Ministry of higher education
University of Babylon
College of science

Some Aspects of Specific Immunity For *Salmonella typhi* In Typhoid Patients and Rabbits

A Thesis
Submitted to the Council of the College of
Science , Babylon University
In Partial Fulfillment of the Requirements for the Degree of
Doctorate of Philosophy in Biology /Microbiology

By
Mahdi Hussain Mheel Al-Ammar
College of Science /University of Kufa ٢٠٠٠

١٤٢٦ ٢٠٠٦

DEDICATION

Dedicated to – Al-Imam – AL- Hessian*

****

My Father & Mother..

*****

My Brothers..

*****

My Wife ……. My Sons..

and

My Friends..

Mahdi Al -Ammar

٢٠٠٦

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية العلوم

بعض أوجه المناعة المتخصصة لـ *Salmonella typhi* في مرضى الحمى المعوية وفي حيوانات المختبر

أطروحة مقدمة إلى مجلس كلية العلوم /جامعة بابل
وهي جزء من متطلبات نيل درجة الدكتوراه /فلسفة
في
علوم الحياة/الأحياء المجهرية

من قبل
مهدي حسين محيل العمار
ماجستير علوم الحياة/كلية العلوم/جامعة الكوفة
٢٠٠١

٢٠٠٦ ١٤٢٤

الخلاصة

في الماضي وفي الحاضر القريب اجريت دراسات عديدة على المرضى المصابين بالتايفوئيد وقد تركزت الجهود على المؤشرات المصلية والبكتريولوجية , فمثل هذا النوع من الدراسات انتشر بشكل جزئي لكنها ليست شاملة و لامقارنة.

ولهذا السبب اجري العمل الحالي لعزل وتشخيص بكتريا الـ *Salmonella typhi* مع اجراء اختبارين من هذه العزلات على الارانب والتي تجرع فمويا .وثلاث اختبارات للحالة المناعية لمرضى التايفوئيد, فضلا عن اربعة فحوصات للمناعة المتخصصة في الارانب المحقونة عن طريق الفم .

تم عزل الـ *S.typhi* من ٣٥ مريضا ,وهي ممرضة للارانب عن طريق التجريع الفموي وانتشرت في الامعاء وخارج الامعاء , مع نفوق الحيوانات خلال ثلاثة ايام . تم تحضير ثلاثة انواع من المستضدات والتي هي مستضد الفوعة والبرتوبلازم بالاضافة الى راشح مزروع الخلية الحر مع استخدام مستضدات الـ *S.typhi* الجاهزة . وتستخدم هذه المستضدات لتحديد الحالات المناعية للمرضى .

خضع ٨٠ مريضا لدراسة مناعية شملت المناعة الخلطية(الموضعية والجهازية) والمناعة الخلوية (الموضعية والجهازية) التي شملت معامل تثبيط هجرة خلايا الدم LIF .

جرى من خلال الدراسة قياس المناعة الذاتية الخلطية للمرضى وشملت قياس بروتين المصل الكلي والضد الافرازي للمرضى المصابين بالتايفوئيد حيث كان معدل تركيز البروتين المصلي الكلي (١٣.٠g/L) فيما كان معدل الكلوبيولين المخاطي (٢.٠٧٥ g/L) . كذلك جرت دراسة المناعة الخلطية للمرضى المصابين بالتايفوئيد في كلا الجهازين المخاطي والجهازي ، حيث ارتفع عيار الضد المصلي في حالة STH , STO كان (٦٤٠ - ١٦٠) على التوالي ، وفي الضد الافرازي وصل الى (٦٤ - ١٦) وكان معدل التلازن الدموي المنفعل لنفس المرضى (١٢٨٠ , ١٢٨) على المستوى الجهازي والمخاطي .

درست المناعة الخلوية غير المتخصصة في المرضى المصابين بالتايفوئيد ، وقد شملت الدراسة . تثبيط هجرة الخلايا البلعمية ، حيث كان معدل LIF في الدراسة الجهازية ٤١٪ بينما كان فى دراسة الاستجابة المناعية المخاطية حوالي ٤٠ % .

ايضاً تم تحديد الوقاية المناعية في حيوانات المختبر ، حيث كان معدل الوقاية المناعية المعاملة بالمساعد المناعي IFA ٨٠٪ ، بينما مع مستضد , Vi والبرتوبلازم ١٠٠٪ . كذلك تم تحديد فرط الحساسية المتأخر ، حيث لوحظت الاعراض بعد (٧٢ hr – ٦) ساعة من الحقن بمستخلص البرتوبلازم .

من الدراسة النسيجية ، تم تحديد التغيرات النسيجية الطارئة على انسجة الحيوان المختبري (الكبد والطحال والرئة) مع معاملتها بالعالق البكتيري او المساعد المناعي IFA ومستضدات البكتريا المتمثلة بـ Vi وبروتوبلازم الخلايا .

Abstract

In the past as well as recently works have been in Iraq on the vast majority of carried out typhoid patients which mainly focused on clinical aspects, serology and bacteriology. Such works are scattered, fragmentary as well as rarely comprehensive or comparative.

Thus, the present work has established to perform the following: ١.proper *Salmonella typhi* isolate diagnosis; ٢. checks Koch's postulate on this Isolate in rabbit oral model ; ٣. plot the immune status of typhoid patients as well as ٤. check typhoid specific immune protection in rabbit oral model .

Thus ,one isolate was elected among five proven *S-typhi* isolates that were recovered from ٣٥ patients. It was pathogenic for rabbit by oral rout giving intestinal and extra intestinal spread terminated by death within three days.

From this isolate , three types of antigens were prepared and assuaged . Namely Vi and protoplasmic sonicate antigens as well as cell free culture filtrate antigen besides the ready prepared *S. typhi* agglutinogens. Such antigens were made available for plotting the immune status of the patients .

Eighty patients were subjected to this immunological study, in which circulating and mucosal *S.typhi* agglutinins and haemagglutinins as well as systemic and mucosal leukocyte inhibition factor were determind .

Natural humoral immunity of these patients was assayed for total serum protein and secretory Ig concentration . The total protein concentration of serum was variable with mean value of (١١٣.٠ g/L) , and the mucosal Ig concentration was also variable in most cases , with mean value of (٢.٠٧٥ g/L) .

In patients, *S. typhi* specific agglutinins titers were measured and found to be within the range of ١٦٠-٦٤٠ in peripheral blood and١٦-٦٤ at the mucosal surfaces.

Likewise, *S.typhi* specific haemagglutinins titers were with the range of ١٦٠-٢٦٥٠ sera and ١٦٠-١٢٨ at mucosal surfaces.

To determine the cellular immune response , one parameter was performed , leucocyte inhibitory factor (LIF) . In *S. typhi* the LIF percentage raised up in systemic LIF to (٤١%) ant the mucosal which was (٤٠٪) when CFCF were used as a sensitizer .

S. typhi immune protection was determined in animals ; the protection rate with IFA was ٨٠% , while the immune protection rate with Vi vaccine and protoplasmic sonicate was ١٠٠٪ respectively . The hypersensitivity of *S. typhi* was measured by useing purified protein derivative sonicate; erythema and indurations were noted during ٦-٧٢ hrs.

Examination Committee

We. The examination committee , after reading this thesis and examing the student Mahdi Hussian Meheel , in its contents , have found it meets and standard and requirements as a thisis in fulfilttment for the degree ph. D.M Biology /Microbiology .

Name : Dr. Ibrahim M. S. Shnawa
Supervisior
Title : Professor
Address : Deportment of College of Science

Signature:
Name:
(Member):
Title:Addres

Signature:
Name:
(Member):
Title:Addres

Signature:
Name:
(Member):
Title:Addres

Signature:
Name:
(Member):
Title:Addres

Signature:
Name chairman
(Member):
Title:Address

Approved for the Collage Committee of Graduate Studies.
Signature:
Name:
Title:Address

CERTIFICATION

I certify that this thesis was prepared under my supervision at the Department of Biology, College of Science, Babylon University, in partial fulfilment of the requirements for the Degree of Doctorate of Philosophy of Science in Microbiology and this work has never been published any where.

Signature:

Name: Dr. Ibrahim M.S.Shnawa

Title: Professor

Address:Department of Biology
College of Science
Babylon University

Date:

In the view of the available recommendations, I forward this thesis for debate by the examination committee.

Signature:

Name: Dr. Kharim H. Rashed

Title: Professor

Address: Department of Biology
College of Science
Babylon University

Date:

Examination Committee

We, the examination committee, after reading this thesis and examining the student Mahdi Hussain Mheel Al Ammar, in its content, have found that it meets the standard and requirements as a thesis in fulfillment for the Degree of Ph.D.in Biology/'Microbiology.

Signature :

Name: Dr. Ibrahim M.S.Shnawa
(Supervisor)
Title : Professor
Address:

Signature :
Name:
(Member)
Title :
Address:)
College of Scienc , Univ.Of Babylon

Signature:
(Member)
College of medicine, University of Kufa

Signatue

Name:
Member)
Assistant Professor
Kufa Technical Institute

Signature :

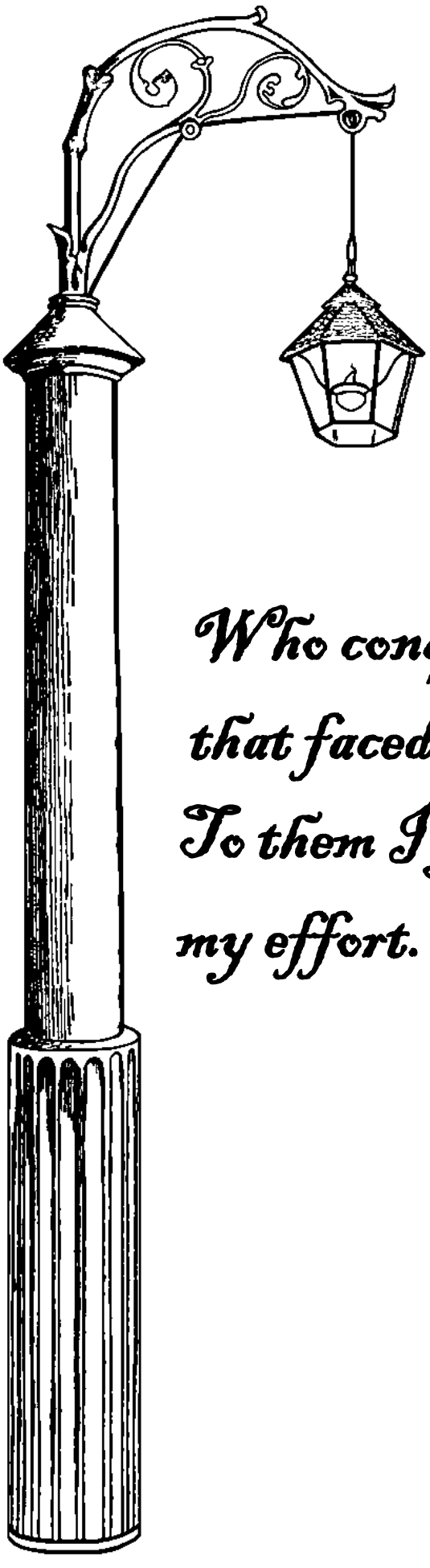
(Member)Asistant Professor
College of Science For Girls, Univ. of Baghdad

Signatue
Name:
C H A I R M A N
Title : Professor
Address: Department of Biology
College of Scienc , Babylon University

Approved for the College Committee of Graduate Studies.

Signature:
Name: Dr. Oda M.Y. Al-Zamely
Title: Assistant Professor
Address: Dean of College of Science
Babylon University

Date:



To all my loved ones,

Who conquer all the hardships
that faced me
To them I present the fruit of
my effort.

ACKNOWLEGMENTS

MOST IMPORTANTLY,I WISH TO THANK GOD FOR GUIDING MY PATH IN LIFE ACCORDING TO HIS WILL

I would like to express my most sincere thanks to my supervisors,Professor Dr.Ibraheem M.S.Shnawa and Professor Dr.,Kareem R.Hameed for their invaluable guidance, encouragement, and criticisms throughout this study.

My thanks also go to Dr.Asad, A.H.Al-Janaby,head of Pathology Dept.in Kufa Medicine College and Manager of Pathology Lab. Of Najaf Teaching Hospital for his assistance to use facilities of this lab.and for his helpful and reading histopathological slides, and also staff of lab for their technical assistance.

My sincere appreciation and respect goes to my brother Dr.Jaafar K.Naama , head of Dept. of Microbiology ,Kufa Medicine College for his help and encouragement .

Special gratitudes also go to Professor Dr. Sabah, N.K.,Deen of Pharmecy College, and Dr.Majid.K.,from Kufa Medicine College for their helpful suggestions and their kind supplement of chemicals, without their help this study would not have been possible.

My great indebtedness is also due to the following persons, who have in one way or another been very helpful during the course of present investigation and in the preparation of the thesis :

Miss M.S.Abdul-Said, Miss I.A.H.Al-Hilaly for their technical assistance.

Special appreciation goes out to my family, for their unending support, love and belief in my abilities.

Ali

٢٠٠٤

List of Contents

List of Tables

List of Figures

List of Abbreviation

No.	Abbr.	Title
١.	*S. typhi*	*Salmonella typhi*
٢.	IFA	Incomplete freund's adjuvant
٣.	Vi-Ag	Virulence antigen
٤.	MHC	Major histocompatibility complex
٥.	٢- Me	٢- Marceptoethanol
٦.	LPS - Ag	Lipopolysaccharide antigen
٧.	PEG	Polyethyleneglycol
٨.	TNF	Leukocyte inhibition factor
٩.	W.H.O	World health organization
١٠.	LIF	Leukocyte inhibition factor
١١.	O.D	Optical density
١٢.	ONPG	O-Nitrophenyl β-galactopyranoside
١٣.	PHA	Passive heamagglutination test
١٤.	STO	O- antigen
١٥.	STH	H- antigen
١٦.	CFCF	Cell free culture filtrate
١٧.	PPD$_{antigen}$	Purified protein derivative of protoplasm sonicate
١٨.	APC	Antigen presenting cell

١٩.	HLA	Human leukocyte antigen
٢٠.	D.T.H	Delayed type hypersensitivity
٢١.	Th-cell	T-helper cells
٢٢.	IL	Interlukin
٢٣.	GALT	Gut associated lymphocyte tissues
٢٤.	MALT	Mucosa associated lymphocyte tissues
٢٥.	N.O	Nitric acid
٢٦.	PIgR	Polymeric Ig receptor
٢٧.	GT	Gut tract
٢٨.	UGT	Urogential tract
٢٩.	OLT	Organized lymphoid tract
٣٠.	BALT	Bile associated lymphocyte tissue
٣١.	LPL	Lamina propria lymphocyte
٣٢.	IEL	Intra epithelial lymphocyte
٣٣.	SC	Secretory component
٣٤.	M	Mutation strain
٣٥.	R	Rough strain
٣٦.	BCR	B-Cell receptor
٣٧.	TCR	T- Cell receptor
٣٨.	STF	*Salmonella typhi* flagella antigen
٣٩.	GIT	Gut intestine tract
٤٠.	ICAM-١	Intestinal cell adhesion molecule -١
٤٢.	VCAM-١	Vascular cell adhesion molecule -١

Acknowledgments

I would like to express my deepest gratitude to my supervison , Professor Dr ibrahim shnawa , for introducing me to the field of immunology of bacterial diseases for his great support and expert guidance at various stages of my study . I greatly admire his creative scientific attitude which helped me throughout this study .

I am especially grateful to Dr Assaid . Al-Janaby , Head of the Department of Pathology, College of Medicine , university of kufa , for his support in the histology part of my study .

I would like to express my thanks to all lab staff of Pediatric Hospital in Babylon for their kind help in specimens collection and diagnosis of samples in the laboratories .

I am greatly indebted to the staff of Central Public Health Laboratory /Baghdad for their diagnosis *S.Typhi* bacteria in their laboratories .

Finally , I express my gratitude to the staff of Department of Biology , College of Science, University of Babylon , Who helped me throughout this study .

١.١ Introduction .

Almost all previous published works have focused mainly on the validity of systemic humoral immune responses in typhoid and para typhoid patients (Widal test) .Cellular immunology research so far is concerned with typhoid patients and appeared to be poor in comparison with bacteriology and serology researches on this topic .

So far published paper concerning the full detailed immune status of typhoid patients are scattered and /or incomplete, thus the present work has undertaken to uncover infection in man and lab animals. Seroprofile ,and immune state of the patient population and an elected group together with trials to evaluate protection immunity in lab animal (Ribbits) using Vi and protoplasmic sonicate antigens are applied.

Typhoid fever is mostly an intracellular infection with *S*. *typhi* . The infection form is intestinal and / or extra intestinal (Mckendrick , ١٩٨٤) following ingestion. *S. typhi* penetrate the gastrointestinal tract and are taken up by reticuloendothelial system (RES) when multiplication occurs, there is bimodal incubation period with peaks at about ٨-١٨ days , the duration reflecting the balance between the infecting dose and the host immune defense mechanisms (Mckendrick , ١٩٨٤) .

Ingestion of the organism may result in several days of symptoms of enterocolitis with diarrhea , although , the symptoms usually disappear before the onset of fever , (Hoffman *et al.,* ١٩٧٥ ; Shnawa and Al-Ameedi , ٢٠٠٤) .

Non- specific symptoms such as chills , diaphoresis , headache , anorexia , cough , weakness , sore throat , dizziness and muscle pains are often present in the first days of illness . (Giannella *et al*., ١٩٧٣; Rubin and Weinstein , ١٩٧٧ ; Tomas , ١٩٩٩) .

The symptoms of fever , abdominal pain and prostration tend to persist through the illness , which are in untreated cases lasts months ; abdominal pain occurs in more than half of the patients and is frequently diffused or located in the right lower guardant over the ileum (Giannella *etal.,*١٩٧٣ ; Hoffman ,*etal*.,١٩٧٥) . These symptoms are listed in (Table ١-١)

Diarrhea occurs in about the third of the patients , and consists of either watery stools or semi-sold stools (Krishna , ١٩٨٦) .

Rose spots (Erythematous vacuoles) appear over the upper abdomen and chest of light –skinned individuals . The lesions are papules about ١-٥ mm in diameter and become hemorrhagic (Mors , *et al.,* ١٩٩٣ ; Krishna , ١٩٨٦) .

In about ٥ % of the patients intestinal bleeding occurs usually after the second week of illness , bleeding occurs from ileum ulcers and may be presented as melanin or bright red blood in stool (Krishna , ١٩٨٦) .

The typhoid bacillus is a motile , gram negative rods belongs to the family enterobacteriacea , it possesses a flagellar H antigen , a cell wall (O) lipopolysaccharide antigen and a polysaccharide virulence Vi an additional surface polysaccharide antigen .

The polysaccharide side chain of the O- antigen confer , serologic specificity to the organism and is essential in virulence (Tomas , ٢٠٠٠) .

Salmonella typhi can contain a wide variety of plasmids that encode virulence factors and other biological functions (Briere , *et al.,* ١٩٦٢ ; Goldstein *et al.,* ١٩٩٣) . As with other gram negative bacilli , the cell cell wall of *Salmonella* containing a complex lipopolysaccharide (LPS) structure that is liberated on the lysis of the cell .

The LPS moiety may function as endotoxin and have important role in determining the virulence of the organisms (Chopra *et al.* , ١٩٨٧) .

The incubation period is usually ١٠-١٤ days , about $١٠^{٧}$ bacilli are required to cause infection depending on inoculums size and immune status of the host (Hornick *et al.* , ١٩٧٠) .

The factors that determine the development of lesions are the virulence of the organism, gastric acidity, and the bacterial flora of the upper small intestine (Waddell and Kunze ,١٩٥٦ ; Black *et al.,* ١٩٦٠).

Although , salmonella survives poorly at pH less than ١.٥ (Gorden and Small, ١٩٩٣) , the normal gastric pH , they survive well at pH ٤.٠ or more and have an adaptive acid tolerance that may promote survival at low pH (Agunod *et al .,*١٩٦٩ ;Foster and Hall , ١٩٩٠) .

The bacilli enter through the intestinal epithelial lining of the jejunum and ileum and reach the sub mucosa where they are phagocytosed by the polymorphs and macrophages (Brandtzaeg, ١٩٨٩)

The organisms survive and multiply within the phagocytes and they reach the mesenteric (lymph nodes) . And enter the blood stream to produce a transient bacteremia (Rubin *et al.* , ١٩٩٠ ; Peterson ,٢٠٠٠) .

The bacilli reach the liver , gall bladder , spleen , bone marrow , lymph nodes , lungs and kidneys , where , further multiplication of the organisms occurs (Hornick *et al*., ١٩٧٠ ; Kohbata *et al.*, ١٩٨٦) .

Hornick *et al.,* (١٩٧٠) found that a dose of $١٠^{٧}$ organisms was needed to induce clinical infection in most adults .

A large number of bacilli are discharged from the gall bladder into the intestine (Bimodal pathogenesis); this time they enter the peyer's patches and lymphoid follicles of the ileum (Hacket *et al. ,* ١٩٨٦ ;Wolfe *et al .,* ١٩٩١)

These lymphoid structures undergo inflammation , necrosis and ulceration and peyer's patches enlargement is seen as a result of recruitment of mononuclear cells and lymphocytes(Hacket *et al.,* ١٩٨٦) .

After several weeks of infection peyer's patches enlarged and necrosis can be marked and are likely to be responsible for the abdominal pain that is characteristic of typhoid fever (Rubin and Weinstein , ١٩٧٧) .

Spleen and other lymphoid tissues show proliferation of large mononuclear cells and formation of ill defined nodular collection of reticuloendothelial elements .The enlargement of liver and spleen is typical of typhoid fever and shows cloud swelling (Rubin and Weinstein , ١٩٧٧) .

The peyer's patches become swollen and infiltrated by a large number of mononuclear cells with ingested *S. typhi* .The surface of the peyer's patches undergoes necrosis and sloughing ; when the slough separates , ulcers are found in the terminal portion of the ileum (Krishna , ١٩٨٦) .

Blood vessels may be eroded and this may result in the intestinal hemorrhage , the ulceration may be extended to the muscular mucosa and serosa and result in intestinal perforation (Krishna , ١٩٨٦) .

The cardiac muscle shows areas of degeneration and focal necrosis ;bronchitis ;and pneumonia may be developed, other tissues may be affected , important lesions are meningitis , osteomyetitis and choleclytitis (Calva and Ruiz , ١٩٨٦) .

From the sub-mucosa of small intestine, the organisms pass via the lymphatic to the mesenteric lymph nodes , and after a while they invade the blood stream via the thoracic duct ; the liver , gall bladder, spleen , kidney , and bone - marrow become infected during this primary bacteremia phase in the first ٤ to ١٠ days of the incubation period (Hornick *et al.* , ١٩٧٠) .

Salmonellosis is an infectious disease of human and animals caused by *Slamonella typhi* , the disease can effect all species of

domestic animals ; young animals pregnant and lactating animals are the most susceptible (Williams *et al*. , ١٩٧٦; Copper , ١٩٩٤) .

Van der zee (٢٠٠٠) found that the enteric disease is the commonest clinical manifestation but a wide range of clinical signs ,which include acute septicemia , abortion , arthritis and respiratory disease may be seen . (WHO , ٢٠٠٤) .

Hitoshi *et al.*,(١٩٩٩) , infected mice with $١٠^{٦}$ bacilli which required to cause infection ; after *S.typhi* was ingested , the part of inoculum that survives stomach acid enters the small intestine ; the incubation period ranges from ٨-٢٨ days depending on host immunity, clinical symptoms appeared that is fever , diarrhea and weakness (Robert *et al.,* ١٩٩٨). *S. typhi* infection may also spread into animal organs like spleen , liver and lungs .

Experiment performed on animal model have shown that orally administered antigens can activate intestinal lymphocytes , which migrate from intestinal inductive sites such as peyer's patches to mesenteric lymph nodes and then return via lymphatic and blood to the intestinal lamina propria and to other mucosal sites such as salivary and memory glands (Mestecky , ١٩٨٧) . This migration behavior has been documented mostly for mucosal B- cells in animals (Vassalli *et al.*, ١٩٦٩ ; Cebra and Craig , ١٩٧١; Giannella,٢٠٠١ ; Shnawa ,٢٠٠١) .

Moreover, it has been shown that antigen specific Th cells arising in peyer's patches after in situ peyer’s patches immunization subsequently , appeared in thoracic duct , intestinal epithelial and lamina propria of the gut (Holmgren *et. al.* ١٩٨٩;W.H.O,٢٠٠٤) .

The definitive method of diagnosis is the isolation of *S. typhi* from a blood culture , which is relatively in most patients , and blood culture become less common or the disease progress and faecal culture in more often at the ٢-nd and ٣rd weeks . Urine and stool cultures are positive less

frequently but should be taken to increase the diagnostic yield(Gilman *et al.* ,١٩٧٥; Khourieh *et al.*, ١٩٨٩).

The bone marrow culture is the most sensitive test , positive in nearly ٩٠٪ of cases can be used when bacteriologic diagnosis is crucially needed or in patients who have been pretreated with antibiotics (Gilman *et al.* ١٩٧٥ ; Hoffman *et al*. ١٩٨٥) . Laboratory identification of the genus *Salmonella* is done by biochemical tests ; the serologic type is confirmed by serologic testing (Rubin and Weinstein ,١٩٩٧ ;Giannella, ٢٠٠٢).

Faeces , blood or other specimens should be plated on several non selective and selective agar media (Blood , MacConky ,D.C.A ,X.L.D, eosin methylen blue , Salmonlla - Shigella and brilliant green agars) as well as into enrichment broth such as selenite or tetrathionate .(Minor and Genus , ١٩٨٤ ; Collee *et al.*, ١٩٩٦).

The biochemical reaction of suspicious colonies is then determined on triple sugar iron agar and lysine iron agar and a presumptive identification is made. Biochemical identification of *Salmonella* has been simplified by systems that permit the rapid testing of ١٠-٢٠ different biochemical parameters stimulateously (Farmer and Kelly , ١٩٩١; Giannella,٢٠٠٢) .

Other laboratory findings are anemia of variable severity and white blood cell count that is normal or decreased with an increased percentage of blood components. (Tomasi , ١٩٨٥ ; Collee *et al.*, ١٩٩٦) .

Widal test is the serological test for typhoid fever and other related enteric fever; antibody may be stimulated by disease .However, antibody to somatic O-Ag is usually higher in the patients with disease and the antibody to H-Ag is usually higher in immunized individuals (Shnawa and Al- Ameedi , ٢٠٠٤).

١.٢ The aims of the study

* Isolation and identification of *S.typhi* from typhoid patients serologically and biochemically together with oral rabbit infection study.

* Detection of the humoral systemic and mucosal immune responses among typhoid patients (Widal reaction)

* Study the passive haemagglutination in patients infected with *S. typhi* .

* Detection the immunoglobulin classes and concentration as well as complement among typhoid patients .

* Detected the leukocyte inhibition factor of typhoid patients at mucosal and systemic level.

* Separation of *S. typhi* antigens (Vi and protoplasmic sonicate) and examining their protection on lab animals and skin test.

* Detecting the cellular and humoral immune response on rabbits in pre and post challenge with live *S.typhi* bacteria.

٢. Literature review

٢.١ Synopsis of the immune system

It is a complex system that protects the body from the disease producing organisms and other foreign bodies . This system includes the humoral immune response and the cell mediated response ; the immune system also protects the body from the invasion by making local barriers on inflammation . The humoral response and the cell mediated responses develop if these first line defenses fail to protect the body ; the humoral immune response is especially effective against bacterial and viral invasions . The main organ of the immune response system is the bone marrow , the thymus and the lymphoid tissues ; such as the lymph node , spleen , and the lymphatic vessels . The response may start as soon as the antigen invades or starts as long as ٤٨ hr later (Robert *et al.* ٢٠٠١) .

The mucosal immune system is composed of the lymphoid tissues that are associated with the mucosal surface of gut tract (G.T); respiratory tract (RT) and urogential tract (U.G.T) . It may be distinguished from the systemic immune system by ;

***** A mucosa related immunoglobulin IgA .

***** T cells with specific mucosal properties .

***** Cells initiating induce mucosal follicle migrate to the diffuse mucosal lymphoid tissues underlying the epithelium (Strober and James , ١٩٩٤) .

The mucosal immune system is a quantitivly important part of the immune system , the human GIT contains as much lymphoid tissues as the spleen . The system can be morphologically and functionally subdivided into organized lymphoid tissues (OLT) consisting of the mucosal follicle (GALT) and (BALT) . Afferent areas where antigen enters the system and induces immune response , and diffuses lymphoid

tissues consisting of cells located in the mucosal lamina propria . Efferent area where antigen interact with differential cells causes the secretion of antibody from B- cells or induce cytotoxic reaction by T- cells (Strober and Jame , ١٩٩٤) .

There are two main parts linked by a mucosal homing mechanism so that sensitized cells from the lymphoid follicles travel to the diffused lymphoid area , where they interact with antigen .

٢.١.١ Organized tissues :

٢.١.١.١ M- cells (CD ١٠٣)

It is a flattened epithelial cells characterized by poorly developed brush border ; a thin glycocalyx is a cytoplasm rich and abundant pinocytosis vesicles , but these lack the proteolytic machinery of epithelial cells (Strober and James , ١٩٩٤).

The M- cells represents the site of the entry of antigen into the intestinal tissues , viral binding to and uptake by membrane cells may be an obligatory means of entry of a positive virulence factor organisms whereas uptake leading to antibody formation and immune elimination of the organism is negative virulence factor .(Strober and James , ١٩٩٤ ; Pang *et al,* (١٩٩٦) showed that the M cells does not have MHC-II so does not have a role in antigen presentation .

٢.١.١.٢ Dome area .

This is the area just below the epithelium of the lymphoid aggregate which is rich in cells bearing class II MHC . (Macrophages , dendritic cells and B cells) . The dome areas contain many T cells mostly bearing CD٤ and a few number CD٤ and CD٨ negative
(Strober and James , ١٩٩٤).

٢.١.١.٣ Lymphoid follicles

Below the dome areas is the follicle zone , which contains the germinal center . B cells are predominant in this region with scattered T cells , unlike other germinal centers (which bears IgD a larger population of B cells (up to ٤٠%) bears surface IgA . The enter follicle areas between and around the follicles are rich in T cells most of which are CD٨ T cells (Strober and James , ١٩٩٤) .

٢.١.٢ Diffused mucosal lymphoid tissues

٢.١.٢.١ Intra epithelial lymphocyte (IEL)

A population of cells lying above the basement membrane among the epithelial cells . There are ٦-٤٠ IEL / ١٠٠ epithelial cells which include CD٣ , CD٢ , CD٨ - T cells (IEL) and have special effector (Strober and James , ١٩٩٤) ..

٢.١.٢.٢ Lamina propria lymphocyte

The lymphocyte population beneath the epithelial layer in the lamina propria (LPL) . It is distinguished from IEL in being equally divided between B and T cells . The B. cells are dominant by IgA - B cells , but IgG , IgM and IgE are also present in a descending order of frequency . The mucosal T cells population composed of both CD٤ and CD٨ cells with the former , being twice as the latter , just as in peripheral blood (Strober and James , ١٩٩٤) .

٢.١.٣ Mucosal S.IgA synthesis and transport

Mucosal surfaces constitute a first line of defense against microbial invasion of the body . A part from the mucus layer, the barrier is maintained by the continuous production and transport of antibodies , formed by plasma cells in lamina propria .

The predominant type is IgA . It is actively transported via epithelial cells into the lumen (Brandtzaeg and Farstad , ١٩٩٩) .

The plasma cells produce IgA (dimeric form) , whereas the epithelial cells produce secretory component (SC) which binds then with IgA to form S. IgA . After the binding of the J. chain Ig polymer to the polymeric immunoglobulin receptor , the complex is transported through the epithelial cells to the luminal side (Mostov and Kaetzel , ١٩٩٩) .

٢.٢ *S. typhi* immunogens

The typhoid bacillus is a motile Gram negative rods, belong to the family Enterobacteriaceae .It possesses a flagellar (H) antigen ,a cell wall O- Lipopolysaccharide(LPS) antigen and polysaccharide virulence (Vi) antigen located in the cell capsule (Kauffman , ١٩٥٠ ; W.H.O , ١٩٨٠)

The somatic antigens represent the side chains of repeating sugar unit projecting outwards from the lipopolysaccharide layer and the surface of the bacterial cell wall; they are hydrophilic and heat stable . It is used for serological diagnosis .

An O- agglutinin titer of ≥١:١٦٠ or a four fold rise supports a diagnosis of typhoid fever , whereas the flagellar (H) antigen represent determinant groups on the flagellar protein ; they are heat labile ; the H agglutinins are more often non specifically elevated by immunization with other bacteria (Kauffman , ١٩٥٠ ; W.H.O , ١٩٨٠) .

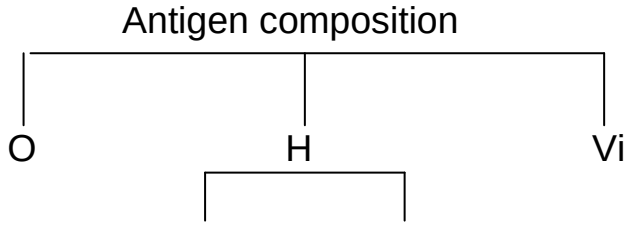
Almost all recently isolated Vi-antigen of *S. typhi* as a covering layer outside the cell wall . This antigen is an acidic polysaccharide ,

which is about ٣ X ١٠٦ Dalton and is a linear homopolymer of N-acetylated groups , which are essential for both antigenicity and immunogenicity of Vi antigen. (Robbins *et al.* , ١٩٩٩) .When fully developed it renders that bacteria agglutinable by antibody and inagglutinable by anti- O antibody. Antigens similar to the Vi-antigen of *S. typhi* have been found in *para typhi C*, and *S. dublin* (Table ٢-١) .

The combination of expressed antigens is used in the kauffman – white classification to define the species. The diversity of both O and H antigens and wide range of combination in which they occur has permitted over ٢٢٠٠ species to be distinguished (Taylor , ١٩٨٣) .

The M antigen is loose extra cellular polysaccharide slime consisting of colonic acid ; it resembles the Vi- antigen in preventing agglutination by O antibody . (Coffman *et al .,*١٩٨٩) .

Table (٢-١) Kauffman- white scheme (Collee *et al.*, ١٩٩٦)



Bacteria	Group		Phase I	Phase II	
١- *S. typhi*	D	٩.١٢	d	-	+
٢-*S. typhi*-A	A	١.٢.١٢	a	١.٥	-
٣- *S. typhi*-B	B	١.٤(٥)١٢	b	١.٢	-
٤- *S. typhi*-C	C_1	٦-٧	c	١.٥	+
٥- *S. typhimurium*	B	١.٤(٥)١٢	i	١.٢	-
٦- *S. cholere-suis*	C_1	٦.٧	c	١.٥	-
٧- *S. dublin*	D	١,٩.١٢	a .p	-	+
٨- *S. enteritidis*	D	١.٩.١٢	g m	١.٧	-

Kauffman and white used cross – adsorption and antisera cross – reaction with different bacterial O- and H antigens to classify a wide range of *Salmonella* (Kauffman , ١٩٥٠ ; W.H.O , ١٩٨٠) .

Other antigens present at the bacterial surfaces which determine agglutination with homologous antibodies , include the fembrial antigen (F) .. In smooth and rough S ⟶R mutation the O- antigen is lost and new R antigens are revealed at the bacterial surfaces (Collee *et al.*, ١٩٩٦) .

Earlier, Pryjma *et al.* , (١٩٩٩) showed that incubation of human peripheral blood mononuclear cells with soluble antigens of *S. typhi*, flagella antigen (STF) , produce a rapid pro inflammatory cytokines tumor necrosis factor alpha (TNFά) and interlukin ١ - IL_1 as well as the

production of IL٦ ,IL١٠ and gamma interferon INF γ (Wyant *et al.*, ١٩٩٦ ;Goldsby *et al* .,.٢٠٠٠)

Bacterial endotoxin is one of the most potent initiators of the inflammatory response , which is a cell wall component of Gram negative bacteria that activates monocyte and macrophages , and it results in the induction of NF- B and initiation of pro inflammatory cascade of cytokines , including TNFά , IL١ and ١L-٦ in macrophages (Bone , ١٩٩١ ; Camenisch *et al.*, ١٩٩٩) .

٢.٣ *S. typhi* processing

Penetration of pathogens into a susceptible host triggers an array of immune responses which involve all the cells of immune system , (T,B-lymphocytes , phagocytes and natural killer cells) . The role of lymphocytes in processing and the entire bacteria have been poorly investigated and bacterial components are able to activate lymphocytes (Kochi *et al.,* ٢٠٠٣).

Monocyte and macrophages and other antigens presenting cells (APC) play crucial roles in the protection of the host from invading pathogen (Germani , ١٩٩٣) .The functions of these cells are to take up , process and present antigen to both naive and memory T- cells (Germani , ١٩٩٣ ; Silva , ١٩٩٦ ; Lanzavecchia , ١٩٩٦; Jonuleit *et al.* , ١٩٩٧) .

The mechanism of antigen uptake by these cells includes phagocytosis , pinocytosis and macropinocytosis (Germani , ١٩٩٣) . These processes allow macrophage to take up particulare antigens , such as bacteria and soluble antigens , including proteins and protein - antibody - complexes (Lanzavecchia , ١٩٩٦) .

Germani (١٩٩٣) found that *S. typhi* antigen are processed into smaller peptides in specialized compartment become loaded with major histocompatability complex MHC II antigen molecules which are transported to the cell membranes where they become available to be presented to T cells (Germani , ١٩٩٣ ; Sliva , ١٩٩٦ ; Lanzavecchia , ١٩٩٦) .

٢.٤ *S. typhi* antigen presentation

The immune system of human and other mammalians evolved mechanisms for sampling the proteins in their environments and cleaving them into small peptides (Ag Processing) ,and then making their peptides accessible to recognition by T. lymphocyte (Ag - presentation) . These processes are early indispensable steps in nearly all acquired immune response to antigenic challenge (Brodisky , ٢٠٠١)

When used in vaccination studies , smooth and rough strains can both induce protective cell- mediated immunity and the influence of LPS of *S.typhi* on antigen presentation . (Muotiala *et al*., ١٩٨٩) .

Timothy *et al*., (٢٠٠٠) demonstrated that the role of monocyte and macrophage is to present antigens to T cells and *S. typhi* flagella (STF) decrease $CD_{١٤}$ expression and are potent inducers of pro inflammatory cytokine protection by human peripheral blood mononuclear cells .

Kauffman (١٩٩٣) demonstrated that the $CD_{٤}$T- cell is only stimulated in the presence of $IL_{١٢}$, which is produced by monocytes, macrophage and dendritic cells in response to *S. typhi*-LPS . $IL_{١٢}$ production is also induced by the activated T cells that interact with APC via $CD_{٤٠}$ ligand($CD_{٤٠}$L) .

The ability of monocyte / macrophage to produce IL -١٢ is regulated by several cytokines with an activating or suppressing effects . The mechanism of *S. typhi* antigen presentation involves distinctive mechanism : First , Major histocompatability complex (MHC) class II molecules present in $CD_{٤}$ T- cells .These antigens must be processed in phagolysosome compartments of the professional cells , antigen presenting cell (APC) .Second MHC class I molecule, expressed on all nucleated cells are able to present *S. typhi* proteins to antigen specific $CD_{٨}$ - T cells . This mechanism allows for the presentation of *S. typhi* antigens by which important antigens may somehow scope the phagosome .

Geppert *et al.,* (١٩٩٠) demonstrated that binding MHC -antigen - peptide complex to T- cell receptors , and the presence of adhesion molecules increased APC T- cell interaction and increased the secondary signals generated by costimulatory molecules e.g. $CD_{٨}$. $CD_{٨٦}CD_{٤}$. and cytokines (Sztein and Mitchell , ١٩٩٧) .

Recently ,Pryjma *et al*., (١٩٩٩) showed a reduction in the expression of surface molecules $CD_{١٤}$ and $CD_{٥٤}$ by human monocyte and macrophage following phagocytosis of many whole –cell bacteria including *S. typhi*.

٢.٥ *S. typhi* antigen recognition

After ingestion , the organisms colonize the ileum and colon and invade the intestinal epithelial and proliferate within the epithelium and lymphoid follicle The mechanism by which *S. typhi* invade the epithelium is partially understood and involved an initial binding to the specific receptors on the epithelial cell surface followed by invasion .

Invasion occurs by *S. typhi* induced the enterocyte membrane to undergo riffling and thereby stimulates pinocytosis of organisms (Giannella , ٢٠٠١) .

S. typhi antigen can be recognized by B cell receptor (BCR) or T cell receptor (TCR) . However ,it will be recognized and bind only a limited protein of such molecule and this binding site is called epitope . The region recognized by immunoglobulin called B. cell epitope is usually different from those recognized by TCR (T cell- epitope) . The concept of T cell epitope is somewhat competed in that T cells recognized only their allied epitopes in association with major histocompatability complex (MHC) molecule on the macrophage cell surfaces (Davies and Cohen, ١٩٩٦) .

B-lymphocytes recognize *S. typhi* antigen by binding it to their surface immunoglobulin or other receptors . Antibodies are able to capture a very broad immunogens (Finlay and Falkow, ١٩٨٨) .

The immune system as a whole is able to recognize many different antigens because it is made up of a vast number of lymphocyte clones . The antigen specificity of *S. typhi* of given clone applies not only to its ability to recognize but also to its effectors .

Formation of cytotoxic effector T cells attack a target cell only if it bears the particle surface antigens recognizing their T-cell receptors . B cell clone has exactly the same binding specificity as the surface immunoglobulin expressed on the clone (Brandtzaeg , ١٩٨٩)

After the invasion of *S. typhi* to the tissues , dendritic cells initiate most immune response , which is presented in most tissues including surface epithelia and lymphocyte cells these cells provide a large surface area for contacting immunogen . They expressed surface receptors for bacterial LPS (Camille *et al.*, ٢٠٠٤) .

Macrophages that can recognize pathogens , need to be activated to–antigen specific phagocyte .T- helper cell function play important roles in generating antigen - specific humoral and cell mediated immunity in both systemic and mucosal compartment .

After ingesting of *S. typhi* , inducing INFγ production in natural killer (NK) cells , which in turn drives the differentiation of Tho cells - Th١ type response are associated with cell mediated immunity , such as delayed – type – hypersensitivity and Ig٢ antibody responses . The Tho cells are differentiated into Th٢ type cells when soluble exogenous antigen is administered , triggering CD٤+ , NK١ , T cell types to produce lL٤ . Thus , either Th٠ , or Th٢ cells or a combination of these cells can support antigen – specific S. IgA – Ab responses .In this respect , Th٢ type cytokines play a role in terminal differentiation of B- cells that are already committed to IgA , (Briere *et al.*, ١٩٩٤) .

While Th١ -type cytokine INF. γ has been implicated in the induction of the polymeric Ig receptor (pIgR) needed for transport of S. IgA . (Wira *et al.*, ١٩٩٤) .

٢.٦ *S. typhi* B - cell activation

Mucosal membranes in the conjunctive upper or lower respiratory , intestinal and genital tracts cover an extensive portion of the body surfaces . The mucosal immune system represents the first line of defense against pathogens (Brandtzaeg , ١٩٨٩) .

The endotoxin (LPS) is a component of Gram negative bacterial cell wall that activate monocytes and macrophages by binding CD١٤ (Cohen and Schifferli , ٢٠٠٠) and it is potent stimulus to B-lymphocyte and macrophage (Frederick *et al.* , ٢٠٠٠; Kochi *et al*.,٢٠٠٤).

Although B- cells play a significant role in immunity to *S. typhi* infection , the antibody produced by plasma cells following salmonella infection has several roles . Secretory IgA and intestinal mucosa may also play role in preventing *S. typhi* penetration to the enterocytes that line the intestinal wall (Michetticle *et al.*, ١٩٩٢) . On crossing the bowel mucosal layer salmonella interact with both enterocyte and M cells .The M-cells are the initial target of salmonella infections .

S. IgA in mucosal secretion can bind antigens, thereby, limiting their absorption and inhibiting bacterial attachment to mucosal surface .The IgA in serum is able to bind and neutralize antigens . (Cassidy *et al.*, ١٩٦٩ ; Frederick *et al.*, ٢٠٠١). Another role of IgA is the induction of the IL-٥ IgA-B cell differentiation of intestine and lL-٤ has been shown to support TGF-B١ (Transforming growth factor) (Whittle and Verma ,١٩٩٧).

In addition , the IgA enhancing cytokines that have shown to induce differentiation of IgA committed B-cell to plasma cell such as IL٢ , lL٦ (Brandtzaeg and Frastad , ١٩٩٩) .

٢.٧ *S. typhi* T. cell activation

Naive CD٤ T cells initially stimulated in the presence of IL-١٢ tend to develop in CD٤ - Th cells producing INF γ (Trinchieri and Scott, ١٩٩٤). These are essential for the host defense against infection by intracellular bacteria such as *S. typhi* and *Mycobacteria* . (Kaufman , ١٩٩٣) .

A virulent strain of *S. typhi* has a great potential not only as a vaccine against virulent *S. typhi* infection , but also carries expression of foreign protein as derived from unrelated pathogen. Moreover ,the *S. typhi* gained increasing interest as recombinant antigen delivery systems against infectious disease that require T. cell response for pathogen elimination , Although , *S. typhi* have a Th , dependent immune response (Trinchieri and Scott , ١٩٩٤ ;Lo *et al* .,١٩٩٩) .

Wei *et al*., (١٩٩٩) , demonstrated that the T cell mediated immunity has proven to be a critical factor in the effective clearance of intracellular bacterial pathogen as *S. typhi* , both $CD_{٤}$- MHC class II restricted and $CD_{٨}$ - MHC class I restricted T. cells play a key role in anti microbial immunity (Kaufman ١٩٩٣ ;Kaufman ,١٩٩٥) .

Studies indicate that $CD_{٤}$ and $CD_{٨}$ T. cells act synergistically to control infection with virulent *S. typhi* . Elimination of *S. typhi* infection depends on the success of the interaction between infected macrophage and T lymphocyte , $CD_{٤+}$ T. cells which exert their protective effect by the production of cytokines primarily INF γ after stimulation with typhoid antigens (Kauffman , ٢٠٠١) .

Nauciel ,(١٩٩٠)showed that the activation of *S. typhi* infected macrophage is mainly mediated by CD٤+ T cells (MHC restricted) which known to contain multiple subpopulation ,of CD٨+ T cells and α,γ T-cells.

Functional diversity of T lymphocytes may also be revelant to T helper (Th) lymphocyte which can be divided into two subset $Th_{١}$ clones which are characterized by the production of INFγ and $Th_{٢}$ clones which are characterized by the production of IL-٤ , IL-٢ produced by activated macrophages and dendritic cells , is th١ principle in inducing cytokine , while IL-٤ promotes induction of $Th_{٢}$ cells (Frederick *et al.* , ٢٠٠١) .

The major role for INF γ in host defence is acting as macrophage activator and may also improve antigen presentation , leading to recruitments of $CD_{٤+}$ T cells and or cytotoxic T cells which may participate in *S.. typhi* killing (Timothy *et al*., ٢٠٠٠) .

In addition to the production of INF γ, and other mediators $CD_{٤+}$ T cells can function as class II MHC restricted cytotoxic cells , destroying monocyte / macrophage infected with *S. typhi* (Robert *et al.,* ٢٠٠١) . The major role of α,γ T cells in human diseases is their ability to act as cytotoxic for macrophages pulsed with *S. typhi* antigens , then induced by *S. typhi* antigens vaccination (Lee *, et al.,* ٢٠٠٤) .

The LPS induced cellular activation mediated through two host proteins , LPS - binding protein (LBP) and leukocyte receptor CD١٤ (Lee *et al*. , ١٩٩٢) . The role of these proteins as pro inflammatory

effecter molecules , LBP and CD١٤ also act in the cellular clearance of endotoxin from the body fluid . The role of LBP is an opsonin for whole gram negative bacteria and CD١٤ is a receptor for both bacteria opsonized with LBP(Grunwald *et al*. , ١٩٩٨ , Schiff *et al*. , ١٩٩٨) .

Binding these particles leads to cellular internalization followed by degradation and inactivation of LPS (Luchi and Manford , ١٩٩٣) .

٢.٨ *S. typhi* Infection and cytokines production

S. typhi can multiply and grow immediately after infection , causing clinical disease known as typhoid fever . After invading the intestine ; most salmonella induce an acute inflammatory response , which can cause ulceration (Giannella , ١٩٧٧) .

Antigen stimulation resulted in the induction of both , $T_{h١}$,$T_{h٢}$ types of cytokines among different $T_{h٢}$-type cytokine. A group of IgA enhancing cytokines that have shown to induce differentiation of IgA committed B cells to plasma cell such as $IL_{-٥}$, $IL_{٦}$ and $IL_{١٠}$ (Cohen and Schifferli , ٢٠٠٠) were preferentially produced by frimbria stimulated $CD_{٤-}$ T cells .

In addition to $T_{h١}$ type cytokines including INF γ and $IL_{٢}$ by the antigens specific $CD_{٤-}$ T cells , INF γ has been shown to enhance the induction of S. IgA which is important (PsIgA) for formation and transport of S. IgA by epithelial cells(Mestecky, ١٩٨٧) while $IL_{٢}$ can synergistically support S. IgA enhancing cytokines e.g. IL-٥ inducing IgA –B cell differentiation (Kauffman *et al.* , ١٩٨٩) .

Kauffman (١٩٩٥) Showed that the interaction of host cells with an invading *S. typhi* or stimulation by bacterial products such as (LPS) of gram negative bacteria . (Kenneth , ٢٠٠٢) demonstrated that the LPS of *S. typhi* released into the blood stream by lysing cell wall is first bond by plasma proteins identified as LPS-binding proteins which interact with $CD_{١٤}$ receptors of monocytes and macrophages and other receptors of

endothelial cells . LPS also acts as B- cell mitogen stimulating the poly - colonal differentiation and multiplication of B- cell and secretion of immunoglobulin IgG and IgM (Kennth , ٢٠٠٢) .

S. typhi LPS activate macrophage and expressed a large variety of cytokines including lL١ , lL٨ , TNF.٢ , TNF α , MCP١ and GM.CSF (Liautard *et al.*, ١٩٩٦ ; Sarmento and Appelberg , ١٩٩٦). These evoke acute inflammatory responses and may also be responsible for damage to the intestine (Giannela , ٢٠٠١). LPS activated macrophages to enhance phagocytosis and cytotoxicity , macrophages stimulated to produce lysosomal enzymes , IL١ and TNF α . (Kenneth , ٢٠٠٢) .

Several transcription factors have been identified as playing a role in regulating the macrophage activation release NF-lL٦ and NF- B, these are expressed at a high level in LPS induced macrophage (Akira *et al* ., ١٩٩٣) . The generation of nitric oxide (NO) and reactive mitogen intermediates is known to play an essential role in bacterial killing , the production of (No) induced by LPS or cytokines , the effect of NF-IL٦ is independent of (N**o**) or may control the expression of factors required in activated macrophage for bactericidal activity(Kamijo *et al.*, ١٩٩٤) .

٢.٩ *S. typhi* immuno protective antigens:

Parental vaccines elicited primarily a humoral antibody immune response (Levine , ١٩٩٩) . Although successful for a variety of systemic pathogens , it has hoped that mucosal vaccines may provide a more physiological and effective immune response , since it is estimated that ٩٠٪ of human infections are initiated at mucosal surface (Kochi *et al.* ٢٠٠٣) . Bacterial vaccine vectors induce the production of multiple cytokines , including (TNF α) , tumor necrosis factor , gamma, interferon INF γ and interlukin . ١٢ (lL-١٢) and pro inflammatory mediators such as nitric oxide , which are early innate immunity (Dietrich *et al.* , ٢٠٠٣) .

Other studies with *serovar typhi* – (Ty ٢١) demonstrated that immunization elicits a specific CD٨+ cytotoxic – T- lymphocyte response , and also elicits both a CD٤+ and CD٨+ cellular immune response . Attenuated serovar *typhi* vaccines delivered intranasally in mice also stimulate CD٨+ major histocompatability complex MHC class I restricted . (Pasetti *et al.*, ٢٠٠٢ ; Salerno *et al.* , ٢٠٠٢) .

The Vi-capsular polysaccharide of *S. typhi* is a licensed vaccine for typhoid fever in individuals ≥ ٥ years old . An increase in specific serum (Abs) is the predominant immune response elicited by the injection of capsular polysaccharides . Vi-vaccine confers significant protection against typhoid fever based on the production of measurable antibodies predominantly of the IgG class (Robbins and Robbins , ١٩٨٤).

The protective efficacy against typhoid fever of single intramuscular injection of ٢٥ Ul of the *S. typhi* Vi-capsular polysaccharide vaccine was assessed in South Africa (Hoffman *et al*.,١٩٨٥ ; Zuzana , *et al.*, ١٩٩٧).

The single dose of Vi capsular polysaccharide vaccine resulted in a ٤ fold increase in antibody titer in between ٨٣٪ and ٩٦٪of patients .

A four- fold rise in antibody-level occurs after one week in ٦٠٪ ,after two weeks in ٨٠٪ , and after one month in ٩٣٪. A second dose of Vi-vaccine at ٢٧-٣٤ months following initial immunization elicited antibody levels similar to those observed following the first dose (Klugman , ١٩٨٧) .

Serum IgG capsular polysaccharide antibodies confer immunity in healthy individuals to respiratory pathogens including pneumococci and *S. typhi* , the causative agent of typhoid fever (Robbins *et al.* , ١٩٩٩) .

Induction of mucosal responses is achieved by the deposition of antigen via the mucosa ; further mucosal (Oral route) has been shown to induce antigen specific immune responses in both mucosal and systemic compartment ; for the development of effective mucosal vaccine , it is

essential to consider the use of adjuvants (CT) cholera toxin which elicits responses by including antigen specific $T_{h٢}$ type . CD٤+ T cells producing lL٤ , lL٥ and lL٦ which are responsible for supporting antigen specific IgA and IgG, antibody production (Manaby *et al.*, ١٩٩٩) .

Robbins *et al.,* (١٩٩٩) shows that the Vi. conjugate , induced booster responses with levels that were higher than those of children ٥ – ١٤ years of age injected with Vi vaccine -alone .

The protection of *S. typhi* Vi. Ag in mice was studied , and after one dose , the Vi-conjugates were more immunogenic than Vi vaccine -alone ; the second dose of Vi- conjugates induced booster responses with a ٥ to ٢٥-fold rise in Vi antibody levels revere the first dose (Zuzana *et al.*, ١٩٩٧) .

Recently, it was demonstrated that *S. typhi* soluble protoplast antigen decrease CD١٤ expression and a potent inducers of pro inflammatory cytokine production , However , it alters the macrophage activated T cells to proliferate to Ags and mitogens (Timothy *et al.* ,٢٠٠٠) .

٢.١٠ *S. typhi* hypersensitivity

An immune response evokes a battery of effecter molecules that acts to remove antigen . Generally , these effecter molecules induce a subclinical , localized inflammatory response that eliminate antigen without damaging the host tissues . Under certain circumstances , however , this response can have deleterious effects , resulting in a significant tissue damage or even death ; this immune response is termed hypersensitivity (Goldsby *et al.*, ٢٠٠٠) .

An individual immune to a *S. typhi* will develop a skin reaction at the site of the local administration of soluble protein antigen (Kauffman , ٢٠٠١)

The DTH to antigens of *S. typhi* is mediated by T. cells primarily of the CD٤ . Allergen penetrates the skin and is taken up by dendritic cells , following capture of allergen . The dendritic cells differentiate into potent APC , and migrate to regional lymph nodes . There they encounter naive CD٤ T- cells triggering the activation of T cells whose antigen receptors are specific for the allergen in the form of processed antigen bound to MHCII and displayed on surface of antigen presenting cell . (Frederick *et al.*, ٢٠٠١) .

As a mechanism of reaction antigen specific CD٤+ T- cells recognize the antigen and secrete cytokines such as INFγ, α which stimulate changes in the local tissue macrophages (Kauffman ,٢٠٠١).

Protoplast antigen stimulates macrophages which produce lL١ and this stimulates the secretion of ١L٢ and expression of lL٢ receptors on T cells in addition to lL١, they are responsible for many of the characteristics of DTH inflammation (Kauffman , ٢٠٠١ ; Frederick *et al.*, ٢٠٠٢) .

٢.١١ .*S Typhi* Vaccines

The use of vaccines against infectious diseases has been one of the true successful stories of modern medicine . This is best exemplified by the fact that there has been a ٩٠ to ١٠٠٪ decline in mortality and morbidity in several childhood infections since the introduction of vaccines and their universal use in children (Robert *et al.* , ٢٠٠١) .

Enteric fever can be prevented by vaccination with several commercially available vaccines , where they require multiple doses to achieve about ٧٠٪ efficacy for only several years (Hornick *et al.* , ١٩٧٠; W.H.O,١٩٨٤) .

Since the nineteenth century the heat - killed whole organisms of *S. typhi* vaccine has been the mainstay of prophylaxis against typhoid fever . Variable efficacy of heat - killed phenol treated *S. typhi* (٥١-٧٠٪ protection compared to tetanus) was demonstrated in several studies (Miller and phop , ١٩٩٦) .

Acetone inactivated vaccine provided greater ٨٠-٩٥٪ protection but is only available to the military in USA . Although the acetone vaccine has been more efficacious in endemic population , both local and systemic adverse reaction occurs frequently with the heat killed – phenol parental vaccine, fever ١٥٪ , headache ١٠ %, local pain ٣٠-٥٠٪ . Reaction occurs within hours after vaccination and can persist for ٧٢ hrs (Miller and phop , ١٩٩٣) .

A Vi - polysaccharide vaccine that is administered parentally has been demonstrated to provide about ٨٠٪ protection in an endemic population (Acharya , ١٩٨٧)and is presently used in France ; side effects are less frequently reported with this vaccine ; its immunogenes in naive population(Taylor ,١٩٨٣; Robbins *et al*.,١٩٨٤ ; Frederick *et al.,* ٢٠٠٥) .

An increase in specific antibody occurs. Antibody is the predominant immune response elicited by the injection of Vi - polysaccharide antigen. . The Vi-vaccine confers significant protection

against typhoid fever based on the production of measurable antibodies , predominantly of the I g A class (Robbins *et al.*, ١٩٨٤ ; W.H.O , ١٩٨٤) .

Both , the sero conversion rate (≥ ٤-fold rise in serum- antibodies) and the protective efficacy induced by Vi vaccine were about ٧٠٪ . The result of the immunogenicity and effectiveness studying provide evidence that serum antibodies to Vi-antigen confer immunity to typhoid fever (Acharya , ١٩٨٧; Robbins *et al*.,١٩٩٩ ; Frederick *et al* ., ٢٠٠٥) .

Robbins *et al.*, (١٩٩٩) found that the Vi conjugates were more immunogen than Vi vaccine alone , which induce booster response with ٥-٢٥ fold rise in antibodies levels .

Although , both serum and intestinal antibody responses have been documented after vaccination (Forrest *et al*., ١٩٩١) ,little is known about the important protective antigen against which an immune response must be generated ; vaccine studies indicated that the Vi-polysaccharide antigen should be an important immune target since parental immunization with this antigen has led to an increased protection (Acharya, ١٩٨٧) . Chronic carriers of *S. typhi* are immune to active infection and have very high antibody titres to salmonella surface proteins including Vi antigen (Lanata *et al*. , ١٩٨٣) . In addition to ,antibodies directed antigens salmonella surface proteins, lymphocytes proliferation assays have documented that cell- mediated immune response occurs after *S.typhi* bacilli infection (Wasserman *et al.,*١٩٨٩).

From the studies in Indonesia ,an adult individual who received a booster dose of Vi- vaccine ٢٧ to ٣٤ months following the initial dose was more likely to develop erythemea and /or indurations , than that given a first dose , but the rate of systemic reaction was increased (Robbins *et al*.,١٩٩٩ ; W.H.O,٢٠٠٤) .

As a diverse reaction from a trial in Indonesia in children of ١ to ٧ years , no severe or unusual side effect was observed

Aserkoff and Bennett , (١٩٦٩) and W.H.O (١٩٨٤) found that the acetone killed and preserved in the dry state , can give a very high degree of immune protection in endemic areas e.g Guyana, Nepal and Poland .Vi- conjugates for typhoid fever , elicit higher levels of antibodies than Vi alone in individuals older than ٥ years of age . (Robbins *et al*., ١٩٩٤). Vi conjugate induced booster response with levels that were higher than those in children of ٥-١٤ years of age injected with Vi - vaccine alone (Zuzana , ١٩٩٧ ; Robbins *et al*.,١٩٩٩) .

Travelers wishing immune protection should receive either live oral vaccine typhoid ٢١ a(Ty ٢١a) given as one capsule every other day for a total of four capsules or typhoid vaccine which is administered as two subcutaneous injection (Levine and Hornick, ١٩٨٧) . In a volunteer study of immunologically naive individuals ٥-٨ doses of $١٠^{١١}$ freshly harvested(Ty ٢١a)provided a high level of protection against *S- typhi* (Hone , ١٩٨٨)

Herman (١٩٩٩) found that the mucosal immunization with soluble protein or peptide immunogen in the absence of mucosal adjuvant may induce a start of antigen- specific immunological tolerance (Mowat , ١٩٩٤) .The mucosal immunization with soluble protein antigens administered with mucosal adjuvant such as a cholera toxin (CT) induce potent systemic and mucosal and cell mediated immune responses (Jakson *et al.* , ١٩٩٣ ; Willson *et al.* , ١٩٩٣) .

٣. Materials and methods

٣.١ Solutions

٣.١.١ Normal Saline (NS)

This solution was prepared at a concentration of ٠.٨٥٪ by dissolving ٠.٨٥ g of sodium chloride (NaCl) , (BDH) in small amount of distilled water , then completed to ١٠٠ ml , autoclaved for ١٥ min at ١٢١C. Normal saline was used in titration of antibodies in systemic and mucosal with causative agent antigens of *S. typhi.* (Garvey *et al.*, ١٩٧٧) .

٣.١.٢ Azide Saline (AS)

Sodium azide at a concentration of ٠.٠٨ % was added to physiological saline . The azide acts as a preservative preventing microbial contamination and used for the dilution of serum (Frei *et al.* , ١٩٩٥)

٣.١.٣ Formal Saline (FS)

The Solution was prepared by adding ٠.٥ ml of formaldehyde (H-CHO) , (BDH) , to ٩٩.٥ml sterile N.S to final concentration of formalin in this solution ٠.٥٪ . The solution was used as a solvent for mucosal I g and in the preparation of Gram negative bacterial antigen (Lehman *et al.* , ١٩٦٨; Pears , ١٩٨٥) .

٣.١.٤ Triss buffer solutions

It was prepared by dissolving (١٢ g) Triss substance $NH_2C(CH_2OH)_3$ in a small amount of D. W. ; then it completed to ١ liter ; the pH of this solution was adjusted to(٧) by using HCl ٠.١ N . The solution used for the preparation of polyethylenglycol PEG (Johnston and Thrope , ١٩٨٢) .

٣.١.٥ Basal Medium Eagle (BEM)

This medium was prepared according to instruction as follows : one gram of Eagle medium dissolved in sterile ١٠٠ ml D.W . This solution was filtered through ٠.٤٥ membrane filter followed by ٠.٢٢ µm sterile millipore filter by syringe filter device . The solution was kept in sterile plastic universal AFMA-Disposable in refrigerators (٤C˚). This BEM was used as cell nutritive solution helpful for measuring migration inhibitory factors of leucocytes in blood and feacal material (Soberg , ١٩٦٨) .

٣.١.٦. Polyethyelenglaycol ٦٪ concentration (BEG)

PEG ٦ g (HO(C_2H_4 O) NH), M.W.(٦٠٠٠) was dissolved in a suitable volume of triss buffer solution , The suspension completed to (١٠٠ ml) . pH adjusted to ٧.٣ , it was used for the separation the secretory Ig (Johnston and Thrope , ١٩٨٢) .

٥.١.٧. Alsever's solution (As)

The solution was prepared by dissolving ٢٤.١٠ g glucose with ٩.١٠ gm trisodium citrate (dehydrated) and ٥.٠٤ gm NaCl in ١٠٠٠ ml D.W . The pH was adjusted to ٦.١ by ١٥٪ citric acid . The solution was membrane filtered through ٠.٢٢ µm millipore filter in syringe type device . It was used as sheep red blood cells preservative (Garvey *et al*.,١٩٧٧)

٣.١.٨ Biuret solution

This solution prepared by dissolving ٣.g of copper sulfate ($CuSo_4$, ٥ H_2O) MW ٢٤٩.٥ in ٥٠٠ ml of D.W then ٩.g of sodium potassium tartarate $NakCu_4H_4$ $C_{6.4}H_2O$ and ٥ g of potassium lodide were added and after complete dissolving ١٠٠ ml of sodium hydroxide ٠.٦ N was added and the volume was completed to final volume ١ L by adding D.W. This solution was used for measuring the total protein , serum and secretory I g (Bishop *et al.* , ١٩٨٥) .

٣.١.٩ Standard albumin solution

The solution was prepared by dissolving ٦٠ g of bovine albumin in a small amount of sodium hydroxide ٠.٦ N and then the volume was completed to liter by using the same solution to final concentration of albumin ٦٠ g/L . A serial double dilutions were prepared as ١:١ , ١:٢ , ١:٤ , ١:٨ , ١:١٦ , ١:٣٢ , ١:٦٤ respectively .

The dilution were made by ٠.٦ N Sodium hydroxide for the preparation of standard curve to determine the total protein and lmmunoglobulin concentrations (Bishop *et al.*, ١٩٨٥) .

٣.١.١٠. ٢-Mercaptoethanal

This solution with a concentration of (٠.٠٥ m) was prepared by adding ٣.٩ml from ٢-me ($H_٢$-$CH_٢$-OH) to normal saline , and then the volume was completed to ١ liter by adding D.W. It was used as a reducing agent for S. Ig and serum Ig (Cruickshank *et al.*, ١٩٧٥) .

٣.١.١١ Tannic acid solution

It was prepared by dissolving (٠.٥ g) of $C_{٧٦}H_{٥٢}O_{٤٦}$) , M.W. (١٧٠١) in a small amount of distilled water , then the volume was completed to ١٠٠ ml . The solution was used for the removal of antigens that are present in sheep red blood cells . (Garvey *et al.* ,١٩٧٧) .

٣.١.١٢ Sugar solution

The solution were prepared at a concentration of ١٪ of (Glucose , Lactose , Xylose and Sucrose) . The solution were membrane filtered through ٠.٢٢m millipore using syringe type device . It was used for detection sugar fermentation and gas production .

٣.٤ : Stains

٣.٤.١ Gram stain

The stain was prepared as in Cruickshank *et al.* , (١٩٧٥) .

٣.٤.٢ Haematoxylin stain and eosin

The stain was prepared according to Fredreick *et al.*, (٢٠٠٢) . It was used for tissue staining .

٣.٥.١ Determination of *S. typhi* infection dose

The bacteria from the culture of the identified species emulsified in saline to a visible density about ١.٥ X ١٠^{٥} cell /ml bacteria , as Judged by comparison with opacity standard (Shnawa and Thwaini,٢٠٠٢).

٣.٥.٢ Lab animal inoculation .

Rabbits of body weight (١٠٠٠-١٥٠٠ g) were orally infected using orogaolne tube (Shnawa and Thwaini , ٢٠٠٢) .

٣.٥.٣. Histopathology

Animal tissues (Liver , Spleen and Lung) were fixed in formalin and embedded in paraffin sections which stained with hematoxylin / eosin .

Light microscopic examination was conducted by a trained pathologist unaware of the experimental condition of the samples (Frederick *et al.*, ٢٠٠٢) .

٣.٦. Ready prepared agglutinogens .

Standard bacterial suspension of known agglutinogens wae used for the two types of antigens involving *Salmonella typhi* O. Ag and *Salmonella typhi* H antigens . (Plasmetic company) .

٣.٧. Preparation of leukocyte sensitizer .

Cell free culture filtrate was prepared by cultivation of the causative bacteria *S. typhi* in brain heart infusion broth for ٢٤ hr; and the liquid media was centrifuged at ٥٠٠٠ Rpm for ١٥ min.. The culture supernatant was membrane filtered through millipore filter in syringe device , the filtrate was collected and distributed in sterile bottles and preserved in refrigerator(٤C) until use as sensitizer (Shnawa and Thwaini , ٢٠٠٢) .

٣.٨. Preparation of *S. typhi* protoplasmic sonicate .

Brain – heart infusion broth was inoculated by *S. typhi* using shaking incubator at ٣٧c^{o}; for ٢٤ hr ; formaline (٠.٥ ml) was added on liquid media . This suspension was centrifuged at ٥٠٠٠ Rpm ١٠ min. .The pellet was washed by ٥ ml of normal saline , and centrifuged at ٣٥٠٠ Rpm for ٥ min. after mixing by vortex , ٥ ml of sterile normal saline was added to the pellet and the suspension was fractioned by sonicator system with oscellation ١٨-٢٠ mm for ١٥ min., in cool conditions . A stepwise examination was done microscopically . However , apart of this suspension centrifuged at ٤٠٠٠ Rpm for ٥ min. , the supernatant was taken and tubed in a tube of a pacimeter W.O.H. International References and the volume ١ml was completed by adding normal saline until the opacity of suspension became equal to opacity of the standard tube ; the concentration of tested suspension was ١٠ lu .

٣.٩. Preparation of *S.typhi* protoplasmic sonicate protein

A volume of ٥ ml of sterile normal saline was added into the protoplasm suspension ; this suspension was centrifuged at ٣٠٠٠ Rpm for ٥ min after mixing by vortex . It was washed twice. Biuret method was used in the measuring the protein in protoplasm according to Bishop *et al.* ,(١٩٨٥).The purified protein derivative was used in skin test of protoplasmic *S. typhi* .

٣.١٠ . Coating-antigen on tanned sheep red blood cells

The antigen coating of tanned erythrocyte was done as in Garvey *et al.*, (١٩٧٧) .

٣.١١ . Vi-Antigen extraction and purification

٣.١١.١ preparation of bacterial dry weight

Brain - heart infusion broth was inoculated by *S. typhi* using shaking incubator overnight incubation to obtain high density growth . The culture was cooled centrifuged at ٥٠٠٠ Rpm for ٢٠ min. , the precipitate which represents the cells of bacteria was dried in an incubator(٣٧ c) for ٤٨ hr .

The dried cells were mixed with ethanol alcohol , and centrifuged at ٥٠٠٠ Rpm for ٢٠min.; the precipitate was mixed with acetone and centrifuged again . The precipitate was washed twice with ether and the precipitate was dried in incubator or oven at ٤٠ C^{o} for ٤٨ hr and this represented the dry weight of bacterial cells.

٣.١١.٢. Preparation of Vi-antigen

The method of Vi-preparation devised by (Websters *et al.*, ١٩٥٢) was followed . Bacteria (١٠ g of dry weight) suspended in ٠.٩٪ of sodium chloride and shake for ٣٠ min .; the suspension were centrifuged at ٣٠٠٠ Rpm for ٣٠ min. ; the supernatant were withdrawn and dialyzed in running tab water for ١٢-١٦ hr . NaCl then was added to dialyzeing ٩٪ concentration and this solution was precipitated with ethanol added slowly to the final ٠.١ , ٠.٢ and ٠.٣ M. Precipitates formed at each of these alcohol concentrations they were collected by centrifugation and dissolved in D.W. Acetic acid were then added to ٠.١m , followed by ethanol at ٠.١ , ٠.٢ and ٠.٣ M . The purified precipitates thus obtained should be redissolved in D.W. and treated with acetic acid added to ١m concentration . The solution were refluxed for ٢٤ hr , then dialyzed and precipitated with ethanol . Precipitates can be stored in refrigerator at ٤C^{o}(Kwapinski , ١٩٧٢) .

٣.١٢ . Disposable equipments

***** Syringes : sterile disposable syringe (٥ml) was used for blood collection and vaccination (BDA company) .

***** Blood collection tube : sterile without anticoagulant tubes were employed for serum collection , and tubes with coagulant (AFMA. Disposable DTA tube) were used for LIF test .

***** Plastic cup : A plastic jar with a screw cap were used for faeces samples collection .

٣.١٣ : Patients and controls

The typhoid patients, and control samples (Blood and faeces) are listed in(Table ٣-٣) .

Table (٣-٣) Samples of *S. typhi* patients and control

Test	No. of patients samples		Control sample	
	Blood clot	Faeces	Blood clot	Faeces
١-Bacteriological* diagnosis	٣٥	٣٥	٢٠	٢٠
٢- Immunology test	٨٠	٨٠	٢٠	٢٠
٣- Ig class &conc.	٢٠	٢٠	٤	٤

***** Samples were collected from Babylon Pediatric Hospital

٣.١٤ . Bacteriology

Laboratory diagnosis of salmonella infection depends mainly on the isolation and identification of salmonella from the specimens of patients blood and faeces (Figure ٣.١.a;٣.١.b).

٣.١٤.١ Blood culture

Samples clot were collected from ٣٥ patients using sterile disposable syring immediately cultured on blood agar , MacConkey and S.S. agar for isolation and biochemical identification of the bacteria (Collee *et al.* ١٩٩٦) .

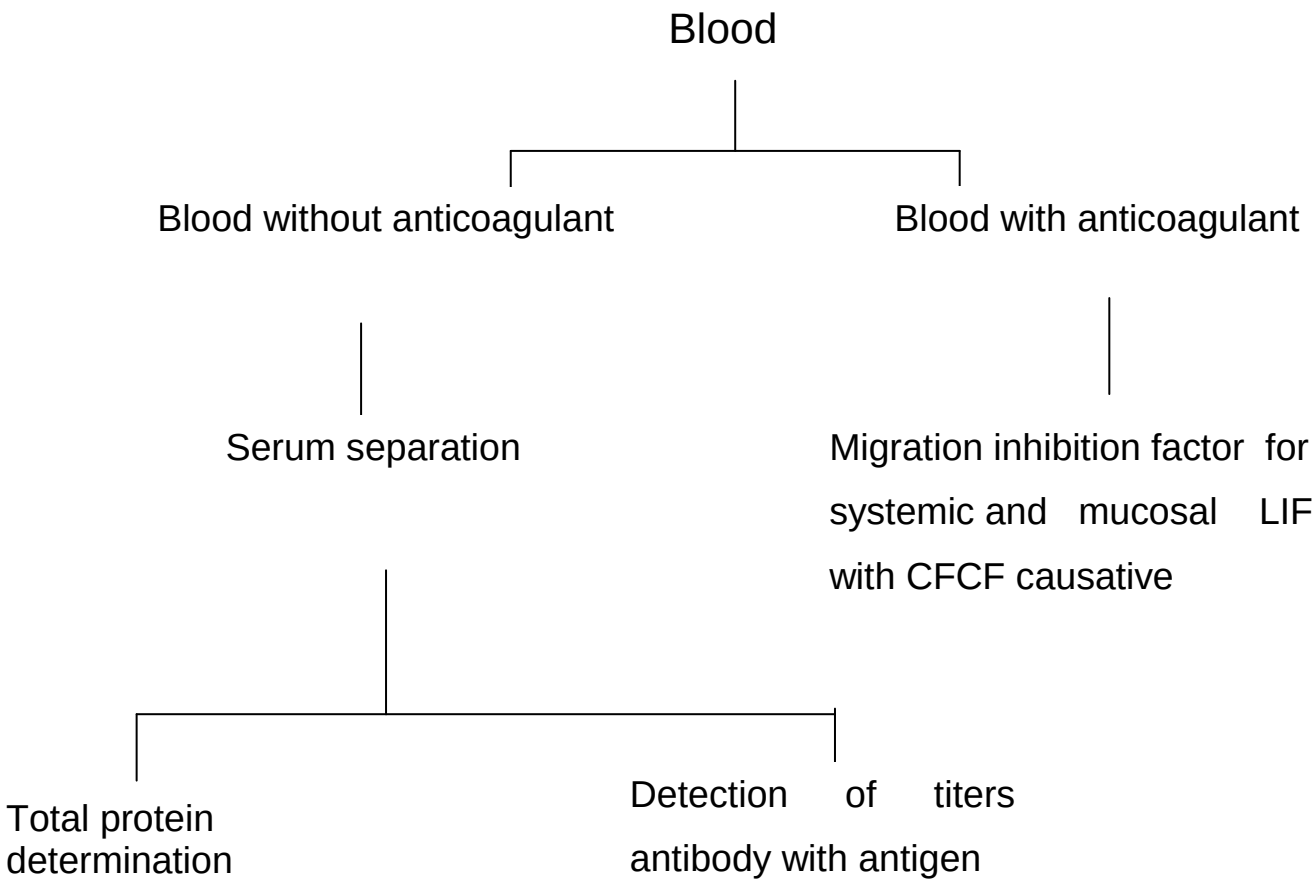


Figure (٣-١.a) Flow chart for the investigation of typhoid patient samples (Shanwa and Thwaini, ٢٠٠٢)

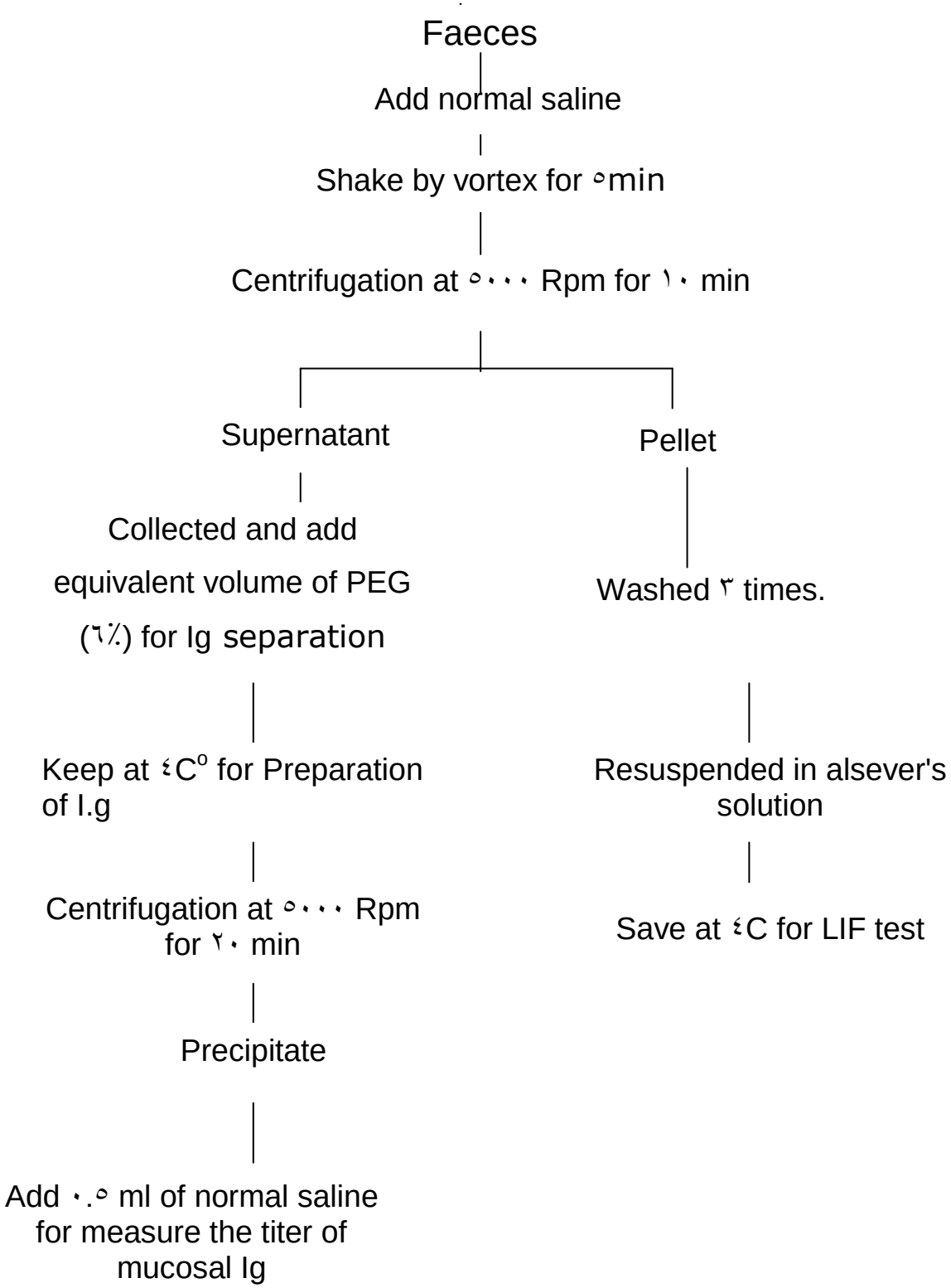


Figure (٣-١.b) Flow chart for the investigation of typhoid patient samples

(Shanwa and Thwaini, ٢٠٠٢)

٣.١٤.٢ Faeces culture

The faeces were cultured on plates of one or more kinds of selective media , both directly and after preliminary culture in a liquid enrichment medium (Tetrathionate broth); the plates are observed for the presence of *salmonella* like colonies . A well separated colony is picked to obtain a pure culture and the pure culture is identified first by a selection of biochemical test and finally by agglutination tests with antisera (Collee *et al.*, ١٩٩٦) .

٣.١٥ . Immunology of typhoid patient

٣.١٥.١ . Separation of sera

Half of the of the healthy and patients blood samples were collected from veins by disposable syringes in ٣ml volume without anticoagulant . The blood was left in room temperature for ١٠ min., and then centrifuged for ٥ min at ٥٠٠٠ Rpm . The serum was collected by pasture pipette and preserved at freeze in tube at ١٨ C^{o} until used (Frei *et al.* ١٩٩٥) .

٣.١٥.٢ . Blood without anticoagulant

The second half of the blood sample collected previously (٣.١٥.١) was tubed in AFMA . Dispo -EDTA tube contains EDTA as anticoagulant. The samples were mostly processed for LIF(Soberg , ١٩٦٨,)

٣.١٥.٣. Separation of mucosal I.g

The separation of secretory I.g from patients feaces was using polyethyleneglycol method according to (Shnawa and Al-Saadi , ١٩٩٢) as following :

*****Feacal sample (٢g) was taken and immediately emerged in universal bottle containing ٥ ml of sterile normal saline shake well by vortex .

*The suspension was centrifuged at ٣٥٠٠ Rpm for ٣٠ min .

*The precipitate was washed three times with normal saline and preserved in at ratio ١:١ in Alsever's solution , for studying LIF .

*The supernatant was taken and equal volume of ٦ % PEG was added and left at room temperature for ١٠ min .

*The suspension was centrifuged at ٣٥٠٠ Rpm for ٣٠ min. , the precipitate was dissolved in ١ml normal saline and kept at refrigerator ٤c˚ for precipitation of immunoglobulin .

*The mucosal I.g was dialyzed for ٢- to ٣ days against tap water and ١day against distilled water ; to removal the residue of I.g suspension (Garvey *et al*., ١٩٧٧) .

٣.١٦ Measurement of total protein concentration

The Biuret method was used in measurement of total protein in serum according to Bishop *et al*., ١٩٨٥ .The concentration of protein was calculated from simple linear regression equation as follows:

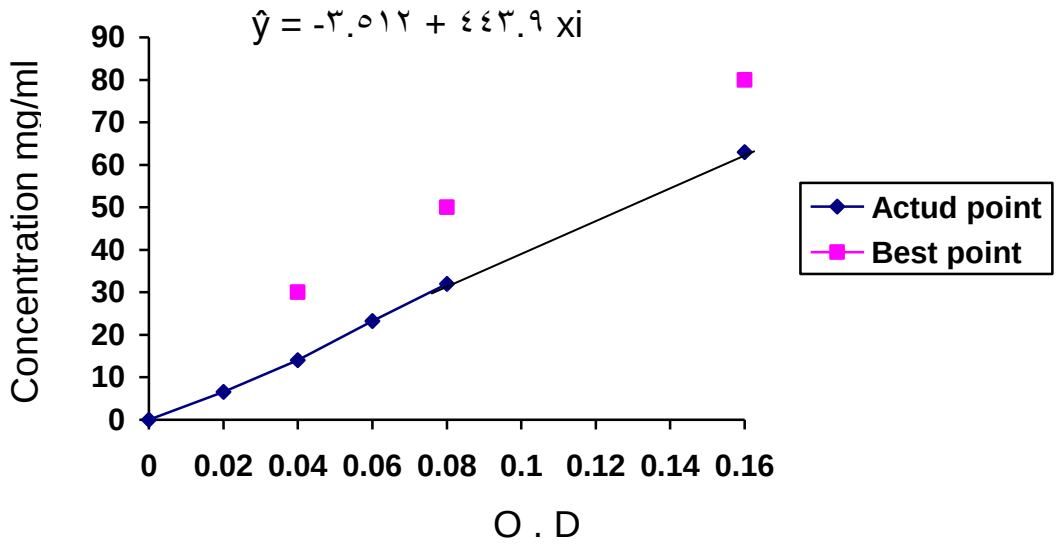


Figure (٣-٢) Standard curve of albumin concentration and optical density

٣.١٧. Measurement of secretory I.g concentration

Biuret method was used in measuring the concentration of S.Ig as in serum protein with modification of using ٠.٢ ml of S.Ig instead of ٠.١ ml serum . (Shnawa and Al-Saadi , ٢٠٠١) .

Mucosal immunoglobulin was calculated from the following formula (Figure ٣-٢) .

٣.١٨ . Studying of standard curve for protein concentration

Standard albumin was used at a concentration of ٦٥ g/L and series of dilution was made and the optical density was estimated by Biuret method (Table ٣-٤)

Table (٣-٤) Statistical features for standard curve equation of albumin concentration and their dilution .

$xi-\bar{x}$	$(xi-\bar{x})^2$	$Yi-\bar{y}$	$(yi-\bar{y})^2$	$(xi-\bar{x})(yi-\bar{y})$
٠.١٠٢	٠.٠١٠٥	٤٢.٦٥	١٨١٩.٩٠	٤.٣٥٩
٠.٠٢١	٠.٠٠٠٥٠	١٢.٦٥	١٦٠.٢٧	٠.٢٢٣
-٠.٠٠٧	٠.٠٠٠٤	-٩.٨٤	٩٦.٨٢٥	٠.٢٣١
-٠.٠١٧	٠.٠٠٠٢٨	-١٣.٥٩	١٨٤.٦٨	٠.٢٣١
-٠.٠٢٧	٠.٠٠٠٧٢	-١٥.٤٦	٣٣٩.٠١	٠.٤١٧
-٠.٠٣٧	٠.٠٠١٣٦	-١٦.٤٦	٢٦٩.٠٤	٠.٦٨٦
-٠.٠١٨	٠.٠٠١٠٥	-٢٠٠٥	١٦٠.٠١	٠.٨٢٤
	Σ=٠.٠١٣٥٦٧		∑=٢٧٦٩.٧٢٠	∑=٦.٠٦٠٢

$$b = \frac{\Sigma (xi - \acute{x})(yi-\acute{y})}{\Sigma (xi-\acute{x})^2} = \frac{٦.٠٦٠٢}{٠.٠١٣٥٦٧} = ٤٤٣.٩$$

$\acute{Y} = ١٧.٣٥$

$\acute{X} = ٠.٠٤٩$

$a = \acute{y} - b\acute{x} = ١٧.٤٥ - (٤٤٣.٩ \times ٠.٠٤٩) = -٣.٥١٢$

$\hat{y} = -٣.٥١٢ + ٤٤٣.٩\ xi$

٣.١٩ Serology

٣.١٩ .١ Agglutination

Direct slid semi quantitative slide as well as standard tube agglutination using serial decimal double dilution for sera and serial double dilution for mucosal I.g solutions were reacted with the test ready prepared and laboratory prepared antigens (Garvey *etal*., ١٩٧٧) . ٢ -ME agglutination studies were done as in Cruickshank *et al*.,(١٩٧٥).

٣.١٩.٢. Passive haemagglutination microtitration method .

Microtitration plate was used for dilution of serum Ig and secretory I.g. ٥٠ µl normal saline was added to each of ١-٩ wells , well No.٩, as a control .

The serum Ig or mucosal Ig (٥٠µl) was added to the first well , mixing the contents and transfer ٥٠ µl from well ١ into well ٢ by micropipette , repeat the process to well ٩ , from which , after mixing discard ٥٠ µl.

Tanned sheep red cells (٥٠ µl) was added to each well , except well ٩ . Then read the result after plate incubation at ٣٧c˚ for ٤٥ min . and the titer was recorded .

٣.٢٠ Single radial diffusion test

Many Immunological methods depend on the ability of soluble antigen to diffuse through agar gel , for readial diffusion , procedure based on the method of Mancini *et al*., (١٩٦٥) as follows :

*****Open the plate and let it stay for about ٥ min at room temperature allowing any condensation to evaporate .

*****Fill wells with ٥ml of serum of control sample using a suitable device

*****Put a wet cotton in a plate center to avoid agaros dehydration ; close plate tightly .

*****Allow plate to stay flat at room temperature along the time stated in reference table .

*****The trays are stored in a box ١-٢ days and examined for zones of Ag-Ab-Precipitation around the well .

*****The squares of diameters of zones are proportional to the concentration of the antigen in the wells (Biomaghreb. Company).

٣. ٢١ Leucocytes inhibitory factor LIF

٣.٢١.١ Peripheral blood LIF

Measurement of migration inhibitory factor in systemic blood:

*****Preparation of agar-A medium in sterile plastic plates and ٢ wells were made with ٢cm in diameter.

*****Capillary tube containing systemic blood from patients with *S.typhi* was placed in each well after being centrifuged by haematocrite centrifuge for ١٠ min. (Leucocytes and Buffy coat) .

*****Eagle basal medium ٠.١ ml was put in each well; one of the wells kept as control.

*****٠.١ ml of antigen (Cell free culture filtrate) of causative bacteria was added in one well .

*****Incubation was done at ٣٧c˚ for ٢٤ hr in Jar humid environment .

*****Measurement of LIF by ocoulometer, same steps were used for control and normal saline was added instead of CFCF as mentioned (Soberg , ١٩٦٨) .

LIF was measured as follows :

$$\text{Factor inhibition} = \frac{\text{Diameter of circle of migration of cell with antigen}}{\text{Diameter of circle of migration of cell without antigen}}$$

٣.٢١.٢ Mucosal LIF

Measurement of LIF in *S. typhi* discharge system (mucosal) was done for measuring the factor that inhibited the migration of leucocytes in case of typhoid caused by salmonella following Soberg (١٩٦٨) method as follows :

*****Agar A medium was prepared in sterile plastic plate ٢ % agar , and two wells were done with ٢ cm in diameter.

*****From cells of exudates of typhoid , in step (٣.١٥) that preserved in Al'severs' solution , capillary tube filled and put in the well after centrifugation by haematocrit centrifuge for ١٠ min .

*****In each well ٠.١ ml of Eagle Basal medium was added and one of the wells was used as control .

*****Addition of ٠.١ml of CFCF prepared in step (٣.٧) for one well and the other wells left as control .

*****The plate incubated at ٣٧c˚ in a jar with humid condition for ٢٤ hr and the inhibition of migration was measured(Shnawa and Al- Saadi , ٢٠٠١) .

٣.٢٢.Experimental antigen dependent immunomodulation

٣.٢٢.١ IFA Immunization (Incomplete Freund's adjuvant)

Adjuvant , Incomplete Freund;s adjuvant was supplied from (Difico) . Five rabbits were elected and adapted to laboratory condition , then housed under adlibitum standardized conditions . The first group of animals were injected (١ml) of adjuvant subcutaneously; the second group received saline as control . After one week of injection , both dosages ١x١٠٥ CFU (١ml) of *S. typhi* injection were diluted with ٤ml normal saline . The clinical symptoms were recorded after ٢٤ hr . The IFA protocols are listed in (Table ٣-٥).

Table (٣-٥) IFA immunization protocol

Group	Suspension	Dose	Route
٥ Rabbits	IFA	١ml	Sc
one rabbit	Saline	١ml	Sc
٦ Rabbits	Live infection	١ml ١x١٠٥cfu	Orally

٣.٢٢.٢ Vi-immunization

Five rabbits , exo-endo and haemo parasite free as well as anti. *S. typhi* antibodies pre rabbits were kept adlibitum condition and assigned in three groups(Table ٣-٦) .

Vi-suspension was administrated in five doses respectively at four week intervals , while the saline injected once , blood was collected for immunological investigation . (Shnawa and Thwaini , ٢٠٠٢) .

Table ٣-٦ Vi immunization protocol

Animal group	Antigen	Dose	Route	Period
٥ Rabbits	IFA	١ dose with ١ml	Skin IM	One week
The first one	Vi-vaccine	٥ doses with ٥ml/B.W (٣mg/ ٥cc)	Orally	٣٠ days
As group one	Live infection	٥ml (١ml of ١.٥x١٠^٥cfu	Orally	٢-٣days
One rabbit	saline	٥ ml	Orally	One week

٣.٢٢.٣ . Protoplast Immunization .

Five rabbits , exo-endo and haemo parasite free as well as anti-*S-typhi* antibodies pre rabbits were kept adlibitum condition and assigned in three groups (Table ٣-٧) .

Protoplasmic antigen was dosaged orally in five doses respectively , at four week intervals , and control received saline once. The blood was collected by heart puncture for immunological investigation .

Table ٣.٧ protoplast immunization protocol

Animal groups	Antigen	Dose	Route	Period
٥ Rabbits	Protoplast sonicate	٥ doses with ٣mg/ ٥cc/B.w	Orally	٣٠ days
The first one	Live infection	٥ml of ٠.١ml (cfu)	Orally	٢٤-٤٨ hr
One rabbit	Saline	٥ml	Orally	One week

*BW = Body weight

٣.٢٢.٤ Skin test .

To investigate allergies for *S. typhi* protoplast , ٠.١ ml of purified protein derivative of protoplast antigen was injected into protoplast primed . A positive reaction within ٢٤-٧٢ hr, the lesions will be recorded , such as erythemea , indurations and necrosis occures.

٣.٢٣ Quality control

The quality control of immune response was detected by using antigen- antibody reaction , The sensitivity index and specificity index were determined as follows (Stites ,*et al*., ١٩٩٤; Abid ,٢٠٠٠).

$$\text{Sensitivity index} = \frac{\text{True positive value-False negative value}}{\text{True positive value}}$$

$$\text{Specificity index} = \frac{\text{True negative value-False positive value}}{\text{True negative value}}$$

*****True (+) value = Immune substance was detected by test .

*****True (-) value = Immune substance absent .

*****False (-) value = false reagent to investigation on immune substance .

*****False (+) value = the value a crying by factors not pathogens .

٣.٢ Chemical reagents. Culture media are mentioned in Table (٣-١) .

Table (٣-١) Chemical reagents of *S.typhi* enteric fever

Seq.	Reagent name	Use for detection of	Method	Reference
١-	Voges - proskour reagent	Aceton production	Add ١ml of ٤٠٪ potassium hydroxide and ٣ml of ٥٪ ά naphthol into media ,positive reaction is eosin pink color at ٢-٣ min	Collee *et al.* ١٩٩٦
٢-	Methyl red reagent	Acid production during fermentation of glucose	Add about five drops of methyl red into the media , positive reaction and bright red color formation	MacFadin , ٢٠٠٢
٣-	Kovacs reagent	Decomposition of the tryptophan to indol	Add ٠.٥ml kovacs reagent to the medium , red color appears as in positive reaction	Collee *et al*.,١٩٩٦
٤-	Catalas reagent	Gas production	١ml of H_2O_2 is poured over to ٢٤hr nutrient agar slope culture of the test organism , production of gas bubbles indicate positive reaction.	Collee *etal.*,١٩٩٦

٥-	Oxidase reagent	Catalas test the transport of electron between electron donars and a redox dye phenyleneamine	Strips of whatman no .١ filter paper are soaked in freasly ١٨ of solution positive reaction in deep – purple color	Collee *etal.*,١٩٩٦
٦-	Nessler's reagent	decomposed urea	Add ٠.١nl of Nessler's reagent , into ٢٤hr culture purple –pink colour indicates positive reaction	MacFadin ,١٩٩٠
٧-	ONPG test	Late lactose - fermentation	Add *O*. nitrophenyl *. B.* glactopyranoside phosphate buffer into the media production of yellow color indicates positive reaction	Collee *et al.* ١٩٩٦
٨-	Iodine reagent	Vi polysaccharide	٢٠g of potassium iodide dissolving in ٥ml D.W. red colour indicates positive reaction	Collee *et al.* ١٩٩٦

٣.٣ Culture Media :Culture media are mentioned in Table (٣-٢) .

Table (٣-٢) Media for culture and biochemical tests

Seq	name	company	Class	The use in this study	Preparation
١-	(B.H.I.) Brain heart infusion broth	Oxoid	Enrichment	Cultivation of gram negative bacteria for preparation of dry weight and activation	Dissolve ٣٨.٧ g per liter following the instructions of company and autoclave with cups tight at ١٢١C^{o} for ٢٠min
٢-	Tetrathionate broth	Oxoid	Enrichment selective media	Enriches *Slamonella* , including typhi	Dissolve ٤٠ g of media in ١ liter of D.W. , Autoclave with cups tight at ١٢١C^{o} for ٢٠min
٣-	MacConkey agar Brilliant green	Oxoid	Differential and selective media	Cultivation of enteric bacteria	Dissolve ٣٠ g of the media in ١ liter D.W. , Adjust the pH to ٧.٥ Distribute in flasks and heat in autoclave ١٢١C at ١٥ min
٤-	S.S. Agar *Salmonella Shigella* agar	Biolife	Selective media	Isolation salmonella	Prepared according to the instruction of the company .Dissolving ٣ g per liter and sterilizeing by autoclaveing at ١٢١C for ٢٠ min.

٥-	D.C.A Deoxycholate citrate agar	Difico	Differential media	Distinguish between the lactose fermenting and non.	The medium was prepared according to the instruction of the company by dissolving ٣١ g per ١٠٠٠ ml D. W. and autoclaveing at ١٢١C for ٣٠ min
٦-	Stock media	Oxoid	General media	Preservation	Prepared steritized medium in screw-capped bottles including B.H.I. with ١٨ to ٢٤ hr bacteria and ٥٪ glycerol was added into the media
٧-	Blood agar	Oxoid	Enrichment media	Hemolytic properties of bacteria	The medium is prepared by adding sterile blood to sterile nutrient agar that has been melted and cooled to ٥٠c^{o}
٨-	Kiliglar iron agar	Oxoid	Differential media	H٢s production and sugar fermentation	Dissolve ١٠ g of medium in ١٠٠ ml D.W. sterilize at ١٢١C^{o} for ١٥ min and cool to form slopes with butt

٩-	Cimmon citrate agar	Difico	Indicat٠r	Citrate utilization	It was prepared by dissolving ٢ g of medium in ١٠٠ D.W. and sterlise in autoclave at ١٢١C for ٣٠ min
١٠-	Peptone water	Oxoid	General propose media	Cell free culture filtrate	Dissolve the ingredients in warm water , adjust the pH to ٥.٥-٧.٥ , distribute as required and autoclave at ١٢١C^{o} for ١٥ min
١١-	Mast agar A	BDH	Agar	Background media for detection of LIF activity of systemic and mucosal responses	٢ g powder is added to distilled water and dissolve by placing the mixture in steamer ١٠٠C^{o} for ١ hr , sterilize by autoclave ١٢١C^{o} for ١٥ min

٤. Results

٤.١ Bacteriological and clinical analysis:

Thirty five clinically proven typhoid patients were subjected to bacteriological examination for blood and feces. Fecal cultures were positive in ٣٥ cases, while blood cultures were positive in ١٥ cases of typhoid patients (Table ٤-١).

Table (٤-١) culture study

Blood culture	Fecal culture
١٥	١٥
٠	٢٠
Total	٣٥ samples

These cultures showed a *Salmonella* like character. Fourteen were showing character of salmonella biochemicaly (Table ٤-٢).

Table (٤-٢) Biochemical test of *S. typhi* enteric fever

Seq.	Tests	Standard results		Experimental result
١.	Gram stain			-
٢.	V-P- test	Pink color appearance		-
٣.	Methyl red	Bright red color appearance		+
٤.	Citrate utilize	Blue color appearance		-
٥.	Indol	Red color appearance		-
٦.	Catalase	Gas bubbles formation		-
٧.	Oxidase	Deep purple color appearance		-
٨.	Urease	Purple-Pink color		-
٩.	Motility	Aersol culture		+
١٠.	ONPG test	Late Lactose fermintation		-
١١.	H_2S	Streak heavs inoculum		+
١٢.	Acid	Slant no changes		+

٤.٢. Definitive diagnosis:

The above mentioned ١٤ isolates were transferred to Central Public Health Laboratory, Baghdad, among which, only five were designated as ٢,٤,٨,٩ and ١١ were *Salmonella typhi*.(Table ٤-٣).

One serologic test with mono and polyvalent immune sera salmonella gives the specificity of *S. typhi* by both anti-H and anti-O immune sera.

Table (٤-٣) Blood and fecal cultures enteric fever suspected

Seq.	Isolates No.	Associated enteric pathogen
١.	٢[١]	*S. typhi*[٢]
٢.	٤	*S. typhi*
٣.	٨	*S. typhi*
٤.	٩	*S. typhi*
٥.	١١	*S. typhi*

٤.٣. Criteria for the elections of *S. typhi* infectious and bacterian standardized starter.

Four determinative criteria were followed to elect the isolate possible as starter for preparation of the infectious doses and for bacterian preparation (Table ٤-٤). These were mainly depending on virulence and pathogenicity as well as serologic characters.

Table (٤-٤) Criteria for the election of *S. typhi* Infecious and bacterian standarized starter

١-	Obtained from clinical typhoid human case
٢-	Produce typhoid serum and mucosal *S. typhi* specific Ab titer in patients
٣-	Produce typhoid significant LIF mucosal and peripheral leucocytes in patients using *S. typhi* CFCF senisitizer
٤-	Produce enteric fever like syndromes in lab rabbits

[١] These *Salmonella typhi* obtained from ١٤ enteric fever suspects.
[٢] Identified in Biology department laboratories and confirmed by Central Public Health Laboratories, Baghdad, in June ٢٠٠٤.

٤.٤. *S. typhi* infection in rabbits:

Three days after the oral dosage of ١.٥ x ١٠^٥ CFU live infection of *S.typhi* to test rabbits, the symptoms were recorded as, fever, tenderness, then diarrhea (Table ٤-٥).

Table(٤-٥) *S.typhi* infection in rabbits

Seq.	Groups	Notes
١.	Infecting dose	One milliliter of ١.٥ x ١٠^٥ CFU of live *S. typhi* was the test infecting dose.
٢.	Duration of symptoms	The set of clinically apparent symptoms was ٢٤ hr and the duration of the symptoms was three days.
٣.	Re-isolation	Pure *S. typhi* were re-isolated from the infected rabbits during the second and ٣thd post infection.
٤.	Symptoms	Weakening, tenderness, fever and diarrhea.
٥.	Death record	All of the infected rabbits were dead.
٦.	Postmortem	On evisceration, congested visceral organ were noted
٧.	Histopathology	Slightly enlarged liver and spleen vascular congestion, inflammatory cells was recorded.

The lab animals that were infected with ١.٥ x ١٠^٥ CFU of live bacteria *S. typhi* the infection signs were noted at ٢٤ hr. weakening and illness.

The clinical symptoms usually begin after ٦-٤٨ hr. after ingestion as fever , diarrhea and weakness .

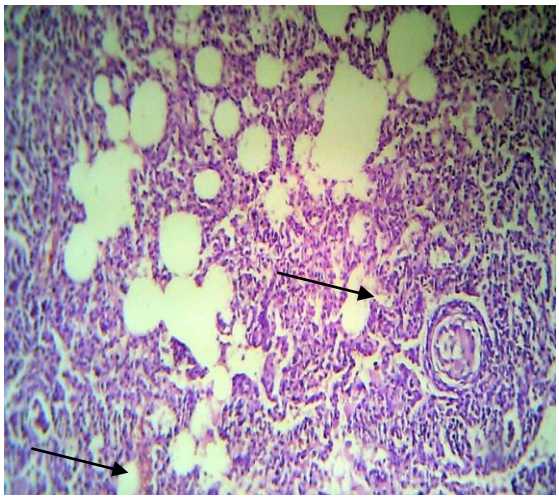
The incubation period for *S. typhi* were ١٠-١٤ days ,depending on the dose of bacteria ; the pure *S. typhi* was isolated from the rabbits during the post challenge of bacteria (Table ٤-٥).

Histopathologic analysis of the organs sections (liver, spleen, lung) taken from rabbits with live infection were done(Figure ٤-١).

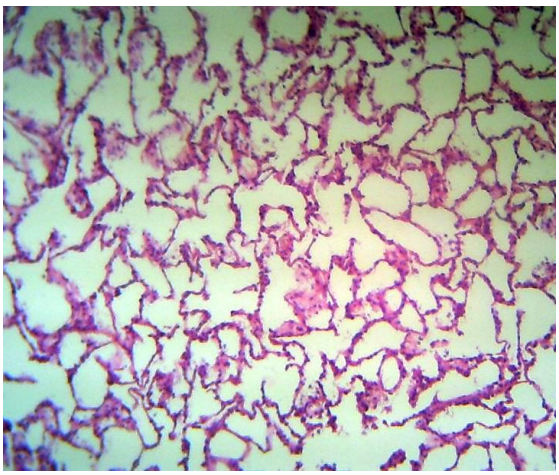
Lung section showing abundant vascular congestion ,chronic inflammatory cells infiltration (mononuclear) in the alveolar spaces and walls, chronic inflammatory cells and infiltration in bronchi as well as bronchioles.

However, liver infection showed hepatitis ,focal necrosis ,postmortem ,as well as the spleen infection showed,reactive follicular hyperplasia, when compared with normal tissues of rabbits.

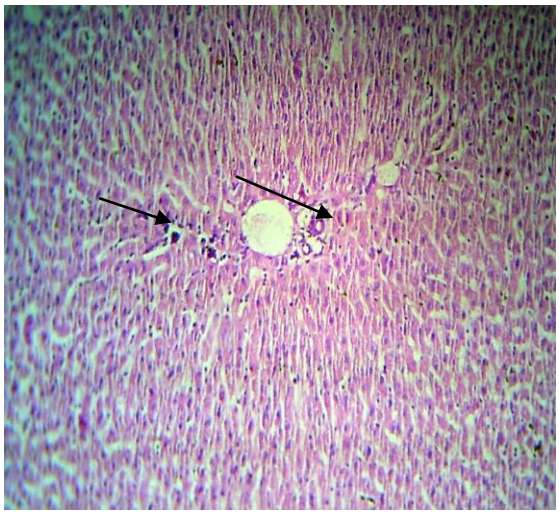
Figure (٤-١) : Histologic sections of lung (a) vascular congestion , mononuclear cell infiltration in alveolar spaces and bronchi and (broncules) (Arrows) ; liver (b) hepatitis and focal necrosis and spleen (c) with reactive hyperplasia . These sections from rabbit orally infected with *S. typhi* H and E stained magnification of ٣٠٠ x.



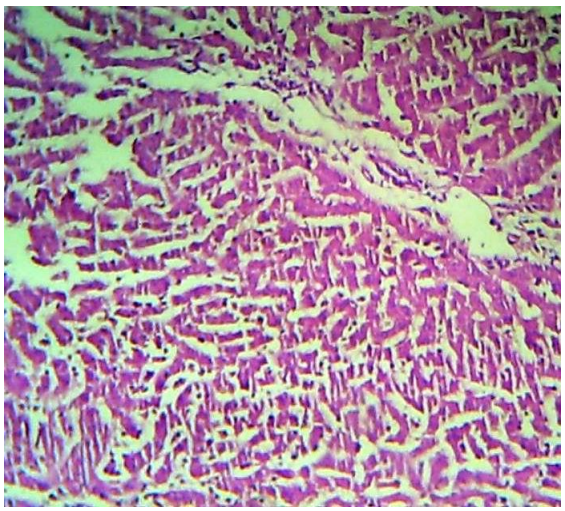
a- Lung infected with *S. typhi*



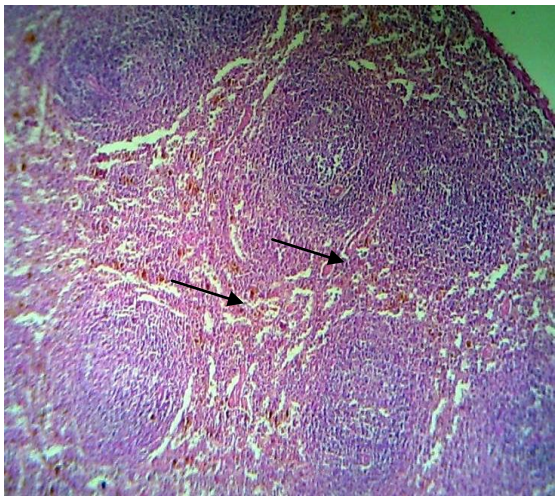
a- Normal lung



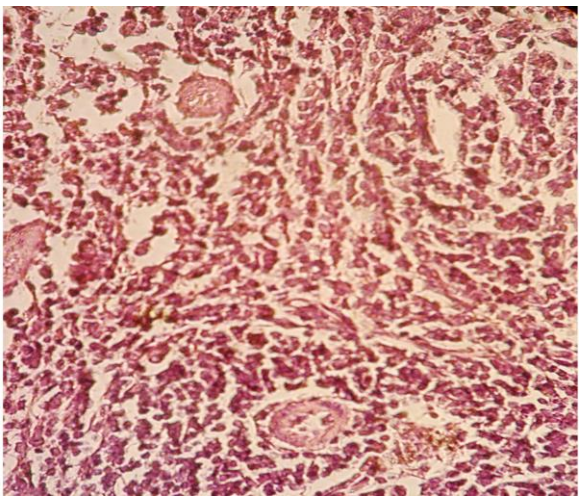
b- Liver infected with *S. typhi*



b- Normal liver



c- Spleen infected with *S. typhi*



c- Normal spleen

Figure (٤-١) Animals organs infected with *S. typhi* and normal

٤.٥. *S. typhi* standardized bacteria.

٤.٥.١ Detection of *S. typhi* Vi-suspension

Chemical and bioassay tests were used to determine the Vi-suspension components. In chemical tests, the O-acetyl and poly-saccharide groups had positive reaction with, its identifying chemical agent.

However, the bioassay test was used for the determination of hypersensitivity reaction of *S. typhi* – Vi. After injection of animal subcutaneously and IM separately, then after one week, no anaphylactic reaction occured, since neither shock nor paralysis or death at ١-٣hr can be scored in both groups; however, the delayed type hypersensitivity reaction was noted on ID injection in primed rates.

٤.٥.٢. Detection of *S.typhi* protoplast-Ag

Chemical test were used for the detection of the protein content in *S. typhi* protoplast, the protein was found as positive reaction with Biuret solution. While the biological assay was examined when animals were injected with protoplasm antigen, . The symptoms appeared after ١٨-٢٤ hr as erythemea and indurations.

٤.٦. Serology:

٤.٦.١- Agglutination

Prozone phenomenon was appeared in the reaction between *S. typhi* agglutinogens and patients' sera and mucosal immunoglobulins. It was apparent in first and/ or second tube in standard tube agglutination .

٤.٦.٢. ٢-ME Effect

The ٢ME treatments of patients' sera as well as mucosal immunoglobulin were of effect on two occasions of STO and STH for both mucosal and serum sample of the patients.

٤.٧. Cellular Immunology

Leucocyte inhibitory factor (LIF) for mucosal and peripheral blood leukocytes, LIF tests were of significant inhibition percentage among patients and non-significant among controls.

٤.٨. Quality control of immunologic test:

The quality control of humoral immunity responses was determined, the index of sensitivity of Widal O-Ag was ٠.٩٨, while the index of specificity was ٠.٩٠. In addition, the SI of protoplasm Ag (PHA) was ٠.٩٨, but the SPI were ٠.٨٥. The specificity index of specific cellular immune response was ٠.٩٥, while the index of sensitivity was ٠.٩٨, (Table ٤-٦).

Table (٤-٦) Quality control of serological test

Seq.	Antigen	Test	SI.١	SPI.٢
١.	Somatic	Widal	٠.٩٨	٠.٩٠
٢.	Protoplasm	PHA	٠.٩٨	٠.٨٥
٣.	Protoplasm	LIF	٠.٩٨	٠.٩٥

١-Sensitivity index
٢-Specificity index

٤.٩. Immune response profile of patients

S. typhi (STO) and (STH) titers of systemic humoral immunity response were higher than titers of mucosal response. The humoral immunity is apparent into three levels of immune responses (low, moderate and high) (Fig ٤-٢, ٤-٣). The ٦٤٠ and ٦٤ titers of both systemic and mucosal responses were more frequenty than other patients.

The data showed inductions of humoral immunity at both systemic and mucosal levels; the patient titers appearance of systemic and mucosal are listed in(Table ٤-٧).

Table(٤-٧) No. of mucosal and systemic responses among typhoid patients

Systemic response				Mucosal response			
STO	NO.*	STH	NO.*	STO	NO.*	STH	NO.*
٦٤٠	٥٠	٦٤٠	٤٨	٦٤	٤٣	٦٤	٤٠
٣٢٠	٢٥	٣٢٠	٢٩	٣٢	٣٠	٣٢	٣٦
١٦٠	٥	١٦٠	٣	١٦	٧	١٦	٤
Total	٨٠		٨٠		٨٠		٨٠

* No = number of patients

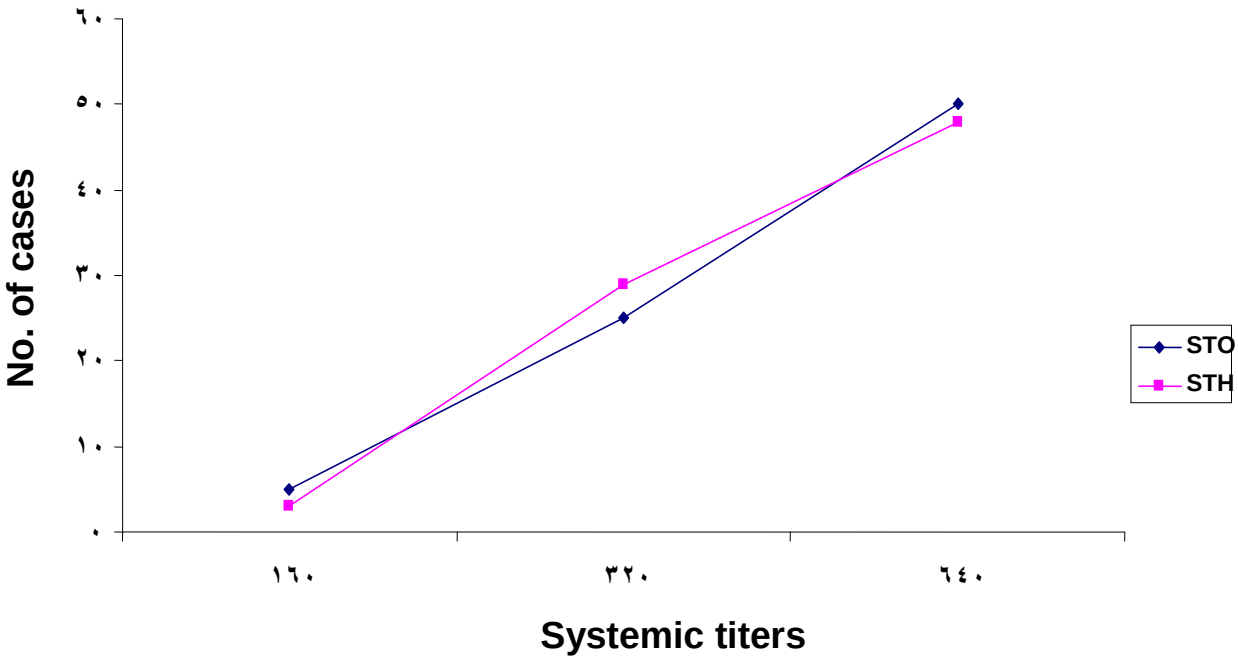


Figure (٤-٢) No. of STO and STH titers of systemic response

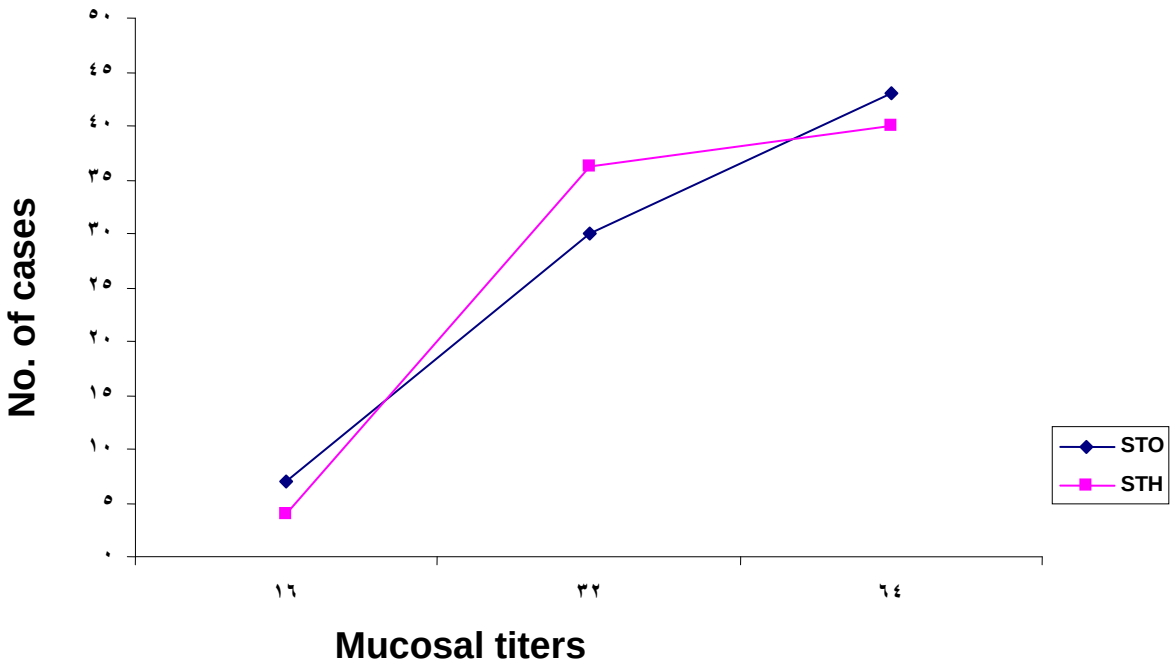


Figure (٤-٣) No. of STO and STH titers of mucosal response

However, the humoral immunity at the systemic and mucosal level, showed increase in the titers of passive haemagglutination, which ranged between ١٦٠-٢٥٦٠ at the systemic and ١٦-١٢٨ at mucosal system. (Figure ٤-٤, ٤-٥).

The systemic response of PHA also increased more than mucosal value; the highest titer was ١٢٨٠ at systemic and ١٢٨ at the mucosal system, low, moderate and high response for *S. typhi* antigen during which natural infection was noted by *S. typhi* sonicate antibody

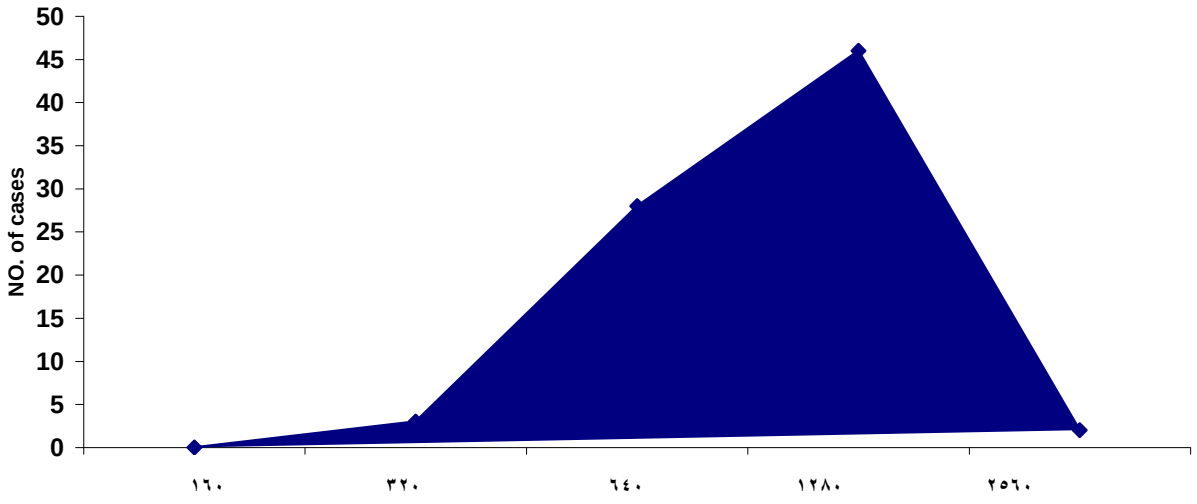


Figure (٤-٤) No.of cases and PHA titers of serum

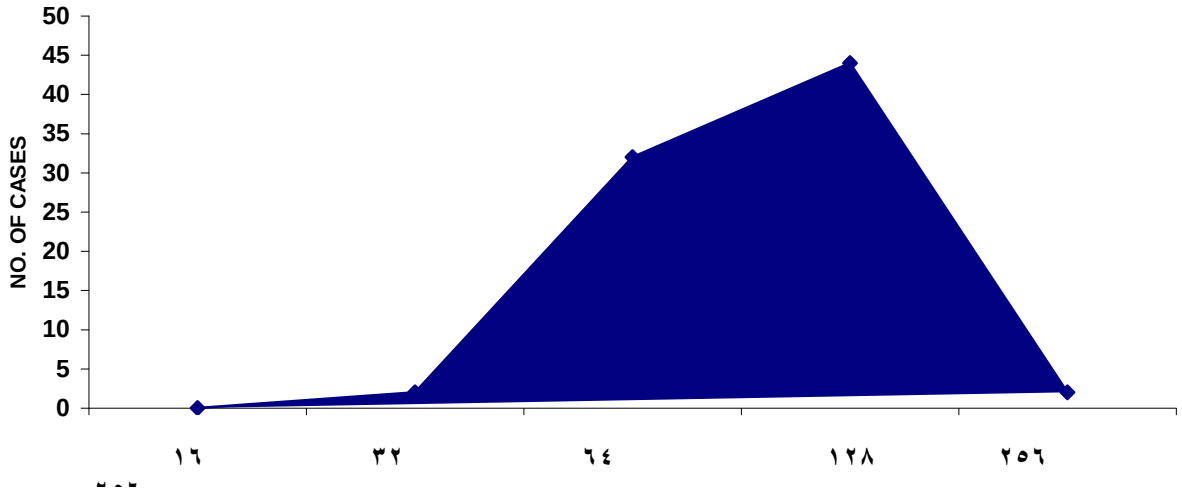


Figure (٤-٥ No. of cases and PHA titers of mucosal

The cellular immunity was determined by using LIF activity .The mucosal LIF assessments showed higher significant LIF indices than in peripheral blood LIF indices (Table ٤-٨) ,while the mean of systemic LIF was ٤٧٪ using CFCF as a sensitizer. The mucosal LIF was ٦٤٪ when CFCF used as a sensitizer respectively. LIF value ranged between (٣٠-٧٠٪) and (٣٠-٦٠٪) for systemic and mucosal LIF respectively (Figure ٤-٦,٤-٧)

Table (٤-٨) Cellular immune profile by LIF activity

Systemic LIF		Mucosal LIF	
LIF test	No. of cases	LIF test	No. of cases
٢٠	٢	٢٠	٢
٣٠	٣٠	٣٠	٣٦
٤٠	٢٥	٤٠	٢٥
٥٠	١٥	٥٠	١٥
٦٠	٤	٦٠	٢
٧٠	٤	٧٠	-
	X'=١٣.٣٣٣		X'=١٦.٠

N.o of patients = ٨٠

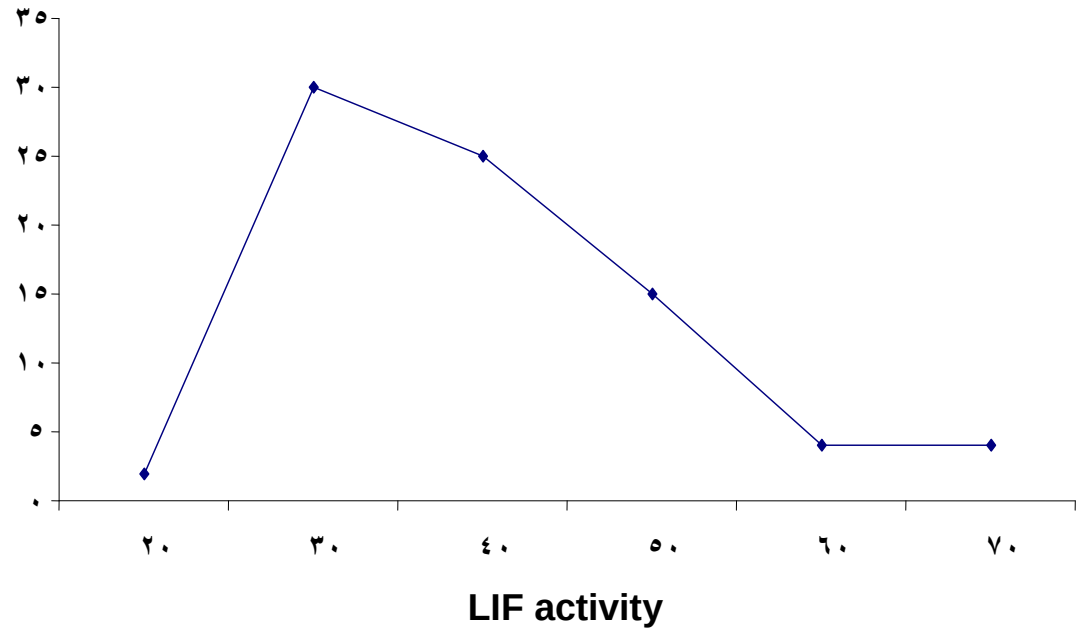


Figure (٤-٦) The relationship between systemic LIF *S. typhi* of and No.of cases

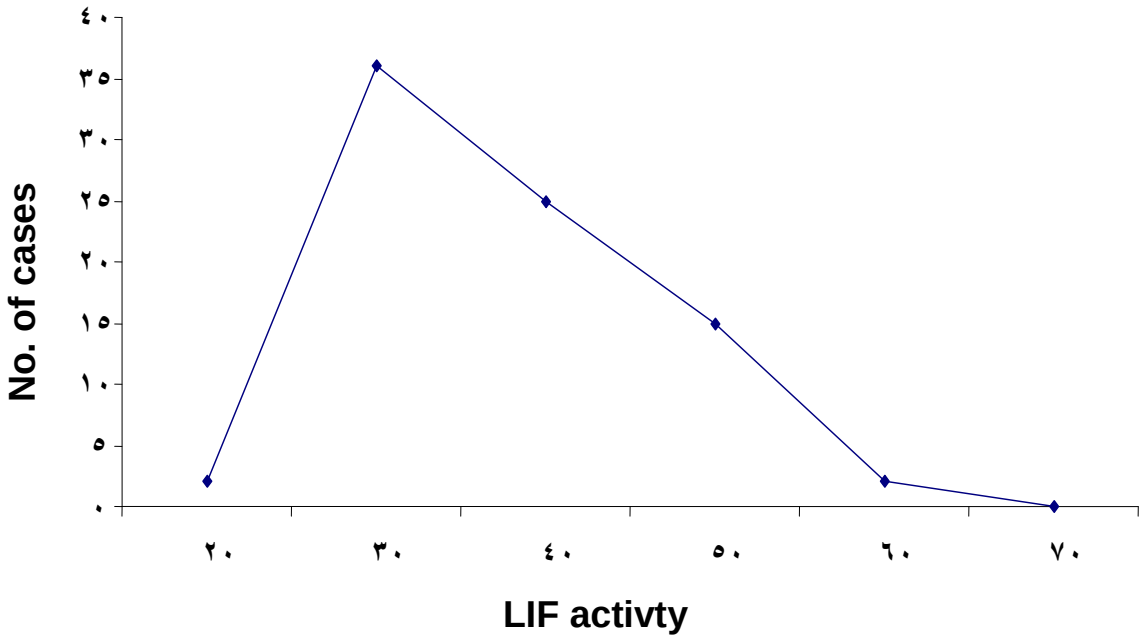
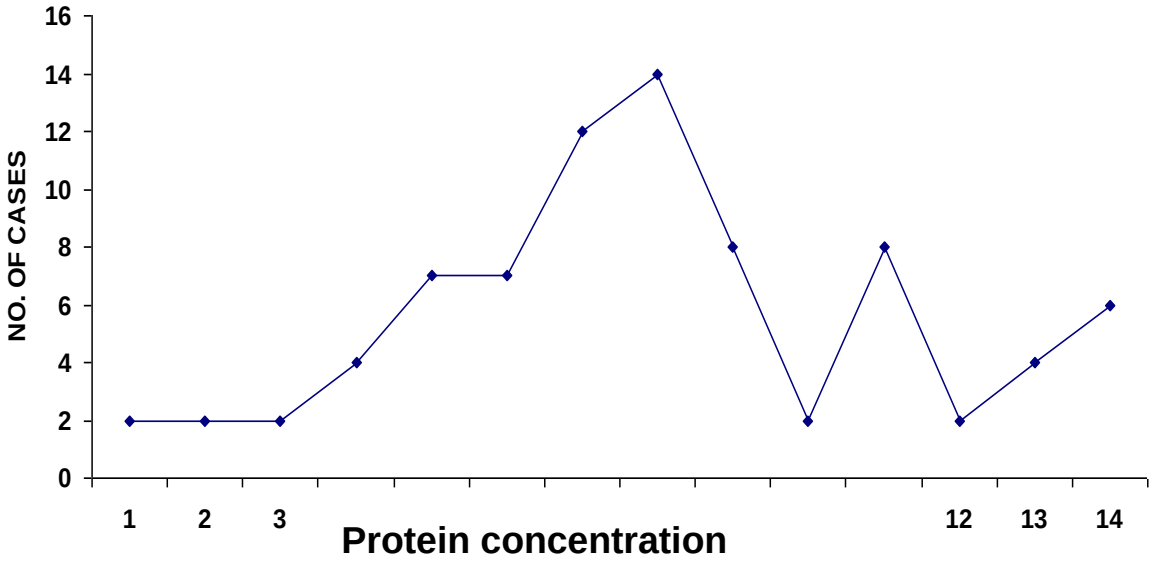


Figure (٤-٧) The relationship between mucosal LIF *S. typhi* of and No. of cases

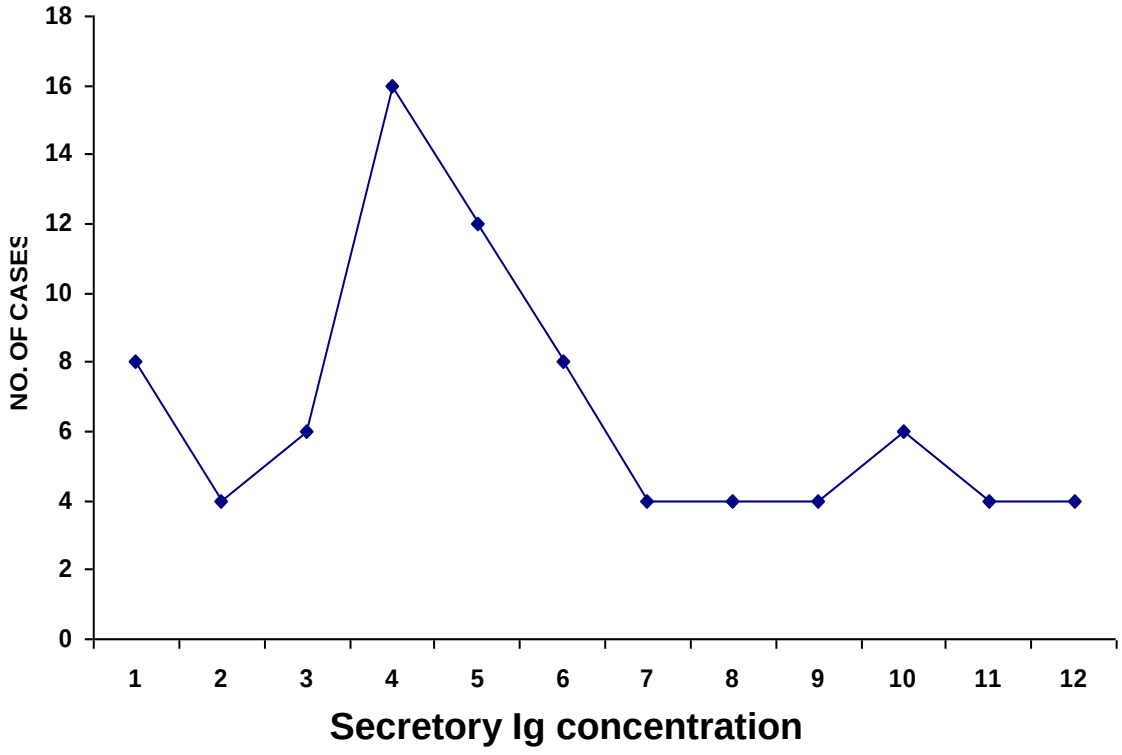
The (S.Ig) and total protein concentration were as a probe for non-specific immunity of typhoid fever patients. From the equation of standard albumin, the total protein among typhoid patients decreased as compared with normal value.

The total protein concentration among patients was found in three groups: (Low, moderate and high). The highest protein values were ١٤. ١٢g/L respectively, while the lowest were ٢ g/L.

However, the I.g concentrations were of various values among patients; it also (low, moderate and high), Level, pattern as compared with normal value (Figure ٤-٨; ٤-٩).



Figure(٤-٨) Protein concentration and No. of cases



Figure(٤-٩) S.Ig concentration and No. of cases

٤.١٠ . Immune status of the patients

The immunology of *S. typhi* was investigated; both humoral and cellular parameters were tested (Table ٤-٩).

The non specific immunity was studied and the value of total protein in serum ranged between ٤٠-١٦٠ g\l ; the normal value was ٧٠-١٢٩ g\l; the mean value was ١١٣.٠٤٥ respectively. While the value of I.g concentration ranged between ٠.١٨- ١١ g/l. the normal value was ٠.١٨-٢.٨٥ g\l the mean value was ٢.٠٧٥ g/L.

The specific humoral immune response was assessed by the determination of antibody (STO, STH) titers in serum and it ranged between (١٦٠-٦٤٠);the highest titer value was ٦٤٠. The local, however, humoral immune parameters were assessed by the determination of mucosal antibody (STO, STH) titers, the titers ranged between (١٦-٦٤); The highest titer was ٦٤ whereas the lowest titer was ١٦ for STO and STH .

The results showed that the serum antibody titers were higher than mucosal antibody titers.

On the other hand, the humoral immune response parameters were assessed by the determination of systemic PHA titers ; the titers ranged between ١٦٠ -٢٥٦٠, while the mucosal PHA titers ranged between ١٦-١٢٨, PHA titers of systemic were more than mucosal response.

The specific cell mediated response was studied through LIF at both systemic and mucosal arms and were between ٣٠-٧٠ % at the systemic and ٣٣-٦٠ % at the mucosal system. The mean values were ٤٢ % and ٤٠٪ respectively.

The immunoglobulin class and concentration of typhoid patients were studied by the determination of concentration values in serum where the IgG value ranged between ٢٥٦.٢ and ١٧١٥.٥, whereas the IgA value ranged between ١٦.٦ – ٤٧٤.٧ and the IgM value was ٣٧.٩ – ٢٤٩.٧.

The immunoglobulin concentrations were distributed into there levels (normal, subnormal and abnormal).

IgA value among patients was normal, but some values are abnormal (٤ cases). The IgA value was also subnormal in lmost of cases, whereas the IgM values were abnormal in all cases (Table ٤-١٠).

The concentration of IgG, IgA and IgM were noted as low IgA concentration in ١١/٢٤ (١, ٣, ٤, ٦, ٩, ١٠, ١٤, ١٦, ١٨, ١٩, ٢٠), combining low IgA and high IgM concentration ١/٢٤ (٨), high IgG concentration in ١/٢٤ (٥), combined high IgG concentration low IgA concentration in ١/٢٤ (٥). High IgG concentration ١/٢٤ (٥) and high IgM concentration ١/٢٤ (٧) were noted among typhoid patients.

The complement $C_٣$ and $C_٤$ in *S. typhi* enteric fever patients was assessed by the determination of concentration value in serum, where the $C_٣$ value ranged between ١٠.٠ -٧٣.٧. Same of $C_٣$ value was normal but in other cases it was subnormal, whereas the $C_٤$ value was normal in almost all cases (Table ٤-١١).

The complement fraction $C_٣$ and $C_٤$ concentration patterns among typhoid patients was high $C_٣$ in ٤/٢٤ (٣, ٥, ٦, ٧), high $C_٤$ in ٢/٢٤ (٥, ٧). High $C_٣$ and $C_٤$ in ٢/٢٤ (٥/٧), high $C_٤$ low $C_٣$ ١/٢٤ (٢٣), low $C_٤$ in ٣/٢٤ (١٩, ٢١, ٢٢) and combined low $C_٣$ and $C_٤$ in ٣/٢٤ (١٦, ١٩, ٢٢).

Table (٤-١٠) Immunoglobulin class and concentration in *S.typhi* enteric fever patients.

seq.	IgG	IgA	IgM
١	٥٢٢.٢	٤٩.٩	٩١.٣
٢	٨٢٩.٩	١١٤.٧	١١٣.٧
٣	٢٥٦.٢	١٦.٦	٣٧.٩
٤	٦٤٩	٨١.٧	٩٦.٧
٥	٢٢٣٧.٦	٣٧٨.٩	١٩٣.٩
٦	١٧١٥.٣	٨١.٧	١٤٣.١
٧	١٤١٧.٥	٤١٦.٩	٢٢١.٦
٨	٧٨٣.٤	١٠٠	٢٠٤.٧
٩	٦٠٥.٨	٥٧.٤	١٧٦.٧
١٠	٤٤١.٩	٣٥.٥	١٣١.٥
١١	١٤٧٥.٣	١٧١.١	٥٩.٤
١٢	١٦٩٠.٥	٤٧٤.٧	٢٠٤.٧
١٣	٨٨٨	١٤٢.٤	٩٨.٣
١٤	٩٧٤.٧	٨١.٧	٧٠.٤
١٥	١٣٤٢.٢	١٨٠.٩	١٣٧.٧
١٦	١٠٧٥.١	٨٩.٨	٢٤٩.٧
١٧	١٣٤٢.٢١	٢٢٢.٤	١٧٦.٧
١٨	٨٧١.٣	٥٨.٤	١٥٦.٧
١٩	٧٣٧.٧	٨١.٧	١١٣.٧
٢٠	٤٤١.٩	٤٩.٩	١٩٠.٥
٢١	١١٧٩.٦١	١٨٠.٩	١٩٠.٥
٢٢	٢٥٦.٢	١٢٣.٦	١١٣.٧
٢٣	٨٢٩.٩	١٧١.١	١٠٧.٩
٢٤	١٠٧٥.٤	٩٧.٤	١٢٥.٥
X' =	٩٨٤.٩٥٠٨	١٤٤.١٣٧٥	١٤١.٩٣٧٥
N.V	٥٦٠-١٥٠٠	١٠٠-٢١٠	٤٠-٢٠٠
R	٢٥٦.٢-١٧١٥.٥	١٦.٦-٤٧٤.٧	٣٧.٩-٢٤٩.٧

Table (٤-١١) Serum C٣ and C ٤ concentration in *S. typhi* enteric fever patients

seq.	C٣	C٤
١	٥٤.٧	٤٠.١
٢	٥٠.٣	١٠
٣	٢٧٠.٥	٢٢.٨
٤	٩٩	٢٥.٢
٥	٢٧٠.٥	٧٣.٧
٦	١٦٩.٢	٣٧.٢
٧	١٧٥.٨	٥٢.٥
٨	١٣٢.٨	٦٤.٥
٩	١٢٦.٩	١٧.١
١٠	٨٣.٤	٢٩
١١	٨٢	٤٠.١
١٢	١٥١	٤٣.١
١٣	٢٢٢٤.٧	٣٥.٨
١٤	٣٣.٥	٣٠.٤
١٥	٥٩.٣	٤٣.١
١٦	٣٣	١٢.٩
١٧	٥.٥	٢٧.٨
١٨	١٢٦.٩	٣٤.٤
١٩	٤٥.٩	١٧.١
٢٠	٥٩.٣	٣٣
٢١	٩٩	١٩.٣
٢٢	٤٥.٩	١٧.١
٢٣	٣٣.٥	٤٧.٧
٢٤	١٣٨.٨	٤٠.١
X'	١٩٠.٤٧٥	٣٣.٩١٦٦٧
N.V	٨٠-١٦٠	٢٠-٤٠
R	٣٣.٥-٢٧٠.٥	١٠-٧٣.١

The humoral immunity at the systemic and mucosal level showed an increase in the titer of specific antibody which ranged between ٦٤٠-١٢٨٠ at the systemic, ٣٢-١٢٨ at the mucosal system with mean value of (٩٨٦.٦, ٥٤) for both.

In case of specific cellular immune response, LIF value ranged between ٠.٣٠- ٠.٦٠ with mean value of (٠.٤٣) respectively at the systemic immunity; LIF ranged at the mucosal immunity between ٠.٣٠-٠.٦٠ with mean ٠.٤٠.

The IgG and IgA value also increased with the highest titers of humoral response, but IgM value was normal in almost all cases among typhoid patients. However, the $C_٣$ and $C_٤$ values in almost all cases were increased with high titer of PHA; the result are listed in (Table ٤-١٢).

Fifteen different immune profiles were noted among these ٢٤ elected patients using protoplasmic sonicate antibody, Ig classes, $C_٣$ and $C_٤$ complement fractions.

The first immune profile that revealed the PHA titers of systemic and mucosal response as (١٢٨٠, ٦٤),then LIF value was significant for both (٠.٣٣, ٠.٣٥).

IgG and IgA value was subnormal (٥٢٢.١, ٤٩.٩) but IgM as normal (٩١.٣) ; however, the $C_٣$ complement value was subnormal (٥٤.٧) while, the $C_٤$ value was normal (٤٠.١).

The second, the PHA titer was ١٢٨٠, ٣٢ for systemic and mucosal; then the LIF value also significant (٠.٣٠, ٠.٤٠), whereas, the IgG, IgA and IgM value was normal (٨٢٩.٩, ١١١.٧, ١١٣.٧) respectively, and the $C_٣$ and $C_٤$ value was subnormal (٥٤.٧, ٤٠.١).

In the third case, moderate titer of PHA for both response (٦٤٠, ٣٢), while, the LIF value was low significant (٠.٦٢- ٠.٥٠). Subnormal value of IgG, IgA and IgM (٢٥٦.٢, ١٦.٦, and ٣٧.٩) respectively, then the $C_٣$ value

were abnormal (٢٧٠.٥) while C_4 normal. In the fourth profile, however, the following cases are observed.

Case No.٥:- High PHA titer (١٢٨٠, ٦٤) and LIF was significant (٠.٣٥, ٠.٣٧), High value of IgG and IgA (٢٢٣٧.٦, ٣٧٦.٩), while IgM value was normal, whereas, the C_3 and C_4 value was high (٢٧٠.٥, ٧٣.٧).

Case No.٦:- The systemic and mucosal immune response of PHA titers was (١٢٨٠, ٦٤) and LIF value was significant for both. High IgG concentration (١٧١٥.٣) while IgA and IgM concentration was normal (١١٤.٧, ١٤٣.١), C_3 and C_4 value was abnormal (١٦٩.٢, ٤٧.٧).

Case No. ٧:- PHA titer was (٦٤٠, ٣٢) with significant LIF (٠.٣٠) for both, IgG was low value (١٤١٧.٥) and IgA moderate value, in addition to, IgM was abnormal value (٢٢١.٦). C_3 and C_4 value at high level (١٧٥.٨, ٥٢.٥).

Case No.٨:- moderate PHA titers (٦٤٠, ٣٢) with LIF value (٠.٣٧, ٠.٥٠) of systemic and mucosal response. Normal value of IgG and IgA, (٧٨٣.٤, ١٠٦.٠), while IgM was high concentration (٢٠٤.٧), the C_3 value was normal but C_4 abnormal (٦٤.٥).

Case No.٩:- High titer of PHA (١٢٨٠, ٦٤) and LIF was significant (٠.٣٧) for both, normal value of IgG and IgM, but subnormal value of IgA (٥٧.٤). Normal C_3 value (١٢٦.٩) with subnormal value of C_4 (١٧,١).

Case No. ١٢:- PHA titer was high value and LIF was significant. IgG, IgA,and IgM, was high value (١٦٩٠.٥, ٤٧٤.٧, ٢٠٤.٧), while the C_3 and C_4 value was normal (١٥١.٠, ٤٣.١).

Case No.١٣:- The PHA titers were (٦٤٠, ٣٢) and LIF was (٠.٤٠, ٠.٣٧) of systemic and mucosal response, Normal value of IgG IgA and IgM, then the C_3 was abnormal and C_4 was of normal value (٣٥.٨).

Case No.١٤:- High titers PHA (١٢٨٠, ٦٤) with significant LIF (٠.٣٨).all immunoglobulin classes and C_3 and C_4 complement was normal value, except IgA was subnormal value (٨١.٧).

Case No.١٦:- PHA titer was ١٢٨٠, ٦٤ and LIF was significant, IgG was normal value (١٠٧٥.١), while, IgA was subnormal value ٨٩.٨, and IgM abnormal value ٢٤.٩. $C_٣$ and $C_٤$ values were subnormal (٩٩.٥, ١٢.٩).

Case No.١٩:- ١٢٨٠ is the highest titer at the systemic and ١٢٨at mucosal level, while the LIF value was (٠.٣٠). IgG and IgM were sub normal values (٧٣٧.٧, ١١٣.٧), while IgA was subnormal value (٨١.٧). $C_٣$ and $C_٤$ were abnormal values ١٥.٩, ١٧.٧ respectively.

Case No. ٢٠:- PHA titers were ١٢٨٠, ٣٢ and LIf was low significant ٠.٥٠, low value of IgG and IgA ٤٤١.٩, ٤٩.٩ and IgM was moderate, $C_٣$ was low value but $C_٤$ value was high.

Case No. ٢٢:- The humoral immunity PHA titers were ٦٤٠, ٣٢ and LIF was low significant (٠.٥٠). IgG was subnormal value (٢٥٦.٢) while IgA and IgM were normal values (١٢٣.٦, ١١٣.٧), $C_٣$ and $C_٤$ were subnormal values ٤٥.٩, ١٧.١ respectively.

.١١. *S. typhi* protective immunity in rabbits

٤.١١.١. A immune protection.(Incomplete Freund's adjuvant)

When lab animals were injected subcutaneously with IFA, the challenged symptoms were mild, the death rate was ٢٠٪ and the protection rate was ٨٠٪.

However, the results showes that in the animals which were not treated with IFA, the symptoms were severe and death ratio was ٥/٥, in addition to, the protection ratio was ٠٪ as compared with control group that received saline (Table ٤-١٣).

Table (٤-١٣) IFA non spesific immune protection against *S. typhi*

Groups	NO. of Rabbits	Infection dose	Symptoms	Death ratio	Protection ratio
IFA Primed	٥	*S .typhi*	mild	١/٥	٨٠٪
Non IFA Primed	٥	*S .typhi*	sever	٥/٥	٠٪
control	١	-	-	٠/٥	١٠٠٪

٤.١١.٢ . Vi immune protection.

Lab animals were injected subcutaneously with IFA, then immunogen in saline containing ٣ mg *S. typhi*-Vi- suspension was dosaged orally; the symptoms were scored as mild, the death ratio was ٠/٥, but the protection rate was ١٠٠%.

Animals with non-Vi- Vaccine treatment become, fever and diarrhea, then death appeared . The death ratio was ٠\٥, while the protection rate was ١٠٠٪,in compared with control group (Table ٤-١٤-a).

Table (٤-١٤-a) *S. typhi* immune protection against live *S. typhi* challenge

Groups	NO. of rabbits	Infection dose	Symptoms	Death ratio	Protection ratio
Vi Primed	٥	*S.typhi*	-	٠/٥	١٠٠ %
Non- -Vi primed	٥	*S.typhi*	Fever and death	٥/٥	٠ %
Control	١	Saline	-	-	١٠٠ %

The findings of evolution of the Vi-immuno protection revealed the similarity between the systemic humoral immune response in pre and post challenge.The antibody titer in serum ranged between (١٦٠-٣٢٠)..

In case of passive haemagglutination, the titers ranged between ٦٤٠_١٢٨٠ in pre and post challenge, the highest titer was ١٢٨٠.

However, at the cellular level, *S. typhi* induced cellular immunity, since the LIF value ranged between ٠.٣٠_ ٠.٥٠ at systemic immunity in pre and post challenge (Table ٤-١٤-b).

Table (٤-١٤-b) Immunologic evaluation of *S. typhi* challenger rabbit primed with Vi- vaccine

	Pre-challenge				Post-challenge			
	Vaccine titers				Vaccine titers			
seq.	STO	STH	PHA	LIF	STO	STH	PHA	LIF
١	٣٢٠	١٦٠	١٢٨٠	٠.٣٥	٣٢٠	٣٢٠	١٢٨٠	٠.٣٥
٢	٣٢٠	٣٢٠	١٢٨٠	٠.٣	٣٢٠	٣٢٠	١٢٨٠	٠.٣
٣	١٦٠	٣٢٠	١٢٨٠	٠.٤٥	١٦٠	٣٢٠	١٢٨٠	٠.٤٥
٤	١٦٠	١٦٠	٦٤٠	٠.٥	١٦٠	٣٢٠	٦٤٠	٠.٤٥
٥	٣٢٠	٣٢٠	٦٤٠	٠.٣٥	٣٢٠	٣٢٠	٦٤٠	٠.٣٥
٦.control	-	-	-	٠.٩٠	-	-	-	٠.٩٠

٤.١١.٣ . Protoplast sonicate immune protection

The result of protoplast immune protection demonstrated that the animals were dosage orally with protoplasm sonicates.

Then challenges were normal. The death ratio was ٠/٥ and ; the protection rate was ١٠٠٪.

In addition, animals with non *S.typhi* sonicate , the symptoms appeared like fever, diarrhea, and death ; the death ratio were ٥\٥ but the protection rate were ٠٪ (Table ٤-١٥-a).

Table (٤-١٥-a) *S. typhi* protoplasmic sonicate immune protection against live *S. typhi*

Group	NO. of rabbits	Infecting Dose	Symptoms	Death ratio	Protection ratio
S.typhi protoplasmic sonicate	٥	*S.typhi*	-	٠/٥	١٠٠٪
Non *S.typhi* protoplasmic sonicate	٥	*S.typhi*	fever, diarrhea, death	٥/٥	٠%
Control	١	Saline	-	-	١٠٠٪

In protoplasmic sonicate protection, the vaccine titers (STO, STH٠in pre and post challenge were similar and the titers (١٦٠-٣٢٠) were more frequent.

However, the PHA titers of both were similar but the LIF is significant in pre and post challenge as about ٣٠-٤٠ %, comparison with control group (Table ٤-١٥-b).

Table (٤-١٥-b) Immunologic evaluation of *S. typhi* challenge rabbit primed with protoplast sonicate vaccinate

Squ.	Pre-challenge				Post-challenge			
	Vaccine titers				Vaccine titers			
	STO	STH	PHA	LIF	STO	STH	PHA	LIF
١	١٦٠	١٦٠	٦٤٠	٠.٤٥	١٦٠	٣٢٠	٦٤٠	٠.٤٤
٢	٣٢٠	٣٢٠	١٢٨٠	٠.٣٣	٣٢٠	٣٢٠	١٢٨٠	٠.٣٣
٣	١٦٠	٣٢٠	٦٤٠	٠.٣٤	١٦٠	٣٢٠	٦٤٠	٠.٣٥
٤	١٦٠	٣٢٠	٦٤٠	٠.٣٦	١٦٠	٣٢٠	٦٤٠	٠.٣٥
٥	٣٢٠	٣٢٠	١٢٨٠	٠.٣١	٣٢٠	٣٢٠	١٢٨٠	٠.٣١
٦	-	-	-	٠.٩٠	-	-	-	٠.٩٠

٤.١١.٤. *S. typhi* skin test

To investigate the allergies, a small amount of purified protein derivatives were separated from protoplast sonicate, then dermally was injected into the protoplast primed. Local damage result and the positive reaction took ٣٤-٧٢ hr; the lesions begin to appear e.g. erythemea, indurations followed by necrosis.

The LIF value was significant at ٣٠٪ in both pre and post challenge of protoplasm sonicates (Table-٤-١٦).

Table (٤-١٦) Skin delayed hypersensitivity test in rabbits vaccinated with protoplasmic vaccine of *s. typhi*

Group	Erythemea	Indurations	Necrosis	Pre LIF	Post LIF
Ribbits	Positive reaction after ٦-١٨ hr	Positive reaction after ٢٤-٤٨ hr with ١٢mm diameter	Occurring after ٧٢ hr	X'= ٠.٣٦	X'= ٠.٣٧
Control with saline	-	-	-	٠.٩٠	٠.٩٠

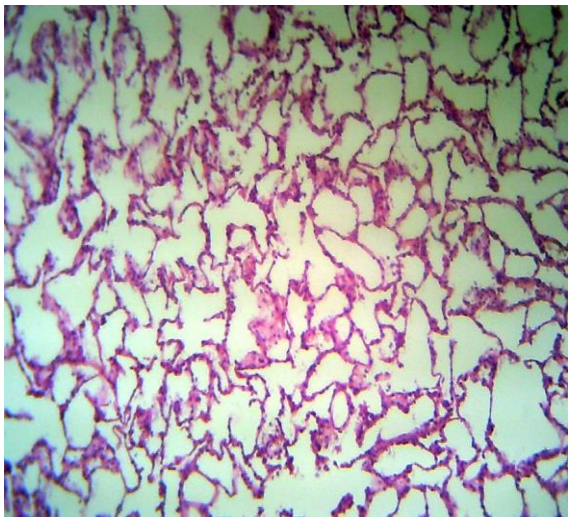
٤.١٢ . Histopathology;

The course of *S. typhi* infection in rabbits without adjuvant or vaccine was intestinal and extraintestinal.

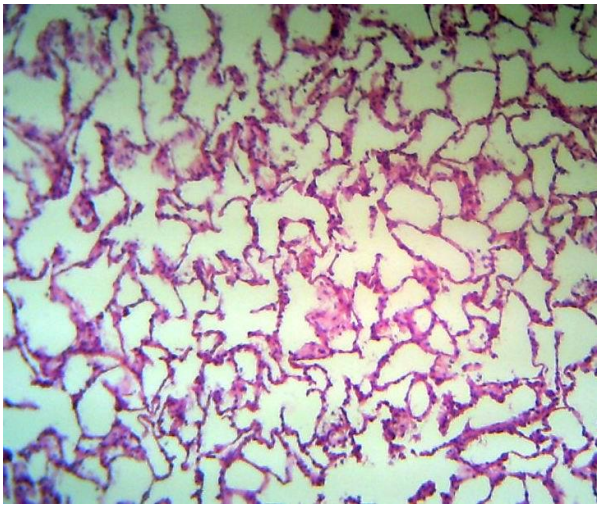
In IFA treated rabbits , the death rate was ١/٥ (٢٠٪) while protection percentage was ٨٠ %.Gross lesions were found only in lungs. Histologic changes were evident in both lung and spleen. The spleenic sections showed reactive hyperplasia while the lungs showed vascular congestion and mononuclear infiltration.

In Vi treated rabbits, the protection percentage was ١٠٠ %.No death rate .No gross lesions in lung, spleen and liver.(Figure ٤-١٠ ;٤-١١)

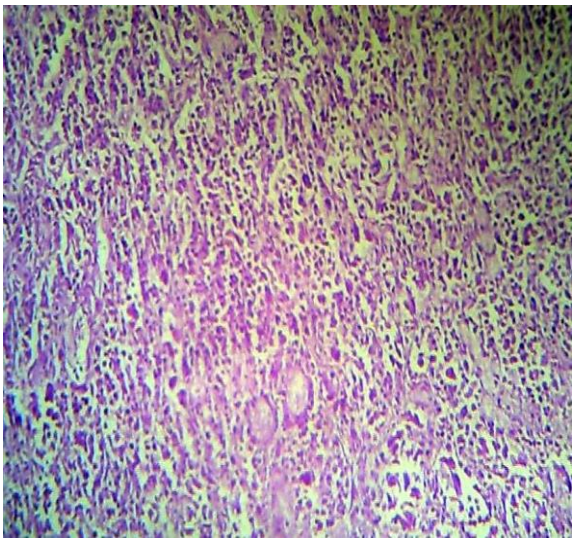
Figure (٤-١٠) Histologic sections of; lung (a), vascular congestion, mononuclear cells infiltration in alveolar spaces, bronchi and broncules (arrows); spleen(b) with reactive hyperplasia. These section were form rabbit orally treated with IFA primed, H and E stained, magnification of (٣٠٠ x).



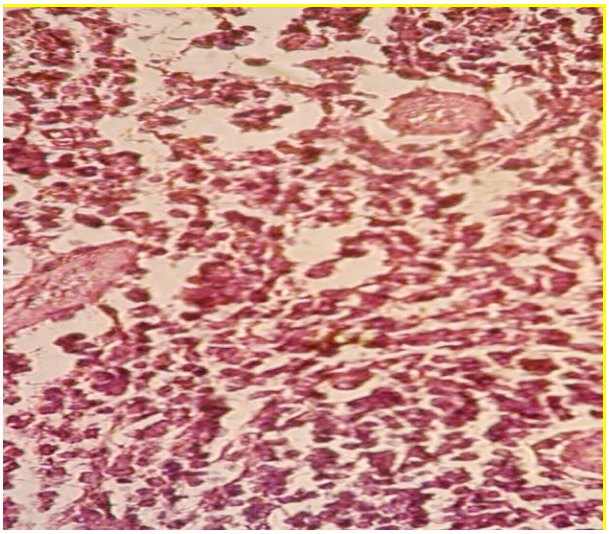
a- Lung treated with IFA



a- Normal lung



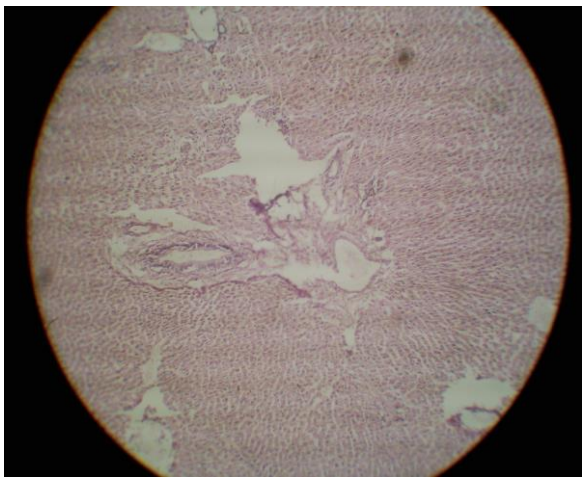
b- Spleen treated with IFA



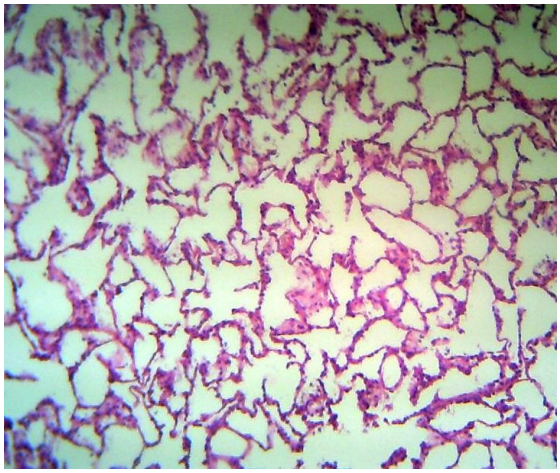
b- Normal spleen

Figure (٤-١٠) Animal organs treated with IFA

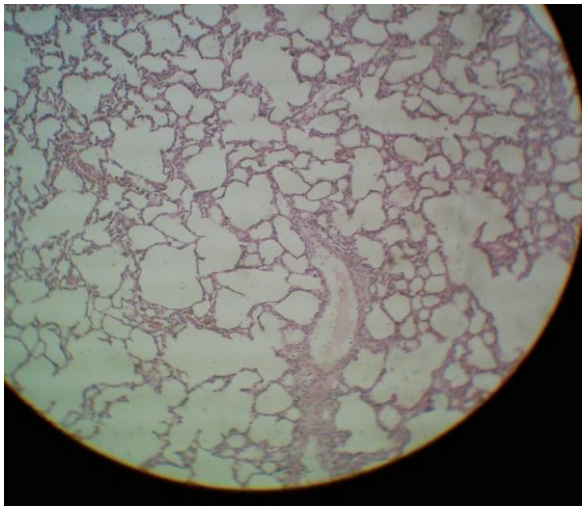
Figure (٤-١١) Histologic sections of; lung (a), liver (b) and spleen (c), no changes occurs. These sections were from rabbits orally dosaged with Vi and protoplasmic sonicate vaccine H and E stained, magnification of (٣٠٠ x).



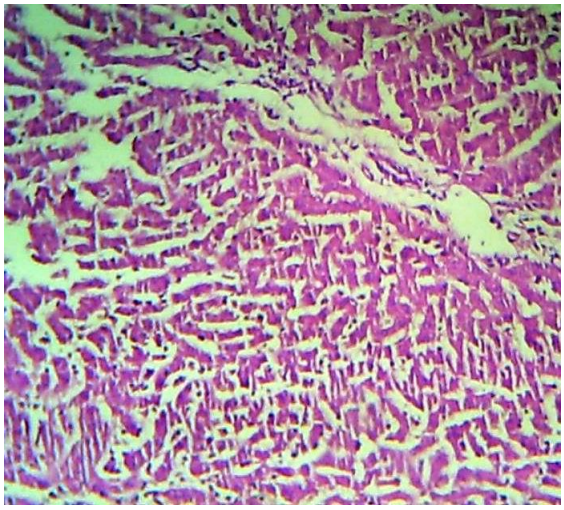
a- Lung treated with Vi and protoplasmic



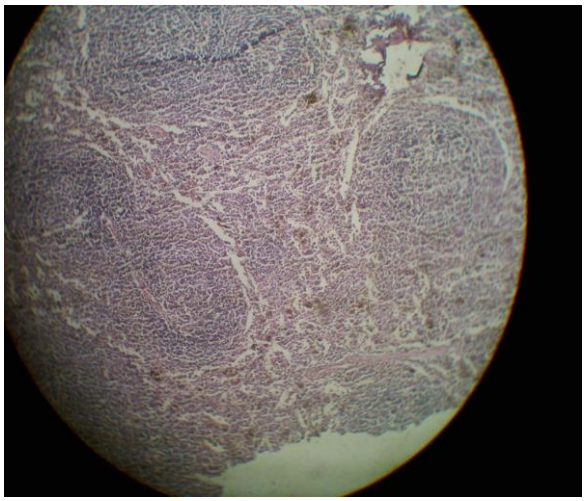
a- Normal lung



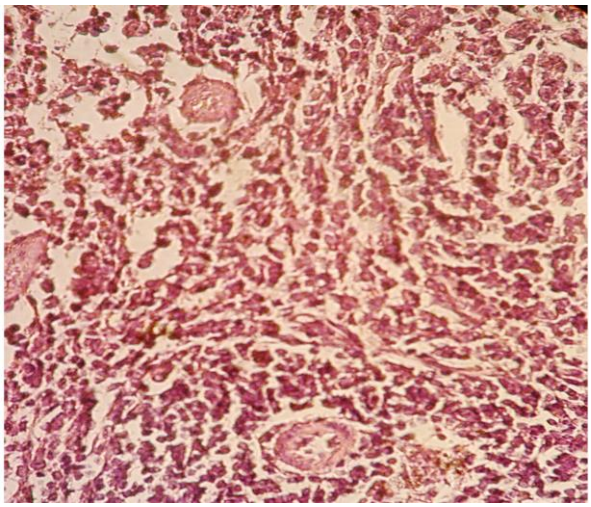
b- Spleen treated with Vi and protoplasmic



b- Normal liver



c- Liver treated with Vi and protoplasmic



c- Normal spleen

Figure (٤-١١) Animal organs treated with Vi and protoplasmic vaccines

Table (٤-١٢) The Ig classes complement fractions, LIFand anti *S. typhi* sonicate Ab titers among ٢٤ elected typhoid patients

Seq.	PHA		LIF		IgG	IgA	IgM	C٣	C.٤
	S	M	S	M					
١	١٢٨٠	٦٤	٠.٣٣	٠.٤٢	٥٢٢.٢	٤٩.٩	٩١.٣	٥٤.٧	٤٠.١
٢	١٢٨٠	٣٢	٠.٣٨	٠.٤١	٨٢٩.٩	١١٤.٧	١١٣.٧	٥٠.٣	١٠
٣	٦٤٠	٣٢	٠.٦٢	٠.٥	٢٥٦.٢	١٦.٦	٣٧.٩	٢٧٠.٥	٢٢.٨
٤	٦٤٠	٣٢	٠.٥	٠.٣٥	٦٤٩	٨١.٧	٩٦.٧	٩٩	٢٥.٢
٥	١٢٨٠	٦٤	٠.٣٥	٠.٣٧	٢٢٣٧.٦	٣٧٦.٩	١٩٣.٩	٢٧٠.٥	٧٣.٧
٦	١٢٨٠	٦٤	٠.٣٥	٠.٣٥	١٧١٥.٣	١١٤.٧	١٤٣.١	١٦٩.٢	٤٧.٧
٧	٦٤٠	٣٢	٠.٣	٠.٣٣	١٤١٧.٥	٤١٦.٩	٢٢١.٦	١٧٥.٨	٥٢.٥
٨	٦٤٠	٣٢	٠.٣٧	٠.٥١	٧٨٣.٤	١٠٦	٢٠٤.٧	١٣٢.٨	٦٤.٥
٩	١٢٨٠	٦٤	٠.٣٧	٠.٣٧	٦٠٥.٨	٥٧.٤	١٧٦.٧	١٢٦.٩	١٧.١
١٠	١٢٨٠	٦٤	٠.٤٥	٠.٣٧	٤٤١.٩	٣٥.٥	١٣١.٥	٨٣.٤	٢٩.٦
١١	٦٤٠	٦٤	٠.٤٦	٠.٤٦	١٤٧٥.٣	١٧١.١	٥٩.٤	٨٢	٤٠.١
١٢	٦٤٠	٦٤	٠.٣٣	٠.٣٧	١٦٩٠.٥	٤٧٤.٧	٢٠٤.٧	١٥١	٤٣.١
١٣	٦٤٠	٣٢	٠.٤١	٠.٣٧	٨٨٨	١٤٢.٤	٩٨.٣	٢٢٤.٧	٣٥.٨
١٤	٦٤٠	٦٤	٠.٣٨	٠.٣٨	٩٧٤.٧	٨١.٧	٧٠.٤	٨٣.٥	٣٠.٤
١٥	١٢٨٠	٦٤	٠.٤١	٠.٤١	١٣٤٢.٢	١٨٠.٩	١٣٧.٧	٨٩.٣	٤٣.١
١٦	١٢٨٠	٦٤	٠.٣٦	٠.٣٣	١٠٧٥.١	٨٩.٨	٢٤٩.٧	٩٩.٥	١٢.٩
١٧	١٢٨٠	٣٢	٠.٣٧	٠.٣٧	١٣٤٢.٢	٢٢٢.٤	١٧٦.٧	٥.١	٢٧.٨
١٨	٦٤٠	٦٤	٠.٥	٠.٣٨	٨٧٧.٣	٥٨.٤	١٥٦.٧	١٢٦.٩	٣٤.٤
١٩	١٢٨٠	١٢٨	٠.٣	٠.٣	٧٣٧.٧	٨١.٧	١١٣.٧	٤٥.٩	١٧.١
٢٠	١٢٨٠	٣٢	٠.٥٢	٠.٥	٤٤١.٩	٤٩.٤	١٩٠.٥	٥٩.٣	٣٣
٢١	١٢٨٠	١٢٨	٠.٥	٠.٤٥	١١٧٩.٦	١٨٠.٩	١٩٠.٥	٩٩	١٩.٣
٢٢	٦٤٠	٣٢	٠.٥	٠.٥	٢٥٦.٢	١٢٣.٦	١١٣.٧	٤٥.٩	١٧.١
٢٣	١٢٨٠	٦٤	٠.٤١	٠.٣٧	٨٢٩.٩	١٧١.١	١٠٧.٩	٣٣.٥	٤٧.٧
٢٤	١٢٨٠	٦٤	٠.٥٤	٠.٣٧	١٠٧٥.٤	٩٧.٤	١٢٥.٥	١٣٨.٨	٤٠.١
x'	١٠١٣.٣	٥٤	٠.٤١٧٠	٠.٣٩٧٥	٩٨٥.٢	١٤٥.٦٥٨	١٤١.٩٣٧٥	١١٣.٢٢٩	٣٤.٣٧٩١
N.V	٢٠-٨٠	٢٠-٨٠	٠.٣	٠.٣	٥٦٠-١٥٠٠	١٠٠-٢١٠	٤٠-٢٠٠	٨٠-١٦٠	٢٠-٤٠
R	٦٤٠-١٢٨٠	٣٢-١٢٨	٠.٣٠-٦٠	٠.٣٠-٦٠	٢٥٦.٢-١٧١٥.٥	١٦.٦-٤٧٤.٧	٣٧.٩-٢٤٩.٧	٣٣.٥-٢٧٠.٥	١٠-٧٣.٧

Table (٤-٩) Mucosal and systemic *S. typhi* specific immunity among(٨٠) patients

Systemic						Mucosal				
No.	Serum protein con.	STO	STH	PHA	LIF	S. Ig con.	STO	STH	PHA	LIF
١	٤٠.٨٥	٦٤٠	٦٤٠	١٢٨٠	٠.٣٣	٣.٧٣٤	٦٤	٣٢	٦٤	٠.٤٢
٢	١١١.٨٢	٦٤٠	٣٢٠	١٢٨٠	٠.٣	٠.١٨٥	٣٢	٣٢	٣٢	٠.٣٣
٣	١٠٧.٣٩	٦٤٠	٦٤٠	١٢٨٠	٠.٦٢	٦.٨	٦٤	٦٤	٦٤	٠.٥
٤	١٠٧.٣٩	٦٤٠	٦٤٠	٦٤٠	٠.٥	٣.٢٩	٦٤	٦٤	٣٢	٠.٥
٥	١١١.٨٢	٦٤٠	٣٢٠	١٢٨٠	٠.٥	١.٥١	٣٢	٣٢	٦٤	٠.٣٧
٦	١٣٨.٤	٣٢٠	٦٤٠	١٢٨٠	٠.٣٣	٠.٦٢٦	٣٢	٦٤	٦٤	٠.٣٧
٧	١٥١.٧٥	٣٢٠	٣٢٠	٦٤٠	٠.٣	٠.١٨	٣٢	٣٢	٣٢	٠.٣٣
٨	٩٤.٠٨	٣٢٠	٣٢٠	٦٤٠	٠.٣٧	٠.٦٢	١٦	١٦	٣٢	٠.٥١
٩	١٦٠.٦	٦٤٠	٦٤٠	١٢٨٠	٠.٣٧	٢.٨٤	٦٤	٦٤	٦٤	٠.٣٧
١٠	١٠٧.٣٩	٦٤٠	٦٤٠	١٢٨٠	٠.٤٥	١.١٦	٦٤	٦٤	٦٤	٠.٣٧
١١	١١١.٨٢	٦٤٠	٦٤٠	٦٤٠	٠.٤٦	١.١٦	٦٤	٦٤	٦٤	٠.٤٦
١٢	٩٤.٠٨	٦٤٠	٦٤٠	٦٤٠	٠.٣٧	١.١٦	٣٢	٣٢	٣٢	٠.٣٧
١٣	١١١.٨٢	٦٤٠	٦٤٠	٦٤٠	٠.٤١	١.٥١	٦٤	٦٤	٣٢	٠.٣٧
١٤	١٥١.٧٥	٦٤٠	٦٤٠	٦٤٠	٠.٣٨	٠.١٨	٦٤	٦٤	٦٤	٠.٣٨
١٥	١١١.٨٢	٣٢٠	٣٢٠	١٢٨٠	٠.٤١	١.٠٧	٣٢	٣٢	٦٤	٠.٤١
١٦	١٦٠.٦	٣٢٠	٣٢٠	١٢٨٠	٠.٣٦	٢.٨٤	٣٢	٣٢	٦٤	٠.٣٣
١٧	٩٨.٥١	٦٤٠	٦٤٠	١٢٨٠	٠.٣٧	٠.٦٢	٣٢	٦٤	٣٢	٠.٣٧

١٨	١١٦.٢	٣٢٠	٣٢٠	٦٤٠	٠.٥	٣.٧٣	٦١	٨	٦٤	٠.٣
١٩	٩٨.٥١	٦٤٠	٦٤٠	١٢٨٠	٠.٥٣	١.٠٧	٦٤	٦٤	١٢٨	٠.٥
٢٠	١٣٨.٤	٣٢٠	٣٢٠	١٢٨٠	٠.٥٢	٠.٦٢	٣٢	٣٢	٦٤	٠.٥
٢١	٧٦.٣	٣٢٠	٣٢٠	١٢٨٠	٠.٥	١.٥١	٣٢	١٦	١٢٨	٠.٤٤
٢٢	٨٤.٣	٦٤٠	٣٢٠	١٢٨٠	٠.٥	٤.٦	٦٤	٣٢	١٢٨	٠.٥
٢٣	٨٥.٢١	٣٢٠	٣٢٠	١٢٨٠	٠.٤١	٢.٦	٣٢	٣٢	٦٤	٠.٣٧
٢٤	١٢٩.٥٧	٦٤٠	٦٤٠	١٢٨٠	٠.٥٤	١.٥١	٦٤	٦٤	٦٤	٠.٣٧
٢٥	١٢٩.٥٧	٣٢٠	٦٤٠	١٢٨٠	٠.٥	١.٩٥٩	٣٢	٦٤	١٢٨	٠.٤
٢٦	١١١.٨٢	٦٤٠	٦٤٠	٦٤٠	٠.٤٥	١.١٦	٦٤	٦٤	٦٤	٠.٤
٢٧	٨٤.٨٥	٦٤٠	٣٢٠	١٢٨٠	٠.٤	١.٥١	٦٤	٣٢	١٢٨	٠.٣
٢٨	١٢٩.٥٧	٣٢٠	٣٢٠	١٢٨٠	٠.٤١	٣.٧٣	٣٢	٣٢	٦٤	٠.٣
٢٩	٨٥.٢١	٦٤٠	٣٢٠	١٢٨٠	٠.٤	٣.٧٣	٦٤	٣٢	٦٤	٠.٥
٣٠	٨٥.٢١	٦٤٠	٦٤٠	٣٢٠	٠.٥	٣.٢٩	٦٤	٣٢	٣٢	٠.٣٦
٣١	١٠٧.٣٩	٦٤٠	٦٤٠	١٢٨٠	٠.٤	٠.١٨	٦٤	٦٤	١٢٨	٠.٤
٣٢	١٠٣.٣	٦٤٠	٣٢٠	١٦٠	٠.٤٥	٠.١٨	٣٢	٣٢	١٢٨	٠.٣٦
٣٣	١٠٧.٣	٣٢٠	٣٢٠	١٢٨٠	٠.٣٣	٠.١٨	٣٢	٣٢	١٢٨	٠.٣٧
٣٤	١١١.٨٢	٣٢٠	٣٢٠	١٦٠	٠.٣	١.١٦	٣٢	٣٢	١٢٨	٠.٥
٣٥	٧٦.٣	٦٤٠	٦٤٠	١٢٨٠	٠.٥	١.١٦	٦٤	٦٤	٦٤	٠.٤
٣٦	١٢٩.٥٧	٦٤٠	٣٢٠	٦٤٠	٠.٣٦	١.٥٩	٦٤	٣٢	٦٤	٠.٣
٣٧	١٢٩.٥٧	٣٢٠	١٦٠	٦٤٠	٠.٤	٠.١٨٥	٣٢	١٦	٦٤	٠.٣٧
٣٨	١١٦.٢	٣٢٠	١٦٠	٦٤٠	٠.٤	١.٥٩	٣٢	١٦	٣٢	٠.٤
٣٩	١١١.٨٢	٦٤٠	٦٤٠	١٢٨٠	٠.٤١	٢.٦	٦٤	٦٤	١٢٨	٠.٣٣

٤٠	١٢٩.٥٩	٦٤٠	٦٤٠	٦٤٠	٠.٢٧	٠.١٨	٦٤	٦٤	٦٤	٠.٤
٤١	٥٨.٥١	٣٢٠	٣٢٠	٦٤٠	٠.٣٣	٣.٧٣	٣٢	٣٢	٦٤	٠.٣
٤٢	١٢٩.٥٧	٦٤٠	٣٢٠	١٢٨٠	٠.٣٧	١.١٦	٦٤	٣٢	١٢٨	٠.٣٧
٤٣	١١١.٨٢	٦٤٠	٦٤٠	١٢٨٠	٠.٣٣	١.٥	٦٤	٦٤	١٢٨	٠.٤
٤٤	١٦٠.٦	٦٤٠	٣٢٠	٦٤٠	٠.٣٧	١.١٦	٦٤	٣٢	٦٤	٠.٣٣
٤٥	١١٦.٢	٣٢٠	٣٢٠	٦٤٠	٠.٣	١.١٦	٣٢	٣٢	٣٢	٠.٣٧
٤٦	١٠٧.٣	٣٢٠	٦٤٠	٦٤٠	٠.٣	٣.٧٣	٣٢	٦٤	٦٤	٠.٣٧
٤٧	١١٦.٢	٦٤٠	٦٤٠	١٢٨٠	٠.٣٣	٣.٨٤	٦٤	٦٤	١٢٨	٠.٢
٤٨	٩٨.٥١	٦٤٠	٣٢٠	١٢٨٠	٠.٤١	٣.٢	٦٤	٣٢	١٢٨	٠.٥
٤٩	١١١.٨٢	٣٢٠	٣٢٠	٦٤٠	٠.٣٦	٣.١٦	٣٢	٣٢	٦٤	٠.٤٢
٥٠	١٠٧.٣٩	٦٤٠	٦٤٠	١٢٨٠	٠.٤١	٤.٦	٣٢	٦٤	١٢٨	٠.٤
٥١	٨٤.٣	٣٢٠	٣٢٠	٣٢٠	٠.٥٨	٢.٦	٦٤	٣٢	٣٢	٠.٤
٥٢	١٠٧.٣٩	٦٤٠	٣٢٠	١٢٨٠	٠.٣٦	١.١٦	٣٢	٣٢	١٢٨	٠.٣٧
٥٣	١١١.٨٢	٦٤٠	٦٤٠	٦٤٠	٠.٥٤	١.٠٧	٦٤	٦٤	٣٢	٠.٤٤
٥٤	١٦٠.٦	٦٤٠	٦٤٠	١٢٨٠	٠.٤	١.٥١	٦٤	٦٤	١٢٨	٠.٣٧
٥٥	١١٦.٢	٦٤٠	٦٤٠	١٢٨٠	٠.٤	١.١٦	٦٤	٦٤	١٢٨	٠.٢٨
٥٦	٩٨.٥١	٣٢٠	٣٢٠	١٢٨٠	٠.٣٦	١.٩٥	٣٢	٣٢	١٢٨	٠.٣٣
٥٧	١٦٠	٦٤٠	٦٤٠	١٢٨٠	٠.٤٣	٢.٦	٦٤	٦٤	١٢٨	٠.٤٤
٥٨	١١٦.٢	٦٤٠	٦٤٠	٦٤٠	٠.٤١	٦.٨	٦٤	٦٤	٦٤	٠.٤
٥٩	٩٤.٨	٦٤٠	٦٤٠	١٢٨٠	٠.٣	٢.٨	٦٤	٦٤	١٢٨	٠.٣٧
٦٠	١٠٧.٣٩	٦٤٠	٣٢٠	٦٤٠	٠.٥٨	١.١٦	٦٤	٣٢	٦٤	٠.٤٥
٦١	٩٨.٥١	٣٢٠	٣٢٠	١٢٨٠	٠.٤	١.٠٧	٣٢	٣٢	٦٤	٠.٥

٦٢	١١٦	٦٤٠	٦٤٠	١٢٨٠	٠.٥٤	١.٩٥	٦٤	٦٤	١٢٨	٠.٥
٦٣	١٢٩.٥	٣٢٠	٣٢٠	١٢٨٠	٠.٥	١.٥١	٣٢	٣٢	٦٤	٠.٥
٦٤	١٥١.٧٥	٦٤٠	٦٤٠	١٢٨٠	٠.٢	٦.٨	٦٤	٦٤	١٢٨	٠.٥
٦٥	٩٤.٠٨	٦٤٠	٦٤٠	٦٤٠	٠.٣	١.١٥١	٦٤	٦٤	٦٤	٠.٣٧
٦٦	١٠٧.٣١	٣٢٠	٦٤٠	١٢٨٠	٠.٣٣	٦.٨	٣٢	٣٢	١٢٨	٠.٥
٦٧	١٥١.٧٥	٦٤٠	٦٤٠	٢٥٦٠	٠.٣٣	١.١٦	٦٤	٣٢	١٢٨	٠.٣٣
٦٨	٩٨.٥١	٣٢٠	٣٢٠	١٢٨٠	٠.٤٥	٣.٢٩	٣٢	٣٢	١٢٨	٠.٤
٦٩	١١٦	٦٤٠	٣٢٠	١٢٨٠	٠.٣٣	١.٩٥	٦٤	٦٤	١٢٨	٠.٣
٧٠	١١١.٨٢	٦٤٠	٦٤٠	١٢٨٠	٠.٤١	١.١٦	٦٤	٦٤	٦٤	٠.٣٦
٧١	٩٤.٠٨	١٦٠	١٦٠	٦٤٠	٠.٦	١.٠٧	١٦	١٦	٦٤	٠.٣
٧٢	١٥١.٧٥	٣٢٠	٣٢٠	١٢٨٠	٠.٤٥	١.١٥١	٣٢	٣٢	٦٤	٠.٤٤
٧٣	١٠٧.٣	٦٤٠	٣٢٠	١٢٨٠	٠.٣٦	١.٠٧	٦٤	٣٢	١٢٨	٠.٤
٧٤	٨٥.٢١	٣٢٠	٣٢٠	١٢٨٠	٠.٤١	١.٩٥	٣٢	٣٢	١٢٨	٠.٤٤
٧٥	١٢٩.٥	٣٢٠	٣٢٠	٦٤٠	٠.٣٣	٤.٦	٣٢	١٦	٦٤	٠.٤
٧٦	١٢٩.٥	٦٤٠	٦٤٠	١٢٨٠	٠.٤١	١.١٦	٦٤	٦٤	١٢٨	٠.٦
٧٧	٩٤.٠٨	٣٢٠	٣٢٠	١٢٨٠	٠.٣٦	٤.٦	٣٢	٣٢	٦٤	٠.٤
٧٨	٩٨.٥١	٦٤٠	٦٤٠	٦٤٠	٠.٥	١.٩٥	٦٤	٦٤	٦٤	٠.٦
٧٩	٩٤.٨	٣٢٠	٣٢٠	١٢٨٠	٠.٥	١.٩٥	٣٢	٣٢	١٢٨	٠.٤
٨٠	١٦٠	٦٤٠	٦٤٠	١٢٨٠	٠.٤	١.٩٥	٦٤	٦٤	١٢٨	٠.٤
X'	١١٣.١	٥١٤	٤٧٠	١٠٤٤	٠.٤٠٩٨٨	٢.٠٩٨١	٤٩.١٦٢٥	٤٥.٠٦٢٥	٨٤.٨	٠.٣٩٩٧٥
R	٧٠-١٢٩	١٦٠-٦٤٠	١٦٠-٦٤٠	١٦٠-٢٥٦٠	٠.٣٠-٠.٧٠	٠.١٨-٢.٨٥	١٦-٦٤	١٦-٦٤	١٦-١٢٨	٠.٣٠-٠.٦٠
N.V.	٤٠-١٦٠	٤٠-٨٠	٤٠-٨٠	٨٠-١٦٠	٠.٨٥-٠٩٥	٠.١٨-١١	٤-٨	٤-٨	٨-١٦	٠.٢٥-٠.٩٠

٥. Discussion

٥.١. A. Synopsis

Humoral serum antibody response in typhoid patients had been tackled in this area and utilized as an infection probe (Shnawa and Hindi , ١٩٩٦) . Gut mucosal antibody responses in typhoid patients had been evaluated an infection probe (Shnawa and Al-Saadi ,٢٠٠١) .

The utility of both mucosal and serum antibodies for diagnosis of typhoid patients was attempted (Abid and Shnawa , ٢٠٠٢) . The seroprofile and infection forms of typhoid were reported in this area (Shnawa and Al-Amidi , ٢٠٠٢) .

The humoral mucosal and systemic as well as cellular mucosal and systemic responses are being attempted to plot the immune status of typhoid patients , mediated by *S. typhi* natural infection together with laboratory animal infection and immune models were verified .

٥.١.B. Human *S. typhi* infection

The clinically diagnosed typhoid fever patients were proved by culture and serologic diagnosis (Collee *et al*., ١٩٩٦ ; Ciannella , ٢٠٠١) .

The extracted and proved to be *S. typhi*. Vi and protoplasmic sonicate antigens were assayed biologically and immunologically and found to be constant with the criteria of Vi and protoplasmic sonicate antigens (Taylor *et al.*, ١٩٨٣ ; Zuzana *et al.*, ١٩٩٧ ; Timothy *et al.*, ٢٠٠٠) .

٥.٢ . *S. typhi* infection in rabbits

The oral *S. typhi* lapin infection model (Section ٤ - ٤ - Figure ٤-١) was shown to have intestinal and extra-intestinal manifestations ,where liver , spleen and lungs are mosetly affected . Such finding may be in general agreement with Taha and Al-Hindawi (١٩٧٩) , working on rabbit and Gunia pigs in salmonella other than *S. typhi*, but in our *S. typhi* oral

rabbit model was more severe . Taha and Al-Hindawi (١٩٧٩) had shown that rabbits were more sensitive to infection than Guina pigs , and the infection had been mainly intestinal . Salmonella are pathogenic in animals that are acting as reservoir for human infection like rodents and pets (Brooks *et al.*, ٢٠٠٤).

Experience on other lab animals models such as mice strains defective in induction of endocytosis by epithelial cells have virulence defects compatible with a defect in crossing the intestinal mucosal barriers (Galan and Curtiss , ١٩٨٩ ; Miller and Phop , ١٩٩٣) .

٥.٣ Serology

The circulating mucosal *S. typhi* specific haemagglutinins was higher than agglutinins . Circulating haemagglutinins and circulating agglutinins are higher than mucosal haemagglutinins and agglutinins (Table , ٤-٩) . However , they are can delineated then an is the following (Table ٥-١).

Table (٥-١) Agglutinins and haemagglutinins at mucosal and systemic titers

No.	Titer	Haemagglutinins		Agglutinins	
		Systemic	Mucosal	Systemic	Mucosal
١	Clinical	٣٦٠-١٢٨٠	٣٢-١٢٨	١٦٠-٦٤٠	١٦-٦٤
٢	Sub Clinical	> ١٦٠	> ١٦	٤٠-٨٠	٤-٨
٣	Base line	> ١٦٠	> ١٦	١٠-٢٠	٢-٤

The above mentioned titer limits were in agreement with (Shnawa and Al-Saadi , ٢٠٠١ ; Shnawa and Mehdi , ٢٠٠٤ as well as Abid and Shnawa , ٢٠٠٢) .

٥.٣.١ Prozone phenomenon

This phenomenon occurred in the second tube in standard tube agglutination test of *S. typhi* because the inhibiting effect of the antibody concentration excesses the optimal ratio of antibody to antigen called antibody excess ,or it may be due to the presence of blocking antibody (Al-Saadi , ١٩٩٨) .

٥.٣.٢ . ٢-ME effect

٢ME was effective on two cases of STO and STH for both fecal and serum samples among typhoid patients . In both cases the agglutination reaction was reduced by ٢-ME .

The failure of ٢ME to reduce antibody clearly implies that there is some I.g resistance to ٢-ME action , while others are sensitive (Al-Saadi , ١٩٩٨) .

٥.٣.٣ . Clinical titers

S. typhi antigens O and H were used for the determination of Abs titers indicated , at the systemic level .The STO and STH were ٦٤٠ as highest titers in almost all patients , while the mucosal response were ٦٤ for both antigens (Table ٤-٩) . This results were in an agreement with Abid and Shnawa (٢٠٠٢) . The mean values of STO and STH were ٥١٤ and ٤٧٤ respectively , at systemic level , while the mean values of STO , and STH were ٤٨.٦ , ٤٤.٦ respectively at mucosal level among typhoid patients .

The results as in table (٤-٩) showed that the passive hemagglutination titers increased and the titers of Abs also increased in typhoid patients with PHA to reach ١٢٨٠ at systemic response and ١٢٨ at mucosal response , which were more than the base line titers in normal subjects .

The mean values of PHA titers were ١٠٣٦ at systemic level ,but the mean values of PHA titers were ٨٤.٨ at the mucosal level , in general ; the titer in systemic response is higher than the mucosal response.

٥.٣.٤ . Quality control of immunological test

The quality control was calculated by two indices which are specificity index and sensitivity index .

In this study , we compared the quality control of humoral immunity (Widal , PHA and LIF) with cellular immunity .

The sensitivity index value of Widal – O - Ag or PHA was increased about ٠.٩٨ , in addition , the LIF test ٠.٩٨ . However , the specificity index values were decreased in humoral and cellular response , it is about ٠.٩٠ for Widal test , ٠.٨٥ for PHA test and ٠.٩٥ for LIF test . These results are similar to (Al-Saadi , ١٩٩٨ ; Abid , ٢٠٠٠) .

The sensitivity index is refer to *S. typhi* antigens ability to react with little amount of specific antibodies. Also , the specificity index referrs that the agglutination reaction was affected by biological factor as an antigen ;formed by pre exposure in mucosal and systemic responses .

The results showed that the mucosal response which was used for typhoid diagnosis by Widal test , and does not differentiate between mucosal and systemic responses among typhoid patients except in titers ratio .

٥.٤ . Cellular immunology

A cell - mediated response is characterized by a sequence of reaction triggered by T - lymphocytes coming into site where antigen is present .The cell becomes activated by interaction with the antigen through the presence of specific cell receptors (Sensitization) following subsequent contact with antigen (Challenge) .

The cell produces and releases a variety of effectors molecules called lymphokines (Weinberg ,١٩٨٤ ; Weir,١٩٩٤).

There are biochemical mediators of a number of widely studied cases in vitro phenomena , but it is believed that a similar activity is responsible for the immune response seen in tissues , the best characterized lymphokin is LIF the subject of present assay (Table . ٤-٩). Upon release from lymphocytes , LIF can be identified by this ability to trap macrophage and inhibiter their migration . The macrophage is the target cell responding to the product LIF of lymphocyte which is the effectors cell (Lo , ١٩٩١ ; Adoose , ٢٠٠٥) .

٥.٤.١ . LIF test

The cell free culture filtrate of *S. typhi* as a sensitizer induces secretion of leukocyte inhibition factor (LIF) from the already sensitized lymphoid cells in peripheral blood and in the inflammatory cells of faecal matter.

The data presented in (Table . ٤-٩) indicate that *S. typhi* induce specific cellular immunity when culture filtrate were used as a sensitizer , the mean values of LIF were ٤١٪ and ٤٠٪ at systemic (Peripheral blood) and mucosal system respectively .The results showed that the LIF formation increased in both peripheral blood and faecal leukocyte and more in patients than in those normal subjects .

Such increase was statistically significant at ٣٠٪ level . It we found higher LIF at systemic than at mucosal level ,when comparing the systemic and mucosal LIF value with control .

٥.٥ . The immune status of typhoid patients

The immune specific to infection is dynamic and not static state . It depends first the genetically controlled responses (Innate) which are either non , moderate with high responsive and this appeared to be linked to MHC complex and to its immunologic functions (Abid , ٢٠٠٠ ; Robert *et al.* ٢٠٠١) .

Second environmental exposure such as natural infection and /or vaccination (Robbins *et al.*, ١٩٩٩ ; Giannella , ٢٠٠١; W. H. O. ٢٠٠٤) .

While the third position is the nutritional and field status and fourth an the extent of the occurrence of the other underlying chronic metabolic and infectious diseases ,finally as well as age of effects .

However, have effect on the immune statue of typhoid patients , these in every of the applied immune function tests on the patients and control . There were normal , subnormal and abnormal level of the immune function parameters . Hence , when all of them , are viewed together , they showed (١٢) different immune profiles in those tested typhoid patients (Table . ٤-١٢) . Such finding may be rarely studied .

٥.٥.١ . Typhoid Herd Immunity

Herd immunity is multifactorial state , namely immunologic , genetic and environmental (Kauffman ,١٩٩٥) .

Typhoid herd immunity is determined by natural exposure to *S. typhi*, vaccination history and the host genetic make up (Miller *et al.* ١٩٩٩) . Thus , B cell distribution curve of low , medium and high responses are obtained (Kauffman,١٩٩٣)

٥.٥.٢. Global immune state

Our data (Table . ٤-٩) indicates an increase of titers of antibodies in systemic and mucosal for STO and STH among typhoid patients . *S. typhi* antigen agglutinins titers were bound to elevate during the infection process at both systemic and mucosal antibody responses in comparison with that of normal subjects .

The total protein of serum and mucosal Ig concentration was distributed in (low , moderate and high) level in *S. typhi* enteric fever , the protein and I.g concentration associated with STO and STH titers .

Meanwhile , *S. typhi* induce a rise of antibodies titers ; it is associated with high PHA titer and significant LIF with CFCF in both peripheral blood and faecal leukocyte and more in patients than those of normal subjects .

The *S. typhi* specific antigens are rather high systemic and serum antibodies and higher in serum in comparison to mucosal and LIF activity which is more significant in systemic response comparison to mucosa .

In general *S. typhi* stimulate both systemic and local humoral immune response as indicated by agglutinins STO , STH-antigens and haemagglutinins (PHA) .Haemagglutinin titers were higher than those of agglutinins at mucosal and systemic levels . Significant migration inhibition was noted in primed as compared to non . *S. typhi* inhibition control subjects .

The important of the mucosal immune system was quickly realized after the discovery of that IgA was the most abundant isotype of antibody in secretion as evidenced by both I.g levels and presence of IgA.IgM producing cells (Brandtzeag and Farsted , ١٩٩٩) .

The result may indicate the presence of *S. typhi* antigen epitope , either of the direct *B.* lymphocyte activation or an epitope that activates

the helper T-cells ($T_{h٢}$) which activates *B*. lymphocyte to proliferate . The local mucosal epitope stimulation leads to both mucosal and systemic antibody responses (Johansen *et al.*, ١٩٩٩ ; Adoose , ٢٠٠٥) .

٥.٥.٣ Immune status of an elected cases

The twenty four typhoid patients showed an evidence of individual variation in their immune responses ; this may be attributed to effect in HLA etitis and MHC complex nature in each of them (Strober and Kelsall , ١٩٩٩ ; Strober *et al.*, ١٩٩٩) .

Test for B . cell functions showed increase in antibody synthesis as well as secretion . Test for T- cell function such as the LIF indicates that there was an antigenic epitope that activatd T-cells to release LIF cytokines that mediate in hypersensitivity (Mowat and Fergusoh , ١٩٨١; Strober *et al.*, ١٩٩٩).

The decrease of immunoglobulins and complement levels among elected typhoid patient can be attributed to either more catabolic action or less synthesis (Ogawa *et al.*, ١٩٨٩) .

Such variation in immune functions , within some patients reflect variation in immune recognition systems like antibody , complement , phagocyte and /or MHC (Brooks *et al*., ٢٠٠٤) .

٥.٦ . *S. typhi* protective immunity in rabbits

٥.٦.١ . IFA immunomodulatory effect

Non specific immune protection against live *S. typhi* as guessed by histological evidence protection percentages and death rates . Such findings were parallel to those reported by other workers (Taylor , ١٩٩٤ ; Zuzana *et al.* ١٩٩٧) .

This can be explained on the basis of one or more of the following :

i- Increase of antigen uptake by macrophages .

ii- Increase of phagocyte antigen presenting to T-cell .

iii- Trigger more antibody synthesis by B- cells .

Thus IFA acts here as non specific immune stimulate or non specific immune modulator . Through antigen targeting , cytokine not working activation and enhancing antibody production (Bachmann , ١٩٩٧ ; Robert *et al.*, ٢٠٠١) .

٥.٦.٢ . Immunology

The immune state as traced by circulating agglutinin and LIF was parallel to that case of human typhoid patients (Miller *et al*., ١٩٩٦) .

The results of post vaccine and post challenge immune state evaluations , death rate and protective rate as well as histopathology investigation indicate that Vi and protoplasmic sonicate antigens were immunoprotective against live - *S. typhi* challenging in a lapin model (Robbins and Robbins , ١٩٨٤ ; Zuzana *et al.*, ١٩٩٧; Timothy *et al.*, ٢٠٠٠).

Detailed discussions about immune protection are stated in the followings sections .

٥.٦.٢.١ IFA Immune protection

The effective protection was observed when the IFA was administered within ٢٤ hr after the bacterial challenge . The protection rate of IFA primed was ٨٠٪ while the non IFA primed was ٠% .

IFA influences virtually every aspect of immune response to antigen , which affect serum IgG and mucosal IgA responses to unrelated antigens administered orally at the same time (Taylor, ١٩٩٤ ; Robert *et al*., ٢٠٠١) .

٥.٦.٢.٢. Vi-immune protection .

Immunity to *S. typhi* requires both cell mediated and humoral immune responses and is achievable by vaccination (Forrest *et al*., ١٩٩١) .

S. typhi. Vi-Ag used as oral vaccine elicits immune response (Table-٤-١٣ a) and the protection rate of Vi - vaccine was ١٠ % , while the death rate was ٠/٥ . The results demonstrated that the oral immunization with Vi-antigen elicits strong systemic cell mediated immunity to live infection bacteria .

The data presented in(Table-٤-١٣-b) showed that the titer of antiserum to Vi-Ag was the highest in both pre and post challenge with - *S- typhi .*

Moreover , the PHA titers was highest when treatment with Vi-vaccine in both challenge . Then inducing an antibody and cellular immune response, significant LIF were ٣٠ % and the mean value were ٤٠ for both challenge .

Very effective protection was observed when Vi- vaccine administered within ٢٤ hr after challenge . This effect could be increased with repeated injections .

The result showed that the Vi-antigen with IFA, could be more effective than Vi-antigen alone . This finding agrees with Zuzana *et al.,* (١٩٩٧) and Sharn , (٢٠٠٤) .

Vaccine studies indicate that Vi- polysaccharide antigen should be an important immune target , since parental immunization with this antigen has lead to increased protection against *S-typhi* (Acharya *,* ١٩٨٧; Klugman, ١٩٨٧) .

٥.٦.٢.٣ . Protoplast sonicate immune protection .

The human restricted intracellular pathogen *Salmonella typhi* is the causative agent of typhoid fever .It' survives within macrophage by a number of mechanisms, including suppression of macrophage activity . They have recently shown that upon incubation of human peripheral blood with *S- typhi* soluble protoplast , there is a rapid production of the proinflammatory cytokines TNFα , IL, as well as the production of IL-٦ and IL١. and INF γ (Timothy *et al.,* ٢٠٠٠) .

The data presented in Table (٤-١٥.a) indicate that protoplast sonicate immune protection was ١٠٠ % in lab animals without symptoms when compared with control . Vaccination of rabbits , with *S-typhi* protoplast antigen gives partial immunity following the administration in animals .

The enlisted data (Table-٤-١٥-b) indicate an increase of titer of antibodies at systemic level for S TO and STH antigens. PHA, titer of antibodies also increased in animals with protoplast sonicate to reach ١٢٨٠ which were more than the base line titers in normal animals at both pre and post challenge .

The results showed the *S-typhi* soluble antigens induce specific cellular immunity when culture filtrates were used as a sensitizer . The mean values were ٣٦ and ٣٥ respectively at the systemic level (Peripheral blood of animals) . There is a significant increase in the inhibition of migration when comparing the systemic LIF value with control . This indicates that the protoplast antigen induces a cellular immune response at the pre- and post challenge of live infection bacteria .

This finding agrees with Mowat and Fergusoh (١٩٨١), who noted that an immune response to a protein antigen of *S-typhi* administered via the mucosal route can be expressed as mucosal immune response with

S-IgA antibody systemic immune responses priming with development of serum IgG, IgM and specific cellular immunity .

٥.٦.٢.٤ . *S-typhi* skin test .

The purified protein derivative of *S-typhi* protoplast sonicate is used to determine the *S-typhi* skin test on lab animals; the immune system will recognize and interacts with the protein as cellular antigens because it is similar to *S-typhi* that causes infection . This will cause the appearance of a delayed –type hypersensitivity (DTH) at the site of injection (Mowat and Fergusoh,١٩٨١; Mostove and Kaetzel,١٩٩٩)) . As a reaction , *S-typhi* skin test is mediated by T-cells following intradermal injection of protein purified , T-cell derived TNFα and TNFβ act on endothelial cell in dermal blood vessels to induce the sequential expression of the adhesion molecules E- selectin, ICAM١ , and VCAM-١ . These molecules aid in the bringing of leukocyte to the site of the reaction resulting infiltration of leukocyte after ٤ hours , but monocyte and T-cells mainly CD ٤ T-cells are replaced after ١٢ hours , erythemea and indurations develop and reach their peak ٢٤-٧٢ hr (Parslow and Bainton ٢٠٠١) .

Depending on the results (Table ٤-١٦) of *S-typhi* skin test . The Judgment for the positively of this is best based on the measurement of the diameter of indurations area at the site of injection after ١٢ hr, erythemea (Redness) around the indurated area in positive reaction appeared at ٦-١٨hr . The dimeter of ≥ ٥ mm was considered to be positive result (Cohen and Schifferli , ٢٠٠٠) . A positive *S-typhi* skin test was correlated with significant LIF value at pre and post challenge;

the mean values for both were ٣٦ and ٣٥ % respectively , when compared with normal values .

Delayed hypersensitivity reactions are characteristically induced by intracellular infections agents including *Salmonella*, *brucellae* and a wide range of viruses (Brostoff, ١٩٧٣) .

The *S. typhi* protoplast sonicate response which occurs when an intradermal injection of ٠.١ ml of a ١ in ١٠٠٠ dilution of protein extract of protoplast (Purified protein derivative , PPD) , ٢٤- to ٤٨ hr later an indurated inflammatory reaction of variable size can be seen in the skin . The injection site is infiltrated with a large number of mononuclear cells , mainly lymphocytes (١٠-٢٠%) and macrophages .

٥.٦.٣ Histopathology.

The animal tissues with IFA treatment , showed changes. The lung section showed vascular congestion with hemorrhage in the alveolar space wall , while the spleen infection showed reactive follicular hyperplasia . The liver section was normal (Figure ٤-١٠).

Results showed that the rabbits tissues (liver, , spleen and lung) when treated with Vi-vaccine and protoplast sonicate vaccine were normal; this finding agrees with Fredrick *et al*., (٢٠٠٢) .

Miller *et al*., (١٩٩٩) referred to the major of *S. typhi* appearing to be important in pathogenisis ; the lipid A components of LPS are potent toxin for mammalian cells and infection of mice .

٥.٧ . *S. typhi* infection ; Major immune features

S. typhi clinical infection was proved bacteriologically and immunologically in men. Laboratory animal infection model was demonstrating susceptible lapin model .

Circulating and mucosal haemagglutinin and agglutinin titres were classified as clinical , sub clinical , suspect as well as base line titer limits.

The immune status of typhoid patients were delineated with the following major immune features :

***** High circulating and mucosal *S. typhi* specific antibody .

***** Mucosal antibody both locally produced and transducted fractions from serum .

***** Fecal non specific leukocyte infiltrate .

***** Significant production of leukocyte inhibitory cytokines .

Herd or population immunity were low , moderate and high responses . *S. typhi* Vi and protoplasmic sonicate antigens were found immune protected against live *S. typhi* infections .

This infection lab model stimulates the case in men and the immune status in lab model which is equivalent with that in typhoid in men .

Protoplasmic (PPD) preparation produces classical tuberculin type is delayed type of hypersensitivity .

Conclusions

***** *S. typhi* antigenic epitopes are T cell dependent epitopes th_1 and th_2 besides an allergic epitopes activity T_{dth} .

***** The *S. typhi* clinical isolates bear Vi antigens .

***** Somatic as well as flagellar antigens were inducing humoral mucosal as well as system specific antibody responses with ratio of ١٠ : ١ , ٥ : ١.

***** The immune status profiles are different in different patients . It approrches ٢١ profiles .

***** *S. typhi* isolate produce a degree and immune state in rabbit somewhat similar to that in men . It was intestinal and extraintestinal .

***** Vi - antigen and protoplasmic sonicate antigen induce immune protection are experimental challenge .

***** ٢-ME sensitive and ٢-MF resistance antibody fraction was detected in the patients .

***** Prozone phenomena was detected .

***** Typhoid patients , exhibit with low , moderate and high immune response to *S. typhi* immunogen .

***** Vi and protoplasmic sonicate is of equal immune protection ability in rabbits.

- Recommendations

***** Detection of proinflammatory , inflammatory and post infection cytokine in typhoid patients where is clinical benefical.

***** Prepare more purified Vi – antigen and use it in vaccination program after vaccine evaluation studies .

***** Abid ,F.J., (٢٠٠٠). Comparative study between local and systemic humoral immune response in typhoid patient . M. Sc. Thesis . Babylon University.

*****Abid , F.G and Shnawa I.M.S. (٢٠٠٢) Comparative study between local and systemic immune response typhoid patients . Iraq J. of Micro .

***** Acharya , l.L., (١٩٨٧) . Prevention of typhoid in Nepal with Vi – capsular polysaccharid of *S. typhi* . N ,Engl . J. Med ., ٣١٧, P:١١٠١-١١٠٤ .

***** Adoos , S.A., (٢٠٠٥). The correlation between mucosal and systemic immune response to antigen of bacteria associated with chronic ear discharge .PH.D .Thesis, College of Science. Babylon University.

***** Agunod , M. ; Yamaguchi , N. ; Lopez , R. (١٩٦٩) . Correlative study of hydrochloric acid , Pepsin , and intrinsic factor secretion in newborns and infants . Am, J. Dig. Dis., ٩ (٤٥) : ١٤-٢٦ .

***** Akira ,S. H.; Sugita ,T.; Nishio, Y. and Kishmoto, T.. (١٩٩٣) . A nuclear factor lL-٦ expression (NF-lL٦) is a member of ac IEBP family .J. MBO.,٩,P : ١٨٩٧ .

*****Al-Saadi,M.A.(١٩٩٨) Separation of Intestinal Secretory Immunolglobulins. M.Sc., Thesis .Babylon University . Iraq .

*****Aserkoff, B. and Bennett ,J.V., (١٩٦٩).Effect of antibiotic therapy in acute Salmonellosis an the fecal secretion of *Salmonella* .N.J .Med. ٢٨١,PP : ٦٣٦ – ٦٤٠ .

*****Bachmann M.F., *et al.*, (١٩٩٧) The role of antibody concentration and avidity in p rotection . J .Science ,٢٦٧:٢٠٢٤.

*Bishop ,M .C;Dben-Laufer ,J.L.; Fody, E.p and thirty three contributors (١٩٨٥). Clinical Chemistry Principles , Procedures and Correlations, P:١٨١-١٨٢.The Murray Printing Company, PhiIadelphia .

***** Black ,P.H.; Kunz, K.Land Swartz, M.n,.(١٩٦٠).Salmnonelosis a review of some unusual aspects .N .Engl., ٢٦٢,P : ٨١١.

***** Bone , R. C., (١٩٩١) . The pathogenesis of sepsis . Ann. Med. ,P:١١٥ - ٤٥٧. (Internt).

***** Brandtzaeg , P. (١٩٨٩) . Overview of the mucosal immune system . Carr. Corbiol .J. Immunol . , ١٤٦, : ١٣ .

***** Brandtzaeg,P. and Farstad I.N., (١٩٩٩) The human mucosal B-cell system . In Ogra, P.L .; Strober, W.; Mestecky, J.; McGhee, J.R,Lamm,M.E. and Bienenstock, J. (eds) Mucosal Immunology . Academic Press . San Diego . C.A.P.٤٣٩ . PP:٦٢١-٦٢٨.

***** Briere , F. ; Bridon , J.M. ; Chevet , D. and Guvet , C. (١٩٩٤) . Interleukin ١٠ induces *B.* lymphocytes from IgA - deficient patients to secret IgA . J. Clin .Invest ., ١٩٤ (١٧): ١٠٤ .

*****Brodisky ,F.M.,(٢٠٠١) Antigen presentation and the major histocompatibility complex .In Parslow,T.G;Stites,D.P ;Terr,A.J.,and Imboden ,J.B.Midecial Immunology.١٠th (ed) ,PP:٨٢-٩٤.Lang Medical Brooks, McGraw .Hill / Medical Publishing Division , New York.

***** Brooks G.F., Butel, J.S .and Mores A.A (٢٠٠٤). In Jawetz, Melnick and Adleberg . Medical Microbiology ٢٣ th ed .P: ٢٧٩-٢٨٩ . A Lang Midecial book .Middle East Edition, A. elton and lang California..

***** Brostoff M. J., (١٩٧٣) . A topic allergy , British Journal of hospital Medicine , ٩ :٢٩ .

***** Calva , J. J. ; Ruiz G. M., (١٩٨٦) Salmonella hepatitis detection of *Salmonella* antigen in the liver of patients with typhoid fever . J. Infect. Dis ., ١٥٤,: ٣٧٣ -٣٧٤.

***** Camenish , T.D; Kohler, B .H,;Earp, H.S., and Matsushima,M., (١٩٩٩) . A novel tyrosin kinase , inhibits TNF α production and LPS induce endotoxic shock , J.Immunol.,١٦٢ ,P:٣٤٩٨-٣٥٠٣..

***** Camille , N. ; Kotton,K.I Elizabeth , L. and Hohmann,L.H . (٢٠٠٤) . Enteric pathogens as vaccine vectors for foreign antigen delivery . J.Immunol., ٧٢ (١٠), PP:٥٥٣٥-٥٥٤٧.

***** Cassidy , J. T. A. ; Burt , R. ; Petty,K. and Sullivan, D. (١٩٦٩) . Selective IgA in connective tissue . N. Engl. J. Med. ٥٣: ٢٧٥ - ٢٨٠ .

***** Cebra , J.J and Graig , S. W., (١٩٧١) . Peyer's patches an enriched source of precursors for IgA-producing immunocytes in the rabbits . J. Exp. Med.,٧١,: ١٣٤ - ١٨٨ .

***** Cohen,H .,and.Schifferli, D. M., (٢٠٠٠).Mucosal and systemic immune response to dimeric fimbriae expressed by *Salmonella* enterica serovar *typhimurium* vaccine strains. J. Immunol., ٦٨,:٣١٢٩-٣١٣٩.

*****Chopra, A.K.; Houston, C.W.; Peteson, J. W., and others (١٩٨٧) .Cloning and expression of the *Salmonella* enterotoxin gene. J. bacterial ., ١٦٩ (٥٠) :٩٥ -١٠٠.

***** Coffman , R.L. , Lebman , D.A., Sharder. B.,(١٩٨٩). Transforming growth factors – b٨ specifically enhance I, A production by LPS stimulate murine . B-lymphocytes ,J.Ex.Med.,١٧٠ : ١٠٣٠ -١٠ ٤٤.

***** Collee , J. ; Barrie , P. ; Marmion, A. ; Fraser ,G. and Simmons ,A. I (١٩٩٦) . Practical Medical Microbiology . ٤th.ed. , New York .

***** Copper , G. L. ,(١٩٩٤) . Salmonellosis - infection in man and the chicken : Pathogenesis and the development of the live vaccines . Review . Vet. Bull . , ٦٤, ١٢٣-١٤٣ .

***** Cruickshank ,R., Duguid, J.P. , Marmiom,B.P., and Swain R.H., (١٩٧٥) Medical Microbiology. ٢.١٢ th (ed) , :٤٥٩.The English Language Book Society .

***** Davies , D. R. ; Cohen , G. H. (١٩٩٦) . Interaction of protein antigens with antibodies . Proc. Nait. Acad .Sci ٩٣,: ٧ . USA.

***** Dietrich, G.; Spreng, S. ;Faver, D.,and Viret, J.F (٢٠٠٣).Live attenuated bacteria as vectors to deliver plasmid DNA vaccine. Curr.Opin.Mol., ٥:١٠-١٩.

***** Farmer,J.J.and Kelly,H., (١٩٩١). Enterobacteriaceae Manual of Clinical Microbiology V. Washington , D.c .Amerrican Socity for Microbiology ٣٧١ - ٣ .

***** Finlay, B.B. and Falkow , S. (١٩٨٨) . Common themes in microbial pathogen-city . Microbial Rev. , ٥٣ ,: ٢١٠ .

***** Finlay , B. B. ; Gumbiner , B. ; and Falkow , S. (١٩٩٢) . Penetration of *Salmonella* through a polarized MDCK epithelial cell monolayer . J. Cell Biol. ,١٠٧, P: ٢٢١-٣٠ .

***** Forrest, B. D ; Labrooy ,J.T;Breyer ,L. and others (١٩٩١) . The human humoral immune response *to S. typhi* Ty٢١. J. Infect. Dis.,١٦٣,P: (٣) ٦- ٤٥.

***** Foster , J.W and Hall , H.K., (١٩٩٠) Adaptive acidification tolerance response of *Salmonella typhimurium* . J. Bacteriol ., ١٧٢ ,: ٧٧١-٧٧٨ .

***** Frederick , M. ; Fadie T. ; Vincent , J. ; Carey and Gerald , B. P., (٢٠٠٢) . Hypersusceptibility of cystic fibrosis mice to chronic *P. auroginosa* , PNA., ١٠٠ (٤);١٩٤٩-١٩٥٤ .

***** Frederik , W. ;Van, G. ; Huan , H. Nguyen and Jerry R.J., (٢٠٠٥) . Vaccines for Mucosal Immunity to Combine Emerging Infections Diseases . J. Immunol ,. ٦ , (٢).:١٢٥-١٢٩.

***** Frederick , W.; Van, G.; Sharon, M.; Wahl , J . F. and others (٢٠٠١) . Partial IgA- Deficiency with increased $Th_٢$ type cytokines TNF.γ. knochout mice. J. Immunol .,١٦٣, : ١٩٥١-١٩٥٧ .

***** Frei ,J.; Heuch, C.; Riesen ,W.; Lang, I.T .; Hill P.G.; El- Nagteh, M.M and Poller ,L., (١٩٩٥) Production of Basic Dignostic Laboratory Reagents, WHO regional publications, Eastern Meditterranean series .

***** Garvey, J.S; Cremer, N.E and Sussdrof, D.H., (١٩٧٧) Methods in Immunology ٣th ed , pp ٥٣-٢٦٧. Addition- Wesley Publishing Company Inc, Reading .

*****Galan J.E., Curtiss R.,(١٩٨٩) Cloning and molecular characterization of gene whose products allow *S.typhimurium* gene . Proc. Natl. Acad Sci ;٨٩ :٦٣٨٣-٧.

***** Geppert, T.D.; Davis ,L.S. ; Gu r,H.; Wacholtz, M.C and Lipsky, P.E., (١٩٩٠). Accessory cell singnals involved in T-cell activation.J. Immunol . Rev., ١١٧,:٥٥-٦٦ [Medline] .

***** Germani, R.N., (١٩٩٣). Antigen processing and presentation . P:٦٢٩-٦٧٦. In W.E. Paul (ed.), Fundamental Immunology. Raven Press, New Yourk, .

***** Giannella , R.A. ; Broitman , S.A. ; Zamchec, K.N., (١٩٧٣) . Gastric acid barrier to ingested microorganisms in man : studies in vivo and in vitro Gut.J.Immunol., ١٣, : ٢٥١-٢٥٦ .

***** Giannella , R. A., (١٩٧٧).Important of the intestinal inflammatory reaction in *Salmonella* mediated intestinal secretion .J. Infect .Immunol., ٢٣,P:١٤٠.

***** Giannella,A. (٢٠٠١) *Salmonella.* Med. Micro .,Chapter (٢١):١-١٠ (Internet) .

***** Gilman, R.H. ;Terminel, M.; Hernandez – Mendoza ,P. and others (١٩٧٥). Relative efficacy of blood , urine, rectal swab, bone marrow and rose spote culture of recovery of *S .typh*i in typhoid fever. Lancet., ١, :١٢١١ -١٢١٣.

***** Goldsby, R. A.; Kindt, T.J. and Osborne, B.A., (٢٠٠٠) Kuby Immunology, ٤th ed .:٩٥-١٠١. W.H. Freeman and Company, New York .

***** Goldstein, F. W.; Champitaze J. C.; Guevara,H. and others .(١٩٩٣). Plasmid mediated resistance to multiple antibiotics in *S. typhi ,* J.Infect., ١٥٣,:٢٦١ -٦.

***** Gorden, J. and Small, P.L.C.,(١٩٩٣) . Acid resistance in enteric bacteria . Infect. Immune., ٦١,:٣٦٤-٣٦٧.

***** Grunwald , U.X ;Fan, R.S.Jak, G.; Workalemaha , A., and Schutt. D.E., (١٩٩٨). Monocyte can phagocytosis gram negative bacteria by a CD١٤ dependent mechanism J.Immunol.,١٥٧,: ٤١١٩.

***** Hackett , J. ; Kotlarshi , I. ; Mathan , V. and others .(١٩٨٦) . The colonization of peyer paches by *S. typhimrium* cured of the cryptic plasmid . J. infect. Dis., ١٥٣, : ١١١٩-١١٢٥ .

***** Harriman, G .R. ; Bougue, M.;Rogers, P.; Finegold, M.;Pacheco S;Bradley, A.;Zhangy and Mbawaike, I.N .,(١٩٩٩). Targeted deletion of the IgA constant region in mice leads to IgA deficiency with alterations in expression of other Ig isotypes. J. Immunol.,١٦٢, : ٢٥٢١-٢٥٢٩ .

***** Histoshi, N.; Kawano,H.; Tanaka ,T.K.; Shikgeyoshi, G.U and others , (١٩٩٩) .Regulatory role of N.K. T – cells in IL- ١٢ production during *Salmonella* infection. J .Immunol . ٦ (٤): ١٠٤- ١٩٣.

***** Hoffman , S. L. ; Punjabi , N. H. ; Rockhill , P. C. and others (١٩٨٥) . Duodenal string - capsule culture. Med.Micro., ٦٠,:٤٨١ .

***** Hoffman , T. A. ; Ruis , G. T. and Conts . G. W. (١٩٧٥) . Water borne typhoid fever in Dade county . FL . Clinical and therapeutic evaluations of ١٠٥ bacteremic patients . Am. J. Med., ٥٩, :٤٨١ .

***** Holmgren , J. J. ; Frykluna,H. and Larsson,M. , (١٩٨٩) . γ. interferon mediate down regulation of electrolyte secretion by intestinal epithelial cells . J. Immunol. , ٣٠, : ٤٩٩ .

***** Forrest, B., Hone, D.M.; Attridge, S.R. ; (١٩٨٨).Agel Evia(Vi antigen - negative) mutant of *Salmonella typhi* Tyi retains virulence in humans.٥٦,P:١٣٢٦-١٣٣٣.

***** Hornick , R.B. ; Greisman, S.E. ; Woodward,T.E.,and others. (١٩٧٠) . Typhoid fever : pathogenesis and immunologic control . N. Engl J. Med., ١٠ (٢٨٣),PP:٦٨٦-٦٨٩ .

***** Jackson , R. J. ; Fujihashi , J. ; Xu-Amano ; H. Kiyono , C. O. ; Elson,K. and MeGhee,J.R . (١٩٩٣) . Optimizing oral vaccines : induction of systemic and mucosal B-cell and antibody responses to tetanus toxoid by use of cholera toxin as an adjuvant. Infect. Immun ., ٦١, : ٤٢٧٢ .

***** Johansen , F.E; Pekna, M.; Norderthany, I.N.; Haneberg, B.; Hietala, M.A (١٩٩٩). Absence of epithelial IgA transport with increased mucosal leakiness , in polymeric IgR\SC deficient in mice . J. of Exp . Med. ١٩٠ (٧).October ٤ .

***** Johnstone,A.and Thrope, R. (١٩٨٢) .Immunochemistry in Practice , pp ١-٧٥. Black Well Scientific Publication .

***** Jonuleit, H.; Kuhn,U.; Muller, G.; Steinbrink, K.; Paragnik, L.; Schmitt, E.; Knop, J. and Enk, A.H. (١٩٩٧). Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. Eur. J.Immunol., ٢٧,P : ٣١٣٥-٣١٤٢ [Medline] .

***** Kauffman F. (١٩٥٠). The diagnosis of *Salmonella* types Springfield, I.L,Charles; C. Tomasi .Med.,Micro, :١٢٦-١٣٢.

***** Kauffman S.H , Hohan E. , Beryer R. and Kirchner H.,(١٩٨٩) Clonal listera monocytogenes- specific T cell active in antimicrobial immunity. J. Immunol.١٣:٢٦٥.

***** Kauffman , S. H. E .,(١٩٩٣ and١٩٩٥) . Immunity to intracellular bacteria. Ann , Rev. ,J. Immunol. , ١١: ١٢٩ and ٢٠٥.

***** Kauffman , S. H. E., (٢٠٠١).Immunity to intracellular bacteria .In Paul ,W. E., Fundamental Immunology- ٥ th ed .: ١٢٢٩-١٢٦٠.Lippinocott Williams and Wilkians, London.

***** Kamijo , R. H. ; Harada , D. ; Shapiro , J. Lee, S. ; Green and others, (١٩٩٤) . Requirement fro transcription factor IRF. ١ in macrophages , Science . ٢٦٣ : ١٦١٢ .

***** Kenneth Todar University of Wisconsin – Madison Department of Bacteriology. (٢٠٠٢) . Mechanisms of Bacterial Pathogenicity : Endotoxins , Bacterial Endotoxin ,:١-١٠.(Internet)..

***** khourieh , M. ;Schlesinger, M,; Tabachnik, E., and others ١٩٨٩). Typhoid fever diagnosis by isolation of *S- typhi* from gastric aspirate- Acta pdiatr and scand ., ٧٨,:٦٣٥-٦٣٩.

***** klugman, K.P., (١٩٨٧). Protective activity of Vi – capsular polysaccharide vaccine against typhoid fever.The Lancet., PP:١١٦٥-١١٦٩.

***** Kochi ,S.K, ;Killeen, P. and Ryan, U.S.,(٢٠٠٣).Advances in the development of bacteria vector technology. Expert,Rev. vaccines ٢,PP:٣١-٣٤.(medline).

***** Kohbata , S. ; Yokabta , H. and Rabuuchi , E. (١٩٨٦) . Cytopathogenic effect of *Salmonella typhi* : GIFU ١٠٠٠٧ on M cells of peyer pathes. In ligated ileal loops . Microbiol .J. Immunol., ٣٠, : ١٢٢٥-١٢٣٧ .

***** Krishna, D. K.V. (١٩٨٦) A Short Text Book of Medicine ١st .,:١٦٤-١٦٨ . Jaypee Brothers .

*****Kwapinski, J.B.G. (١٩٧٢) Methodology of Immunochemical and Immunological Research.: ٢٦٧-٣١٦. Wiley-Inter Science , New York .

***** Lanata ,C.F.; Levine, M.M.; Ristori, C. and others (١٩٨٣). Vi serology indetection of chronic of *S. typhi* carrier in an epidemic area .Lancet .,٢,:٤٤١.

***** Lanzavecchia, A.(١٩٩٦).Mechanisms of antigen uptake for presentation . Curr.Opin.J. Immunol., ٨, : ٣٤٨-٣٥٤ [Medline] .

***** Minor,I. and Genus, II. , (١٩٨٤) *Salmonella* ; Bergey's Manual of Systematic Bacteriology . Baltimor : William and Wlkins ,.: ٤٢٧-٤٢٨ .

*****Lee, S.F. (٢٠٠٤). Oral colonization and immune response to *streptococcus gordonii* :Potential use as a vector to induce antibodies against respiratory pathogens. Curr .Opin .Infect., Dis.,١٦:٢٣١-٢٣٥.

***** Lee, J.D.; Kato, K.; Tobias, P.S ; Kirkland, T.N and. Ulevitch, R. J (١٩٩٢) .Transfection of CD١٤ into cells dramatically enhance the sensitivity of LPS and LPS binding protein. J.Exp. Med.,١٧٥ ,PP:١٦٩٧.

***** Lehman, J.D.; Smith, J.W.; Miller , T.E.; Bornett, J.A-Stanford . J.P. (١٩٦٨). Local immune response in experimental pyelonephritis . J. Clin. Invest., ٤٧ : ٢٥٤١-٢٥٥٠ .

***** Levine, M. and Hornick ,R.B. (١٩٨٧) Comperhensive Immunology of Human Infection .,PP: ٢٤٩-٢٩٠. Plenum Press, New York .

***** Levine, M.M.(١٩٩٩).Typhoid fever vaccines. In,S .A Plotkin and W.A Orenstein Vaccines ٣ rd . The W,B Saunders Company, Philadelphia,P: ٧٨١-٨١٤.

***** Liautard ,J.P .A.; Gross,J. D. and Kohler, G.,(١٩٩٦). Interaction between professional phagocytes and *Brucella Spp*. Microbiology, ١٢, ١٧٩.

***** Lo ,O. H.; Metcalf, E .S. and Mark, J.S (١٩٩٩). T cell responses to gram negative intracellular bacterial pathogens : a role for CD ٨- Tcell in immunity to *Salmonella* infection and the involvement of MHC class Ib molecules.J. Immunol.,١٦٢, : ٥٣٩٨-٥٤٠٦ .

*****Luchi, M. and Munford, R.S. (١٩٩٣). Binding internalization and deaylation fo bacteria LPS by human neutrophil .J.Immunol., ١٥١, :٩٥٩.

*****Macfaddin , J.F(٢٠٠٠). Biochemical tests for identification for medical bacteria ٣ed. Lippnocot Williams and Wilkins.

***** Manaby, Y.; Takachika, H.T ; Noriko, K.N.;Okada,H. and others. (١٩٩٩) . Nasopharyngeal – Associated lymphoreticular Tissue (NALT) , Th١ , Th١ cell regulated IgA responses for inhibition of bacterial attachment to epithelial cells . ٢١(٦) March, ١٥ .

***** Mckendrick , M. W., (١٩٨٤) . Typhoid . Med .Micro., ٢ ,: ٦٩ -٧٢ .(Internet).

***** Mestecky , J.,(١٩٨٧) . The common mucosal immune system and current strategies of induction of immune response in external secretions. J. Clin. Immunol. , ٧,:٢٦٥ .

***** Michetticle , P. ; Mahan , M.J ; Slauch , J.M ; and others (١٩٩٢) . Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen *Salmonella tyhimurium* . Infect. Immune., ١ (٦٠): ١٧٨-١٧٩ .

***** Miller , S.I and Phop, A., (١٩٩٣) .Respressed gene promotes *Salmonella* invasion of epithelial cells . J. Bacteriol . , ١٧٥, : ٤٧٧٥-٤٧٨٥ .

***** Miller, S.I.;Hohmann, E.L and Pegues, H. (١٩٩٦). *Salmonella* (including *Salmonella typhi*) . In Mandell, G.L.;Bennett ,J.E and Dolin, R. (es) Principles and Practice of Infections Disease ,PP:٢٠١٣-٢٠٣١, Churchill Livingstone **.**

***** Morse, D.L. ;Birkhead, G.S.; Levine, W.C.,and others(١٩٩٣).Typhoid fever at a resort hotel in New York:A large outbreak with unusual vehicle .J.Infect. Dis., ١٦٧,:١٢٢٨-٢٣٢.

***** Mostov , K . and Kaetzel , S. C. , (١٩٩٩) . Immunoglobulin transport and (pIgR) . In mucosal immunology . Orga, P. L.; Strober , W. J.; Mestecky,N. and Lamm , M. E. Acadmic press . San Diego. CA. PP: ٣١٩ .

***** Muotiala , A. ; Hovi, M. and Makela, P. H. (١٩٨٩) . Protective immunity in mouse Salmonellosis ; Comparison of smooth and rough live and killed vaccines . Med . Micro ., ٦, ٥١-٦٠ .

*****Mowat A. M. and Fergusoh M.,(١٩٨١) Hypersenistivtiy in small intestine mucosa .Induction of cell mediated immunity to a destroy .Clin .Exp.Immunol. ٤٣: ٥٧٤-٥٨٩

*****Nauciel, C. (١٩٩٠) . Role of $CD_{٤}^{+}$ T-cells and T- independent mechanisms in acquired resistance to *S. typhimrium* infection . J. Immnol ., ١٤٥ ,: ١٢٦٥-١٢٦٩ .

*****Pang, G.; Buret. A.; Oloughlin, E. ;Smith, A. ;Batey, R.X. and Clancy, R.(١٩٩٦).Immunologic functional and morphological characterization of three new human small intestinal epithelial cells ., ١١, ٨-١٨.

*****Parslow ,T.G., and Bainton ,D.F., (٢٠٠١) Innate immunity. In Parslow, T.G.; Stites ,D.P. ; Terr ,A.J., and Imboden J.B. Med. Immunology ,١٠ ed . : ١٩-٣٩ Lang Medical Brooks , McGraw-Hill/ Medical Puplishing Division, New York .

***** Pasetti , M. F. ; Salerno, G. and Sztein, M. B. , (٢٠٠٢) . *Salmonella* enterica serovar *typhi* live vector vaccines delivered intranasally elicit regional and systemic specific CB_{λ}^{+} major histocompatibility class I- restricted cytotoxin T lymphocytes . Infect. Immun., ٧٠, : ٤٠٠٩- ٤٠١٨ .

***** Pears, A. (١٩٨٥).Histochemistry (٨ th ed): ٤٥٠ Churchill-Livingston, New york .

***** Peterson, J. (٢٠٠٠) . Bacterial pathogenesis.Med-Micro., Ch. ٧ . (Intenet)

***** Pryjma. J.J ; Baran ,M.; Ernst, M. and Flad, H. D. .(١٩٩٩). Altered antigen presenting capacity of human monocyte after phagocytosis of bacteria . Infect. Immunol., ٦٢ ,PP:١٩٦١ – ١٩٦٧.

*****Robbins, J. B; Schneerson, R. and Szu, S.C..(١٩٩٤).Perspective hypothesis: Serum IgG antibody is sufficient to confer production against infectious disease by inactivating the inoculum. J. Infect .Dis.,١٧١:١٣٨٧-١٣٩٨.(Medline)

*****Roberts, M. A.; Bacon ,J. L. and Sichatield,S.(١٩٩٨).Prior immunity to homologous and heterologous *Salmonella* serotypes suppresses local and systemic anti – fragment antibody responses and protection from tetanus toxin in mice immunized with *Salmonella* strains expressing fragment C١.Infect. Immunol.,٦٧,PP:٣٨١٠-٣٨١٥.

***** Robbins, J. D. and Robbins ,J.B.(١٩٨٤). Re-examination of the protective role of the capsular polysaccharide(Vi antigen) of *S.typhi*.J.Infect. Dis., :١٥٠:٤٣٦-٤٤٩.

***** Robbins,J. B.;Schaeerson ,R. ;Andersan, P. and Smith, D.H.,(١٩٨٤). Conjugates for typhoid fever .J. Infect . Dis., ١٦١: ٨٢١ – ٨٣٢.

***** Robbins, J.B.; Schencerson, R. ; Czuis ,S. and Vince ,. (١٩٩٩) . Bacterial polysaccharide – protein conjugate vaccines – Pure Appl . Chem. ٧١ (٥) P:٧٤٥-٧٥٤ .

***** Robert , C. W; pearay ,L. ;Orga ,J. and Howard,P., (٢٠٠١). Vaccination /strategies for mucosal immune response. Clini. Microbiology Reviw. ٣٤٠ – ٤٤٥ .

***** Rubin , R. H. and Weinstein , l. (١٩٧٧) Salmonellosis Microbiologic , Pathologic and Clinical Feature , Stration inter-Continental Medical Book Crop. ,P:٢٣٥-٢٣٩. New York .

*****Rubin, F.A. ;Whirter,M.C. ; Burr,P. D, and others .(١٩٩٠) .Rapid diagnosis of typhoid fever through identification of *S. typhi* within ١٨ hrs of specimen acquisition by culture of the mononuclear cell –platelet fraction of blood. J. Clin .Microbiology., ٢٨,: ٨٢٥-٨٢٧.

***** Sarmento , A. and Appelberg , R. (١٩٩٦) . Involvement of reactive O_2 intermediate in TNF- α dependent bacteriostatics of *mycobacterium avium* infected .J. Immunol , ٦٤, : ٣٢٢٤ .

***** Salerno, G.R. ;Pasetti ,M.F and Sztein,M.B, (٢٠٠٢).Characterization of CD٨+ effectors cell responses in volunteers immunized with *Salmonella* enterica serovar typhi strain Ty٢١ –a typhoid vaccine J.Immunol., ١٦٩,:٢١٩٦-٢٢٠٣.

***** Schiff, D.E.K ; Soldaa ,K.J.; Lee,J.; pugin ,K.D and Tobia,P.S., ,(١٩٩٨).Phagocytosis of gram negetive bacteria by a unique CD١٤ dependent mechanism. J. Biol., ٦٢,P :٧٨٦.

*****Sharn H.,(٢٠٠٤)Vi of *S. typhi* target , the protection family of molecule is intestinal and suppression of inflammation response , Puplished online.

*****Shnawa I.M.S and Hindi W.A.Z (١٩٩٦) Febrile circulating agglutinins technical research . J. Babylon (Accepeted).

***** Shnawa, I.M.S.,(٢٠٠١) Anuran non specific cellular immune function .Babylon Jonal.٧(٧).:١٢٢-١٢٩.

***** Shnawa, I.M.S. and Thwaini, Q.N. (٢٠٠٢). Lapin mucosal humoral versus systemic humoral and cellular immune response post to intracellular administration of heat killed C . Feutus J. Baby. Univ. ٧ (٣): ٥٣٨-٥٤٣ .

*****Shnawa I.M. S. and AL- Ameedi B.H.,(٢٠٠٢) Humoral immune profile of human serum hepatitis.Babylon J. of science.

*****Shnawa I.M. S. and AL- Saadi A.K (٢٠٠٢) Gut mucosal I.g. Separation, partial characterization and utility as infection probe. Iraq J. Micro .

*****Shnawa I.M.S. and Mehdi M.S (٢٠٠٤) Mucosal agglutinating Ab to Ag of bacteria associated with U.T infection .Iraq J.Science.

***** Silva , R. (١٩٩٦) . Endocytosis and vacuolar function , P. ١٦٨.١-١٦٨.٢ ١٢ In L. A. Herzenberg , D. M. Weir, and C. Blackwell (ed.) Weir's Handbook of Experimental Immunology . Blackwell Science , Cambridge , ٨(٧)P:٢٢٣-٢٢٩.

***** Soberg , M. (١٩٦٨) . Invitro Migration inhibition of peripheral blood leucocytes in delayed type hypersensitivity . Acta Medica. Secand. ١٨٤, : ١٣-٢٥ .

***** Strober,W. and James, S.P (١٩٩٤) The Mucosal Immune System . In Stites, D.P.; Terr, A.I. and Parslow, T.G (eds) Basic & Clinical Immunology ,: ٥٤١-٥٥١. Prentic-Hall International , Inc, London .

***** Strober , W. ;. Lamm ,M. E. and MeGhee j. R. (١٩٩٩) . Mucosal immune response overview In; Orga, P.L.; Mesteky, J.; Lamm, M.E ;Strober, W.; McGhee,J.R., (es),Mucosal immunity,٢nd ed,: ٤٨٥-٥٠٦ . Acadmic, Press ,New York .

*****Strober W. and Kelsall B.,(١٩٩٩) Gut-associated lymphoid tissues : Ag handling and T-helper cell cytokines ,J. Immunol. P.٢٩٣-٣١٧.

***** Sztein, M.B., and Mitchell,G. (١٩٩٧) . Recent advances in immunology : impact on vaccine development., P: ٩٩-١٢٥. In Levine, M.M.;Woodrow, G.C.;Kaper, J.B and Cobon, G.S. (ed.), New generation vaccines. Marcel Dekker, Inc., New York, .

*****Taylor, A. (١٩٨٣) . Purification and Immunochemical properties of *Escherichia coli* B polysaccharide cross – reacting with *Salmonella typhi* Vi antigen : Preliminary evidence for cross – reacting of the polysaccharide with *Escherichia coli* K١ antigen . Infect . Immunol.,PP:٢٠٤-٢٥١.. Gdansk Poland .

*****Taylor G.F.,(١٩٩٥) Cytokines as adjuvant for vaccines. Ag .specific responses differ from polyclonal responses. Infect. Immun., ٦٣: ٣٢٤١-٣٢٤٤.

*****Taha R. and Al –Hindawi N., (١٩٧٩) Symptoms and pathological changes caused by *Salmonella Spp*. isolated from foods in Baghdad. Iraq .J. of science

***** Timothy , L.W. ; Tanner, M.K. and Marcelo, B. , (٢٠٠٠) . Potent immunoregulatory effect of *S. typhi* flagella on antigenic stimulation of human preipheral blood mononuclear cells . Infect .Immunol ., . ٦٧ (٣) .:١٣٣٨-١٣٤٦.

***** Tomas, T. B (١٩٨٥). The discovery of secretory IgA and the mucosal immune system.J. Immunol Today., ١٢ :٤١٦.

***** Tomas T.B.(١٩٩٩ and ٢٠٠٠).Typiod fever .In Bennett. J. C and plum F (eds) Cecil text book of medicine . ٢.٢٠ th. PP:١٦٤٢ - ١٦٤٤ . W.B stanuder company , Adevision of Harcourt Back Company.

*****Trinchieri , G. and Scott, P., (١٩٩٤) . The role of interlukin- ١٢ in the immune response disease and therapy .J. Immunol Today ., ١٠ : ٦٤٠ .

*****Ogawa T.H.,Shimauchi J. and Hamada A.,(١٩٨٩) Mucosal and systemic immune response in BALB/ Mice. Infect . Immun., ٥٧: ٣٤٦٦-٣٤٧١ (Medline).

***** Van, Der Zee. Huis, H. and Veld , H. J., (٢٠٠٠) . Method for the rapid detection of *Salmonella* . Med.Micro. P: ٣٧٣-٣٩١ .

***** Vassalli ,G. P. ; and Meflusky, R. T., (١٩٦٩) . The distribution of large dividing lymph node cells in syrngetic recipient after intravenous injection . J. Exp. Med.,: ١٣٠-١٤٢٧ .

***** Waddell , W. R. and Kunz, L. J. (١٩٥٦) . Association of *Salmonella enteritis* with operation of stomach.N.Engl.J.Med.,PP:٢٥٥.

*****Wasserman , S. ; Murphy , J. and Bagar , S. and others . (١٩٨٩) . Immunity in typhoid - endemic region . Clin. Exp. Imminol., ٧٥ PP: ٢٢٨-٢٣٣ .

***** Wei , F.L. ; Helena ; Eleanor , S. and Mark , J. (١٩٩٩) . T- cell response to gram negative Intracellular bacteria pathogen : A role for CD^{8+} T-cells in immunity to *S.typhi* infection and involvement o f MIC class١ .J.Immunol. ١١(٩) : ١٢٩ .

***** Weinborg , E.D.,(١٩٨٤) . Iron with holding : a defence against infection and neoplasia . Physiol , Rev., ٦٤(٢) PP : ٦٥ .

*****Whittle, B.L., and Verma, N.K., (١٩٩٧).The immune response to T-cell epitope delivred by *Salmonella* enhanced by prior immunological experience.Vaccine١٥ .PP:١٧٣٧-١٧٤٠. (Medline).

* Williams , P. M. ; Camara , A. ; Hardman , S. ; Swift , D. ; Milton , N. J. ; Hope , K. ; Winzer, B. ; Middleton,K. ; Pritchard , D. I., and Bycrofy , B. W. (١٩٧٦) . Quorum sensing the population dependent-control of Virulence. Phil. Tran. R. Soc. Lond. B., ٣٥٥, P: ٦٦٧-٦٨٠ .

***** Willson , A. D. A. ; Robinson , L.; Irons,A. and Stokes , C. R. (١٩٩٣) . Adjuvant action of cholera toxin and pertusis toxin in the

induction of IgA antibody response to orally administered antigen . Vaccine , ١١: ١١٣ .

***** Wira , C.R. ; Richardson , J. ; Prabhala , R. (١٩٩٤) . Endocrine regulation of mucosal immunity . Handbook of Mucosal Immunology . Son Diego : Academic Press .: ٧٠٥-٧١٦ .

***** Wolf , J. L. ; Rubin , D. H. ; Finberg , R. ; Kauffman , R. S. ; Sharpe , A. H. ; Trier , J. S. and others (١٩٩١) . B. N. Intestinal M cells : A pathway for entry of *reovirus* into the host . Science ,٢١٢ P: ٤٧١-٤٧٢ .

***** World Health Organization (WHO), (١٩٨٠). Center of Reference on *Salmonella*. Antigenic formulae of the *Salmonella* .W.H.O international *Salmonella* center.Institute pasture.J. Micro ., P:٢١٥.

***** World Health Organization . (WHO) (١٩٨٤) . Guidelines on detection and monitoring of *Salmonella* infected poultry flocks with particular reference to *Salmonella enteritids* . WHO , Geneva , Switzeland .٢١٢,P:١٢٥-١٢٧.

***** World Health Organization (WHO) ; Reginol Office for Europe (٢٠٠٤) . WHO Surveillance programme for control of foodborne infection and Intoxications in Europe: ١١٣-١٣٣ ,Berlin , Germany .

***** Wyant, T.; Tanner, M.K., and Sztein, M.B.. (١٩٩٦) *Salmonella typhi* flagella are potent inducers of pro-inflammatory cytokine secretion by human monocytes invitro . J.Immunol.,:٢٣٥.

***** Zuzana, K. ; Slavomir,Z.U.and Dolores,J., (١٩٩٧) . Synthesis and Immunological properties of Vi and Di O - Acetyl pectin protein conjugates with adipic acid dihydrazide as the linker .J. Infect . Immunol ,٥٦,: ٢٠٨٨-٢٠٩٨ .