

Available online at [GSC Online Press Directory](#)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsconlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



## Typhoid infection and its effect on liver function assessment among pregnant women in Owerri, Imo State, Nigeria

Ike Christian Chukwuemeka<sup>1,\*</sup>, Akwari Dike Kalu<sup>1</sup>, Ogwuegbu Happiness Odinakachi<sup>2</sup> and Chikezie John Austin<sup>3</sup>

<sup>1</sup> Department of Biological Sciences (Microbiology Programme), College of Basic and Applied Sciences, Rhema University Nigeria, P.M.B. 7021 Aba, Abia State, Nigeria.

<sup>2</sup> Department of Microbiology, Abia State University Uturu, P.M.B. 2000 Uturu, Abia State, Nigeria.

<sup>3</sup> Department of Internal Medicine, Abia State University Uturu. P.M.B. 2000 Uturu, Abia State, Nigeria.

Publication history: Received on 29 May 2019; revised on 21 July 2019; accepted on 25 July 2019

Article DOI: <https://doi.org/10.30574/gscbps.2019.8.1.0096>

### Abstract

Typhoid infection and its effect on liver function assessment among pregnant women were evaluated. Serum and blood samples were collected and processed for liver function assessment, widal test and microbiological identification of *Salmonella typhi* using standard methods. A total of ninety (90) patients were studied. Serum alanine transaminase (ALT) and Serum aspartate transaminase (AST) values for healthy pregnant women and pregnant women with typhoid infection had progressive value increase with increase in pregnancy time, except for the slight reduction in the third trimester with ALT. Mean values of healthy pregnant women and pregnant women with typhoid infection (iu/L) for ALT and AST were (8.93 ±1.07/ 9.95 ±0.19; 7.52 ±0.45/ 8.50 ±0.14) at first trimester, (10.62 ±1.19/11.64 ±0.05; 9.34 ±1.03/ 10.32 ±0.86) at second trimester and (9.81 ±0.16/ 10.83 ±0.76; 10.40 ±0.49/ 11.38 ±0.08) at third trimester, Conversely, total and conjugated bilirubin followed a different trend of retrogressive decrease with increase in pregnancy time. Total and conjugated bilirubin (mg/dL) mean values for healthy pregnant women and pregnant women with typhoid infection at first trimester were (0.56 ±0.87/ 0.69 ±0.09; 0.57 ±0.66/ 0.70 ±0.26), followed by second trimester (0.43 ±1.06/ 0.56 ±0.05; 0.48 ±0.77/ 0.61 ±0.03) and third trimester (0.32 ±0.57/ 0.45 ±0.11; 0.39 ±0.54/ 0.52 ±0.41). Values of LFT results among pregnant women with typhoid infection were higher than that of healthy pregnant women (p<0.05). Therefore, pregnancy alterations and infections could cause significant alterations in the results of LFT among pregnant women.

**Keywords:** Typhoid infection; Liver function test (LFT); Physiological changes; Aspartate transaminase (AST); Alanine transaminase (ALT); Bilirubin

### 1. Introduction

Typhoid infection is among the most endemic diseases in the tropics. The disease has been associated with poverty and underdevelopment with significant morbidity and mortality [1]. Typhoid fever is an acute febrile infectious disease whose causative organism is the bacterium "*Salmonella enterica* serovar *typhi*." [2]. The incubation period of typhoid and paratyphoid infections is 6-30 days. *Salmonella* represents a group of Gram-negative, facultative anaerobic pathogenic bacteria. *Salmonella enteric* serovar *typhi* (*S. typhi*) is a facultative intracellular pathogen that causes typhoid fever in people. *Salmonellae* are individuals from the family of *Enterobacteriaceae*. The organism is non-capsulated, non-sporulating, flagellated, Gram-negative anaerobic bacilli and with external coat antigens. The bacterium is serologically positive for lipopolysaccharide antigens, protein flagellar antigen, and polysaccharide capsular antigen [3-4]. Typhoid bacteria interfere with the liver and its functions. The invasion of the liver cells by

\* Corresponding author

E-mail address: [christian\\_ike@rhemauniversity.edu.ng](mailto:christian_ike@rhemauniversity.edu.ng)

typhoid bacterium can cause organ congestion, sinusoidal blockage and cellular inflammation [5-6]. When these happen, the parenchyma transaminase and membranous alkaline phosphatase and gamma glutamyl transpeptidase enzymes of the liver leak out and find their way into the circulation, leading to increased enzyme activity [7]. The liver is enlarged and the bacteria cause inflammation of the liver. Untreated typhoid infection can lead to damage of the liver. Typhoid infection is widely recognized as a major public health problem in most developing tropical countries [8]. It is a systemic infectious disease characterized by an acute illness; the first typical symptoms are fever, headache, abdominal pain etc. Human being is the only reservoir and host for typhoid infection and is transmitted by fecal contaminated water and food in endemic areas especially by carriers handling food [9].

The liver is our body's most important organ after the heart, performing many important functions including metabolism, detoxification and formation of important compounds including blood clotting factors and albumin [10]. It is the largest organ in the human body, located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm and lies to the right of the stomach. In a normal pregnancy, many physiological and hormonal changes occur within the human body, some of which can mimic those seen in women with liver disease. The pregnant woman experiences physiological changes to support fetal growth and development [11-12]. Pregnancy does not change size of liver but in the third trimester the enlarging uterus displaces the liver superiorly and posteriorly, which suggests significant hepatomegaly [13]. Routine liver function tests (LFT) usually include alanine and aspartate transaminases, total and conjugated bilirubin, alkaline phosphatase, and prothrombin time. Alanine (ALT) transaminase is predominantly found in liver, unlike aspartate (AST) transaminase. A typical hepatic activity comprises an ALT rise, accompanied by lesser elevation in AST [14-15]. Uterine muscle contractions during labor may increase AST or ALT activity levels [16]. In some studies, results showed slight increase in ALT during the second trimester of pregnancy compared to non-pregnant women, but all the values remained below the upper normal [11-12]. An increase in ALT or AST levels during labor might be due to contractions of the uterine muscle [17-18].

Bilirubin is a waste product from the breakdown of red blood cells, which passes through the liver to become processed, where it is mixed with sugars to become water-soluble and is called direct or conjugated bilirubin. Before bilirubin passes through the liver for processing, it is water insoluble and is called indirect or unconjugated bilirubin. A damaged liver may be unable to process bilirubin, causing an increase in the total bilirubin levels. Bilirubin comes from haemoglobin (Hgb) as red blood cells (RBCs) breakdown either through physiological regeneration at the end of normal lifespan or as a consequence of pathologic hemolysis. Hgb releases heme which is converted inside macrophages to biliverdin which is then converted into unconjugated bilirubin (indirect bilirubin) which travels through the liver where it combines with glucuronic acid to form conjugated bilirubin (also known as bilirubin diglucuronide or direct bilirubin [19-21]. The unconjugated bilirubin is water insoluble which impairs its ability to be excreted in bile, while conjugated bilirubin is water soluble. However, bilirubin is reported on an LFT as total and conjugated bilirubin [14]; [22-24]. Total bilirubin concentrations decreased during all three trimesters of pregnancy [12]; [25-26]; while conjugated bilirubin concentrations also decreased during the second and third trimesters [12]. Hyperbilirubinemia, total bilirubin levels greater than 1.3mg/dL, can cause jaundice and icterus. Continued hyperbilirubinemia can cause kernicterus or brain damage [16]; [27]. Therefore, this study was targeted at evaluating the effect of typhoid infection on liver function assessment among pregnant women in Owerri, Imo State, Nigeria.

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## 2. Material and methods

### 2.1. Study area, sample size and distribution

The study area was in Owerri, Imo State, while the study was conducted at Federal Medical Centre (FMC) Owerri, Imo state, Nigeria. The study was carried out between February, 2017 to November, 2017. A total of ninety (90) patients attending hospital were distributed as follows: thirty (30) healthy pregnant women, thirty (30) typhoid-infected pregnant women and another thirty (30) healthy non-pregnant women (serving as control) were studied. The study criteria included healthy pregnant women, pregnant women with typhoid infection and healthy non-pregnant women; all within the age range of 18 - 38 years and excluded pregnant women with gestational diabetes mellitus, hypertension (blood pressure equal to or greater than 140/90 mm Hg) and malaria. The study was among three groups of pregnancy time (first trimester, second trimester and third trimester), and each result was compared with control. All of these women were examined within a framework of preventive medicine for a routine clinical and biological checkup and none of the women included had evidence of liver disease.

## 2.2. Sample collection and processing

Blood samples were taken from antecubital vein by plastic disposable syringes. The blood was transferred into a glass tubes. After an hour incubation at room temperature (clot retraction), centrifugation of the blood was done at a relative centrifugal force for 5 minutes.

## 2.3. Microbiological analysis for *Salmonella typhi* identification

About 10-15 ml of blood was collected with standard aseptic precautions from each patient and was inoculated into 40-45 ml of brain heart infusion broth. Incubation was done at 37°C. Sub culturing was performed on MacConkey agar plates which were incubated at 37°C for 18-24 hours. Lactose non-fermenting colonies, if any, on subcultured plates were picked up and examined by microscopy after Gram-staining of the smears. Standard biochemical tests including motility test were performed for identification of *Salmonella typhi*.

## 2.4. Liver function assessment and Widal test

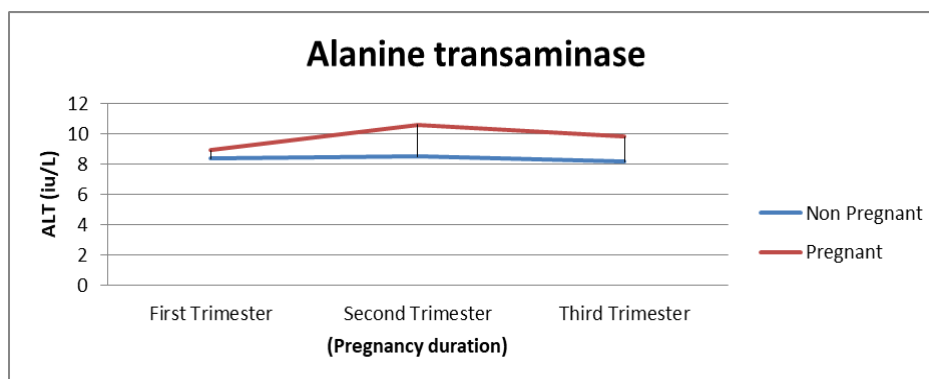
The centrifuged sera from 2.2 above were removed by disposable pasture pipettes and separated into sterile bottles and were stored in the refrigerator at 20°C until it's been used for the widal test and liver function assay within one week interval. Estimation of liver function assessment using kit method was done to include alanine transaminase (ALT), aspartate transaminase (AST), total and conjugated bilirubin during pregnancy period (first, second and third trimester) among healthy pregnant women, typhoid-infected pregnant women and comparing with a control group of healthy non-pregnant women.

## 2.5. Statistical analysis

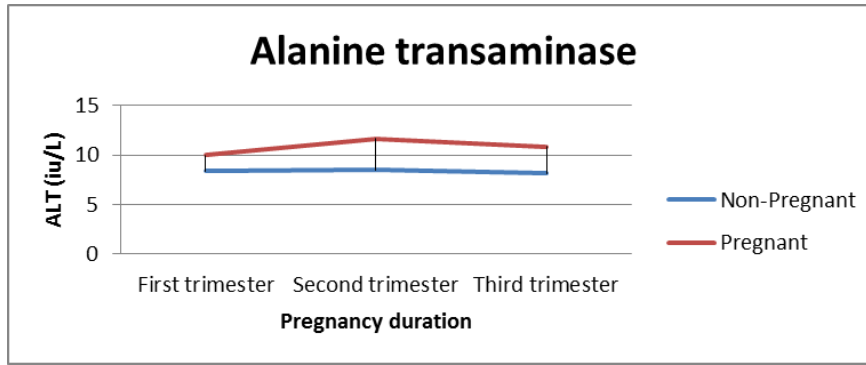
Analysis of variance (ANOVA) was employed and used to analyze all data obtained from the three trimesters and compared with control group of healthy non-pregnant women. Results are expressed as mean  $\pm$  SD (standard deviation).

## 3. Results

The results of liver function tests (LFT) are shown in Figures 1-8. Serum alanine transaminase (ALT) for healthy pregnant women and pregnant women with typhoid infection are shown in Figures 1 and 2. Serum alanine transaminase (iu/L) mean value for healthy pregnant women was (8.93  $\pm$ 1.07) in the first trimester, followed by (10.62  $\pm$ 1.19) in the second trimester, and with a slight reduction in the third trimester (9.81  $\pm$ 0.16), which was higher than that of the first trimester. This trend is applicable to all ALT result obtained for healthy pregnant women, pregnant women with typhoid infection and healthy non-pregnant women. Highest ALT values were observed in pregnant women with typhoid infection, with the highest value recorded in the second trimester (11.64  $\pm$ 0.05). ALT (iu/L) values obtained with healthy pregnant women (1st trimester -8.93  $\pm$ 1.07, 2nd trimester -10.62  $\pm$ 1.19, 3rd trimester -9.81  $\pm$ 0.16), pregnant women with typhoid infection (1st trimester -9.95  $\pm$ 0.19, 2nd trimester -11.64  $\pm$ 0.05, 3rd trimester -10.83  $\pm$ 0.76) and healthy non-pregnant women (1st trimester -8.36  $\pm$ 0.88, 2nd trimester -8.50  $\pm$ 0.76, 3rd trimester - 8.15  $\pm$ 1.34) when compared are statistically significant ( $p < 0.05$ ).

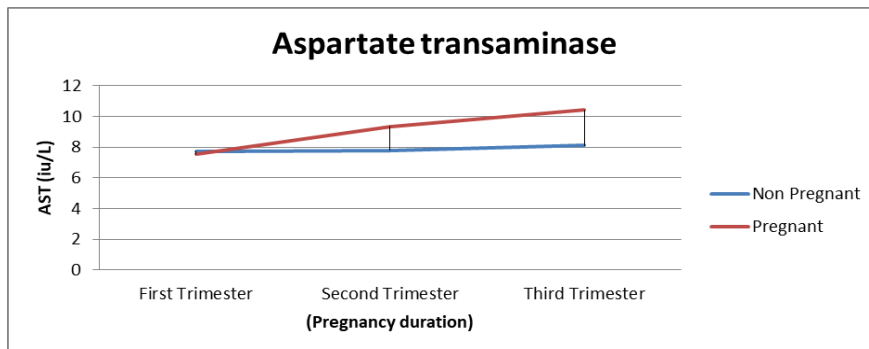


**Figure 1** Serum alanine transaminase result for healthy pregnant women

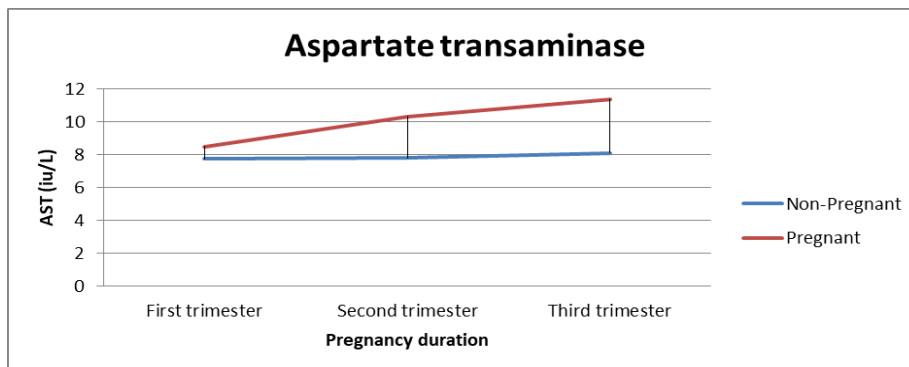


**Figure 2** Serum alanine transaminase result for pregnant women with typhoid infection

Serum aspartate transaminase (AST) activity followed the trend of progressive value increase with increase in pregnancy time and is shown in Figures 3 and 4. Although, results obtained in the study showed insignificant increase in AST (iu/L) values among healthy non-pregnant women ( $p > 0.05$ ). There was no decline phase at any time during pregnancy period. Mean AST (iu/L) values for healthy pregnant women at first trimester was ( $7.52 \pm 0.45$ ), followed by second trimester value ( $9.34 \pm 1.03$ ) and third trimester ( $10.40 \pm 0.49$ ). Highest values of AST (iu/L) were obtained among pregnant women with typhoid infection; first trimester ( $8.50 \pm 0.14$ ), followed by second trimester ( $10.32 \pm 0.86$ ) and third trimester ( $11.38 \pm 0.08$ ). The results obtained in each of the trimesters for healthy pregnant women (1st trimester  $-7.52 \pm 0.45$ , 2nd trimester  $-9.34 \pm 1.03$ , 3rd trimester  $-10.40 \pm 0.49$ ), pregnant women with typhoid infection (1st trimester  $-8.50 \pm 0.14$ , 2nd trimester  $-10.32 \pm 0.86$ , 3rd trimester  $-11.38 \pm 0.08$ ) and healthy non-pregnant women (1st trimester  $-7.74 \pm 0.99$ , 2nd trimester  $-7.80 \pm 1.81$ , 3rd trimester  $-8.11 \pm 0.65$ ) when compared are statistically significant ( $p < 0.05$ ).



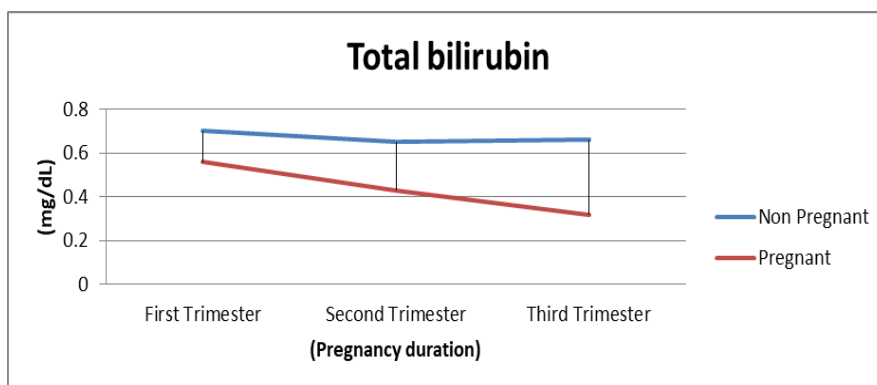
**Figure 3** Serum aspartate transaminase result for healthy pregnant women



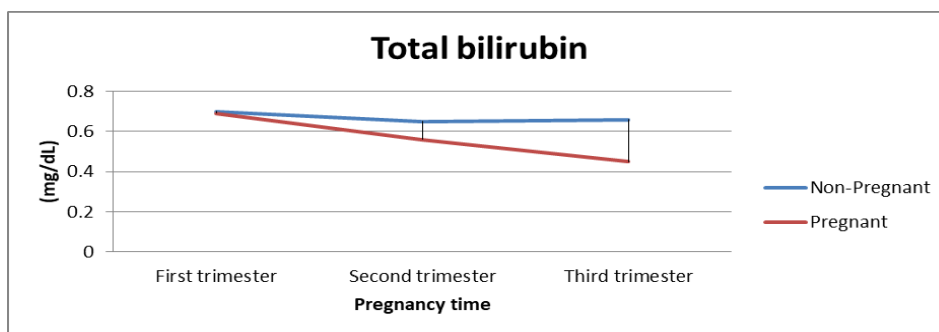
**Figure 4** Serum aspartate transaminase result for pregnant women with typhoid infection

Conversely, total and conjugated bilirubin followed a different trend with that of AST and is shown in Figures 5, 6, 7 and 8 respectively. In Figures 5, 6, 7 and 8, values of both total and conjugated bilirubin obtained for healthy pregnant women and pregnant women with typhoid infection were retrogressively decreasing with increase in pregnancy time.

However, results from healthy non-pregnant women did not follow a pattern rather were variable and insignificant for both total and conjugated bilirubin ( $p > 0.05$ ). Mean total bilirubin (mg/dL) for healthy pregnant women at first trimester was ( $0.56 \pm 0.87$ ), followed by second trimester ( $0.43 \pm 1.06$ ), and third trimester ( $0.32 \pm 0.57$ ). Also, values of total bilirubin obtained from pregnant women with typhoid infection was higher than that of healthy pregnant women, with highest value in the first trimester ( $0.69 \pm 0.09$ ), followed by second trimester ( $0.56 \pm 0.05$ ) and lowest in the third trimester ( $0.45 \pm 0.11$ ). The values obtained in each of the trimesters for healthy pregnant women (1st trimester -  $0.56 \pm 0.87$ , 2nd trimester -  $0.43 \pm 1.06$ , 3rd trimester -  $0.32 \pm 0.57$ ), pregnant women with typhoid infection (1st trimester -  $0.69 \pm 0.09$ , 2nd trimester -  $0.56 \pm 0.05$ , 3rd trimester -  $0.45 \pm 0.11$ ) and healthy non-pregnant women (1st trimester -  $0.70 \pm 1.01$ , 2nd trimester -  $0.65 \pm 0.98$ , 3rd trimester -  $0.66 \pm 1.22$ ) when compared are statistically significant ( $p < 0.05$ ).

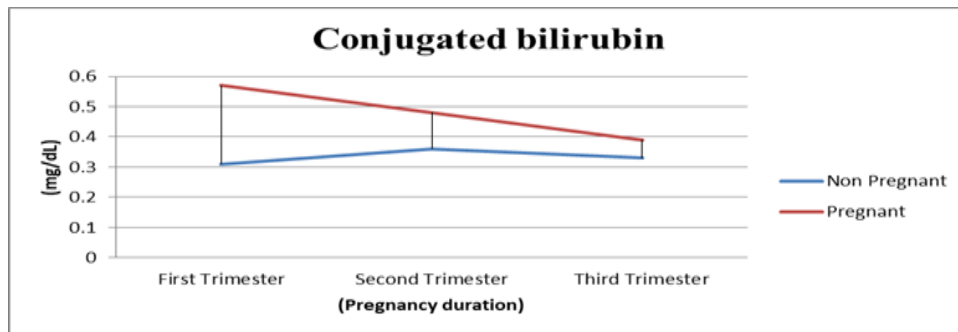


**Figure 5** Total bilirubin result for healthy pregnant women

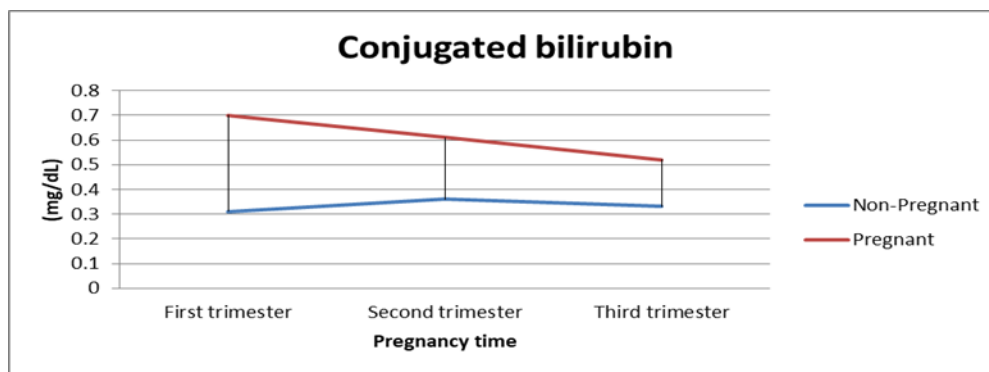


**Figure 6** Total bilirubin result for pregnant women with typhoid infection

Conjugated bilirubin (mg/dL) for healthy pregnant women (Figure 7) recorded mean value at first trimester ( $0.57 \pm 0.66$ ), followed by second trimester ( $0.48 \pm 0.77$ ), and third trimester ( $0.39 \pm 0.54$ ). Values of conjugated bilirubin obtained from pregnant women with typhoid infection (Figure 8) were higher than that of healthy pregnant women (Figure 7), with highest value in the first trimester ( $0.70 \pm 0.26$ ), followed by second trimester ( $0.61 \pm 0.03$ ) and lowest in the third trimester ( $0.52 \pm 0.41$ ). Mean values obtained in each of the trimesters for healthy pregnant women (1st trimester -  $0.57 \pm 0.66$ , 2nd trimester -  $0.48 \pm 0.77$ , 3rd trimester -  $0.39 \pm 0.54$ ), pregnant women with typhoid infection (1st trimester -  $0.70 \pm 0.26$ , 2nd trimester -  $0.61 \pm 0.03$ , 3rd trimester -  $0.52 \pm 0.41$ ) and healthy non-pregnant women (1st trimester -  $0.31 \pm 0.15$ , 2nd trimester -  $0.36 \pm 0.61$ , 3rd trimester -  $0.33 \pm 0.07$ ) when compared are statistically significant ( $p < 0.05$ ).



**Figure 7** Conjugated bilirubin result for healthy pregnant women



**Figure 8** Conjugated bilirubin result for pregnant women with typhoid infection

#### 4. Discussion

Liver disease is measured by determining various liver function tests to include alanine transaminase (ALT), aspartate transaminase (AST), total and conjugated bilirubin levels in the serum. Increased serum levels of the various enzymes in the liver function tests carried out on patients with typhoid fever only are indicative of a hepatocyte disorder. The liver function tests are used for evaluation of hepatic involvement during typhoid fever. According to [28], elevated serum enzymes (ALP, AST and ALT) in his work were discovered in 85% of patients with typhoid fever. [29] and [30] in their findings reported that 62 and 70%, respectively of patients with typhoid fever had elevated AST and ALT.

Serum alanine transaminase - ALT (iu/L) levels progressively increased from the first trimester to the second semester, before a sudden decrease in the third trimester. Although, ALT values in the third trimester was higher than that of the first trimester. Values of AST in healthy pregnant women, pregnant women with typhoid fever and healthy non-pregnant women when compared were statistically significant ( $p < 0.05$ ). This result was similar to the reports of [11-12]; [28-30], while two other studies by [31-32] found that serum ALT activity was significantly higher during the third trimester than in controls.

Aspartate transaminase - AST (iu/L) activity followed a trend of progressive value increase with increase in pregnancy time. It could be deduced that there is a correlation between pregnancy time and AST values, which would be said to be directly proportional. Although, same trend existed even with healthy non-pregnant women. But AST values were much higher during pregnancy periods with typhoid infection than during healthy pregnancy periods. Therefore, there is a clear correlation of AST values with pregnancy time on one end and typhoid infection on another end. The first trimester value was high, followed by second trimester and the highest was recorded in the third trimester. AST values among healthy pregnant women, pregnant women with typhoid infection and healthy non-pregnant women when compared were statistically significant ( $p < 0.05$ ). At the third trimester, mainly during labour, highest AST values were observed and could be linked with contraction of uterine muscles [17-18]. The result obtained in this study is in agreement with the assertions of [12]; [28-30] and [33]. Other studies found significant that supported the results obtained are the works of [26] and [34].

During the study, total and conjugated bilirubin activities decreased with pregnancy time. Total bilirubin levels had consistent decreases that are lower in expectant mothers than in healthy non-pregnant women in all three trimesters.

However, it was different for conjugated bilirubin as though there was consistent trend of retrogressive value decrease along pregnancy duration line but values obtained were high in expectant mothers than in non-pregnant women. The least value for total bilirubin and conjugated bilirubin for healthy pregnant women were recorded during the third trimester. Values of total and conjugated bilirubin obtained in this study for pregnant women with typhoid infection were higher than that of healthy pregnant women. The values obtained for total bilirubin and conjugated bilirubin among healthy pregnant women, pregnant women with typhoid infection and healthy non-pregnant women when compared were statistically significant ( $p < 0.05$ ). This result is in agreement with the reports of [12]; [28-30]; [33] and [35]. Meanwhile, hemodilution with pregnancy age had explained in part the cause for corresponding decrease in bilirubin concentration [36-40].

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## 5. Conclusion

Typhoid still remain a major public health problem in many developing countries of the world and if untreated can lead to increase in the serum levels of alanine transaminase, aspartate transaminase, total bilirubin and conjugated bilirubin.

Liver function test (LFT) results among pregnant women in most cases are altered by the normal changes that occur during pregnancy and also when infections like typhoid occur. Serum ALT and AST increase significantly but progressively with pregnancy time and at occurrence of typhoid infection, while total and conjugated bilirubin decreases significantly with pregnancy time and at occurrence of typhoid infection. These pregnancy and infection-related alterations are usually considered when evaluating LFT values in pregnant woman, otherwise results can be misinterpreted.

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## Compliance with ethical standards

### *Acknowledgments*

The authors are grateful to the management and staff of Federal Medical Centre (FMC), Owerri for the support provided to carry out the research.

### *Disclosure of conflict of interest*

No conflict of interest.

### *Statement of ethical approval*

The study was approved by the Ethical Committee of Federal Medical Centre (FMC), Owerri, Imo State, Nigeria.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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### How to cite this article

Ike CC, Akwari DK, Ogwuegbu HO and Chikezie JA. (2019). Typhoid infection and its effect on liver function assessment among pregnant women in Owerri, Imo State, Nigeria. *GSC Biological and Pharmaceutical Sciences*, 8(1), 105-113.

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