Impact of Salmonellosis on Antineutrophil Cytoplasmic Antibodies, Alkaline Phosphatase and Lactic Dehydrogenase

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Abstract

The study was carried out during a period of March 2018-July 2019 for the detection of Salmonellosis in 106 suspected patients with age group range from 17 - 69 years, who attended to Baghdad teaching hospitals, that had been examined and defined as suspected cases by specialized physician with the recording of clinical manifestation .The diagnosis done by immunochromatography method, a blood sample was taken from each patients as well as other 30 healthy control matching in age and gender. The study included measurement of the level of antineutrophil antibodies, activity of Alkaline phosphatase , and Lactic acid dehydrogenase in sera of patients and healthy control. The result indicated that anti –salmonella IgM positive in 54 cases, anti-salmonella IgG positive in 40 cases and 12 positive cases with both IgM and IgG. The Level of c-ANCA and p-ANCA, Alkaline phosphatase and Lactic acid dehydrogenase increased significantly, with no statistical difference between the gender of patients sera in comparison with healthy control.

Key Words: *Salmonellosis, Alkaline phosphatase, Lactic acid dehydrogenase, c-ANCA, p-ANCA*

Introduction

Salmonella typhoid and paratyphoid are transmitted mainly by the fecal-oral route. In most cases an asymptomatic carrier of S. typhi, or an individual who has recently recovered from the infection, continues to excrete large numbers of organisms in the stool and contaminates food or water, either through direct food handling, through transfer of bacteria by flies and other insects, or by contamination of potable water and raw frozen chickens meat [1-4].

Only 10% of patients recovering from typhoid fever excrete S. typhi in the stool for three months, and in the past 2-3% became permanent carriers. These infections have great potential for epidemic spread [5-7] Salmonella typhi infections are commonly mild and self limiting. Severe disease represents the "tip of the iceberg". In temperate countries persistent carriers are a more important reservoir of infection [8]. A number of host factors increase the risk of Salmonella infections. Disease related (achlorhydria) or iatrogenic (antacids, H_2 blockers, proton pump inhibitors) reduction in stomach acidity or gut pathology (surgery, inflammatory bowel disease, malignancy) and recent antibiotics increase the susceptibility to infection. Disease related or iatrogenic immuno suppression and several other infections, notably schistosomiasis, malaria [4, 5].

The clinical features of typhoid and paratyphoid fever are generally similar, although paratyphoid tends to be a more mild infection [9]. Most patients with enteric fever present with a non-specific gradual onset of an influenza-like illness although Salmonella typhi infection can present with fever and a bewildering array of signs and symptoms ranging from non-metastatic central nervous system syndromes including psychosis and cerebellar ataxia [10], through to focal involvement of bone [11], liver [12, 13], spleen [13], testes [14], menninges [15], vascular prostheses, atheromatous plaques [16].

In general the enteric fevers are sub-acute infections with an incubation period of approximately 7-14 days (range 3-60 days) following exposure. The illness begins insidiously with non-specific signs and symptoms of fever , headache, muscle and joint aches, malaise, lassitude, anorexia, often a dry cough (sometimes associated with a sore throat) [17]. The spleen enlarges, but lymphadenopathy is not usually prominent. Relative bradycardia is considered common in typhoid although

in many series this has not been a feature of the disease. Some abdominal complaints are usual although either diarrhea or constipation may occur.

There is usually some abdominal discomfort, and even in the first week of the disease the patient may notice passage per-rectum of a small amount of blood or melena normal bowel habit is unusual in typhoid. diarrhea [3,18] The clinical evolution of untreated typhoid is divided classically into weeks [19] During the first week the fever rises gradually, and in the second week reaches a high plateau. By the second week the patient has become progressively weaker, has lost weigh. By the third week of infection, if untreated, a dangerous stage is entered upon in which either intestinal perforation or hemorrhage become more likely as the necrotic Peyer's patches either erode through the wall of the terminal ileum [20] or penetrate a large blood vessels .complications in the third and fourth week also include pneumonia, acute psychosis, coma, myocarditis [21], pericarditis, orchitis , meningitis [22], most salmonellae induce an acute inflammatory response, which can cause ulceration. They may elaborate cytotoxin that inhibit protein synthesis. and contribute to the inflammatory response or to ulceration However, invasion of the mucosa causes the epithelial cells to synthesize and release various proinflammatory cytokines, including: IL-1, IL-6, IL-8, TNF-α, IFN-α, MCP-1, and GM-CSF. These evoke an acute inflammatory response and may also be responsible for damage to the intestine, Initial host responses involve neutrophil infiltration, followed by the arrival of Lymphocytes and macrophages. Occasionally, diffuse colitis occur mimicking inflammatory bowel disease [23].

Material and Method

The study carried out during the period from (March 2018- July2019), studied group were involved Suspected patients their age range between 17-69 years. Blood samples were obtained from a total of 106 patients clinically suspected with Salmonellosis that had been examined and defined as suspected cases by specialized physician with the recording of clinical manifestation.

Blood samples

Five mL of venous blood was obtained from each patients and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in eppendorf tubes and then test for Salmonellosis (IgM and IgG) and stored in deep freeze at -20° C until the time for using.

Immunochromatographic assay

About 100 µl of serum from each sample was added to the sample hole of the kit. The colour density is proportional to the antibody titer. The complexes (appears in colour band after 10 minutes) confirm that the test was performed correctly. This CerTest-salmonella kit which determines the salmonella quality in bloods samples. Pre-coating was achieved to the membrane proceeding to test band region to the monoclonal antibodies of the mouse, it was achieved against salmonella antigens. Through test, samples were reacted with conjugated colors' (anti-salmonella of monoclonal mouse microsphere (red antibodies)), the samples were dried before that, the combination then travelled to reach membranes via the act of capillaries. While samples move via the membranes tests, tinted particle were migrated. In positive results, certain antibodies that have existed on the membranes captured these particles which lead to appearance of red tinted line that can clearly observed while the other result appears in a green tinted line (the negative results that represent the control samples.

Immunological and Clinical biochemical tests

The level of Anti protienase-3; Cyclic -anti neutrophil cytoplasmic antibodies (c-ANCA) are examined by Enzyme Linked Immunosorbent Assay (ELISA)according to [24]. Alkaline phosphatase and Lactic dehydrogenase Concentration determined according to manufactures instructions of Biosystem (Spain).

Statistical Analysis

The results were analyzed using statistical system SPSS version -18 (T-testing).

Result

Serological tests

Serum level anti –salmonella IgM present in 54 cases with a percent of 50.9%, also, the level of anti –salmonella IgG present in 40 cases with a percent of 37%.While the level of both IgG and IgM present in 12 cases with a percent of 11.3% (Table 1).

Table 1: Distribution of anti-Salmonella IgG and IgM antibodies using immunochromatography method

Anti- Salmonella antibodies	Total	Positive		Negative	
		No.	%	No.	%
IgG	106	40	37	66	63
IgM	106	54	50.9	52	49.1
IgG + IgM	106	12	11.3	94	88.7

Level of p-ANCA and c-ANCA

Level of anti-PR3 in the serum of patients with Salmonellosis shows a significant increasing (P< 0.05) in comparison to healthy control, while, the results shows no-significant difference (P>0.05) between the gender in both groups. The serum level of anti-PR3 was (24.82 ± 0.28), (23.64 ± 0.36) in males and females of patients group respectively comparing to healthy control group (15.08 ± 0.92) and (15.85 ±0.15) in males and females respectively as shown in table 2.

Table-2: The level of anti –PR3 (Iu/ml) in sera of Salmonella patients and control

Groups	Mean ± SD	
	Males	Females
Patients	24.82 ± 0.28	23.64 ± 0.36
Control	15.08 ± 0.92	15.85 ± 0.15
T – test value	0.51	0.91

The level of anti-MPO shows a significant increasing (P<0.05) in serum of patients with Salmonellosis in comparison with healthy control, while the results shows no significant differences (P>0.05) between the gender in both groups. The level was (23.48 ± 0.16), (22.64± 0.22) in males and females of patients groups respectively in comparison with (16.35 ± 0.28), (16.80 ± 0.21) in males and females of healthy control respectively. Table (3).

Table 3: The level of anti- MPO (Iu/ml) in sera of Salmonella patients and healthy control

Groups	Mean ± SD	
	Males	Females
Patients	23.48 ± 0.16	22.64 ± 0.22
Control	16.35 ± 0.28	16.21 ± 0.19
T – test value	0.55	0.67

Alkaline phosphatase activity

The activity of alkaline phosphatase increased significantly (pd≤.05) in Salmonellosis patients in both gender in comparison with healthy control (Table 4).

Table 4: Alkaline phosphatase activity (Iu/ml) in patients with Salmonellosis and healthy control

Groups	Mean ± SD	
	Males	Females
Patients	186.37 ± 0.73	179.10 ± 0.45
Control	1118.58 ± 0.48	115.90 ± 0.39
T – test value	0.551.42	1.01

Lactic acid Dehydrogenase activity:

The activity of Lactic acid dehydrogenase increased significantly (pd≤.05) in Salmonellosis patients in both gender in comparison with healthy control (Table 5).

Table 5: Lactate dehydrogenase activity (Iu/ml) in patients with Salmonellosis and healthy control

Groups	Mean ± SD	
	Males	Females
Patients	232.08 ± 1.82	228.35 ± 0.38
Control	155.40 ± 0.18	157.09 ± 0.36
T – test value	3.30	1.013

Discussion

Salmonella can both colonize and cause infections in humans and animals. Of interest, some Salmonella species appear to be better adapted to humans, and vice versa. For example, S.typhi does not have an animal reservoir and is solely transmitted by humans. Most human cases of Salmonella involve ingestion of a contaminated food item, in particular, eggs, poultry, ground beef, or dairy products [(25)]. Salmonella can be acquired by direct personal contact, nosocomial transmission, or contaminated drugs/solutions [(26)]. The result indicated anti-salmonella IgG was 40 (37%) of cases while IgM was 54 (50.9%) cases by immunochromatography method (Table 1).

Generally, the prevalence of infection is related to several factors including nutritional habits [(27)]. The level of anti-PR3, in the serum of patients with Salmonellosis shows a significant increasing (P< 0.05) in comparison to healthy control, while, the results shows no-significant difference (P>0.05) between the gender in both groups (Table 2). There are many explanations for the presence of the antibodies in Salmonellosis, one of them is the disruptive effect of salmonella on polymorph nuclear PMN expose, change intracellular proteins and rendering them antigenic city and result in the production of antibodies for epitope of anti -PR3 [28], also the other explanation is the antibody produced as a response to amoebic antigen, this antigen is cross-react with PMNL cytoplasmic components like anti PR3 [29].

The level of anti-MPO shows a significant increasing (P<0.05) in serum of patients with Salmonella in comparison with healthy control, while the results shows no significant differences (P>0.05) between the gender in both groups (Table 3). The results are in line with other studies which proved that PMNL granulocytes have an important role in innate immunity and their programmed cell death & removal are effective for acute inflammation resolution. MPO which is a hem protein generally associated with killing bacteria and tissue injury which oxidative, this property is expressed in

neutrophils, MPO binds to neutrophil [29].

The level of ALP increased significantly (P<0.05) in patients sera with Salmonellosis in comparison with healthy control, while the results showed no-significant difference (P>0.05) between the genders in both groups. The increasing level of alkaline phosphatase in the serum may be as a result to damage of liver cells and other cells infected with bacteria and released of the enzymes to blood stream [28].

The activity of Lactate dehydrogenase increase significantly (P<0.05) in patients sera of Salmonellosis in comparison to healthy control .While the results show no-significant difference (P>0.05) between gender in both groups. (Table-5).The increasing in LDH enzyme level may be due to cellular death and leakage of the enzyme from the infected cells [30].

Conclusion

The result indicated that anti –salmonella IgM positive in 54 cases, anti- salmonella IgG positive in 40 cases and 12 positive cases with both IgM and IgG. The Level of c-ANCA and p-ANCA, Alkaline phosphatase and Lactic acid dehydrogenase increased significantly, with no statistical difference between the gender of patients sera in comparison with healthy control.

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