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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of Liver and Renal Function Tests among Patients with Typhoid Fever in Motta General Hospital, Northwest Ethiopia

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This is to certify that the thesis prepared by Birku Gashaw, entitled: assessment of Liver and Renal Function Tests among patients with typhoid fever in Motta General Hospital, Northwest Ethiopia, complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Background: Typhoid fever is a systemic infection caused by *salmonella typhi*. Oro-fecal contamination is a common route of entry to cause infection of the gastrointestinal system. The bacteria then burrow the walls of the intestines and cause bacteremia through a profusion of bacteria in a person's bloodstream, which invade the liver, kidney, and other organs. In addition to the systemic infection, the liver organ can exposed to the bacteria directly via the gut-liver axis (enterohepatic circulation). Typhoid hepatitis and renal damage are the atypical presentations of typhoid fever. Lack of sensitive and specific diagnosis tests leads to poor clinical management of typhoid fever.

Objective: To assess liver and renal function tests among typhoid fever patients in Motta General Hospital, Northwest Ethiopia 2023.

Method: Institutional-based comparative cross-sectional studies were conducted at Motta General Hospital from May to December 2023 among 90 typhoid fever cases and 90 control groups who were selected with a convenient sampling technique. Laboratory tests were performed by the principle of spectrophotometry method to measure liver and renal function tests. Data entry and analysis were done by SPSS version 26. A p-value of <0.05 was taken as the cut-off point for significant differences.

Results: The mean age of typhoid fever and control subjects were 33.10± 12.11 years and 32.62 ± 7.708 respectively. The levels of serum AST, ALT, ALP, TB, DB, and creatinine showed statistically significant differences between typhoid fever and control groups ($p<0.05$). Duration of typhoid fever illness had an impact on the level of serum liver and renal function tests.

Conclusion: The findings of this study showed that liver function tests(ALT, AST, ALP, TB, and DB) and renal function tests(creatinine) were significantly altered by typhoid fever as compared with the control group, which would play an essential role in the management of typhoid fever.

Keywords: Typhoid fever, liver function test, renal function test

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Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CFAS	Calibrator for automated system
CRE	Creatinine
DB	Direct bilirubin
LFT	Liver function test
MDH	Malate dehydrogenase
MGH	Motta General Hospital
NADH	Reduced nicotinamide adenine dinucleotide
PI	Principal Investigator
RFT	Renal Function Test
RPM	Revolution per Minute
SB	Serum Bilirubin
SD	Standard Deviation
SOP	Standard Oprating Procedure
SPSS	Statistical Package for Social Science
TB	Total Bilirubin
TF	Typhoid Fever
WBC	White Blood Cell

1. Introduction

1.1. Background

Typhoid fever is a common bacterial infection in the tropics (1). It is a systemic infection caused by water and food-borne pathogens such as Salmonella enterica subspecies, *enterica serovar typhi* (S. typhi) *(2).*

The salmonella bacteria is transmitted through contaminated food and water. The bacteria then burrow the walls of the intestines and cause bacteremia through the profusion of bacteria in a person's bloodstream, which invade liver, kidney, and other organs(3).

In addition to the systemic infection, the liver organ can exposed the bacteria directly via the gut-liver axis. The intestine and liver are important organs for nutrient absorption, metabolism, and the immune system. The functions of the two organs influence each other through the gut-liver axis. It represents a bidirectional communication between the intestine and the liver(4). The portal vein is the direct venous outflow from the intestine to the liver. The liver is immediately exposed to a variety of harmful substances originating from the intestine as well as intestinal germs like salmonella when the intestinal barrier is damaged. An increase in intestinal permeability linked to a disordered gut microbiota exposes the liver to bacteria that can directly affect hepatocytes or Kupffer cells, which are cells of the hepatic innate immune system(4, 5).

Pattern recognition receptor (PRRs), specifically Toll-like receptors (TLR), is the first component of the immune system to detect host invasion by pathogens, activate immune responses, and form the link between innate and adaptive immunity. PRRs recognize pathogen-associated-molecular-patterns (PAMPs) and danger-associated molecular-patterns (DAMPs)(6). In invasive salmonella infection, DAMPs and PAMPs stimulate the innate immune system which causes activation of macrophages and produces inflammatory chemicals like interferon-gamma (IFN)-γ, tumor necrosis factor (TNF)-α, IL-1β, and Interleukin (IL)-6. IFN-γ regulates the level of macrophage activation, which is the primary mechanism by which persistent infection is controlled. The release of IFN-γ and immediate host resistance to Salmonella infections depend on IL-18(7, 8).

Kupffer cells are macrophages present in the liver that phagocytose S. Typhi to help it avoid immune surveillance. In addition to antimicrobial peptides, reactive oxygen species (ROS, like hydrogen peroxides, superoxides anion, and hydroxyl radicals), and reactive nitrogen species (RNS, including nitric oxide and peroxynitrite) are produced by the macrophage cells to control the invading pathogen but the salmonella bacteria have superoxide dismutase that encodes an enzyme required for the detoxification of superoxide anion and catalyzes the detoxification of nitric oxide and is needed to preserve a chronic Salmonella infection(9, 10).

Most of the Salmonella species' virulence factors are encoded on Salmonella pathogenicity islands (SPI). Effector proteins necessary for liver invasion are delivered via a type III protein secretion system (TTSS) encoded by the Salmonella pathogenicity island-1 (SPI-1) locus. The SPI-1 effector SipB stimulates caspase-1 in macrophages and activates rapid cell death by a mechanism of both necrosis and apoptosis. SPI-2, which encodes distinct TTSS, is the second specific DNA region necessary for liver infection survival and replication in macrophage cells. The SPI-2 effector proteins are known to give the capacity of Salmonella strains to proliferate within macrophages and cause a persistent infection(11-13).

In case of this, there is uncontrolled stimulation of the adaptive and innate immune response that leads to detrimental inflammation and tissue injury of the liver organ. When these happen, the transaminase enzymes of the liver leak out and find their way into the circulation, leading to increased enzyme levels(14). Due to research showing a significant link between gallstones and the likelihood of developing into a chronic carrier state salmonella infection, S. Typhi can spread from the liver to colonize the biliary system through the ducts or capillaries that connect the liver to the gallbladder which leads to the release of membranous alkaline phosphatase into the circulation (15).

Twenty to forty percent of patients excreted Salmonella in the urine, usually during the second or third week of the disease. Toxic nephrosis, the development of micro-abscesses, or the creation of metastatic foci in the kidney is due to the passage of salmonella bacteria through the intact kidney in typhoid fever. In addition to being a significant predictor of many renal diseases, oxidative stress caused due to bacteria also plays a role in the cause of glomerulonephritis. Glomerulonephritis leads to abnormal function of the kidney in the elimination of waste products including creatinine and urea which causes an increasing those chemicals(16, 17).

1.2. Statement of the problem

Typhoid fever is thought to affect 11–21 million people annually and approximately 128 000–161 000 deaths annually worldwide(18). Developing countries contributed to more than 87% (nearly 12.5 million) of the global cases(19, 20).

Typhoid hepatitis is one of the atypical presentations of TF and can be the involvement of the liver during the course of typhoid fever. Liver organ damage can be involved leading to a variety of presentations from uncomplicated typhoid fever to a complicated one. Abnormal liver function tests suggesting hepatic involvement has been reported as 23 to 60% by various studies(21). Studies reported that the incidence of elevated transaminases and alkaline phosphatase significantly in all the cases in the 2nd and 3rd week of illness. As the liver has a great functional reserve, significant liver damage may have occurred without obvious clinical signs and symptoms(22). The reported incidence of salmonella hepatitis ranges from 0.4-26% of typhoid fever cases and is highest from a study in Vietnam(23).

Study undertaken in India AST was raised in 27 cases and ALT in 25 cases out of 54 cases. Most cases with raised AST and ALT presented in the 2nd week of fever(24). As the study indicated in the United Arab Emirates out of 50 enteric fever cases, elevated AST of more than twofold was seen in 2 cases in 1st week, 13 cases in 2nd week, and 7 cases in 3rd week, elevated ALP levels of more than two-fold was seen in 2 cases in 1st week, 11 cases in 2nd week and 6 cases in 3rd week and hyperbilirubinemia was found in 5/50(10%) patients(25). Hepatic dysfunction is common in typhoid fever. Salmonella hepatitis is seen in patients with prolonged illness and inappropriate use of antibiotics. liver dysfunction in typhoid fever causes hepatomegaly, jaundice, biochemical alterations and histopathological changes(26).

The renal damage caused by the salmonella bacteria is either immunological or directly caused by the invasion of Salmonella typhi which causes pyelonephritis (a sudden and severe kidney infection that causes the kidneys to swell and can cause permanent damage)(27). Even if typhoid fever is a top health challenge have not any published study conducted in Ethiopia on this title, particularly in the area of study. The purpose of this study is to assess the liver and renal function tests of patients with typhoid fever.

1.3. Significance of the study

For policymakers and health administrators, the study findings will be very important, as typhoid fever has no specific and sensitive diagnostic markers it may be used to implement an appropriate and comprehensive diagnosis of patients with typhoid fever. In the clinical management of typhoid fever patients, this finding will also be very important to the community and health professionals. The findings of the current study may serve as baseline information for other researchers who are interested in doing on this and related studies that may follow in the future.

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2. Literature review

2.1. Liver and Renal Function tests in typhoid fever patients

Typhoid fever is the common cause of mortality and morbidity in countries with poor hygienic practices and limited access to safe drinking water. Different studies have been conducted in different parts of the world among typhoid fever patients to assess the effect of typhoid fever infection on liver and renal function tests(28).

In the prospective study conducted in India 31 children of typhoid fever patients participated in the study with elevated levels of serum aspartate aminotransferase (AST) (61.3%), serum alanine aminotransferase (ALT)48.4%), alkaline phosphatase (AP) (22.6%) and serum bilirubin (SB) (6.1%). Therefore; hepatic dysfunction was presented even in cases without hepatomegaly, with high levels of AST (60%), ALT (40%), ALP (20%), SB (6.7%)(29).

Based on the cross-sectional study of MY Hospital, MGM Medical College Indore (2015) a total of 54 typhoid fever patients were included, the majority of the cases 40.74% were age range of 4-8 yrs. Of the 54 patients that were examined, 20 (37.03%) were male and 34 (62.9%) were female. Of the cases, 27 (50%) had high AST and 25 (46%), had raised ALT. Most of the cases with high-value ALT and AST were found in the 2nd week of fever(24).

In the study of India (2015) a total of 50 enteric fever patients with ages ranging from 14yrs to 60 yrs with a mean age of 21.9yrs and male: female ratio being 3.5:2. In the analysis of liver function tests, AST was normal in 28 patients, elevated (More than 2fold) in 22/50(44%) patients. ALP was normal in 31 patients, raised to more than 2fold in 19/50(38%) patients. ($P< 0.05$) and bilirubin range in these patients is 1.8 to 5.6 mg/dl and predominantly had conjugated hyperbilirubinemia. In general, abnormal LFT in enteric fever is seen more commonly in patients presenting in 2nd and 3rd week of illness (25).

According to a comparative cross-sectional study of Iraq (2020) 120 typhoid fever patients aged 20 to 50 years, and 60 healthy individuals of the same age as the comparative subject. The mean value of serum liver enzymes: alkaline phosphatase (ALP), alanine transaminases (ALT) and aspartate transaminases (AST) were (130±9.8IU/l; 31.4±5.9IU/l; 26.8±4.2IU/l), respectively in

Typhoid fever patients. There was significantly higher ($P<0.05$) occurs in the level of liver enzymes ALT, AST, and ALP(26).

Based on the study of Iran (2003) in typhoid fever patients of 107, there were 76 (71.1%) males and 31 (28.9%) females. The median age was 25.5 years (range 18 months to 67 years). Biochemical abnormalities of increased serum bilirubin in 26 (24.2%), 2 to 30 folds rise in alanine transaminase (ALT) in 76 (71.1%) patients, 2 to 22 folds rise in aspartate transaminase (AST) in 56 (52.3%) patients were seen. The serum alkaline phosphatase level was high in 25 (23.3%) patients. Therefore biochemical abnormalities were seen in 22.4% of patients(18).

In the cross-sectional and prospective study in Iran (2007) the study included 118 patients with typhoid fever in the age range of 2-53 years. Hepatomegaly was revealed in 14% of the cases and was correlated with elevated serum bilirubin (5.05 ± 13.03 mg/dl in hepatomegaly subjects). Alanine aminotransferase (ALT) was elevated in 22 of the cases. In conclusion, the elevation of liver enzymes is relatively less common(30).

Out of the sixty children enrolled in the prospective observational study that was carried out in Nepal in 2018–2019, fifty-six percent were girls, and forty–three percent were boys. In most of the instances, there was a fever, lack of appetite, coughing, and vomiting. On admission, AST and ALT levels were found > 35 IU/L in 26 cases (43.33%) and 34 cases (56.66%) respectively.

Based on an observational study of Rashid Hospital, United Arab Emirate (2005-2007) with a total of 52 patients included mean age + SD of participants in the study was 27.4+7.89 years (14-45 years) and males outnumbered the females 45(87%) vs 7(13%). A higher level of alanine aminotransferase (85%) and in 10% of cases the level was more than 10fold of normal value, aspartate aminotransferase (75%), alkaline phosphatase (44%) and serum bilirubin (25%). In conclusion, patients from tropical countries or those who have traveled recently to areas of high incidence of TF should arouse suspicion of clinical diagnosis of enteric fever(31).

A hospital-based descriptive study was conducted in the United Arab Emirates (2007-2008) and a total of 75 typhoid fever patients were included. The biochemical changes included; raised alanine aminotransferase (73.3%), aspartate aminotransferase (62.7%), bilirubin (30.6%), and alkaline phosphatase (44%). In conclusion, typhoid fever causes a significant hepatic dysfunction(32).

Based on the comparative cross-sectional study in Nigeria (2014) the study included 30 typhoid fever cases and 20 healthy groups with an age range of 22-40 years. Mean serum level of alanine transaminases (ALT), aspartate transaminases (AST), alkaline phosphatase (ALP), total bilirubin (TB), and direct bilirubin (DB) 22.8±5.94, 28.33±11.72, 116.69±48.68, 19. 31±5.84, and 5.60±2.50 respectively were obtained for those typhoid fever subjects. While the control subjects have mean serum levels of ALP (71.05±18.18), AST (16.65 ±7.45), ALT (13.85±6.09), TB (10.09±4.85), and DB (3.00±1.67). The results suggest that typhoid infection can elevate ALP, AST, ALT, TB, and DB serum levels and can lead to hepatic dysfunction (33).

According to a comparative cross-sectional study conducted by Cameron (2020) on the biochemical profile of the liver and kidney 112 healthy individuals and 151 patients were diagnosed with typhoid fever. The tests of kidney and liver function (ALT, AST, T-BILI, C-BILI, ALP, GGT, urea, and creatinine) presented a significantly higher level at varying degrees, especially for ALT ($p < 0.001$), AST, ALP, urea and creatinine ($p < 0.01$), T-BILI and D-BILI ($p < 0.05$). The findings of this investigation indicate that typhoid fever adversely affects the liver and kidneys' ability to function depending on the duration of the illness. (27).

According to the study in Nigeria (2019) the result of renal changes associated with male and female Typhoid fever patients, there is a significant increase in Creatinine levels in both male and female patients compared to their control and a significant increase, $p < 0.05$ in Urea level of the Typhoid positive females. Thus, using renal biochemical profiles as a diagnostic tool for early typhoid fever infections may help identify complications associated with the fever early on, improving patient treatment and preventing potential death from such complications(34).

2.2. Conceptual framework

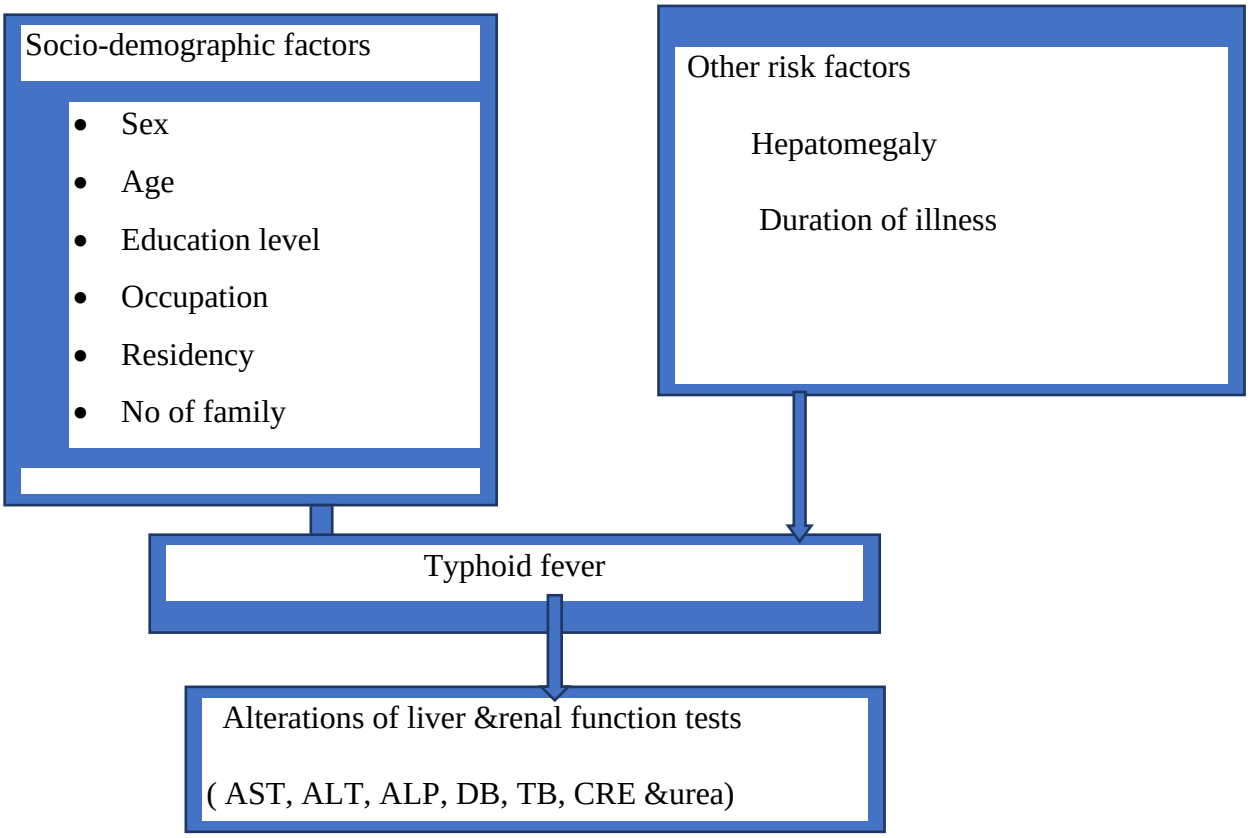

Figure 1: Conceptual framework of the study

3. Objective

3.1. General objective

- ✓ To assess levels of liver and renal function tests among typhoid fever patients in Motta General Hospital, Northwest Ethiopia from May to December 2023.

3.2. Specific objective

- ✓ To assess the value of liver and renal function tests among typhoid fever patients in Motta General Hospital, Northwest Ethiopia.
- ✓ To compare the median of liver and renal function tests in typhoid fever patients with the control group in Motta General Hospital, Northwest Ethiopia.
- ✓ To assess the associated factors for derangement of liver and renal biochemical profiles among typhoid fever patients in MGH, Northwest Ethiopia.

4. Method and Material

4.1. Study area

This study was conducted at Motta General Hospital. Motta is a town in northwest Ethiopia found on the secondary road connecting Dejen and Bahir Dar, it is situated in the east Gojam Zone of the Amhara Region. It is located at latitude 11°5′N, longitude 37°52′E, and is 2,487 meters above sea level. It is located 120 kilometers from Bahir Dar (the capital of Amhara Regional State) and 365 kilometers from Addis Ababa. Around 1.2 million people in the surrounding area were served by the hospital. The hospital has around 250 workers, 18 laboratory professionals assigned to main, emergency, and inpatient laboratories(35).

4.2. Study design and period

An institutional-based comparative cross-sectional study was conducted from May to December 2023.

4.3. Population

4.3.1. Source population

- All febrile patients who were attending MGH during the study period were considered as the source population for the case group.
- All MGH employees and apparent students who were available during the study time served as a source population for the control group.

4.3.2.Study population

- All typhoid fever patients who were attending MGH during the study period were taken as the study population for the case group.
- All apparently healthy staff and students who were available in MGH during the study period and corresponded with cases by age and sex were taken as the study population for the control group.

4.4. Eligibility Criteria

4.4.1. Inclusion criteria

- ✓ Typhoid fever patients who were attending MGH in the study period and accommodating to participate in this study were used as inclusion criteria for the case group.
- ✓ Apparently healthy staff members and apparent students who were available at MGH during the study period, who corresponded with cases by sex and age and volunteered to participate in this study were used as inclusion for the control group.

4.4.2. Exclusion criteria

Individuals who have chronic diseases such as heart disease, liver disease, kidney disease, insufficient samples (less than 3ml), and malaria and individuals who abuse alcohol and pregnant women were not included in this study for both the case and control group.

4.5. Study variable

4.5.1. Dependent variable

- ✓ The level of liver function tests (AST, ALT, ALP, DB, and TB)
- ✓ The level of renal function tests (creatinine and urea)

4.5.2. Independent variables

- ❖ Socio-demographic factors: sex, age, residency, occupation, and number of family.
- ❖ Clinical features: duration of illness, hepatomegaly, headache, abdominal pain, nausea, diarrhea, and BMI.
- ❖ Laboratory test: antibody titer

4.6. Sample size determination and sampling method

4.6.1. Sample size determination

The sample size was determined by using the mean direct bilirubin (DB) value from the Cameroon study which yields the largest sample size.

A two-sided test with significance level α and power 1–β is used to determine the sample size required for comparing the means of two normally distributed samples. use 80% power and a 95% confidence level to determine the right sample size. Based on the study of Cameroon (2020) mean value of DB in typhoid fever and healthy groups (0.29 ± 0.53: 0.11 ± 0.08) was used to determine sample size by the formula: $n = \frac{(S1^2 + S2^2)}{d2} X (Z\alpha+Z\beta)^2$ where n= sample size, s_1= standard deviation of TF group =0.53, s_2= standard deviation of apparently healthy group =0.08, Zα=1.96, Zβ = power = 0.84, d= difference between means = 0.29-0.11=0.18. $n = ((0.53)^2 + (0.08)^2) \times (1.96+0.84)^2/ (0.18)^2 = 69.52$ round to 70.

10% nonresponse rate = 0.1x70= 7 so the minimum sample size is 70+7 = 77 for each group. Increasing sample size important to increase the representativeness of the sample to the entire population and power to show statistical differences between control and typhoid fever groups. Therefore 90 typhoid fever and 90 apparently health participants were included (27).

4.6.2.Sampling method

A convenient sampling method was used to conduct this study.

4.7. Data Collection and Measurement

4.7.1. Data collection

Clinical history and sociodemographic data were gathered using semi-structured pretested and translated questionnaires. Data collectors(nurses) interview study participants directly to complete the questionnaire.

4.7.2. Laboratory analysis

After the collection of three to five milliliters of blood samples from both typhoid fever and healthy groups, serum were separated using a centrifuge running at 5,000rpm for five minutes. The serum was tested for renal function (creatinine and urea) and liver function (ALT, AST, ALP, DB, and TB). The analysis was carried out using the spectrophotometric principles, which measure the analyte's absorption spectrum at each wavelength. The Cobas c311 automated chemistry analyzer and the Roche reagent were used.

4.8. Data quality assurance

4.8.1 Pre analytical

To ensure that the participants could understand the questions during the interview, the questionnaires were translated into the Amharic language. Ten percent of participants were pre-tested before the real data collection. The blood sample was collected in aseptic techniques to avoid hemolysis and other interferences

4.8.2. Analytical

Before the participant sample run, both pathological and normal control samples were tested. The participant sample was analyzed after both control values were accepted.

4.8.3 Post-analytical

All information was recorded in the unique code of each subject and check its completeness and consistency.

4.9. Data analysis and interpretation

The distribution of data was examined by the Kolmogorov-Smorvo test after the data were entered into SPSS version 26. For categorical variables, data were represented by frequency and percentages; for continuous data with a normally distributed distribution, as mean ± standard deviation (SD); and for continuous data with skewed distribution, as median ± IQR. The LFT and RFT levels of the TF and control groups were compared using non-parametric tests, namely the Mann-Whitney U test and the Kruskal-Wals test. A p-value of <0.05 is to be considered as statistically significant.

4.10. Operational definitions

Liver function tests- are sets of blood tests that are important to the assessment of liver dysfunctions, which are ALT, AST, ALP, TB, and DB.

Renal function tests- are sets of blood tests that help assess renal dysfunctions. Urea and creatinine are two of the tests.

Typhoid fever(case group)- a febrile illness patient who has positive for rapid salmonella stool antigen test. and an antibody titer of $\geq$ 1:80.

Apparently healthy (control group): An individual without any symptoms or signs of illness, with a negative rapid stool antigen test result for salmonella and non-reactive for widal test.

4.11. Ethical consideration

Addis Ababa University Department of Medical Laboratory was given ethical clearance through its research and ethics committee. Motta General Hospital Administrators granted additional permission. Written informed consent and assent were given by participants before the commencement of data and specimen collection after the use of the study was explained. Confidentiality of each information collected from the participants was maintained by employing a special code.

4.12. Dissemination of the result

The final result of the study will be submitted to Addis Ababa University College of Health Science Department of Medical Laboratory Science and Motta General Hospital. It will also be accessible to decision-makers, researchers, and clinicians. Additionally, the results will be published in peer-reviewed journals.

5. Workflow

Figure 2: Workflow of this study

6. Result

6.1. Socio-Demographic Characteristics of Study Participants

A total of 90 typhoid fever subjects (53 males and 37 females) and 90 control groups (46 males and 44 females) with the age range of 15-53 were involved in the study. The mean age ± SD of typhoid fever patients and controls was 33.10 ± 12.11 years and 32.62 ± 7.708 years respectively. 58.9% of typhoid fever cases and 11.1% of the control group were live in rural areas. Around 27.8% of typhoid fever was a farmer and 86.7% of controls were government employees. 35.6% of typhoid fever cases had no formal education and 92.2% of control groups were diploma and above(Table 1).

Table 1: Socio-demographic characteristics of TF case and control groups in MGH, Northwest Ethiopia, May to December 2023.

Socio-demographic character		Case(n=90)	Control(n=90)
Sex	Male	53(58.9%)	46(51.1%)
	Female	37(41.1%)	44(48.9%)
Age in year	<25	29(32.2%)	20(22.2%)
	25-34	16(17.8%)	35(38.9%)
	≥ 35	45(50.0%)	35(38.9%)
Mean ±SD		33.10 ± 12.11	32.62 ± 7.708
Residence	Urban	37(41.1%)	80(88.9%)
	Rural	53(58.9%)	10(11.1%)
Occupation	Gov't employee	6(6.7%)	78(86.7%)
	Merchant	11(12.2%)	0
	Farmer	25(27.8%)	0
	Housewife	19(21.1%)	0
	Student	29(32.2%)	12(13.3%)
Educational status	No formal education	32(35.6%)	0
	Primary	30(33.3%)	3(3.3%)
	Secondary/preparatory	13(14.4%)	4(4.4)
	Diploma and above	15(16.6%)	83(92.2%)
Family member	<5	84(93.3%)	83(92.2%)
	≥5	6(6.7%)	7(7.8%)
BMI	<18	3(3.3%)	0
	18-25	87(96.7%)	86(95.6%)
	>25		4(4.4%)
Mean± SD		20.33±1.37	21.93±1.74

BMI-body mass index, SD-standard deviation, n-number of study participant

6.2. Clinical features of typhoid fever patient

The clinical signs and symptoms in TF cases were: headache (100%), abdominal pain (78.9%), diarrhea (14.4%), nausea (87.8%), and hepatomegaly (32.2%) (Figure 3).

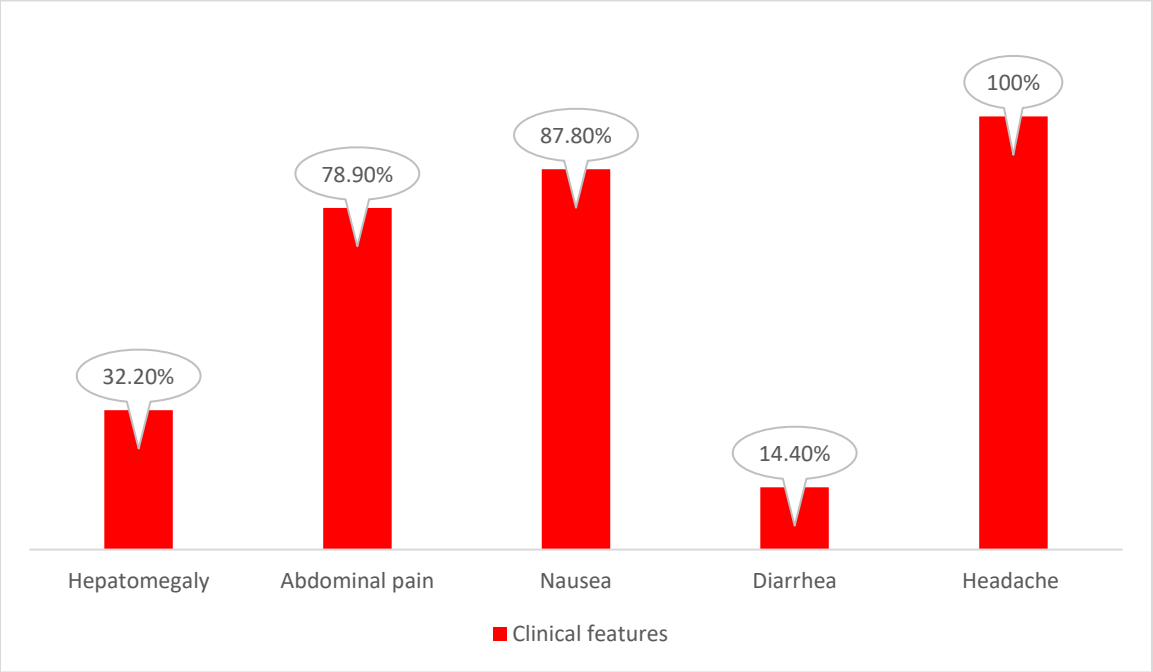

Figure 3: Clinical features of TF patients in MGH, Northwest Ethiopia, May to December 2023.

6.3. Comparisons of Liver and Renal function tests in typhoid fever case and control groups.

The serum levels of AST, ALT, and ALP were significantly higher in typhoid fever cases than control group with median ± IQR (54.35±78.33 versus 25.45 ±11.23, P<0.001), (54.05±78.33 versus 18.10 ±12.27, p<0.001) and (90.50±74.25 versus 80.00 ±54, p<0.001) respectively. Similarly, levels of TB and DB were significantly higher among typhoid fever patients compared to control groups (0.80±1.2 versus 0.504 ±0.302, p<0.001), (0.174±0.76 versus 0.113 ± 0.097, p=0.002) respectively. Renal function test creatinine was significantly higher in typhoid fever cases than in control groups with median ± IQR 1.07±0.69 versus 0.68 ±0.19, p<0.001. The level of urea was no statistical difference between typhoid fever patients and comparison groups (29.20±11.43 versus 29.60 ±13.05, p = 0.171) (Table 2).

Table 2: Comparison of liver and renal function tests among TF cases and control groups in MGH, Northwest Ethiopia, May to December 2023.

Parameter	Case group(n=90) Median ±IQR	Control group(n=90) Median ±IQR	P-value
ALT(U/L)	54.05±78.33	18.10 ±12.27	<0.001
AST(U/L)	54.35±78.33	25.45 ±11.23	<0.001
ALP(U/L)	90.50±74.25	80.00 ±54	<0.001
AST: ALT	1.29±0.38	1.4±0.46	0.005
TB (mg/dl)	0.80±1.2	0.504 ±0.302	<0.001
DB (mg/dl)	0.174±0.76	0.113 ±0.097	0.002
CRE(mg/dl)	1.07±0.69	0.68 ±0.19	<0.001
Urea (mg/dl)	29.20±11.43	29.60 ±13.05	0.171
WBC ($X10^3/\mu L$)	11.73±4.62	7.18 ±3.69	<0.001

IQR-Inter quartile range, WBC-White blood cell and BMI-Body mass index.

- Mann-Whitney U test was used for comparison

6.4. Liver and Renal function test results of age and gender-adjusted on typhoid fever case and controls.

The values of AST, ALP, TB, and creatinine showed significant differences in typhoid fever cases in males and both genders. ALT was significantly difference in males, females, and both genders of TF patients, $p<0.05$ (Table 3), However, DB and urea were not significantly different in both typhoid fever patients and control groups, $p>0.05$ (Table 3&4).

Table 3: Liver and Renal function test results of age and gender-adjusted value on typhoid fever case in MGH, Northwest Ethiopia, May to December 2023.

Age and Sex		ALT Median± IQR	AST Median± IQR	ALP Median± IQR	TB Median± IQR	DB Median± IQR	CRE Median± IQR	Urea Median± IQR
<25(n=29)		23.7±38.5	33.1±33.65	83.0±58.0	0.57±0.69	0.14±0.20	0.75±0.71	30.3±11.4
Male (n=15)		23.7±41.6	29.2±31.3	81.0±47.0	0.47±0.66	0.09±0.27	0.57±0.68	31.4±8.3
Female (n=14)		24±38.3	43.9±37.5	87.5±81	0.67±0.76	0.14±0.14	0.840.73	26.7±14.4
P-value		0.98	0.41	0.27	0.41	0.65	0.26	0.22
25-34(n=16)		51.7±79.9	51.4±82.5	122±79.7	0.58±1.14	0.23±0.47	1.05±0.82	26.1±14.2
Male (n=12)		54.7±72.3	53.1±72.1	125±73.2	0.57±1.10	0.14±0.47	1.07±0.54	26.1±17.7
Female (n=4)		15.9±70.1	20.6±97.3	89.0±130.7	1.11±1.50	0.34±0.93	0.74±1.09	26.0±14.15
P-value		0.27	0.15	0.33	0.22	0.72	0.30	0.81
≥35(n=45)		67.9±75.6	86.1±85.2	131±86	1.29±1.45	0.30±1.16	1.16±0.26	28.4±12.1
Male (n=26)		69.2±94.1	89.1±92.2	90.5±83	1.36±1.45	0.25±1.18	1.15±0.48	30.2±15.4
Female (n=19)		58.5±56.7	69.8±79.8	134±90	1.21±1.46	0.30±1.15	1.16±0.36	26±6.7
P-value		0.92	0.89	0.59	0.81	0.70	0.48	0.21
P-Value	Bothsex(90)	0.001	0.002	0.010	0.032	0.219	0.012	0.547
	Male (n=53)	0.027	0.007	0.021	0.032	0.532	0.032	0.461
	Female (n=37)	0.024	0.058	0.150	0.509	0.245	0.154	0.922

n-number of study participants

- Mann-Whitney U Test to see gender differences in each age group
- Kruskal-Wallis Test to see gender differences in all adjusted age group

Table 4: Liver and Renal function test results of age and gender-adjusted on control groups in MGH, Northwest Ethiopia, May to December 2023.

Age and Sex		ALT Median± IQR	AST Median± IQR	ALP Median± IQR	TB Median± IQR	DB Median± IQR	CRE Median± IQR	Urea Median± IQR
<25(n=20)		19.1±12.3	27.5±15.2	61±41.7	0.44±0.34	0.16±0.20	0.69±0.19	34.9±14.1
Male (n=4)		21.6±11.9	33.1±19	48.5±15.2	0.39±0.54	0.21±0.23	0.65±0.33	30.3±16
Female (n=16)		19.1±13	26.9±9.9	67±46.7	0.44±0.34	0.16±0.13	0.70±0.21	34.9±11.2
P-value		0.85	0.64	0.12	0.67	0.51	0.85	0.85
25-34(n=35)		16.8±18.2	26.7±13.1	73±77	0.55±0.39	0.11±0.15	0.71±0.22	28.9±13.3
Male (n=20)		20.3±17.5	26.3±12.8	80.5±64.5	0.63±0.49	0.14±0.18	0.72±0.19	25±11.2
Female (n=15)		16.7±19	27±13.9	73±93	0.41±0.27	0.08±0.08	0.64±0.19	31.1±18.5
P-value		0.38	0.64	0.76	0.04	0.12	0.34	0.18
≥35(n=35)		17.3±8.0	23.2±11	85±71	0.50±0.19	0.11±0.07	0.66±0.18	28.6±11.3
Male (n=22)		17.9±9.05	27±17.1	87.5±75	0.49±0.21	0.09±0.08	0.64±0.16	27.4±10.6
Female (n=13)		16.6±9.3	22.3±4.0	78±63	0.56±0.25	0.12±0.08	0.71±0.24	31.9±12.7
P-value		0.17	0.09	0.25	0.32	0.19	0.45	0.45
P-Value	Both sex	0.66	0.64	0.24	0.76	0.42	0.92	0.23
	Male(46)	0.85	0.68	0.06	0.23	0.11	0.48	0.42
	Female(44)	0.44	0.46	0.79	0.48	0.52	0.74	0.81

n-number of study participants

- Mann Whitney U Test to see gender differences in each age group
- Kruskal-Wallis Test to see gender differences in all adjusted age groups

6.5. Duration of illness in TF patients and LFT and RFT test results

Duration of illness had effects on liver and renal function tests of typhoid fever patients. ALT, ALP, TB, and Creatinine levels of typhoid fever study subjects of greater than or equal to three weeks and two weeks duration were significantly higher than study subjects exposed for less than or equal to one week (Table 5).

Table 5*:* The impact of duration of illness on LFT and RFT of TF patients in MGH, Northwest Ethiopia, May to December 2023.

Parameters	Duration of illness		
	≤ one week(n=68) (Median± IQR)	Two weeks (n=20) (Median± IQR)	≥Three weeks(n=2) (Median± IQR)
ALT(IU/L)	42.6±51.05[a, b]	89.8±87.8	130.35±1
AST (IU/L)	50.7±61.25[a]	103.05±113.62	121.2±1
ALP (IU/L)	88±67.75[a, b]	158.5±147.25	203±1
TB (mg/dl)	0.71±0.898[a, b]	1.63±1.93	2.41±1
DB (mg/dl)	0.15±0.27[a]	0.79±1.35	1.25±1
Creatinine (mg/dl)	0.97±0.69[a, b]	1.19±0.44	1.38±1
Urea (mg/dl)	28.55±10.4	28.75±15.95	14.15±1
WBC($X10^3/\mu L$)	11.9±4.0 [a]	9.5±8.8	7.4±1

- Independent-sample Kruskal- Wallis test was used for multi-comparison.

➢ a-$p<0.05$ when two week compared with one week

➢ b-$p<0.05$when three week compared with one week

6.6. Titration of typhoid fever cases and LFT and RFT test results

Antibody titer has effects on liver and renal function tests of typhoid fever patients. AST, ALT, and Creatinine levels of typhoid fever study participants of 1:160 and 1:320 antibody titer showed statistically significant differences when compared with study subjects of 1:80(Table 6).

Table: 6 The effect of O-antibody titer on Liver and Renal function tests of TF patients in MGH, Northwest Ethiopia, May to December 2023.

Parameters	1:80 (n=70) (Median± IQR)	1:160 (n=18) (Median± IQR)	1:320 (n=2) (Median± IQR)
ALT(IU/L)	42.6±51.7[a, b]	75.5±75.22	122.85±1
AST (IU/L)	50.6±61.83[a, b]	99.7±88.4	139.30±1
ALP (IU/L)	88.5±63.75[a]	151.5±112.5	199±1
TB (mg/dl)	0.73±0.89	1.52±1.8	2.46±1
DB (mg/dl)	0.15±0.27	0.69±1.31	1.28±1
Creatinine (mg/dl)	1.06±0.7[a, b]	1.16±0.37	1.66±1
Urea (mg/dl)	28.55±9.92	27.05±19.15	16.20±1
WBC($X10^3/\mu L$)	11.8±4.1	10.2±6.2	8.1±1

Independent-sample Kruskal-Wallis test was used for multi-comparison.

- a-$p<0.05$ when 1:160 compared with 1:80
- b-$p<0.05$ when 1:320 compared with 1:80

7. Discussion

Typhoid fever is a common bacterial infection in the tropics with considerable morbidity and mortality. It mostly affects liver and kidney organs which leads to alteration of liver and kidney function tests(14, 28).

According to this study, typhoid fever has a significant impact on the level of liver and renal function tests in comparison to control groups. ALT, AST, ALP, TB, DB, and creatinine were significantly higher in typhoid fever patients as compared to control subjects. The value of urea has not shown a statistically significant difference between typhoid fever cases and control groups.

Based on these findings the values of AST, ALT, and ALP were significantly higher among typhoid fever cases as compared to the control group. The finding is in agreement with past studies conducted in Iraq and Cameroon(26, 27). which reported significantly higher values of ALT, AST, and ALP in patients with typhoid fever compared to the control groups. Furthermore, different case reports conducted in Malaysia, India, and South Africa support this study and report high levels of ALT, AST, and ALP in typhoid fever patients(36-38). and also, a prospective study in India, a cross-sectional study performed in India, a prospective observational study conducted in Nepal, and an observational study conducted in the United Arab Emirates showed that elevated liver enzymes in typhoid fever patients(24, 29, 31, 39). Similarly, a study conducted in Nigeria(33) reported elevated enzyme levels of ALT, AST, and ALP in typhoid fever patients than control groups.

The high value of serum ALT and AST enzymes would be due to hepatocyte necrosis, which is characterized by the destruction of hepatocytes by reactive oxygen species (ROS) initially intended for the destruction of Salmonella bacteria in the liver(40). During the infection of typhoid fever, *Salmonella typhi* leaves the intestinal lumen by infecting lymphoid tissues via the lymphatic circulation to end up in the macrophages of the liver (Kupffer cells) where it multiplies there and spreads into the body(41). The lysis of a part of *Salmonella* bacteria in the lymph nodes releases the endotoxin (lipopolysaccharides) which is carried by the blood to the liver. In the liver, Kupffer cells are activated by these lipopolysaccharides and secrete cytokines (TNF-α, IFN-γ, IL-1, IL-6, IL-18) which induce the production of ROS that destroy *Salmonella*

typhi, which also destroy hepatocytes(42). The increase in serum biochemical parameters of ALT and AST would be indicated by the increase in the dose of endotoxin during the increase in the duration of the disease(43).

Via the ducts or capillaries that connect the liver and gallbladder, S. Typhi can spread from the liver to colonize the biliary system(15). The high level of ALP indicates that obstruction of the bile ducts can be due to the existence of Salmonella typhi, bile duct blockage results in increased production of ALP by epithelial cells and released into the circulation of ALP biochemicals(44).

The levels of TB and DB showed an increment in typhoid fever cases than control subjects. The results of this study were similar to the findings conducted in Cameroon and Nigeria(27, 33). It indicates that the value of bilirubin was significantly higher in typhoid fever cases than in control subjects. Additionally, case reports reported from Malaysia, Pakistan and a cross-sectional and prospective study conducted in Iran, and a descriptive study conducted in the United Arab Emirates showed that elevated levels of serum bilirubin in typhoid fever patients(30, 32, 36, 45). The liver damage that we found via the increased level of AST, ALT, and ALP was further confirmed by the high values of serum bilirubin. When hemoglobin, a component of blood red cells, breaks down naturally during the process of recycling damaged or aged red blood cells, bilirubin is formed. It is then transported via the bloodstream to the liver and eliminated with bile(46). The significant increase in serum bilirubin levels in typhoid fever patients indicated the disturbance of the liver excretion function. It may be due to canalicular occlusion by the swollen hepatocytes(47).

An increase in the level of creatinine in typhoid fever compared with the control group in this study agrees with the past studies conducted in Nigeria and Cameron(27, 34). The increase of serum creatinine level in typhoid fever could be immunological or directly by the invasion of salmonella typhi. As the byproduct of creatine phosphate in muscle and is generated constantly by the body, creatinine is an endogenous measure for the evaluation of glomerular function. It usually gets completely removed from the blood by the kidney(48). The significant increase in the serum level of creatinine indicated impaired kidney function(49).

There is no difference significantly in the level of urea in typhoid fever patients and control groups in this study. About 85% of urea, a nitrogen-containing substance produced in the liver as

a byproduct of protein catabolism, is excreted through the kidney(48). In typhoid fever both liver and kidney functions are altered, the liver has dysfunction in producing urea and the kidney has dysfunction in excreting urea waste products.

In this study, the impact of the duration of illness on the liver and renal parameters of individuals with typhoid fever patients was also analyzed. Liver function tests; ALT, ALP, TB, and creatinine levels of typhoid fever patients in three weeks and two weeks showed significant increases compared with one week of typhoid fever duration, and AST and DB levels of typhoid fever patients in two weeks showed significant increase compared with one week of typhoid fever duration. These findings are similar to the past study conducted in India(24, 25).

8. Strengths and limitations of the study

8.1.Strengths of the study

- This study is the first in Ethiopia, as far as the principal investigator and advisors know.
- The study was done with titration and antigenic tests.
- It offers baseline data for future research.

8.2. Limitation of study

The sample size of the study was small, which is not sufficient to generalize the findings to the general population. Different tests like GGT, albumin, uric acid, electrolytes, LDH, and C-reactive protein parameters were not tested due to scarcity of budget. It is challenging to discuss the results with other findings because there is a dearth of literature for this study.

9. Conclusion and recommendation

9.1. Conclusion

Typhoid fever patients showed significant increments of liver function tests(ALT, AST, ALP, TB, and DB) and renal function tests(creatinine) as compared to control groups. Duration of illness had effects on liver and renal function tests of typhoid fever patients.

9.2. Recommendation

Based on the findings of this study, the inclusion of liver and renal function tests to other integral laboratory tests for comprehensive diagnosis of typhoid fever is recommended with the consideration of further studies.

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11. Annexes

11.1. Annex I: Participants' information sheet

A. English version

Title of the project: Assessment of liver and renal function tests among typhoid fever patients in Motta General Hospital, Northwest Ethiopia.

Principal investigator: Birku Gashaw (BSC, MSC candidate)

Name of organization: Addis Ababa University

Introduction

Dear study participants, you are invited to participate in the study on liver and renal function tests of typhoid fever patients in Motta Hospital, Amhara Region, northwest Ethiopia. This study is approved by Addis Ababa University Department of Medical Laboratory Science research ethics committee. You are voluntarily participating in this study and you have a full right to stop participation if you have something uncomfortable

Purpose of the study: The purpose of the study is to assess liver and renal function tests in typhoid fever patients.

Duration: the duration of this study depends on the availability of study subjects and it may take 2-8 months.

The associated risk with the study: There might be some minimal risk and discomfort when we take venous blood. However, during the collection of blood specimens from you, appropriate precautions will be taken and samples will be collected by an experienced laboratory technologist. If anything happens, appropriate medical care will be provided to you.

Procedure of the study: If you agree to participate in this study, you will give about 3-5 venous blood for clinical chemistry analysis.

Expected Benefit: Dear participants you will have a benefit from this study because this study assesses your liver and renal function test changes. No payment is requested for the clinical

chemistry tests and you will know your health status early. If your result shows any abnormalities in clinical chemistry tests, you will manage and be treated early.

Confidentiality: The confidentiality of your information and laboratory results is respected strictly. A unique identification number is given to you and your name will not be written in the form and the result of laboratory tests can only be accessed by the researcher.

Contact information

If you have additional questions about the study, you can contact the:

Birku Gashaw cell phone-0927617363 E-mail: birkugashaw07@gmail.com

Thank you for your cooperation. If you are a volunteer to participate in the study, I kindly request you to provide your response to the questionnaire on the next page.

B. አማረኛ ቅጅ

የፕሮጀክቱ ርዕስ፡ በአማራ ክልል ሰሜን ምዕራብ ኢትዮጵያ በሞጣ አጠቃላይ ሆስፒታል የታይፎይድ ትኩሳት ህሙማን የጉበት እና ኩላሊት ተግባር ምርመራ

ዋና መርማሪ፡ ብርቁ ጋሻው (BSC፣ MSC እጩ)

የድርጅቱ ስም፡- አዲስ አበባ ዩኒቨርሲቲ

መግቢያ

ውድ የጥናት ተሳታፊዎች በሰሜን ምእራብ ኢትዮጵያ በአማራ ክልል በሸጋው ሞጣ ሆስፒታል የታይፎይድ ትኩሳት በሽተኞች የጉበት እና ኩላሊት ተግባር ምርመራ ላይ እንዲሳተፉ ተጋብዛችኋል። ይህ ጥናት በአዲስ አበባ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ሳይንስ ጥናትና ምርምር የሥነ ምግባር ኮሚቴ የጸደቀ ነው። በዚህ ጥናት ውስጥ በፈቃደኝነት እየተሳተፉ ነው እና የማይመች ነገር ካለ ተሳትፎዎን የማቆም ሙሉ መብት አለዎት

ከጥናቱ ጋር የተዛመደ ስጋት፡- የደም ሥር ደም በምንወስድበት ጊዜ መጠነኛ የሆነ አደጋ እና ምቾት ማጣት ሊኖር ይችላል። ነገር ግን ከእርስዎ የደም ናሙና በሚሰበሰብበት ጊዜ ተገቢውን ጥንቃቄ ይደረጋል እና ናሙና የሚሰበሰበው ልምድ ባለው የላብራቶሪ ቴክኖሎጂ ባለሙያ ነው። የሆነ ነገር ከተፈጠረ, ተገቢ የሕክምና እንክብካቤ ይሰጥዎታል.

የጥናቱ ዓላማ፡- የጥናቱ ዓላማ በታይፎይድ አወንታዊ ታካሚዎች ላይ የጉበት እና ኩላሊት ተግባር ምርመራን ለመገምገም ነው።

የቆይታ ጊዜ፡ የዚህ ጥናት ቆይታ የሚወሰነው በጥናት ርእሶች መገኘት ላይ ሲሆን ከ2-8 ወራት ሊወስድ ይችላል።

ከጥናቱ ጋር የተዛመደ ስጋት፡- የደም ሥር ደም በምንወስድበት ጊዜ መጠነኛ የሆነ አደጋ እና ምቾት ማጣት ሊኖር ይችላል። ነገር ግን ከእርስዎ የደም ናሙና በሚሰበሰብበት ጊዜ ተገቢውን ጥንቃቄ ይደረጋል እና ናሙና የሚሰበሰበው ልምድ ባለው የላብራቶሪ ቴክኖሎጂ ባለሙያ ነው። የሆነ ነገር ከተፈጠረ, ተገቢ የሕክምና እንክብካቤ ይሰጥዎታል።

የጥናቱ ሂደት፡ በዚህ ጥናት ላይ ለመሳተፍ ከተስማሙ ለክሊኒካል ኬሚስትሪ ትንተና ከ3-4 የሚያህሉ ደም መላሽ ደም ይሰጣሉ።

የሚጠበቀው ጥቅም፡- ውድ ተሳታፊዎች ከዚህ ጥናት ጥሩ ጥቅም ያገኛሉ ምክንያቱም ይህ ጥናት የጉበት እና ኩላሊት ተግባርዎ ለውጦችን ስለሚገመግም ነው። ለክሊኒካዊ ኬሚስትሪ ሙከራዎች ምንም ክፍያ አይጠየቅም እና የጤና ሁኔታዎን ቀደም ብለው ያውቃሉ። ውጤትዎ የክሊኒካል ኬሚስትሪ ምርመራዎችን ያልተለመዱ ነገሮችን ካሳየ እርስዎ ቀደም ብለው ይቆጣጠራሉ እና ይታከማሉ።

ምስጢራዊነት፡ የመረጃዎ እና የላብራቶሪ ውጤቶችዎ ሚስጥራዊነት በጥብቅ ይከበራል። ልዩ መለያ ቁጥር ተሰጥቶዎታል እና ስምዎ በቅጹ ላይ አይጻፍም እና የላብራቶሪ ምርመራ ውጤቱ በተመራማሪው ብቻ ሊገኝ ይችላል.

የማንነትህ መረጃ

ስለ ጥናቱ ተጨማሪ ጥያቄዎች ካሉዎት የሚከተሉትን ማነጋገር ይችላሉ፡-

ብርቁ ጋሻው፣ ስልክ ቁጥር-0927617363 ኢሜል፡ birkugashaw07@gmail.com

ሳሙኤል ከንዱ፣ስልክ ቁጥር-+251 975379824

ለትብብርዎ እናመሰግናለን. በጥናቱ ለመሳተፍ ፈቃደኛ ከሆናችሁ በሚቀጥለው ገጽ ላይ ለመጠይቁ ምላሽ እንድትሰጡ በትህትና እጠይቃለሁ።

11.2. Annex II: Consent Form

1. **Adult study participants**

A. English version

I have read, or have had this document read to me in a language that I understand, and I understand the purposes, procedures, and risks of this research project as described within it. I understand that at any time I may withdraw from this study without giving a reason. I know that no special payment for being participating in the study. I freely agree to participate in this study, as described. I understand that I was given a signed copy of this document to keep.

Name of participant. _____ Signature ____ Date_____

B. አማረኛ ቅጅ

የፍቃደኝነት ማረጋገጫ ቅጽ

እኔ ከዚህ በታች የተገለጸዉ በዚህ ጥናት ተሳታፊ ልመሆን ስወስን የጥናቱ ዓላማዎች ኣሳራሮችና ቾድመ ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳታፋይነት ፈቃደኝነቴን በማነኛዉም ግዜ የማስወገድ መብቴን በመራጋገጥ ነዉ። ሰለዚህ በጥናቱ ተሳታፊ መሆኔን በፈርማየ እየራጋገጥኩ ይህንን ስውስን በጥናቱ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማነኛዉም ግዜ ራሴን ለምግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እንደማትነፈጉኝ በማመን ነው። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝመሆኑንበፈርማየኣጋግጣለሁ።

የተሳታፊውስም……

ፊርማ…… ቀን …….

ስለ ትብብርዎ አመሰግናለሁ!

2. **Parental consent form**

A. English version

I_____ parent, after being fully informed about the purpose of this study on the assessment of liver and renal function tests in typhoid fever patients in Motta Hospital Northwest Ethiopia. I, the undersigned, have been told about this research. I have been informed there is no harm related to giving specimens. I have been informed that other people will not know my child's results as it is coded with numbers rather than written names. I understand that there may be no benefit to me personally apart from the clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below, I agree to let my child participate in this research study.

_____ _____ ____/____/___

Name of parent signature Day/month/year

B. አማረኛ ቅጅ

የስምምነት መጠየቂያ ቅጽ

እኔ----------የልጇ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ፡፡ የጥናቱ ርዕስ በሞጣ ሆስፒታል የሚገኙ ታይፎይድ ሕሙማን ላይ የጉበት እና ኩላሊት ተግባር ምርመራ ና ጥናት ነዉ።በጥናቱ ልጇ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል፡፡ ናሙና መስጠት ምንም አይነት ጉዳት ልጇ ላይ እንደሌለዉ ተነግሮኛል፡፡ በጥናቱ ወቅትም የልጇ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ

የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ፡፡ በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ፡፡ በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊ ስም……

ፊርማ…… ቀን …….

ስለ ትብብርዎ አመሰግናለሁ!

3. Assent form

A. English version

I am fully informed about the purpose of this study on the assessment of liver and renal function tests in typhoid fever patients in Motta Hospital Northwest Ethiopia. I have been informed there is no harm related to giving specimens. I have been informed that other people will not know my test results as it coded with numbers rather than written names. I understand that there may be no benefit to me personally apart from the clinical service I get from these results. I have been allowed to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I will participate in this study provided my parents/guardians give their consent to give my blood for the study.

Name of participant signature Day/month/year _____ _____ ____/____/___

B. አማረኛ ቅጅ

በሞጣ ሆስፒታል የሚገኙ ታይፎይድ ሕሙማን ላይ የጉበት እና ኩላሊት ተግባር ምርመራ በሚለው ርእስ ዋና ዓላማዉ ተነግሮኛል. ናሙና ብሰጥ ምንም ዓይነት ችግር እንድሌለዉም ተነግሮኛል፡፡ በጥናቱ ወቅትም የኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘዉ የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የገኘዉ ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ በዚህ ጥናቱ ለመሳተፍ የኔ ፍላጎት እንዳለ ሁኖ ወላጆቼከፈቀዱልኝተስማምቻለሁ፡፡

የተሳታፊስም……

\ፊርማ…… ቀን ……

ስለ ትብብርዎ አመሰግናለሁ!

11.3. Annex III. Screening checklist

A. **English version**

ID:_____

Table 7: Show Screening checklist

No	Questions	Response
1	Do you have taken any medication within this two weeks?	1. Yes 2. No
2	If your answer is yes for Q#1, what type of drug do use?	1. Anti-protozoa 2. Anti-bacterial 3. Anti TB 4. Other
3	Do you have a habit of alcohol drinking	1. Yes 2. No
4	If yes to Q 3, how often do you take it?	1. One per day 2. Two or three per day 3. More than four per week 4. sometimes (holiday)
5	Do you have a history of chronic liver disease?	1. Yes 2. No
6	Do you have a history of chronic heart disease?	1. Yes 2. No
7	Are you pregnant? (For females)	1. Yes 2. No
8	Widal test	O, 1. Reactive 2. Non- reactive H, 1. Reactive 2. Non-reactive
9	Malaria test	_____
10	Salmonella stool antigen test	1. Positive 2. Negative

B. **አማረኛ ቅጅ**

ID_____

ተ.ቁ	የምርመራው አይነት	ውጤት
ነ	ኣልኮል የመጠጣት ልምድ ኣለቦት	1. አዎ 2. የለም
2	ለተራ ቁጥር 1 መልስዎ አዎ ከሆነ ምን ያክል ይጠጣሉ	1.በየቀኑ 2. ሁለት ወይም ሰሶት ግዜ በሳምንት 3. ኣንዳ ኣንድ ግዜ (በባኣል ግዜ)
3	በዚ ሁለት ሳምንት ወስጥ መድሓኒት ወስደው ያዉቆሉ	1. አዎ 2. የለም
4	ለተራ ቁጥር 3 መልስዎ አዎ ከሆነ ለምን ኣይነት በሽታ	1.ለ ፕሮቶዝዋ 2. ለባክቴሪያ 3. ለቲቪ 4. ሌላ
5	ለብዙ ግዜ የቆየ የጉበት በሽታ ኣለቦት	1. አዎ 2. የለም
6	ለብዙ ግዜ የቆየ የልብ በሽታ ኣለቦት	1. አዎ 2. የለም
7	እርግዝና ላይ ነሽ	1. አዎ 2. የለም
8	የዋይዳል ምርመራ	O_____ H_____
9	የወባ ምርመራ	_____
ነ0	የሳልሞኔላ ሰገራ ኣንቲጂን ምርመራ	1. አዎንታዊ 2. ኣሉታዊ

11.4. Annex IV: Structured Questionnaires

Questions on Socio-demographic characteristics of the respondent for both case and control groups

English version

Note: Please Encircle or Write the appropriate answer in the provided space.

Table 8: Show Questions on Socio-demographic characteristics of the respondent

No.	**Questions**	**Response categories**	**Remarks**
1	Identification Number	ID NO_____	
2	Sex	1. Male 2. Female	
3	Age of respondent	In completed years ----------	
4	Where is your residence?	1. Urban 2. Rural	

6	What is your marital Status?	1. Married 2. Single 3. Divorced 4. Widowed 5.. Separated	
7	Educational status of the respondent	1. No formal education 2. Primary educational level (1-8) 3. Secondary and above (above grade 9) 4. Diploma and above	
8	What is your occupation?	_____	
9	Number of family members	_____	

Questions on clinical features for only typhoid fever confirmed patients

ID_____

Table 9: Show Clinical features for only typhoid fever patients

No	Questions	Response
1	Duration of illness	1, One week and less 2, Two weeks 3, Three weeks 4, Four and above
2	Hepatomegaly	1. Yes 2. No
3	Jaundice	1. Yes 2. No
4	Headache	1. Yes 2. No
5	Abdominal pain	1. Yes 2. No
6	Diarrhea	1. Yes 2. No
7	Nausea	1. Yes 2. No

I thank you for your cooperation

አማረኛ ቅጅ

ማሳሰብያ: እባክዎ ትክክለኛውን መልስ ያክብቡ ወይም ይፃፉ።

ተራ ቁ	ጥያቄዎች	የምላሽ ምድቦች
1	መታወቂያ ቁጥር	_____
2	ፆታ	1. ወንድ 2. ሴት
3	ዕድሜ	_____
4	መኖሪያዎ የት ነው?	1.ከተማ 2. ገጠር
6	የጋብቻ ሁኔታ	1. ያገባ 4. መበለት 2. ያላገባ 5. ተለያይቷል 3. የተፋታ
7	የትምህርት ደረጃ	1. መደበኛ ትምህርት የለም 2. ኣንደኛ ደረጃ(1-8) 3. ሁለተኛ ደረጃ (9-12) 4. ዲፕሎማ/ድግሪና ከዛ በላይ
8	ሥራዎ ምንድ ነው?	_____
9	የቤተሰብ ብዛት	_____

ስለ ትብብርዎ አመሰግናለሁ!

11.5. Annex V. Biochemical and anthropometric measurements

ID_____

Table 10: Show Laboratory result format

No	Test	Result
1	Height	_____(meter)
2	Weight	_____(Kg)
3	BMI	_____Kg/m^2
4	ALT	_____U/L

5	AST	_____ U/L
6	ALP	_____ U/L
7	Creatinine	_____ mg/dl
8	Urea	_____mg/dl
9	TB	_____ mg/dl
10	DB	_____ mg/dl
11	AST: ALT	_____
12	Antibody titer	_____
13	WBC	_____X10^3/µl

Table 11: Reference range of Cobas 311 clinical chemistry analyzer at Motta General Hospital

Parameters	Reference range	
	Male	Female
ALT	0-41 (IU/L)	0-33 (IU/L)
AST	0-40 (IU/L)	0-32 (IU/L)
ALP	40-129 (IU/L)	35-104 (IU/L)
Creatinine	0.7-1.2 (mg/dl)	0.5-0.9 (mg/dl)
Urea	16.6-48.5 (mg/dl)	16.6-48.5 (mg/dl)
TB	0-1.2 (mg/dl)	0-1.2 (mg/dl)
DB	0-0.9 (mg/dl)	0-0.9 (mg/dl)

11.6. Annex VI: Materials, Procedure, and principles of tests

SOP for Blood Collection

Equipment

- ✓ 21gauge needle for each participant
- ✓ Blood collection tubes (serum separator tube)
- ✓ Tourniquet
- ✓ Box of gloves
- ✓ 70% alcohol

- ✓ Cotton

Laboratory Blood sample collection procedure and processing

1. Assemble blood collection materials.

2. Identify and prepare the patient

3. Label tubes with the client's name/identification number.

4. Wear a glove and prepare the patient on a comfortable position

5. Tie the tourniquet around the arm of the patient above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.

6. using the tip of the index finger examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture

7. Disinfect the collection site by swabbing the skin with alcohol swab.

8. Insert the needle directly into the vein and withdraw peripheral blood of approximately 3ml in serum separator tube

9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.

10. Properly avoid the used materials in a safe container.

11. Leave for 30 to 45 min. to clot the blood

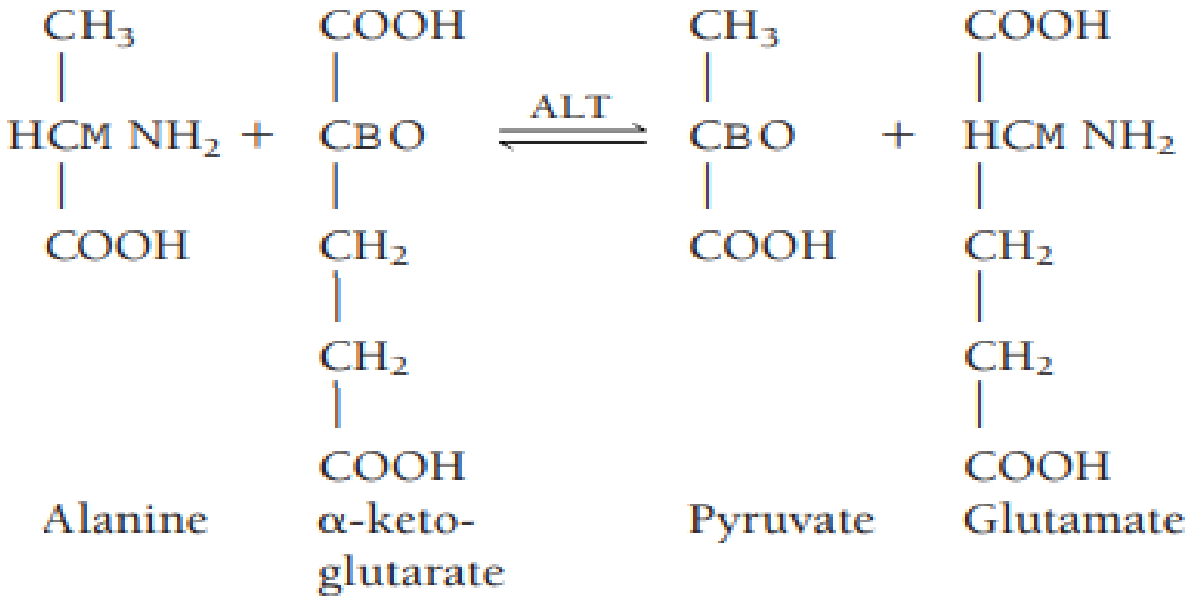
12. Centrifuge at 5000 rpm for 5 minutes and Serum will be separated.

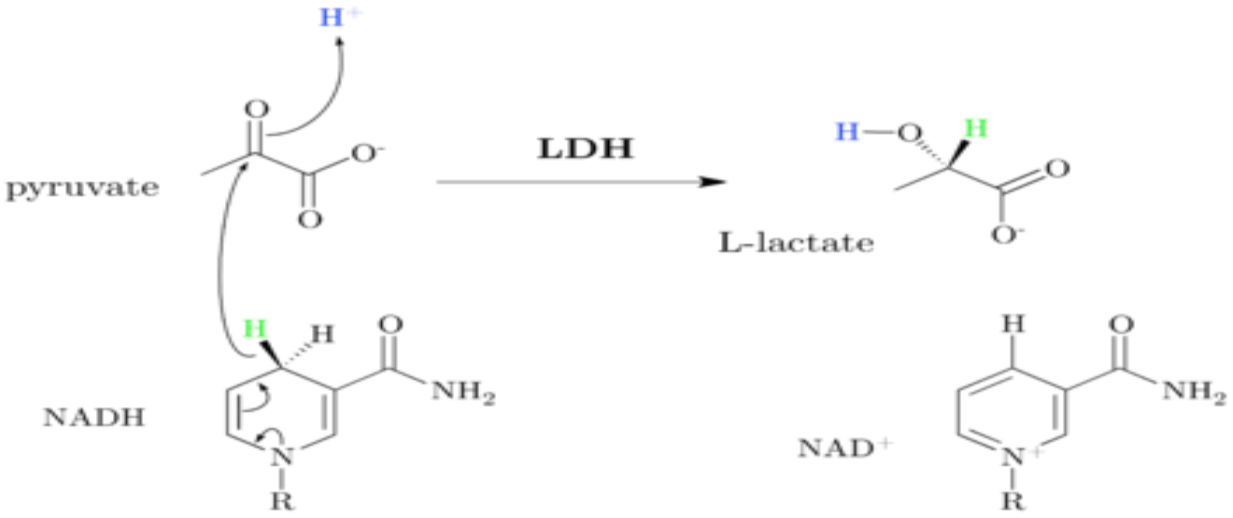
Principles of each test

Alanine aminotransferase

Test principle- Alanine aminotransferase (ALT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The next is reduced to lactate-by-lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of

decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of ALT present in the sample.





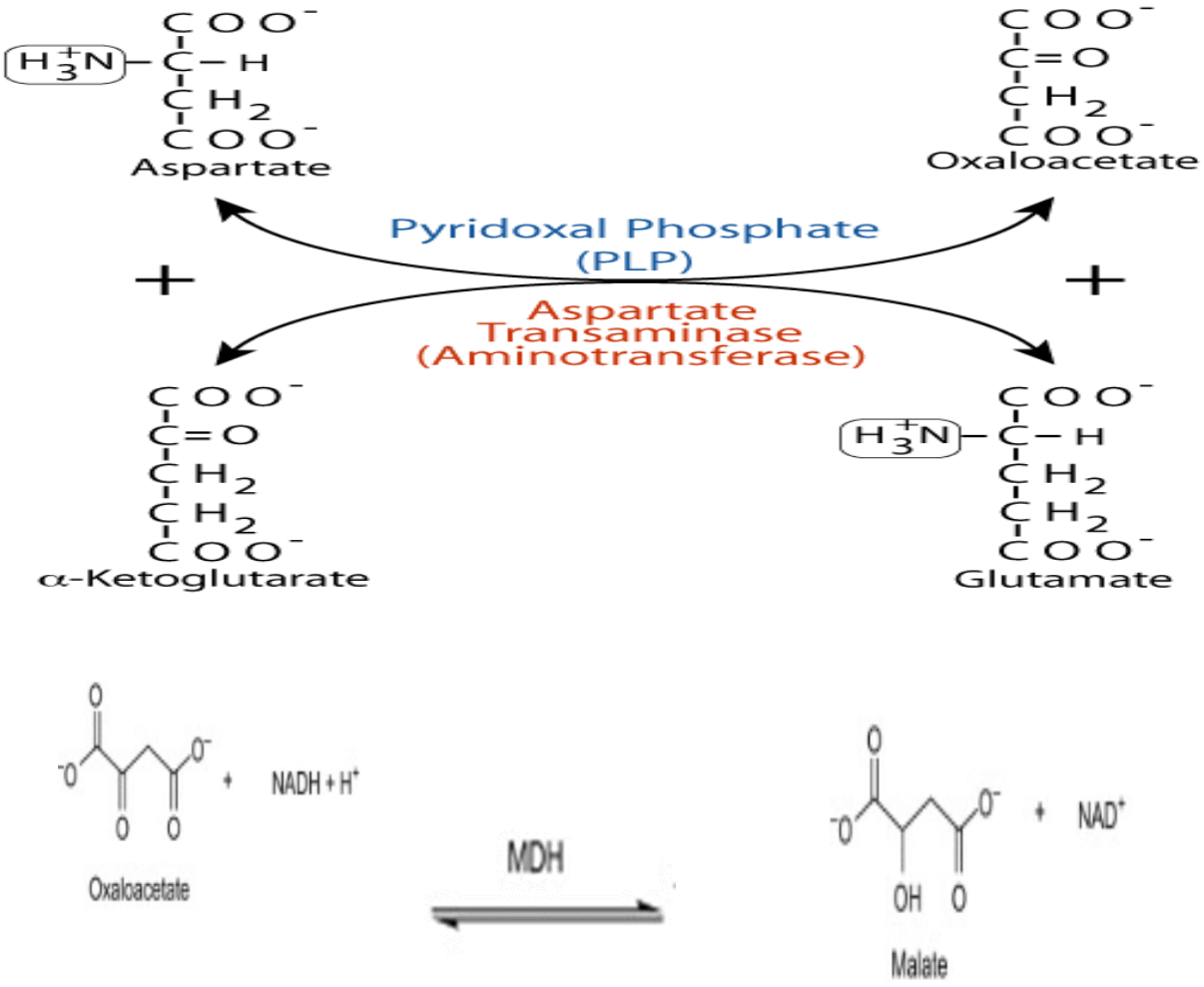
Source: Lactate dehydrogenase. (2023, January 31). In *Wikipedia*

The control should include a set of controls that is normal and abnormal. assay technique is kinetic assay, the method is IFCC, lower detection limit 5U/L, measuring rang 5-500U/L, calibrator c.f.a.s, working solution is R1 59µL and R2 17µL, correlation: Y=10.997X-1.051, r=0.996, reference interval for male 0-41U/L and for female 0-33U/L, company: Roche

diagnostic GmbH, Sandofer Strasse 116, D-68305 Mannheim USA, lot No-62565501,exp date 08/12/2023(50, 51).

Aspartate aminotransferase

Principle- Aspartate aminotransferase (AST) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxaloacetate. The latter is reduced to malate-by-malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decreasing absorbance resulting from the oxidation of NADH to NAD+, directly proportional to the activity of AST present in the sample.

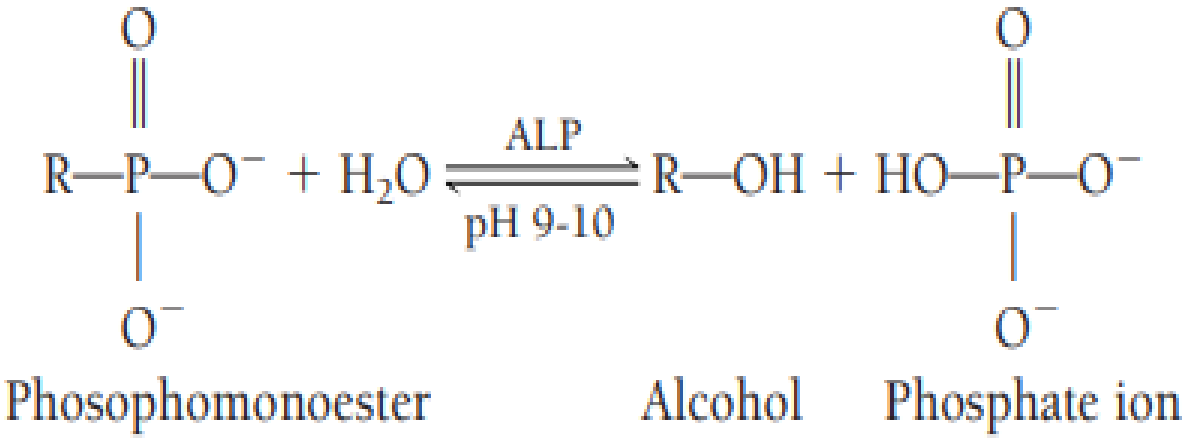


Source: https://education.med.nyu.edu/mbm/aminoAcids/nitrogen.shtml

Control should include a set of control which is normal and abnormal, assay technique is kinetic assay, the method is IFCC, lower detection limit 5U/L, Measuring rang 5-500U/L, calibrator c.f.a.s, working solution R1 40µL` And R2 17µL, correlation: Y=0.991X+1.22, r=0.999, Reference interval 0-40U/L for male and 0-32U/L for female, company: Roche diagnostic GmbH, Sandofer Strasse 116, D-68305 Mannheim USA, lot No-60286301,exp date 08/12/2023(50, 52).

Alkaline phosphatase

Principle of the test- Alkaline phosphatase (ALP) catalyzes the hydrolysis of 4-nitrophenylphosphatase (4-NPP) with the formation of free 4- nitro phenol and inorganic phosphate, acting as the alkaline buffer as a phosphate-group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, which is directly proportional to the activity of ALP in the sample.



Source:
https://iubmb.onlinelibrary.wiley.com/doi/full/10.1002/bmb.2002.494030060138

The Control of each test should include a set of control that is normal and abnormal, assay technique is kinetic assay, the method is IFCC, lower detection limit 5U/L, measuring range 5-1200U/L, working solution R1 75µL and R2 17µL, correlation: Y=0.958x-0.357, r=1.00, reference interval male 40-129U/L female 35-104, interference: hemolysis, drug, company: Roche diagnostic GmbH, Sandofer Strasse 116, D-68305 Mannheim USA, lot No-60572301,exp date 08/12/2023(53, 54)

Total and direct Bilirubin

Test principle- Bilirubin is converted to colored azobilirubin by diazotized sulfonic acid and is measured photometrically. From the two bilirubin fractions in serum –bilirubin-glucuronide and free bilirubin which is bound to albumin– only the former reacts directly, while free albumin reacts after being displaced from protein by an accelerator. The difference between the two measurements total bilirubin (with accelerator) and direct bilirubin (without accelerator) enables the calculation of indirect bilirubin.

Total bilirubin

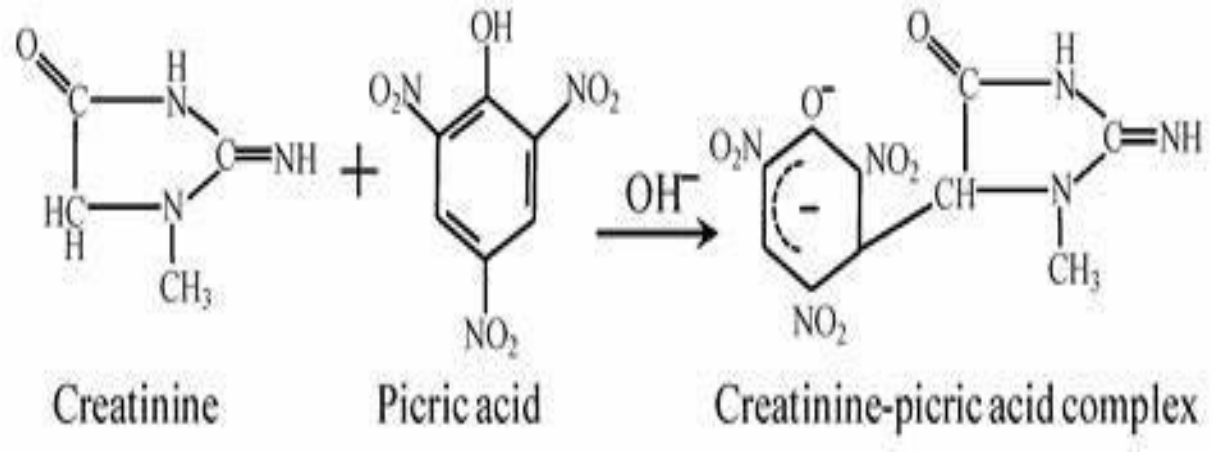
The control of a test should include a set of control which is normal and abnormal, the assay technique is endpoint assay, lower detection limit 0.146mg/dl, measuring range 0.146-38mg/dl, working solution R1 120µL` And R2 24µL, reference interval: adult up to 1.2mg/dl and newborn up to 17mg/dl, correlation: Y=0.993x+1.20, r=1.0, interference-hemolysis, lipemic sample, drug, company: Roche diagnostic GmbH, Sandofer Strasse 116, D-68305 Mannheim USA, lot No-64690501,exp date 21/05/2024.

Direct bilirubin

Control should include a set of control which is normal and abnormal, assay technique is endpoint assay, measuring range 0.08- 13.8 mg/dl, lower detection limit 0.07mg/dl, working solution R1 120µL` And R2 24µL, correlation Y=0.993x-0.158 r=0.999, Reference interval 0-0.2mg/dl, interference: hemolysis, lipemic sample, company: Roche diagnostic GmbH, Sandofer Strasse 116, D-68305 Mannheim USA, lot No- 63690301,exp date 21/05/2024(55).

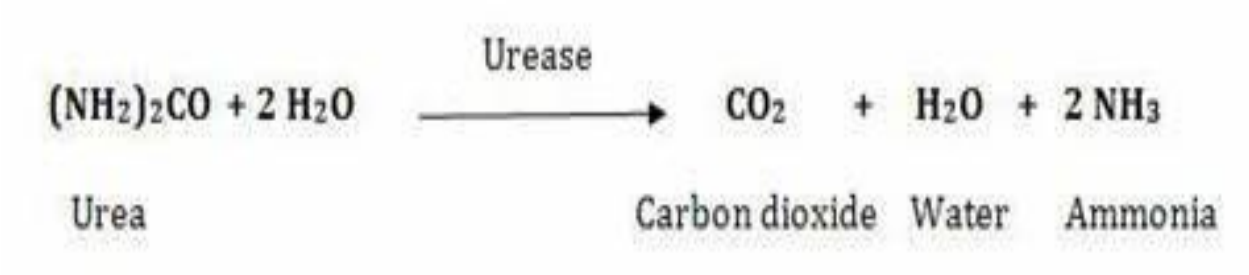
Creatinine

Test principle-creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed time during conversation is proportional to the concentration of creatinine in the sample. Analytical Measurement Range: 0.10-30.51 mg/dL, Reportable Range: 0.10-122.04 mg/d, Limit of Detection: 0.10 mg/dL, Reference Range: Serum, adult male 0.67-1.17 mg/dL adult female 0.51-0.95mg/dL, Lot No-52763201, exp date 14/08/2024.



Urea

Test principle- the first reaction involves the hydrolysis of Urea by urease to yield ammonium and carbonate as a product. During the second Reaction, in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH, 2-oxoglutarate reacts with ammonium to produce L-glutamate. The reaction involves the oxidation of two moles of NADH to NAD+ for each mole of urea hydrolyzed. The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured Photo-metrically, lot No 63490701, exp date 14/08/2024(56).



SOP for widal and salmonella stool antigen test

Equipment-O antigen suspension, H antigen suspension, test tube, mixer, normal saline, Positive and negative control, Widal test card, applicator stick, serum sample, stool antigen test strip, stool cup, timer

Principle of widal test

Widal test is an agglutination test in which specific typhoid fever antibodies are detected by mixing the patient's serum with killed bacterial suspension of Salmonella carrying specific O and H antigens and observed for clumping ie. Antigen-antibody reaction. The main principle of Widal test is that if homologous antibody is present in patient's serum, it will react with respective antigen in the suspension and gives visible clumping on the test slide, card or test tube

Procedure of Widal test

Slide test

- ✓ Place one drop of positive control to one reaction circles of the card.
- ✓ Pipette one drop of negative control on the next reaction circle.
- ✓ Pipette one drop of the patient serum to be tested onto another reaction circles.
- ✓ Add one drop each of O or H antigens to the control and serum sample of the reaction circles.
- ✓ Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
- ✓ Rock the slide, gently back and forth and observe for agglutination macroscopically within one minute.

Tube test (titration)

- ✓ Take 2 sets of 5 test tubes and label them 1 to 5 for O and H antibody detection.
- ✓ Pipette into tube No.1 of all sets 1.95 ml of saline.
- ✓ To each of the remaining tubes (2 to 5) add 1.0 ml of isotonic saline.
- ✓ To tube No.1 tube in each row add 0.05 ml of the serum sample to be tested and mix well.
- ✓ Transfer 1.0 ml diluted serum from tube no.1 to tube no.2 and mix well.
- ✓ Transfer 1.0 ml of the diluted sample from tube no.2 to tube no.3 and mix well. Continue this serial dilution up to tube no.4 in each set.
- ✓ Discard 1.0 ml of the diluted sample from tube No.4 of each set.
- ✓ Tube No.5 in all the sets, serves as a saline control. Now the dilution of the serum sample achieved in each set is as follows: Tube No.: 1 2 3 4 5(control) Dilutions 1:40 1:80 1:160 1:320
- ✓ To all the tubes (1 to 5) of each set add one drop of the respective Widal test antigen suspension (O or H) from the reagent vials, mix well, and observe for agglutination.

Interpretation of Widal Test

Slide test

- ❖ Agglutination is a positive test result and it indicates the presence of clinically significant levels of the corresponding antibody in the patient's serum.
- ❖ No agglutination is a negative result and indicates the absence of clinically significant levels of antibody in the patient's serum

Tube-method

- ❖ The titer in Widal test antigen suspensions is the highest dilution of the serum sample that gives a visible agglutination.

Antibody titer greater than 1:80 is considered significant and usually suggests a positive test for Salmonella infection(57).

Salmonella stool antigen test

The rapid Salmonella typhi card test is a qualitative immunoassay for the detection of Salmonella in fecal samples. During the test, anti-Salmonella antibodies pre-coated on the membrane react with Salmonella antigens in the sample. Approximately 150 mg/ml of fecal samples from different parts were placed in buffer vials and shaken to disperse the samples well. Uncapped the vial and placed exactly 4 drops into the sample well. The result read 10 min after dispensing the sample.

Interpretation

Positive result: A distinct pink-colored band appears on the test line regions and a pink line on the control line region.

Negative result: No line appears in the test line region. A distinct pink line on the control line region.

Invalid result: The control line next to the test line does not become visible within 20 minutes after the addition of the sample(58).

Declaration

The undersigned declares that this proposal complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

MSc candidate: **Birku Gashaw (BSc)**

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisor: **Samuel Kinde (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Gobena Dedefo (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.