

Effect Of Some Enzymes Activity In Liver Diseases From Patients Of Salmonella Paratyphi A With Iraqi Woman.

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Received in : 25 April 2010

Accepted in : 8 February 2011

Abstract

This present study demonstrated that liver was involved in 14 %of typhoid patients manifesting with hepatomegaly. Elevation of serum enzymes in typhoid fever was presumably of a muscular origin, while elevation of liver enzyme was relatively less common.

This study was performed on 30 female patients diagnosed by ultrasound (US) of abdomen, with paratyphoid A, ranged between (20-40) years compared with 30 healthy control .Patients volunteers were treated with appropriate antibiotics for 14 days and investigations were repeated 2-3 week after completion of treatment. Patients had clinical and biochemical evidence of hepatic dysfunction. The spectrum of hepatic involvement included hepatomegaly , jaundice, derangement of various hepatic functions and abnormal US abdomen.

The results revealed a highly significant values increase in serum AST and ALT compared to control was found while a significant increase of serum ALP compared to control was noticed and a significant decrease in serum LDH compared to control was obtained.

Key Words: Enzymes Activity , Liver Diseases and Salmonella Paratyphi A

Introduction

Salmonella paratyphi A tends to produce an illness clinically to produced by S.typhi with prolonged fever and a tendency to relapse .This is the commonest paratyphoid fever in India and Asia[1] .There are three species of salmonella that cause paratyphoid salmonella paratyphi A, S.paratyphi B (or S .Schotmulleri) and S.paratyphi C(S.hirschfeldil) [2].Salmonella Typhi can specifically only attack humans,So the infection nearly always comes from contact another human, either an ill person or a healthy carrier of the bacterium.

The bacterium is passed on with water and foods and can withstand both drying and refrigeration but by keeping food refrigerated correctly this minimizes the production of the bacterium .Significantly unclean food from street vendors and flooding help distribute the disease from a person to person[3].Because of poverty and poor hygiene and sanitary conditions the disease is more common in less-industraized ,principally owing to the problem of unsafe drinking water ,inadequate sewage disposal and flooding [4].

Paratyphoid fever is caused by any of three strains of salmonella paratyphoid [5,6]. Paratyphoid fever is found in large part of Asia, Africa, Central and South America. There are 16 million cases a year ,which result in a bout 25,000 daths world wide[7] Paratyphoid fever resembles Typhoid fever but presents with a more abrupt onsent,milder symptoms and a shorter course. Infection is charactetrized by a sustained fever

,headache,abdominal pain, malaise,anorexia, a non production cough(in early stage of illness),a relative Brady cardia(slow heart rate),and Hepato

Splenomegaly(an enlargement of the liver or Spleen).In adults,Constipation is more common than diarrhea only20 % to 40%of people will initially have abdominal pain[8].

After ingestion of contaminated food or water,S.typhiparatyphi bacilli reach the small Intestine, penetrate the mucosa and then remain viable within the macrophages which ingest them. The bacilli reach the blood stream via the lymphatic system, and are then disseminated to the organ of the reticuloendothelial system within the first 24 hours of infection .After the incubation period(3 days-1-months), usually 8-14 days which is affected by the infective dose, the clinical syndrome appears[1].

Fulminant hepatic is a dramatic clinical syndrome characterized by massive necrosis of liver cells[9]. Although salmonella hepatitis is a rare presentation of typhid fever, fulminant hepatic failure is extremely uncommon, few cases were repoted with such presentation the first reported case of fulminant hepatic failure in the state of Qater, associated with salmonella paratyphi A infection.Enteric fever, that is typhoid and paratyphoid fever , is the common name for infections caused by salmonella enterica serotypes typhi and paratyph. Of the three types of S. paratyphi (A,B and C),B is the most common[10].

Alanine Transaminase (ALT)Serum is present in high concentration in liver and to a lesser extent in skeletal muscle ,kindney and heart [11].

Alkaline phosphatase(ALP) are a group of enzymes with hydrolyse phosphates at an enzymes which hydrolyse phosphates at alkaline PH.The activity measured by routine methods includes that of several isoenzymes. They are found in bone, liver, kidney ,intestinal wall, Lactating mammary gland and placenta. In bone the enzyme is found in osteoblasts and is probably important for normal bone formation. In adults, the normal levels of alkaline phosphatase are derived largely from the liver. Pregnancy raises the normal rangs because of the production of a heat-stable alkaline phosphatase by the placenta[12].

Lactate dehydrogenase (LDH) catalyses the reversible inter conversion of lactate to pyruvate. It is widely distributed, with high concentrations in the heart, skeletal muscle, liver, kidney, brain , erythrocytes and measurement of total LDH is therefore a non-specific index of cell damage.

The aim of the present study was to evaluate the major source of increased serum enzyme level specify these enzymes because liver and serum contain of enzymes in paratyphoid fever and to determine the most relevant clinical entity , hepatitis or myopathy , during paratyphoid fever [13].

Experimental part

Subjects: This study performed on 30 female patients diagnosed by ultrasound (US) of abdomen, with paratyphoid A ranged between (20-40) years, who were selected from patients attending Baghdad Teaching Hospital in addition to 30 female healthy control .

Collection of Blood samples: Six ml of venous blood was withdrawn from all subjectes enrolled in study.The blood were transferred to plain tubes, left to clot at room temperture for 15 min.,centrifuged at 3500 rpm for 10 min. The resulted serum was separated and kept frozen.

Statistical Analysis

Statistical analysis was performed using student t-test for comparison of variables. The p value <0.05considered significant and p<0.01considered highly signif icant. All data in the table (1) are Mean \pm SD [14].

Determination of Serum Aspartate Transaminase (AST) Activity

Serum (AST) activity was measured using colorimetric method according to (Reitman and Frankel, 15) utilizing a ready made kit for determination of serum aspartate aminotransferase.

In this method, the following reaction represents measurement of AST activity



Glutamate-Oxaloacetate transaminase is measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2, 4-dinitrophenyl-hydrazine. The absorbance was read at 540 nm after 5 min.

Calculation :Expressed equation is :

$$\text{AST Activity} = \frac{\text{Test-Blank test}}{\text{Standard Solution-Blank Standard}} \times 67$$

= $\mu\text{ml/M in/Liter}$ blood serum and change to International Unite Per Liter(IU/L)

Normal value:AST Activity in Serum up to 12U/L [15].

Determination of Serum Alanine Transaminase(ALT)**Activity:**

The (ALT) activity was measured using colormetric method according to (Reitman and Frankel, 15)

Utilizing a ready made kit for the determination of serum alanine aminotransferase.

In this method , it represents measurement of ALT activity



Glutamate-pyruvate transaminase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl-hydrazine . The absorbance was read at 540 nm after 5 min.

Calculation :Expressed equation is:

$$\text{ALT Activity} = \frac{\text{Test- Blank test}}{\text{Standard Solution-Blank Standard}} \times 133$$

= $\mu\text{ml/M in/Liter}$ blood serum and change to International Unite Per Liter(IU/L)

Normal value: ALT Activity in Serum up to 12U/L [15].

Determination of Serum Lactate Dehydrogenase Activity (LDH)

Serum (LDH) activity was measured using enzymatic colorimetric method where a ready made kit is used.

The method is based on the reduction of pyruvate to located in the presence of NADH by the action of the lactate dehydrogenase:



The Pyruvate that remains unchanged reacts with 2, 4- dinitrophenyl-hydrazone which is determined by calorimetric.

Calculation: Enzyme activity was determined by reference to the calibration curve.

Mixed and allow to stand for 20min Mixed and allow to stand at room temperature for 10 minutes, read absorbance of all tubes against distilled water at 520nm The corresponding values in U/L are shown in the following table:

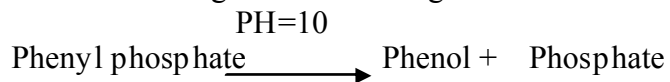
Ordinate= absorbance

Abscissa= activity in U/L

Normal value: 80-190 U/L [16].

Determination of Alkaline Phosphates Activity

Colorimetric determination of ALP activity was performed using a kit from Biomerieux, France according to the following reaction:



The phenol liberated was measured in the presence of amino-4-antipyrine and potassium ferricyanide. The presences of sodium arsenate in the reagent stop the enzymatic reaction. The absorbance of the sample was measured at 510 nm against reagent blank [17].

Results and Discussions

The results of serum AST, ALT, Alkaline phosphate (ALP) and Lactate dehydrogenase (LDH) in paratyphoid patients and their control group are shown in table (1). A highly significant values increase in serum AST and ALT compared to control was found while a significant increase of serum ALP compared to control was noticed and a significant values decrease in serum LDH compared to control was found. The diagnosis of fulminant hepatic failure due to S. Paratyphoid was passed mainly on two facts: First, positive blood culture for S. paratyphoid A elevated alkaline phosphatase level that agreed with this study [18]. The pathogenesis of severe hepatic involvement in Salmonella infection may be multifactorial, involving end toxin, local inflammatory and or host immune reactions [19].

Alkalines phosphatase 150.6 U/L normal(21-92) also had increased levels of AST serum similar observations were made with ALT serum, SA salmonella endotoxin induced consumptive coagulopathy, damage to hepatocytes arteritis [20], direct invasion of the hepatocytes by the organisms, immune complexes and consumption of complement are believed to contribute to hepatic insult [21]. The clinical presentation and extent of hepatic dysfunction in typhoid fever would, therefore, depend upon these contributory. Factors and may or may not be associated with hepatomegaly typhoid [22]. It is concluded that presence of high fever, Jaundic and tender hepatomegaly should arouse suspicion of typhoid hepatitis

.Hepatic dysfunction in these cases, despite its high incidence and serious nature, is transient and responds favorably to appropriate antibiotic therapy.

Myopathy during the course of typhoid fever is reported increasingly [23]. Both clinical entities, myopathy and hepatitis, are usually diagnosed by serum enzymes such as aspartate aminotransferase(AST), alanine aminotransferase(ALT), alkaline phosphatase (ALP),lactate dehydrogenase(LDH),AST and LDH are elevated in myopathy as well as hepatitis, whereas ALT are relatively specific for liver and muscle, respectively[24] . According to the original definition of typhoid hepatitis, it is possible that an over diagnosis for typhoid hepatitis occurred and typhoid myopathy was missed [25].The invasion of intestinal lymphatic tissue is suggested to result in a host reaction with hyperplasia of the liver reticuloendothelial system and infiltration of portal spaces as well as the reduction of the microcirculation causing necrosis[26].Hepatic damage by salmonella typhi appears to be mediated by bacterial endotoxin.The presence of intact bacilli has been demonstrated in the hepatic tissue that may be related to presence of Salmonella bacteria in reticuloendothelial system and hyperplasia of kupffer cell (typhoid nodule) without significant liver injury[27]. In the present study, the pattern of serum enzymes is more comparable to what is found in myopathy (60 %) rather than liver disease (22%) ,as the association of AST and liver enzymes [28].The higher level of serum LDH in all other age groups among cases is more likely due to mycobacterial induced tissue injury as well as other pathophysiological conditions , which remained undiagnosed during this study[29]. The results clearly indicate that adult have higher prevalence and susceptibility to mycobacterium infection as compared to control .Thus, serum LDH still remains as one of the important parameters to assess the cell/tissue specific stress or pathology, caused by mycobacterium tuberculosis the observed correlation between serum LDH level and sputum mycobacterial load alone or in combination with its individual isoforms, especially LDH3 and LDH5 can thus be used as an index for diagnosis of tissue/organ affected by the disease (clinical). It also indicates the severe patho-physiological conditions of the liver of patients [30].

Hepatic dysfunction detected by clinical and or biochemical parameters was noticed in as many as 64.5%of cases in this study which is higher than that reported by others[31]. Hepatomegaly is usually observed in enteric fever after the first week of illness, most often persists throughout the period of marked elevation of temperature, becomes less evident as defervescence progresses and usually lasts for 3-4 week.

Incidence of hepatomegaly is believed to be 2-3 times more common in typhoid fever than paratyphoid fever [22]. Tender hepatomegaly observed in 2 of our cases, suggested a more severe hepatocellular involvement as both of these cases presented with jaundice. Even

though majority of cases with jaundice had hepatomegaly, Liver was not enlarged in one case suggesting that significant hepatic dysfunction can occur in typhoid fever without hepatomegaly. Jaundice associated with typhoid fever tends to occur at the peak of fever which differentiates it from viral hepatitis in which case fever usually comes down after the appearance of jaundice. Jaundice in most of these cases is due to typhoid hepatitis. However, hemolysis resulting in jaundice is a recognized complication of typhoid fever in patients with G-6-P deficiency or thalassemia [32]. Other causes of Jaundice include ascending cholangitis, Salmonella liver abscess, suppurative pyelophlebitis and cholecystitis [21].

Conclusions

From the results of present study, conclusions could be drawn that liver is involved in 14 % of typhoid patients manifesting with hepatomegaly. Elevation of serum enzymes in typhoid fever is presumably of a muscular origin, while elevation of liver enzyme is relatively less common.

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Table (1): AST, ALT, ALP and LDH levels in sera of controls and patients

| Parameter | Control n=30 | Patient n=30 | P Value |
|------------------------|-----------------|-----------------|-----------------------|
| AST(IU/L) Mean±SD | 13 ±1.05 | 28.7± 2.27 | P< 0.01 Highly Sig |
| ALT(IU/L) Mean±SD | 12±0.52 | 23.2±1.25 | P< 0.01 Highly Sig |
| ALP(IU/L) Mean± SD | 49.2 ±3.04 | 150.6± 1.32 | P< 0.05 Sig |
| LDH (IU/L) Mean± SD | 205± 4.67 | 159± 3.11 | P< 0.05 Sig |

تأثير بعض فعالية أنزيمات أمراض الكبد في مرضى سالمونيلا باراتفويد A لدى المرأة العراقية

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استلم البحث في : 25 نيسان 2010

قبل البحث في : 8 شباط 2011

الخلاصة

أظهرت الدراسة الحالية للكبد عند 14% من مريضات التيفويد اللاتي لديهن تضخم الكبد وأرتفاع أمصال أنزيمات الحمى التيفويدية وعلى ما يبدو من منشأ عضلي، بينما أرتفاع أنزيم الكبد نسبيا أدنى بدرجة أقل من الشائع. شملت الدراسة 30 مريضا بالباراتفويد A للجنس الأنثوى اللاتي شخصن بأستعمال الأمواج فوق الصوتية (US) وكانت علامات المرض ظاهرة عليهن فضلا عن 30 من الأصحاء بوصفهن سيطرة ضبط تتراوح أعمارهن بين (20-40) سنة وتم معالجتهم بتناولهن المضادات الحياتية مدة 14 يوما وتكرارها 2-3 أسابيع بعد أكمال العلاج للتحقق من الشفاء برهنت جميع الحالات سريريا وكميويويه عندهن أختلالا وظيفيا في الكبد. تضمن الطيف الكبدي تضخم الكبد، اليرقان، ومختلف الوظائف المشوشة للكبد والصفة الشاذة للبطن من خلال أمواج فوق الصوتية (US).

أظهرت نتائج الدراسة ان هناك زيادة معنوية عالية في مستوى الـ (AST)، و (ALT) في أمصال مرضى الباراتفويد A مقارنة بمجموعة السيطرة، إذ لوحظ زيادة معنوية في مستو مصصل الفوسفاتيز القاعدي (ALP) مقارنة بمجموعة السيطرة، بينما وجد أن هناك أنخفاضا معنويا في مصصل لاكتيت دي هيدروجينيز (LDH) مقارنة بمجموعة السيطرة.

الكلمات المفتاحية: فعالية الأنزيمات، أمراض الكبد، سالمونيلا باراتفويد A