         | 119         | 72.6         | 212        | 62.4         |          |
| <i>Supplemental feeding</i> |               |              |             |              |            |              |          |
| Yes                         | 97            | 55.7         | 160         | 97.6         | 257        | 76.0         | < 0.0001 |
| No                          | 77            | 44.3         | 4           | 2.4          | 81         | 24.0         |          |
| <i>Diarrhoea</i>            |               |              |             |              |            |              |          |
| Yes                         | 9             | 5.2          | 20          | 12.2         | 29         | 8.6          | 0.017    |
| No                          | 165           | 94.8         | 144         | 87.8         | 309        | 91.4         |          |
| <i>Given boiled water</i>   |               |              |             |              |            |              |          |
| Yes                         | 147           | 84.5         | 155         | 94.4         | 231        | 68.3         | 0.009    |
| No                          | 27            | 15.5         | 9           | 5.6          | 107        | 31.7         |          |
| <i>Weight</i>               |               |              |             |              |            |              |          |
| Underweight                 | 10            | 5.7          | 18          | 11.0         | 28         | 8.3          | 0.005    |
| Normal weight               | 96            | 55.2         | 107         | 65.2         | 203        | 60.1         |          |
| Overweight                  | 68            | 39.1         | 39          | 23.8         | 107        | 31.7         |          |
| <i>Anaemia</i>              |               |              |             |              |            |              |          |
| Yes                         | 92            | 52.9         | 114         | 69.5         | 196        | 58.0         | < 0.0001 |
| No                          | 82            | 47.1         | 50          | 30.5         | 141        | 42.0         |          |
| <b>Total</b>                | <b>174</b>    | <b>100.0</b> | <b>164</b>  | <b>100.0</b> | <b>338</b> | <b>100.0</b> |          |

a significant positive association between Met-Hb level and the use of boiled water to prepare formula milk ( $\chi^2 = 6.91$ ,  $P = 0.009$ ),

where 94.4% of infants with high Met-Hb levels were fed by boiled water compared with 84.5% of children with low Met-Hb

Table 3 Methaemoglobin levels (Met-Hb) of the study infants by family's water source

| Water source                    | Met-Hb level  |              |             |              | Total      |              |
|---------------------------------|---------------|--------------|-------------|--------------|------------|--------------|
|                                 | ≤ 5% (normal) |              | > 5% (high) |              | No.        | %            |
|                                 | No.           | %            | No.         | %            |            |              |
| Tap water                       | 88            | 50.6         | 113         | 68.9         | 201        | 59.5         |
| Treated water                   | 46            | 26.4         | 23          | 14.0         | 69         | 20.4         |
| Filtered water                  | 27            | 15.5         | 11          | 6.7          | 38         | 11.2         |
| Other (reservoir, private well) | 13            | 7.5          | 17          | 10.4         | 30         | 8.9          |
| <b>Total</b>                    | <b>174</b>    | <b>100.0</b> | <b>164</b>  | <b>100.0</b> | <b>338</b> | <b>100.0</b> |

$P < 0.0001$ .

levels. There was a highly significant association between the level of Met-Hb and supplemental feeding for infants ( $\chi^2=81.01$ ,  $P < 0.0001$ ) (Table 2).

The findings revealed that there was no statistical association between Met-Hb and any significant health problems such as confusion, chest pain and skin discoloration, but there was statistical significant association between the level of Met-Hb and infants who had diarrhoea ( $\chi^2 = 5.3$ ,  $P = 0.017$ ) (Table 2); 12.2% of children with raised Met-Hb levels suffered from diarrhoea compared with 5.2% with lower Met-Hb.

There was also relationship between Met-Hb level and weight of infants, where the percentage of underweight infants with high Met-Hb (11.0%) was more than the percentage with low Met-Hb level (5.7%) ( $\chi^2 = 10.46$ ,  $P = 0.005$ ).

The percentage of anaemic infants with a high level of Met-Hb (69.5%) was greater than those with low Met-Hb level (52.9%) ( $\chi^2 = 17.36$ ,  $P < 0.0001$ ) (Table 2). This result also was supported by the regression test which showed a negative correlation between Met-Hb level and Hb level ( $F = 25.48$ ,  $P < 0.0001$ ); a 1% increase of Met-Hb level in blood was associated with a 0.27 g/dL decrease of Hb in blood. Moreover, there were differences in mean Hb level

between different age groups, where the lowest mean Hb level was infants aged > 6 months (10.3 g/dL), and the highest in infants aged 1–3 months (10.8 g/dL) (ANOVA test,  $F = 3.21$ ,  $P = 0.042$ ).

There was a large difference in the level of Met-Hb according to the source of drinking water used by the families of infants ( $\chi^2 = 17.77$ ,  $P = 0.000$ ). The percentage of infants with high Met-Hb in the families using treated water (14.0%) was approximately half the percentage of infants with low Met-Hb in the families using the same sources of water (26.4%) and the same was true for filtered water (6.7% versus 15.5%) (Table 3). The mean Met-Hb level of infants whose families using tap-water was 5.61% (SD 2.8%), for treated water 4.54% (SD 2.6%), for filtered water 4.02% (SD 2.4%) and for other (reservoir, private well) 6.13% (SD 2.8%) (ANOVA,  $F = 5.10$ ,  $P = 0.007$ ).

#### Hb and Met-Hb levels

The results showed that the proportion of infants with slightly raised Met-Hb levels (i.e. > 3%) was highest in Khan-Younis (80.2%) compared with Jabalia (79.0%) and Gaza City (74.9%). The percentage of infants with a greatly raised Met-Hb level (> 8%) was 24.0% from the infants of Khan-Younis, 18.5% from Jabalia and 12.1% from Gaza City (Table 5).

Table 4 Family's water source of the study infants by area of residence

| Study area  | Water source |      |               |      |                |      |                    |      |       |       |
|-------------|--------------|------|---------------|------|----------------|------|--------------------|------|-------|-------|
|             | Tap water    |      | Treated water |      | Filtered water |      | Other <sup>a</sup> |      | Total |       |
|             | No.          | %    | No.           | %    | No.            | %    | No.                | %    | No.   | %     |
| Jabalia     | 34           | 79.1 | 6             | 14.0 | 3              | 7.0  | 0                  | 0    | 43    | 100.0 |
| Gaza        | 105          | 52.8 | 53            | 26.6 | 34             | 17.1 | 7                  | 3.5  | 199   | 100.0 |
| Khan-Younis | 62           | 64.6 | 10            | 10.4 | 1              | 1.0  | 23                 | 24.0 | 96    | 100.0 |
| Total       | 201          | 59.5 | 69            | 20.4 | 38             | 11.2 | 30                 | 8.9  | 338   | 100.0 |

<sup>a</sup>Reservoir, private well.  
 $P < 0.0001$ .

Table 5 Methaemoglobin levels (Met-Hb) in the study infants by area of residence

| Study area  | ≤ 3% |      | > 3% to ≤ 5% |      | Met-Hb level |      |      |      | Total |       |
|-------------|------|------|--------------|------|--------------|------|------|------|-------|-------|
|             | No.  | %    | No.          | %    | > 5% to ≤ 8% |      | > 8% |      | No.   | %     |
| Jabalia     | 9    | 21.0 | 12           | 28.0 | 14           | 32.5 | 8    | 18.5 | 43    | 100.0 |
| Gaza        | 50   | 25.1 | 58           | 29.1 | 126          | 33.7 | 24   | 12.1 | 199   | 100.0 |
| Khan-Younis | 19   | 19.8 | 26           | 27.0 | 28           | 29.2 | 23   | 24.0 | 96    | 100.0 |
| Total       | 78   | 23.1 | 96           | 28.4 | 168          | 49.7 | 55   | 16.3 | 338   | 100.0 |

Cut-off level ≤ 3 from World Health Organization [6], ≤ 5 from Skipton [7] and > 8 from data of the Paediatric Hospital, Gaza City.

The overall mean Met-Hb level of the study infants was 5.26% (SD = 2.80%) and differed significantly between the study areas: 5.50% (SD 2.7%) in Jabalia, 4.88% (SD 2.6%) in Gaza City and 5.94% (SD 3.0%) in Khan-Younis (ANOVA,  $F = 5.10$ ,  $P = 0.007$ ).

#### Nitrate levels and Met-Hb

The mean nitrate concentration in the drinking water wells of Jabalia was 124 ppm (range 71–248), in Gaza City 119 ppm (18–244) and in Khan-Younis 195 ppm (18–440) [7] (Table 6). These values exceed the Palestinian standards, which recommend 70 ppm nitrate in the drinking water and the WHO standard of 50 ppm [8].

Table 6 Nitrate concentration of drinking water wells in the study areas. Source: Public Health Laboratory, Palestine [7]

| Study area  | No. of wells in study area | Mean nitrate concentration of wells (ppm) | Range  |
|-------------|----------------------------|---|--------|
| Jabalia     | 12                         | 124                                       | 71–248 |
| Gaza        | 28                         | 119                                       | 18–244 |
| Khan-Younis | 14                         | 195                                       | 18–440 |
| Total       | 54                         | 146                                       | 18–440 |

$F = 5.10$ ,  $P = 0.007$ .

#### Discussion

This study revealed a difference in the percentage of infants with raised Met-Hb levels in the 3 localities; the infants from Khan-Younis had the highest methaemoglobin level compared with Jabalia and Gaza. A study in Palestine in 2000 found a high proportion (92.4%) of surveyed children in the southern hospital in Khan-Younis had Met-Hb levels exceeding the safe level (> 5%) [9].

By reviewing the analytical data from the records of the Public Health Laboratory for the local drinking water wells, it was found that the highest mean nitrate concentration in wells was in Khan-Younis (199 ppm) and the lowest was in Gaza City (125 ppm).

There was a similar pattern between the Met-Hb levels of the infants and the nitrate levels of drinking water in the different residential areas. Khan-Younis had the highest nitrate level in wells and the highest mean Met-Hb level among the infants living in that area (5.94%). The level of nitrates in the drinking water of Gaza City was the lowest, and the mean Met-Hb level of infants was the lowest (4.90%). These results suggest a relationship between the nitrate concentration in drinking water sources and Met-Hb

level in the infant's blood. This result agrees with other studies which suggest that the nitrate level in drinking water is the most important factor that affects Met-Hb level. Ziebarth [10] and Clesceri et al. [11] found that few cases of methaemoglobinaemia were reported with a water nitrate level of less than 50 ppm and most of the cases occurred at a level 90 ppm.

Concerning the age of infants, previous studies concluded that infants are more susceptible to nitrate toxicity than older children or adults. In our study, among infants < 6 months the most risky group was infants > 3 months compared with age 1–3 months. This suggests that the effect of nitrate concentrations on the Met-Hb level in infants begins after starting artificial milk feeding or after starting supplemental feeding. Preparing formula and supplemental feeds needs drinking water, which is the main source of nitrate in this age group. This result is supported by the fact that the average age of introducing supplemental feeding in these infants was 2.5 months. Moreover, infants having artificial feeding have low gastric acidity, where NADH-Met-Hb reductase enzyme is only 50% active, and the fetal Hb is more susceptible to oxidation than adult Hb [12].

The study revealed no relationship between the sex of infants and Met-Hb level. This result is logical, when there are no physiological differences between males and females at this age in general and specifically in the blood system. In addition, the sources of drinking water for both sexes are the same at this age and there is unlikely to be discrimination between the sexes in the quantity of feeding or quality of water used to prepare formula milk.

Our study showed no significant relationship between any socioeconomic factors of families—mother's age, parental

educational level and occupation—and the infants' Met-Hb level. This result could be explained by the fact that there is insufficient awareness about the effects of nitrate contaminated water on the health of infants among most of the population of Gaza society, regardless of educational level or occupation.

The results of the study strongly suggested that the type of water supply of infants' families significantly affects the level of Met-Hb in infants' blood, where the highest proportion of infants with Met-Hb level > 5% belonged to families using tap water (68.9%) or other sources (10.4%) compared to families using treated or filtered water (about 20%). Moreover, the mean Met-Hb of infants from families using tap water or other sources was higher than those who using treated and filtered water (4.54% and 4.02% respectively).

It was also observed in this study that there was a highly significant correlation between the source of water supply and the study area. The infants' families in Khan-Younis and Jabalia used tap water (64.6% and 79.1% respectively) more than the infants' families of Gaza City (52.8%). This explains the higher Met-Hb levels in infants from Khan-Younis and Jabalia than those from Gaza City. This is attributed to 2 main factors: first the high level of nitrates in the sources of water, second, the higher use of other sources or tap water in families from Khan-Younis and Jabalia than those from Gaza City. It is very important to note that these other sources are not monitored by any government or non-government agency, and the public cannot know if these are highly contaminated with nitrate, since nitrate is known to be colourless, odourless and tasteless [13].

The study has shown that a high proportion of infants with high Met-Hb levels



(75.8%) are fed by formula milk or mixed feeding. This indicates a strong positive correlation between artificial milk feeding and high Met-Hb level among infants < 6 months. On the other hand there was a negative correlation between breastfeeding and Met-Hb level that means the breastfeeding can be considered as a protective factor against raised Met-Hb in infants' blood. This finding suggests that the relationship between artificial feeding and high Met-Hb level can be attributed to the correlation between nitrate contaminated water and a high level of Met-Hb. This result agrees with other studies which show that the use of nitrate-contaminated water to prepare infant formula milk is a well-known risk factor for methaemoglobinaemia [14]. Furthermore, artificial feeding decreases stomach acidity to lower levels than adults, thus allowing the growth of stomach bacteria which reduce nitrate-to-nitrite conversion, raising the level of nitrates which react with haemoglobin to form Met-Hb [15].

The use of boiled water was also positively associated with raised Met-Hb, as approximately 95% of infants with high Met-Hb levels were from families who boil water before using it. This result is congruent with studies which revealed that the concentration of nitrate in water increases if the water is boiled [16,17]. This result also explains the absence of a relationship between Met-Hb level and the educational level of infants' parents. Parents with a high educational level may boil water before preparing formula to avoid bacterial contamination. However, if they do not know about correct practices to reduce the level of nitrate concentration in water, the practice of boiling water, paradoxically, may increase the problem for infants.

The findings also showed a high positive statistical significant association between

supplemental feeding and high Met-Hb level, where 97.6% of infants with high Met-Hb level had supplemental feeding compared to 55.7% of those with low Met-Hb level. This could be attributed to using water in this kind of feeding, and the correlation is in reality between the nitrate-contaminated water and Met-Hb level. This indicates that supplemental feeding could be another factor contributing to increasing levels of Met-Hb in infants. This result is supported by the previous result which showed that the water source played a role in determining the level of Met-Hb, and the Met-Hb level increased when the source of water was tap water or other sources rather than filtered or treated water. Moreover, this result is congruent with the previous result which reflected that the level of Met-Hb increased with using formula or mixed feeding as supplemental feeding.

Concerning the relationship between the level of Met-Hb in the infants' blood and the health status of those infants, earlier studies showed that reduced oxygen transport becomes clinically manifest when Met-Hb concentration reaches 10% of Hb concentration and above and that ranges between 15% and 30% are consistent with mild to moderate symptoms, but are not considered life-threatening [14,18]. In our study, the mean Met-Hb level among infants did not exceed 6%, and the highest level was 13%; it is not surprising therefore that complaints of health problems (confusion, chest pain and skin discoloration) among the infants were not very high (around 6%) and there were no cases of cyanosis in this study.

Symptoms of diarrhoea were more common among infants with high Met-Hb levels. This finding agrees with a report of an infant of 8 weeks who became ill with symptoms of vomiting and diarrhoea after their mother stopped breastfeeding and be-

gan feeding with formula reconstituted with water from the family's private well [14]. Artificial feeding is therefore a confounding factor in this study, as the water used in preparing the formula of artificial feeding could be contaminated with both high levels of nitrates and bacteria.

An important finding of our study was the significant association between high Met-Hb levels in infants and anaemia and underweight; 69.5% and 65.2% of infants with high Met-Hb level were anaemic and underweight respectively. This suggests that Met-Hb levels have a negative impact on the nutrition status of the infants. Moreover it was found that each 1% of Met-Hb level decreased the level of Hb in blood by 0.27 g/dL. This result is considered a logical consequence of the formation of Met-Hb, because Met-Hb is an oxidation product of haemoglobin [19] and toxic methaemoglobinemia may be associated with haemolytic anaemia caused by the precipitation of Hb to form Heinz bodies. The other finding is that significantly more infants with raised Met-Hb were underweight than infants with normal Met-Hb (11.0% versus 5.7%).

The high level of Met-Hb leads to diarrhoea, a finding supported by Avery who mentioned that diarrhoea was a prominent symptom in the majority of methaemoglobinemia cases linked to drinking water [15]. The presence of diarrhoea and poor feeding habits decrease further the chance of benefiting from feeding, which in turn leads to growth retardation and underweight. Moreover high Met-Hb level and anaemia are associated with low oxygen in blood and in tissues. The low level of oxygen in tissues is also associated with a decrease in metabolic processes which leads to decreased benefit from the foods in the growth of infants and in turn leads to growth retardation of infants and underweight.

The overall conclusion from the current study is that key factors for the level of Met-Hb in the blood of infants in Gaza community may be the drinking water and the concentration of nitrate in this water. All the variables which were associated with high Met-Hb level could be attributed to nitrate-contaminated water.

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