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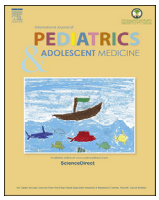

Original research article

New diagnostic biomarker in acute diarrhea due to bacterial infection in children

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ABSTRACT

Background: Diarrhea is a major cause of morbidity and mortality in children, and diarrhea may be due to infection that is bacterial or non-bacterial. Differentiation between diarrhea from a bacterial or non-bacterial infection is not a simple task, and no single method is present to differentiate between these causes of diarrhea.
Objectives: To evaluate the diagnostic accuracy of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and procalcitonin (PCT) in the diagnosis of acute diarrhea due to bacterial infection.
Design: Case control study of forty children with bacterial infection diarrhea diagnosed by stool culture and CRP, 40 children with acute non-bacterial infection diarrhea and 30 age- and sex-matched healthy controls. Stool cultures, serum CRP, PCT and serum sTREM-1 were measured in all children on admission.
Results: Children with acute bacterial infection diarrhea had a significant increase in the serum sTREM-1 and PCT levels on admission compared to patients with nonbacterial infection diarrhea and controls (26.3667 ± 16.8184 ng/ml vs 7.2267 ± 6.4174 ng/ml vs 6.7367 ± 5.6479 ng/ml and 39.9933 ± 22.5260 ng/ml vs 1.8533 ± 1.7123 vs 0.2840 ± 0.1208 ng/ml, respectively; $P < 0.05$). sTREM-1 demonstrated significantly higher sensitivity (93.7%) and specificity (94.3%) in the prediction of bacterial infection as a cause of acute diarrhea in children with an area under the receiver operator characteristic (ROC) curve (95% CI) of 0.94 (0.84–0.99) at a cutoff value of 12.4 ng/ml.
Conclusions: Both serum PCT and sTREM-1 are valuable in the early diagnosis of acute bacterial infection-induced diarrhea in children, and there was markedly higher diagnostic discriminatory power for sTREM-1.

1. Introduction

Although it is a preventable disease, acute diarrhea remains a major cause of morbidity and mortality in children worldwide, resulting in more than 1.8 million deaths per year among those younger than five years. Most of these mortalities occur in developing countries [1]. Diarrhea in children is caused by a wide range of pathogens, including viral, bacterial and protozoal pathogens. These pathogens make overcoming the high disease burden a large challenge [2]. In developed countries, the morbidity and mortality caused by acute diarrhea have become less threatening in recent decades. However, acute diarrhea continues to be an important and frequent cause of hospitalization; it has significant morbidity, especially in young children under 5 years of age in developing countries [3]. The frequency of bacterial and parasitic gastrointestinal infections has declined with improvements in the public health infrastructure (water and sewage management); however, this is not the case with viral gastroenteritis [4]. A rapid, reliable test that predicts bacterial infection is beneficial to improving the outcome through early antibiotic treatment [5]. Markers of bacterial infection include a routine leukocyte count and C-reactive protein (CRP) [6]. During the acute phase response, there is an increase in the blood levels of many proteins, including C-reactive

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protein (CRP) and procalcitonin (PCT). Both showed better performance than other traditionally used markers, such as leukocyte counts, to differentiate between bacterial and viral infections [7–11]. Because they are fast, without requiring time for the bacteriology results, and can rule out bacterial infection, particularly for PCT, they are routinely used in developed countries [12,13]. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) is a newly proposed marker [14].

The molecular weight of CRP is 120 kDa, and its gene location is between 1q21 and 1q23. It is an important component of the innate defense system against infections [15]. It recognizes the phosphocholine on the surface of many bacteria; then, it activates the classical complement pathway and facilitates phagocytosis by neutrophils. Because CRP lacks specificity, it is used as an additional marker in combination with more conventional parameters, such as the number of leukocytes in CSF, blood count and protein level, to help the clinician to narrow down the differential diagnosis [16]. PCT protein (the calcitonin precursor propeptide) is synthesized in C cells of the thyroid gland and secreted from leukocytes in the peripheral blood. Its molecular weight is 13 kDa [17], and its gene is located on the short arm of chromosome 11 (11p15.4) [16]. In bacterial infection, the secretion of PCT is increased up to several thousand-fold, but it remains normal or slightly increased in viral infections and inflammatory reactions that are not infectious [18]. The serum PCT level increases within 2–3 h after infection with a peak value at 6–12 h, which normalizes within 2 days. In contrast, the CRP levels increase between 12 and 18 h after bacterial infections [19,20]. PCT is stable in plasma and its plasma half-life is approximately 22 h. Unlike most cytokines, PCT is stable in vitro, which makes it both a promising new marker for early and sensitive identification of infected patients as well as for titration of the response to treatment [21]. However, PCT is not considered an ideal marker because it is elevated in conditions other than infection, and it may remain low in infections [22]. Additionally, the use of PCT is complicated by variation in the choice for the abnormal cutoff value and the diverse age range.

On the other hand, TREM-1 is a trans-membrane glycoprotein cell-surface receptor of the immunoglobulin superfamily. TREM-1 acts in cooperation with toll-like receptors (TLRs), and this cooperation is controlled by nuclear factor-κβ (NF-κβ) [23]. The expression of TREM-1 is up-regulated on phagocytic cells in the presence of bacteria and fungi, triggering the secretion of the pro-inflammatory cytokines that amplify the host response to the microbial agents [24]. Some data have demonstrated that expression of membrane-bound TREM-1 on neutrophils and monocytes/macrophages is strongly altered during bacterial infection, peaking at 6 h. Therefore, the aim of this study was to evaluate the diagnostic utility of these markers (PCT and sTREM1) in acute diarrhea from bacterial infection and their usefulness in differentiating between acute diarrhea from bacterial and non-bacterial infections.

1.1. Subjects and methods

Subjects: This study was performed on eighty infants and children with acute diarrhea, aged 3–36 months, admitted to the Pediatric Department at Tanta University Hospital, Tanta, Egypt. Another 40 age- and sex-matched, apparently healthy infants and children were enrolled as controls. Diarrhea was defined according to the WHO case definition criteria [1].

Exclusion criteria: Patients with chronic diarrhea, malnutrition, other systemic infections, or those who had received antibiotics in the last 14 days before enrollment or had co-existing morbidities were excluded. Informed consent was obtained from the guardians of the studied infants and children before study participation.

Children with acute diarrhea were further subdivided into the following two groups:

Group 1: children with acute diarrhea due to bacterial infection (no = 40). Bacterial infection was diagnosed by the presence of all of the following: fever, toxic manifestation, leukocytosis and positive stool bacterial culture (the isolated bacterial pathogens included the following: *Escherichia coli* in 47%, *Campylobacter jejuni* in 20%, Shigella in 17% and Salmonella in 16%). **Group 2: children with acute diarrhea due to non-bacterial infection (no = 40)**, including those positive for rotavirus antigen in stool and those with proven protozoal infection (*Entamoeba histolytica* or *Giardia lamblia*) in stool analysis with negative results for stool bacterial cultures. On admission, the following items were recorded for each patient: age, sex, vital signs and clinical symptoms and signs (fever, vomiting and diarrhea). Acute diarrhea was defined as an increase in the number of loose stools to more than the normal number (i.e., an increase to ≥2 loose stools per day) for a period of <15 days. History taking included the following: administration of antibiotics, recent travel abroad, date and duration of admission, duration of illness and previous hospitalization or history of diarrhea. Thorough clinical examination was performed with special emphasis on the assessment of dehydration level following the recommendations of the WHO Program for Control of Diarrheal Diseases. The symptoms were regularly evaluated and recorded daily on the follow-up chart along with the diarrheal episode.

1.2. Stool samples

A single stool specimen was collected from each child with the help of their parents. The specimens were examined for the color and consistency of the stools. Fresh fecal specimens were examined by light microscopy for the presence of parasitic ova, cysts, blood, mucous, pus cells, fatty drops and white blood cells (WBCs) as well as by the modified acid-fast stain for *Cryptosporidium parvum*. All stool specimens were cultured for Salmonella, Shigella, *Campylobacter jejuni*, *Vibrio cholerae*, and *Escherichia coli* by standard methods [25]. Stool samples were tested for rotavirus antigen by enzyme immunoassay (EIA) using a kit (RIDASCREEN® Rotavirus test, R-Biopharm AG, Landwehrstr. Darmstadt, Germany) [26].

Blood samples were used for routine laboratory investigations, including the CRP, leukocyte count, and PCT and sTREM-1 measurement. After 72 h of antibiotic treatment for cases with evidence of acute diarrhea due to bacterial infection, the CRP, serum PCT and sTREM-1 levels were re-estimated.

Serum analysis: Serum was separated from blood samples collected on admission from all patients and after 3 days from patients who received antibiotic treatment for acute bacterial diarrhea. The serum was then stored at −20 °C.

C-reactive protein (CRP): A nephelometric assay (Dade-Behring, France) was used to measure CRP with a detection limit of 0.2 mg/l and intra-assay coefficients of variation at low and high concentrations of 3.3% and 2%, respectively, using the normal value of 6 mg/l [27].

Procalcitonin (PCT): A specific immunoluminometric assay (LUMItest®, Brahms Diagnostica GmbH, Germany) was used to measure the PCT in duplicate. Luminescence was automatically measured on a Berilux Analyzer 250 (Behring Diagnostics, Germany). The detection limit was 0.08 ng/ml, and the intra-assay coefficients of variation at low and high concentrations were 12% and 5%, respectively. The normal serum procalitonin with this is < 0.5 ng/ml [28].

Soluble triggering receptor expressed on myeloid cell-1 ELISA: According to the manufacturer's instructions (Quantikine Human TREM-1 Immunoassay, R&D Systems, USA), TREM-1 was measured with a commercially available human ELISA kit using a

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