

Possible Hematological Changes Associated with Acute Gastroenteritis among Kindergarten Children in Gaza

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Abstract

Background: Gastroenteritis is considered one of the leading causes of morbidity and mortality in children especially in developing countries. It is a major childhood problem in Gaza and one of the most common etiologic agents of iron deficiency anemia (IDA). **Aim:** This study was conducted to investigate possible changes in blood parameters that are associated with gastroenteritis infection among kindergarten children in Gaza. **Subjects and Methods:** A cross-sectional case-control study was performed including kindergarten children suffering from gastroenteritis and matched healthy control group. Types of etiological agents were identified using standard microbiological and serological procedures. Blood samples were collected for estimation of complete blood count and for determination of serum iron, total iron binding capacity (TIBC), and transferrin saturation. Independent sample *t*-test was used for comparisons and performed using SPSS software version 17 (Chicago Illinois USA). **Results:** The prevalence of enteric pathogens among cases (88.5% [85/96]) was significantly higher than in asymptomatic controls (11.1% [6/54]). The most common enteric pathogens isolated were *Entamoeba histolytica* (28% [42/91]) and *Giardia lamblia* (26.7% [40/91]). Blood tests revealed that 21.8% (21/96) of cases and 14.8% (8/54) of controls had IDA, which were not significantly different. Meanwhile, a significant difference was found between the TIBC and hemoglobin in cases compared to controls. **Conclusion:** This study indicates that gastroenteritis infection could be considered as a common health problem in kindergarten children in Gaza, and it is possibly associated with changes in hemoglobin concentration and TIBC.

Keywords: Acute gastroenteritis, Gaza, hematological changes, Iron deficiency, Kindergarten

Introduction

Worldwide, diarrheal diseases are continued to be a common health problem that increase the financial burden on the health systems particularly in developing countries. Acute gastroenteritis is one of the leading causes of morbidity and mortality in children <5-years,^[1-3] especially in developing countries.^[4-7] Gastroenteritis is a serious burden to public health. Its etiologic agents include a wide variety of bacteria, viruses, and parasites.^[8-10] Serious outcomes are associated

with these intestinal pathogens include malnutrition, impaired physical development, and reduced school achievement in children.^[11] In Gaza, acute gastroenteritis is a common infection among children, associated with high morbidity and mortality rates when left without a proper treatment.^[12]

Anemia is a major public health problem in both developed and developing countries that needs a national and international interventions to correct it and its underlying causes.^[13,14] It is currently estimated that anemia affects about one-quarter of the world's population and estimated that 36% of developing world's population suffers from this health condition.^[13-16]

Iron deficiency is recognized as the primary cause of anemia worldwide and is the most prevalent form of micronutrient malnutrition in the world. The etiology of anemia is multifactorial including nutritional habits, the bioavailability of micronutrients, parasitic infections, inflammation, and genetic

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factors;^[15-18] however, non-nutritional causes of anemia are numerous and diverse. In the developing countries, common parasitic infections associated with blood loss can result in iron depletion, iron deficiency, and ultimately anemia.^[19-23] The negative effects of some protozoan and helminthes parasites on the absorption of some micronutrients leading to nutritional anemia have been reported in different scientific works.^[18,24]

Gaza Strip (geographic coordinates 31°25'22" N, 34° 20' 2" E) is a narrow region (365 km²) of land along the Mediterranean coast, just 41 km long and 6–12 km wide. It is one of the most heavily populated areas (4383 persons/km²) of the world with a total population about 1.7 million people.^[25] In the present work, a prospective cross-sectional case–control study was conducted in Gaza city to determine the type and prevalence of enteropathogens causing acute gastroenteritis among kindergarten children and to assess their possible effect on blood parameters and body iron status.

Subjects and Methods

Study population and sample collection

This study is a cross-sectional case–control study conducted in Gaza from 1st of January to the 30th of June 2011. Sample selection was randomly done for 15 kindergarten in Gaza but covering all different localities and population clusters in Gaza. Sample size was determined using Sample Size Calculator according to the number of enrolled children in Gaza kindergartens (<http://www.surveysystem.com/sscalc.htm>) to define the enteropathogens causing gastroenteritis among kindergarten children and to find out their possible effect on blood parameters.

One hundred and ninety written requests were sent to the parents or guardians of the symptomatic cases and asymptomatic carriage (apparently healthy controls) in 15 kindergartens to have their approval to involve their child in the study. Thankfully, 96 of 115 (response rate 83.5%) and 54 of 75 (response rate 72.0%) of symptomatic cases and asymptomatic carriage, respectively, agreed to participate in the study and to provide stool and blood samples for further investigations. The 15 kindergartens are in geographically different localities and population clusters in Gaza.

The study protocol was approved by the ethical committee of our biology department and performed in accordance with the ethical standards established in the 1964 and 1975 Declaration of Helsinki and the modifications thereafter. All ethical considerations were followed including respect for the privacy of the subject. A signed consent form was obtained from the parents or guardians providing their acceptance and full understanding of this study and its aims. Stool samples were collected from cases and controls in clean, dry plastic containers in their kindergarten by the help and under the supervision of the investigator. They were tested using standard microbiological procedures according to the guidelines

of Clinical and Laboratory Standards Institute (CLSI).^[26] Furthermore, blood samples were collected on the same time by expert laboratory technologist for estimation of complete blood count (CBC) and serum samples were tested for determination of iron concentration and total iron binding capacity (TIBC).

Inclusion and exclusion criteria

All samples were collected from different kindergarten regions representing all parts of Gaza. Any child who is suffering from any chronic disease or was hospitalized was excluded. Children with gastroenteritis symptoms including watery, nonbloody or bloody diarrhea, abdominal cramps, nausea, vomiting or low-grade fever considered as cases and apparently healthy matched controls (asymptomatic) were included.

Microbiological methods

Standard microbiological procedures and guidelines, as described by CLSI^[26], were used. For the identification of parasites, fresh stool specimens prepared with saline and/or iodine were directly examined. In addition, flotation and sedimentation techniques were performed on all samples that gave negative result using direct microscopic examination. For detection of bacterial agents, fresh stool samples were plated directly or after enrichment step onto *Salmonella-Shigella*, Hecktoen enteric, and Sorbitol MacConkey agars (HiMedia, India) and incubated for 24 h at 37°C. Suspected colonies were identified by standard laboratory procedures. For detection of viral agents, enzyme immunoassay (GastroVir-Stripcolor kit) was used for the detection of Group A *Rotavirus* and *Adenovirus* serotype 40 and 41 according to the manufacturer's instructions (Cori BioConcept, Belgium).

Complete blood count

About 2½ ml of venous blood were drawn into sterile ethylenediaminetetraacetic acid tubes, mixed and processed within 1 h by (Sysmex-Kx-21). The following parameters were calculated: White blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), and MCH concentration (MCHC). Serum iron (SI) and TIBC were performed using Mindray Bs-200, chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics, Shenzhen, China) on samples with hemoglobin concentration <11 g/dL.

Evaluation of iron deficiency anemia

In the present research, and due to the inadequate financial and laboratory resources, as well as the politically constrictive environment in the Gaza Strip, which negatively affects the availability of medical, laboratory equipment, chemicals and diagnostic kits to perform laboratory investigations, iron deficiency anemia (IDA) were considered in the kindergarten children; when transferrin saturation (SI/TIBC) was <16%^[27] in combination with low hemoglobin

concentration <11 g/dL for ≤ 4 -year-old children, and <11.5 g/dL for 5-year-old children.^[28]

Determination of serum iron

Serum iron was determined by the colorimetric method according to the manufacturer instructions of DiaSys, Diagnostic Systems GmbH, Germany kit by using computerized chemical chemistry auto analyzer equipment (Mindray BS-200).

Determination of total iron binding capacity

Total iron binding capacity was determined by the colorimetric method according to the manufacturer instructions of Linear Chemicals, Spain kit by using computerized chemical chemistry auto analyzer equipment (Mindray BS-200).

Calculation of transferrin saturation

Transferrin saturation was calculated as a percentage of the ratio of SI to total TIBC.

Data analysis

The results that obtained from laboratory investigations were tabulated, encoded and statistically analyzed using Statistical Package for Social Sciences (SPSS) program version 17 (Chicago, IL, USA). Descriptive analysis and independent *t*-test were performed. The differences were considered statistically significant at $P \leq 0.05$.

Results

General characteristics of the study population

The present study is a prospective cross-sectional case-control study included 150 kindergarten children from Gaza city. The

stool and blood samples were collected from children of both genders (56% [84/150] males and 44% [66/150] females). The ages of children enrolled in this study were 3 (8% [12/150]), 4 (34% [51/150]), and 5-years (58% [87/150]). According to the study protocol, 96 diarrheal stool samples were collected from 51 boys (53.1% [51/96]), and 45 girls (46.9% [45/96]) and they considered as symptomatic cases. The asymptomatic carriage (controls group) included 33 boys (61.1% [33/54]) and 21 (38.9% [21/54]) girls.

Prevalence and type of enteropathogens isolated from cases and controls

Overall, there were 91 samples (60.7% [91/150]) positive for parasitic, bacterial, and/or viral enteric pathogens. The parasitic etiologic agents had the highest prevalence rate (54.7% [82/150]) in comparison to the bacterial (4% [6/150]) and viral (2% [3/150]) etiologic agents. More than half of samples collected were positive for one or more parasitic agent. The highest isolated enteric pathogen was *Entamoeba histolytica* (28% [42/91]), followed by *Giardia lamblia* (26.7% [40/91]). *Adenovirus* and *Salmonella* were not isolated from all tested samples [Figure 1]. The overall prevalence of positive stools from symptomatic cases was significantly higher (88.5% [85/96]) than that isolated from asymptomatic controls (11.1% [6/54]).

Hematological parameters of cases and controls

Table 1 shows the CBC parameters of blood samples that collected from all enrolled children in this study. There was no significant differences between the mean score results of blood parameters tested for both, cases (diarrheal) and controls (nondiarrheal), except for the WBC which was significantly higher in cases (symptomatic) compared to control

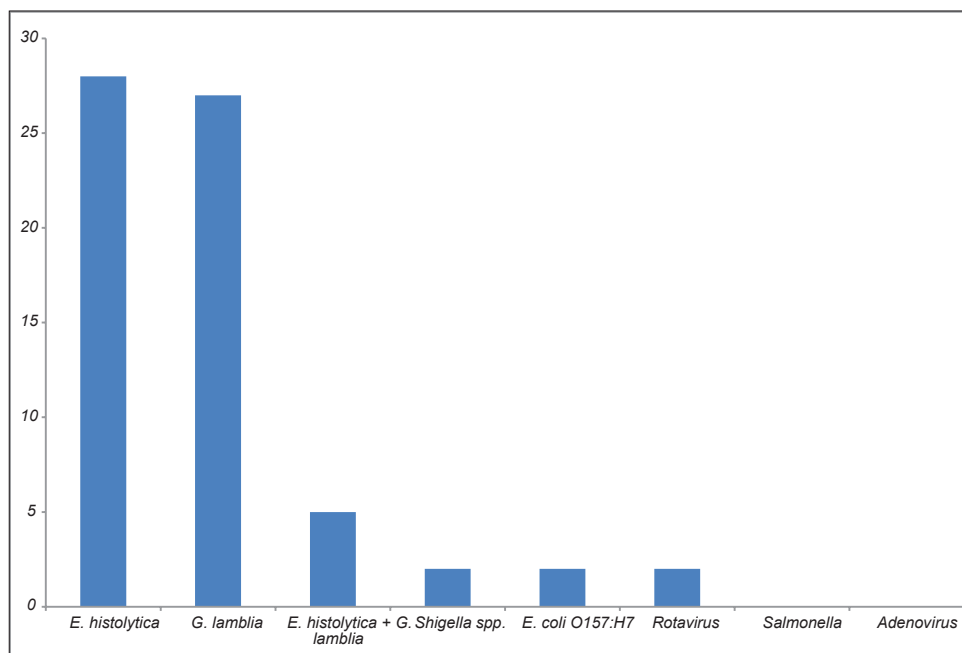


Figure 1: Prevalence of parasitic, bacterial, and viral enteric pathogens causing acute gastroenteritis among tested stool samples

group (asymptomatic) ($P < 0.01$). However, the mean score result of hemoglobin concentration is slightly lower in cases compared to controls, but this difference did not reach statistical significance ($P = 0.32$). The other blood parameters (RBC, HCT, MCV, MCHC, and PLT) had almost the same mean score results.

Serum iron, TIBC, and transferrin saturation level were performed only on blood samples that have hemoglobin concentration <11 g/dL. SI level divided by the TIBC multiplied by 100 gives a transferrin saturation level. There were 21 (21.9% [21/96]) and 8 (14.8% [8/54]) children from the cases (diarrheal samples) and controls (no diarrhea) showing IDA with hemoglobin level <11 g/dL.^[27-29] Decreased hemoglobin, SI, and transferrin saturation levels were found in cases compared to controls, but this difference was not statistically significant. Nevertheless, the results showed a

statistically significant difference between the level of TIBC of cases compared to controls ($P < 0.01$) [Table 2].

Possible effect of parasitic infection on blood parameters

The mean level of blood parameters tested from children with different parasitic infections showed almost the same values irrespective of the type of parasite or even when double infection with two parasites were existed. Furthermore, comparing these results with the blood parameters values tested from children with negative parasitic samples revealed no clear differences. Yet, the mean value of WBC was found to be higher in blood samples of children with *E. histolytica* infections compared to blood samples of children with *G. lamblia* infection alone or children without any parasitic infection [Table 3]. These differences were statistically significant.

Table 1: Differences in blood parameters among cases (diarrheal) and controls (no diarrheal)

Blood parameter	Presence of diarrheal				P
	Diarrheal samples		Non-diarrheal samples		
	Mean (SD) (n=96)	95% CI	Mean (SD) (n=54)	95% CI	
RBC (×10 ¹² /L)	4.5 (0.3)	4.5-4.6	4.6 (0.4)	4.5-4.7	0.39
Hemoglobin (g/dL)	11.5 (0.9)	11.3-11.7	11.6 (0.8)	11.4-11.9	0.32
HCT (%)	35.3 (2.3)	34.8-35.7	35.8 (2.2)	35.2-36.4	0.18
MCV (fl)	78.1 (4.1)	77.2-78.9	78.5 (5.7)	76.9-80.0	0.6
MCH (pg)	25.4 (1.8)	25.1-25.8	25.5 (2.3)	24.9-26.1	0.8
MCHC (g/dL)	32.5 (1)	32.3-32.7	32.5 (1.1)	32.2-32.8	0.64
PLT (×10 ⁹ /L)	388.5 (99)	368.4-408.6	385.4 (92.4)	360.2-410.7	0.85
WBC (×10 ⁹ /L)	9.7 (3.2)	9.0-10.3	8.3 (2.7)	7.6-9.0	<0.01*

*Significant at $P \leq 0.05$. RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, WBC: White blood cell, SD: Standard deviation, CI: Confidence interval, HCT: Hematocrit

Table 2: Hemoglobin, SI, TIBC, and transferrin saturation results of cases and controls having hemoglobin concentration <11 g/dL

Blood parameter	Presence of diarrheal among anemic children				P
	Diarrheal samples		Non-diarrheal samples		
	Mean (SD) (n=21)	95% CI	Mean (SD) (n=80)	95% CI	
Hemoglobin (g/dL)	10.3 (0.6)	10.2-11.6	10.4 (0.6)	11.5-11.8	0.71
SI (µg/dL)	34.4 (10.5)	29.6-39.2	40.3 (2.4)	19.5-61.0	0.38
TIBC (µg/dL)	316.6 (23.6)	306.9-328.4	363.7 (57)	317.6-413.1	<0.01*
Transferrin saturation (%)	11.0 (3.8)	9.3-12.7	11.4 (7.2)	5.4-17.4	0.84

*Significant at $P \leq 0.05$. SI: Serum iron; TIBC: Total iron-binding capacity; SD: standard deviation, CI: Confidence interval

Table 3: Mean values of blood parameters in association with different parasitic pathogens

Blood parameter	Negative	<i>G. lamblia</i>	<i>E. histolytica</i>	<i>E. histolytica</i> and <i>G. lamblia</i>
	Mean (SD) (n=61)	Mean (SD) (n=40)	Mean (SD) (n=42)	Mean (SD) (n=7)
RBC ($\times 10^{12}/L$)	4.6 (0.4)	4.5 (0.3)	4.4 (0.3)	4.7 (0.2)
Hemoglobin (g/dL)	11.6 (0.9)	11.6 (.8)	11.4 (1.0)	12.0 (0.6)
HCT (%)	35.7 (2.3)	35.3 (2.1)	35.2 (2.5)	36.1 (1.7)
MCV (fl)	78.1 (5.4)	79.1 (3.5)	77.7 (5.0)	77.6 (2.2)
MCH (pg)	25.2 (2.4)	25.9 (1.4)	25.2 (2.2)	25.9 (0.2)
MCHC (g/dL)	32.3 (1.1)	32.7 (0.9)	32.5 (1.1)	33.3 (0.7)
PLT ($\times 10^9/L$)	385.5 (95.3)	390.2 (102.3)	379.9 (90.3)	432.7 (114.6)
WBC ($\times 10^9/L$)	8.7 (3.1)	8.8 (2.3)	10.0 (3.4)*	10.6 (2.6)*
SI ($\mu g/dL$)	37.3 (22.8)	36.9 (14.4)	34.3 (7.4)	ND
TIBC ($\mu g/dL$)	358.5 (53.2)	311.4 (20.8)	319.8 (25.9)	ND
Transferrin saturation (%)	10.7 (6.5)	12.0 (5.1)	10.9 (2.8)	ND

*Significant at $P \leq 0.05$. RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, WBC: White blood cell, SI: Serum iron, TIBC: Total iron binding capacity, SD: Standard deviation, ND: Nondetermined, HCT: Hematocrit, *Giardia lamblia*: *G. lamblia*, *E. histolytica*: *Entamoeba histolytica*

Discussion

Gastrointestinal diseases are a major public health problem for children in Gaza Strip, as in other developing communities around the world, and are among the most common causes of IDA due to gastrointestinal tract blood loss.^[20,30] Gastroenteritis can also be accompanied by malnutrition with reduced iron intake further contributing to the IDA.^[19,20,22,23] Many previous and most recent studies from Gaza Strip and other parts of Palestine (West Bank) revealed a significant prevalence of IDA among kindergarten, pre- and age-school children due to gastrointestinal infection and other nutritional factors.^[31-38]

In this study, CBC, SI, and TIBC were performed to investigate the possible effect of gastrointestinal infections on these blood parameters among kindergarten children suffering from community gastroenteritis and their comparable healthy controls. The findings of our study showed that 21.8% (21/96) of the diarrheal cases also had iron deficiency with hemoglobin concentration <11 g/dL, compared to only 14.8% (8/54) of non-diarrheal controls. The hemoglobin level with other blood parameters was slightly lower in diarrheal cases than in non-diarrheal controls. In the iron deficient children, SI, TIBC, and transferrin saturation were also slightly lower in diarrheal cases. Assuming no interference from other etiological factors like inflammation, the results suggest that IDA is increased in diarrheal cases, although this difference did not achieve statistical significance.

This could be due to the fact that the infection was not chronic enough to cause malabsorption or sufficient blood loss to effect iron levels. The results of this work are inconsistent with those noted in other studies, where the authors found that the high prevalence of enteropathogens was associated with anemia.^[32,39,40] Al-Zain studied the impact of socioeconomic conditions and intestinal parasitic infection on hemoglobin level among children aged 2–15 years in Um-Unnasser village, North Gaza, Palestine. He found that 25% were anemic, and the prevalence rate of anemia was highest in children aged below 6 years.^[41] In another study in Gaza Strip, Al-Agha and Teodorescu studied the prevalence of anemia, nutritional indices and intestinal parasitic infestation in primary school children aged 6–11 years. They found an association between anemia and intestinal parasitic infestation in those children who manifested mainly by intestinal parasites *E. histolytica* and *G. lamblia*. Furthermore, they showed a high prevalence of anemia in children suffered from malnutrition along with intestinal parasitic infestation.^[31]

Iron deficiency anemia seen in diarrheal and non-diarrheal cases suggests that nutritional anemia may be prevalent in this population, which may aggravate the clinical symptoms.

Infections are a well-recognized cause of mild to moderate anemia. Several studies have demonstrated that even

mild infections can induce a significant decrease in iron levels.^[42-45] The reduction in hemoglobin level during and after acute or chronic inflammatory diseases is due to many factors such as blocking the iron release, reduction in the intestinal absorption of iron and inhibition or inappropriate erythropoietin production.^[21] Levy *et al.* 2005 in a study, conducted in Israel examined the association between anemia and infection among Bedouin infants. They concluded that anemia is a significant and independent risk factor for diarrhea. In Ecuador, Sackey *et al.* found that children suffered from intestinal parasitic infections had significantly reduced mean hemoglobin levels compared with their noninfected counterparts. Their data indicated that *G. lamblia* infection had an adverse impact on child linear growth and hemoglobin levels.^[39] Moreover, in a recent study performed in Egypt, the frequency of parasites was significantly higher in cases with IDA compared to controls.^[46] Furthermore, a study conducted recently to compare hematological indices in cases with *Blastocystis hominis* infection against healthy controls revealed that the frequency of SI was significantly lower in cases with *B. hominis* infection compared to controls.^[45]

Ali and Zuberi in Pakistan investigated the presence or absence of any association between low birth weights, recurrent diarrhea or recurrent acute respiratory infections with IDA in Pakistani children aged 1–2 years. They found that there was no statistically significant difference of recurrent diarrhea between anemic and nonanemic children at 1–2 years age.^[47] Another study in Pakistan conducted to determine the clinical pattern of infections in admitted malnourished children. A total of 112 admitted malnourished children with various types of infections aged 3–60 months were enrolled in the study. The authors found that the association of malnutrition and infections among children is an important health issue and the common infections found in malnourished children were acute gastroenteritis (44.6%), followed by enteric protozoa and helminthic infections (23%).^[48]

Study limitation

The generalizability of above results is subject to certain limitations. For instance, the difficulty in measuring serum ferritin levels and using low transferrin saturation as the marker of iron deficiency could interfere with anemia of inflammation.

Conclusion

Diarrhea remains a health problem in children in Gaza. This study highlights quite a number of the enteric pathogens that cause community gastroenteritis among kindergarten children in Gaza and their possible effect on hemoglobin concentration and TIBC. Another cohort study, on infected and healthy population is recommended to determine other causes of community gastroenteritis and their possible effect on hematological indices.

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