Original Article

Estimation of IL-8 and TNF-α Levels in Pediatric Diarrhea Patients Infected with Enterohemorrhagic *E. coli O157:H7*

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Abstract

Background: Verotoxins are bacterial virulence factors produced by *E. coli O157:H7*, transmitted by the fecal-oral route. **Objectives:** The aim of this article was to diagnose *E. coli O157:H7* which causes diarrhea and sometimes develops into HUS, which considers pig health problems and estimates the levels of interleukin (IL)-8 and tumor necrosis factor (TNF)-α in the sera of pediatric patients infected with Enterohemorrhagic *E. coli* compared to the control group. **Materials and Methods:** Stool and blood samples were collected from 421 pediatric patients with diarrhea, ranging in age from birth to 13 years old, from March to October 2022. Samples were collected from Al Noor Teaching Hospital, Babylon Hospital for Pediatric and Gynecology, Hilla, Iraq. *E. coli O157:H7* was cultured on eosin methylene blue (EMB) and Sorbitol MacConkey agar (SMA), confirmed by biochemical test and cultured on HiCrome *E. coli O157:H7* selective medium which was an agar base supplemented with cefixime tellurite agar. Serum from 30 pediatric diarrhea patients infected with *E. coli O157:H7* compared with 30 healthy children as control group used to determine serum levels of IL-8 and TNF-α by sandwich ELISA. **Results:** The results revealed that out of the total 421 samples used in this study, *E. coli O157H:7,* represented 7% (30 of 421) stool samples. This 30serum samples of infected children as well as 30 samples from healthy children subjected to the estimate serum level of IL-8 and TNF-α which record significant differences $P \leq 0.01$ and $P \leq 0.05$ to this cytokines in different age group; the mean of IL-8 level was 283.62 ± 17.8 pg/mL (7–9 years), and the mean of TNF-α was 208.62 ± 28.7 pg/mL (10–13 years) comparative with the control group of 80.58 ± 15.4pg/mL and 32.50 ± 7.5 pg/mL, respectively, and also result showed an increased mean level of IL-8 than TNF-α in the male comparative with female 195.19 ± 10.4 pg/mL and 159.05 ± 12.4 pg/mL, respectively, comparative with the control group. The result showed no significant differences in IL-8 and TNF-α between watery diarrhea (192.43 ± 24.3 pg/mL and 136.05 ± 20.4 pg/mL) and bloody diarrhea (189.02 ± 22.5 pg/mL and 123.80 ± 13.5 pg/mL), and also result showed significant increase of mean sera level of IL-8 than TNF-α in formula feeding children comparative with breastfeeding children (187.87 ± 19.5 pg/mL and 119.93 ± 17.4 pg/mL, respectively). **Conclusion:** The finding of this study suggested that increased levels of IL-8 and TNF-α are present in all age groups, in male comparative with female, and also in pediatric diarrhea feeding by formula than breastfeeding and no differences of this cytokine according to consistency of diarrhea. These results contribute to using the immune profile as a serological marker for diagnosing diarrhea caused by *E. coli O157:H7* in comparison with the control group.

Keywords: Enterohemorrhagic *E. coli*, IL-8, pediatric diarrhea, TNF-α

Introduction

Escherichia coli (*E. coli O157:H7*) is classified as a serotype species within the *E. coli* genus. It is also recognized as one of the strains of *E. coli* capable of producing the Shiga toxin (Stx). Since its initial isolation in 1982, *E. coli O157:H7* has been identified as a significant pathogen.[1] Shiga toxin-producing *E. coli* (STEC), also called verotoxin-producing *E. coli*, can be transmitted between animals and humans through contaminated food.[2] These pathogens are known to cause a range of symptoms, from mild cases of diarrhea

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to more severe conditions such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and in rare cases, even death.

It is important to note that children are particularly vulnerable to these severe manifestations. During the initial stages of pathogenesis, the Enterohemorrhagic *E. coli O157:H7* strain interacts with the intestinal epithelial cells' apical surface. This interaction leads to the release of various virulence factors, such as Stxs and lipopolysaccharide (LPS). The components under consideration include H7 flagellin, long polar fimbriae (Lpf1/Lpf2), hemorrhagic coli pili, and effector proteins. The injection of virulence components into host cells occurs via a type 3 secretion system (T3SS).[3]

The mammalian host possesses two primary categories of immune response, namely innate and adaptive, which play a crucial role in effectively managing and eradicating pathogens. The body's natural immunological response serves as the first barrier to protection for the host against microbial infections, mostly located in the epithelium that lines the mucosal surface of the gastrointestinal tract.[4] In addition to their crucial involvement in the transfer of ions, fluid absorption, as well as secretion, gastrointestinal epithelial cells also play a vital role in regulating both the phrase and enhancement of compounds that kill bacteria produced when a person gets sick. These substances include the chemoattractant cytokines interleukin (IL)-8, macrophage inflammatory protein (MIP)-1, monocyte chemoattractant protein (MCP-1), the proinflammatory cytokines tumor necrosis factor (TNF-α), and IL-1.

The primary effect of TNF-α in the biological context is to combine and coordinate both innate and adaptive immune responses. However, it is important to note that this classification is primarily for educational purposes, as the reciprocal activation of these two types of immunities is so intertwined that they cannot be effectively studied in isolation.[5] At the outset, it is seen that TNF-α can enhance the activation of T and B leukocytes. Additionally, it exerts an influence on macrophages and natural killer (NK) cells by feedback chemotaxis. The cells in question, commonly referred to as antigen-presenting cells (APCs), are integral constituents of the innate immune system. The process of cell-to-cell contact gives rise to a cascade of consecutive occurrences that afterward trigger a reaction from the adaptive immune system. The immune response is predominantly modulated by T and B cells, which are essential in the production of antibodies.[6] TNF-α is known to stimulate the production of prostaglandins (PG), which in turn leads to an elevation in body temperature and the initiation of fever. It also plays a role in the upregulation of cytokines and chemokines, as well as the activation of endothelial cells.[7-10] These processes collectively contribute to vascular alterations that promote enhanced blood circulation at the site of infection.[11,12]

IL-8 is a CXC chemokine, and according to the new naming systems, it is called CXCL8 and is part of the CXC chemokine family. CXCL8 is known to attract neutrophils and other granulocytes to sites of infection. In addition to facilitating phagocytosis and angiogenesis, IL-8 also stimulates the release of histamine and the mobilization of calcium within cells. Macrophages and other innate immune cells can produce IL-8 to recruit additional immune cells to the scene. Proinflammatory IL-8 is widely known to contribute to inflammation.[13]

The aim of this study is to determine whether the levels of these cytokines increase or decrease following infection with *E. coli O157:H7* and to shed light on the cellular immune response.

Materials and Methods

Collection of samples

Blood and stool samples were obtained from 421 individuals who were admitted to Al Noor Teaching Hospital and Babylon Hospital for Pediatric and Gynecology in Hilla City due to the presence of diarrhea. Data was collected on patients aged from birth to 13 years old over the period from March to October 2022. The acquired data includes information on the patients. This information was gathered using a questionnaire that included age, gender, type of feeding, and consistency of diarrhea.

Isolation and identification of *E. coli O157:H7*

Following the collection of a fecal sample in a sterile container, the samples underwent culturing subsequent to first isolation on eosin methylene blue (EMB) agar and sorbitol MacConkey agar (SMA) which distinguish between sorbitol fermenting (SF) bacteria which appear pink colonies and non-sorbitol fermenting (NSF) pale colonies. The identity of the bacteria was subsequently validated by the use of biochemical testing.[14] Out of 421 stool samples, 91 (21.6%) were NSF. Only 30 (7.1%) isolates tested positive result for *E. coli O157:H7* depending on the results obtained from the cultivation of bacteria on the HiCrome *E. coli O157:H7* selective medium, which consisted of an agar base supplemented with cefixime tellurite agar (all media manufactured by HiMedia, India). This medium was utilized to differentiate between *E. coli O157* and *E. coli* non-*O157 STEC* strains. The incubation process was carried out aerobically at a temperature of 37°C for a duration of 24 h. The colonies exhibiting a mauve coloration were indicative of the presence of *E. coli O157:H7*, while the colonies exhibiting a blue coloration were indicative of the presence of *E. coli* strains other than *E. coli O157:H7*.

Assay for cytokines

A total of 30 patients who were infected with *E. coli O157:H7* (excluding other infections such as viral and parasite infections) and 30 healthy children participated in the study. Each participant had 3 mL of venous blood collected using labeled gel tubes. The blood samples were allowed to accumulate at room temperature for 30 min and then subjected to centrifugation at 3000 revolutions per minute (rpm) for 10 min. Following centrifugation, the serum samples were transferred to Eppendorf tubes using sterile micropipettes. These samples were then stored at a temperature of −20°C for further analysis, specifically to measure the levels of IL-8 and TNF-α.

The topic of discussion is a sandwich. The concentration of human IL-8 and TNF-α cytokines in serum samples was determined using an ELISA kit. The instructions provided by the manufacturer (BT Lab, Korea) were followed for the assay.

Statistical analysis

Data was statistically analyzed to determine the likelihood (P value) of a correlation between IL-8 and TNF-α. Statistical analysis was carried out using SPSS version 20. Continuous variables were presented as mean ± SE. An independent t test was used to compare potential differences between the two groups. One-way ANOVA and Duncan test were used to discover associations between categorical variables. The values of $P \leq 0.05$ and $P \leq 0.01$ were considered significant.

Ethical approval

The research followed the ethical guidelines outlined in the Declaration of Helsinki. The procedure was carried out after receiving the patient's verbal and analytical consent. Documented as M220105 dated 17-01-2022, a local ethics committee assessed the research protocol and the subject information and consent form before granting approval.

Results

A total of 421 fecal samples were collected in this study from children suffering from diarrhea. Of these, only 91 (21.6%) NSF colonies were cultured on chromogenic agar diagnosed as *E. coli O157:H7*, with 30 isolates (7.1%) [Tables 1–4 and Figure 1].

Discussion

Cytokines play a significant role in pathogenesis, with many researchers studying cytokines as serological markers to diagnose several infections. The TNF-α and IL-8 play an important role in the process of inflammation and various infections. TNF-α is needed for the proper function of the

Table 1: Mean difference of biomarkers between patients and control according to age group

Groups parameters	Age group	Control	Patients	P value
		Mean ± SE		
IL-8	1–3 years	75.03 ± 10.2	165.51 ± 22.1 b	≤0.0001**
	4–6 years	70.97 ± 12.6	227.56 ± 15.6 c	≤0.0001**
	7–9 years	80.58 ± 15.4	283.62 ± 17.8 d	≤0.0001**
	10–13 years	88.50 ± 16.0	70.97 ± 5.9 a	0.411
TNF-α	1–3 years	25.61 ± 2.3	130.28 ± 11.2 a	≤0.0001**
	4–6 years	24.50 ± 1.9	119.23 ± 20.3 a	≤0.0001**
	7–9 years	31.12 ± 3.4	181.28 ± 18.9 b	≤0.0001**
	10–13 years	32.50 ± 7.5	208.62 ± 28.7 b	≤0.0001**

** Significant difference at $P \leq 0.01$

Different letters refer to significant differences at $P \leq 0.05$

Table 2: Mean difference of biomarkers between patients and control according to gender

Groups parameters	Gender	Control	Patients	P value
		Mean ± SE		
IL-8	Male	86.05 ± 5.2	195.19 ± 10.4	≤0.0001**
	Female	74.58 ± 9.1	177.21 ± 6.6	≤0.0001**
P value			0.562	
TNF-α	Male	31.49 ± 2.2	159.05 ± 12.4	≤0.0001**
	Female	26.31 ± 3.7	85.35 ± 8.5	≤0.0001**
P value			≤0.0001**	

** Significant difference at $P \leq 0.01$

Table 3: Mean difference of biomarkers between patients and control according to diarrhea type

Serum conce. consistency of diarrhea	IL-8	TNF-α
Watery	192.43 ± 24.3	136.05 ± 20.4
Bloody	189.02 ± 22.5	123.80 ± 13.5
P value	0.919	0.751

* Significant difference at $P \leq 0.05$

Table 4: Mean difference of biomarkers between patients and control according to feed

Parameters feeding	IL-8	TNF-α
Breast	144.21 ± 10.3 a	95.25 ± 9.6 b
Formula	187.87 ± 19.5 b	119.93 ± 17.4 a
Food	232.19 ± 13.2 c	165.79 ± 15.5 c

Different letters refer to significant differences at $P \leq 0.05$

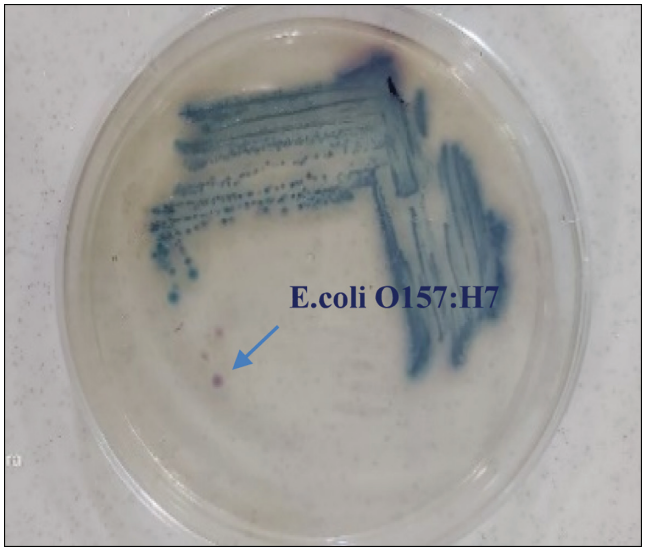

Figure 1: Growth of *E. coli O157:H7* isolates on chromogenic agar medium

immune system by regulating balance in the gut. In the present study, the serum level of TNF-α was significantly higher in children infected with *E. coli O157:H7*. Across all age groups, there was a significant difference observed when comparing the experimental group to the healthy group ($P \leq 0.01$). The highest percentage was recorded in the 10–13 years (208.62 ± 28.7). Several previous studies on other diseases have found similar to those of study.[15] Several authors have previously demonstrated that elevated systemic levels of this cytokine are present in the study.[16,17] Also, the result of this study showed a significant association between IL-8 and *E. coli O157:H7* infected children and age. The highest prevalence rate was in the 7–9 years age group (283.62 ± 17.8) followed by the 4–6 years age group (227.56 ± 15.6), with lowest in the 10–13 years age group (70.97 ± 5), as shown in Table 1. This result agreed with those recorded by the study.[18,19]

The statical analysis revealed significant difference between the serum levels of TNF-α and IL8 in infected children regarding gender with *E. coli O157:H7*. A higher level was reported in male at $P \leq 0.01$. IL-8 increased significantly higher than TNF-α in male (195.19 ± 10.4) compared to the female cytokine serum level (159.05 ± 12.4), which agrees with other studies.[20,21]

This can be explained by the exposure of males to the external environment more than females, leading to infection with pathogens by contact and a lack of attention to personal hygiene by mothers. These variations are frequently attributed to a multitude of variables, including functional and environmental influences, with hormonal origins being a significant contributor.[22] The present study detected no significant differences in sera level of IL8 and TNF-α according to the consistency of diarrhea. This result was compatible with the study.[23,24]

The relevance of IL-8 in the resolution of infections is a topic of ongoing debate, as the overexpression of IL-8 by the epithelium has the potential to exacerbate infections after neutrophil infiltration, inflammation, and other pathological processes. The observed contradiction may be indicative of the innate immune system's capacity to elicit a defensive reaction. However, it is important to acknowledge that this activation can also lead to adverse consequences, such as heightened tissue deterioration.[25]

Recent research has elucidated the contribution of cytokines in the pathogenesis of diarrheal conditions. The function of the intestinal lining epithelia is influenced by numerous cytokines, and the alteration of these cytokines during infection may exacerbate intestinal secretion, permeability, and motility, as well as induce diarrhea symptoms.[26] The findings from Table 4 indicate a reduced concentration of cytokines in breastfed infants, which aligns with the conclusions drawn by the previous researchers who observed a greater incidence of gastroenteritis in formula-fed children and those attending childcare facilities. Infants who are breastfed exhibit the development of a gut microflora that is abundant in probiotics and has less harmful bacteria compared to those who are formula-fed. This phenomenon has been regarded as one of the factors that contributes to the reduction in the incidence of infectious diarrhea among infants who are breastfed. Research findings have shown that human milk serves as a reservoir of lactic acid bacteria in the gastrointestinal tract of infants.[27-29]

Conclusion

This study found the importance of using SMA and chromogenic agar for diagnosing *E. coli O157*. The study also found an association between higher IL-8 and TNF-α production and *E. coli O157* diarrhea in all age groups, with higher levels in male compared to female. Serum cytokine concentrations were elevated in patients

with watery diarrhea than bloody diarrhea, and these cytokines were elevated in pediatric patients feeding by formula, highlighting the importance of breastfeeding in enhancing innate immunity and fighting pathogenic bacteria.

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Conflicts of interest
There are no conflicts of interest.

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