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Evaluate the efficiency of Widal test in diagnosis of typhoid Fever in arriving patients to Kirkuk hospitals

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Abstract
The aim of this study was to investigate the accuracy of widal test by compared with enzyme linked immunosorbent assay (ELISA) as standard method and for this purpose 150 blood samples were pulled from patients suspected infected with typhoid fever coming to Azadi Teaching Hospital and Children's Hospital in Kirkuk Governorate from 1-09-2016 to 1-11-2016, and 20 sample of blood were pulled from healthy people considered as a control group. The sample tested by tube titration method of Widal test and also by using ELISA test (antibodies IgM and IgG). The results showed that the percentage of infection in patients with typhoid fever were 74% (111:150) when diagnosed by widal test, and IgM antibodies increasing in ratio 32.6% (49:150) Which indicate as acute phase. IgG antibody recorded increasing with ratio 2.7% (4:150) this indicate as chronic phase. Both types of Ab (IgM and IgG) increasing in ratio 12% (18:150). the accuracy of widal test showed that the Positive and negative predictive value were 49.45%, 87.17% respectively, While the percentage of sensitivity and specificity were 92.95% and 43.03% respectively. We conclude from this study that widal test have high sensitivity for diagnosis typhoid fever but its haven't good Specificity and it must be replace with more accurate testing such as ELISA .

Keyword: Typhoid fever, Widal test, ELISA

Introduction
Typhoid fever is a life-threatening disease, It is a major health problem and is endemic in developing countries due to pollution of drinking water, poor health awareness, increased illegal immigration, and antibiotic resistance to antibiotics **[1,2]**. The confirmation of the diagnosis of typhoid fever is not based solely on clinical symptoms, which are not specialized and similar to other pathological symptoms such as malaria, dengue fever, rickettsiosis, etc. **[3,4]** .Some studies have focused to the importance of using more than one method to confirm typhoid fever, and that the most important of these methods are blood culture and DNA detection tests, which are sensitive and high Specificity **[5]**. The ELISA technique which detects the immune response of the body, is diagnosed infection by detecting the presence of antibodies in the body in the acute and chronic phases **[6]**. Another method that used to diagnosis typhoid fever was Widal test which is one of the oldest and most widely used diagnostic tests for typhoid fever in developing countries, because that it is easy to perform and is inexpensive **[7]** However, one of the disadvantages of this test is apossible to give wrong results in the case of taken antibiotics and vaccines, because the antibodies remain in the body for a long time as well as the infection with members of Enterobacteriaceae family which cross reactions leads to false positive results **[8]** .

Material and methods
A- Samples collection
A total of 170 blood samples were collected, 20 sample were belonged to healthy people who were considered as control and 150 samples of persons suspected infected with typhoid fever. All patients were attended to Azadi Teaching Hospital and Children's Hospital in Kirkuk Governorate. Blood samples were left for 20 minutes to coagulate in the tubes without anticoagulant and then placed in a centrifuge for the purpose of obtaining the serum for the performance of serological examination **[9]** .

B- Widal test
The production kit was used by the British Plasmatic Company and samples were tested according to the company's instructions .

C- *Salmonella typhi* IgM and IgG ELISA
The production kit was used by the American MyBiosource company and samples were tested according to the company's instructions .

D- statistical analysis
The results were statistically analyzed using the SPSS program. The efficiency of the test was measured and reported as reported in **[10]** as following:
-Positive predictive value (PPV) = true positive / (true positive + false positive) x 100
-Negative predictive value (NPV) = (real negative) / (real negative + false negative) x 100
-Sensitivity = (true positive) / (true positive + false positive) x 100
- Specificity = (real negative) / (real negative + false positive) x 100

Results and discussion:
The percentage of infection was 74% (111:150) by widal test depending on tubes titration method of the somatic antigen with or without flagellate antigen, and 30% (6:20) of control group was positive (Table 1). This result of present was higher than that recorded by Rada (2013) **[5]** and Abass (2012) infection ratio was 53.7% and 46.1% **[6]** respectively, but less than study of Al-Asadi (2011) which recorded 94.37% **[11].** This may due the location, time of collection samples and number of samples.

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Table (1): Result of widal test depending on somatic and flagellate antigens

Samples	Total number	Positive cases	Percentage
patients	150	111	74%
Control group	20	6	30%
Total number	170	117	68.8%

By using ELISA assay, the incidence of typhoid fever was different (Table 2). The results showed that the acute incidence of typhoid fever based on IgM antibody, it was 32.6% (49:150)), while chronic incidence of IgG antibody was 2.7% (4:150), Both types of antibodies was 12% (18:150) in patients, and no positive case were detected with high antibodies in control group.

Table (2): ELISA test result among febrile patients

Samples	Total number	IgM (%)	IgG (%)	IgM / IgG (%)	Total positive
Patient	150	49 (32.6%)	4 (2.7%)	18 (12%)	71 (47.3%)
Control group	20	0	0	0	0
Total number	170	49(32.6%)	4 (2.7%)	18 (12%)	71 (47.3%)

These results not compatible with many studies, Nirmala and Asha (2015) which recorded 71.9%, 11.57%, 16.53% in acute infection, chronic infection and co-infection respectively**[12]**. Kumar and his colleagues indicated 104 and 116 infections per 197 suspected cases of acute infection, chronic infection and co-infection, respectively **[13]**. This may suggest that the differences may return to difference in the area and the season of study, stage of disease, the cultural and health level, and the number of samples in each study .

In present study, ELISA test was used to evaluate widal test. The sensitivity and specificity of the test was 92.95% while the specificity of the test was 43.03%. The positive and negative predictive value was 49.45% and 87%, respectively (Table 3). The sensitivity was nearly to study **[13]** which recorded 98% of sensitivity, while the sensitivity ratio recorded in this study differed from study **[13,16]** which recorded 84% and 85.71% respectively. on the other hand the Specificity in our study was agreed with **[13]** which record 43.7%. While the percentage of Specificity recorded in this study decreased from many studies which recorded Higher percentage of specificity of widal test **[15,17,13]** .

The value of positive and negative predictive were 49.45% and 87.17%, respectively. These values in the current study are agreed with previous study **[17]** that achieved 46.6% and 88.5% for the value of positive and negative prediction, respectively. In previous studies **[13]** and **[14]** showed it showed high prediction values of positive and negative.

Table (3): Comparison of Widal test with ELISA

Test		ELISA		
		Positive	Negative	Total
Widal test	Positive	66	45	111
	Negative	5	34	39
Total		71	79	150

*PPV= 49.45%, NPV= 87.17%, Sensitivity = 92.95%, Specificity = 43.03%

These differences may return to the company equipped with the equipment used. In addition, some studies were adopted on blood culture and PCR to evaluate widal test. The Widal test showed was little sensitivity being required (12-6) days to detect the presence of antibodies after infection **[18]** this is due to delayed immune response to the body **[19]**. The negative results of widal test may be due to early collection of blood during the disease or the development of microbes insufficient to produce antibodies **[20]** or because of taking antibiotics. On the other hand, the presence of agglutination of the test without the diagnosis of malaria with negative blood culture and with the absence of pre-immunization of the disease indicates the presence of an infection involved with the antigen of *Salmonella typhi* **[21]**. The involvement of somatic and flagellate antigens with the other serotype of Salmonella and other members of Enterobacteriaceae made widal test less effective, therefore it is doubtful to diagnose typhoid fever **[22]**. In many countries, including Iraq, the slide method is used to determine whether the result is positive or negative of typhoid fever, this method may be positive only for 1:20 or 1:40 when using tube titration method, thus this type of method is misleading and give to a wrong diagnosis of the disease **[23]**. In contrast to the ELISA test, which is highly sensitive when compared with other immunological tests, it also has the flexibility of its method of implementation and performance **[24]**, and a security test does not require the use of radioactive materials, in addition the presence of antigens can be detected, even if there are few concentrations in the patient's sample, as it can diagnose the infection during the first ten days of infection as a cute phase **[6]** .

We conclude from this study that widal test have high sensitivity for diagnosis typhoid fever but its haven't good specificity, which may indicates negative false results when the disease is diagnosed.

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تقييم اختبار ويدال لتشخيص حمى التيفوئيد للمرضى الوافدين الى مستشفيات كركوك

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الملخص

هدفت الدراسة لمعرفة دقة تشخيص اختبار الويدال بالمقارنة مع اختبار الاليزا كطريقة تشخيصية قياسية، ولهذا الغرض تم اخذ 150 عينة دم من الاشخاص الوافدين والمشكوك بإصابتهم بحمى التيفوئيد الى مستشفى ازادي التعليمي ومستشفى الاطفال من1-9-2016 الى 1-11-2016 واخذت 20 عينة دم من الاشخاص الاصحاء كمجموعة سيطرة. وتم تشخيص العينات باختبار الويدال بطريقة المعايرة في الانابيب واختبار الاليزا للضد المناعي IgM و IgG. اظهرت الدراسة ان نسبة الاصابة بحمى التيفوئيد كانت 74% (150:111) عند تشخيصها باختبار ويدال. وعند تشخيصها باختبار الاليزا سجلت اضداد IgM ارتفاع بنسبة بلغت 32.6% (150:49) من العينات والتي تمثل الاصابة في المرحلة الحادة، وسجلت اضداد IgG نسبة 2.7% (150:4) والتي تمثل المرحلة المزمنة للمرض، وسجل كلا النوعين من الاضداد (IgM & IgG) نسبة بلغت 12% (150:18). وعند تقييم اختبار ويدال اعتماداً على نتائج اختبار الاليزا بلغت قيمة التنبؤ الايجابي والسلبي 49.45% و87.17% على التوالي، في حين بلغت قيمة الحساسية والنوعية له 92.95% و43.03% على التوالي. لذا استنتج من هذه الدراسة ان اختبار ويدال له حساسية عالية لكن لايمتلك نوعية جيدة لتشخيص الاصابة بحمى التيفوئيد ويجب تأكيد نتائج اختبار الويدال بأختبار اكثر دقة كاختبار الاليزا .

الكلمات المفتاحية: حمى التيفوئيد، اختبار الويدال، الاليزا