

Serum Levels of Interleukins 6 And 8 In Malnourished Children with Acute Diarrhea: A Case-Control Study

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ABSTRACT

Background: Acute gastroenteritis continues to be a prevalent global health issue among children. The release of interleukin-6 (IL-6) and interleukin-8 (IL-8) from the gastrointestinal tract's epithelial cells is essential for protection against mucosal infections because it triggers the body's inflammatory reactions to infectious pathogens both locally and systemically.

Objectives: To investigate serum levels of IL-6 and IL-8 in malnourished children with acute diarrhea.

Patients and Methods: A case-control study that included three groups of 30 children each: children with severe acute malnutrition (SAM) and acute diarrhea (SAM-AD), well-nourished children with acute diarrhea (AD), and healthy controls (HC). Participants underwent clinical evaluation and routine laboratory investigations. Serum levels of IL-6 and IL-8 were measured within 72 hours of diarrheal onset using Luminex[®] Human Premixed Multi-Analyte kit.

Results: Serum level of IL-6 in the SAM-AD group (median 1.0 [IQR 1-4.3] pg/ml) was comparable to the HC group (2.2 [1-4.6] pg/ml) but significantly lower than the AD group (12 [3.9-175.6] pg/ml). The SAM-AD group had significantly lower IL-8 levels (16.2 [11-39] pg/ml) than the AD (612 [84.8-800] pg/ml) and HC (157.5 [23.8-298.7] pg/ml) group. IL-6, IL-8, and CRP showed significantly strong correlation with one another ($r > 0.67$; $p < 0.001$). The presence of fever was significantly associated with higher levels of IL-6, IL-8, and CRP.

Conclusion Our study found significantly lower serum levels of IL-6, IL-8, and CRP in malnourished children with acute diarrhea compared to well-nourished children with acute diarrhea, which indicates that malnutrition may impede the acute phase inflammatory response during acute diarrhea.

Keywords: Acute diarrhea, SAM, Cytokines, IL-6, IL-8.

INTRODUCTION

Around the world, children still frequently suffer from acute gastroenteritis, which has a high morbidity, mortality, and financial cost. Each year, more than 500,000 children under the age of five die as a result of diarrheal infections, with the majority of these deaths occurring in low- and middle-income countries. Additionally, diarrheal illnesses are a major reason for hospital stays and emergency room visits⁽¹⁻⁴⁾.

The majority of cases of acute diarrhea in children (70–90%) are caused by viral infections (rotavirus, norovirus, adenovirus, etc.); the remaining cases are caused by bacteria (Shigella, Salmonella, Campylobacter, enterotoxigenic E. coli, etc.) and parasites (Cryptosporidium, Giardia, and E. histolytica)⁽⁴⁻⁶⁾.

In the defence system during severe diarrhea, cytokines are crucial. IL-6 and IL-8, in particular, are essential for immunisation against mucosal infections; they are generated by the gastrointestinal tract's epithelial cells to elicit an inflammatory response to infectious pathogens both locally and systemically^(7,8).

A number of research looked at the function of cytokines, namely IL-6 and IL-8, as biomarkers for acute diarrhea in children who were fed well. The blood levels of IL-6 and IL-8 in children with acute diarrhea have often been shown to be considerably greater than those in healthy individuals. Furthermore, compared to viral diarrhea, bacterial diarrhea has much higher levels

of IL-6^(9,10). But malnourished youngsters were not included in any of these investigations.

A major public health issue is severe acute malnutrition (SAM), especially in poorer nations, and up to two-thirds of children with SAM present with diarrhea. SAM is associated with increased severity and risk of mortality from acute diarrhea^(11,12). This could be attributed to multiple vulnerabilities associated with SAM, including impaired immunity, disruption of gastrointestinal barrier, reduced exocrine protective secretions, altered intestinal microbiota, and deficit of essential nutrients⁽¹¹⁻¹⁴⁾.

SAM has a significant role in immune system impairment in children, resulting in abnormalities related to opsonization, complement system and phagocytosis, chemotactic activity of neutrophils and monocytes, and antigen-presenting cell function⁽¹⁴⁾. SAM has a detrimental effect on the immune system and may hinder cytokines and the acute phase inflammatory response^(15,16). Studies on the levels of IL-6 and IL-8 in malnourished children experiencing severe diarrhea have not been conducted, as far as we are aware.

In this study, we aimed to evaluate serum levels of IL-6 and IL-8 in children with SAM and acute diarrhea compared to well-nourished children with acute diarrhea as well as healthy controls.

PATIENTS AND METHODS

This case-control study was conducted from December 2021 to December 2022 at the Department of Pediatrics, Sohag University Hospital and the Department of Clinical Pathology at South Egypt Cancer Institute (southern Egypt).

The study included three groups of children (30 for each): The first group included children aged between 6 months and 5 years who presented with SAM and acute diarrhea (SAM-AD). The second group included age-matched well-nourished children presented with acute diarrhea (AD group). The third group included age-matched healthy control children presented for regular check-up (HC group).

Exclusion criteria were antibiotic therapy in the last 72 hours, systemic infections, malignancy, autoimmune and autoinflammatory diseases, chronic liver/kidney disease, and diabetes mellitus.

Study participants underwent comprehensive history taking and clinical evaluation, including sociodemographic data, nutritional assessment, anthropometric measures, and details of acute diarrheal illness (e.g., onset, fever, vomiting, abdominal pain, frequency of diarrhea, and presence of blood in diarrhea). Laboratory investigations included hematological count, C-reactive protein (CRP), serum electrolytes, serum IL-6 and IL-8, and liver and kidney function tests.

SAM was defined among 6- to 60-month-old infants and children according to the diagnostic criteria of the WHO as having a mid-upper arm circumference (MAUC) < 115 mm, weight for height/length < -3 Z-score of the WHO growth standards, and/or bilateral edema⁽¹⁷⁾.

With or without a fever or vomiting, and lasting less than 14 days, acute diarrhea was defined by the WHO as "passage of loose or watery stools at least three times in a 24 h period". However, this definition took into account the significance of the change in stool consistency rather than frequency and the value of parental insight in determining whether or not children have diarrhea⁽⁵⁾.

Serum IL-6 and IL-8 levels were measured using serum separator tubes after 5-ml blood samples were taken from research participants (within 72 hours after diarrheal start). Blood samples were allowed to clot at room temperature for 30 minutes before being centrifuged at 1000 rpm for 15 minutes. Following this, serum was immediately separated and stored at -70°C until analysis. Serum IL-6 and IL-8 levels were determined using the Luminex Human Premixed Multi-

Analyte kit (Catalogue No. LXSAHM-02; R&D Systems Inc, Minneapolis, MN, USA) as directed by the manufacturer. The test is based on analyte-specific antibodies that are precoated onto magnetic microparticles that are embedded with fluorophores in specified ratios for each individual microparticle area. The findings were analysed using the Luminex xPonent® software version 4.2 (Austin, TX, USA).

Ethical approval:

The Medical Ethics Committee of the Faculty of Medicine at Sohag University granted approval for this study (approval number. Soh-Med-21-12-06). All of the research participants' parents or authorised legal guardians provided us with written informed permission. The Helsinki Declaration was followed throughout the study's conduct.

Sample size:

We calculated the sample size using STATA/BE 17 (StataCorp, College Station, TX, USA) to detect a 50% mean difference in IL-6 or IL-8 levels between SAM-AD and control groups at a significance level of 0.05 and power of 80%. This resulted in 22 subjects per group, which was finally increased to 30 subjects per group.

Statistical analysis

An Excel file containing the obtained data was exported to STATA/BE 17 for analysis. Categorical variables were defined as frequencies or proportions, and the Chi-square/Fisher exact test was used to compare them between research groups. The quantitative variables were not normally distributed and were expressed as the median (IQR); for the three- and two-group comparisons, the Kruskal-Wallis and Wilcoxon rank-sum tests were utilised, respectively. The IL-6, IL-8, and CRP measurements were correlated using the Spearman Rho rank test. P values < 0.05 were deemed statistically significant.

RESULTS

The three studied groups had comparable age and gender. The SAM-AD group had significantly lower BMI, head circumference, and MUAC compared with the AD and HC groups. Both SAM-AD and AD groups had higher pulse rate and lower systolic blood pressure than the HC group. Regarding diarrhea, the SAM-AD and AD groups had comparable frequency of motions and proportions of bloody diarrhea, vomiting, abdominal pain, and fever (**Table 1**).

Table (1): Demographic and clinical features of study participants

Characteristics	SAM-AD group (n= 30)	AD group (n= 30)	HC group (n= 30)	P value (within groups)	P value (SAM-AD vs. HC)	P value (AD vs. HC)	P value (SAM-AD vs. AD)
Age, months	13.5 (10-30)	12 (8-20)	12 (10-36)	0.650*	0.100 [#]	0.410 [#]	0.436 [#]
Gender, no., male/female	18/12	15/15	14/16	0.561 ^{\$}	0.301 ^{\$}	0.796 ^{\$}	0.436 ^{\$}
Body mass index, Kg/m ²	13 (12.5-13.9)	17.4 (14.9-19.5)	16.4 (15.4-17.4)	0.001*	<0.001 [#]	0.191 [#]	<0.001 [#]
Head circumference, cm	42.5 (41-46)	48 (45-50)	47 (45-52)	<0.001*	<0.001 [#]	0.755 [#]	<0.001 [#]
Mid-upper arm circumference, cm	9 (8.3-9.5)	13 (12-14)	13.5 (13-14.5)	<0.001*	<0.001 [#]	0.099 [#]	<0.001 [#]
Frequency of diarrhea, no.	7.5 (6-9)	7 (5-9)	–	–	–	–	0.429 [#]
Bloody diarrhea	5 (16.7%)	4 (13.3%)	–	–	–	–	1.000 [‡]
Vomiting	8 (26.7%)	6 (20%)	–	–	–	–	0.542 ^{\$}
Abdominal pain	5 (16.7%)	6 (20%)	–	–	–	–	0.739 ^{\$}
Fever	16 (53.3%)	19 (63.3%)	–	–	–	–	0.432 ^{\$}
Pulse, beat/min	107 (102-110)	105 (100-110)	99 (96-104)	0.004*	0.001 [#]	0.074 [#]	0.087 [#]
Respiratory rate, cycle/min	26 (24-28)	25 (22-25)	24 (24-26)	0.003*	0.008 [#]	0.422 [#]	0.001 [#]
Systolic blood pressure, mmHg	70 (70-80)	80 (70-90)	90 (80-90)	<0.001*	<0.001 [#]	0.041 [#]	0.005 [#]

Data are presented as median (IQR) or number (%)

* Kruskal-Wallis test, [#] Wilcoxon rank-sum test, ^{\$} Chi-square test, [‡] Fisher’s exact test

AD, acute diarrhea; HC, healthy control; SAM; severe acute malnutrition,

The laboratory features of the study participants are shown in **table 2**. The SAM-AD group had significantly higher total leucocytic count and lower hemoglobin level than the AD and HC groups. Both SAM-AD and AD groups had significantly higher platelet count than the HC group. The CRP level in the SAM-AD group was significantly higher than the HC group but significantly lower than the AD group. The SAM-AD group had significantly higher creatinine but lower albumin, calcium, and potassium levels than the AD and HC groups. IL-6 in the SAM-AD group was comparable to the HC group but significantly lower than the AD group. Last, the SAM-AD group had significantly lower IL-8 levels than the AD and HC groups.

Table (2): Laboratory features of study participants

Variables	SAM-AD group (n= 30)	AD group (n= 30)	HC group (n= 30)	P-value (within groups)*	P-value (SAM-AD vs. HC) [#]	P-value (AD vs. HC) [#]	P-value (SAM-AD vs. AD) [#]
WBCs (×10 ⁹ /L)	11.2 (9-12.5)	8 (7-9.2)	6.6 (5.3-8.1)	<0.001	<0.001	0.008	0.005
Hemoglobin (g/dL)	9.2 (8.3-9.8)	12.1 (11.9-13.2)	12.8 (12-13.1)	<0.001	<0.001	0.588	<0.001
Platelets (×10 ⁹ /L)	304 (235-335)	314 (248-381)	296 (223-312)	0.042	0.048	0.026	0.420
CRP (mg/dL)	0 (0-12)	18 (6-48)	0 (NA)	<0.001	<0.001	<0.001	<0.001
Sodium (mmol/L)	135.5 (130-140)	138 (135-140)	137.5 (135-140)	0.248	0.125	0.817	0.182
Potassium (mmol/L)	3.45 (3.1-3.9)	3.95 (3.7-4.4)	4 (3.7-4.2)	0.001	<0.001	0.732	0.008
Calcium (mg/dL)	8.5 (8.1-8.7)	9.2 (9.1-9.5)	9.3 (9-9.5)	<0.001	<0.001	0.853	<0.001
ALT (IU/L)	22 (12-40)	34 (20-40)	20.5 (17-36)	0.195	0.689	0.058	0.273
AST (IU/L)	18 (16-42)	29 (18-35)	20 (17-30)	0.515	0.982	0.153	0.620
Albumin (g/dL)	3.4 (2.1-3.6)	3.8 (3.5-4.1)	4 (3.7-4.1)	<0.001	<0.001	0.288	<0.001
Creatinine (mg/dL)	0.57 (0.4-0.7)	0.41 (0.22-0.65)	0.4 (0.32-0.5)	0.004	<0.001	0.847	0.024
IL-6 (pg/ml)	1.0 (1-4.3)	12 (3.9-175.6)	2.2 (1-4.6)	<0.001	0.738	<0.001	<0.001
IL-8 (pg/ml)	16.2 (11-39)	612 (84.8-800)	157.5 (23.8-298)	<0.001	<0.001	0.017	<0.001

Median and IQR: Non parametric test. *Kruskal-Wallis test, [#] Wilcoxon rank-sum test

AD, acute diarrhea; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HC, healthy controls; IL-6, interleukin-6; IL-8, interleukin-8; SAM, acute severe nutrition; WBCs, white blood cells.

As shown in **tables 3, 4, and 5**, IL-6, IL-8, and CRP showed significantly strong correlation with one another ($r > 0.67$). Moreover, the presence of fever was significantly associated with higher levels of IL-6, IL-8, and CRP. Last, children with bloody diarrhea tended to have higher, but not statistically significant, levels of IL-6, IL-8, and CRP.

Table (3): Correlations among IL-6, IL-8, and CRP among study participants*

Variables	IL-6	IL-8	CRP
IL-6	1		
IL-8	0.814 ($p < 0.001$)	1	
CRP	0.692 ($p < 0.001$)	0.671 ($p < 0.001$)	1

*Using Spearman's Rho test

Table (4): Association of fever with IL-6, IL-8, and CRP among study participants

Variables	Fever	No fever	<i>p</i> value*
IL-6 (pg/ml)	8.3 (1.02-56.9)	2.5 (1-7.02)	0.031
IL-8 (pg/ml)	98.4 (23.1-800)	21.4 (11.5-163.6)	0.033
CRP (mg/dl)	12 (6-24)	0 (0-6)	0.002

Median and IQR: Non parametric test.

*Wilcoxon rank-sum test

Table (5): Association of bloody diarrhea with IL-6, IL-8, and CRP among study participants

Variables	Bloody diarrhea	No bloody diarrhea	<i>p</i> value*
IL-6 (pg/ml)	30.8 (2.5-56.9)	3.7 (1-12.5)	0.116
IL-8 (pg/ml)	196.4 (60.7-800)	46.02 (13.2-561.7.6)	0.087
CRP (mg/dl)	12 (12-24)	6 (0-24)	0.051

Median and IQR: Non parametric test.

*Wilcoxon rank-sum test

DISCUSSION

SAM is a significant public health issue in underdeveloped nations, where it is linked to increased acute diarrhea severity and mortality risk ⁽¹²⁾. In this case-control study, we found that malnourished children with acute diarrhea had significantly lower serum levels of IL-6 and IL-8 as well as CRP compared with well-nourished counterparts who had acute diarrhea. This indicates that malnutrition may impede the acute phase inflammatory response to acute diarrhea.

Intestinal epithelial cells secrete several cytokines (e.g., tumor necrosis factor- α [TNF- α], IL-1 β , IL-6, IL-8, and IL-10), either innately or in reaction to an inflammatory or infectious stimuli⁽¹⁸⁾.

Proinflammatory cytokines, such as IL-6 and IL-8, are important players in the regulation of inflammatory processes. Both lymphoid and non-lymphoid cells generate IL-6, which is widely recognised for its function in the defence mechanism against acute infectious diarrhea. IL-6 also plays a significant role in the control of immunity, the acute-

phase response, the maturation of B and T lymphocytes, and hematopoiesis. IL-8 has a role in inflammatory cells' chemotaxis—the movement of neutrophils and lymphocytes towards the site of inflammation. The gastrointestinal tract's epithelial cells emit IL-6 and IL-8, which are essential for protection against mucosal infections. These cytokines create inflammatory responses to infectious pathogens both locally and systemically, facilitating the elimination of bacteria ⁽⁷⁻⁸⁾.

In the present study, well-nourished children with acute diarrhea had significantly elevated serum levels of IL-6 and IL-8 compared with healthy children. Our findings are in agreement with prior case-control studies, showing IL-6 and IL-8 levels in the serum and/or feces of well-nourished children with acute gastroenteritis were considerably greater than those in healthy controls ^(9,10,18).

Among children with acute diarrhea under study, 58.3% had fever and 15% had bloody diarrhea. While acute viral and bacterial diarrhea can not be definitively differentiated on clinical grounds alone, the presence of high-grade fever and blood/mucus in diarrhea tend to be associated with bacterial etiology^(6,9,10).

Our findings revealed significantly higher serum levels of IL-6, IL-8, and CRP among febrile than non-febrile children with acute diarrhea. Furthermore, serum levels of these inflammatory markers tended to be elevated (although not statistically significant) in association with bloody diarrhea. These findings are in line with earlier research that showed children with bacterial gastroenteritis had noticeably higher blood levels of IL-6 and CRP than those with viral or non-bacterial gastroenteritis ^(9,10,19-21). Conversely, some research shown that infants with bacterial gastroenteritis had noticeably greater amounts of IL-8 ^(10, 21). Others reported no significant role of IL-8 level in discriminating bacterial from viral gastroenteritis^(9,18,20). However, serum IL-8 may be more sensitive than IL-6 in the differentiation between rotavirus and norovirus infections⁽⁹⁾.

Children with SAM were not included in any of the aforementioned research looking at the effect of IL-6 and IL-8 in acute diarrhea. Our study's key conclusion is that, in comparison to their well-nourished peers, malnourished infants with acute diarrhea had far lower blood levels of CRP, IL-6, and IL-8. SAM is a significant contributor to children's compromised immune systems, resulting in abnormalities related to opsonization, complement system and phagocytosis, chemotactic activity of neutrophils and monocytes, and antigen-presenting cell function. As part of its detrimental effects on the immune system, SAM may reduce the amount of amino acids and other cofactors needed for the production of cytokines and other acute phase reactants, which might impede the acute phase inflammatory response, including cytokines ^(13,15,22). However, previous studies have reported inconsistent findings concerns children with SAM's capacity to

produce an appropriate acute phase immune response (9,10,18,20).

Our results go in line with a number of studies showing impaired acute phase inflammatory response in children with SAM. For instance, an animal study demonstrated that protein depletion leads to a significantly slower IL-6 response⁽²³⁾. Moreover, using whole blood from extremely malnourished children, an in vitro investigation found reduced levels of TNF- α and IL-6 production⁽²⁴⁾.

According to another in vitro study, peripheral blood mononuclear cells from malnourished children had a decreased capacity to generate cytokines, such as IL-1, IL-6, IL-8, and TNF- α ⁽²⁵⁾. Similarly, **Rodríguez et al.**⁽¹⁶⁾ demonstrated a reduced production of IL-2 and IFN- γ from peripheral blood CD4⁺ and CD8⁺ lymphocytes of malnourished in comparison to well-nourished children. Furthermore, **González-Martínez et al.**⁽²⁶⁾ found that the IL-6 gene was expressed less in children who were malnourished than in those who were well-nourished. Last, **Mărginean et al.**⁽²⁷⁾ reported lower serum levels of IL-6 and IL-8 in malnourished compared with well-nourished children.

Conversely, a number of studies have found that malnourished children had comparable or even greater levels of proinflammatory cytokines than their well-nourished peers⁽²⁸⁻³¹⁾. **Malavé et al.**⁽²⁸⁾ reported a similar increase in serum levels of IL-6 in malnourished and healthy children with overt infections. Furthermore, studies from Kenya, Ghana, and Turkey showed a significantly higher proinflammatory response, including elevated serum IL-6 levels, when comparing children with protein-energy deficiency to healthy counterparts⁽²⁹⁻³¹⁾. Such increase in proinflammatory cytokines, including IL-6, was also described in malnourished patients in the intensive care unit, which was associated with higher mortality risk⁽²²⁾. Of note, **Manary et al.**⁽¹⁵⁾ described higher serum cytokines, including IL-6, but, marasmic toddlers had lower amounts of acute phase proteins than well-nourished children who had infections. This inconsistency across studies might be attributable to variations in the type and severity of malnutrition, the associated type and severity of infections, and several confounding factors (e.g., measurement accuracy, time of sampling along the disease course). For instance, the extent of acute phase immune response may vary between edematous and non-edematous malnourished children and is affected by blood concentrations of certain micronutrients⁽³¹⁻³³⁾. Moreover, children with respiratory infections may mount higher acute phase proteins than those with diarrhea⁽³⁴⁾.

The nature of the presented antigen impacts the cytokine profile produced by the inflamed bowel mucosa of the gastrointestinal tract⁽²⁰⁾. In addition, based on the reduced production of IL-6 shown in vitro studies, it is possible that the higher serum level of IL-6 in malnourished children results from its diminished clearance rather than increased synthesis⁽¹⁵⁾. Another

explanation could be the stimulation of IL-6 synthesis by other substances, such as IL-1 β , which are more prevalent in malnourished children, whether they have an acute illness or not⁽¹⁵⁾. Further studies are required to investigate such possible confounding factors and the complex interactions among cytokines in malnourished children with acute diarrhea.

The study's strength is its case-control methodology, which enables comparisons between the blood levels of IL-6 and IL-8 in malnourished children with acute diarrhea and both of well-nourished children having acute diarrhea and healthy controls. However, we acknowledge some limitations. First, serum levels of IL-6 and IL-8 may be affected by the timing of blood sample collection and storage duration⁽³⁵⁾.

However, we obtained blood samples from all children in this study within the first 3 days of the onset of acute diarrhea. **Vaisman et al.**⁽¹⁸⁾ showed no significant differences in serum IL-6 and IL-8 levels when measured within 36 hours and between 36 and 72 hours of diarrheal onset. Therefore, the timing of blood sampling likely has a limited confounding effect on the results of our study. Moreover, the study participants were not subjected to microbiological examinations in order to distinguish between diseases caused by bacteria and viruses. The difference between the two etiologies is critical since various studies have found a link between bacterial gastroenteritis and greater serum levels of IL-6 and IL-8^(9,10,19-21).

Furthermore, we measured IL-6 and IL-8 levels in the serum; fecal IL-6 may be a more direct predictor of intestinal inflammation in children with infectious diarrhea. However, various factors may affect fecal IL-6 levels, leading to undetectable or low measured concentrations, such as the timing of sample collection, degradation in stool, and dilution with diarrhea⁽⁹⁾.

Another limitation is related to the relatively small sample size, which might reduce the study power to detect some statistically significant differences and limit the generalizability of study findings. Last, our study has a case-control design with inherent limitations, including selection bias, information bias, and confounding.

CONCLUSION

Our study found that malnourished children with acute diarrhea may have lower serum levels of IL-6 and IL-8 as well as CRP compared with well-nourished children with acute diarrhea. This indicates that malnutrition may impede the acute phase inflammatory response during acute diarrhea. However, to validate the findings of our study, further extensive studies with a greater number of patients are advised.

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