International Journal of TROPICAL DISEASE & Health
8(2): 79-89, 2015, Article no.IJTDH.2015.080
ISSN: 2278–1005

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Evaluation of the Serum Liver Enzymes Markers, Lipid Profile and Kidney Function Parameters in Typhoid Patients

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Authors' contributions

This work was carried out in collaboration between all authors. Author OYN designed the protocol, carried out the literature search and also wrote the first draft of the manuscript. Author EUE screened the patients and performed statistical analysis. Authors EAM and NOC conducted biochemical analysis. All authors were also involved in gaining ethical approval and all author made corrections to the manuscript and final version submitted was approved by all. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2015/17322
Editor(s):
(1) Viroj Wiwanitkit, Department of Laboratory Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.
Reviewers:
(1) Anonymous, Brazil.
(2) Anonymous, India.
(3) Ricardo José Anselmo, Department of Basic Sciences, National University of Lujan, Argentina.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1133&id=19&aid=9180

Original Research Article

Received 8th March 2015
Accepted 16th April 2015
Published 8th May 2015

ABSTRACT

Aim: Typhoid fever is a life-threatening infection caused by the bacterium *Salmonella enterica serotype Typhi* occurring frequently in underdeveloped regions of the world due to overcrowding and poor sanitation. However, the biochemical changes induced by typhoid fever have not been fully understood as a guide to finding a possible counter measure to combating the disease. The study was designed to determine the biochemical changes associated with typhoid disease.
Place and Duration of Study: The study was carried out at the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria, in the year 2014.

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Methods: The study evaluated kidney electrolyte, lipid profile, liver marker enzymes in the serum of typhoid patients. 140 subjects were recruited for this study, 70 of which were normal individual control group and 70 individual with typhoid test group with age range of 18 to 28 years. Blood samples were analyzed to determine the lipid profile, kidney function and liver enzyme concentration spectrophotometrically.
Results: Urea and creatinine concentration in typhoid patients significantly increased *(P = .05)* when compared with the control group. Sodium ion, potassium ion, chloride ion and bicarbonate ion significantly decreased *(P = .05)* when compared to the control group. Result for liver function test revealed a significant increase in the concentration of AST, ALT and ALP in test group. HDL, LDL, Cholesterol, triacyglycerols and VLDL concentration significantly increased *(P = .05)* in typhoid patients when compared with normal individuals. Concentration of total protein, albumin, globulin significant decrease *(P = .05)* in test group when compared with the control group.
Conclusion: The study gave a clear indication of the changes induced by typhoid fever in humans. This study also revealed some physiological and biochemical conditions in typhoid patients that varies from normal individual, which explains the influence of typhoid morbidity.

Keywords: Typhoid fever; electrolytes; urea; creatinine; cholesterol; blood.

1. INTRODUCTION

Typhoid fever is a systemic disease caused by *Salmonella typhi*, affecting only humans [1]. In humans and animals, *Salmonella* infections are a significant cause of morbidity and mortality [2]. Typhoid fever is communicated by polluted food and water by feces and urine of patients and carriers [1].

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 [3].

Typhoid fever, also known as typhoid is a common worldwide bacterial disease transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella enterica* serovar Typhi [2].

The bacterium that causes typhoid fever may be spread through poor hygiene habits and public sanitation conditions, and sometimes also by flying insects feeding on faeces. Diagnosis is made by any blood, bone marrow or stool cultures and with the Widal test (demonstration of salmonella antibodies against antigens O-somatic and H- flagellar).

The term *enteric fever* is a collective term that refers to typhoid and paratyphoid [2]. With an estimated 16–33 million cases of typhoid fever annually resulting in 216,000 deaths in endemic areas, the World Health Organization identifies typhoid as a serious public health problem.

The serological test (widal test) is a widely used tool to detect the presence of salmonella groups the agent for typhoid fever. However, the most accepted standard is the isolation of the organism from stool or blood sample culture, which is widely used today. Hamze et al. [4] evaluated the use of widal test in Lebanon and concluded that the test is still a valuable tool for typhoid fever control. During the course of typhoid fever, the patient develops a high titre of agglutinins. The detection and estimation of these agglutinins by widal reaction is done routinely in many countries.

With the high mortality rate associated with typhoid patients, the study was designed to determine the various biochemical parameters such as liver enzymes and kidney function in typhoid patient's serum to possibly recommend management measures for the disease condition.

2. MATERIALS AND METHODS

2.1 Blood Collection and Serum Preparation

Blood sample of typhoid patients were obtained from Medical Center of the Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. For the test group, prior to blood collection, our team had visited the medical centre and informed the director about the research. We visited the centre daily speaking with typhoid patients and informed them about the research work. Those who get interested were given consent form to seek their approval to

release blood samples to our team. Those that approved and signed the consent form duly, we drew blood samples from them and kept refrigerated till time for biochemical analysis. A total of 70 typhoid patients who were receiving treatment from the medical centre were surveyed for the purpose of this work. The study was approved by the ethical committee of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 Control Group

For the control group, which comprises individuals of Michael Okpara University of Agriculture Umudike, within the age 25-40yrs, the study announcement was made open the subjects nominated from those who replied to the announcement. A one day session was planned for the subjects giving them thorough details of the purpose of the research work. Afterward the participants were given informed consent forms and those who returned correctly filled form and signed were screened for enrolment. Prior to biochemical analysis, typhoid test (Widal test) was carried out on the control group to ensure they do not carry the typhoid vector. The control group was also screened to ensure that two weeks before the experimental day, they were not on any anti malaria therapy, typhoid treatment, not diabetic, obese and for female absence of pregnancy and were nonsmokers and alcoholics. We ensured that the control and test group were within the same age limit and were made up of both male and female.

2.2.1 Determination of serum urea concentration

This was done using the method of Chaney and Marbach [5].

2.2.2 Determination of serum creatinine concentration

This was done using the method of Taussky [6].

2.2.3 Determination of sodium concentration

This method was done using the method of AOAC [7] as contained in Teco test kit (U.S.A).

2.2.4 Determination of potassium concentration

This was done using the method of AOAC [7] as contained in Teco test kit (U.S.A).

2.2.5 Determination of bicarbonate concentration

The determination of bicarbonate ion was by the method described by Tietz et al. [8] as contained in Teco bioassay test kit (U.S.A).

2.2.6 Determination of chloride concentration

The colometeric determination of chloride in human was done by the method described by Tiezt et al. [8] as contained in Teco bioassay test kit (U.S.A).

2.2.7 Determination of alkaline phosphatase activity

The activity of alkaline phosphatase was determined using the method described by King and King [9].

2.2.8 Determination of Aspartate and Alanine Aminotransferase (AST and ALT)

The activities of AST and ALT were determined using Reitman and Frankel [10].

2.2.9 Determination of Conjugated Bilirubin (CB) and Total Bilirubin (TB)

The conjugated and total bilirubin were determined using the Max Discovery ^{Tm} total bilirubin Assay Kit (U.S.A.) and Diazyme's Direct Bilirubin Vanadate Oxidation assay by the colorimetric method described by Jendrassik and Grof [11].

2.2.10 Determination of total protein concentration

This was done using the method of Wiechselbum [12] as contained in the Quimica Clinica Applicada (QCA) Test kit (Spain).

2.2.11 Determination of albumin concentration

The determination was by the method of Grant [13] as contained in the Randox test kit (U.K).

2.2.12 Total cholesterol estimation

The serum total cholesterol was determined using the enzymatic method of Allain et al. [14].

2.2.13 High Density Lipoprotein (HDL) Cholesterol Estimation

The High Density Lipoprotein (HDL) cholesterol was estimated using the method of Grove [15].

2.2.14 Triacylglycerol estimation

The Serum Triacylglycerol (TAG) was estimated via colorimetric method of Tietz [16].

2.2.15 Low Density Lipoprotein (LDL) Estimation

The low density lipoprotein (LDR) cholesterol was estimated using the method described by Assman [17].

2.2.16 Very Low Density Lipoprotein (VLDL) Estimation

Very low density lipoprotein (VLDL) cholesterol was estimated using the formula, VLDL = total cholesterol – (HDL+LDL).

2.3 Statistical Analysis

Statistical analysis was performed using Student T- test at 95% confidence level (SPSS VERSION 17) Windows 7 Operating System.

3. RESULTS

The results obtained from biochemical analysis are shown in bar chart below, and are expressed as mean and standard deviation for both the control and test group.

4. DISCUSSION

Typhoid fever presents with continuous fever, abdominal pain, constipation as symptoms and rose spot and relative bradycardia as signs usually. But sometimes it may present with variation in clinical finding such as diarrhea [18]. Several stages are seen in the pathophysiology of typhoid fever which is a complex process [19]. As the symptoms of typhoid fever are often nonspecific, they result in frequent diagnostic confusion with malaria, dengue fever, influenza or other febrile illness [1]. In the present study, evaluation of kidney markers (urea, creatinine, electrolytes and liver enzymes were assayed for the purpose of selecting a marker with high sensitivity and specificity. These parameters were also assayed in healthy subjects as a control study.

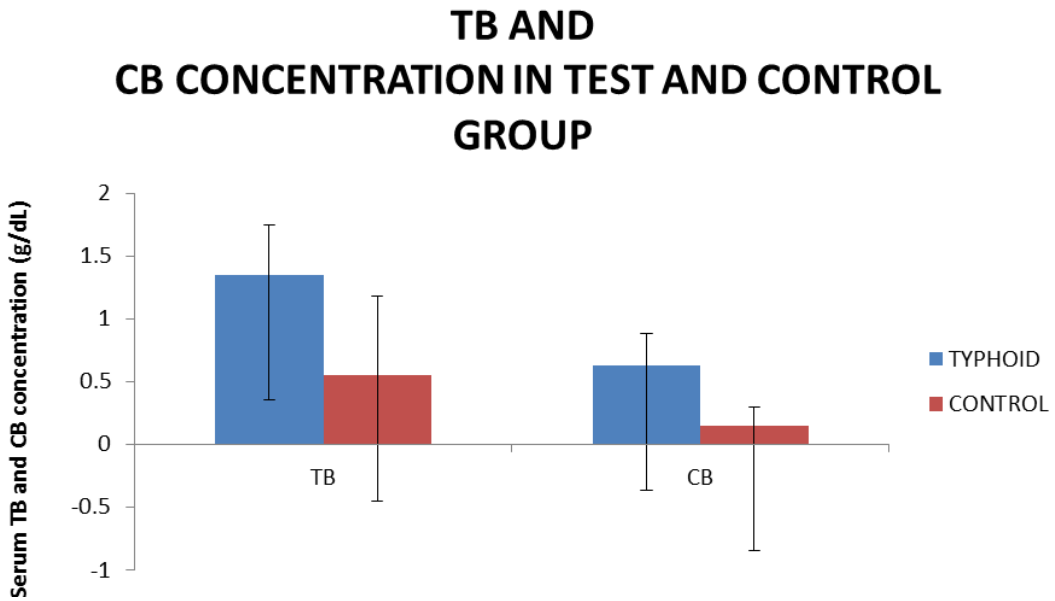

Fig. 1. The mean serum total bilirubin and conjugated bilirubin concentration of typhoid and normal individuals represented in Fig. 1

There was a significant (P=.05) difference between the mean total serum Bilirubin concentrations of the typhoid infected individuals when compared with normal individuals. There was no significant (P=.05) difference between the mean of serum Conjugated Bilirubin concentrations in typhoid infected individuals when compared with normal individuals

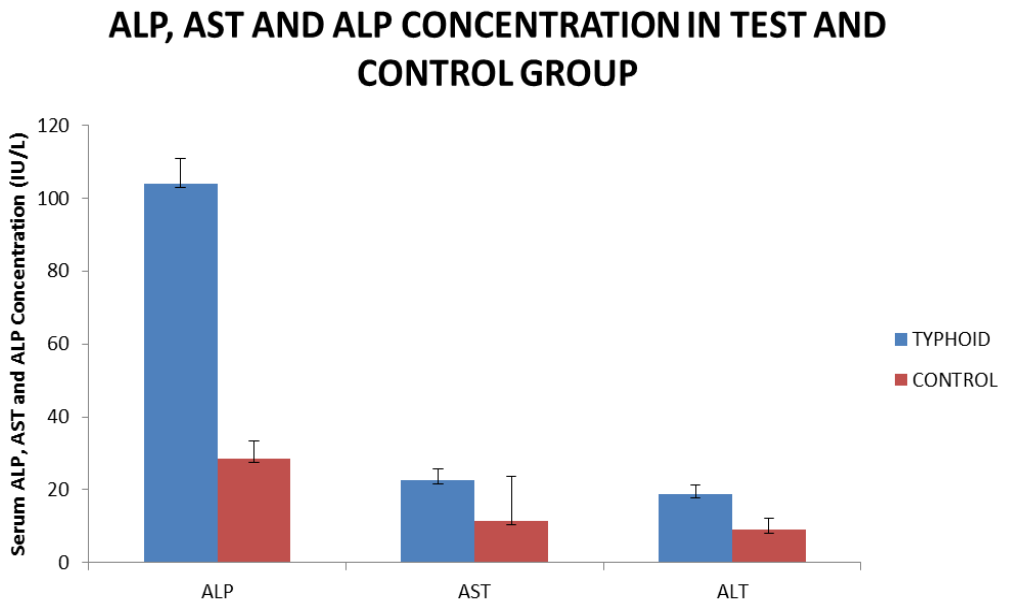

Fig. 2. The mean serum ALP, AST and ALP concentration of typhoid and normal individuals represented in Fig. 2

There was a significant (P = .05) difference between the mean of serum ALP concentrations in typhoid infected individuals when compared with normal individuals. Mean serum AST concentration was significantly (P=.05) higher in typhoid infected individuals when compared with normal individuals. Mean serum ALT concentration was significantly (P = .05) higher in typhoid infected individuals when compared with normal individuals

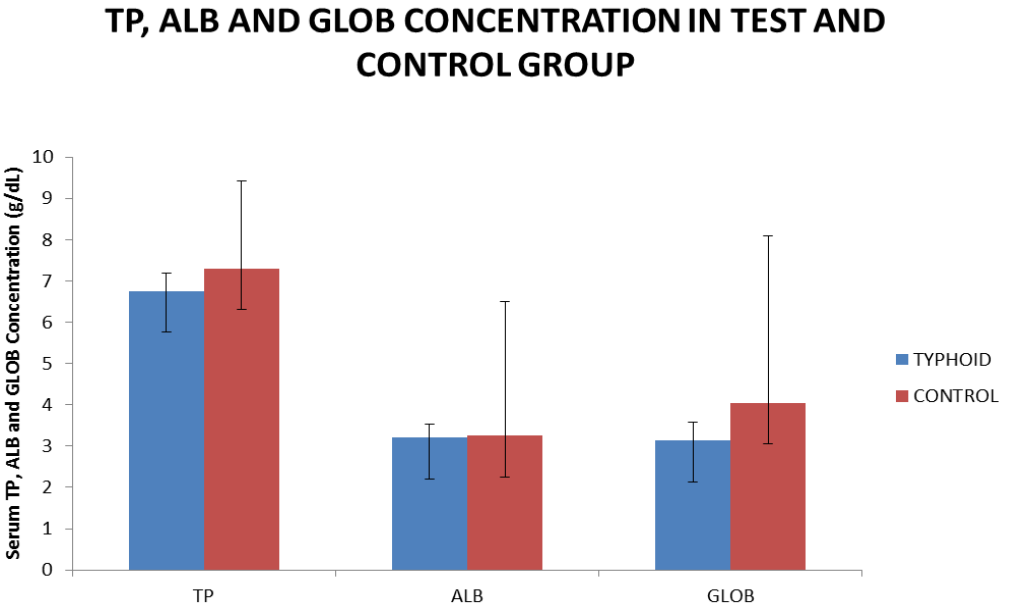

Fig. 3. The mean serum TP, ALB and GLOB concentration of typhoid and normal individuals represented in Fig. 3

Mean serum TP concentration was significantly (P=.05) lower in typhoid infected when compared with normal individuals. Mean serum Albumin concentration was significantly (P=.05) lower in typhoid infected individuals when compared with normal individuals. Mean serum Globulin concentration was significantly (P=.05) lower in typhoid infected individuals when compared with normal individuals

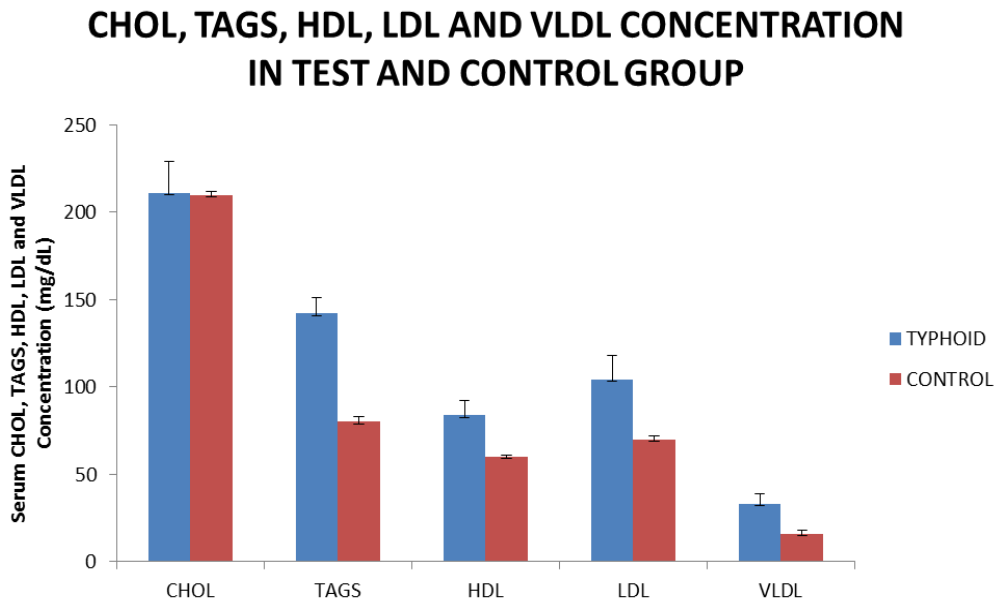

Fig. 4. The mean serum CHOL, TAGS, HDL, LDL and VLDL concentration of typhoid and normal individuals represented in Fig. 4

Mean serum cholesterol concentration was significantly (P = .05) higher in typhoid infected individuals when compared with normal individuals. Mean serum triacyglycerol concentration was significantly (P = .05) higher in typhoid infected individuals when compared with normal individuals. Mean serum HDL concentration was significantly (P = .05) higher in typhoid infected individuals when compared with normal individuals. Mean serum LDL concentration was significantly (P = .05) higher in typhoid infected individuals when compared with normal individuals. Mean serum LDL concentration was significantly (P = .05) higher in typhoid infected individuals when compared with normal individuals

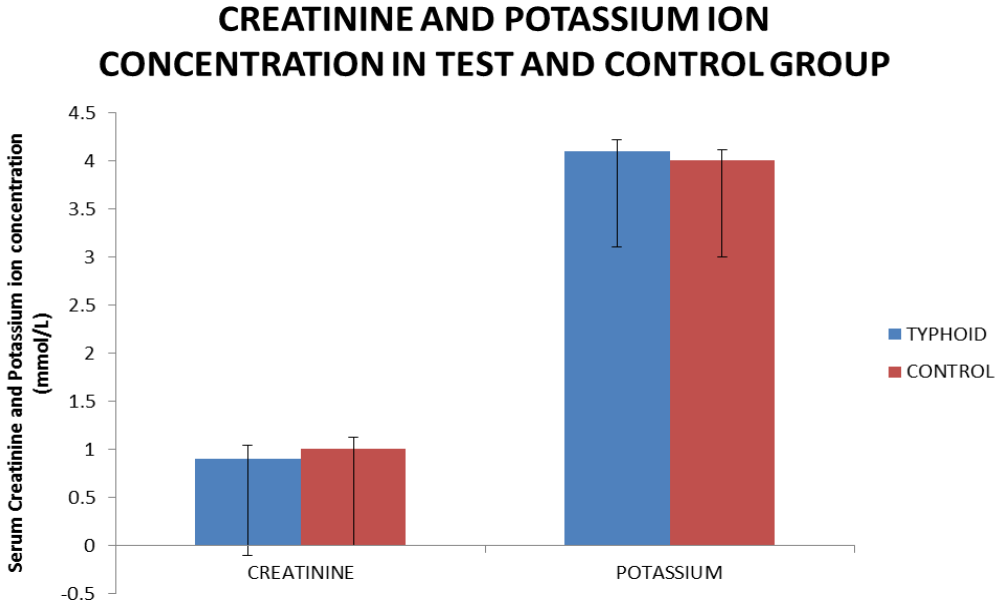

Fig. 5. The mean serum creatinine and potassium concentration of typhoid and normal individuals represented in Fig. 5

Creatinine ion and potassium ion concentration in typhoid and normal individual revealed no significant difference (P = .05)

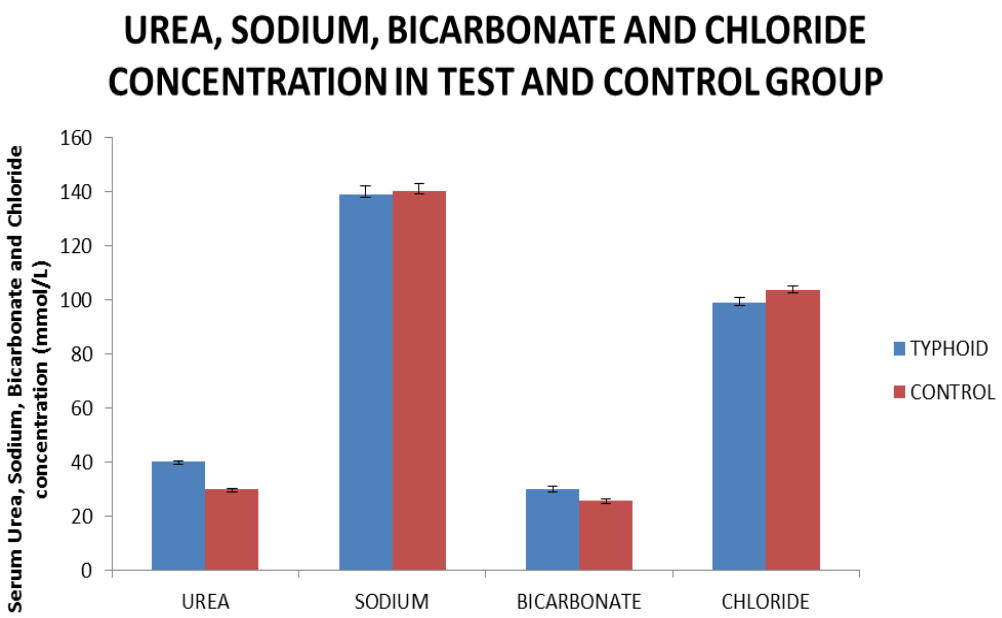

Fig. 6. The mean serum creatinine and potassium concentration of typhoid and normal individuals represented in Fig. 6

Mean value of urea concentration was significantly (P = .05) higher in the typhoid patient when compared with the normal individuals. Mean values for Chloride were not significantly (P = .05) different between the test and control. Mean values for Sodium were not significantly (P = .05) different between the test and control. Mean values for bicarbonate were not significantly (P = .05) different between the typhoid individual and normal individual control

Creatinine a breakdown product of creatine phosphate in muscles and is fairly constant rate by body, it only varies depending on the body muscle mass. From the result of this study, there was a significant difference between the creatinine concentrations of typhoid patient when compared to normal individual. The result revealed a significant increase of serum urea and creatinine of the typhoid patients *(P = .05)* when compared to the control. This is in contrast to the work of Sameera et al. [20]. Elevated serum urea and creatinine level is often as a result of renal dysfunction due to the death of nephrons. There is a unique relationship between S. *typhi* and macrophages in the liver, spleen, intestinal lymphoid follicles, and mesenteric lymph nodes which could be responsible for the pathogenesis, [19]. Functionally active cytokines (TNF alpha, IL-1, interferon alpha and beta) are synthesized by macrophage cells and are an important source of arachidonate metabolites and reactive oxygen intermediates. These products can lead to cellular necrosis, other inflammatory cells recruitment, immune system stimulation, vascular instability, initiation of the clotting mechanism, and other abnormalities associated with typhoid fever [21].

Etim et al. [22] revealed that increase in creatinine level is mostly likely as a result of impaired glomerular filtration of urea and creatinine. Creatinine level has also been shown to increase as a result of muscle injury. High creatinine level has been well documented in diseases such as hepatitis, typhoid, urinary infections, kidney infections, diabetes, which reveals that there is acute renal failure.

This study reveals high level of urea in the typhoid patients when compared with normal individuals, which can be associated with increased catabolic rate which characterize the disease. Ogbadoyi and Tsado [23] have reported increased serum urea level in malaria and malaria-typhoid ailments. Etim et al. [22] reported that increased Urea: Creatinine ratio in malaria or malaria-typhoid patients also indicate that the causes of Uraemia in these patients are largely pre-renal and may be due to reduced renal blood flow, rather than organic renal involvement. Reduced blood flow to the glomeruli due to malaria-associated hypotension may be responsible for the reduced glomerular filtration rate and hence decreased renal excretion of the analytes.

Potassium is one of the mineral that is crucial for life, potassium is necessary for the heart, kidney and other organs to work normally. Result of this study indicated that there was no significant difference in potassium level between the test and control group, this finding corresponds with the report of Sameera et al. [20]. This also gave a good implication that typhoid fever does not affect the serum potassium. Potassium is an essential macro-mineral in human nutrition; it is the major cation (positive ion) inside animal cells, and it is thus important in maintaining fluid and electrolyte balance in the body.

Table 1. Normal range of value for studied parameters (Wiki reference range for blood test) represented in Table 1

Parameters	Normal Value
ALT (IU/L)	8.0-56
ALP (IU/L)	53-128
AST (IU/L)	8.0-40
TP (g/dL)	6.0-8.4
ALB (g/dL)	3.5-5.5
GLOB (g/dL)	2.3-3.5
HDL (mg/dL)	40-86
LDL (mg/dL)	94-130
TAGS (mg/dL)	54-110
CHOL (mg/dL)	140-250
VLDL (mg/dL)	2.0-30
CREATINIE (mmol/L)	0.5-0.9
POTTASSIUM (mmol/L)	3.5-5.1
SODIUM (mmol/L)	135-147
CHLORIDE (mmol/L)	95-110
BICARBONATE (mmol/L)	18-23
UREA (mmol/L)	3.0-7.0

Sodium is useful in controlling blood pressure and blood volume; it is also needed for proper functioning of the nerves and muscle [24]. From this study, there was no significant change in serum sodium concentration of the typhoid patient when compared with the normal individual. This result was in disagreement with the findings of [25]. This bacteria *Salmonella typhi* did not affect serum sodium level in the test patient.

Bicarbonate is a major element in our body secreted by the stomach, it is necessary for digestion, when ingested for example with mineral water, it helps buffers lactic acid generated during exercise and also reduce the acidity of dietary compound. Bicarbonate is present in all body fluid and organs; it plays a major role in acid base balance in the human body [26]. Our findings also showed that there is no significant difference between the typhoid patient and normal individual bicarbonate ion concentration.

Chlorine is a major anion that is important in the maintenance of the cation / anion balance between intra and extra cellular fluids. This electrolyte is therefore essential to the control of proper hydration, osmotic pressure and acid-base balance [27]. In this study, there was no significant difference between the chloride ion concentration of the test and control group. For the studied electrolyte our result indicated that typhoid fever does not affect electrolyte concentration.

Serum total bilirubin revealed significant difference in test group when compared with normal group, while conjugated bilirubin also revealed no significant difference between the test and control group. Our finding reports significant increase *($p = .05$)* in the level of bilirubin in the typhoid patients and our findings correlates with the findings of Adeosun et al. [28], who reported elevated bilirubin (Jaundice) in malaria and also typhoid fever patients which is as a result of hemolysis and in severe conditions can develop to liver damage. Paul and Christopher [29], Mazumder et al. [30], Attwood [31] also revealed presence of jaundice (high amount of bilirubin) in cases of malaria, typhoid and some other disease causing infections. Etim et al. [22] reported significantly raised bilirubin levels in untreated malaria patients.

Our result indicated a significant decrease in total protein concentration in test group when compared with the control group and this agrees with the work Adeosun et al. [28] who reported significant decrease of total protein in infected patients. The decrease in total protein corresponds to the severity of the infection.

Alkaline phosphatase revealed significant difference in typhoid patient when compared with normal individual. The concentration of Aspartate amino transferase (AST) and Alanine amino transferase (ALT) is for detecting hepato cellular 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, amino-transferases are considered useful in differentiating hepatocellular from cholestatic forms of liver injury. AST activity is related to damage of cell in kidney, pancrease and erythrocytes. ALT and AST were significantly higher in typhoid patients than normal individual.

This was in contrast to the result obtained from Shamin et al. [27] finding. In general, mechanism relating to association between liver marker and in typhoid fever may reflect elevations in ALT and AST. Serum total protein was significantly lower in typhoid patients as compared to normal counterparts.

Albumin and globulin were also significantly lower in typhoid patients as compared to normal individuals from the result of this study. This also disagrees with the finding of Shamin et al. [32]. If the globulin levels fall below the normal ranges, it can be assign of several serious health conditions which are tonal diseases, hepatic dysfunctions; celiac disease etc. gammaglobulenemia and hypogamma-globulinemia can cause the globulin levels to drop. This is also a sign that proteins taken in by the digestive system are not being broken down or absorbed properly. High levels of globulin can be caused by an overproduction of globulin, an underproduction of albumin or a loss of albumin. Albumin can be lost due to kidney disorders which lead to an excessive amount of protein being shed from the body.

For lipid profile, our study revealed distinction in the lipid profile of the test group when compared with the control group as indicated by the result. The typhoid patients had increased cholesterol concentration when compared with the control group. This finding opposes the result of Amal [33], who reported a decrease in cholesterol concentration in typhoid patients.

HDL, LDL, VLDL and triacylglycerols also increased in typhoid patient when compared with normal individuals. Unbalances in lipoproteins fractions can be attributed to lipid peroxidation attack which may affect their regular metabolism and the successive delivery of lipid to peripheral organs in the body [34].

The result revealed a significant increase in the level of total cholesterol in typhoid patients. Total cholesterol (TC), triacylglycerol (TAG), and the lipoprotein system (HDL, LDL) concentrations can function as marker or indicators of cardiovascular status.

In the studied typhoid group, there was an abnormal cholesterol level (hypercholes-terolemia) that is, higher concentrations of LDL when compared with the control group, which is strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke, and peripheral vascular disease [35]. LDL particles are often termed "bad cholesterol" because they have been linked to atheroma formation [36].

In the typhoid group, Increase in TAGs concentrations indicated an increase in total cholesterol level. High levels of TAGs in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease and stroke.

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. Increase in HDL levels and increase in LDL-cholesterol is observed in patients with typhoid at the peak of fever.

The association between increased LDL cholesterol levels and increased risk of heart disease has been well established and documented, independently of TG levels and other risk factors [37]. Intravascular ultrasound studies demonstrate that patients with low HDL cholesterol and high TG levels have more extensive coronary atheromas than those with an isolated elevation of LDL cholesterol [38]. Any substance or condition that increases the LDLs concentration and lowers the HDLs tends to increase the probability of plaque formation and a heart attack [39]. Generally speaking our study gave a clear indication that typhoid fever affects lipid metabolism in the test group.

5. 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 enzymatic changes but these changes are covered up by antibacterial therapy. The study revealed increase in liver enzymes and blood lipid imposed by typhoid fever. Creatinine and urea concentration also increased with reduction in electrolytes. For proper management and possibly treatment of typhoid, more emphasis should be targeted towards increased excretion of the blood nitrogen based products, initiation of early treatment through early diagnosis can considerably decrease the morbidity and mortality caused by typhoid and drugs which have the ability to increase the rate at which urea and creatinine are eliminated from

the body should be administered. Patients who suffer from typhoid have persistent fever, fatigue, loss of appetite, severe headache, swollen spleen, constipation or diarrhea situation. Such as lack of treatment, mortality may reach one-tenth, but if early treatment is administrated then the mortality rate can be dropped to one per cent or less.

ETHICAL APPROVAL

This study was approved by the ethical committee of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike. It was given approval number MOUAU/COLNAS/BCM/2014/005, specific for human study.

COMPETING INTEREST

All the authors acknowledge that there is no competing interest and final version of manuscript was approved by all authors.

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