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Investigative Impact of Serum IL-6 and TNF-α Levels in Pediatric Diarrhea Patients with *listeria monocytogenes* Infection

Hind AL-Hammami
Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Iraq.
***E-Mail**: hind.f.al.hammami@jmu.edu.iq*

ABSTRACT

Background: *In children, diarrhea is a significant cause of morbidity and severe mortality. Infection with pathogens as Listeria monocytogenes is one of the etiological reasons that can result in diarrhea. Important inflammatory markers in bacterial diarrhea in children include the IL-6, TNF-α, blood markers CRP, WBC, and Absolute Neutrophil Count, It can be important in the initial diagnosis of an infection*

Objectives: *This investigation intended to measure some immunological markers (such as IL-6 and TNF-α and other blood markets such as CRP, WBC, and Absolute Neutrophil Count) in children with diarrhea, especially with the emergence of Listeria monocytogenes bacteria isolates to the considerate corresponding immune system to get rid pathogen.* ***Methods****: From November 2022 until the end of July 2023, 480 suspected patients with acute diarrhea infection who attended the AL-Zahraa Teaching Hospital were registered, encompassing both sexes, and children aged 1 to 5 years. Patients' stool samples were cultivated on certain conditions, and bacterial isolates were subsequently identified using morphological, biochemical, and API techniques. After receiving consent from patients and ethical advice from hospitals, the blood and serum levels of immunological markers were evaluated in various groups.*

Results: *Present study revealed that L.monocytogenes .recovered at 8.3% of cases(no=40 total isolate), Mean age of the disease was 3.3±2.32 years, the percentage of infection in males(72.5%) more than females(32.5%), There were notable increasing in P-value of CRP, serum IL-6, serum TNF-α, WBCs and ANC in children with diarrhea associated with L.monocytogenes compared to the control group ($p<0.05$) with highly sensitivity and specificity.*

Conclusion: *Data revealed the coordinated role between study immune markers responding to acute diarrhea in children and consider suitable markers.*

Keywords*: IL-6, TNF-α, diarrhea, Listeria monocytogenes.*

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1- INTRODUCTION

One of the most prevalent disorders in children is acute diarrhea, within the first five years of life, it is estimated that 12 or more episodes of diarrhea occur per child year in underdeveloped nations. Lack of published research on childhood diarrhea in Iraq, together with the effects of war, a broken health system, and a continuous public health emergency, are all having an impact on the population's most vulnerable groups, especially children. The pathogen spectrum linked with diarrheal disease has to be investigated since a number of pathogens like viruses, bacteria, and infection by parasites are amongst the most frequent sources agents of acute diarrheal state in babies. According to 121,266 household surveys, the

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cause of morbidity, with morbidity was diarrheal sickness occurrence of 7.8 per 1000 per year. Additionally, diarrheal sickness was the main reason for baby deaths in Indonesia, accounting for 24.1% of all child fatalities and 40% of all fate causes in the initial two years of existence. The pathophysiology of acute infectious diarrhea is mediated by a diverse group of enteric pathogens, including bacteria. Untreated bacterial gastroenteritis can lead to serious consequences including septicemia[1].

Pathogenic bacteria like *Listeria monocytogenes* are one of the etiological causes that can cause diarrhea, Listeria bacteria are gram-positive (G+ve) bacillus, motile, non spore-forming, and catalase-positive bacteria. *L. monocytogenes* is the major microbial species that source listeriosis in individuals out of the seven distinct strains that make up this genus. Humans who are infected with listeriosis can experience a wide range of symptoms, including gastroenteritis, granulomatous uterine infections, bacterial infection in newborns, endocarditis, myocarditis, encephalitis, meningitis, liver necrosis, and spontaneous abortion[8]. Stool samples from children should be laboratory tested *L. monocytogenes* later diarrhea in children can have catastrophic consequences [2]. In the immune system, the cytokine IL-6 possesses both pro- and anti-inflammatory properties. Neutrophil development and migration to infection sites are regulated by IL-6 [11]. L-6 shows an important role in both the acute phase response and the adaptive response. Either directly or by promoting the development of T follicular helper (TFH) cells, IL-6 promotes the generation of antibodies in B cells. Additionally, the pro-inflammatory cytokine TNF-α, which is mostly generated by macrophages, can cause tissue inflammation by activating macrophages, drawing in neutrophils, and up-regulating additional pro-inflammatory mediators In some types of inflammatory colitis, the TNF- is raised in the gastrointestinal tract and plays a key role in mucosal inflammation [3].

The body's response to infection is coordinated by a force of cytokines, such as Tumor necrosis factor (TNF-α), (IL-6), (IL-8), (IL-4), and INF-β, these acute cytokines release into the serum. This leads to a noticeable increase in their concentrations when measured laboratory[14], and this has been studied in just a few investigations, Serum levels of TNF-α and IL-6 were shown to be elevated in a study of children with difficult bacterial infections such as *Shigella dysenteriae*, *E. coli, and Listeria monocytogenes*[15].

IL-6 concentrations in plasma were More than 44 hours after infection, whereas TNF-α was only detectable for up to 18 hours after bacterial activation [6] this may be a helpful marker to diagnose bacterial gastrointestinal tract infections because a prior study found that CRP and serum IL-6 levels increased midst severe bacterial infections. Serum cytokine levels in acute diarrhea caused by the Listeria monocytogenes bacterium have hardly ever been investigated in Iraq to date.

2. Objectives

The goal of this investigation was to determine whether there was any relationship between the levels of serum IL-6, TNF-, and other blood markers in babies with severe diarrhea and bacterial intestine contagion.

3-Methods

Study population

From November 2022 to the end of July 2023, 480 children with clinical diarrhea were admitted to AL-Zahraa Teaching Hospital and private clinics; among them were (356) boys and (124) girls between the ages of one to five years, and 50 healthy children from the Consulting clinic center in the infirmary were election as the control group at the selfsame time.

For inclusion in the study, questionnaires and consent forms were given to each patient's family. There was a statistically significant difference between the two groups measured at value ($p \leq 0.05$).

Listeria monocytogenes identification

Diarrheal stool specimens were achieved by taking two ml(2 ml) of each sample directly and inoculating it into Broth of Listeria Enrichment Broth Base (Himedia, India) In as little as 10 minutes after specimens were gathered, then, stool specimens were kept at 4°C for Forty-eight hours. Following the manufacturer's directions, feces specimens of the patient were planted in a special culture medium of Listeria (Oxford, UK) with Oxoid Listeria Selective Enrichment Supplement(Thermo Fisher Scientific, Waltham. MA, USA), after that, it was brooded at thirty-seven degrees for 72hours [7].

After this identification by some biochemical test, and processed by another test to ensure identification, Indole and Motility (SIM) medium (Merck, Germany) tests were performed on prospective *L. monocytogenes* colonies. Finally, API testing was used to further diagnosis the found of *L.monocytogene s.* A prepared clinical isolate of *L. monocytogenes* from microbiology laboratories of the biology department, collage of Sciences was used as a positive control.

Blood sample collection

Five-milliliter(5 ml)of blood specimens were obtained from each of the children in the 1 to 4 days of the beginning of a severe phase of diarrhea and split into two parts, one of which was placed in a tube containing a substance to count white blood cells and neutrophils. The second group was a serum used for immunological tests as C-reactive protein (CRP) in this procedure involves latex particles in the solution will adhere in the entity of C-reactive protein with a level of CRP in the sample ≥ 6 mg/L, normal levels are <6 mg/L.[8] If don't found adhere this means the non-attendance of CRP..After that, sera were kept at 80 °C for the following evaluation of IL-6 and TNF-α by ELISA method based on the industrialization's index.

Immunological Investigations

An immunological study including C-reactive protein CRP, count white blood cells WBC, and ELISA method to evaluate IL-6 and TNF-α based on the company instructions.

Data Analysis

Software statistics tools for social science were used to conduct statistical analysis utilizing the Student's t-test (SPSS). Values were given as mean SD and were considered statistically significant if the value was $p \leq 0.05$.

4-RESULTS

Forty isolates (8.3%) of the 480 diarrheal samples were determined to be L. monocytogenes positive, conducting the biochemical differential assays for catalase test (+ ve), sodium hippurate test (+ ve),

camp test (+ ve), bile-esculin test (+ ve) and motility test (+ ve), by using the differential culture media. Children with diarrhea infection at a Mean of 3.3±2.32 years in comparison with the mean age of healthy control was 2.9 ± 1.15 years. Statistical differences were found between the diarrhea patients with *L.monocytogenes* and controls approximately age at a significance value $p \leq 0.05$. All these results found in this search were demonstrated in Table 1, which shows also the sex divide among children patients with diarrhea the main of patients with diarrhea associated with *L.monocytogenes* were males (72.5%) with a non-significant difference at ($p \leq 0.05$) related for dominance of males were (25.0%)

whilst in females 32.5% of diarrhea patients (males exposed to danger more than females) in comparison with control of females were (12.5%) and with non-significant at P-value ($p = 0.73$). As shown in the same Table(1), There were significant increases in WBCs, ANC(Absolute Neutrophil counts), CRP (C-reactive protein), serum interleukin 6 (IL-6), and serum Tumor necrosis factor(TNF-α) in children with diarrhea related with *L.monocytogenes* in comparative with the dominance set ($p<0.05$). (Table 1). vulnerability, specificity, negative oracular value (NPV), and positive predictive value (PPV) of a deliberate signal are listed in Table(2).

Table (1): Comparison in General data and biomarkers results between children patients and controls.

Parameters	Patients group (N=40)	Control group (N=40)	p-value
Sex			
Male	29(72.5%)	10(25.0%)	**0.245**
Female	11(27.5%)	5(12.5%)	**0.73**
Age	2.32 ±3.3	1.15±2.9	p<0.05**
Total leucocytes count ×103/cm3	8.6±1.81	5.1±1.22	p=0.05 **
CRP(C-reactive protiens), mg\dl	7.8±2.4	0.2±0.07	**p<0.05****
Absolute neutrophil /cm3×10cou	8.1±2.31	3.6±1.13	**p<0.05****
IL-6, pg/mL	49.8±12.12	0.3±0.11	**p<0.05****
TNF-ά, pg/mL	37.2±18.51	1.0±0.2 2	**p=0.01****

** significant at value($p \leq 0.05$), Values are expressed as mean ± SD.

Table(2): Predictive values of measured indicators.

	IL-6	TNF-α	WBC count	CRP	ANC
Sensitivity	78.3	80.9	44.2	82.7	**38.8**
Specificity	92.5	89.4	80.4	88.2	**75.1**
PPV	76.4	74.6	62	79.5	**50.3**
NPV	60.5	55.4	40.1	54.2	**43.7**

5-Discussion

In comparison to other bacteria linked to pediatric diarrhea, our findings show that *L.monocytogenes* is more rarely found in infectious diarrhea samples from patients in the city of AL-Diwaniyah. Of the 480 patient samples analyzed, 40 cases (8.3%) were identified through the culture method and various biochemical tests, and they were then verified by the API kit method. Similar studies investigating the frequency of *L. monocytogenes* in human diarrheal samples discovered that this bacteria was rather uncommon. Separate investigations revealed 0.2%[9] and 4.6%[10] of patients in China and Iran, respectively. Differences in the current study results of the diagnosis methods, richness, and culture steps use age to detect the presence of *L. monocytogenes* and the cause of the rise in *L. monocytogenes* in the diarrheal specimen of the infected children in investigation in AL-Diwaniyah city and the variations from earlier studies may be resulted from some factors such as the diet, level of immune factors against *L. monocytogenes* bacterium contagion and close of contact to normal container of *L. monocytogenes* microbe in the climate, as interaction with brute and state of drinking water, may also vary among the various geographical regions [11].

In this study, individuals with *L. monocytogenes* were an average of three years and three months old. Similar earlier studies indicate that patients with *L. monocytogenes* often present at ages of 2.8 years four [12] years and six months [13], respectively, in Los Angeles and Iran. In our study, 29 instances of *L.monocytogenes* infection (72.5% of patients) were male, while 11 cases (27.5% of patients) were female. In Iran, there were four cases (44.4%) of females and 5 states (55.5%) of males with *L. monocytogenes* infections. In Sweden, 25 instances (52%) were girls and 23 cases (47.9%) were boys, while in Los Angeles, the presence of some patients were boys[14].

the current study, it was investigated if IL-6, TNF-, and other acute markers of inflammation could be used as quick distinguishing markers in children who had diarrhea brought on by an infection *with L. monocytogenes*. The findings demonstrated that serum IL-6 and TNF- levels were significantly increased in all diarrheal patients than in healthy children, with a sensitivity of 78.3% and 80.9%, respectively, and specificities of 92.5% and 89.4%, respectively, as opposed to WBC and ANC, which had a sensitivity of 44.2%, 38.8%, respectively, and values of 80.4% and 75.1%, respectively. According to other investigations, children with multisystemic bacterial infections in Bangladesh were display, to have acutely high serum levels of (IL-1_, IL-6, IFN-_, GM-CSF, and HMGB1)[15]. However, Azim et al. found that infants with bacterial and viral diarrhea infection had greater levels of numerous cytokines, including TNF-, IL-8, IL-6, IL-10, and INF-[16]. Various cytokines are produced during infection by bacteria and their byproducts. In numerous infectious illnesses, the conflicting effects of cytokines on hosts have been documented For instance, the immunity that occurs by the cell, which is governed by cytokines, controls body strength versus Listeria monocytogenes Tumor necrosis factor-(TNF-) and interleukin-6 (IL-6) contribute in body strength versus L. monocytogenes. Reactive oxygen species are generated by macrophages and neutrophils in response to the pro-inflammatory cytokines, which help

fight infection. The intestinal epithelium releases certain cytokines, like IL-6, which the body uses to trigger inflammatory responses to infections both locally and systemically. The rise in pro-inflammatory cytokines like IL-6 and TNF- during infections is reflected by the direct mucosal release of these cytokines, which aids in the killing or suppression of microorganisms by enhancing acute phase responses with longer persistence in the circulation [17]. The sensitivity and specificity of the common acute phase reactant (CRP), which is frequently evaluated in kids with acute infections, were 82.7% and 88.2%, respectively. These results were consistent with earlier studies conducted in Saudi Arabia, which showed that in patients with acute bacterial gastroenteritis, CRP level measurement had the greatest value compared to other cytokines and blood indicators such INF- and IL-1-, WBC, and ANC [18, 19].

Conclusion

Data revealed the coordinated role between studying immune markers responding to acute diarrhea in children and considering suitable markers.

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