Assessment of the Diagnostic Value of Antigen Detection in the Sera of Patients with Enteric Fever

Thesis

Submitted for the partial Fulfilment of the Master Degree in Microbiology and Immunology

by

Samira kamel Estafanos

(M.B., B.Ch.)

Ain Shams University

Supervised by

Assist.Prof. **Mohammed Salah M. Ibrahim**

Assist Prof. of Microbiology and Immunology

Prof. Dr. **Mohammed Fathy Abdel Wahab**

Professor of Tropical Medicine

Dr. **Taghreed El-Khashab**

Lecturer of Microbiology and Immunology

Faculty of Medicine

Ain Shams University

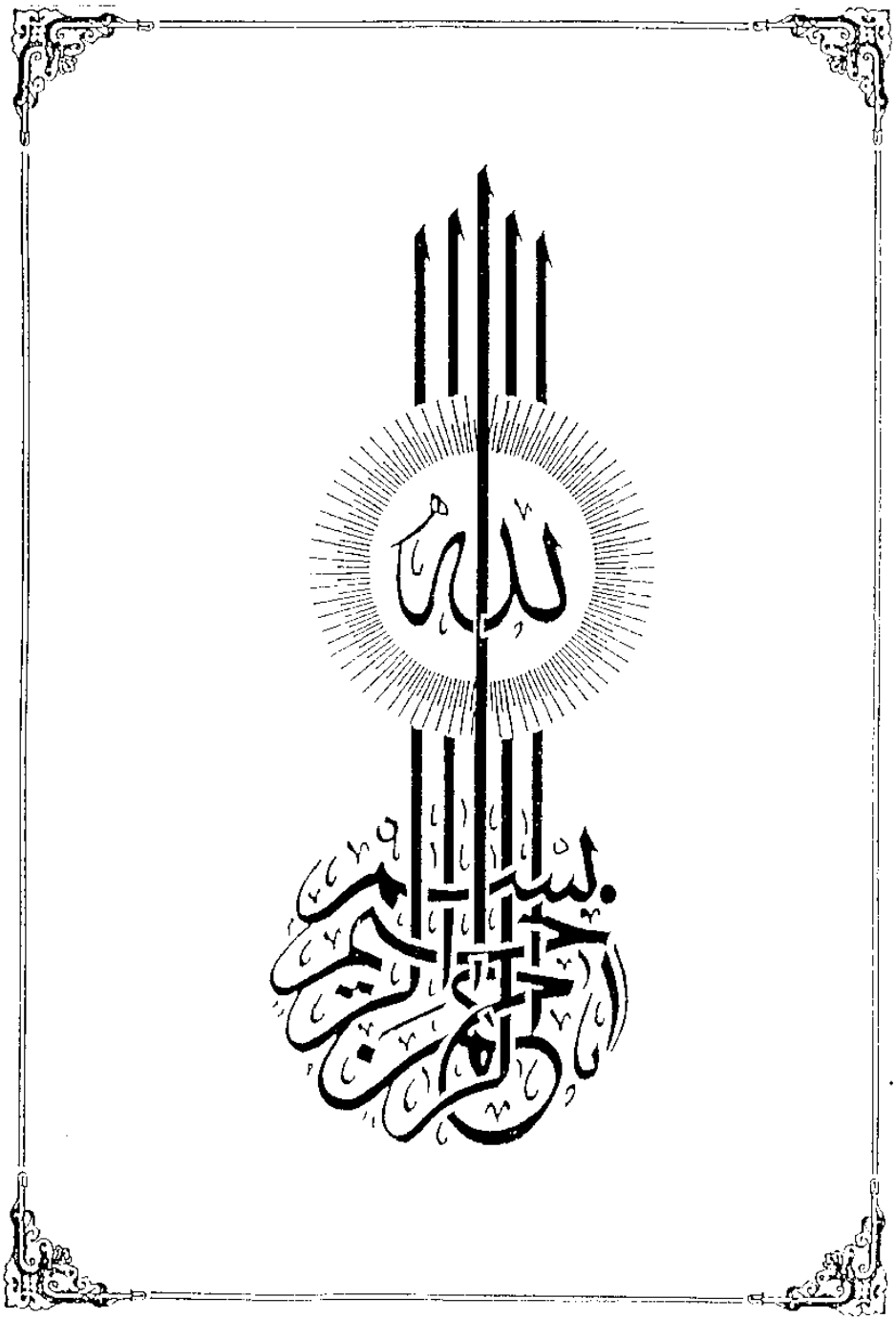
1993

Acknowledgment

I wish to express my deep respect and gratitude to Prof. Dr. Mohamed Salah, Assist Prof. of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, who kindly suggested and supervised this thesis.

Also my sincere thanks and appreciation to Prof. Dr. Fathy Abdel Wahab, Prof. of Tropical Medicine, Faculty of medicine, Ain Shams University, for his unfailing support and continuous encouragement.

Special thanks to Dr. Taghreed El Khashab, Lecturer of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for her kind supervision, guidance and valuable advice that made this work come to light.



ABBREVIATION LIST

AH agglutinins	: Salmonella paratyphi A.agglutinins.
BH agglutinins	: Salmonella paratyphi B agglutinins.
CH agglutinins	: Salmonella paratyphi C agglutinins.
CIE	: Counter immunoelectrophoresis.
CMIR	: Cell mediated immune response.
CSF	: Cerebrospinal fluid.
ELISA	: Enzyme linked immunosorbent assay.
FIA	: Fluorescence immunoassay.
LAT	: Latex agglutination test.
LPS	: Lipopolysaccharide.
PBA	: Passive bacterial agglutination.
PBS	: Phosphate buffered saline.
RIA	: Radioimmunoassay.
SCT	: Staphylococcal coagglutination test.
Table I	: Table introduction.
Table R	: Table result.
TH agglutinin	: Salmonella typhi H agglutinin.
TH antigen	: Salmonella typhi H antigen.
TO agglutinin	: Salmonella typhi O agglutinin.
TO antigen	: Salmonella typhi O antigen.

CONTENTS

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Enteric fevers are endemosporadic in Egypt. The reported number of enteric fever cases are below the real one as many cases are treated in the private practice without notification *(Abdel Wahab et al; 1982)*.

The traditional methods for laboratory diagnosis of typhoid fever are Widal test and bacterial culture.

Widal reaction is well known to be most misleading evidenced by large number of its positivity in other disease in the same extent as its negativity in typhoid cases *(Abu Ghareeb 1968)*.

Also, a positive reaction is first detected about the seventh to tenth day of the illness in enteric fever, so a negative result at an early stage is inconclusive *(Pang and Puthucheary, 1983)*.

Bacterial culture may give false negative results and is time consuming because of the need for repeated subculture which may take about (3-4) days. Also, it is expensive and needs withdrawal of antibiotics.

Recently, another methods were introduced for rapid and early diagnosis of enteric fever depending on antigen detection in the body fluids. These include latex agglutination, Staphylococcal

coagglutination, readioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA) ...etc.

Aim of the work

The aim of this work is to evaluate the diagnostic values of antigen detection in sera of patients with enteric fever by latex agglutination and staphylococcal coagglutination in comparison with the classical cultural and widal tests.

REVIEW OF LITERATURE

Enteric Fever

Enteric fevers are at the upper end of the virulence spectrum of many diseases caused by Salmonella. The enteric fever include two main disease: typhoid fever most commonly caused by S.typhi although other strains have been occasionally implicated such as S.typhimurium and S.heidelberg. The other disease is paratyphoid fever caused by S.paratyphi A, B, and C.

Salmonellae

Morphology and Staining Properties:

Salmonellae are gram negative bacilli that belong to the family Enterobacteriaceae. Most serotypes are motile by peritrichous flagella, but S.gallinarum and S.pullorum are non motile.

Salmonellae are non sporing, non capsulated, non acid fast, aerogenic, non-lactose fermenting, urease negative, citrate utilizing, voges-proskauer negative and KCN negative *(Dugid et al; 1984).*

Cultural Character :

Salmonellae are aerobic and facultatively anaerobic, grow on simple laboratory media in temperature ranging from (15°C - 41°C), optimally at (37°C). Many strains are prototrophic, i.e, capable of growing on a glucose-ammonium minimal medium such as that of Davis and Minigioli's, but some strains are auxotrophic and require enrichment of the medium with one or more amino acids or vitamins, e.g. most S.typhi require tryptophan *(Stockes and Bayne, 1958).*

Antigenic Character of Salmonella

The genus Salmonella is subdivided in Kauffman and White classification into more than 1000 serotypes containing different combinations of antigens *(Cruickshank et al, 1982).*

The antigenic diversity among numerous Salmonella species resides in the structural differences of the lipopolysaccharide (LPS) component of the outer membrane. These molecules are the main target for anti-Salmonella antibodies. The polysaccharide moiety contains the antigenic determinants whereas the lipid moiety is responsible for endotoxin effects.

The structure of LPS is divided into three regions:

Region (I):

Contains the antigenic O-specific polysaccharide units which vary widely among different strains.

Region (II):

Contains an oligosaccharide "common-core" shared among many different strains. The failure to synthesize region (I) results is R (rough) mutants which have a rough colony morphology and lack the O-antigens *(Paul, 1984)*. Antibodies against this R-core have been found by some investigators to protect against infections caused by a wide variety of gram negative bacteria *(Nys et al., 1990)*.

Region (III):

Is the lipid part called lipid A, which is shared among all Salmonellae and serves to anchore LPS on the outer membrane *(Paul, 1984)*.

According to Kauffman and White, the Salmonellae are grouped into major groups based on common somatic (O) antigens, sharing a common antigenic determinant and flagellar (H) antigens. The O group first defined were designated by capital letters (A to Z) and those

discovered later by the numbers (51 to 64) of the characteristic O-antigen. Over 60 different O-antigens have been recognized.

O-antigens are heat stable, being unaffected by heat at (100°C) for 2.5 hours, alcohol stable and unaffected by suspending the bacteria in 0.2% formaldehyde.

O-antigens are liable to be changed in character by the process of form variation and lysogenic conversion, and to be lost from the bacteria in "S-R"mutation *(Cruickshank et al., 1982).*

The flagellar (H) antigens are the proteins that make up the peritrichous flagella of these bacteria. In contrast to the O-antigens, the H-antigens are heat-labile, alcohol-labile and well preserved in 0.04% - 0.2% formaldehyde.

The H-antigens of Salmonella are diphasic; i.e they can exist in either of two phases, phase I or (specific phase), and phase II or (non specific phase). The phase I antigens are shared by only a few organisms and react only with homologous antisera, whereas, the phase II antigens are shared by many organisms and will cross-react with heterologous antisera *(Joklik et al., 1984).*

A third antigen (V_i antigen) is found in a few strains occurring as a heat-labile polysaccharide surface antigens, and is functionally analogous to K-antigen in other genera. Because this antigen is thought to be associated with virulence, it is presumably superficial to the O-antigens. V_i antigen is destoroyed by heat. It is present in practically all strains of S.typhi on primary isolation, but in only few strains of S.paratyphi *(Burrow,1985).*

Table (I_1): Antigens of some representatives of the genus Salmonella (Kauffman-White classification)

O-group	Serotype (Species)	O-antigens (and V_1)	H. antigens	
			Phase 1	Phase 2
A	S.paratyphi A	1, 2, 12	a	-
	S.paratyphi A var. durazzo	2, 12	a	-
B	S.paratyphi B	1, 4, 5, 12	b	1, 2
	S.paratyphi B var. odense	1, 4, 12	b	1, 2
	S.java	1, 4, 5, 12	b	(1, 2)
	S.limete	1, 4, 12, 27	b	1, 5
	S.typhimurium	1, 4, 5, 12	i	1, 2
	S.typhimurium var. copenhagen	1, 4, 12	i	1, 2
	S.agama	4, 12	1	1, 6
	S.abortus-aqui	4, 12	-	e, n, x
	S.abortus-ovis	4, 12	e	1, 6
	S.agona	4, 12	f, g, s	-
	S.brandenburg	4, 12	1, v	e, n, z15
	S.bredeney	1, 4, 12, 27	1, v	1, 7
	S.derby	1, 4, 5, 12	f, g	-
	S.heidelberg	1, 4, 5, 12	r	1, 2
	S.saint-pual	1, 4, 5, 12	e, h	1, 2
	S.salinatis	4, 12	d, e, h	d, e, n, z15
	S.stanley	4, 5, 12	d	1, 2
C1	S.paratyphi C	6, 7, v1	c	1, 5
	S.cholerae-suis	6, 7	c	1, 5
	S.cholerae-suis var. kunzendorf	6, 7	(c)	1, 5
	S.decatur	6, 7	c	1, 5
	S.typhi-suis	6, 7	c	1, 5
	S.bareilly	6, 7	y	1, 5
	S.infatis	6, 7	r	1, 5
	S.menstom	6, 7	g, s, t	-
	S.montevideo	6, 7	g, m, s	-
	S.oranienburg	6, 7	m, t	-
	S.thompson	6, 7	k	1, 5
C2	S.bovis-morbificans	6, 8	r	1, 5
	S.newport	6, 8	a, h	1, 2

- *Numbers in blood type indicated the antigens characterizing the O-group.*
- *Numbers in brackets are antigens that are rarely expressed.*
- *After Cruickshank, et al., 1982.*