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Isolation and Detection of Salmonella and Enterobacteriaceae in Typhoid Patient and their Effects in Blood Smear in Kirkuk City

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Abstract

Typhoid fever is one of the important health problems that affects Kirkuk city population. Therefore, this study conducted on the bacteria implicated in patients suffered from typhoid fever symptoms.

Objetivo: Salmonella typhi is an important intracellular pathogen, cousedEnteric fever is a disease of developing countries associated with poor public health and low socio-economic indices

Method: The stool and blood samples were collected from 300 males and females patients at different age stages (2-72) years old who visited Azadi-teaching hospital , Kirkuk general hospital, pediatric hospital, Alnaser hospital and general health laboratory in Kirkuk's city for the period from December 2019 to March 2021

Coclusio:

Salmonella typhi and some entereobacterioceae Females are more affected than males, and age has a clear effect on the severity of the infection. Typhoid fever also has a clear effect on the blood cell shapes of infected patients.

Typhoid fever is one of the important health problems that affects Kirkuk city population. Therefore, this study conducted on the bacteria implicated in patients suffered from typhoid fever symptoms.

Keywords: Typhoid fever, Enterobacteriocea, Widal test, immunochromatographic, Blood smears

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عزل وتشخيص السالمونيلا والبكتيريا المعوية لدى مرضى التيفوئيد وتأثيراتها على مسحات الدم في مدينة كركوك

اسما سميع كرومي [1] ، كولجمين مدحت عبدالله[2] ، ماجدة ابراهيم [3]

المستخلص

الخلفية: عتبر حمى التيفوئيد من المشاكل الصحية الهامة التي يعاني منها سكان مدينة كركوك. لذلك، أُجريت هذه الدراسة على البكتيريا المتورطة في المرضى الذين يعانون من عراض حمى التيفوئيد.

الهدف: تُعد السالمونيلا التيفية مُمرضًا خلويًا مهمًا، وتُسبب الحمى المعوية مرضًا شائعًا في الدول النامية، ويرتبط بضعف الصحة العامة وانخفاض المؤشرات الاجتماعية والاقتصادية.

الطريقة: جُمعت عينات البراز والدم من 300 مريض من الذكور والإناث في مراحل عمرية مختلفة (2-72 عامًا) ممن زاروا مستشفى آزادي التعليمي، ومستشفى كركوك العام مستشفى الأطفال، ومستشفى النصر، ومختبر الصحة العامة في مدينة كركوك، خلال الفترة من ديسمبر 2019 إلى مارس 2021.

النتائج:

تُصاب الإناث بالسالمونيلا التيفية وبعض أنواع البكتيريا المعوية بشكل أكبر من الذكور، وللعمر تأثير واضح على شدة الإصابة. كما أن لحمى التيفوئيد تأثير واضح على أشكال خلايا الدم لدى المرضى المصابين.

الخلاصة

تُعتبر حمى التيفوئيد من المشاكل الصحية المهمة التي تُصيب سكان مدينة كركوك. ولذلك، أجريت هذه الدراسة على البكتيريا المتورطة في المرضى الذين يعانون من أعراض حمى التيفوئيد.

الكلمات المفتاحية: حمى التيفوئيد، البكتيريا المعوية، اختبار فيدال،- اللوني المناعي، مسحات الدم

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Introduction

Enteric fever is a disease of developing countries associated with poor public health and low socio-economic indices [1].It can result in complications in different organ systems and delay in identification and prompt treatment can be fatal. The important gastrointestinal complications of enteric fever include hepatitis, intestinal ulcers, bleeding and bowel perforation [2]. Salmonella typhi (S. typhi) causes typhoid fever which is a disease characterized by high mortality and morbidity worldwide. In order to curtail the transmission of this highly infectious disease [3]. Salmonella typhi is an important intracellular pathogen, among the more than 2,300 closely-related Salmonella serovars bacteria recognized, S. typhi is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever [4]. S. typhi is an enteric bacillus which belongs, to the genus Salmonella in the family Enterobacteriacaea and it is a multi–organs pathogen which inhibits the lymphatic tissues of the small intestine, liver, spleen, and blood stream of infected humans. This bacteria has a mixture of features that make it an efficient pathogen. This species contains an endotoxin that is characteristic of Gram-negative organisms, as well as the virulence-enhancing Vi antigen. Many of the S. typhi virulence factors are clustered in some areas of the chromosome known as Salmonella pathogenicity islands (SPI), such as adhesion, invasion, and toxin genes. A protein known as invasion that permits non-phagocytic cells is also produced and excreted by the bacterium. Where it is capable of intracellular living. The oxidative burst of leukocytes may also be inhibited, making innate immune reaction ineffective[5]. Hematologic changes included increases in, RBC count, hemoglobin concentration, associated with a transitory leukopenia characterized by neutropenia and lymphopenia[6]. Conventional serotyping or antigenic classification of Salmonella was traditionally founded upon antibody reaction with 3 types of surface antigens, somatic O antigens, flagellar H antigens, 3 and Vi capsular antigens. The O antigen determines the group of Salmonella isolate belongs to, while the H antigen determines the serovar. The capsular antigen occurs only in S. Typhi, S. Paratyphi C. [7] Salmonella has the capacity to form biofilms on both biotic and abiotic surfaces. The family Enterobacteriaceae includes many genera (Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus, and others[8].

Materials and Methods

A total of 275 stool and blood samples were collected from patients aged between 2to 72 years old with a clinical approved of typhoid fever by physicians in Kirkuk Hospitals , sample collocated in the period from December 2019 till march 2021 and 25 apparently healthy control individuals. stool and Blood samples were collected from patients with a clinical suspicion of typhoid fever and screened for the presence of S.typhi infection in stool culture, hematological test and serological test[9]..The blood sample was drained on the first day of management at the same time with stool culture sample collection. The blood sample analyzed for various hematological parameters including red blood cells (RBC, hemoglobin concentration (Hb), total white blood cells(WBC), platelet count (PLT), Lymphocytes and Monocytes ,serological test including widal test and immunochromatographic test[10];[11].

Results and Discussion

Stool and blood specimens of 300 febrile patient

collected and examined for the presence of Salmonella typhi and some Entereobacterioceae that effect on blood cells counts ,shapes and their maturation as shown in Table (1).

Table (1) Results of positive and negative Salmonella growth from stool samples

Groups	total	Positive culture		Negative culture	
	No.	NO.	%	NO.	%
Cases	275	18	6	257	91.67
Control	25	0	0	25	8.33
Total	300	18	6	282	94
		ns Chi-Square = 1.741 P-Value = 0.187			

The results refer that (18) patients at ratio (6 %) were showed positive stool culture and(257) patient at ratio (91.67%)were showed negative stool culture from total 275 febrile patients clinically suspected for having typhoid fever, also the stool samples from control group (25 persons) showed non growth as indicated in table(1) .The samples taken in the second week from people suffering of disease to certainly the effect of Salmonella typhi , By culturing on MacConkey agar , S.S.agar ,XLD agar, and the bacterial isolates according to the morphological characteristics, Gram stain, biochemical tests and other methods. Culture remains to be the gold standard in the diagnosis of typhoid fever. [12] ; disagree with the previous studies shown that the stool culture is not the best test to isolate Salmonella typhi bacteria in comparison with blood culture.In a previous study a stool was cultured from 300 individuals, the sensitivity of Salmonella typhi detection from stool samples is low, [13] in a study mention that Salmonella typhi was not isolated from stool samples at either site, but Nontyphoidal Salmonella (NTS) prevalence in stool samples was (24.1) (95%) per 1000 population and 10.3 (95%) per 1000 population as shown in Table (2).

Table (2) Percentage of infected patient with typhoid fever according to gender

	Male		Female		Total	
	NO.	%	NO.	%	NO.	%
Cases	135	49.09	140	50.91	275	100
Control	10	40	15	60	25	100
Total	145	48.33	155	51.67	300	100
	ns Chi-Square = 0.758 P-Value = 0.384					

The study showed variation in the ratio between males and females of typhoid fever distribution, the results showed that the ratio in the females was (50.91%) while the ratio in the males was (49.09 %) as show in the table (2). , were agree with previous study in Kirkuk reported that females were more infected with typhoid fever than males also same results reported in Basrah by [14]; [15], showed that the females are more infected than males in Iran. While a study in Kufa carried out by[16]., [17]., 2014 reported that males are more infected than female with ratio 1.5:1. They boost their score for the nature of males working out the home and most there meal in the restaurant or in hawkers or they deal with affected or carrier people ,this tends to provides a greater immunity to this infection to the male subjects than in woman , occurs more among the male subjects, probably as a result of their occupational and social aspects. Current study results with respect to higher typhoid ratio in the females than males may related to the fact that the female don't interesting there health but care only on her family and the main cause to delayed there to visiting doctor or the healthy center is there economic and we studied and tested female more than male. In some studies they thought there is no different between male and female in the affection of typhoid fever disease and this differentiation due to the number of sample tested. (16)reported that the male-female ratio of typhoid cases was found to be 1.36, suggesting that in this population males are either more susceptible to typhoid, or more likely to present for hospital treatment, than females. as shown in Table (3).

Table (3) Percentage of infected patients with typhoid in different age groups

Age	Infected		Not affect		Total	
	NO.	%	NO.	%	NO.	%
2-12	14	5.09	1	4	15	5
12-22	41	14.90	3	12	44	14.66
22-32	58	21.09	2	8	60	20
32-42	59	21.45	10	40	69	23
42-52	53	19.27	5	20	58	19.34
52-62	32	11.63	2	8	34	11.34
62-72	18	6.54	2	8	20	6.66
Total	275	100	25	100	300	100
	ns Chi-Square = 5.924 P-Value = 0.432					

As shown in the table (3) this study showed age dependence distribution ratio for the infected patient with typhoid fever. The age range of typhoid infection was between(2 to 72) years old, this indicates the wide range age distribution of typhoid fever that affect all stage of age .The results revealed that the most infected age group with typhoid fever was (32 to 42)years old, that ration of this age group ratio was (21.45 %). This high ratio of infection for this age group may be attributed to that in this age most people spend half times outside of home and may be take an contamination water and food from hawkers or travels to endemic area. [18]; [19], agreement with the a previous studies that shown the ages group (30-40) were showed the highest range of infection. However another study showed that the lowest percentage of typhoid infection was at years between (2 to 12) years old at ration of (5.09 %) [20]; [21], this lowest percentage indication of this group are rarely eat their meals outside the home and not work more of them were pupils. While the age group(22 – 32) were (21.09%) the nearest percentage to highest rate because they are considered a class that most of them are employed or self-employed and eating street vendor food or they worked in areas where proper health services are no available. Also in Duhok city, [22]; , shown that the age group (21-30) years is the vulnerable group for typhoid fever. The age stages (62-72) showed the second lowest percentage (6.54%) of infected subjects in studied population , this low rate of infection in this age group may be due to that these age category are rarely depend on outside nutrient because most of them are don't work and retired. as shown in Table (4).

Table(4) Percentage of positive Widal test to positive Immunochromotography ICA test for patient with positive *Salmonella typhi*

ICA test	Positive		Widal Test positive		Widal Test Negative	
	No.	%	NO.	%	No.	%
IgG	74	24.67	35	47.30	39	52.70
IgM	95	31.67	73	76.84	22	23.16
IgG & IgM	109	36.33	96	88.07	13	11.93
Negative ICA	22	7.33	4	18.18	18	81.82
Total	300	100	208	69.33	92	30.67
			** Chi-Square = 64.496 P-Value = 0.0003			

Widal test and ICA test are tests to diagnosis typhoid fever disease but they different in the cost , time and accuracy in action. On the bases a differentiation between them according to number of infected person, the table (4) shown that widal test are positive in most tested samples with a percentage (67.34%). However there are some studies considered that widal test is a false positive study [23]. Also shown that Widal test had low specificity, but the study of House, 2005 disagree

with the last study. This study compared among IgG that it is ratio are (24.67%) , positive widal test that are (47.30%) and negative widal test that are (52.70 %). IgG refers to chronic disease . The acute cases IgM that recorded (31.67%) for the suspected people that were positive to widal test as a ratio (76.84%) while negative cases were (23.16 %). The highest range of the infection are positive IgG & IgM as a percentage(36.33%) compared with positive widal test that are also the highest rate as (88.07%).The last detected IgM antibody is suggestive of recent infection and IgG indicated a current or previous infection . as shown in Table (5).

Table(5) Distribution of Gram negative bacteria that isolate in stool

	S.typhi		E.coli		K.pneumonia		P.merabilis		No growth	
	NO.	%	NO	%	NO	%	NO	%	NO	%
Cases	18	6.54	115	41.8	68	24.7	52	18.90	56	20.36
Control	0	0	2	8	1	0.36	1	0.36	20	80
	** Chi-Square = 53.844 P-Value = 0.0009									

Enterobacteriaceae consist of a large family include various type of gram negative bacteria some of them pathogenic and some not[24].In recent study focused on only four kind of pathogenic bacteria that isolate from stool culture with various percentage after certain the result with biochemical testes.Table(5) shown these percentage , from 275 cases suffered from sign and symptoms of typhoid fever there were only 18 case (6.54 %) isolated as S.typhi that is the causative agent of typhoid fever and this is the lowest percentage among the isolated bacteria while the highest percentage of bacteria are E.coli as 115 cases (41.81 %)and this bacteria conceder a normal flora when it is present in stool with a small amount but when it is present with a large amount it is causes Enteric disease and symptoms [25]. The last bacteria isolate was K.pneumonia that was isolate as a percentage (24.72 %)it is also normal flora 105 cell /1 gm and more that causes Enteric disease due to immunity disease ,ingestion contamination food ,transport from affect person , alcohol and take antibiotic without asking professional. Other bacteria studied are Proteus merabilis with rate (18.90 %) and there are cases no growth as a rate 56 (20.36%). But the control group different in result it shown no growth of S.typhi bacteria from 25 control cases and 2 case only (8%) of E.coli, 1(0.36%) case of K.pneumonia and 1 case (0.36%) but there are no growth in 20 case (80%)of control group. as shown in Table (6).

Table (6): The relation among positive typhoid infection and Histopathological change in blood film

	Change in N.		No change in N.		Total	
	No.	%	No.	%	No.	%
Cases	182	60.67	118	39.33	300	100
Platlate	89	29.67	211	70.33	300	100
Band cell	171	57	129	43	300	100
Roealeux	163	54.33	137	45.67	300	100
	** Chi-Square = 71.322 P-Value = 0.00002					

Table (6) showed relation among shape of blood cells and typhoid fever infection ,This disease change the cells shape or effect on their maturation in (182)cases (60.67%) from(300) febrile patient and there is no change in (39.33%) cases. In this case series thrombocytopenia was present in (29.67%) cases,higher than reported by Joseph,1997 (10% and 9.1%)this due to the effect of the bacterial infection on the blood. It is shown change in the shape of neutrophil that shown in band shape that is mean the neutrophils nucleus without segmented lobes and stay in immature stage in (57%)cases, while it is shape are one peace like band,or change it is lobs shape to S shape like figure (4.29) .In (54.33%) c the RBC form Roealeux formation or toxic granulation in(45.67%) aren't seems any chang in the RBC 6. The current study includes more than one change in blood cells shapes and this is clear in 183 cases (60.67 %) that shown present rate of change in the blood film for the patient that suffered from typhoid fever while there are no appear any change in the 118 cases (39.33 %) from 300 febrile patient.

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