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Liver Enzymes and Blood Lactate Profile of Patients Diagnosed with Typhoid Fever in Abuja, Nigeria.

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ABSTRACT

Salmonella enterica serotype Typhi antibiotics resistance is on the increase and this frustrates efforts at treatment. Persistence of drug-resistant typhoid fever leads to higher mortality rate because treatment is evasive. Lactate is a marker of the severity of stress response in illnesses and liver function enzymes are indicative of the health of the liver. This study intended to identify the effect of drug resistant typhoid fever infection on liver enzymes and blood lactate levels of patients diagnosed with typhoid fever. Fifty subjects were recruited, forty-five were positive for Widal test and further subjected to stool culture examination for the identification of *Salmonella* Typhi. All patient's blood were analysed for lactate, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). *Salmonella* Typhi were identified in eight out of forty-five Widal positive test patients. The eight positive *Salmonella* Typhi isolates showed resistance to the Amoxicillin (30 mcg), Tetracycline (30 mcg), Cotrimoxazole (25 mcg), Ceftriazone (30 mcg), Levofloxacin (5 mcg), Gentamicin (30 mcg) and Netillin (30 mcg) but showed susceptibility to ofloxacin (5 mcg). ALP (158.1±8.32 IU/L), AST (55.1±6.78 U/L), ALT (65.2 ±4.96U/L) and blood lactate (10.5±2.4 mmol/L), were elevated in all drug resistant patients when compared to reference standard ALT (7-56 U/L), AST (10-40 U/L), ALP (20-120IU/L), lactate (0.8-2.2 mmol/L). ALT, AST and ALP enzyme levels increased with increasing number of resistances to antibiotics. Untreated typhoid fever infection exerts metabolic toll on liver functions.

Keywords: Antibiotics, liver enzymes, lactate, typhoid fever, drug resistance, infection.

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INTRODUCTION

Typhoid fever is an endemic infection caused by *Salmonella* serotype Typhi. It is acquired through oral-faecal transmission and is often diagnosed through blood, urine, stool and bone marrow culture [1]. It is estimated that there is about 14.3 million typhoid fever infections and more than 135,000 deaths worldwide annually [2]. However, the true incidence of typhoid fever is difficult to evaluate in Nigeria because of the lack of a proper coordinated epidemiological surveillance system and inadequate laboratory diagnosis status of Widal test which overestimates the incidence of typhoid fever in Nigeria and encourages unnecessary antibiotics consumption. Nevertheless, information on typhoid fever prevalence has been reported by several researchers in some states in Nigeria ranging from 0.071% in Oyo to 47.1% in Osun [3].

Antimicrobial resistance is the ability of microbes to resist the effects of medication previously used to treat them. Resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from another [4]. The antibiotic resistance crisis has been attributed to the overuse and misuse of these medications as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements [5]

In a bid to cope with the several justified and unjustified antibiotic use as treatment against *Salmonella* Typhi, the bacterium has developed defenses against some of the antibiotics employed [6]. Antibiotic resistance could range from minimal to moderate, with multi-drug resistance being defined as resistance to at least three first-line antibiotics [7].

Hepatic involvement is one of the earliest reported complications in the course of typhoid fever according to studies conducted in 1974 [8]. Salmonella hepatitis has been reported as a secondary life-threatening complication of untreated typhoid fever with increase in reports on myopathy during the course of the disease [9]. Both hepatic involvement and increases in reports on myopathy during the course of typhoid are responsible for release of the enzyme lactate dehydrogenase. The rise in serum lactate dehydrogenase in typhoid occurs early during the disease and may be attributed to cell necrosis of intestinal lymphatic tissue. It is believed that there is a unique relationship between macrophages in the liver, spleen and intestinal lymphoid follicles which could be

responsible for this pathogenesis [10] Liver biopsies conducted on typhoid fever patients demonstrate focal Kupffer cell hyperplasia and mononuclear cell infiltration of the portal space [11] The Kupffer cells tend to aggregate and the aggregations have even been termed typhoid nodules [12]. Elevated lactate levels have been observed among typhoid fever patients. However, the presence of elevated lactate levels is seen to manifest in most sepsis-related diseases and its presence in typhoid fever is not unexpected, especially because it is related to hepatomegaly and some mildly deranged liver function [9]. The elevated levels of lactate during typhoid fever have also been attributed to the inability of the liver to clear out excess lactate produced via gluconeogenesis. This may be due to a liver defection by Salmonella endotoxin induced consumptive coagulopathy, damage to hepatocytes, [13], direct invasion of the hepatocytes by the organisms [14], immune complexes and consumption of complement [15].

In this study we investigated the levels of blood lactate and some liver enzyme in antibiotics resistant typhoid fever outpatients.

METHODS

Study area and population

Study population included male and female outpatients aged 7 - 75 years reporting to Karu General Hospital Abuja, laboratory from May-June, 2019. Sample size for this study was 50 samples.

Ethical consideration

This study and procedures followed were in accordance with the ethical standards of the of National Health Research Ethics on human experimentation and the Helsinki Declaration of 1975, as revised in the year 2013. The ethical approval (BHU/REC/19/H/0001) was obtained from Research and Ethics Committee Bingham University, Karu, Nigeria. Written informed consent was obtained from patients before inclusion into the study.

Experimental Design

Patients directed to the laboratory by the consulting doctor for Widal tests (which is widely used in Nigeria for typhoid fever diagnosis) were given written informed consent forms and questionnaires after explaining the research to them. A semi-structured questionnaire was employed in obtaining information from participants in this study about their age, sex, duration of illness, pre-treatment information, and their disease recurrence rate. Trained phlebotomist collected blood samples used for lactate and liver enzymes test. Only patient samples positive for

Widal test were considered eligible as test subjects because most were diagnosed and treated only based on the Widal test results. Eligible patients were given stool sample bottles to bring freshly-passes morning stool the next day. Healthy individuals not suspected to have typhoid fever were recruited as control.

Blood sample collection

Using standard phlebotomy techniques, 5 ml of venous blood was collected from subjects. 3ml transferred to Ethylene Diamine Tetra-Acetic Acid (EDTA) containers for Widal test and gently mixed, while 2ml was transferred to lithium heparin bottle for lactate, ALT, AST and ALP analysis.

Stool sample collection and culture

Freshly passed early morning stool was collected in sterile wide mouth sample containers. Stool specimen was inoculated into Selenite F broth in McCartney bottles, and incubated for 18 hours at 37°C to encourage selective enrichment of *Salmonella spp*. After 18 hours, the inoculum was streaked directly onto Salmonella-Shigella Agar and incubated at 37°C for 24 hours. The resulting colonies were observed for colorless colonies with black centre and sub-cultured to obtain pure cultures.

Identification of isolates

Isolates were identified based on their cultural characteristics, Gram stain reaction, cell morphology, slide agglutination and biochemical tests like triple sugar iron, urease test and lack of oxidase [16].

Blood Lactate Analysis

An amount of 0.5 ml of whole blood taken from the lithium heparin bottle was used to analyse lactate levels using rapid Accutrend Plus diagnostic meter and BM Lactate test strips (Roche Diagnostics) according to manufacturer's directions. The lactate test strip was inserted into the Accutrend plus meter and blood was applied to completely cover the test area of the strip. The meter counted down and displayed result in 60 seconds. The results were displayed in mmol/L and measured plasma value of lactate. Accutrend Plus diagnostic meter employs colorimetric lactate oxidase/mediator reaction and has a record of high reproducibility when compared with laboratory diagnosis [17].

Liver enzymes analysis

1.5ml of blood sample in lithium heparin bottle was centrifuged at 3000rpm for 3 minutes and then 1 ml of sera was pipetted into sample cups and used to analyse for ALT, AST and ALP levels using an automated biochemical analyser (Selectra pro S 6003-500). The biochemical

analyser was calibrated as per manufacturers protocol using commercial standards provided. 1 ml of sera was pipetted into sample cups and reagents were loaded into designated regions in the analyser. The tests ALT, AST and ALP were keyed in and after 60 minutes, results were displayed on the screen.

Antibiotics Susceptibility Testing

The antibiotics susceptibility testing was done by Kirby-Bauer disk diffusion method according to NCCLS (2000) guidelines using 30 mcg of Amoxicillin, 25 mcg of Cotrimoxazole, 5 mcg of Levofloxacin, 5 mcg of Ofloxacin, 30 mcg of Tetracycline, 30 mcg of Netillin, 10 mcg of Gentamicin and 30 mcg of Ceftriaxone. A bacterial suspension was made using a single colony of organism emulsified in normal saline and turbidity adjusted to McFarland standard suspension. A sterile non-toxic cotton swab on a wooden applicator was dipped into the inoculum and the soaked swab rotated to drain excess liquid. The entire Muller Hinton agar surface of the plate was streaked with the swab containing the inoculum and allowed to dry for 5-10 minutes with the lid in place. Using sterile forceps, the antimicrobial disks were then placed on the agar. The plate was incubated at 37˚C for 18 to 48 hours. Using a millimeter ruler on the underside of the plate, the radius of each inhibition zone was measured in millimeters and the value doubled to get the diameter. The zones of inhibition were read and results interpreted using standardized thresholds for defining susceptibility [18].

Statistical analysis

Antimicrobial susceptibility frequency data was presented in percentages. Lactate and liver enzymes analysis data was presented as Mean ±SD and compared with theoretical/hypothetical values using the one sample student t-test. Two-way ANOVA with multiple comparisons was used to compare lactate and liver enzymes levels based on increasing antimicrobial resistance profile. $P<0.05$ was considered statistically significant. All data were statistically evaluated using Graph pad Prism 10.

RESULTS

Out of the 50 patients tested, 32% were male while 68% were female. Subjects within the age range of 20-40 years had the highest cases of Widal positives (58%). Out of the 45 Widal positives, prevalence of *Salmonella* Typhi in patient's stool was 8 isolates (18%) and 37 isolates (82%) were identified as negative for growth of *Salmonella enterica* ser. Typhi in their stool. The five patients that served as

control did not show any observable growth in their stool culture.

Data obtained from the patient's questionnaires on disease duration, disease frequency, pre-treatment and dosage of drugs are shown in Table 1.

Table 1: Frequency of demographics/data from *Salmonella* Typhi positive subject's questionnaires

Data	Subjects (n=8)
Disease frequency ≥ **3** times	8 (100%)
Disease frequency < 3 times	0 (0%)
Disease duration ≥ **3** weeks	7 (87.5%)
Disease duration < 3 weeks	1 (12.5%)
Self-treatment with local remedy	2 (25%)
Self-treatment with ethno-medicinal drugs	6 (75%)
Adherence to drug regimen	4 (50%)
Inconsistency to drug regimen	4 (50%)

The antibiotics resistance rates in Table 2 shows that 75% of the stool culture positive for *Salmonella* Typhi were resistant to Tetracycline, 62.5% resistant to Ceftriaxone, 100% resistant to amoxicillin, 87.5% resistant to Cotrimoxazole, 87.5% resistant to Gentamicin, 67.5% to Netillin, 12.5% resistant to Levofloxacin and 0% resistant to Ofloxacin.

The lactate values, AST, ALP, ALT and antibiotics resistance type in *S*. Typhi positive subjects are depicted in Table 3. The mean value for lactate was 10.5±2.24 mmol/L, 65.15±4.96 U/L for ALT, 55.08±6.78 U/L for AST and 158.10±8.32 U/l for ALP.

Table 2: Frequency of Antimicrobial resistance patterns of *Salmonella* Typhi positive patients (n=8)

S/N	Antimicrobial agent	Disk potency (mcg)	Sensitive	Intermediate	Resistant
1.	Tetracycline	30	1 (12.5%)	1 (12.5%)	6 (75%)
2.	Ofloxacin	5	7 (87.5%)	1 (12.5%)	0 (0%)
3.	Ceftriaxone	30	2(25%)	1 (12.5%)	5 (62.5%)
4.	Levofloxacin	5	7(87.5%)	0 (0%)	1 (12.5%)
5.	Amoxicillin	30	0 (0%)	0 (0%)	8 (100%)
6	Cotrimoxazole	25	0 (0%)	1 (25.5%)	7 (87.5%)
7.	Gentamicin	10	0 (0%)	1 (12.5%)	7 (87.5%)
8.	Netillin	30	3 (37.5%)	0 (0%)	5 (62.5%)

Table 3: Comparison of mean Lactate, AST, ALT and ALP values with control

Parameter	**Actual mean**	**Control means**	**Reference standard upper limit**	**P value**
Lactate (mmol/L)	10.50±2.40	1.58±0.52	2.2	<0.0001
AST (UI/L)	55.08±6.78	33.30±0.67	40	0.0004
ALT (UI/L)	65.15±4.96	45.50±2.25	56	0.012
ALP (UI/L)	158.10±8.32	84.25±8.69	120	<0.0001

Table 4: Mean lactate, ALT, AST and ALP levels based on number of antibiotics resisted

Number of Antibiotics resisted	Frequency in population	Mean Lactate mmol/L	Mean ALT U/L	Mean AST U/L	Mean ALP IU/L
2	1	9.0±0.00 ^{abc}	58.0±0.00 ^{a}	42.2±0.00^{a}	141.9±0.00 ^{a}
4	1	6.0±0.00^{ab}	62.4±0.00 ^{abc}	49.2±0.00 ^{bc}	149±0.00 ^{b}
5	3	10.4±1.12 ^{acd}	63.5±3.95 ^{abc}	55.5±3.07 ^{bc}	160.5±2.33 ^{c}
6	3	12.3±0.85 ^{cd}	70.3±1.00 ^{abc}	60.9±1.01 ^{d}	164.1±1.35 ^{d}

Values are Mean ± SD; Values with different superscript down the column are significantly different ($p < 0.05$).

There was obvious increase in liver function enzymes measured based on increasing number of antibiotics the bacteria were resistant to (Table 4). All liver enzymes (with exception of ALT levels in patient's resistant to four and five antibiotics) showed statistical increase between lower and higher number of antibiotics resistance profile.

DISCUSSION

In most developing countries like Nigeria, typhoid fever is widely diagnosed with only Widal test and this formed the basis of our inclusion criteria however typhoid fever cases reported in this study were diagnosed using the positive *Salmonella* Typhi stool culture analysis. The absence of *Salmonella* Typhi growth in the stool of 82% of the patients after testing positive for Widal test could be due to cross reacting antibodies from serum of febrile patients other than typhoid fever, [19] and other infections sharing a common antigenic determinant with *Salmonella* Typhi [20] due to low specificity of Widal tests [21], further justifying the error in complete dependence on Widal test as basis for diagnosis of typhoid fever. Also, patients in the clinical relapse phase as observed by Miller and Peuges [11] may be infected with *Salmonella* Typhi but have absence of *Salmonella* Typhi growth in their stool culture.

Resistance to Amoxicillin, Tetracyline, Cotrimoxazole, macrolides (Gentamicin and Netillin and cephalosporin (Ceftriaxone) was observed in all eight *Salmonella* Typhi positive isolates. Maximum resistance was observed in

amoxicillin; 8 (100%) isolates were resistant, Cotrimoxazole; 7(87.5%) isolates were resistant, 1(12.5%) isolate was intermediately susceptible and with Gentamicin; 7(87.5%) isolates were resistant and 1(12.5%) isolate was intermediately susceptible. The fluoroquinolones (Ofloxacin and Levofloxacin) were seen to be effective treatments with more susceptibility rates. Ofloxacin showed higher efficiency in 7(87.5%) isolates and intermediate susceptibility seen in 1(12.5%) isolate. 7(87.5%) isolates showed susceptibility to Levofloxacin with only 1(12.5%) isolate resistant to it. This is consistent with the report of Alam et al. [22]. who observed high susceptibility to Levofloxacin and Ofloxacin (Table 2). Similar trend has also been reported by Willey et al. [23] where high resistance to first line drugs, macrolides and 3rd generation cephalosporins and susceptibility to fluoroquinolones were observed. The multiple drug resistance observed in all *Salmonella* Typhi positive isolates could be due to abuse of these drugs. Self-treatment for typhoid fever by patients in cases of other febrile diseases such as non-typhoidal Salmonellosis, endocarditis and urinary tract infection could result to development of antibiotics resistance [24]. Diagnosis of typhoid fever in its absence also leads to misuse of these antimicrobials hence encouraging the bacteria to evolve defense mechanisms, such that the bacterium becomes resistant to the intended drug effect [25].

Data obtained from *Salmonella* Typhi positive patient's questionnaire (Table 1) revealed that they have had typhoid fever for 3 weeks or more, and evidently supported by the presence of *Salmonella* Typhi in the stool as *Salmonella* Typhi is excreted in the stool 2-3 weeks post infection [26]. All *Salmonella* Typhi positive patients underwent self-medication using various antibiotics, which included amoxicillin, ceftriazone, and tetracycline in a bid to treat the disease, of which 50% didn't complete the self-treatment regimen. Those drugs may have been abused by constant ingestion irrespective of being prescribed or not and this could have led to the development of resistance [27]. The antibiotics resistant typhoid fever seen in 75% of the eight isolates could also be as a result of pre-treatment with ethno-medicinal therapy (Table 1). Fifty percent of *Salmonella* Typhi positive subjects who claimed to have always adhered to their drug regime but were resistant may have contracted drug resistant strain of *Salmonella* Typhi prior to treatment. Multi-drug resistance to some antibiotics observed in 50% of patients that failed to adhere to their drug regime could be because of incomplete dosage of drugs which causes the destruction of

some bacteria while the undestroyed ones go on to develop more resistance properties [28].

Elevated lactate levels we observed in *Salmonella* Typhi positive patients could be due to typhoid fever as well as other factors since prevalence of elevated lactate levels was observed in all *Salmonella* Typhi positive subjects with a mean concentration of 10.5±2.24 mmol/L way above the normal range of 1.8-2.2 mmol/L. The highest lactate levels were observed in the patient with highest number of drug resistance which may be related to difficulty in successfully treating the infection at the onset. There was a significant difference ($P<0.0001$) observed when mean lactate levels of *Salmonella* Typhi positive subjects were compared with theoretical normal (Table 3). There may not be adequate reports on elevation of lactate levels in typhoid fever, however it was discovered and reported by Sameera et al. [29], that there is a statistically significant increase in lactate dehydrogenase (an enzyme that catalyzes the conversion of pyruvate to lactate) in typhoid fever patients when compared with healthy controls. This elevated lactate dehydrogenase levels in typhoid fever cases emphasizes the usefulness of lactate levels data in the determination of clinical and prognostic feature of typhoid fever. Typhoid fever results in an increase in lactate levels when the cytotoxins from *Salmonella* Typhi cause necrosis to lymphoid tissues and injury to the Kupffer cells of the liver. When this occurs, *Salmonella* Typhi release endotoxins that cause toxemia [30]. As the liver is clearly implicated in this process, it is not surprising to find liver enzymes elevated during and active typhoid infection.

Alanine amino transaminase (ALT), Aspartate amino transaminase (AST) and Alkaline phosphatase (ALP) are referred to as diagnostic liver enzymes because in elevated states, they can be indicative of liver malfunction. Studies have associated elevated liver enzymes with active typhoid fever infection [31, 32]. As expected with most liver related dysfunctions, AST 55.08±6.78U/L, ALT 65.15±4.96U/L and ALP 158.10±8.32U/L, were elevated in all patients with active typhoid infection when compared with reference standards of ALT (7-56 U/L), AST (10-40 U/L), ALP (20-120IU/L). There was a significant increase observed in the comparison of mean ALT ($P<0.0001$), AST ($P<0.0001$) and ALP ($P<0.0001$) levels of *Salmonella* Typhi positive patients with their respective control values (Table 3). This increase in liver enzymes may be due to hepatomegaly caused by endotoxins produced from *Salmonella* Typhi and direct invasion of the hepatocytes by *Salmonella* Typhi. A large population of *Salmonella* Typhi attach

themselves to the Kupffer cells such that Kupffer cells which are phagocytic are unable to destroy the bacteria population [33]. Invasion of *Salmonella* Typhi in the liver causes injuries and release of liver enzymes in large quantities indicating that hepatomegaly is not a complication of typhoid fever but a feature of the disease [34].

The elevation of liver enzyme levels observed (Table 4) as number of antibiotics resistance increases may be because the typhoid fever infection remains untreatable, hence the liver is continuously burdened. This also is the case for untreated typhoid fever cases caused by even antibiotics-susceptible *Salmonella* Typhi [35]. However, further studies on larger populations may be necessary to corroborate this.

CONCLUSION

Sustained typhoid fever infection due to antibiotics resistance may encourage secondary metabolic burden on the liver and consequently impairing its optimum function. Analysing these liver function enzymes and blood lactate level may be relevant in ascertaining typhoid fever disease burden in patients with history of antibiotics resistance as liver function tests could indicate underlying salmonella hepatitis, a rare complication of typhoid fever.

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Contribution to research and manuscript: OA and RO researched literature and conceived the study. OA and RO were involved in protocol development, gaining ethical approval and acquisition of material, patient recruitment, lab analysis and OA analysed the data. RO wrote the first draft of the manuscript. OA reviewed and edited the manuscript and both authors approved the final version of the manuscript.

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