Evaluation of Rapid Test in Comparison With Widal Test and Polymerase Chain Reaction For Diagnosis of Typhoid Fever

Thesis

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By

Ahmed Samir Abd el Hafez

M.B.B.Ch.

Faculty of Medicine - Alexandria University

Supervised by

Professor/ Mohamed Khairy Mostafa Elnagar

Professor of Tropical Medicine
Faculty of Medicine - Ain Shams University

Doctor/ Runia Fouad El-Folly
Lecturer of Tropical Medicine
Faculty of Medicine - Ain Shams University

Doctor/ Marwa Saad Mohamed Fathy
Lecturer of Medical Microbiology & Immunology
Faculty of Medicine - Ain Shams University

Faculty of Medicine

Ain Shams University

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Introduction:

Typhoid fever, also known as enteric fever, is a potentially fatal multisystemic illness caused primarily by Salmonella typhi. The variety of manifestations of typhoid fever make this disease a true diagnostic challenge ***(Wicks et al., 1999)***. S. typhi has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation and overcrowding ***(Giannella, 1996).***

Salmonella is a Small Gram-negative bacillus, Usually motile (H antigen), possess polysaccharide capsule (K antigen on most Salmonella spp. or Vi antigen of Salmonella typhi and Salmonella paratyphi), has a specific O antigens, S. typhi is a non-gas producer and produces minimal H_2S, salmonella spp. are relatively resistant to bile acids and this trait is utilized for selective isolation media ***(Grimont and Weil, 2007).***

The genus Salmonella contains over 2,000 sero-species and is one of the most important pathogens in the family Enterobacteriaceae, all strains of Salmonella fall within one species, S. enterica, but this nomenclature has not caught on and the genus continues to be recognized by the popular species names, many named on the basis of serotyping and outbreaks:- Salmonella enteritidis (enteritis), Salmonella typhimurium (enteritis), Salmonella cholerasuis (septicemia), Salmonella typhi (enteric fever; asymptomatic

carriage), Salmonella paratyphi (enteric fever; asymptomatic carriage ***(CDC, 2011)***.

Typhoid fever begins 7-14 days after ingestion of S typhi. The fever pattern is stepwise, characterized by a rising temperature over the course of each day that drops by the subsequent morning. The peaks and troughs rise progressively over time.

S. typhi has no nonhuman vectors. It can be transmitted by oral transmission via food or beverages handled by an individual who chronically sheds the bacteria through stool or, less commonly urine. Hand-to-mouth transmission after using a contaminated toilet and neglecting hand hygiene. Oral transmission via sewage-contaminated water or shellfish ***(Singh, 2001).***

The clinical symptoms of ***typhoid fever*** are nonspecific, and several other febrile diseases may be simulated. The incubation period is usually one to two weeks, and the duration of the illness is about four to six weeks. The patient experiences poor appetite, headaches, generalized aches and pains, fever, lethargy, constipation or diarrhea. People with typhoid fever usually have a sustained fever as high as 39 C-40 C. Chest congestion develops in many patients, and abdominal pain and discomfort are common. The fever becomes constant. Improvement occurs in the third and fourth week in those without complications ***(Azad et al., 1997)***. About 10% of patients have recurrent symptoms (relapse) after feeling better for one to two weeks. Relapses are actually more common in individuals treated with antibiotics ***(Joshi, 2001).***

Due to its heterogeneous and poorly specific clinical symptomatology, the diagnosis of ***typhoid fever*** always requires laboratory confirmation, either by isolation of the pathogen or by demonstration of specific antibodies. Culture provides direct evidence of the presence of the pathogen and is ***the gold standard*** but blood-culture sensitivity is often low, ranging from 50 to 90 % depending on the disease stage, *salmonella* species, culture medium, quantity of circulating bacteria and the blood-culture technique employed ***(Greenberg et al., 1996)***.

Hence, serological tests play a major role in cases when the disease cannot be detected by blood culture. Serological tests include widal test, rapid test, complement fixation test, indirect Coombs test, and ELISA. Previous studies found ELISA to be an effective method for diagnosis of typhoid fever. ELISA, However, the interpretation of these tests is often difficult, particularly in patients with chronic typhoid fever, in re-infections and relapses, and in areas of endemicity where a high proportion of the population has antibodies against typhoid fever ***(Schroeder, 1968).***

The PCR assays provide a rapid and highly sensitive method of differentiating the major ***Salmonella*** groups that will be valuable for clinical and forensic applications. Also, it had excellent sensitivity and specificity and was able to detect all of the cases of acute disease**.** PCR is a very useful tool for the rapid diagnosis of acute typhoid fever and a good marker for the post treatment follow-up and the early detection of relapses ***(Haque et al., 1999)***

Febrile syndromes with no apparent focus are a cause of great concern in patients. They, therefore require a fast and precise etiological diagnosis as

typhoid fever is one of the causes of febrile syndromes.

Aim of Work:-

The aim of the present study is:

1- To analyze the diagnostic yield of rapid test in comparison with widal test and polymerase chain reaction for diagnosis of typhoid fever.

2- To evaluate the sensitivity, specificity, accuracy, the cost and the time consuming of rapid test in comparison with widal test and polymerase chain reaction for diagnosis of typhoid fever.

Patients & Methods:

Patients:

The patients enrolled in our study will be selected from Military Fever Hospital. They will be about 30 cases that will be suspected to be typhoid fever. They will be selected according to;

Inclusion criteria:

1. Clinical manifestations:

Symptoms; are similar to those of the flu and include:

- Fever, often rising to (40 C) or more a rising and falling (undulating) fever is one of the hallmarks of the disease.
- Chills, weakness, fatigue, poor appetite, headaches, generalized aches and pains, lethargy, constipation or diarrhea.
- Chest congestion develops in many patients, and abdominal pain and discomfort are common.

Signs

- Rose spots, which are salmon-colored, blanching, truncal, maculopapules usually 1-4 mm wide and fewer than 5 in number.
- Splenomegaly, hepatomegaly, coughing and bronchitic chest pain are sometimes seen.

2. Positive Widal test (titre1≥320)

Exclusion Criteria:

Negative Widal test (titre1<320)

Methods:

The following will be done for all patients:

A- Clinical study including:

1- Full medical history:-

- Exposure to potentially contaminated foodstuffs or travel to an area where the disease is endemic.
- Somatic complaints (weakness, fatigue, malaise, body aches, constipation, abdominal pain and anorexia) may often predominate.

2- Through clinical examination:-

- Compatible signs and symptoms included hepatosplenomegaly, lymph gland enlargement and localization typical of this infection, white coated tongue, skin rash or bronchitic chest.

B- Complete blood picture (with differential count).

- Leukopenia in acute typhoid fever.
- Anemia.
- Thrombocytopenia.

C- Serological tests;

1) Widal test.

2) Rapid test: lateral chromatographic immunoassay ***(Hatta et al., 2002)***

D- PCR: multiplex PCR using multiple primers for different species of salmonella ***(Kumar et al., 2002)***. It will be presenting our reference point.

E-Abdominal ultrasonography:

For detection of hepatomegaly and or splenomegaly.

Ethical Considerations:

An informed consent will be obtained from each of the participants or one of the responsible relatives before recruitment in the study.

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List of Abbreviations

ALT.....alanine aminotransferase

Anti-LPS HA test..Anti LipopolysacharideHaemAgglutenation tests

APTT .....Activated Partial Thromboplastin Time

AST.....Aspartate aminotransferase

Bp.....Base pair

CBC .....Complete blood count

CDC.....Centers for Disease Control and Prevention

CIE .....Countercurrent Immunoelectophoresis

DA .....Diagnostic accuracy

DNA .....Deoxyribonucleic acid

EDTA.....Ethylenediaminetetraacetic acid

EIAtest .....Enzyme Immunoassay Test

ELISA .....Enzyme-linked immunosorbent assay

ESR .....Erythrocyte sedimentation rate

FAE .....Follicleassociated epithelium

FDPS .....Fibrin degradation product

Fg .....Famtogram

fliC-d .....Flagellingene of salmonella

GALT .....Gut-associated lymphoid tissues

GMF.....Graphical modeling framework

HA TESTS .....HaemAgglutenation tests

HB .....Haemoglobin

HIV.....Human immunodeficiency virus

List of Abbreviations(Cont.)

HLA-B27 Ag.....Human leukocyte antigen B27

ICT .....ImmunochromatographicTEST

IEC .....International Electrotechnical Commission

IgG.....immunoglobulin G

IQR.....Inter Quaratile Range

IgM .....Immunoglobulin M

INTS .....Invasive non- typhoid salmonella

IQR.....interquartile range

LPS.....Lipopolysacharide

M-cell .....Microfold cell

MDR..... Multi Drug Resistant

MHz.....megahertz

MIC ..... Minimum Inhibitory Concentration

MNC.....Mononuclear Cells

NARST.....Nalidixic-Acid-Resistant S. typhi

NPV .....Negative Predictive Value

NTS.....Non- Typhoid Salmonella

OMP.....Outer Membrane Protein

PCR .....Polymerase Chain Reaction

PLT.....Platelet

PPV.....Positive Predictive value

PT.....Prothrombin time

PV.....Portal vien

PVD .....portal vein diameter

R.....Reverse primers

RNA .....ribonucleic acid

List of Abbreviations(Cont.)

RPHA.....Reverse Passive Haemagglutination Test

rpm.....Revolutions per minute

SIADH.....Syndrome of Inappropriate Release of Antidiuretic Hormone

SPI-1.....Salmonella Pathogenecity Island 1

SPS.....Sodium PolyanetholSulfonate

SPSS.....Statistical Package for Social Sciences

SPV.....Salmonella Plasmids Virulence

SS agar.....Salmonella-ShigellaAgar

SSA, 320A.....Toshiba JustVision SSA-320A ultrasound machine

T.bilirubin.....Total bilirubin

TNF-U.....Tumer necrosis factor-u

TPTest.....typhoid/paratyphoid fever diagnostic test

U.V-Vis.....ultraviolet-visible spectrophotometry

US.....ultrasonography

UVp.....under votage protection

VICPS.....capsular polysaccharide vi antigens

WBC.....White blood cell

WHO.....World Health Organization

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