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"Determination of humoral and cellular immune response at systemic and mucosal levels in rabbits after intranasal administration of heat killed *H. pylori* antigen"

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Abstract

The nasal compartment , the common mucosal immune system was attempted as a model for providing the immunological opinion that mucosal immunization induces mucosal as well as systemic immune responses specific to the stimulating antigen. Four successive doses of heat killed *H. pylori* (HpHK) antigen were intranasal administered in four successive weeks a part to rabbits.

HpHK antigen was stimulated humoral haemagglutinins titers as well as to the significant increased in the total protein concentrations at serum and mucosal secretions in rabbits , in addition to the significantly increased in the concentrations of serum IgA and mucosal anti *H. pylori* IgA with INF-γ at serum and mucosal levels . Thus *H. pylori* antigen was B and T cells dependent type.

Introduction:

The effective protective immunity against *H. pylori* can be induced in experimental animal models after immunization using various routes of delivery. The best manner to induce strong immune responses both at local and systemic levels was to prime animals mucosally either orally or intranasally **(Giudice and Michetti, 2004 ; Arora and Czinn, 2005)**. Immunity against *H. pylori* is characterized by a strong Th1 response and innate immunity is an indispensable step for generation of adaptive response against *H. pylori* infection **(Vorobjova *etal*., 2008)**.

H. pylori cause infiltration of gastric epithelium and the underlying lamina propria by neutrophils , T and B lymphocytes , macrophages and mast cells. The rapid determination of the presence of serum and mucosal anti *H. pylori* antibodies in clinical practice settings would assist in defining appropriate further investigations and management steps in the evaluation of patients with possible *H. pylori* infection **(Abbas *etal*., 2005).** It cause mucosal as well as systemic humoral and cellular immune responses , till now little references regarding the immune status of the lapin animal primed with this bacteria and the time of development of these responses **(Kuster *etal.*, 2006 ; Aguemon *etal*., 2004)**.

Aim of study:

The aim of this study is to compare mucosal and systemic humoral and cellular immune responses in rabbits after intranasal administration of heat killed *H.pylori* (HpHK) antigen .

Material & Methods:

1- Antigens:

Heat killed *H.pylori* (HPHK) antigen was prepared from 24 hour brain heart infusion agar plate culture. Then was added 6 ml of normal saline to the plat's scrape , The collected solution centrifuged at 4000 rm\ mint for 5 mints. Double wash were placed with normal saline then compared with standard opacimeter (WHO) to obtain

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the concentration equals to 10 IU\ml . The suspension tubes were used after placing them in a water path at 60 degrees for 30 mints to kill the bacteria and obtaining the antigen that was used for the immunization of rabbits after examinate for the sterility test. **(Svanborg-Eden *etal*., 1985 and Sachse *etal.*, 2005)**.

2- Animals:

Two groups, each of two rabbits *O. canniculus* were used , adapted to laboratory conditions and housed under Ad libitum standardized conditions , one group served as test an the other as control group **(Schneider *etal*., 1990)**.

3- Immunization protocol:

Four successive doses of (HpHK) antigen were administered via intranasal rout into rabbits through four successive weeks followed by one week then bled by cardiac puncture. Each dose was about 3 ml of antigen that had 10 IU\ml concentration. Control animals received sterile normal saline in the same protocol .

4- Mucosal samples and immunoglobulines separation :

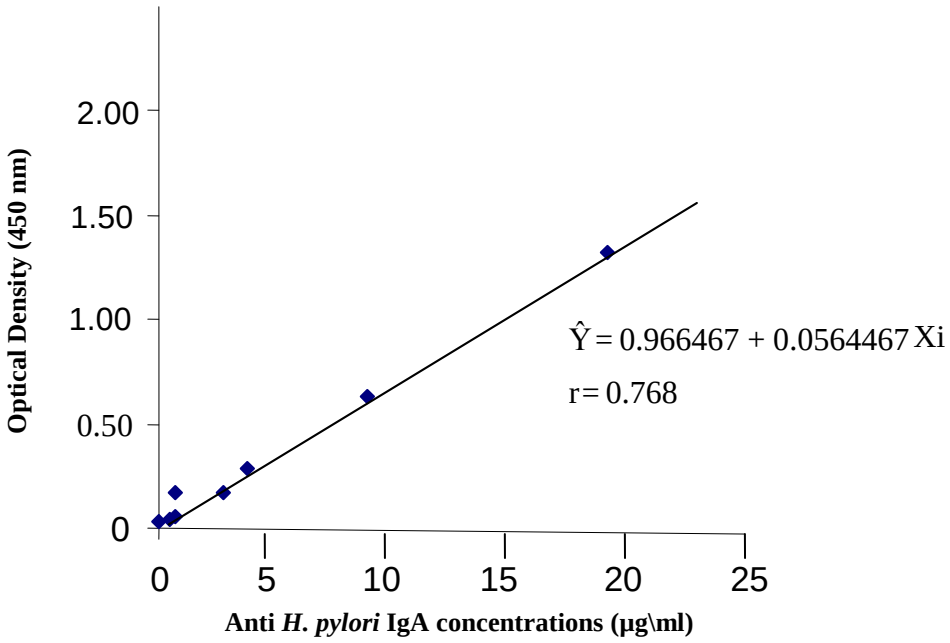
Gut mucosal samples were obtained from two parts of gut mucosa included stomach (antrum) and duodenum . Then the immunoglobulines were separated from these parts according to **(Shnawa and Abid, 2005).**

5-Blood Samples:

Serum was collected for the immunological tests that include: micro haemagglutination test , total protein concentrations , measure the concentrations of serum IgA and mucosal anti *H.pylori* IgA and INF-γ **(Garvey *etal*., 1977)**.

6-Immunology:

Passive haemagglutination test was performed by microtiteration method, serum and mucosal total protein concentrations according to colorimetric method using readily prepared solutions provided by Biolabo company, France and Randox – Laboratories Ltd , UK. Company , serum IgA using single radial immunodiffusion assay and mucosal anti *H. pylori* IgA antibodies were evaluated by using specialized



ELISA kit (provided from the Diagnostic Automation , INC Company, USA) as in

standard curve that explained in (Figure 1) , INF-γ cytokine was assayed using ELISA kit (provided from Immuno. tech. A. Beckman, Coulter Company) as in standard curve that explained in (Figure 2)**.**

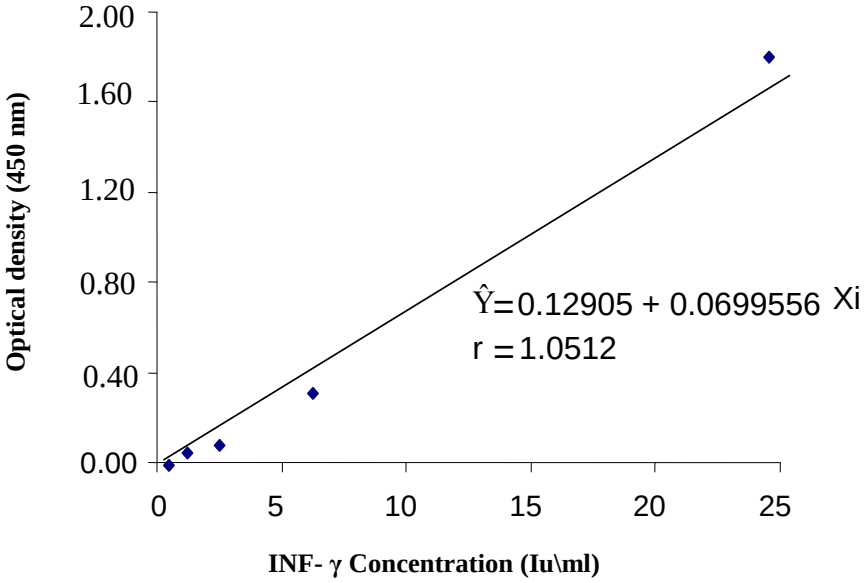


Figure (2) Standard curve of INF-γ

i IgA concentrations (

Results and Discussion

H.pylori possess several immunogenic subfractions , such immunogens stimulate B cells , T cells as well as the subset Tdh responsible for hypersensitivity **(Velin *etal.*, 2004 and Choi *etal.*, 2011)** . The serological diagnosis of *H. pylori* infection , immune responses against the relevant microorganism can be analyzed in experimental animal models and the host immune responses have been linked to susceptibility to gastroduodenal diseases in *Helicobacter pylori* infection **(Islam *etal.*, 2007)**. Most data suggest that a mucosal and systemic T helper type 1 (Th1) response is associated with peptic ulceration , but the role of Th2 cells is less clear **(David *etal.*, 2004)**.

The immune response was studied at the end of the immunization period after administration of (HpHK) antigen via intranasal route which stimulates *H. pylori* specific humoral systemic and mucosal haemagglutinins titers (Table 1). It played an important role in the significant increase of the total protein concentrations (Table 2) which correlate with the increase in the number of stimulating B cells responsible for antibodies production in addition to their correlation with the induction of cytokines from stimulating T cells **(Reddy, 2010)** .

Serum IgA and mucosal anti *H. pylori* IgA antibodies concentrations at mucosal secretions of immunized rabbits were also significantly increased (Table 3 and 4), Because *H. pylori* is a mucosa associated organism , it was initially thought an IgA

type anti *Helicobacter* antibody response would be essential for protective immunity **(Kuster *etal.*, 2006 ; Islam *etal.*, 2007).**

The cellular systemic and mucosal immune responses were represented by significant increased in the concentrations of cytokine especially INF-γ (Table 5) .Hence, their epitopes can be of T dependent type through the activation of Th1 **(Michetti, 2011).**

The correlation between local and systemic immune responses affected by several factors includes : immune gene nature , host susceptibility to replicating or non replicating immunogens, nature of immunization protocol, nature of the host immune system and type of experimental design (**Brandtizage and Frasted, 1999)**.

From all of the above we can conclude that nasal compartment is being induction compartment of mucosal immune system . HpHK antigen induce both humoral and cellular immune responses both at mucosal and systemic levels.

Table (1) Haemagglutinins of anti *H. pylori* antibody at mucosal and systemic titers

Samples	Rabbits Sequence	Haemagglutinins titers	Range
Serum	R_1	10240	10240
	R_2	10240	
Mucosal			
Antrum	R_1	2048	1536
	R_2	1024	
Duodenum	R_1	1024	1024
	R_2	1024	
Serum control	R_1	10	10
	R_2	10	
Mucosal control	R_1	1	1
	R_2	1	

Table (2) Total protein concentrations at serum and mucosal secretions

Samples	Rabbits Sequence	Total protein concentrations (mg/dL)	M±S.D.	P - value
Serum	R_1	11.20	11.00±0.35	0.000[a]
	R_2	10.80		
Mucosal				
Antrum	R_1	0.68	0.67±0.04	0.030[b]
	R_2	0.66		
Duodenum	R_1	0.63	0.61±0.03	0.000[c]
	R_2	0.59		
Serum control	R_1	5.50	5.65±0.15	0.000[d]
	R_2	5.80		
Mucosal control	R_1	0.20	0.19±0.01	0.010[d]
	R_2	0.18		

Table (3) Concentrations of serum IgA antibodies

Serum samples	Rabbits Sequence	Concentrations of IgA (mg\dl)	M±S.D.	P - value
	R_1	524.70	514.55±11.95	0.030[a]
	R_2	504.40		
Control	R_1	176.40	173.35±5.8	0.010[b]
	R_2	170.30		

Table (4) Concentrations of mucosal anti *H. pylori* IgA antibodies

Mucosal samples	Rabbits Sequence	Concentrations of IgA (µg\ml)	M±S.D.	P - value
Antrum	R_1	20.00	19.55±0.17	0.000[a]
	R_2	19.10		
Duodenum	R_1	15.20	16.20±2.01	0.010[b]
	R_2	17.20		
Control	R_1	8.20	7.85±0.54	0.000[c]
	R_2	7.50		

Table (5) Concentrations of INF-γ

Samples	Rabbits Sequence	Concentrations of INF-γ (Iu\ml)	M±S.D.	P - value
Serum	R_1	18.25	19.04±1.09	0.000[a]
	R_2	19.83		
Mucosal				
Antrum	R_1	25.00	24.15±1.69	0.010[b]
	R_2	23.30		
Duodenum	R_1	21.20	22.72±2.01	0.000[c]
	R_2	24.25		
Control	R_1	7.46	8.25±1.67	0.000[d]
	R_2	9.04		

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