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Study of Some Proinflammatory Cytokines and Biochemical Parameters in Patients with Typhoid Fever in Salah Al-Din Governorate

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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 150 patients with typhoid fever (males and females) aged 15 to 55 years, and 20 healthy individuals in the same age as a control group, from salahaldin hospital in salahaldin-tikrit/Iraq, from 1stAugest 2020 to the end of february 2021 to assess pro-inflammatory cytokines (IL-6 andIL-8)levels and biochemical parameters in their serum. Patients showed significant increaseCompared with the control group in the concentration of IL–6 (18.9±4.0pg/ml), IL–8 (34.5±7.3pg/ml). Mean value to serum levels of liver enzymes: alkaline phosphatase (ALP), alanine transaminases (ALT) and aspartate transaminases (AST) were (35.2±9.2IU/l; 18.4±3.0IU/l; 57.9±4.2IU/l), respectively in Typhoid fever patients (P<.001)

Keywords: Typhoid fever, Liver enzymes, IL-6, IL-8

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] In some areas, it has been estimated that typhoid fever is responsible for 2 to 5% of all deaths. Hospital case fatalities are still high (1 to 30%), in many parts of the developing world1 . In the US, the incidence was 400 cases/year, mostly among travelers2 . In Egypt, estimated the incidence of typhoid fever to be 13/100000 persons

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].S. typhi, the etiologic organism of typhoid fever, is similar to other Salmonellae in that it is a gram-negative, flagellated, non-capsulated, nonsporulating, facultative anaerobic bacillus. It has a somatic (O) antigen (oligosaccharide), flagellar (H) antigen (protein), and an envelop (K) antigen (polysaccharide); and has a lipopolysaccharide macro-molecular complex called endotoxin that forms the outer portion of the cell wall[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

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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]. [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 IL8 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. The present study was designed to assess liver enzymes and bacterial interaction (S.typhi) with pro-inflammatory human cytokines and typhoid patients.

Methodology: Sample collection and processing. Five ml of blood samples were withdrawn from 150 patients with Typhoid fever (male and female) aged 15 to 55 years (mean age:41.3±14.3),and 20 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 Salahaldin hospital in Salahaldin-Tikrit/Iraq, from 1 stAugest 2020 to the end of March 2021. 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 Co in order to calculate 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 (Kombotech/china). 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 45pg/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

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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. The reagents and the ELISA protocol were prepared according to the manufacturing company (Kombotech/china). ELIZA readers are computer programs capable of generating a standard curve. A standard curve generated for each set of samples

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 ($p<.001$),$P< .05$ was considered significant.

Result:

The results of the investigation of patients with typhoid fever in correlation with liver function are presented in figure(1-3),(1-4),(1-5). 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

(35.2±9.2, 18.4±3.02, 57.9±4.2 IU/l), respectively compared with control group.Concentrations of pro-inflammatory cytokines (IL-6and IL-8) were significantly elevated in the serum of patients with typhoid fever compared to the control group as shown in figure (1-1),(1-2).

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 (35.2±9.2, 18.4±3.02, 57.9±4.2 IU/l), respectively (1-3),(1-4),(1-5).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

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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) were analyzed by EALSA 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 figure(1-1),(1-2) indicate the higher concentrations of pro-inflammatory cytokines IL-6 and IL-8 in typhoid patients compared to healthy subjects (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. 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]. The immune system slows down the rate of multiplication of disease germs to give the acquired immune system time to stimulate, so infection with S. typhi usually confers a certain degree of humoral immunity Ouyang 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 (34.5±7.3) compared to the control group (4.8±2.0) figure(2-2).. These results are consistent with the results of previous studies [5,23].This cytokine is considered non-hormonal. Since, Andrew et al [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].

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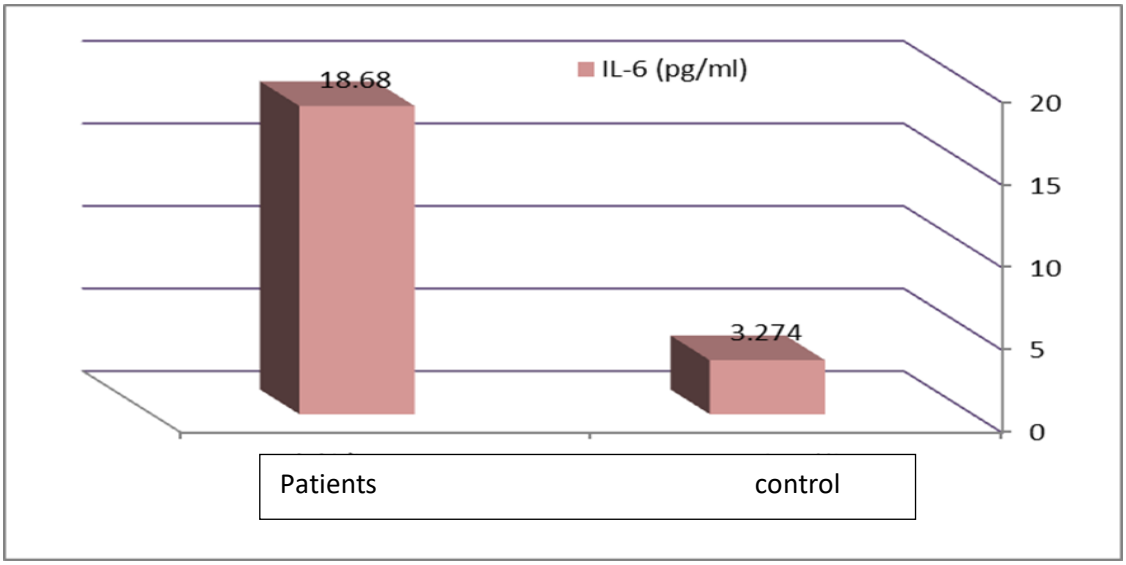

Figure (1-1) shows the comparison in measuring the average IL-6 concentration between typhoid patients and healthy controls

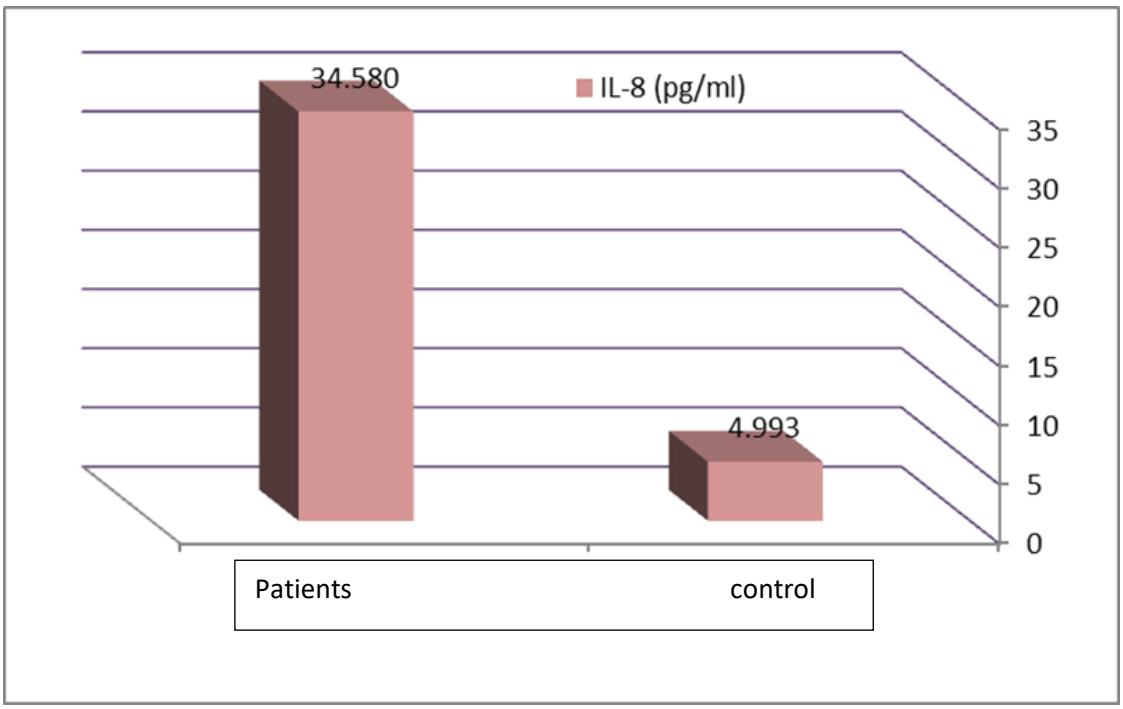

Figure (1-2) shows the comparison in measuring the average concentration of IL-8 between typhoid patients and healthy controls

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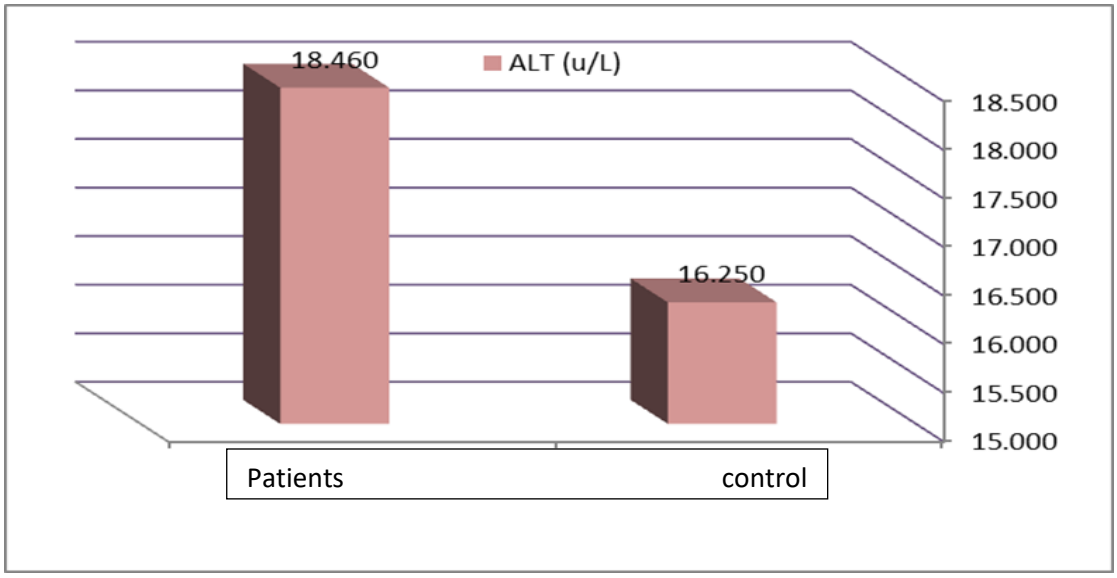

Figure (1-3) shows the comparison in ALT measurement between typhoid patients and healthy controls

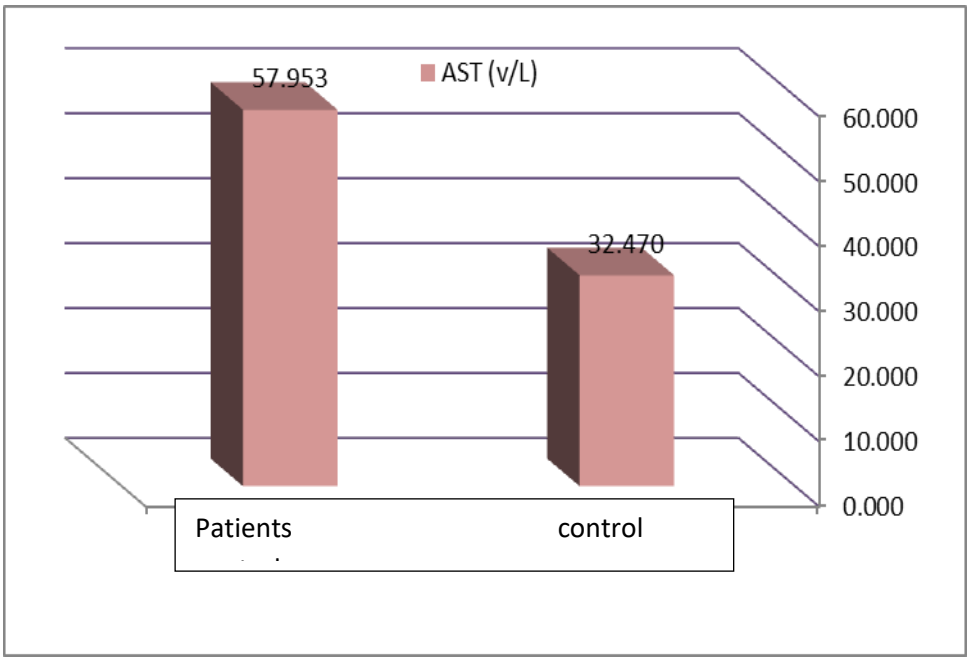

Figure (1-4) shows the comparison in AST measurement between typhoid patients and healthy controls

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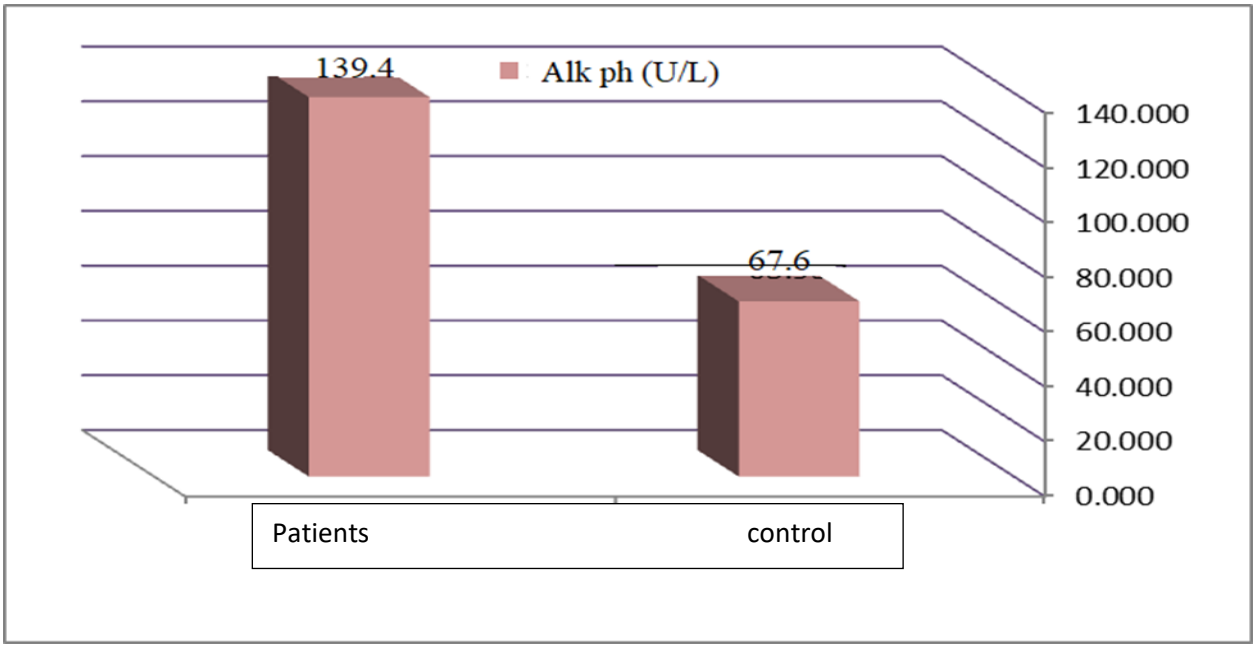

Figure (1-5) shows the comparison in ALK measurement between typhoid patients and healthy controls

Consulation

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 also, abnormal liver function test in enteric fever.

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