AIM OF THE WORK

The present study is designed to:

1. Screen acute Leukemia and Malignant Lymphoma patients for Helicobactor pylori antibodies to six virulence antigens by a new line immunoassay and correlate the clinical status to epidemiological markers of bacterial virulence factors.
2. Screen the same cases to HCV antibodies to verify the role of HCV infection as a possible risk cofactor.
3. Correlation between the HCV antibodies and six virulence antigens.

SUBJECTS AND METHODS

Patients:

This study was conducted on one hundred patients referred to Internal Medicine Department Haematology unit in Alexandria Main University Hospital presented with dyspeptic complaints in the form of dyspepsia, abdominal pain, loss of weight, nausea, vomiting. A written informed consent was taken from all enrollees. Clinical selection and sampling of cases started in June 2012 and was completed by the end of September 2013.

Inclusion criteria for patient selection included:

- Age group between 15 and 60 years old.
- Being positive to acute leukemia and malignant lymphoma (proven by CBC and Biopsy).

Exclusion criteria for patient selection included:

- Previous eradication treatment for Helicobacter pylori infection and Hepatitis C virus.
- Antimicrobial treatment two months preceding the study (concomitant medication with bismuth preparations, proton pump inhibitors, H2 receptor antagonists, or non steroidal ani-inflammatory drugs).
- Other serious illness and history of gastric surgery.
- Extremity of age
- Positive for Hepatitis B virus and HIV , hypertension , diabetes mellitus, renal and liver failure .

History sheets for one hundred patients included the following data:

- Patient number:
- Patient name:
- Sex:
- Age:
- Residence:
- Number of family members:
- Smoking:
- Complaints of: heart burn, dyspepsia, hematemsis, melena, abdominal pain, loss of weight, and vomiting.
- Any drug intake:
- Past history of previous endoscopy :
- Past history of previous gastrointestinal surgery:
- Family history of similar conditions in other family members.
- Previous history or exposure to radiotherapy or chemotherapy treatment

METHODS:

For each case enrolled the following items were fulfilled:

1. Full history taking and clinical examination.
2. Routine laboratory tests:
   - Complete blood picture
   - Liver function tests.
   - Renal function tests.
3. Bone marrow examination for cases.

❖ **Testing the samples for presence of HCV antibodies:**

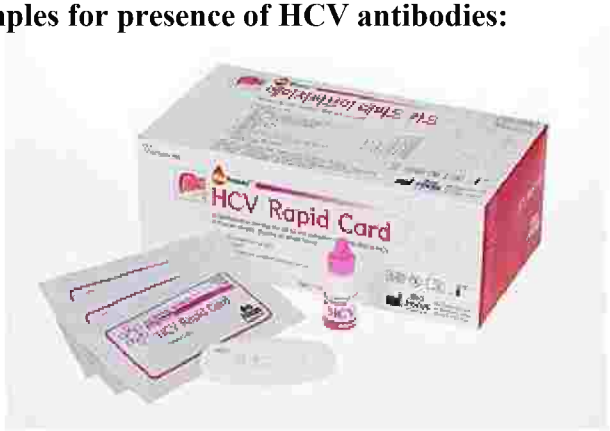

Fig. (19): The kit used for diagnosis of HCV

Principles: The HCV test device (serum/plasma) detects antibodies to HCV through visual interpretation of development in the internal strip . Protein A is immobilized on the test region of the membrane

Specimen collection & storage :

- The HCV test device (serum/plasma) was used with human serum or plasma specimens only .
- Only clear , non hemolysed specimens were used with this test . Serum or plasma were seperated as soon as possible to avoid hemolysis and placed in epindorphs labeled with the patient's name and number.
- The testing was immediately done after specimen collection .
- The samples were brought to room temperature prior to testing .Frozen specimens were completely thawed and mixed well prior to testing .

Procedures:

- The tests, specimens , and/ or controls were brought to room temperature (15-30'C) before use .

1. The test and device were labeled with patient or control identification . For best results , the assay was preformed within one hour .
2. Using the provided disposable, pippette, the narrow portion of the pipette was filled with the serum or plasma (approxim. 10ul), and the liquid was transferred to the specimen well (S) , then 2 drops of buffer were added and the timer started. As the test begins to work, the colour migrates across the membrane.
3. The coloured band (s) appeared . The result was read at 10 minutes.

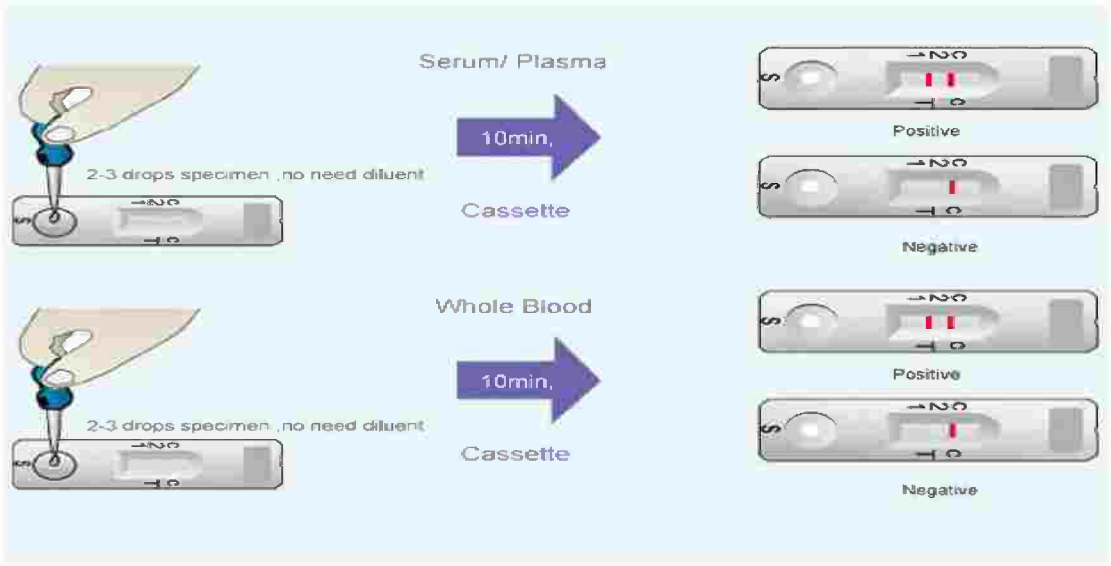

Fig. (20): The procedure of the rapid commercial HCV test

Line immunoassay (LIA) for antibody detection to six virulent *H.pylori* antigens:
Summary of technique preparation:

Pathogen antigen proteins were transferred onto a nitrocellulose membrane by a special spraying process. Nitrocellulose membrane was then cut up into individual strips.

Incubation of antigen-carrying nitrocellulose-strips with human samples allowed the detection of specific antibodies that may be present in serum. These antibodies develop immune complexes with antigen fixed on test strip.

After removing the unbound antibodies by washing steps, single nitrocellulose –strips were incubated with alkaline phosphatase conjugated anti-human IgG-antibodies. After unbound conjugated antibodies were removed by a further washing step, a visualization of antigen/antibody –complex (of the bound antibodies) is accomplished by the addition of a non-coloured substrate, which forms blue- violet precipitates at each site (antigen bands) where the conjugated anti-human antibodies had bound.

Enzyme /substrate –reaction was stopped through washing nitrocellulose –strips with distilled or deionized water. Depending on the observed band pattern one can interpret the presence of specific IgG- antibodies.

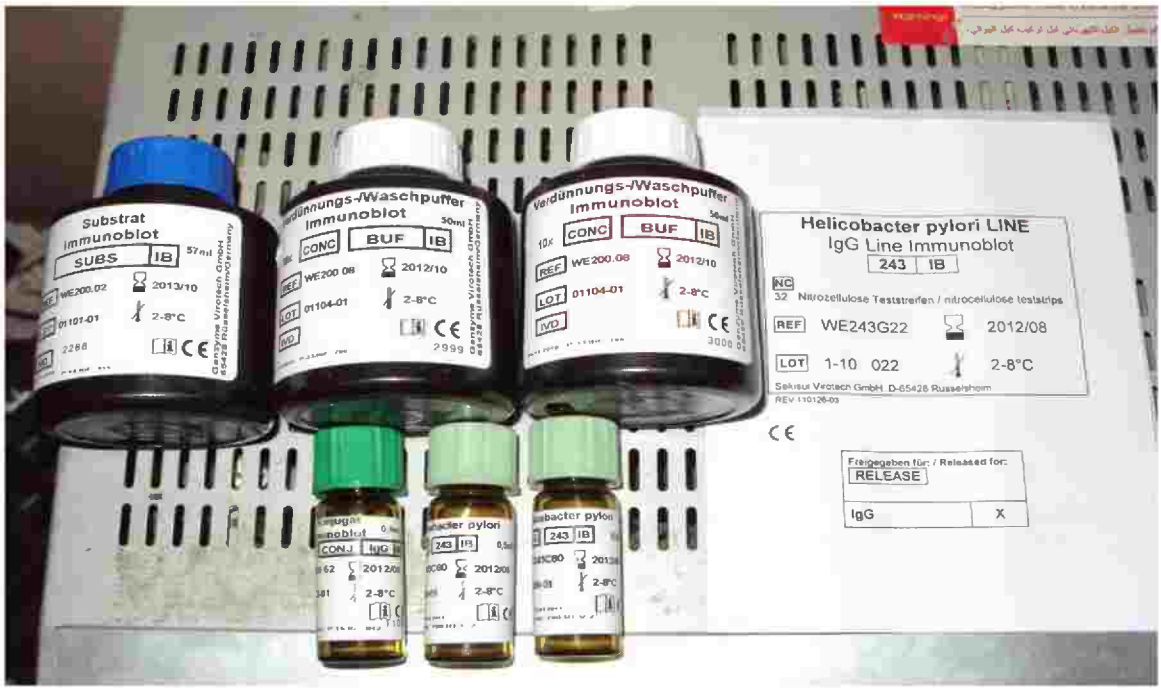

Fig. (21): The Line immunoassay kit

Preparation of reagent:

1. The corresponding concentrate was brought to room temperature (20-25°c) before preparing the dilution. High quality distilled water was used and brought up to room temperature before usage.
2. Dilutions were mixed well before starting the test.
3. Dilution/wash buffer: Dilution wash buffer was 10x concentrated. It was diluted 1:10 with distilled water and mixed well before use.
4. IgG conjugate: The conjugate was diluted 1+100 with finally diluted dilution/ wash buffer and mixed thoroughly. Conjugate working solution (1.5ml) was required for each serum sample.
5. Substate solution was ready to use.

Test procedure:

1. Test was proceeded at room temperature.
2. For each sample 1strip was put into each channel of a clean incubation tray. Each strip was held only at the marked upper end.
3. Ready serum dilution buffer (1.5 ml) was pipetted on each and put onto the rocking platform. Antigen strips were consistently covered with liquid.
4. The solid antigen strips were moistured completely within one minute and were incubated in supine lateral position.
5. Microlitre portions (15ul) of patient serum in IgG was applied , by pipetting at the upper ,marked end of the strip. Patient serum was incubated for 30 minutes on the rocking platform.
6. The liquid was aspirated or poured away out of the channel completely. During the pour away of the liquid ,the antigen strips remained at the bottom of the channel. The remaining liquid was drained onto a filter paper.

7. Washing of strips: 1.5 ml of ready to use dilution\wash buffer was incubated each for 3x5 minutes on the rocking platform. Washing buffer was then poured away completely.
8. The liquid was then poured away out of the channels completely as before .
9. The prepared conjugate dilution (1.5 ml) was pipetted each in the corresponding incubation channel and incubated for 30 minutes on the rocking platform.
10. The liquid was again aspirated out of the channels completely as before.
11. Washing of the strips: 1.5 ml of ready to use dilution wash buffer was incubated each for 3x5 minutes on the rocking platform. The wash buffer was poured away completely then rinsed 1x1minute with distilled water.
12. The liquid was then poured away out of the channels.
13. Ready to use substrate solution (1.5 ml) was pipetted into the channels and developed for 10 ± 3minutes on the rocking platform.
14. Colour reaction was then stopped by pouring away the substate solution. The strips were then washed without incubation in between for 3 time with 1.5 ml distilled water each.
15. Distilled water was then poured away and the strips were left to dry on a clean filter paper.
16. Results were then recorded in the enclosed evaluation sheet.

Interpretation of results:

For a secure interpretation of results each LINE strip was filtered out with two controls:

1. Serum control:

Only after incubation with patient serum, serum incubation band appeared below the mark line.

2. Conjugate control:

The LINE strip was filtered out with a conjugate control band which appeared after incubation with the respective conjugate. The test procedure was valid, if serum control as well as internal conjugate control appeared clearly visible on the developed nitrocellulose test strip.

Fig. (22): The bands appearing on the test strip

Interpretation criteria:

Table (V): The interpretation table for the H.pylori test strip

The number of Visible bands	Interpretation
No band or only one band of p30.p19	Negative
Only one band of VacA,UreA,p25	Borderline
CagA Or Arise from ≥ 2 bands of the following: VacA,p30,UreA,p19,p25	Positive

RESULTS

Cases of the present study were selected from Haematology unit (Internal Medicine) of Alexandria Main University Hospital during the year 2012.

A hundred Leukemia/ Malignant Lymphoma cases who were fully investigated by clinical and laboratory tests were the candidate of our screening serological study. Acute leukemia cases were previously diagnosed by CBC and bone marrow examination.

Malignant lymphoma cases were previously diagnosed by lymph node biopsy.
Method of statistical analysis used was

All cases were subjected to the following:

- Microbiologic investigations as:
  1. Helicobactor line immunoassay to detect six virulent antibodies in sera of all patients following the manufacturer's instructions.
  2. Screening for HCV antibodies will be done by Enzyme Linked Immunosorbent Assay (ELISA), a rapid commercial test following the manufacturer's instructions.

Confidentiality of the patient's data was assured.

Table (VI) shows sociodemographic data of the two studied groups. Age in patients group ranged 18-51 with mean value 36.9±10.19 and in control group ranged 19-52 with mean value 37.2±9.84. Males in patients group were 58 (58%) and in control group 15(60%), female in patients group were 42(42%) and in control group 10(40%). There was no statistical significant difference regarding age and sex of the two studied groups (P > 0.05).

Table (VI): Sociodemographic data of the two studied groups.

	Patients group "n=100"		Control group "n=25"		P
Age					
Range	18 – 51		19 - 52		
Mean	36.9		37.2		0.365
S.D.	10.198		9.84		
Sex					
Male	58	58.0	15	60.0	0.425
Female	42	42.0	10	40.0	

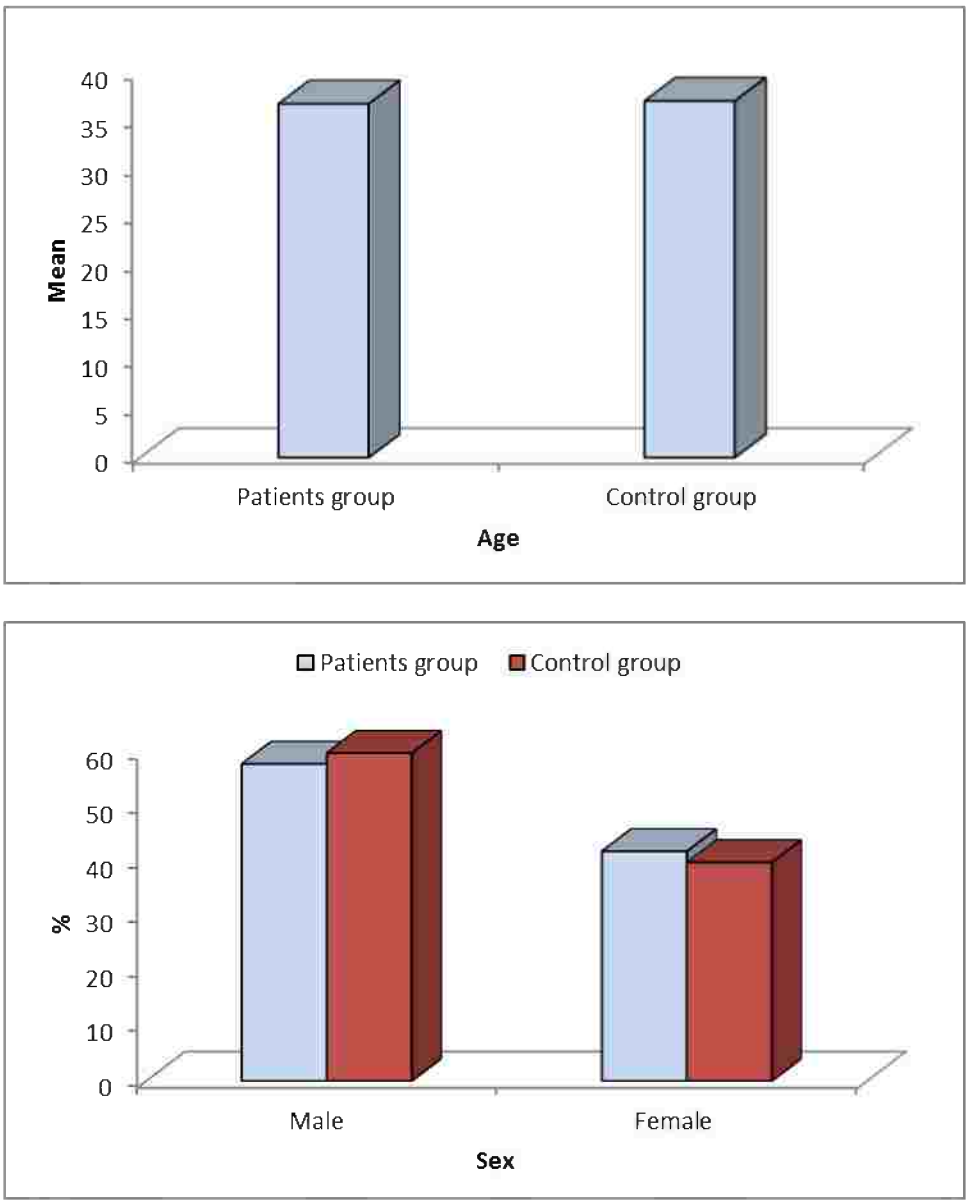

Fig. (23): Sociodemographic data of the two studied groups.

Table (VII) shows distribution of the studied patients groups regarding diagnosis. ALL was 30 (30%), AML 32(32%), NHL 22(22%), AHL 1(10%) and HL 6(6%).

Table (VII): Distribution of the studied patients groups regarding diagnosis.

	Number	Percent
ALL	30	30.0
AML	32	32.0
NHL	22	22.0
AUL	10	10.0
HL	6	6.0
Total	100	100.0

N.B: ALL: Acute lymphocytic leukemia, AML: Acute myeloid leukemia, NHL: Non–Hodgkin lymphoma, AUL: Acute undifferentiated lymphoma, HL: Hodgkin lymphoma

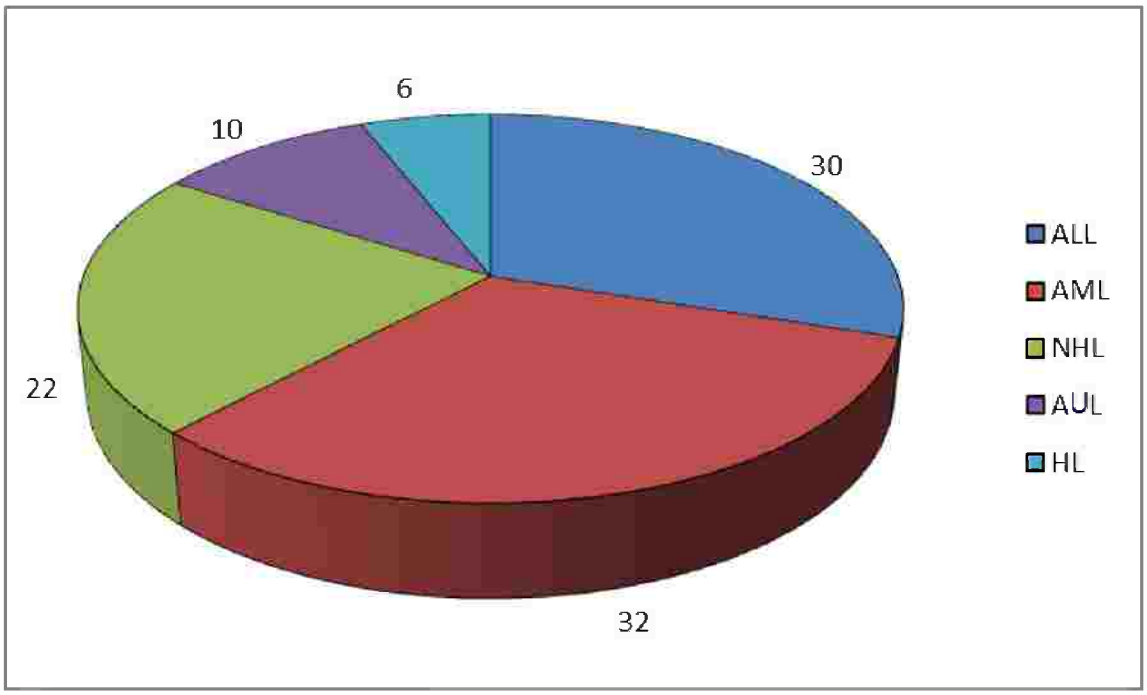

Fig. (24): Distribution of the studied patients groups regarding diagnosis.

Table (VIII) shows distribution of the studied patients groups regarding the incidence of HCV. Positive HCV was 47(47%) and negative HCV was 53(53%).

Table (VIII): Distribution of the studied patients groups regarding the incidence of HCV.

HCV	Number	Percent
Positive	47	47.0
Negative	53	53.0
Total	100	100.0

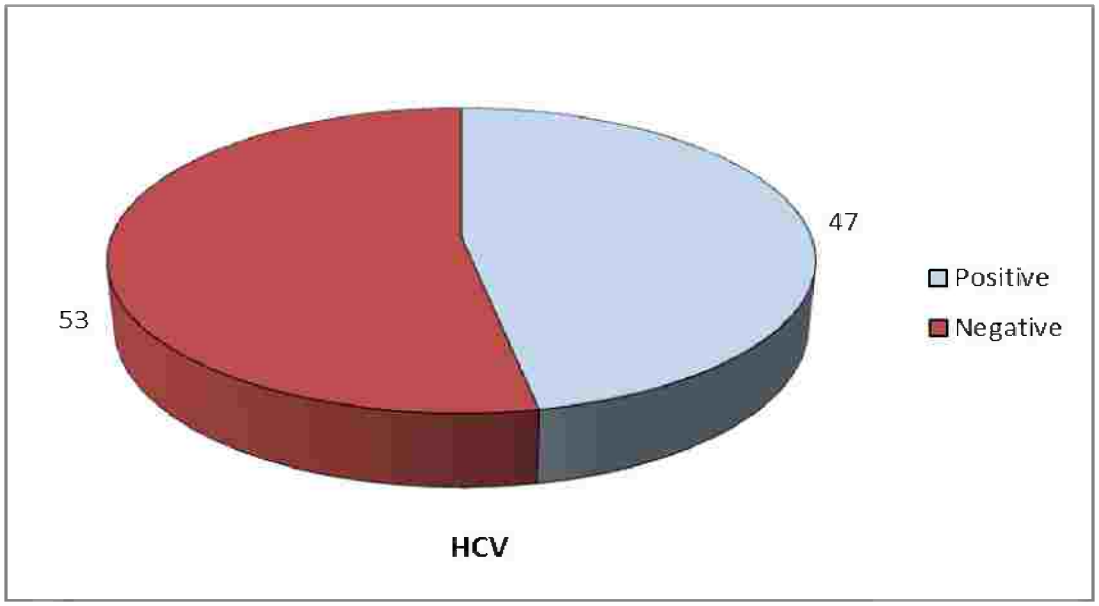

Fig. (25): Distribution of the studied patients groups regarding the incidence of HCV.

Table (IX) shows distribution of different bands present on H. pylori strip. in the studied patients. CagA was 90 (90%), VacA 50 (50%), p30 80 (80%), urea A 78(78%), p25 was 100 (100%), p19 was 100 (100%).

Table (IX): Distribution of different bands present on H. pylori strip. in the studied patients.

	Number	**Percent**
Cag A	90	90.0
VacA	50	50.0
p30	80	80.0
Ure A	78	78.0
p25	100	100.0
p19	100	100.0
Total	100	100.0

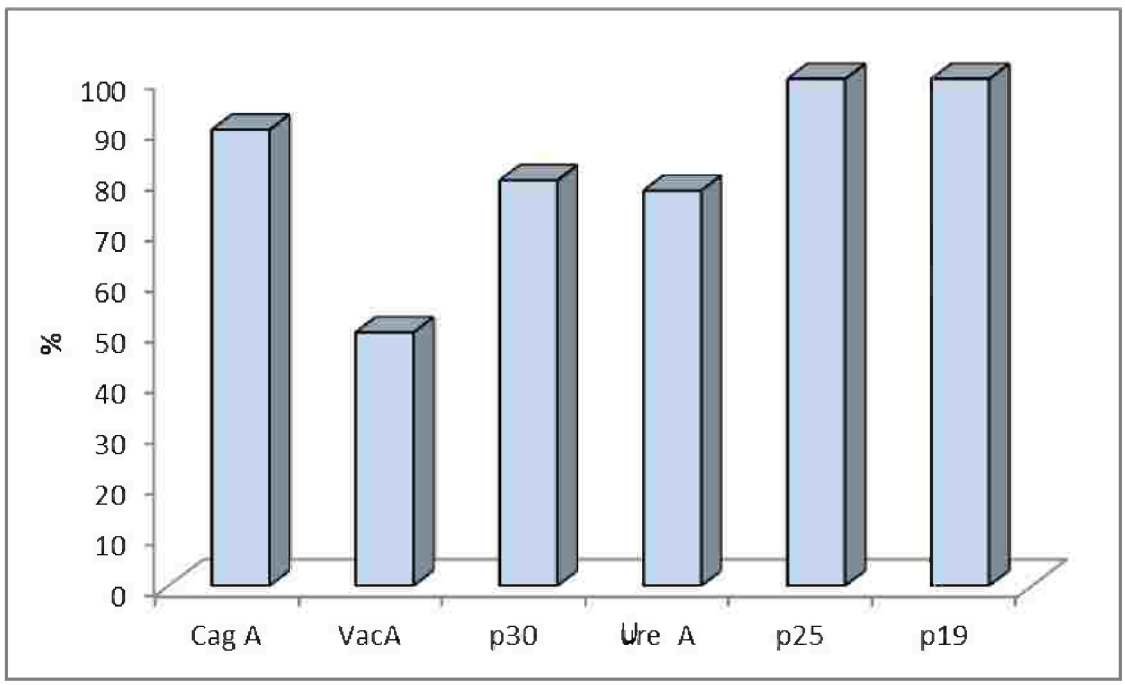

Fig. (26): Distribution of different bands present on *H. pylori* strip. In the studied patients

Table (X) shows relation between incidence of HCV and different bands present H. pylori strip. There was no statistical significant difference between incidence of HCV with CagA, p30 and urea A ($P > 0.05$), while there was statistical significant difference between incidence of HCV and VacA ($P < 0.05$).

Table (X): Relation between incidence of HCV and different bands present *H. pylori* strip.

	Total	HCV				p
		Positive "n=47"		Negative "n=53"		
		No.	%	No.	%	
Cag A	90	44	48.9	46	51.1	0.621
VacA	50	31	62.0	19	38.0	0.048*
p30	80	42	52.5	38	47.5	0.452
UreA	78	38	48.7	40	51.3	0.652
p25	100	47	100.0	53	100.0	-
p19	100	47	100.0	53	100.0	-

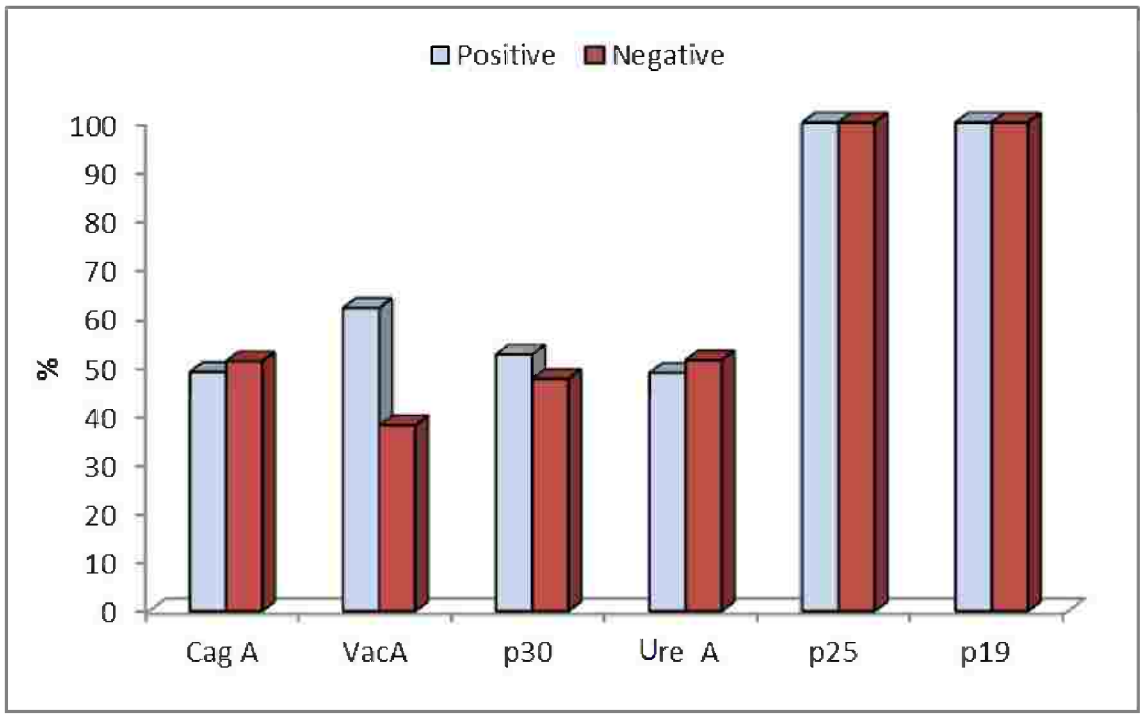

Fig. (27): Relation between incidence of HCV and different bands present *H. pylori* strip.

Table (XI) shows relation between diagnosis and different bands present *H. pylori* strip. There was no statistical significant association between diagnosis and different bands present *H. pylori* strip (P > 0.05).

Table (XI): Relation between diagnosis and different bands present H. pylori strip.

	Total	Diagnosis										p
		ALL "n=30"		AML "n=32"		NHL "n=22"		AUL "n=10"		HL "n=6"		
		No.	%	No.	%	No.	%	No.	%	No.	%	
Cag A	90	29	96.7	28	87.5	20	90.9	8	80.0	5	83.3	0.23
VacA	50	12	40.0	18	56.3	12	54.5	5	50.0	3	50.0	0.165
p30	80	27	90.0	20	62.5	19	86.4	10	100.0	4	66.7	0.15
UreA	78	13	43.3	24	75.0	22	100	6	60.0	5	83.3	0.254
p25	100	30	100.0	32	100.0	22	100.0	10	100.0	6	100.0	0.311
p19	100	30	100.0	32	100.0	22	100.0	10	100.0	6	100.0	0.25

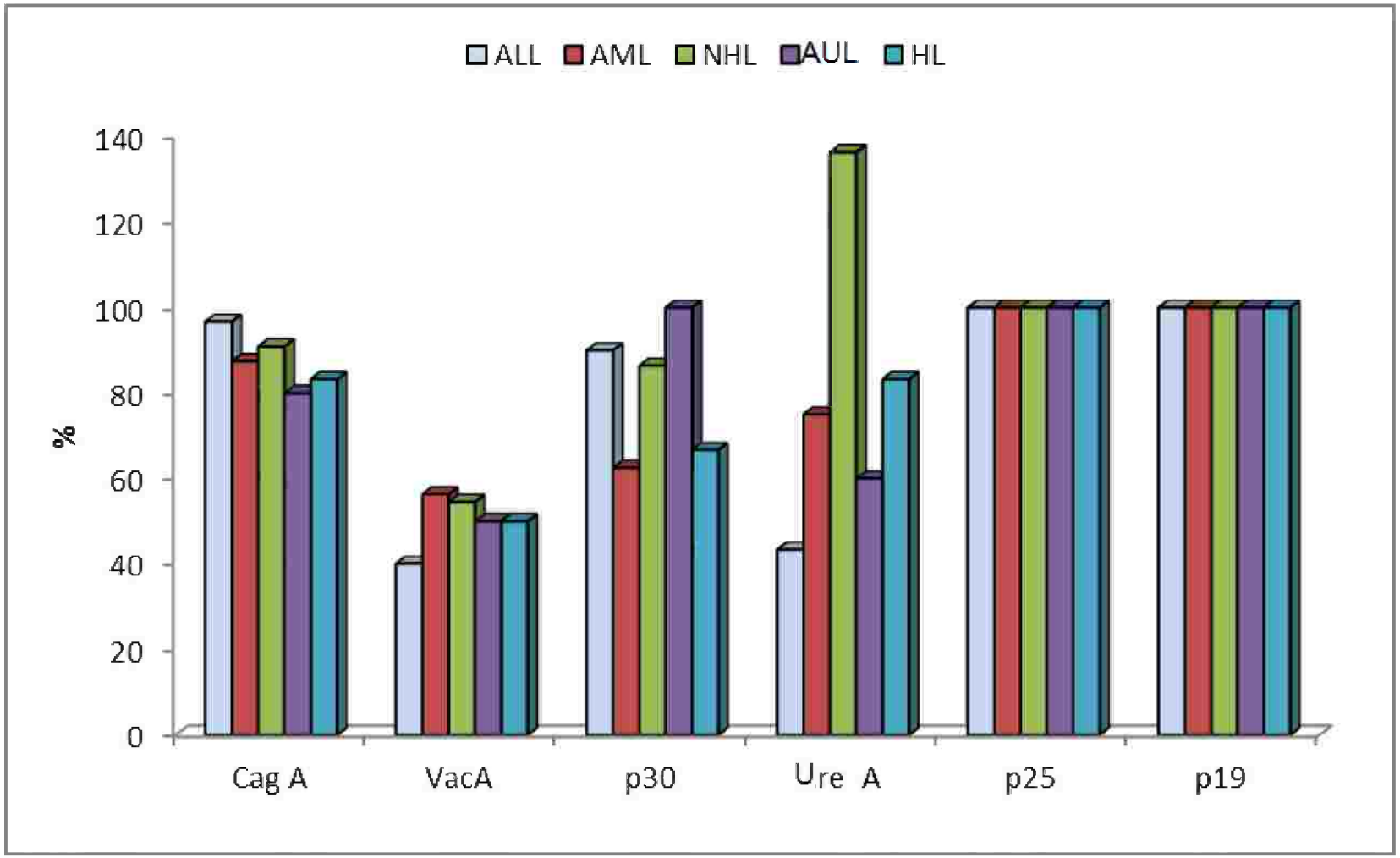

Fig. (28): Relation between diagnosis and different bands present *H. pylori* strip.

Table (XII) shows relation between diagnosis and blood picture. There was statistical significant difference between diagnosis with WBCS, Hb and platelet ($P < 0.05$), while there was no statistical significant difference between diagnosis and RBCs ($P > 0.05$).

Table (XII): Relation between diagnosis and blood picture

	Diagnosis				**P**
	ALL "n=30"	**AML "n=32"**	**NHL "n=22"**	**AUL & HL "n=16"**	
WBCs					
Range	4.71-38.74	7.5-22.1	4.53-39.3	4.5-23	
Mean	22.62	15.89	16.68	10.39	**0.0133***
S.D.	12.89	4.90	10.36	5.17	
RBCs					
Range	3.32-8.71	3.63-7.14	3.1-8.91	2.35-7.27	
Mean	5.89	5.24	5.13	5.20	**0.254**
S.D.	1.73	1.32	1.90	1.13	
Hb					
Range	8.8-10.20	7.1-11.3	6.9-20	6.9-18	
Mean	9.18	10.16	11.95	12.65	**0.042***
S.D.	2.05	2.43	3.91	2.62	
Platelet					
Range	124-1967	216-743	110-1985	159-765	
Mean	952.30	449.60	766.45	356.20	**0.001***
S.D.	674.72	181.66	588.70	155.65	

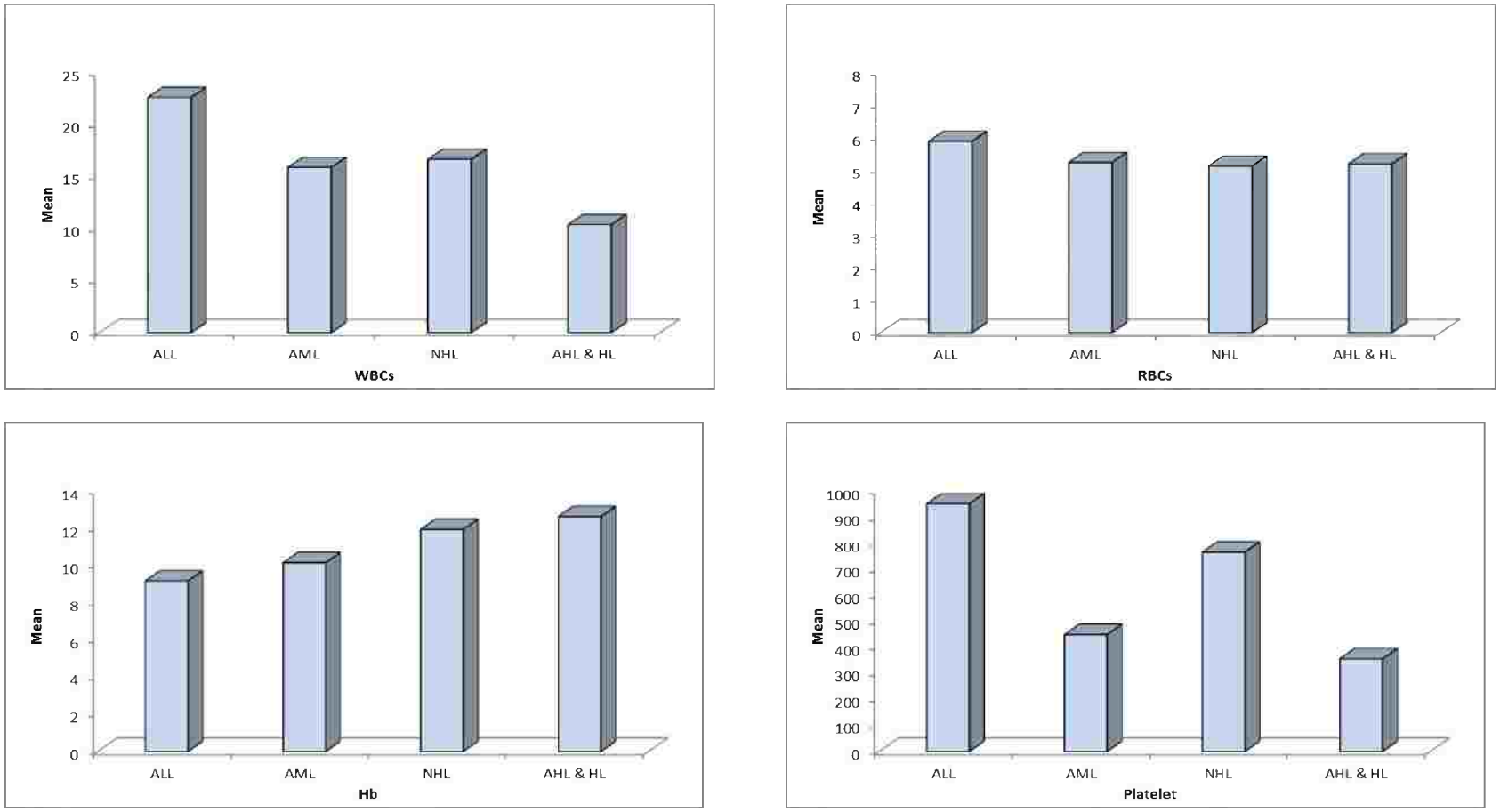

Fig. (29): Relation between diagnosis and blood picture