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Assessment of Liver Enzymes and Cytokines in Typhoid Fever

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ABSTRACT
Typhoid fever is one of the most prevalent diseases in developing countries with high significant morbidity and mortality rates. In this study 120 patients with typhoid fever (males and females) aged 20 to 50 years, and 60 healthy individuals in the same age as a control group, from Al-Sader teaching medical city in Najaf/Iraq, from 1stMarch 2019 to the end of September2019 to assess their liver function and pro-inflammatory cytokines (IL-6 andIL-8)levels in their serum. Mean value to serum levels of liver enzymes: alkaline phosphatase (ALP), alanine transaminases (ALT) and aspartate transaminases (AST) were (130±9.8IU/l; 31.4±5.9IU/l; 26.8±4.2IU/l), respectively in Typhoid fever patients (P<.05 for each). Patients also showed significant increase in the concentration of IL–6 (157±5.2pg/ml), IL–8 (136±7.1pg/ml), and C-reactive protein (CRP) 41±3.4 mg/l (P<0.001 for each) compared to healthy subjects 40±6.3 pg/ml; 37±5.9 pg/ml and 9±2.2 mg/l, respectively (P<.001).
Keywords: Typhoid fever, Liver enzymes, IL-6, IL-8, C-reactive protein.
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INTRODUCTION
Typhoid fever (Enteric fever) is an acute fever disease, which is a severe life-threatening disease and is considered a major health problem in the world and in many developing countries affects about 16 million Persons and causes the death of nearly 600,000 people annually [1-3].
S.typhi is the causative agent of typhoid fever. It is most frequently occurred through consumption of contaminated water or foods with feces of infected patient [4,5]. Clinical studies have shown that *S.typhi* infection stimulates both the intestinal mucosal response and humoral and systemic immune response, which have an important role in controlling and clearing *S. typhi* infection [6].
The prevalence of pro-inflammatory anti-inflammatory cytokines in typhoid patients increases their levels compared to patients with another severe disease [7].
IL-6 is a cytokine initially described as a potent catalyst for the acute phase protein. It was evident that IL-6 triggered fever when injected into rabbits and that IL-6 concentrations were associated with fever in patients with burns [8].
IL-8 is a low molecular weight protein binds to heparin and has a role in sepsis [9]. IL-8 can be produced by immune cells or by non-immune cells such as endothelial or epithelial cells. macrophages secret IL–8 within a few hours after coming into contact with bacteria, or bacterial toxins. High concentrations are isolated to IL-8 in conjunction with high levels to IL-6 [10]. Raffatella et al [11] demonstrate that S.typhi possesses virulence mechanism that induces the induction of host respondents leading to neutrophil recruitment in the intestinal mucosa, only S.typhimurium works on stimulating the release of IL-8 from the polarized cell. Abnormal liver function test in enteric fever is seen more commonly in patients presenting in 2nd and 3rd week of illness. Hepatic dysfunction is common in enteric fever. Salmonella hepatitis was seen in patients with prolonged illness and inappropriate antibiotic use [12].
Several researchers [13,14] have studied liver function in adults suffering from typhoid fever. C–reactive protein (CRP) has been described as an acute-reactive substance that is synthesized by liver cells in response to pro-inflammatory interleukin-6 and thus uses CRP as a more common biomarker [15].The present study was designed to assess liver enzymes and bacterial interaction (*S.typhi*) with pro-inflammatory human cytokines and CRP in typhoid patients.

METHODOLOGY
Sample collection and processing.
Five ml of blood samples were withdrawn from 120 patients with Typhoid fever (male and female) aged 20 to 50 years (mean age:41.3±14.3),and 60 healthy individuals in the same age(mean age:39.3±14.4) as a control group, using disposable syringes. From all patients were included from Al-Sader teaching medical city in Najaf/Iraq, from 1stMarch 2019to the end of September 2019. The collected blood was allowed to clot at room temperature for 30 min, then centrifuged for 5 min.at 3000 rpm. Sera were separated into sterile tubes and stored at -20 C° in order to calculate liver enzymes and cytokines.

Liver enzymes measurement.
Liver function assays which were conducted includes; aspartate transaminase(AST), alkaline phosphatase (ALP),and alanine transaminase (ALT).We performed liver function assays were carried out on automated analyzer Automatic Hitachi/ERBA/ARCHETEC according to manufacturer company.

Cytokines measurement.
IL-6 and IL-8 levels in patient serology and controls were measured using the enzyme-linked immunosorbent assay (ELISA) kits (Sigma, USA). Both cytokines were done according to kit protocol described by company. The detection limit for both IL-6 and IL-8 was 20 pg/ml and 45

pg/ml, respectively, and the normal values were below the detection limit. All samples from the same patient were analyzed in the same range in two versions to reduce analytical errors.

Principle and calibration curve of cytokines.

The wells of micro titer plates were coated by specific antibodies to human cytokines. Human cytokine samples and standards are pipette into the wells to binding to the coated antibody. After washing, an enzyme-linked ab specific to human cytokine is added to the well. Substrate solution is added to the wells after washing, as the color develops according to the amount of cytokine. The reaction was stopped (color development) by adding the suspension solution and reading the partial titer plate (color intensity measurement) at the appropriate wavelength, as the absorption is proportional to the concentration of cytokines.

IL-6 and IL-8 standard preparations.

All preparations were thoroughly and warmly mixed at room temperature prior to use. 55µl diluent buffer was reconstituted for lyophilized Human protein Standard (55ng) for a final concentration of 1ug/ml. A 275ul diluent buffer was reconstituted for lyophilized antibody detection (2.75µg) to a final concentration of 5ug/ml. The reagents and the ELISA protocol were prepared according to the manufacturing company (Sigma, USA). ELIZA readers are computer programs capable of generating a standard curve. A standard curve generated for each set of samples.

C-reactive proteins (CRP) Measurement.

Use the (Latex Test Kit) to perform this test according to [16] by testing the suspension of latex particles coated with anti-human CRP antibodies against unknown serum.

Statistical analysis.

For the statistical analysis of the results, it was shown as Means ± SD. Data were analyzed using SPSS (T-test) version 17 and microsoft excel computerized programs and taking P-value (p<.05) and (P<.001),P< .05 was considered significant.

RESULTS

The results of the investigation of patients with typhoid fever in correlation with liver function are presented in Table 1. The result data were suggested that there was a significant increase (P<.05) occurs in the serum level of liver enzymes ALP, ALT and AST with mean value (130±9.8, 31.4±5.9, 26.8±4.2 IU/l), respectively compared with control group.

Concentrations of pro-inflammatory cytokines (IL-6and IL-8) and CRP were significantly elevated in the serum of patients with typhoid fever compared to the control group as shown in Table 2.

DISCUSSION

Abnormal liver function tests indicate hepatic involvement, one of them caused by typhoid fever. In the present investigation, we observed abnormal liver function tests and elevation of ALP, ALT and AST with an average value of twice more than usual (130±9.8; 31.4±5.9; 26.8±4.2 IU/l), respectively Table 1.These findings are consistent with a number of studies that reported a significant increase in the rate of transaminases and alkaline phosphatase in all cases in the 2nd and 3rd week of the disease, as well as mild elevations in the levels of these enzymes in the first week [12,17].

High level of liver enzymes were also reported in patients suffering from typhoid fever in many studies [15,18,19]. Haleem et al [14] demonstrated the correlation between liver enzymes and disease progression, and concluded that among liver enzymes, ALP showed a higher level in typhoid status compared to malaria and dengue. They also noted that other enzymes such as ALT, AST, TB and CB are predominant in the case of dengue infection. In other wise, Gitin [20] illustrated the transminase elevation will be usually more than 1000 U/L with elevated bilirubin levels in patients with viral hepatitis. These features can make us easily differentiate between salmonella hepatitis and viral hepatitis.

The rise of transminase along with alkaline phosphates indicates involvement of hepatic disorder which may be secondary to endotoxin effect on hepatic cells causing edema and biliary stasis [21]. Damor et al [22] concluded that values of serum ALT is more raised in patients of viral hepatitis as compared to those of enteriche patitis, While the serum levels of LDH are more raised in enteric hepatitis as compared to viral hepatitis and serum ALT:LDH ratio along with clinical parameters can help for early diagnosis of enteric hepatitis.

To study the interaction of *S. typhi* with human cytokines (IL-6, IL-8) and C-reactive protein were analyzed by EALSA and the Latex Titer kit. Interleukin IL-6 is an important mediator in the host's response to the disease and has been proposed based upon circumstantial evidence, being a major endogenous pyrogen is responsible for activating CNS mechanisms in fever during infection and inflammation [8].

The results in Table 2, indicate the higher concentrations of pro-inflammatory cytokines IL-6 and IL-8 in typhoid patients compared to healthy subjects (p<.001 for both).These results are similar to those recorded by other researchers[15,23]. Also, Hamada et al [24] reported that the concentration of IL-8 and IL-6 in typhoid fever patients was higher than in healthy individuals (P>.0001). Furthermore, Al-Dahhan [2] and Muhammed Ali [25] reported that concentration of IL-6 was significantly raised in all groups of typhoid fever in comparison to healthy control. During the acute phase of infection, IFN-γ is significantly induced in addition to increasing IL-6, IL-8, IL-10, IL-15, and TNF-α. However, the number of white blood cells for paratyphoid fever patients does not increase significantly during the acute phase compared to other infectious diseases [26].Interleukin-6 is the only inflammatory cytokine that can be measured in large quantities in blood circulation during fever [23]. Also, Cartmell et al [8] noted that the increase in rats temperature was accompanied by a significant increase in

the concentrations of IL-1 and IL-6 at the site of inflammation, but only IL-6 in circulation and cerebrospinal fluids.Patients with typhoid fever have persistent fever, Hence, pyrogenic cytokines would be expected tobe present in the circulation during the acute phase of the disease[23].The cytokines IL–6 is an important medium for the markers and symptoms of infectious diseases [27]. Yamashita et al [28] they noted that the average concentration of plasma cytokine (IL-6) was significantly higher in patients, demonstrating the predictive value of clinical outcomes of treatment in typhoid fever. The results of the present study indicate that IL-8 levels are higher in patients (136±7.1) compared to the control group (37±5.9) Table 2. These results are consistent with the results of previous studies [5,23].This cytokine is considered non-hormonal. Since, Van Damme [29] detected concentrations of IL-8, but this cytokine is considered non-pyrogenic. IL-8 has the ability to induce an oxidative explosion, leading to the formation of oxygen radical species, it has recently been shown that IL-8 is a potent catalyst of cytochrome C type III in neutrophils [9].

C-reactive protein production is part of the nonspecific acute phase response to most forms of inflammation, infection and tissue damage [30].CRP regulates its synthesis in the liver by pro-inflammatory cytokine (IL-6), showing a significant increase in serum concentration of patients with typhoid fever compared to the control group (P<.001) Table2.These results are consistent with those of other researchers [15,23]. Therefore, CRP was described as an acute phase reactant synthesized by hepatocytes in response to inflammatory cytokine(interleukin-6)[15].On the other hand, Venugopal et al[31] indicated that CRP may have a causal role in vascular disease and thus be a target for therapy. Moreover, elevated levels of CRP lack specificity, for example, acute and chronic infections, smoking, acute coronary syndromes, and active inflammatory states that are often associated with high levels of this protein. Typhoid is still an outstanding unresolved problem and poses a major health risk in many developing countries in the world. The development of this disease is often associated with elevated liver enzymes such as ALP, AST and ALT.

Table 1. Serum levels of three liver enzymes in patients with typhoid fever and control group.

Parameter	Patient (n=120) Mean ± S.E.	Control (n=60) Mean ± S.E.	P-value
ALP(IU/l)	130±9.8	25±92.0	<.05
ALT (IU/l)	31.4±5.9	3 ±18.0	<.05
AST(IU/l)	26.8±4.2	6 ±21.0	<.05
ALP, alkaline phosphatases;ALT, alanine transaminases;AST, aspartate transaminases; Mean value±SD.			

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Table 2. Concentration of pro-inflammatory cytokines (IL-6and IL-8),and CRP in patients with typhoid fever and control group.

Parameter	Patient (n=120) Mean ± S.E.	Control (n=60) Mean ± S.E.	P -value
IL-6 pg/ml	157±5.2	40±6.3	<.001
IL-8 pg/ml	136±7.1	37±5.9	<.001
CRP mg/l	41±3.4	9±2.2	<.001

CONCLUSION

This study showed the relationship between elevated liver enzymes than normal range is considered abnormal and progression of the disease. If not properly treated, these enzymes cause severe liver damage. In addition, typhoid patients showed significant elevated in the concentrations of pro-inflammatory cytokines and C-reactive protein, and also, abnormal liver function test in enteric fever.

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