1-INTRODUCTION

Typhoid fever is One of the most important health problems in Sudan and other developing countries. It is a systemic infection caused by the gram–negative bacterium enteric, subspecies *enterica, Serotype typhi*. (Sinha *et al.*, 1999).Typhoid fever is widespread and potentially lethal infection which follows ingestion of *Salmonella typhus*. Over 1000 species of Salmonellae have now been described. *Salmonella typhi* B are distributed throughout the world (Geddes, 1981).

Rationale

It was only in the mid-19th century that physicians began to distinguish it from typhus and malaria (Cunha, Cunha 2004).

The disease is endemic in south Asia and parts of south east-Asia, the Middle East, Central America and Africa, the global annual incidence of typhoid fever was estimated to be around 21.7 million cases with 216,510 deaths per year (CFR1%) (Crump Luby and Mintz, (2004).

In Sudan there are lacks of information's about the history of the disease and it may be no studies of the disease were conducted.

There were significant decreases in albumin, while increases in total protein and globulin. The increases in total protein and globulin were in agreement

with previous reports and these are consistent with humoral immune response, however, the low albumin concentration may suggest increased loss of albumin through renal tubules due to possible damage (Emenuga, *et al.*, 2014). Mean total serum protein, serum globulin were high in typhoid patients as compared to that in their normal counterparts. Mean albumin level was low in typhoid patients as compared to that in normal individuals (Amen Shamim . *et al.*, 2012).

General objective

To identify variations in biochemical parameters (serum total protein, albumin and globulin), in addition to albumin: globulin ratio patients with typhoid fever, compared to healthy ones. And to determine correlation between typhoid titers and biochemical parameters.

Specific objective

1- Estimation of serum total protein.

2- Estimation of serum albumin.

3- Estimation of serum globulin.

4- To correlate typhoid titers with estimated parameters.

5- To compare studied participants with estimated parameters.

6- To compare typhoid positive participants estimated parameters between different genders.

2-LITERATURE REVIEW

2.1 Endemic of typhoid

At the beginning of the 19Th century, typhoid was defined on basis of clinical signs and symptoms and pathological (an atomically changes), however, at this time all sorts of enteric fevers were characterized as "typhoid". In 1880s, the typhoid bacillus was first observed by Eberth in spleen sections and mesenteric lymph node from a patient who died from typhoid. Robert Koch confirmed a related finding by Gaffky and succeeded in cultivating the bacterium in 1881, but due to lack of differential characters, separation of the typhoid bacillus from other enteric bacteria was uncertain (Todar's online).

In 1896, it was demonstrated that the serum from animal immunized with typhoid bacillus agglutinated (clumped) the typhoid bacterial cells, and it was shown that the serum of patients afflicted with typhoid likewise agglutinated the typhoid bacillus , serology diagnosis of typhoid was thus possible by 1896. The genus salmonella is a member of the family enter bacteria, it composed of bacteria related to each other both phenotypically and genotypically .salmonella DNA base composition is 50-52 mol% G+C, similar to that of *Escherichia, shigella,* and *citrobacter,* the bacteria of

genus salmonella are also related to each other by DNA most closely related to salmonella are *Escherichia*, *shigella*, and *citrobacter*, similar relationships were found by numerical taxonomy and 16s ss RNA analysis (Todar' s online).

Salmonella nomenclature has been controversial since the original taxonomy of the genus was not based on DNA relatedness, consideration e.g *Salmonella abortus-pvis* and into the Kauffmann–white schemes in 1946 a salmonella species. was defined as" a group of related fermentation phage-type" with the result that each *Salmonella serovar* was considered as species since host-specificity suggested by some of these earlier names does not exist (e.g S. *typhi-murium* and S. *cholerae-suis* are in fact ubiquitous). Names derived from the geographical origin of the first isolated strain of the newly discovered *serovars* were next chosen e.g S. *London*, *S. panama* and *S. stanlryville* Todar' s online).

Salmonella are ubiquitous human and animal pathogens. They colonize virtually all animal including poultry, birds, livestock, reptiles, and human salmonella infections in humans typically produce of three clinical syndromes such as gastroenteritis enteric fever, or focal disease in addition, Salmonella infection in animals causes substantial losses of livestock. Salmonella spp. include the bacteria which gram negative flagellated and

facultative an aerobic *bacilli* characterized by the presence of O, H and VI antigens. The taxonomic classification of the genus salmonella is complex and problematic based on DNA homology and host range, the genus salmonella is classified into two species, *Salmonella enteric* and *Salmonella Bongori. Salmonella enterica* is further sub divided into six subspecies l, II, IIa, IIIb, IIIv, and v. Most of the Salmonella that are pathogenic to human beings belong to the sub group 1 of *S. enterica* subsp. Enteric. This includes the typhoid and paratyphoid bacilli and most other serotypes are named as for example, S enteric sub sp enteric serotype *enteritidis* however for sake of convenience, it is abbreviated as *S. enteritidis*. Todar's online).

2.2. History and epidemiology of typhoid

History and epidemiology of the disease typhoid is predominantly a disease of countries with inadequate sanitation and poor standards of personal and food hygiene. The disease is endemic in south Asia and parts of south east-Asia, the middle east, central America and Africa, out breaks of typhoid have been reported from countries eastern Europe in 2000, the global annual incidence of typhoid fever was estimated to be around 21.7 million cases with 216,510 deaths per year (CFR1%) (Crump Luby and Mintz, 2004).

Typhoid is rare in resources –rich countries where standard of sanitation are high typhoid and paratyphoid in England and Wales are usually imported disease associated with foreign travel or contact with somebody who has travelled between 1990 and 1004 there were an average of 374 laboratory reports of typhoid and paratyphoid each year in England and Wales.

The most frequently reported region of foreign travel for typhoid and paratyphoid A was south Asia, the Mediterranean and Middle East were the most frequently reported regions for paratyphoid B (HPA, 2004).

Occasional outbreak of indigenous typhoid occurred in the UK, the best community outbreak was in 2001 in new port, Wales and involved five cases (Puplic health laboratory services, 2001).

Prevention of typhoid and paratyphoid depends primarily on improving sanitation and water supplies in endemic areas and on scrupulous personal, food and water hygiene immunization may be considered for individual at risk from typhoid fever there is no vaccine available to prevent paratyphoid infection (Sinha *et al.*, 1999).

Following ingestion of contaminated food water, *S. typhi* penetrates the intestinal mucosa replicates and enters the blood stream. The prompt antibiotic therapy, but may be as high as 20% in untreated cases of typhoid has previously been thought to be a milder disease in children ,recent

information however indicates that typhoid can cause significant morbidity in children aged one to five years who reside in endemic countries (Sinha *et al.*, 1999).

Unlike other *Salmonella* sp., both *S. typhi* and *S. paratyphi* only colonies humans, most of the more than 2000 other serotypes of salmonella cause only local infection of the gastro-intestinal tract (gastroenteritis) or food poisoning) and are commonly found in many mammalian hosts transmission is primarily via the oral route following ingestion of food and water contaminated by faces and occasionally the urine of persons acutely ill with typhoid or those who are chronic carriers. Direct fecal-oral transmission can also occur. In healthy individuals, one million or more organisms may be required to cause the illness; however ingestion of fewer organisms may still result in illness especially in susceptible individuals the incubation period varies from one to three weeks, depending on host factors and the size of the infecting dose (Glyna and Bradley, 1992).

The risk of contracting typhoid fever is highest for travelers to areas of high endemicity in the Indian subcontinent, a region of high incidence of typhoid fever (more than 100 cases per 100,000 peoples per year (crump *et Al.*, 2004). The attack rate for travelers has been estimated at 1 to 10 per 100,000 journey All patients with typhoid and Para typhoid excrete the organisms at

some stage during their illness about 10% of patients with typhoid following the acute illness and 2 to 5 % become long-term carriers (more than one year) (Mermin, *et al.*, 1998) and Stein berg *et al.*, 2004).

The likelihood of becoming a chronic carrier increases with age, especially in females and those with biliary tract abnormality. Typhoid can successfully treated with antibiotic therapy and general medical support strain of *S. typhi* have become increasingly resistant to antibiotics, particularly in south Asia this has implication for the treatment of typhoid fever as traditional antibiotic therapy (Chloramphenicol , Co-trimoxazole and amoxicillin) may not be effective. Treatment is usually with Fluoroquinolones, third generation Cephalosporins or Azithromycin may need to be given in resistant cases following natural infection with typhoid and immune response develop that may practically protect against re-infection and severity of disease (WHO, 2000).

2.3. Biochemical *Changes* in typhoid

Serum total protein was significantly increased in typhoid fever patients irrespective of their gender. Protein loss probably is the result of changes in the integrity of the small intestinal mucosa Serum albumin concentration was reported to be decreased during infection, in this study the proteins loss may be due to intestinal infection (Arroyove, Calcano, 1979).

2.4. Pathogenesis

As was reported by Rubin, and Weinstein, (1977). Infection in man is induced by the ingestion of salmonella organism derived directly or indirectly from a human source, man being the only reservoir of the disease. A large number of organisms must be swallowed To produce symptomatic infection in health y previously unvaccinated person ingestion of 10^7 organisms causes disease in 50% of people while 10^9 organisms causes symptomatic disease in more than 95 % of cases . However in the event of un usually virulent organism or patients with reduced resistance and body's poor–defense mechanism event very small doses can cause symptomatic infection (Rubin, and Weinstein, 1977). In the stomach, *Salmonella* are exposed gastric acidity and low ph which kills of the organism, viable bacilli which survive pass into the small intestines and here they multiply. Multiplication of *Salmonella* in the intestinal tract may be asymptomatic associated only with transient excretion of organisms in stools or symptomatic producing clinical manifestations of the disease in addition to gastric acidity, a number of non-specific factors play in determining the outcome of ingestion of organisms and those include:

(a) Physical barrier to epithelial penetration imposed by intestinal mucus.

(b) The cleansing effects of the secretions.

(c) Lysozyme present in the secretions

(d) Bacteriostatic activity of the lactoferrin present in the stomach and gut.

(e) Beta, glycoprotein of gastric juice known to act as pro activator in the alternating pathways of activation of complement.

(f) Lastly, perhaps the nutritional state of the individual exposed to *Salmonella* (Rubin, and Weinstein, 1977).

Another factor is the section of cationic antimicrobial peptides secreted by the Paneth of the intestinal crypts .these peptides bacterial membranes (Michettip, *et al.*, 1992).

Salmonella have the ability to induce non-phagocytic cells including small intestinal epithelial cells to be internalize them and this is associated with the production of LTD4 and phospholipase2.The main target cell of entry is the microfolod or the "M" cells which virtue of their location provide rapid entry into the systemic cells that over lie the peyer's patches (Hoffman, Punjab, and kumala, 1984).

Endotoxin is obviously an important component of any gram negative organism such as *Salmonella typhi* vi antigen appears important in enhancing human virulence of typhoid stains it has protective properties for the organism by:

(1) Preventing immune antisera indicated killing.

(2) Increase in peroxide resistance.

(3) Resistance to complement mediated lysis and antiphagocytic properties although circulating endotoxin did not appear to account for the sustained fever and toxemia of typhoid fever, the end toxin component of S. *typhosa* could contribute importantly to the pathogenesis of typhoid fever by enhancing inflammatory responses at the tissue sites of multiplication of *S. typhi*. Endotoxin stimulates macrophages to produce monokines, arachidonic and it is possible that toxic macrophage produce released as a result of the interaction of *S. typhi*. Endotoxin and macrophages are responsible for major manifestation of typhoid fever (Hoffman, Punjab, and kumala, 1984).

2.5. Diagnosis

As was reported by, Johnson, *et al.*, 2011. Infection with typhoid or paratyphoid fever result in a very low-grade septicemia a single blood culture is positive in only half of cases stool culture is not usually positive during the early phase of the diseases the diagnostic field to about 80% of cases. The widal test is an old serologic assay for defecting lgM and lgG to the O and H antigens wild used in most developing countries because of its low cost. Newer serologic assays for *S. enterica* and *Serotype typhi* infection are occasionally used in outbreak situations and are somewhat more are not

an adequate substitute for blood stool, or bone marrow culture because there is no definitive serologic test for typhoid often has to be made clinically, the combination of history of risk for infection and gradual onset of fever that increases in severity over several days should raise suspicion of typhoid or paratyphoid (Johnson, *et al.*, 2011).

2.6. Alteration of serum total protein due to typhoid infection

In the 1960s, Beisel and his colleagues at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) began a systematic study of the metabolic responses to infection. They studied volunteers who were to be exposed to the bacterium, Francisella tularensis, or a rickettsia, Coxiella burnetti (Q fever) or a virus, sand fly fever virus (Beisel, *et al.*, 1967).

Volunteers who became acutely ill after exposure to F. tularensis were treated with streptomycin. All these subjects recovered quickly without complications or squeal. In those exposed to C. burnetti, upon the onset of fever and symptoms, tetracycline therapy was begun. Again, all subjects recovered (Beisel, *et al.*, 1967).

The volunteers were begun on a formula diet for 2 wk before exposure. Balance data were collected for 8 d before exposure and for 14–20 d after exposure. An estimated tissue loss based on nitrogen loss and the average weight loss recorded for these volunteers. For comparison, values for these

parameters in patients with either severe or moderate typhoid fever from the Coleman and Dubois study are shown. Average fever days have been included as a rough index of the severity of the infection. There appears to be a linear response between the amount of nitrogen lost and the persistence of fever (Beisel, *et al.*, 1967).

Beisel observed that the daily excretion of creatinine rose and fell roughly parallel with fever index (product of h × *F* > 100°F). In individuals exposed to sandfly fever, who suffered only 3 d of fever above 100°F, it took 10 to 11 d to recover from negative nitrogen balance when they were offered only normal amounts of dietary nitrogen and calories. To assess how much of the increased nitrogen excretion was attributable to reduced appetite/food intake and how much could be attributed to infection itself, healthy individuals were pair-fed to match exactly the daily food intake observed in tularemia-infected individuals (Beisel, *et al.*, 1967).

Beisel, *et. al.*, 1967 found that dietary restriction accounted for only 35% of the cumulative nitrogen loss that occurred during infection. They also found that the changes in glucocorticoid metabolism that occur during infection appeared inadequate to explain the extent of the catabolic response observed. Looking at both the early data and those from Beisel, *et al.*, 1967 a number of conclusions can be offered. Negative nitrogen balance is not observed

until after fever began, but increases and persists for days to weeks after the febrile phase. Negative nitrogen balance appears to correlate with net loss in body weight. Negative nitrogen balance and weight loss are both the product of reduced food intake as well as of infection-induced increased nitrogen excretion. Diets high in both calories and nitrogen can ameliorate the development of negative nitrogen balance (Beisel, *et al.,* 1967). Asymptomatic individuals do not incur negative nitrogen balance. The catabolic nature of infections has long been known, but more recently there has been evidence of concomitant anabolism. With regard to nitrogen, the translocation of amino acids from muscle to liver not only leads to increased amino acid degradation and increased urinary nitrogen excretion, but it also permits an increased synthesis of plasma proteins, that of the acute-phase proteins (Beisel, *et al.*, 1967).

2.6.1 Protein contents

Proteins are important building blocks of all cell and tissues. Proteins are necessary for your body's growth, development, and health blood contains two classes of protein albumin and globulin, albumin proteins keep fluid from leaking out of the blood vessels globulin proteins play an important role in your immune system (Janice T. Busher 1990).

2.6.2 Purpose of the total protein test

Total protein test is completed as part of your routine health checkup. It is one of the tests that make up your comprehensive medical panel (CMP). It may be specifically ordered if you are experiencing unexplained weight loss, fatigue, edema, or have symptoms of kidney or liver disease. The total protein test will take a measurement of total protein in your blood. Specifically looking for amount of albumin and globulin. This also looks at the ratio of albumin to globulin in your blood. This is known as the a/g ratio (Janice T. Busher 1990).

2.6.3Total protein range

As was described by (Janice T. Busher 1990), the normal range for total protein is between 6 and 8.3 gm/dl this range may vary slightly among laboratories. These ranges also vary on other factors such as age, gender, population and test method your total protein is abnormal additional tests must be performed to identify which specific protein is low or high before diagnosis can be made. Elevated total protein may indicate inflammation or infections (such as viral hepatitis B or C HIV). Bone marrow disorder (such as multiple myeloma or waldenstorm's disease).

Low total protein may indicate:

- Bleeding .

-Liver disorder.

-Kidney disorder (such as nephritic or glomerulonephritis).

-Malnutrition malabsorption condition (such as celiac disease or inflammatory bowel disease).

-Extensive burns.

-Gamma globulinemia.

2.7. Serum Albumin and Globulin

Hundred of protein are dissolved in the plasma by measuring the concentration of these proteins the clinician can obtain information regarding disease states in different organ system.

The measurement of protein is done by serum which is the fluid that remains after plasma has clotted thus removing fibrinogen total protein content provides some information regarding a patients general status fractionating the total protein the normal serum protein level is 6 to 8 g/dl. Albumin makes up 3.5 to 5.0 g/dl , and remainder is the total globulin these values may vary according to the individual laboratory (Janice T. Busher 1990).

2.7.1. Clinical significance of Serum Albumin

The serum albumin concentration is a function of its rates of synthesis and degradation and its distribution between the intravascular and extravascular

compartments. The total body albumin pool measures about 3.5–5.0 g kg^{-1} body weight (250–300 g for a healthy 70 kg adult) (Carter, 1994).

The plasma compartment holds about 42% of this pool, the rest being in extravascular compartments. Some of this is tissue-bound and is therefore unavailable to the circulation. Each day, 120–145 g of albumin is lost into the extravascular space. Most of this is recovered back into the circulation by lymphatic drainage. Albumin is also lost into the intestinal tract (about 1 g each day), where digestion releases amino acids and peptides, which are reabsorbed. There is minimal urinary loss of albumin in healthy subjects. Of the 70 kg of albumin that passes through the kidneys each day, only a few grams pass through the glomerular membrane. Nearly all of this is reabsorbed, and urinary loss is usually no more than 10–20 mg day^{-1}.

The distribution of albumin between body compartments can be examined by injecting radiolabelled albumin into the venous circulation. A typical biexponential plot of log plasma concentration versus time shows a first-order process two-compartment model can be constructed (Carter, 1994). There is a rapid phase of disappearance from the plasma over the first 2 days. This represents the transcapillary exchange rate of 4.5% h^{-1}, giving a distribution half-time of about 15 h. Then there is a slower exponential decay, representing the fractional degradation rate (FDR), of about 3.7%

day^{-1} with elimination half time of about 19 days. The FDR closely parallels the rate of synthesis in steady state (3.8% day^{-1}) (Carter, 1994).

The mechanism of the escape of albumin into the extra vascular compartment has come under review recently. Albumin must cross capillaries. Most organs in the body have continuous capillaries, but in some there are wide-open sinusoids (liver, bone marrow) or fenestrated capillaries (small intestine, pancreas, adrenal glands). Starling's theory holds that the rate of escape depends on the permeability of the wall and hydrostatic and oncotic pressures on either side of the wall(Ganong, 1995).

Half of the escaping albumin does so through the continuous capillaries, and there appears to be an active transport mechanism to facilitate this (Peters, 1996).

Albumin binds to a surface receptor called albondin, which is widely distributed in many capillary beds, except in the brain(Schnitzer, *et. al.*, 1988). Bound albumin enters vesicles within the endothelial cell and is discharged on the interstitial side within 15 s. The rate of transfer is increased with the addition of long-chain fatty acids (LCFAs) to albumin, and with the cationization and glycosylation of the molecule (Peters, 1996).

2.7.2 Serum Albumin Nature

In humans, albumin is the most abundant plasma protein, accounting for 55%- 60% of the measured serum protein (Gosling, 1995).

It consists of single polypeptide chain of 585 amino acids with a molecular weight of 66500 Da. The chain is characterized by having no carbohydrate moiety, a scarcity of tryptophan and methionine residues, and an abundance of charged residues, such as lysine, arginine, glutamic acid and aspartic acid (Peters, 1996).

Circulating molecule is arranged in a series of α- helices, folded and held by 17 disulphide bridges. The folding creates sub domains face each other to form domains. These can be seen as cylindrical structures with polar outer walls and hydrophobic central core (Doweiko, Nompleggi, 1991).

Albumin serves in the transport of bilirubin, hormones, metals, vitamins, and drugs; it has an important role in fat metabolism by binding fatty acids and keeping them in a soluble form in the plasma. This is one reason why hyperlipemia occurs in clinical situations of hypoalbuminemia. The binding of hormones by albumin regulates the amount of free hormones available at any time. Because of its negative charge, negative charge albumin is also able to furnish some of an ions needed to balance the cations of the plasma.

Albumin is synthesized in the liver the rate of synthesis is constant in normal individuals at 150 to 250 mg/kg/day, resulting in the production of 10 to 18g of albumin daily in a 70 kg man (Janice T. Busher 1990).

The liver produces albumin at less than half of its capacity. The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease states. Fasting or a protein-deficient diet cause a decrease in albumin synthesis as long as the deficiency state is maintained. In the normal individual the liver increases albumin synthesis in response to the increased albumin synthesis in response to the increased availability of amino acids provided by the portal blood following each protein–containing meal. Decrease in extra-vascular colloidal pressure serves as a stimulus for albumin synthesis and though to act within the liver, thyroid hormone, corticosteroid, growth hormone and insulin all can increase albumin synthesis (Janice T. Busher 1990).

The main site of albumin degradation is not known, albumin appears to be catabolized in locations that are capable of rapid equilibration with the blood stream, it is degraded into amino acids that are utilized for energy requirements of the cell or secreted into the pool of extracellular amino acid (Janice T. Busher 1990).

2.8. Globulin

The globulin fraction includes hundred of serum protein including carrier proteins, enzymes, complement, and immunoglobulin; most of these are synthesized in the liver although the immunoglobulin is synthesized by plasma cells. Globulins are divided into four groups by electrophoresis the four fractions are α_1, α_2, β and y, depending on their migratory pattern between the anode and cathode. Increase in globulin fraction usually result from an increase in immunoglobulins. Malnutrition and congenital immune deficiency can be cause a decrease in total globulins due to decrease synthesis, and nephrotic syndrome can cause a decrease due to protein loss through the kidney (Janice T. Busher 1990).

2.9. Albumin Globulin ratio

This ratio is dependent on the albumin and total globulin levels and normally used as a rough guide due to the variability in albumin and globulin levels the globulin portion of the ratio is considered to have the most impact and is therefore the most clinically relevant. An increased ratio can be attributed to either decreased globulin or an increased albumin level, which occurs with dehydration (Pagana Kathleen, pagana timothy, 1998).

The above test is done to:

- investigate inflammatory and for immunological disturbances.

- To ascertain digestive sufficiency.

- Factors which can cause increased level.

- Dehydration.

- Factors which can cause decreased levels when sample is taken while lying dawn high levels indicate.

- Other conditions.

- Hypothyroidism.

- Adrenal hypo-function.

- Blood viscosity may be too high due to blood state is

Low level indicates:

- Liver dysfunction.

- Immune activation.

- Other condition blood may be too thin (Weatherby Dicen, Ferguson scott, 2002).

3- MATERIALS AND METHODS

3.1Materials

3.1.1 Study design:

This is descriptive analytical case and control study.

3.1.2 Study area:

The study was done in El-Bar-Omdarag, Gelass, El-Brsa, Karema and Merowe (Merowe locality), northern Sudan. Data collection was collected in period between April to June 2014.

3.1.3 Study population:

One hundred adult individuals were invited to play a rule in this study. Fifty positively diagnosed with typhoid (25 male and 25 female) will be defined as on zero day of treatments cases of this study, while fifty individuals (25 male and 25 female) negatively diagnosed with typhoid, apparently healthy as obtained by physical examination and disease history will be defined as control in this study.

Inclusion criteria:

Those positively diagnosed with typhoid fever on zero day of treatment

Exclusion criteria:

Those with DM, hypertension, renal dysfunction or those with any disease that could affect serum protein or under constriction diet.

3.1.4 Ethical consideration:

My university had given me the permission to do this study. Moreover, all of the selected patients were fully agreed by taking typhoid samples from them.

3.1.5 Data Analysis:

Collected data analyzed by using S.PSS 11.5. Descriptive Statistics, Independent samples test, T- Test, Group Statistics, mean, SD, Correlation

3.2Methodology

3.2.1Sampling:

About 5 ml of venous blood were collected from each patient randomly. The samples collected under aseptic condition and placed in sterile plane containers and after clotting centrifuged for 4 minutes at 3000 RPM to obtain serum

3.2.2 Estimation of biochemical parameters:

(a) Reagent for measurement of total protein concentration.

The reagent was supplied by Biosystems Company and was composed of:

1-Copper (11) acetate 6mmol/L

2- Potassium iodide 12mmol/L

3- Sodium hydroxide 1.15 mol/L

4- Protein standard. Bovine albumin with concentration of 60.2g/L (6.02g/dL).

Reagent was ready to use (appendix11).

(b) Reagent of Albumin for measurement of albumin concentration.

The reagent was supplied by Bio systems Company and it was composed of:

1- Reagent acetate buffer 100 m mol/L.

2- Bromocresol green 0.27 m mol/L.

3- Detergent.ph 4.1.

4- Albumin standard (Bovine albumin with concentration of 48.7g/L(4.87g/dL). Reagent and standard were ready to use.

3.2.3 Estimation of serum total protein concentration:

Using Biuret method (appendix11).

Principle of the method:

Protein in the sample reacts with copper (11) ion in alkaline medium forming a colored complex that can be measured by Colorimeter at 545 ± 10 nm

Procedure:

In the three labeled tubes (blank, standard and sample) 1.0 ml of reagent was added in each tube. Twenty µl of sample was added to the sample tube and

20 µl of standard was added to the standard tube. Twenty µl of distilled water was added to the blank tube. All tubes was mixed thoroughly and left to stand for 10 minutes at room temperature. The absorbance of the standard (ASTD) and the sample (AS) was measured at 545 nm against the blank within maximum 2 hours.

Calculation:

The protein concentration (g/dL) = AS/Astd × concentration of standard(g/dL).

Protein concentration in g/L= Protein concentration in g/dl×10.

3.2.4 Estimation of serum albumin:

Using Bromocresol green (BCG). The principles of the method are appendix 111.

Albumin in the sample reacts with Bromocresol green in acid medium forming a colored complex that can be measured at 630 nm (610 ---- 670 nm).

Procedure:

In the three labeled tubes (blank, standard and sample) 1.0 ml of reagent was added in each tube. Ten µl of sample was added to the sample tube and 10 µl of standard was added to the standard tube. Ten µl of distilled water was added to the blank tube, then all tubes was mixed thoroughly and left to stand for 1 minute at room temperature. The absorbance of the standard

(ASTD) and the sample (AS) was measured at 360 nm against the blank within maximum 30 minutes.

Calculation:

Albumin concentration (g/dL) = (As/Astd) × concentration of standard (g/dL)

Albumin concentration g/L = Albumin concentration in g/dL×10.

3.2.5 Globulin calculation:

Globulin calculated by subtracting serum albumin from total protein.

Serum globulin = total protein- Serum albumin (g/dl)

3.2.6 Albumin: Globulin Ratio = Serum albumin (g/dl) /serum globulin (g/dl)

4- RESULTS

4.1 Results

At, P. value = 0.000 this study showed highly significant differences in all estimated biochemical parameters for serum total protein with mean ± S. D. (8.4 ± 0.07,6.8 ± 0.41), serum albumin (3.5 ± 0.05, 4.1 ± 0.19),serum globulin (4.9 ± 0.08, 2.8 ± 0.46),A/G ratio (0.7, 1.5) for typhoid positive and negative, respectively (Table 4-1).

Comparison of estimated biochemical parameters between studied participants. Table(4-1)

Variables	Typhoid positive Mean ± SD	Typhoid negative Mean ± SD	P. value
Serum total protein	8.4 ± 0.07	6.8 ± 0.41	0.000
Serum albumin	3.5 ± 0.05	4.1 ± 0.19	0.000
Serum globulin	4.9 ± 0.08	2.8 ± 0.46	0.000
Albumin: globulin ratio	0.7	1.5	0.000

At, P. value < 0.05 this study showed no significant difference, P. value was 0.632, 0.832 and 0.760 with mean ± S.D. serum total protein (7.5 ± 0.92, 7.6 ± 0.79) serum albumin (3.8 ± 0.38, 3.8 ± 0.37) and serum globulin (3.8 ± 1.16, 3.9 ± 1.15) for males and females respectively (table 4-2).

Comparison of biochemical parameters between different genders in typhoid positive participants. Table(4-2)

Variables	Male group Mean ± SD	Female group Mean ± SD	P. value
Serum total protein	7.5 ± 0.92	7.6 ± 0.79	0.632
Serum albumin	3.8 ± 0.38	3.8 ± 0.37	0.832
Serum globulin	3.8 ± 1.13	3.9 ± 1.15	0.760

At, P. value < 0.05 this study showed no significant difference, P. value was 0.11, 0.60, and 0.06 with mean ± SD serum total protein (8.4 ± 0.06, 8.4± 0.08) serum albumin (3.5 ± 0.05, 3.5 ± 0.06) and serum globulin (5.0 ± 0.06, 4.9 ± 0.08) for titer 1/160 and titer 1/320 (Table 4-3).

Comparison study of estimated parameters regarding typhoid titers. Table(4-3)

Variables	Titer=1/160 Mean ± SD	Titer=1/320 Mean ± SD	P. value
Serum total protein	8.4 ± 0.06	8.4 ± 0.08	0.11
Serum albumin	3.5 ± 0.05	3.5 ± 0.06	0.60
Serum globulin	5.0 ± 0.06	4.9 ± 0.08	0.06

Correlations of typhoid titers against estimated parameters. Table(4-4)

Estimated parameters	Regression	P. value
Serum total protein	-0.231	0.106
Serum albumin	0.077	0.595
Serum globulin	-0.273	0.055

At P< 0.05 this study showed no correlations between typhoid titers and estimated parameters.

5- DISCUSSION

Comparison of estimated biochemical parameters and between infected males and healthy, and infected females and healthy were significantly different. On the other hand, comparison between estimated parameters and typhoid titers and between estimated parameters and genders in typhoid positive participants, in addition to correlations between the biochemical parameters and typhoid titers were not significantly different.

Enteric fever was significantly increased total serum protein and globulin over the control in patients as compared to healthy irrespective of genders. This may be due to inflammation or infection. Increase in serum globulin may be due to immune activation which increases immunoglobulin. The fever decreased serum albumin and albumin : globulin ratio in infected patients compared to healthy ones, irrespective of genders, this may be due to acute inflammation or to loss of albumin in urine, the decreased in serum albumin affected liver synthesis and in detoxification of the liver. The same findings were obtained by the authors, Abro, *et al.*, 2009); Emenuga, *et al.*, 2014); Amen Shamim, *et al*, 2012); Reinoso.*et al.*, 1998); Arroyove and Calcano 1979). No significant differences between genders in serum total protein, albumin, and globulin in the studied patients; the result was

supported by Abro, *et al.*, 2009). And Amen Shamim *et al*, 2012). Correlations between the titers and serum total protein, albumin and globulin were negatives, the findings was confirmed by Emenuga, *et al*, 2014).

Conclusion

- Enteric fever increased serum total protein and globulin while decreased albumin and albumin: globulin ratio, irrespective of genders. Highly significant differences in estimated biochemical parameters for healthy and infected males and females. Comparison of biochemical parameters and different genders in typhoid positive participants, moreover, and between typhoid titers and biochemical parameters were not significant. Correlations between the titers and serum total protein, albumin and globulin were negative.

Recommendations

1- Hospitals and health centers must include laboratories qualified technicians.

2-Drinking water supplies tanks should be clean weekly to avoid the contamination by the bacteria.

3- Prevention of accumulation of food remains to avoid reproduction of house flies.

4- Prevention of stool excretion in plains to avoid transmission of the bacteri

by house flies.

5-Continuous control of house flies.

6- Patients after diagnosis should start treatment as soon as possible to avoid Complications.

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