RESULTS

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This study included 25 patients suffering from symptoms of dyspepsia. All of them showed signs of gastroduodenal disease on endoscopy i.e. (gastric ulcer, duodenal ulcer, gastritis, duodenitis and/or gastric carcinoma) (group I) and 10 patients suffering from the same symptoms but without endoscopic pathological findings served as control group (group II).

This study was done from April 1998 to January 1999.

I- Results of history data

Table (1): Comparison between cases and controls as regards age.

Age (years)	Cases (Group I) (n = 25)	Controls (Group II) (n = 10)
Range	20 – 60	25 – 50
Mean	36.08	33.50
± SD	12.96	8.68
t	0.68	
P	> 0.05	

Their was no significant difference (P > 0.05) between cases and controls as regards age.

Table (2): H. pylori infection in relation to age.

Age (years)	Culture	
	Positive (n = 16)	**Negative (n = 9)**
Range	20 – 60	21 - 55
Mean	36.31	35.67
± SD	13.44	12.87
t	0.12	
P	> 0.05	

There was no significant relation (P > 0.05) between H. pylori infection and the age of patients.

16 out of 25 patients were positive for H. pylori by culture, their age ranged from 20-60 years with mean age of 36.31 year. 9 out of 25 patients were negative for H. pylori by culture, their age ranged from 21-55 year with mean age of 35.67.

Table (3): H. pylori infection in different age groups.

Age groups		Culture		Total
		Positive	Negative	
20-40 years	No.	2	3	5
	%	40.0	60.0	100.0
41-60 years	No.	14	6	20
	%	70.0	30.0	100.0
Total	No.	16	9	25
	%	64.0	36.0	100.0
P		> 0.05		

There was no statistically significant relation (P >0.05) between H. pylori infection and age.

Table (3) showed that 2 (40%) out of 5 patients with age ranged between 20-40 years were H. pylori positive and 14 (70%) out of 20 patients with age ranged between 41-60 years were H. pylori positive.

Fig 1 : H.pylori infection in different age groups

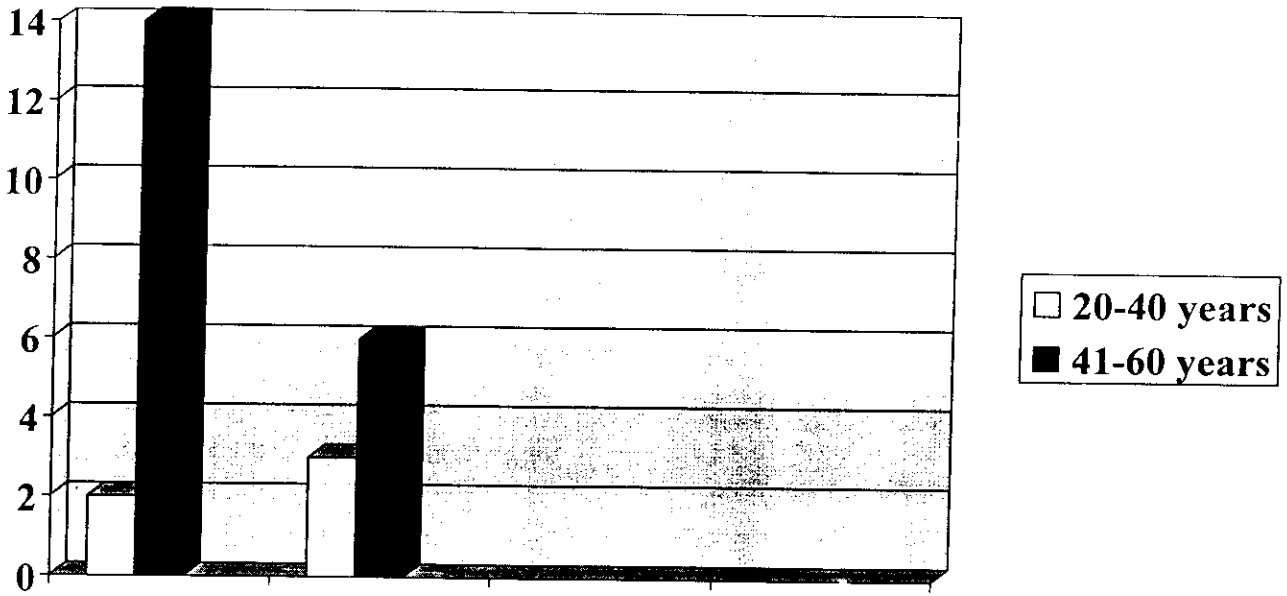

Table (4): H. pylori infection in both sex.

Sex	+ve culture		-ve culture		Total cases	
	No.	%	No.	%	No.	%
M	15	75.0	5	25.0	20	100.0
F	1	20.0	4	80.0	5	100.0
Total	16	64.0	9	36.0	25	100.0
P:	< 0.05*					

H. pylori infection in males was significantly higher ($P < 0.05$) than in females.

Table (4) showed that 15 (75%) out of 20 male patients were H. pylori positive by culture, and 1 (20%) out of 5 female patients was H. pylori positive by culture.

Fig 2 : H.pylori infection in both sex

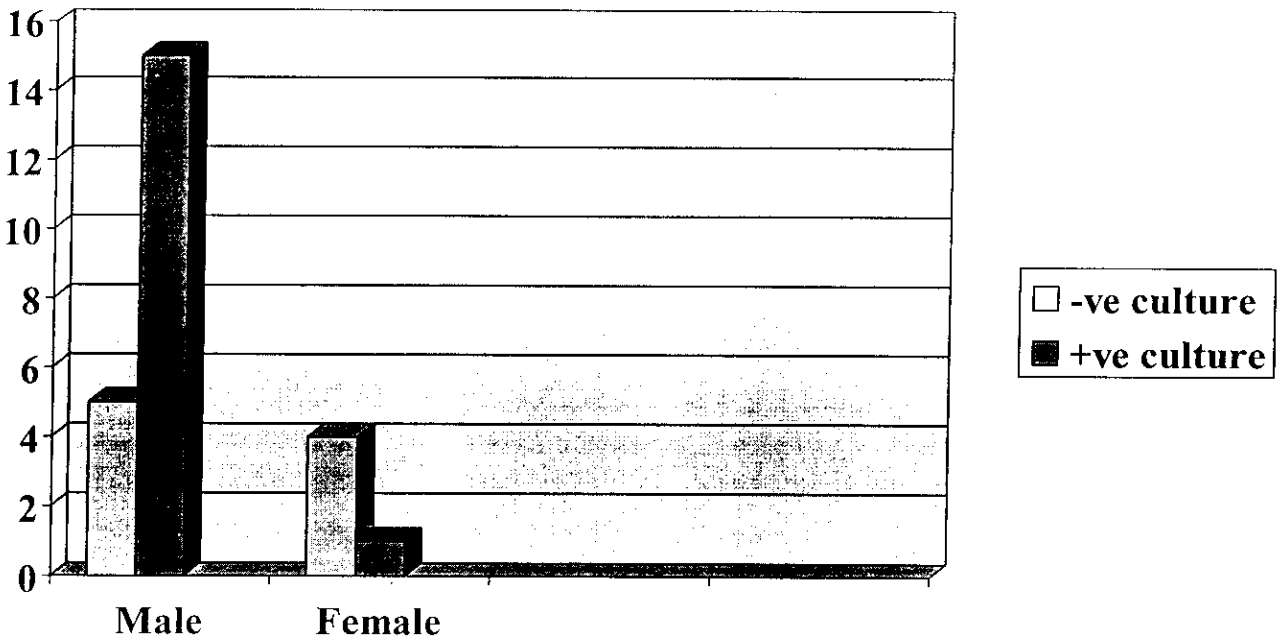

Table (5): H. pylori infection and its relation to smoking.

Smoking	+ve culture		-ve culture		Total cases	
	No.	%	No.	%	No.	%
-	10	58.8	7	41.2	17	100.0
+	6	75.0	2	25.0	8	100.0
Total	16	64.0	9	36.0	25	100.0
P:	> 0.05					

There was no significant difference ($P > 0.05$) between the smokers and non smokers according to the presence of H. pylori infection.

10 (58.8%) out of 17 non smoker patients and 6 (75%) out of 8 smoker patients were H. pylori positive by culture.

Fig (3) : H.pylori infection and its relation to smoking

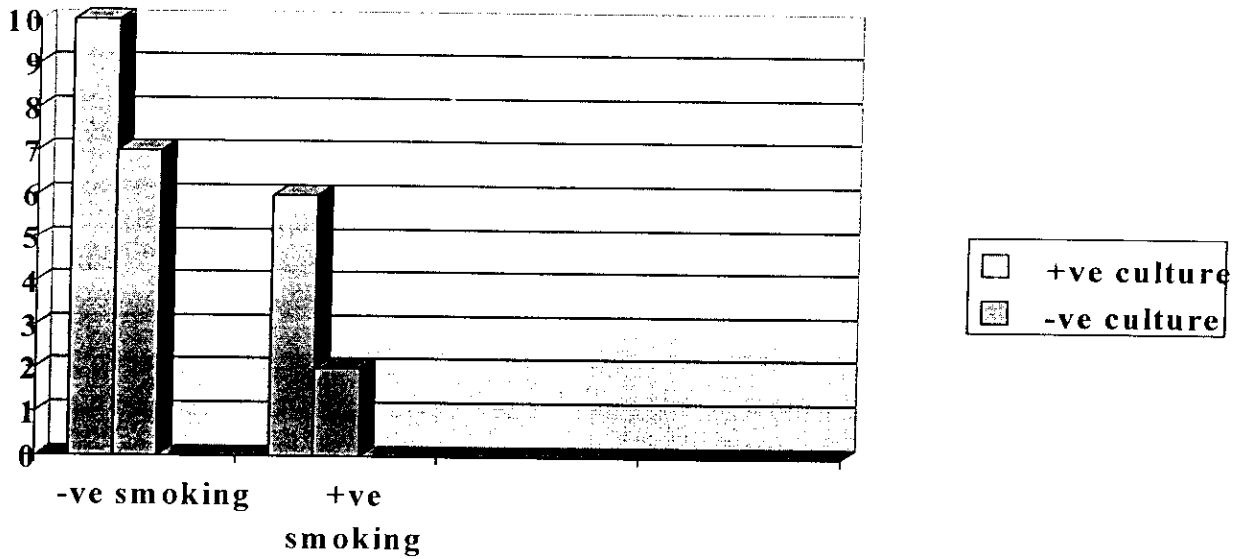

Table (6): Relation between H. pylori infection and socioeconomic status.

Socioeconomic state	+ve culture		-ve culture		Total cases	
	No.	%	No.	%	No.	%
Low	15	78.9	4	21.1	19	100.0
High	1	16.7	5	83.3	6	100.0
Total	16	64.0	9	36.0	25	100.0
P:	< 0.05*					

* Significant

There was significantly higher rate ($P < 0.05$) of H. pylori infection among patients with low socioeconomic status.

15 (78.9%) out of 19 patients with low socioeconomic status were H. pylori positive while 1 (16.7%) out of 6 high socioeconomic state patients was H. pylori positive.

Fig 4 : Relation between H.pylori infection and socioeconomic status

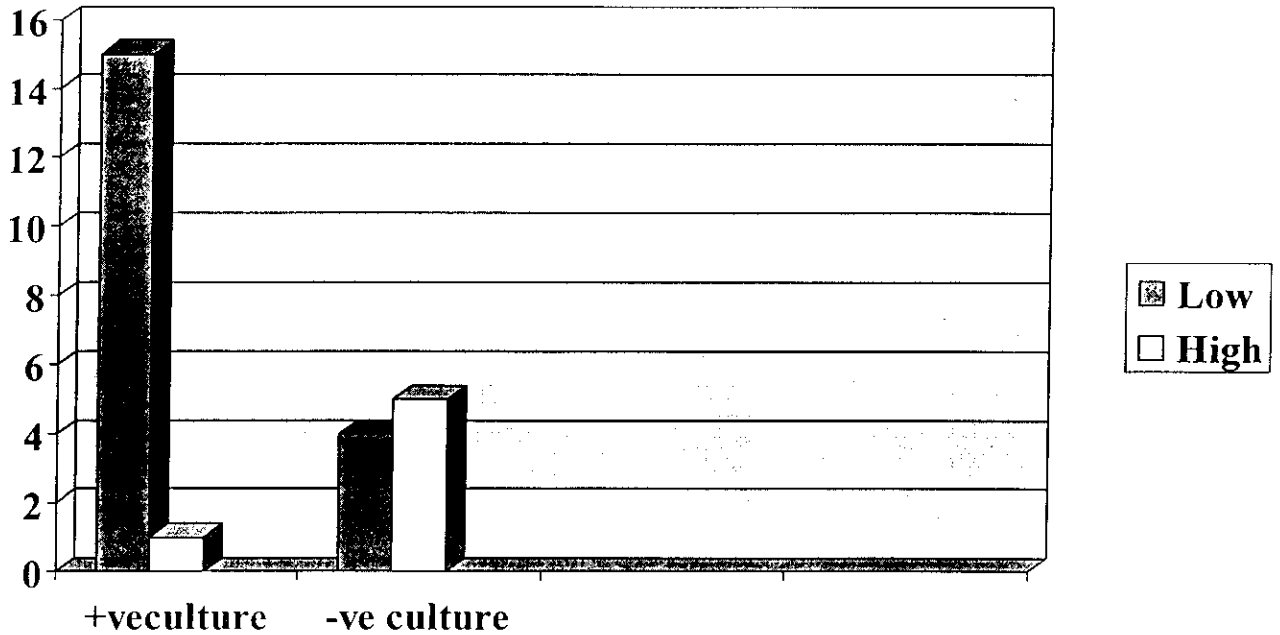

Table (7): Relation between H. pylori infection and history of previous treatment.

History of treatment	+ve culture		-ve culture		Total cases	
	No.	%	No.	%	No.	%
-	3	33.3	6	66.7	9	100.0
+	13	81.2	3	18.8	16	100.0
Total	16	64.0	9	36.0	25	100.0
P:	< 0.05*					

* Significant

H. pylori infection was significantly higher ($P < 0.05$) in patients with past history of treatment than in patients without such history.

13 (81.2%) out of 16 patients with positive past history of treatment were H. pylori positive culture. And 3 (33.3%) out of 9 patients without past history of treatment were positive for H. pylori by culture.

Fig 5 : Relation between H.plylori infection and history of previous treatment

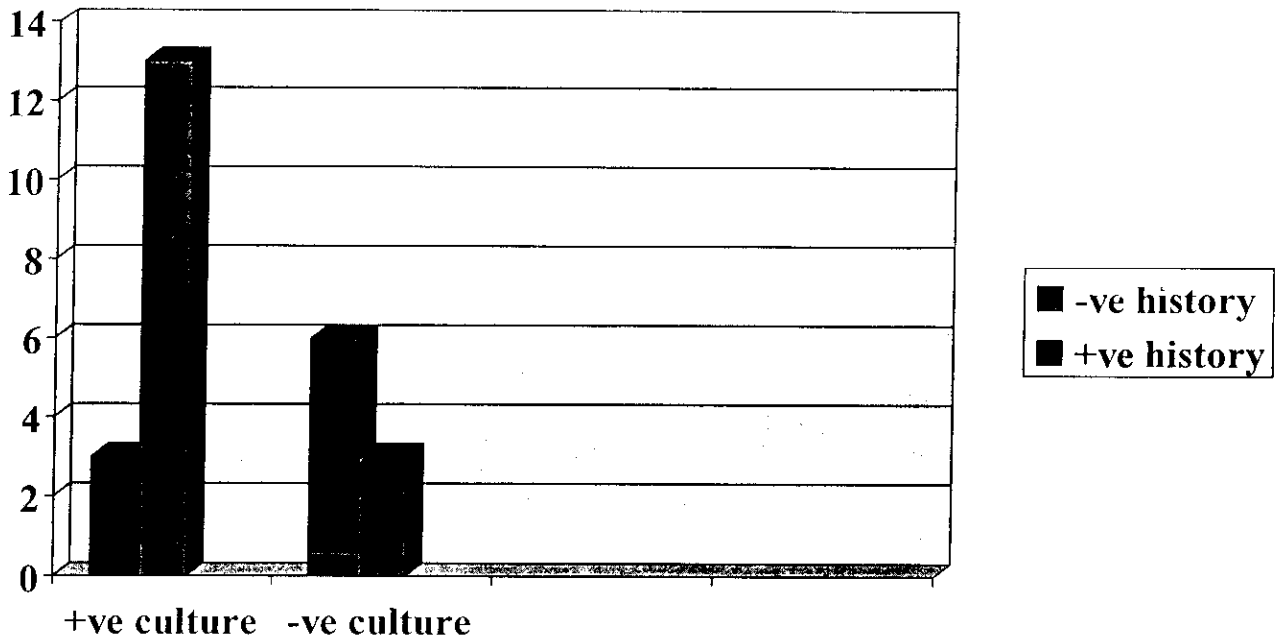

Table (8): H. pylori infection in relation to family history.

Family history	+ve culture		-ve culture		Total cases	
	No.	%	No.	%	No.	%
-	12	60.0	8	40.0	20	100.0
+	4	80.0	1	20.0	5	100.0
Total	16	64.0	9	36.0	25	100.0
P:	> 0.05					

There was no statistically significant difference ($P > 0.05$) between H. pylori infection among patients with and without family history of similar condition.

12 (60%) out of 20 patients without family history of similar condition and 4 (80) out of 5 patients with family history of similar condition were H. pylori positive culture.

Table (9): Relation between H. pylori infection and different complaints.

complaints	-ve culture		+ve culture		Total cases	
	No.	%	No.	%	No.	%
Anorexia	2	50.0	2	50.0	4	100.0
H. burn	3	30.0	7	70.0	10	100.0
Epi. Pain	2	50.0	2	50.0	4	100.0
Vomiting	0	0.00	4	100.0	4	100.0
Nausea	2	66.7	1	33.3	3	100.0
Total	19	36.0	16	64.0	25	100.0

Table (9) showed that:

- 2 (50%) out of 4 patients complaining of Anorexia showed H. pylori positive culture.
- 7 (70%) out of 10 patients complaining of heart burn showed H. pylori positive culture.
- 2 (50%) out of 4 patients complaining of Epigastric pain showed H. pylori positive culture.
- 4 (100%) of patients complaining of vomiting showed H. pylori positive culture.
- 1 (33.3%) out of 3 patients complaining from nausea showed H. pylori positive culture.

Table (10): Comparison between cases (+ve and –ve cultures) and controls as regards endoscopic findings.

Endoscopic finding	Cases						Controls	
	-ve culture		+ve culture		Total cases			
	No.	%	No.	%	No.	%	No.	%
Normal	0	0.0	0	0.0	0	0.0	10	100.0
Gastritis	4	44.4	5	55.6	9	36.0	0	0.0
D. ulcer	5	41.7	7	58.3	12	48.0	0	0.0
G. ulcer	0	0.0	2	100.0	2	8.0	0	0.0
G. cancer	0	0.0	1	10.0	1	4.0	0	0.0
Duodenitis	0	0.0	1	100.0	1	4.0	0	0.0
Total	9	36.0	16	64.0	25	100.0	10	100.0

Table (10) showed that:

- 5 (55.6%) out of 9 patients with gastritis showed positive culture for H. pylori.
- 7 (58.3%) out of 12 patients with duodenal ulcer showed positive culture for H. pylori.
- 2 (100%) of patients with gastric ulcer showed positive culture for H. pylori.
- 1 (100%) of patients with gastric cancer showed positive culture for H. pylori.
- 1 (100%) of patients with duodenitis showed positive culture for H. pylori.
- Regarding the comparison between cases (group I) and controls (group II).
- There were 10 (100%) control patients with normal mucosa on endoscopy and all of them showed negative culture for H. pylori.

Table (11): Comparison between H. pylori culture and urease test.

Ureases	Culture		Total
	Positive	Negative	
Positive:			
No.	14	6	20
%	70.0	30.0	100.0
Negative:			
No.	2	3	5
%	40.0	60.0	100.0
Total:			
No.	16	9	25
%	64.0	36.0	100.0

$P > 0.05$

There was no significant relation ($P > 0.05$) between culture of H. pylori and direct urease test as method for diagnosis of H. pylori.

- 14 (70%) out of 20 cases positive by direct urease were positive for H. pylori by culture.
- 2 (40%) out of 5 cases negative direct urease test were H. pylori positive culture.

Results of tissue culture:

- This study included 25 patients (group I) 16 (64%) of them were H. pylori positive culture.
- The cytopathic effect of H. pylori lysates on vero cell line was studied and all H. pylori lysate were cytotoxic to vero cell line.
- The cytotoxin production by the 16 H. pylori isolates was tested by the Vero, BHK-21, HEP-2 and LLC-MK2 cells. Positive cytotoxic activity was demonstrated by the presence of > 25% cell rounding and detachment. The highest dilution of the cell-free filtrate producing cytopathic effects was designated the cytotoxic titre (reciprocal of dilution).

Table (12): Titre and cytotoxic activity of H. pylori isolated strains on different cell lines.

Titre	Cytotoxic activity	Vero		BHK 21		HEP 2		LLC-MK2	
		No.	%	No.	%	No.	%	No.	%
1	-ve	4	25.0	8	50.0	7	43.75	8	50.0
2	+ve	3	18.75	6	37.5	6	37.5	8	50.0
4	+ve	4	25.0	2	12.5	3	18.75		
8	+ve	3	18.75						
16	+ve	2	12.5						
Total		16	100.0	16	100.0	16	100.0	16	100.0

Table (12) showed that:

- 75% of H. pylori isolates were cytotoxic to Vero cells.
- 50% of H. pylori isolates were cytotoxic to BHK-21 cells.
- 56.25% of H. pylori isolates were cytotoxic to HEP2 cells.
- 50% of H. pylori isolates were cytotoxic to LLC-MK2.

Table (13): Vero titre versus endoscopic findings.

Endoscopy / Titre	Gastritis	D. ulcer	G. ulcer	G. cancer	Duodenitis
1	3	0	1	0	0
%	60	0.0	50	0.0	0.0
2	1	0	0	1	0
%	20	0.0	0.0	100.0	0.0
4	1	2	1	0	0
%	20	28.6	50	0.0	0.0
8	0	3	0	0	0
%	0.0	42.9	0.0	0.0	0.0
16	0	2	0	0	1
%	0.0	28.6	0.0	0.0	100.0
Total	5	7	2	1	1

Table (13) showed that:

- 2 (40%) out of 5 H. pylori strains isolated from patients with gastritis were cytotoxic to vero cells.
- 7 (100%) of H. pylori strains isolated from patients with duodenal ulcer were cytotoxic to vero cells.
- 1 (50%) out of 2 H. pylori strains isolated from patients with gastric ulcer were cytotoxic to vero cells.
- 1 (100%) of H. pylori isolated from patients with gastric carcinoma were cytotoxic to vero cells.
- 1 (100%) of H. pylori isolated from patients with duodenitis were cytotoxic to vero cells.

Table (14): BHK21 titre versus endoscopic findings.

Endoscopy / Titre	Gastritis	D. ulcer	G. ulcer	G. cancer	Duodenitis
-	4	2	2	0	0
%	80.0	28.6	100.0	0.0	0.0
2	1	4	0	0	1
%	20.0	57.1	0.0	0.0	100.0
4	0	1	0	1	0
%	0.0	14.3	0.0	100.0	0.0
Total	5	7	2	1	1

Table (14) showed that:

- 1 (20%) out of 5 H. pylori strains isolated from patients with gastritis were cytotoxic to BHK-21.
- 5 (71.4%) out of 7 H. pylori strains isolated from patients with D. ulcer were cytotoxic to BHK-21.
- Non of H. pylori strains isolated from G. ulcer patients were cytotoxic to BHK-21.
- 1 (100%) of H. pylori isolated from G. carcinoma patients was cytotoxic to BHK-21.
- 1 (100%) of H. pylori isolated from patients with duodenitis was cytotoxic to BHK-21.

Table (15): HEP2 titre versus endoscopic findings.

Endoscopy / Titre	Gastritis	D. ulcer	G. ulcer	G. cancer	Duodenitis
-	3	2	1	1	0
%	60.0	28.6	50.0	100.0	0.0
2	0	4	1	0	1
%	0.0	57.1	50.0	0.0	100.0
4	2	1	0	0	0
%	40.0	14.3	0.0	0.0	0.0
Total	5	7	2	1	1

Table (15) showed that:

- 2 (40%) out of 5 H. pylori strains isolated from patients with gastritis were cytotoxic to HEP-2 cells.
- 5 (71.4%) out of 7 H. pylori strains isolated from patients with D. ulcer were cytotoxic to HEP-2.
- 1 (50%) out of 2 H. pylori strains isolated from patients with G. ulcer were cytotoxic to HEP-2.
- Non of the stains isolated from G. cancer were cytotoxic to HEP-2.
- 1 (100%) of H. pylori isolated from patients with duodenitis was cytotoxic to HEP-2.

Table (16): LLCMK2 titre versus endoscopic findings.

Endoscopy / Titre	Gastritis	D. ulcer	G. ulcer	G. cancer	Duodenitis
1	3	3	1	0	1
%	60.0	42.9	50.0	0.0	100.0
2	2	4	1	1	0
%	40.0	57.1	50.0	100.0	0.0
Total	5	7	2	1	1

Table (16) showed that:

- 2 (40%) out of 5 H. pylori strains isolated from patients with gastritis were cytotoxic to LLC-MK2.
- 4 (57.1%) out of 7 H. pylori strains isolated from patients with D. ulcer were cytotoxic to LLC-MK2.
- 1 (50%) out of 2 H. pylori strains isolated from patients with G-ulcer were cytotoxic to LLC-MK2.
- 1 (100%) of H. pylori isolated from patients with G. cancer were cytotoxic to LLC-MK2.
- Non of H. pylori isolated from patients with duodenitis were cytotoxic to LLC-MK2.

Table (17): Correlation between effect of H. pylori toxins on different tissue cultures.

	BHK-21	HEP-2	LLCMK2	VERO
BHK-21	1.000			
HEP-2	- 0.354	1.000		
LLCMK-2	0.538*	-0.221	1.000	
VERO	0.316	0.113	0.028	1.000

* Significant

There was significant correlation between BHK-21 cytopathic findings and LLC-MK2 cytopathic findings.

This table showed the correlation between different cell lines used as regard the cytopathic effect of H. pylori toxin.

III- Results of electrophoresis:

- SDS electrophoresis of H. pylori broth culture filtrate (which contain the toxin) revealed several similar protein bands that were present in all the examined samples as well as in the control sample.
- There was a protein band between 66 KDa and 97 KDa, this band was nearer to 97 KDa molecular weight.
- The molecular weight of this band was corresponding to the same molecular weight of H. pylori vaculating cytotoxin (87-94 KDa).
- The culture media for H. pylori [Brucella broth with 10% F.C.S.] was used as negative control to compare its bands with those of the broth culture filtrate of H. pylori.

In this study a molecular weigh marker with the following bands was used:

* 97 KDa (phosphate b)
* 66 KDa (bovine serum albumin).
* 45 KDa (ovalbumin).
* 17 KDa (lysozyme).