Study of Biochemical Changes and Elevated Levels of Enzymes in *Salmonella typhi* Infected Patients in Pakistani Population

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Abstract: *Typhoid fever causes significant biochemical changes and hepatic complications. As many studies have indicated several biochemical parameters that are involved in developing the risk of typhoid fever. The current study was designed to evaluate these risk factors in general Pakistani population. Serum biochemistry and liver enzymes were studied to investigate the relationship of these risk factors to Typhoid fever. Total 100 subjects were studied, 50 healthy individuals and 50 typhoid patients. Blood samples were collected from Allied and National Hospital, Faisalabad, Pakistan. In this study, Nested PCR was used to test the samples. Elevated level of ALT (P < 0.0001) and AST (P < 0.0001) were observed in typhoid patients. Typhoid patients had significantly higher concentrations of Triglyceride (P = 0.0044), Globulin (P = 0.0004) and Total protein (P = 0.0978) while LDL (P = 0.0197), Albumin (P < 0.0001), Glucose (P = 0.0006), HDL-cholesterol (P < 0.0001) and Cholesterol (P = 0.04) were significantly lower than those of healthy individuals. This study appears to be ample evidence based on the physiological and biochemical parameters in typhoid patients to explain influence of typhoid morbidity. Extensive research in this field would enable us to make modern drugs to treat typhoid fever patients.*

Keywords: *Biochemical, Typhoid fever, Hepatic enzyme.*

Introduction

Typhoid fever is a systemic disease caused by *Salmonella typhi*, affecting only humans [5]. In humans and animals, *Salmonella* infections are a significant cause of morbidity and mortality [19]. Modern literature on human typhoid that includes immunological and molecular techniques is limited. The knowledge of the pathogenesis of *S. typhi* is very limited because *S. typhi* infects only humans, resulting in a lack of virulence assays [9]. Typhoid fever is transmitted by contaminated food and water by feces and urine of patients and carriers [5].

Typhoid fever is endemic in developing countries and may cause very different clinical findings. Although hepatic involvement and abnormal liver function tests may be seen in 50% of the patients [18]. The current study was designed to evaluate these risk factors in general Pakistani population. This study was undertaken to determine the frequency and severity of the biochemical changes and hepatic dysfunction in typhoid patients. Extensive research in this field would enable us to make modern drugs to treat typhoid fever patients.

Materials and methods

Sample collection

A total of seventy (70) blood samples were collected between 06-09 h in the morning in a gel and heparin (anticoagulant) tubes from normal and typhoid infected patients. Samples were centrifuged at 769 × g for 15 minutes. Serum was separated and stored in to a small aliquot at –20°C till analysis. Samples with anticoagulant using heparin (1%) were also taken for PCR.

Place of sample collection

The blood samples of male and female patients were collected from Allied and National Hospital, Faisalabad. Informed consents were obtained from all subjects. Those typhoid patients who visited the hospital for checkup and were picked up randomly from in and around Faisalabad rural areas. Age, gender, weight, height, blood pressure, fever or vomiting was recorded. A complete physical examination of each child and adult/old individual was performed by one of the physician attending the patients.

Research station

The samples were analyzed in duplicate for various tests in the Human Enteric Laboratory of the Department of Health, National Institute of Biotechnology and Genetic Engineering, Faisalabad.

Analysis of samples

Serum glucose test was performed by commercially available kit (Chemhouse). Cholesterol was determined by commercially available kit (Lot.Q115 Ref.4248). Triglycerides were determined by Human kit (Lot.11001 Ref.10720P). HDL-and LDL-cholesterol were determined by commercially available kits (Cat No. D1H20-400, Diasis Diagnostics). Serum proteins, albumin and globulin were determined by using Crescent (Cat No. CS 610, Crescent Diagnostics) kits. ALT and AST were determined by using commercially available kit (Human Gmbh 65205 Wiesbaden Germany). All tests were performed in triplicate following strict external and internal quality control protocols.

PCR diagnosis

PCR was performed by the protocol of Haque et al. [11].

PCR conditions

For regular PCR, master mixer was 20 µL and sample added 5 µL (Table 1). T-buffer was added to make up the volume. Using a thermal cycler, the reaction mixture was subjected to 32 cycles (Table 2).

Table 1. Master mixer for PCR

Mixture	Volume
Taq buffer	2.5 µL
Mgcl2	1.5 µL
dNTP	0.75 µL
S1(Primer)	1 µL
S2(Primer)	1 µL
Taq Polymerase	0.2 µL

Table 2. PCR cycles and timings

Temperature	Time	Cycle
94°C	5 min	1 cycle
94°C	1 min	
50°C	1 min	30 cycles
72°C	1 min	
72°C	5-7 min	1 cycle

For nested PCR, conditions were the same, except that amplified product was used as template. S3 and S4 primers were used in nested PCR.

Detection of PCR products

A reaction mixture of 10 µL was fractionated electrophoretically in 1.5% agarose gel and 0.5X TBE buffer 80 µL containing 1 µL of ethidium bromide per ml, and was photographed by using Eagle Eye (Strategene).

Statistical analysis

Analysis of variance was applied to determine the difference between groups. Graphpad Instat version 3.0 was applied to test the difference between means. Results were expressed as mean ± standard deviation. Paired sample t-tests were used for comparisons of variables between typhoid and healthy individuals.

Results

The detection of *S. typhi* in human blood sample was confirmed by nested PCR.

In this research, only PCR positive samples were included. Bright band showed positive sample while no band showed negative sample. Fig. 1 shows that all the samples were positive.
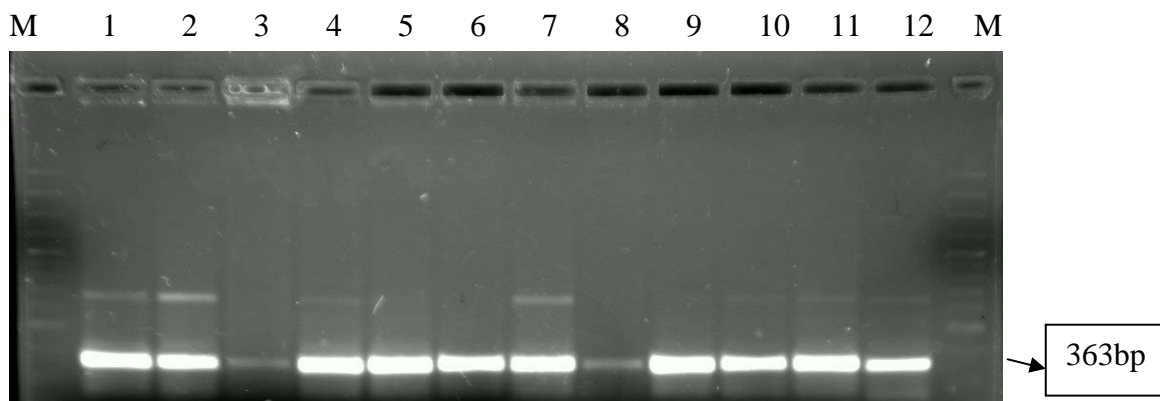

Fig. 1 PCR based confirmation of *Salmonella Typhi* fliC gene
Lane M: DNA Ladder (Fermentas SM 0323) showing bands of 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100 bps.
Lane 2-12: Positive samples showing the 363 bp product.
Lane 1: Showing positive control.

Mean anthropometric parameters of normal and typhoid infected patients with gender and age have been done. Body weight, body mass index and blood pressure of normal and typhoid infected individuals with age and gender has been analyzed. Body weight of individuals was significantly different with age, gender and groups. Typhoid infected, individuals did show a significantly low body weight as compared to normal individuals. Mean body mass index was significantly lower ($P < 0.0001$) in individual suffering from typhoid as compared to normal individuals.

Mean systolic blood pressure was significantly lower ($P < 0.0001$) in typhoid infected individuals as compared to that of normal (Table 3). The mean systolic blood pressure was lower in children as compared to young typhoid infected individuals. Mean diastolic blood pressure was lower in typhoid infected individuals as compared to that of normal individuals.

Mean body temperature was higher in typhoid infected individuals as compared to normal individuals.

Table 3. Serum Anthropometric profiles of normal and typhoid individuals

Serum Anthropometric profiles of normal and typhoid individuals			
Parameter	**Normal**	**Typhoid**	***P***
Weight (kg)	78.5 ± 1.09	61.55 ± 0.78	< 0.0001
Systolic BP (mm/Hg)	106.6 ± 0.71	136.83 ± 3.97	< 0.0001
Diastolic BP (mm/Hg)	87.17 ± 0.6	75.55 ± 2.9	0.0003
Temperature (°F)	98.6 ± 0.30	98.93 ± 0.33	0.02

Mean serum glucose concentration was significantly lower ($P = 0.0006$) in typhoid infected individuals as compared to normal individuals. The mean glucose levels were 82.8 ± 0.07 in typhoid patients and 172.6 ± 7.5 in normal individuals (Fig. 2(a)). Mean serum cholesterol was lower in typhoid patients as compared to normal individuals Fig. 2(b). The mean cholesterol levels were 145 ± 0.02 in typhoid patients and 176.7 ± 1.70 in normal individuals.
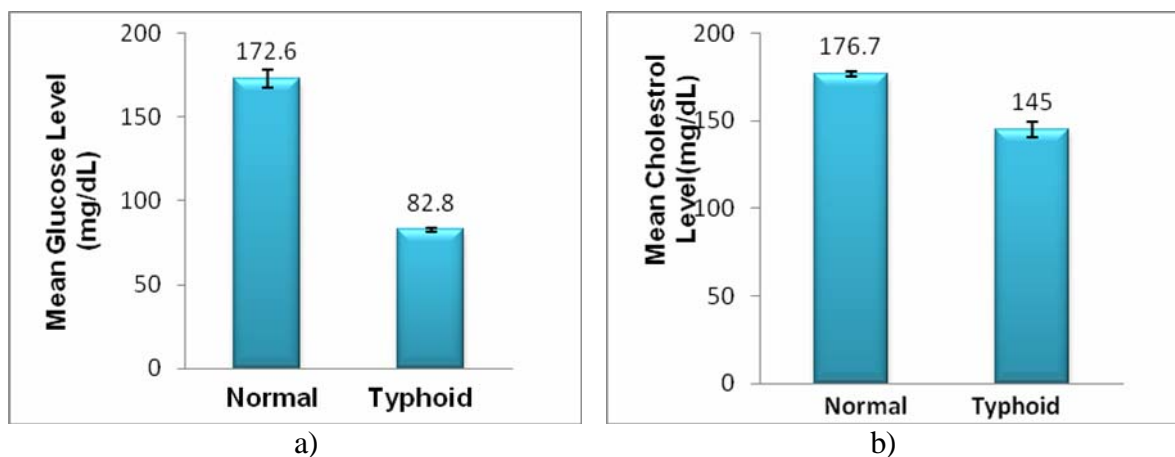

Fig. 2 (a) The mean serum glucose (mg/dL) concentration of normal and typhoid individuals. (b) The mean serum cholesterol (mg/dL) concentration of normal and typhoid individuals.

The mean triglyceride levels were 160.6 ± 5.67 in typhoid patients and 115.1 ± 6.53 in normal individuals (Fig. 3(a)). The mean serum triglyceride was higher in typhoid infected individuals as compared to that in normal individuals. The mean HDL-cholesterol levels were 35.93 ± 2.3 in typhoid patients and 49.3 ± 0.7 in normal individuals (Fig. 3(b)). A significant decrease in mean serum high density lipoprotein was observed in typhoid individuals as compared to normal individuals.

Mean total serum proteins were significantly higher ($P = 0.09$) in female child as compared to male child (Table 4). In serum total proteins was high in typhoid patients as compared to that in their normal counterparts. Mean serum albumin did differ significantly between age and age x gender interaction however; serum albumin concentration was low in young female as compared to young male. Serum albumin concentration was significantly higher ($P < 0.0001$) in male child compared to female child and mean albumin level was low in typhoid patients as compared to that

in normal individuals (Fig. 4). Serum globulin concentration was higher in typhoid patients as compared to that of normal individuals.
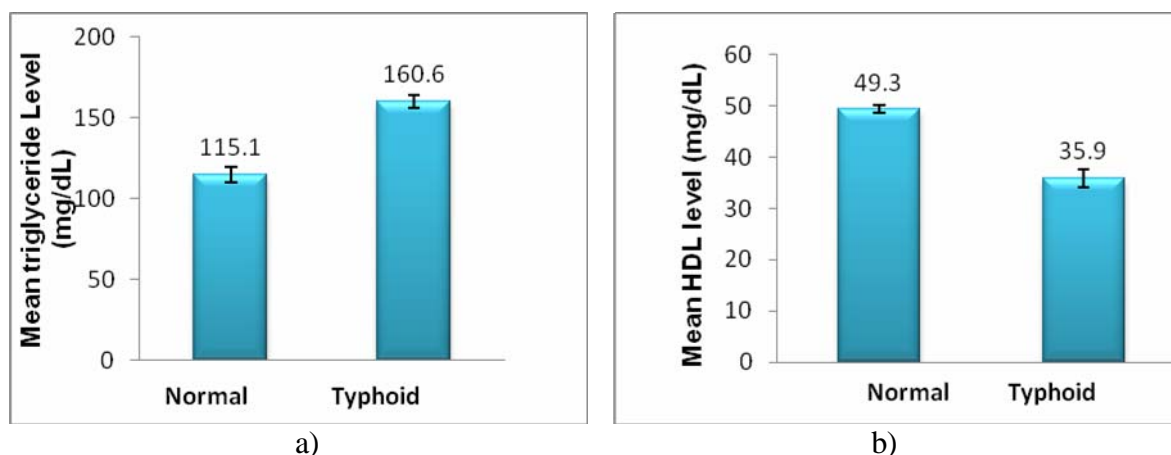

a)

b)

Fig. 3 (a) The mean serum triglyceride (mg/dL) concentration of normal and typhoid individuals. (b) The mean serum HDL Cholestrol (mg/dL) concentration of normal and typhoid individuals.

Table 4. Serum biochemical profiles of normal and typhoid individuals

Serum anthropometric and biochemical profiles of normal and typhoid individuals			
Parameter	**Normal**	**Typhoid**	**P**
BMI (kg/m²)	23.55 ± 1.2	19.15 ± 0.4	< 0.0001
Glucose (mg/dl)	172.6 ± 7.5	82.92 ± 0.07	0.0006
Cholestrol (mg/dl)	176.7 ± 1.70	153.9 ± 0.02	0.0473
Triglyceride (mg/dl)	115.1 ± 6.53	160.6 ± 5.67	0.0044
HDL-cholestrol (mg/dl)	49.3 ± 0.7	35.93 ± 2.3	< 0.0001
LDL-cholestrol	110.28 ± 5.50	75.05 ± 8.5	0.0197
Total protein (g/dl)	7.82 ± 0.11	8.65 ± 0.38	0.0978
Albumin (g/dl)	5.74 ± 0.08	4.09 ± 0.16	< 0.0001
Globulin (g/dl)	2.08 ± 0.12	4.52 ± 0.32	0.0004
AST (U/L)	50.50 ± 0.7	55.24 ± 0.14	< 0.0001
ALT (U/L)	47.49 ± 0.21	56.40 ± 1.9	< 0.0001

a)   b)
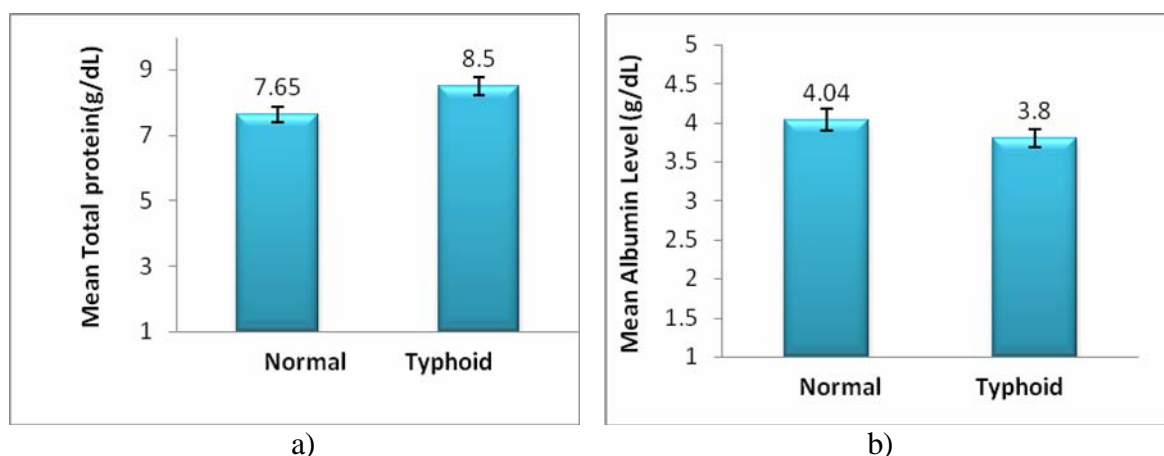
Fig. 4 (a) The mean serum total protein (g/dL) concentration of normal
and typhoid individuals. (b) The mean serum albumin (g/dL) concentration
of normal and typhoid individuals.

Mean serum alanine aminotransferase (ALT) concentration was higher in typhoid infected individuals as compared to control individuals. Mean serum alanine aminotransferase concentration was significantly higher in female as compared to male typhoid patients. The mean ALT levels were 56.40 ± 1.9 in typhoid patients and 47.49 ± 0.21 in normal individuals (Fig. 5(a)).

Mean serum aspartate aminotransferase (AST) concentration was higher in typhoid individuals as compared to that of normal individuals. Mean serum aspartate aminotransferase concentration was significantly higher in female as compared to that of male individuals. The mean AST levels were 55.24 ± 0.14 in typhoid patients and 50.50 ± 0.7 in normal individuals (Fig. 5(b)).

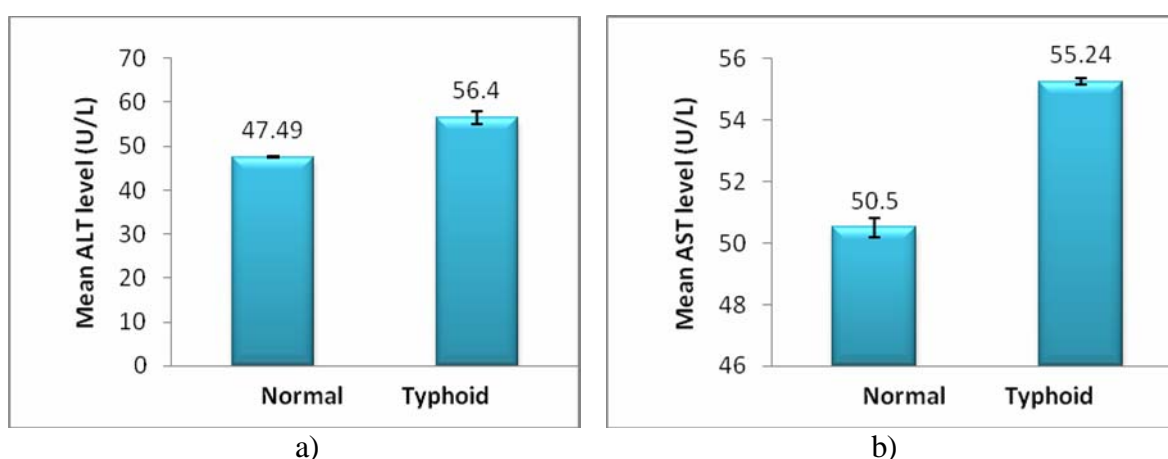a)   b)

Fig. 5 (a) The mean serum ALT (U/L) concentration of normal and typhoid individuals.
(b) The mean serum AST (mg/dL) concentration of normal and typhoid individuals.

Discussion

S. typhi is the most wide spread pathogen in developing countries and can lead to fatal, if left untreated. One of the most important health problems in Pakistan and other developing

countries is Enteric Fever [13]. Individuals at either end of the age spectrum (neonates and the elderly) are at increased risk of bacterial infections [4].

Body weight as well as Body mass index (BMI) was significantly low in typhoid fever as compared to normal healthy individuals. Children that were suffering from typhoid fever did show low body weight, blood pressure, BMI, and high body temperature as compared to young male or female that was suffering from typhoid. Thinness due to an inadequate caloric intake is a serious condition, especially in young people. Diseases like typhoid fever are quite common among them [2]. Being underweight or lean was independently associated with having low blood pressure, less body mass index and decrease serum cholesterol concentration. In case of report of typhoid fever, the blood pressure of patients was lower from normal range [6, 10-11]. In typhoid fever, Physical examination upon second admission revealed a blood pressure of 110/70 mm/Hg [22]. In typhoid fever BMI, body weight, blood pressure are significantly lower because of repeated episodes of illness, limited and poor quality of food. *S. typhi* requires glucose in macrophages for replication so level of glucose decreases in the body and due to this blood pressure, body weight and body mass index also decreases.

Blood glucose level is an essential part of our body. Serum glucose concentration was significantly lower in typhoid patients as compared to that in their normal healthy individuals. Hypoglycemia has rarely been described as a clinical sign of severe bacterial sepsis [17]. Plasma glucose level increased with age indicating 0.15 mmol/L increase in fasting and 0.26 mmol/L increase in 2 hour post-prandial plasma glucose level [16]. Glucose level was significantly lower ($P = 0.0006$) in typhoid patients as compared to normal individuals. *S. typhi* requires glycolysis and glucose for successful infection of macrophages that cause infection. By this glucose level is low in typhoid fever patients.

No significant difference was observed in serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride among healthy and typhoid patients. Mean serum cholesterol level did decrease significantly, however, cholesterol was significantly lower ($P = 0.04$) in male child as compared to that of female child and male young from female young. In our results range of cholesterol was 140 - 167. Low level of cholesterol may be due to low intake of food or low fat in the diet which eventually led to deficiency of cholesterol [7, 12]. This decrease may also be attributed to malabsorption of fat from intestine. The decrease in the level of total cholesterol concentration in typhoid patients [14] in which low levels of HDL-cholesterol where observed in enteric fever patients [8].

Severe and protracted hypertriglyceridemia, decrease in HDL-cholesterol levels and increase in LDL-cholesterol is observed in patients with enteric fever at the peak of fever [15]. In our results, HDL level was decreased in typhoid infected individuals as compared to that of normal individuals. Mean serum triglyceride was high in typhoid infected individuals as compared to that of normal individuals. Typhoid fever is associated with inflammation and ulceration of the gut and liver of which oxidative lipid modification plays an important role in exacerbating the disease.

Serum total protein was significantly higher in typhoid fever patients irrespective of their gender. In young and old individuals serum total proteins were higher in typhoid patients as compared to those of their normal counterparts. Total proteins did not differ significantly in children with acute diarrhea. Hypoproteinemia in children with diarrhea is due to the enteric

protein loss and probably is the result of changes in the integrity of the small intestinal mucosa [20]. In the present study the proteins loss may be due to intestinal infection.

Serum albumin was found to be low in typhoid infected patients [1]. Serum albumin level is observed low in typhoid patients but no significant difference is present Mean serum albumin did differ significantly between age and age x gender interaction; however, serum albumin concentration was low in young female as compared to that of young male. Serum albumin concentration was reported to be lower during infection [3]. Mean serum globulin concentration was high in young male and female as compared to that in their old individuals. Typhoid patients had significantly higher ($P = 0.0004$) serum globulin concentration in old individuals. Due to inflammatory conditions the level of globulin is high in typhoid fever patients.

The concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is for detecting hepatocellular injury and may help in monitoring the status of liver. Both enzymes increased in many hepatic diseases and have limited value in differential diagnosis. However, aminotransferases are considered useful in differentiating hepatocellular from cholestatic forms of liver injury. AST activity is related to damage of cell in kidney, pancreas and erythrocytes. ALT and AST were significantly higher ($P < 0.0001$) in some typhoid patients. In general, mechanism relating to association between liver marker and in typhoid fever may reflect elevations in ALT and AST.

Conclusion
This study appears to be ample evidence based on the physiological and biochemical parameters in typhoid patients to explain influence of typhoid morbidity. Typhoid Fever causes high incidence of biochemical and enzymatic changes but these changes are cover up by antibacterial therapy.

Acknowledgements

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