PRIMARY MALIGNANT GASTRIC NEOPLASMS, A STUDY OF THEIR ASSOCIATION WITH HELICOBACTER PYLORI INFECTION AND CORRELATION WITH HAEMOGLOBIN LEVEL AND BLOOD GROUP

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Abstract

The main objective of this study was to see the various histopathological variants of malignant gastric neoplasms, and to correlate them with Helicobacter pylori infection, blood grouping, hemoglobin level and clinical presentation. 40 cases of primary gastric malignancies were taken as a study group. Gross and microscopic features of the specimens, sent to the Department of Pathology, TUTH were studied. Giemsa stained sections were prepared for detection of H. pylori. Blood from the patients were collected and used for blood grouping & hemoglobin estimation. Serum was used to detect IgG against H. pylori.

Among 40 cases of gastric malignancies, 38 (95%) cases were adenocarcinomas, 1 (2.5%) case was non-Hodgkin's lymphoma and 1(2.5%) case was leiomyosarcoma. Among adenocarcinomas; 28 (73.69%) cases were of tubular type; 6 (15.79%) cases were of signet-ring cell type; 2 (5.26%) cases were of papillary type and 2 (5.26%) cases were of mucinous type. Gastric malignancies were more common in male (70%) and after the age of 50 yrs(75%). The most common complaints were abdominal discomfort and abdominal pain which were seen in 95% cases. Hematemesis & melaena were present only in 15% and 25% cases respectively.

Grossly, most of the tumors were located at antrum (78.95%) with ulcerating growth (89.48%).Giemsa staining revealed H.pylori in 30 cases(78.95%)of adenocarcinomas; in a case of non- Hodgkin's lymphoma and not in a case of leiomyosarcoma. H.pylori seropositivity (IgG) was seen in 29 cases (76.31%) of adenocarcinomas, in a case of non- Hodgkin's lymphoma and not in a case of leiomyosarcoma.

Both histological method and serological test showed positivity for H. pylori in 26 cases (65%) of gastric malignancies.

Both blood groups A and O were found in 14 cases (36.84%) each; group B was found in 8 (21.05%)cases and group AB in 2 (5.27%) cases of gastric carcinomas.

Most of the patients (52.63%) were mildly anaemic while 15.79% cases were found to be moderately anaemic, and 31.58% cases were non-anaemic.

CONTENTS

INTRODUCTION

Malignant tumors of stomach are very common forms of malignant neoplasms. Among these, gastric carcinoma is the most common. Gastric cancer is sixth most common fatal malignancy in UK and accounts for 10% of all deaths from malignant diseases, in France it holds fifth place. In TUTH, it is found to be the most common malignancy, constituting 16.05% of all malignancies. Carcinoma of the stomach is one of the ' captains of the men of death'. It is more common in male and after the age of 50 years, and more frequent in populations with blood group A. Gastric cancer patients complain of epigastralgia, loss of weight, vomiting, occasionally hematemesis & melaena. In asymptomatic patients detection may be too late, even metastasis to other organs may be present.

Helicobacter pylori is gram negative, spirally shaped, unipolar multiflagellate bacteria; discovered by Warren & Marshall. It colonizes antral gastric epithelium. Its association with gastritis and peptic ulcer has already been established. A Working Group of the International Agency for Research on Cancer would have concluded that H.pylori is carcinogenic to human. Association of gastric carcinoma & MALT lymphoma with Helicobacter pylori infection has been reported from different parts of the world.

Helicobacter pylori infection can be diagnosed by histological method (on gastric tissue), serological method (detection of antibody against H. pylori), biopsy urease method, urea breath test and culture. In our country, histological method is feasible and economical.

The correlative study between different malignant gastric tumors, H.pylori infection, blood group and anaemia has not been carried out. So, this prospective study has been designed to correlate the different types of malignant gastric tumors with H. pylori infection, blood group, anaemia and the clinical symptoms. It is hoped that the outcome of this research would be useful for early management and prevention of gastric cancers and diseases associated with H. pylori.

Classification of primary malignant gastric tumors[1]

I. Epithelial tumors

1. Adenocarcinoma
2. Adenosquamous carcinoma
3. Squamous cell carcinoma
4. Undifferentiated carcinoma.
5. Unclassified carcinoma

II. Carcinoid tumors.

III. Non- epithelial tumors.

1. Smooth muscle tumors.

a) malignant leiomyoblastoma

b) Leiomyosarcoma

2. Others.

IV. Haemopoietic and lymphoid neoplasms.

1. Non- Hodgkin's lymphomas (MALT oma).
2. Hodgkin's disease
3. Others

V. Miscellaneous tumors.

VI. Unclassified tumors.

Classification of gastric carcinoma[2]

I. Stout (Atlas of tumor Pathology), 1953.

1. Fungating.
2. Penetrating.
3. Spreading.
4. Superficial spreading.
5. Linitis Plastica.
6. No special type.

II. Lauren, 1965.

1. Intestinal.
2. Diffuse.

III. Ming, 1997.

1. Expanding.
2. Infiltrative

IV. WHO, 1990.

1. Papillary.
2. Tubular.
3. Mucinous.
4. Signet ring.

V Japanese Society for Gastric Cancer , 1981

1. Papillary.
2. Tubular.
3. Poorly differentiated.
4. Mucinous.
5. Signet ring.

V. Goseki system[3]

1. Group I
2. Group II
3. Group III
4. Group IV
5. Others.

VI. According to depth of invasion[4]

1. Early gastric carcinoma.
2. Advanced gastric carcinoma.

TNM system for the staging of gastric carcinoma[5]

T stage	Tumor status in relation to penetration of gastric wall
T1	Confined to mucosa
T2	Involving all layers to serosa
T3	Penetrating serosa, with or without direct invasion of adjacent tissues and organs

T4	Diffuse infiltration of gastric wall.
TX	Degree of involvement unknown

N Stage	Lymph node involvement
No	No lymph nodes involved.
N1	Perigastric nodes adjacent to tumor involved.
N2	Metastasis to nodes on both curvatures or regional areas.
NX	Lymph nodal status unknown.

M stage	Distant involvement
Mo	No metastasis
M1	Distant metastasis

Staging system for gastric cancer[5]

Stage	Definition
Ia	Tumor confined to gastric mucosa only (T1, No, Mo)
Ib	Tumor extending to but not through the serosa (T2,No, Mo)
Ic	Tumor extending through the serosa with or without local invasion (T3,No,Mo)
II	Diffuse involvement of gastric wall (T4, No, Mo) or any involvement of gastric wall in association with tumor deposits in the perigastric lymph nodes (T1-T4, N1, Mo)
III	Any degree of gastric wall with involvement of perigastric lymph nodes distant from the tumor or on both gastric curvatures (T1-T4, N2, Mo)
IV	Distant metastasis.

INCIDENCE

Among the malignant gastric tumors, carcinoma is the most common (90 - 95%)[4]. Next in order of frequency are lymphomas (4%), carcinoids(3%) and malignant spindle cell tumors (2%)[4].

The occurrence of gastric cancer varies considerably both within and between different countries. In a study Shrestha et al. also found gastric cancer as the most common malignancy in TUTH, representing 16.05% of all malignancies[7]. In a study of cases from 2048 Baishak to 2055 Poush (7 years 9 Month) Manohar et al. found gastric cancer to be the commonest cancer in TUTH constituting 15.24% of all malignancies[6].

Carcinoma of the stomach is one of the "captains of the men of death'. In 1959, 14076 people died from this cause in England and Wales[8]. Gastric cancer is sixth most common fatal malignancy in UK and accounts for about 10% of all deaths from malignant diseases[9]. In France, stomach cancer holds fifth place among cancers and there are 8700 new cases each year[32].

There is high incidence of gastric cancer in Japan, Chile, CostaRica, Colombia, China, Portugal, Iceland, Finland, and Scotland and considerably lower in the United states, United Kingdom, Canada, Australia, New Zealand, Greece, Honduras, and Sweden[4]. There is an approximately 30 fold greater incidence of the disease in Japan and several Asian republics of the USSR compared to large parts of rural Africa South of the Sahara[10].

In Iceland, stomach cancer accounts for 35-45% of all malignant tumors [10]; While in the United States gastric cancer accounts for about 3% of all cancer deaths[4].

Most patients are over 50 years of age [11], but cases in younger individuals and even children are on record[12] [13] [14]. Men are more often affected by the disease than women, the ratio varying from

approximately one in young adults to a maximum of two or more around the age of 60 and falling thereafter to approach unity again at advanced ages[10].

There is a relationship of socio- economic factors to gastric cancer mortality. In general, the lower the income, social class or living standard the higher the mortality from gastric cancer[10].

PREDISPOSING AND RISK FACTORS

1.) Infection.

a. H. pylori infection.

H. pylori infection of the stomach is an important risk factor for the development of gastric cancer. A Working Group of the International Agency for Research on cancer would have concluded that H. pylori is carcinogenic to humans[15]. Many authors have found a strong association between H. pylori infection and the occurrence of gastric carcinoma[10][17][18][19][20].

Infection due to H. pylori appears to be a major risk factor for primary gastric Non-Hodgkin's lymphoma[49]. It is strongly associated with low grade B cell mucosa associated lymphoid tissue (MALT) lymphoma; which regresses in about half of cases, when H. pylori infection is eradicated with antimicrobial agents[21][22][50].

b. Epstein-Barr virus (EBV):

The gastric epithelium is frequently infected with EBV and suggests that prolonged EBV persistence may contribute to the development of gastric carcinoma[33][34]. A strong association between Epstein-Bar virus (EBV) and gastric carcinoma has been demonstrated by the uniform presence of EBV in all carcinoma cells, episomal monoclonality, elevated antibodies, and a unique "lace pattern" in the mucosa.[35]. Epstein- Bar virus- associated gastric carcinomas appear as

superficial depressed or ulcerated lesions in the upper part of the stomach and have a diffuse- type histology with lymphoid infiltration[67].

2.) Dietary factors:-

The possible effects of diet in the causation of gastric cancer are the presence of carcinogens in food; introduction of carcinogen in the preparation of food and the absence of protective factors. General conclusions show an association of gastric cancer with a high consumption of starches and a reduced consumption of fat, fresh fruits and green leafy vegetables[31]. The high incidence in Japanese could be explained by their consumption of rice to which commercial talc has been added to improve its flavour[10] . Cigarette smoking[31] and alcohol consumption increase the risk of stomach carcinoma, notably the distal segment; while green tea drinking reduces the risk[23].

Vitamin C is associated with a protective effect for only the diffuse histologic subtype[24]. High intake of vitamin B6, folate, niacin and iron are associated with decreased risk for only the intestinal type[24].

Nitrosatable compounds which occur naturally in many foods, water are potent carcinogens in the pathogenesis of cancer[10].

3.) Geographical factors:-

There are geographic variations in the incidence of gastric cancer. Japan, Chile, Costa Rica, Finland and Iceland have highest recorded death rate from gastric cancer, while the incidence is considerably low in the USA, UK and Canada. The higher incidence is likely to be the result of environmental influences.

4.) Genetic factors:-

There is clear evidence of familial clustering of carcinoma of the stomach, the most famous being that of the Bonapartes , Napoleon, his father, his grandfather, brother and three sisters died of gastric carcinoma[5]. Family history of gastric carcinoma is found to be clearly associated with the multifocal occurrence[27].

Defective RNA transcription sometimes by aberrant DNA methylation might be one of the pathways of inactivation of P^{16} gene that leads to the development of gastric carcinoma[25]. Human telomerase catalytic subunit (hTERT) expression is up-regulated during an early stage in the carcinogenic process, and telomerase activation may be a critical step in gastric carcinogenesis[26].

In 1953, Aird made the observation that blood group A was more frequent in patients with gastric cancer[28]. This was later confirmed by other studies.

5.) Premalignant changes in the gastric mucosa.

Precancerous lesions are:

a.) Hypo- or achlorhydria in atrophic gastritis.

b.) Epithelial dysplasia.

c.) Adenomatous polyps.

d.) Chronic gastric ulcer.

e.) Stump carcinoma in patients who have undergone partial gastrectomy.

Mild dysplasia in atrophic gastritis seldom progresses to carcinoma; while moderate dysplasia may progress relatively rapidly to severe dysplasia, which seems to be highly predictive of gastric malignancy[29]. Juvenile polyposis of the stomach has also malignant potential[30]. Stomach cancer is stated

to be three to four times more common in patients with pernicious anaemia[31] than in the general population[10].

CLINICAL PRESENTATION[8]

Patients with gastric malignancy may be asymptomatic; however the following symptoms are frequently encountered:

1) Gastric distention : Inability to take a normal meal, vomiting.
2) Anorexia leading to loss of weight.
3) Anaemia, tiredness, weakness, pallor.
4) Persistent pain, no response to treatment, no periodicity.

The following clinical groups are distinguished:

1) The 'new dyspepsia' after 40:

   Vague but persistent indigestion occurring in a patient who has never previously had ' stomach trouble.'

2) Insidious onset:

   Especially in a man, he feels tired and weak.

3) The obstructive types: Carcinoma of the cardia presents with dysphagia; carcinoma of the pylorus with fullness, belching and then vomiting.

4) Lump : the incidental discovery of a lump in the epigastrium in asymptomatic patients. In about 30% cases a lump can be palpated.

5) Silent : Carcinoma of the body of the stomach may be silent; but gives rise to features in other organs e.g. obstructive jaundice, ascites, Krukenberg tumor.

ADENOCARCINOMA

Gastric adenocarcinoma is of major importance worldwide as a cause of death from malignant disease.

Gross features:

Advanced gastric cancer may take the form of polypoid, fungating, ulcerated, diffusely infiltrating (so called linitis plastica) types or may show combination of these[37]. Ulcerated tumors are commonest in the antrum on the lesser curvature.

Microscopic features:

The WHO subdivides gastric carcinoma into following types:

1) Tubular : is composed predominantly of neoplastic tubules often showing irregular branching and anastomosis.
2) Papillary : is characterized by numerous papillary processes with fibrovascular cores.
3) Mucinous : is characterized by conspicuous amount of extracellular mucin(more that 50% of the tumor).
4) Signet-ring cell: consists predominantly of single cell or small clusters of cells containing intracytoplasmic mucous vacuoles and accounting for more than 50% of the tumor.

Lauren divided gastric adenocarcinoma into two main types:

1) intestinal: It shows well-defined glandular structures with papillae, tubules or even solid areas. The epithelium lining the neoplastic lumina consists of intestinal-type tall columnar cells.
2) Diffuse : It consists mainly of scattered individual cells or clusters of cells.The majority of the cells are small, uniform and poorly cohesive and usually have a signet-ring morphology.

Early gastric cancer

It is defined as a carcinoma which is limited to the mucosa or to the mucosa and submucosa only, regardless of the status of the lymph node metastasis.

Gross features:

Japanese Gastroenterological Endoscopic Society divided early gastric cancers into three main types[109]:

Type I : the protruded type

Type II : the superficial type. This is further subdivided into three subgroups:

- Type IIa :elevated
- Type IIb: flat
- Type IIc: depressed

Type III: the excavated type

Microscopic features:

As advanced gastric carcinoma, early gastric cancers may also be grouped into papillary, tubular, mucinous and signet-ring cell carcinomas. The majority have been of tubular or signet-ring cell type with a minority of papillary and mucinous forms.

Histologic grade[110]:

Histologic grades for gastric adenocarcinomas are as follows:

Grade X - Grade can not be assessed.

Grade 1 - Well differentiated (>95% of tumor composed of glands).

Grade 2 - Moderately differentiated (50% to 95% of tumor composed of glands)

Grade 3 - Poorly differentiated (5% to 49% of tumor composed of glands)

Grade 4 - Undifferentiated (<5% of tumor composed of glands)

Tubular carcinomas are assigned grade 1. Signet-ring cell carcinomas are assigned grade 3. Small cell carcinomas and undifferentiated carcinomas are assigned grade 4.

Spread of gastric carcinoma

1) Direct : Gastric carcinoma may directly spread to pancreas, liver, spleen, transverse colon and omentum, and often leads to early transperitoneal dissemination.
2) Lymphatic :Lymph node metastases are present in 90% of autopsies on gastric carcinomas and in 70% of surgical resections[10]. Lymph nodes along the lesser and greater curves, para-aortic nodes, pancreatic and splenic nodes, mediastinal nodes may be involved. Spread by way of the thoracic duct to the left supraclavicular nodes
(the ' sentinel' or 'signal ' nodes of Troisier and of Virchow) is not common.
3) Bloodstream : The most common sites for distant metastatic spread are the liver (49%), lung (33%), Ovary (14%) and bone (11%).
4) Transperitoneal : Secondary deposites in omentum, peritoneum, mesentery and ovaries (Krukenberg tumor) are seen.

Prognosis

The prognosis for gastric carcinoma has been found to be related to patient's age, depth of invasion , tumor size, microscopic type and grading , perineurial invasion , regional lymph node involvement , type of surgery etc. The experience of the Japanese has shown that surgery of early gastric cancer results in 5 years survival rates of the order of 90%. The 5 years survival rate in advanced carcinoma after gastrectomy ranges between 20- 30%.

The expression of transforming growth factor B[38]; urokinase-type plasminogen activator[39], nm 23 [40], Cyclin E and p53[41], T antigen[42], vascular endothelial growth factor[43] may be prognostic indicator for patients with gastric carcinoma and may contribute to the progression of gastric carcinoma. Serum concentration of CD 44 variant 6 is related to the progression of diffuse type gastric carcinoma[44]; while

serum CA 125 titer may be a powerful predictor of peritoneal metastases in patient with gastric carcinoma[45].

Non- Hodgkin's lymphoma

The stomach is the most common extra nodal site for non- Hodgkin's lymphoma; primary gastric lymphoma is uncommon, constituting only 2% to 5 % of malignant gastric lesions[46][47].

Gross Features:

Many gastric B-cell lymphomas simulate gastric carcinomas grossly, being polypoid, fungating or ulcerating tumors; most commonly located in the antrum.

Microscopic Features:

Most of the low grade tumors are of the so- called MALT type. Focal or extensive plasmacytoid differentiation, Dutcher bodies (true intranuclear eosinophilic inclusions made up of immunoglobulin) and infiltration of the glandular epithelium by the neoplastic lymphocytes, resulting in so-called lymphoepithelial lesions are usual features.

In high grade B-cell lymphomas of the stomach, a significant proportion of the neoplastic cells are blast cells, usually resembling centroblasts but sometimes immunoblasts.

The prognosis for gastric large cell lymphomas is substantially better than for gastric carcinoma. The overall 5 year disease free survival rate is approximately 60%[48].

Gastric Stromal tumors

(Leiomyoma, leisomyosarcoma, leiomyoblastoma, neurilemmoma, schwannoma, neurofibroma)

Gastric stromal tumors may affect any part of the stomach. Most occur in adults aged over 30 years.

Gross features:-

Tumors may be single or multiple and vary in size from tiny intramural microscopic lesions to bulky tumor masses. Most tumors grow as an endophytic polypoid submucosal growth ; some as exophytic subserosal lesions and some in both directions.

Microscopic features :-

Gastric stromal tumors show two basic cell types: spindle and epithelioid. Typical spindle cell tumors occur in the gastric corpus and are composed of interlacing bundles or whorls of spindle shaped cells with elongated blunt-ended nuclei and eosinophilic cytoplasm with paranuclear vacuole.

Epithelioid stromal tumors occur more commonly in the antrum and consist of round vacuolated or clear cells, often arranged in sheets or nests. Nuclei are oval or round and contain finely dispersed chromatin, small to large eosinophilic nucleoli.

Carcinoid Tumors

They are not common in the stomach and usually present in middle-aged adults.

Gross features:

Most carcinoids occur as smooth, firm, well- circumscribed polypoid mass with yellow- grey cut surface.

Microscopic features:

Gastric carcinoids are composed of small, uniform, polygonal or cuboidal cells with regular, round or oval nuclei, stippled chromatin, very infrequent mitoses and show minimal nuclear pleomorphism. These cells usually show a mixed growth pattren, with nests or trabeculae of cells separated by a loose connective tissue.

Immunohistochemistry of gastric tumors

Adenocarcinoma

Reactivity of gastric adenocarcinoma cells for keratin, EMA, and CEA is the rule. Immunoreactivity for M1 (a mucin antigen) , cathepsin E, lysozyme, alpha-1 – antitrypsin, alpha-1- antichymotrypsin, alpha-2-macroglobulin may be seen.

MALT lymphoma

Tumor cells are immunoreactive for common leukocyte antigen, CD 19 and CD 20.

Carcinoid tumor

Tumor cells are immunoreactive for neuron- specific enolase, chromogranin A and PGP 9.5. Immunoreactivity for serotonin, gastrin, somatostatin,pancreatic polypeptide and human chorionic gonadotrophin may also be present.

Smooth muscle tumors

Immunoreactivity for desmin, alpha- actin, myosin is seen.

Helicobacter Pylori

History

Team work between 4 departments was the secret of the first successful culture, in Royal Perth Hospital, Western Australia, of human gastric spiral bacteria, now called Helicobacter pylori[1]. Warren and Marshall (1983) were the first to describe and isolate the organism and to assoiciate it with gastritis[51].

Spiral gastric bacteria are not new. In 1893 Bizzozero described spiral bacilli colonizing the mucus and glands of the stomach of healthy dogs. Salmon (1896) described them in more detail and found them also in cats and rats but not in man. Krienitz (1906) described 3 types of `spirochaete' in the stomach of a patient with carcinoma of the lesser curve, one of which may have been H. Pylori[2]. Doenges (1938) described `spirochaetes', probably H. pylori in 43% of stomach sections taken at necropsy, but he was unable to relate their presence to gastric disease. Freedberg and Barron (1940) reported similar organisms in 37% gastrectomy specimens.

Steer and Colin-Jones (1975) came near to identifying H. pylori and its association with gastritis in a study of biopsy specimens, but misinterpretation of culture results led to the conclusion that the organisms seen were Pseudomonas aeruginosa.

Within few years of publication of Warren and Marshall's work many reports appeared from all parts of the world confirming the association of H. Pylori with chronic active gastritis and peptic ulcer. The name Helicobacter was given by C.S. Goodwin in 1989.

Morphology

It is gram negative spirally shaped bacterium, unipolar, multiflagellate (6-7), rod like with bluntly rounded ends: 0.5 to 0.9 micrometer wide by 2-4 micrometer long. It is micro-aerophilic and it requires carbon dioxide for growth. It undergoes coccal transformation in adverse condition.

Culture

True spiral forms may be few or absent in culture media. It occurs in the form of straight or slightly curved rods. Four to six sheathed flagella are normally attached at one pole, each 2.5 micrometer long and about 30 nm in thickness. Coccoid forms emerge from prolonged culture.

Primary isolation of H. pylori has been achieved with brain heart infusion agar (BHIA) and 20% horse serum. Luxuriant growth occurs with egg yolk or casein in BHIA. Cultivation of H. pylori in brain-heart infusion broth is recommended for studies on the physiology, metabolism and enzyme expression of the organism[3]. In liquid media a thin layer of fluid yields growth after 3 days, heavy growth for primary isolation can be obtained after 48 hours on BHIA plus 7% horse blood lysed with saponin plus 1% isovitalex. H. pylori grows between pH 6.6 and 8.4 and between 33°C and 40.5°C.

Genome and Plasmids

The DNA composition of 32 strains of H. Pylori was found to yield an average of 35.2 mol%; G+C with range of 34.1-37.5 (mol%). Not all strains contain plasmid and there may be two subtypes of H. Pylori with different plasmids in same patient.

Toxins

1. Vacuolating toxin (VacA) - This is an extracellular 87 KD toxin; product of Vac-A gene.
2. Cytotoxin - This is a 120-140 KD protein; product of Cag A gene.

Diagnosis

Methods to diagnose patient with H. Pylori infection can be divided as follows:[4][5]

1 Histological method

H. Pylori can be detected in gastric biopsy and resection specimens. The tissue is processed by usual methods & embedded in paraffins. Several staining methods for H. Pylori have been proposed: Haematoxylin and Eosin, Giemsa, Warthin-Starry, H. pylori silver Genta, Triple (Carbol fuchsin/Alcian blue/Hematoxylin-Eosin) stains and immunohistochemistry. Claudio Doglioni et al. concluded that H. pylori silver stain (HpSS) is highly sensitive and simple method in detecting H. pylori[6]; while Ashton-Key et al. concluded immunohistochemistry using an immunoperoxidase technique is highly sensitive and easy to use[58]. The carbol fuchsin/alcian blue/hematoxylin-eosin stain is suitable for simultaneous visualization of H. pylori infection and gastric morphology[59].

2 Culture

Culture is no more sensitive than microscopy. Specimens must be cultured within 2 hours of being taken. Brain heart infusion agar or Brucella agar supplemented with activated 0.2% charcoal or 1% corn starch, chocolate agar or campylobacter-selective agar containing vancomycin, polymyxin B and trimethoprim (Skirrow 1977) can be used. Plates are incubated at 37°C under the microaerobic conditions. Plates may need to be examined after 3 days and 5 days. Colonies of H. pylori are translucent and 1-2 mm in diameter.

Presumptive and Confirmatory identification

a Gram stain of colonies show curved gram negative bacilli.

b Biochemical tests : Catalase +ve, oxidase +ve, urease positive results strongly indicate that the colonies are Helicobacter pylori.

c Reaction for hippurate hydrolysis and nitrate reduction are negative.

3 Biopsy urease test

H. pylori produces such abundant urease that the enzyme can be detected in specimens of colonized gastric mucosa placed directly in a solution of urease with a suitable indicator. In the original test, Christensen's urea broth was used. A number of modifications have been described and kits are available commercially.

4 Urea breath test

Patients whose stomach is colonized with H. pylori respond to a dose of urea by producing excess gastric ammonia and CO2. If the urea is tagged with an isotope of carbon, tagged CO2 will appear in the breath where it can be measured.

5 Serodiagnosis

Serology represents non-invasive test for determining colonization of H. pylori. ELISA, particularly when used to measure specific antibiodies of different immunoglubulin classes, is the most widely used. It's sensitivity 93.8% and specificity 79.3% by ELISA[60]. Other methods are : latex agglutination, complement fixation, Helico blot etc.

For research purposes

Polymerase chain reaction

DNA of H. pylori has been identified in faeces, saliva, and dental plaque by polymerase chain reaction[61]. PCR procedure is a valuable technique for detection of H. pylori and its high sensitivity and specificity should make it a reference test for detection of H. pylori[62].

In situ hybridization

Using both RNA and DNA probes is a sensitive and specific method for detecting H. pylori in tissue sections. It shows strong specific straining of bacteria and permits direct comparision with histology[63].

Epidemiology of H. pylori

Helicobacter pylori has been isolated from persons in all parts of the world. Human are the major sole of reservoir for H. pylori; which spread from person to person by oral-oral, fecal-oral or gastro-oral routes[101]. On occasion, transmission occurs from person to person through improperly cleaned endoscopes. H. pylori has now been isolated from faeces especially in children.

The prevalence of H. pylori is related to age and geographical location. Males and females are equally affected. Infection is many times more common in developing countries than in developed ones[64]. Within a given population, infection is more common in lower socio-economic groups[64]. In all populations, infection is most commonly acquired in childhood possibly from parents or other children and usually lasts for the lifetime of the individual. There is a steady increase with age.

In a preliminary study, 80% of the general population were infected with H. pylori[65]. In another study, it was found that there was a significant regional difference in the seroprevalence of H. pylori within Nepal, which showed lower prevalence in an isolated rural village[66].

There is increasing evidence that incidence of infection has progressively decreased in the United States and other developed countries probably as a result of decreased crowding and improved sanitation. The age related increase in prevalence reflects both a cohort phenomenon (with persons born earlier having higher infection rates in childhood) and continuing exposure and infection into adulthood.

Helicobacter pylori infection and gastric cancer[70]

It is now established that H. pylori is one of the commonest chronic bacterial infections of humans worldwide and causes chronic gastritis and peptic ulceration[68][69][71]. One of the dilemmas to be explained in attributing gastric cancer to H. pylori infection is the occasional finding of low cancer rates in some populations with very high rates of infection. The link between bacterial infection and carcinoma has a long and illustrious history. In this century, associations have been recognized between salmonella infection and carcinoma of the gall bladder, secondary bacterial infection in achalasia and carcinoma of the oesophagus. It is known that Helicobacter pylori colonizes gastric epithelium and a Working Group of the International Agency for Research on Cancer would have concluded that H. pylori is carcinogenic to humans.

Patterns of gastritis and cancer risks

Each individual will react in a unique way to H. pylori infection in terms of the inflammatory response and acid production. There are two principal routes:

a. The elevated acid route

Individuals with elevated acid production have an antral predominant gastritis with little inflammation in the corpus. they have an increased risk of duodenal ulceration; but a low risk of gastric cancer.

b The low acid route

Individuals with hypochlorhydria have a pan-gastritis, leading to glandular atrophy and metaplasia. Such individuals are at an increased risk of gastric ulcer and cancer.

The route will be determined by host factors including genetic make-up, age at infection, nutritional status and the presence of intercurrent infections, environmental factors such as diet and virulence of H. pylori. Glandular atrophy may be a consequence of lethal damage to stem cells resulting from H. pylori-mediated inflammation; as polymorph activity is concentrated on the proliferative compartment in the pit-isthmus zone in active chronic H. pylori gastritis.

Genetic alteration in gastric cancer

Genetic alteration is the result of DNA damage. Potentially oncogenic DNA damage is manifest by the activation of oncogenes, deletions, and mutations in tumor suppressor genes. In one study, in young Italian patients with gastric carcinomas, it was shown that cagA positive H. pylori has an etiologic role in both diffuse and intestinal-type gastric carcinoma[72]; however, most of other studies concluded that links between H. pylori and cancer will be strongest for the intestinal type.

Genetic alterations in the precursors of gastric cancer are

1. demonstration of the tpr-met rearrangement, which involves the fusion of a translocated promotor region (tpr) locus on chromosome 1 to the 5 region of the met gene on chromosome 7.
2. Cripto and TGFa overexpression.
3. Telomerase reduction.
4. P53 and K-ras over expression.
5. Aberrant bcl-2 expression.

Genetic alterations in gastric cancer are

1. Mutations of apc gene
2. Loss of heterozygosity on chromosome 5q.
3. Loss of heterozygosity of the putative tumor suppressor gene dcc on chromosome 18q.
4. Loss of heterozygosity of the c-met gene on chromosome 7q 31.

5 Overexpression of bcl-2.

6 Overexpression of c-erbB-2.

Damaging agents: Bacterial products and Carcinogenesis.

A Toxins

1 Vacuolating toxin - 87 KD toxin, product of the vac-A gene is expressed by only 50-60% of strains. This toxin inhibits human gastric cell Na+/K+ ATPase activity and causes cell vacuolization. An increased prevalence of vacuolating toxin-positive strains has been observed in patients with preneoplastic lesions.

2 Cytotoxin - 120 KD toxin, product of the Cag A gene induces a rapid secretion of interleukin-8. Shimoyama et al. found that CagA seropositivity was associated with increased risk of gastric cancer in Japanese population[73]; while in Mexico CagA (+) status was found as predictor of risk for gastric carcinoma [74].Cytotoxin is also toxic to somatostatic cells.

B Urease and Ammonia

H. pylori is rich in urease; which releases ammonia from a urea substrate; thus increasing intragastric ammonia concentration of about 0.015% (normal < 0.005%). Ammonia is cytotoxic; inhibits mitochondrial and cellular respiration and potentiates vacuolization. In addition, ammonia may interact with hypochlorous acid to produce highly toxic mono-N-chloramine.Moreover ammonia also causes backflow of hydrogen ions into the cells. The role of urease and ammonia in the pathogenesis of gastric carcinogenesis could be significant.

C Acetaldehyde

H. Pylori possesses the enzyme alcohol dehydrogenase, which generates acetaldehyde from ethanol substrate. Acetaldehyde is a highly reactive moiety with the potential for DNA damage.

D Mucolytic factors

H. Pylori produces proteases and lipases capable of degrading gastric mucus and could thereby compromises barrier function of gastric mucus gel. This allows greater access of luminally generated carcinogens to the target epithelial cells and increased ingress of bacterial antigens could augment the inflammatory response and lead to increased production of endogenous carcinogenic agents.

Damaging agents : Products of the host response

H. pylori produces a wide variety of antigens including the 87 KD cytotoxin, the Cag-A and heat shock proteins, and pro-inflammatory mediators such as platelet activating factor, urease and lipopolysaccharide. These induce a vigorous immune and inflammatory reaction in the gastric mucosa. During this host response, there is free radical generation such as superoxide (O_2^-), nitric oxide (NO) and their adducts peroxynitrite (ONOO-), H_2O_2 hydrooxyl radical (OH), HClO, and NO_2Cl as well as alkylperoxy radicals; which may be involved in carcinogenesis.

H.Pylori and intraluminal nitrosation

Multifocal atrophic gastritis is known to carry an increased risk of gastric cancer and is clearly related to H. pylori infection. Progressive corpus atrophy leads to hypochlorhydria and proliferation of nitrosating bacteria in the gastric juice. Dietary nitrates are converted into nitrites by these bacteria, which are also capable of catalysing the reaction between nitrites and dietary amines and amides at neutral pH to create N-nitroso compounds. Such compounds include known carcinogens.

H. Pylori and cell proliferation

Gastric epithelial cells proliferate more rapidly in patients with H. pylori infection compared with uninfected subjects. In hyperproliferative states, opportunities for DNA repair are minimized. Thus, by increasing mitogenesis, H. pylori can be said to be indirectly mutagenic, because it increases the chances of converting DNA damage caused by endogenous breakdown of DNA or by ingested carcinogens, into stable mutations. Hyperproliferation will also have a tumor-promoting effect. Possible mechanisms underlying the observed hyperproliferation include a hyperplastic response to either direct cellular damage or damage by neutrophils and inflammatory products. This concept of 'chronic mitogenesis' facilitating mutagenesis may be a universal phenomenon in carcinogenesis.

Heliocobacter pylori and gastric lymphoma

The gastrointestinal tract is the most commonly affected primary site in extranodal non-Hodgkin's lymphoma, and the majority of primary gastrointestinal lymphomas arise in the stomach.

It is believed that the normal gastric mucosa contains no lymphoid tissue and that lymphoid follicles develop by H. pylori-induced chronic active gastritis[75]. However, the mechanism of the evolution from chronic gastritis to malignant lymphoma has not yet been fully explained, and the role of H. pylori in the pathogenesis of gastric lymphoma still remains unclear. Only one explanation has been offered by Hussell et al.[76], who showed that cellular proliferation in low grade gastric MALT lymphoma might be dependent on H. pylori specific T cells and their products. The presence of H. pylori- associated chronic gastritis may be essential but not sufficient to induce malignant lymphoma and that some unknown factors, such as gene abnormalities, may therefore be necessary for malignant transformation.

Xu WS et al.[77] have described that the role of H. Pylori in pathogenesis of gastric lymphoma may vary in different populations and not all low-grade gastric MALT lymphomas are H. pylori-dependent. In a study of ECK M. et al.[100] in 98% of the patients with MALT- type lymphoma, H. pylori-specific IgG serum

antibodies were detected; and on histological examintion, H. pylori was found only in 78% of the patients. Some recent studies have described the regression of low grade gastric MALT lymphoma after the eradication of H.pylori[50] [78] [79]. Shotaro N et al.[80] have suggested that H. pylori is more likely to be associated with early states of primary gastric lymphoma than with advanced states.

Helicobacter pylori and extra-gastric diseases[107] [108]

Recently, a potential role of H.pylori infection in several extraintestinal pathologies has been suggested. The postulated role of H.pylori in the pathogenesis of extraintestinal manifestations is based on the facts that: (1) local inflammation has systemic effects;(2) H.pylori gastric infection is a chronic process lasting for decades,and (3) persistent infection induces a chronic inflammatory and immune response able to induce lesions both locally and remote to the primary site of infection.

The causative mechanisms,proposed for the associations between H.pylori infection and extradigestive diseases can be divided into:

1 Direct effects: Direct effects of infectious agent on the vascular wall include endothelial injury or dysfunction related to circulating endotoxins,smooth-muscle proliferation,and local inflammation.

2 Indirect effects: Indirect effects are likely more frequently involved and include production of inflammatory mediators with proinflammatory,procoagulant,and atherogenic action;changes in risk factors (lipid profile,coagulation,oxydative metabolites),cross-reactive antibodies,nutrient and vitamin malabsorption,as well as metabolic factors,such as overproduction of ammonia.

Helicobacter pylori infection and vascular diseases

Helicobacter pylori has been found to be associated with vascular diseases like atherosclerosis[102], coronary artery disease[104](acute myocardial infarction[103] [106]);primary Raynaud phenomenon and primary headache.

Mendall et al. reported for the first time a higher seroprevalence of H. pylori infection in male patients with established coronary artery disease compared with age- and sex-matched controls. Pasceri et al. also reported significantly higher prevalence of H.pylori infection in patients with ischemic heart disease than in controls. The pathogenetic mechanisms involved in coronary heart disease are: i.) development of procoagulant status. ii.) action through inflammatory mediators e.g. C-reactive protein,TNF-alpha. iii.) decrease in the level of antioxidants.

In a recent Italian study,72% of patients affected by primary Raynaud phenomenon were infected by H.pylori. After eradication of the infection, complete disappearance of the clinical attacks of Raynaud phenomenon and reduction in the intensity,duration and frequency of the attacks were noticed.
Gasbarrini et al. found H.pylori infection in 40% of patients with primary headache.Clinical attacks of headache completely disappeared in 17% of the successfully treated patients.

Helicobacter pylori infection and autoimmune diseases

H.pylori infection has been found to be associated with some autoimmune diseases like Sjögren's syndrome, Schönlein-Henoch purpura, autoimmune thyroiditis, idiopathic arrhythmias, Parkinson's disease and nonarterial anterior optic ischemic neuropathy.

The pathogenetic mechanism relating H.pylori to any of these immunological diseases is unknown.H.pylori might be responsible for a clinical expression of latent autoimmune pathology or the production of autoantibody through a cross-reaction mechanism. H.pylori strains have similar epitopes to those found on gastric epithelium, salivary gland ducts, and endometrium.

Helicobacter pylori infection and skin diseases

H. pylori infection has been linked to several skin diseases like chronic idiopathic urticaria, rosacea, alopecia areata etc.

An association with chronic idiopathic urticaria has been proposed with the pathogenetic mechanism related to an increase in gastric vascular permeability during H. pylori infection, resulting in a greater exposure of the host to alimentary allergens. H. pylori infection was reported in 84% of patients with rosacea. A recent report showed a higher prevalence of H. pylori infection in patients with alopecia areata compared to general population.

Helicobacter pylori infection and other diseases

A possible association between H. pylori infection and diseases like sideropenic anaemia, growth retardation, late menarche,extragastric MALT lymphoma, diabetes mellitus,periodontal diseases[105], hepatic encephalopathy (liver cirrhosis) etc has been suggested.

Idiopathic sideropenic anaemia in H. pylori infection may be developed secondary to an active hemorrhagic gastritis and due to utilization of iron and human transferrin by the bacterium for its growth. In a case control study, 55.2% of children being investigated for short stature proved to be infected by H. pylori. Growth retardation relates to the increased levels of circulating cytokines,such as TNF-alpha associated with H. pylori infection. A Danish study showed that H. pylori is related to late menarche;the mechanism may be a retardation in endocrine development through a relative malabsorption of nutrients. A case control study showed an increased prevalence of H. pylori seropositivity in patients with noninsulin dependent diabetes mellitus. Finally,H. pylori has been implicated as a factor contributing to hepatic encephalopathy by increasing the amount of circulating ammonia.

Lacuna of Knowledge

There are various reports from other parts of the world regarding the morphological variants of gastric cancer and its association with Helicobacter pylori infection. No such type of study has been carried out so far in Nepal. This research was carried out in order to correlate different malignant gastric tumors with prevalence of H. pylori infection, blood group and anaemia.

Aims and Objectives

1. To study the incidence of various histopathologic variants of primary malignant gastric neoplasms.
2. To study the incidence of morphological variants of gastric carcinoma.
3. To assess the association between H. pylori infection and gastric carcinoma.
4. To assess the frequent blood group in patients with gastric carcinoma.
5. To study the haemoglobin level in cases with the gastric malignancy.
6. To correlate the findings with clinical presentation.

MATERIALS AND METHODS

This is a prospective study, which included 40 cases with primary malignant gastric neoplasms during the research period of twelve months. Specimens (gastric biopsy and/or gastrectomy specimen) from these patients referred to Department of Pathology, T.U. Teaching Hospital were included in this study.

Inclusion criteria

All patients with histopathologically proved gastric malignancy; either in gastric biopsy or in gastrectomy specimen irrespective of age and sex were included.

Study Procedure

Gastric biopsy or resection specimens fixed in 10% neutral buffer formalin were received in Dept. of Pathology, TUTH. All tissues of gastric biopsy specimens were kept for processing; while in relation to gastrectomy specimens sections from the following sites were taken.

- growth (ulcerative, infiltrative or others)-
- resection margins
- normal looking mucosa
- lymph nodes

Then, the tissues were processed by the usual graded alcohol dehydration in the automated histokinette. An overnight schedule of 16-18 hours was used. After processing tissues were embedded in paraffin wax and microtome sectioning was done with a thickness of 3-4 micrometer. These sections were fixed in glass slides, dewaxed and made ready for staining.

Haematoxylin & Eosin stain was done in all cases. Giemsa stain was done in all cases of endoscopic biopsy specimens and in a section from normal looking mucosa in a case of gastrectomy specimen. Special stains like Alcian blue and Periodic acid Schiff (PAS) stain were done when required.

Haematoxylin and Eosin stain was done by following procedure

1. Dewaxation in Xylene
2. Treatment through graded alcohol of decreasing concentration i.e. 100%, 90%, 80%, 70% to water.
3. Staining with Harris Haematoxylin for 15 minutes.
4. Wash in tap water for 5 minutes.
5. Treatment in 1% acid alcohol (1% hydrochloric acid in 70% alcohol) for 5-10 seconds.
6. Again wash in tap water for 5 minutes.
7. Treatment with Lithium carbonate for 1 minute.
8. Wash in tap water.
9. Stain with 0.5% Eosin for 10 minutes.
10. Treatment through graded alcohol of increasing concentration i.e. 70%, 80%, 90%.
11. Treatment with Xylene for clearance.
12. Mounting with DPX.

Giemsa stain was done as described below

1. Dewaxation in xylene.
2. Treatment through graded alcohol of decreasing concentration i.e. 100%, 90%, 80%, 70% to water.
3. Pouring of Giemsa stain (1:10 working solution) to cover the tissue and kept for 15-20 minutes.
4. Wash with water.
5. Dry the tissue & mounting with DPX.

Blood samples (5ml.) were taken from these patients to determine blood group, haemoglobin and for Helicobacter pylori serology.

Blood grouping was done by using monoclonal antibodies (Eryclone).

Haemoglobin estimation was done by coulter counter.

The following two methods were performed to detect H. pylori.

1 Giemsa staining of histological sections

The presence of curved or a spiral form bacilli in tissue section was taken as positive histological test to diagnose the H. pylori. The density of H. pylori colonisation was graded as follows [36]:

0	-	None (Negative)
1+	-	Spiral form micro-organism(H. pylori) found only in one place after a careful search.
2+	-	Only a few spiral form micro-organism (H. pylori) found.
3+	-	Scattered spiral form micro-organism (H.pylori) found in separate areas/foci.
4+	-	Numerous spiral form micro-organism (H.pylori) in separate areas/foci.
5+	-	Nearly complete gastric surface covered by a layer of spiral form micro-organism (H.pylori).
6+	-	Continuous gastric surface coverage by a thick layer of spiral form micro-organism (H. pylori).

2 Serological test:

The presence of antibodies (Ig G) to H. pylori was estimated by the use of Helicobacter pylori IgG Enzyme Immunoassay method(ELISA) [using kit (catalog Number: GB - 1051) from General Biologicals Corp. #6, Innovation first road, Science - based industrial park, HSIN CHU 30077, Taiwan, R.O.C.]. The test was carried out as per the manufacturer's instruction.

Statistical analysis

Statistical analysis was done whenever appropriate, using the chi-square test.

Table No.1

Total Number of cases and Histopathologic Variants of Primary Malignant Gastr Tumors

	Epithelial				Smooth muscle	Lymphoid
	Tubular	Signet-ring cell	Papillary	Mucinous	Leiomyosarcoma	Non-Hodgkin's lymphoma
No.	28	6	2	2	1	1
%	70.0	15.0	5.0	5.0	2.5	2.5
	Total	38(95.0%)			Total -1(2.5%)	Total -1(2.5%)

Total Number of cases were 40, among which 38 (95%) were epithelial tumors (adenocarcinoma); 1(2.5% was smooth muscle tumor and 1(2.5%) was lymphoid neoplasm.

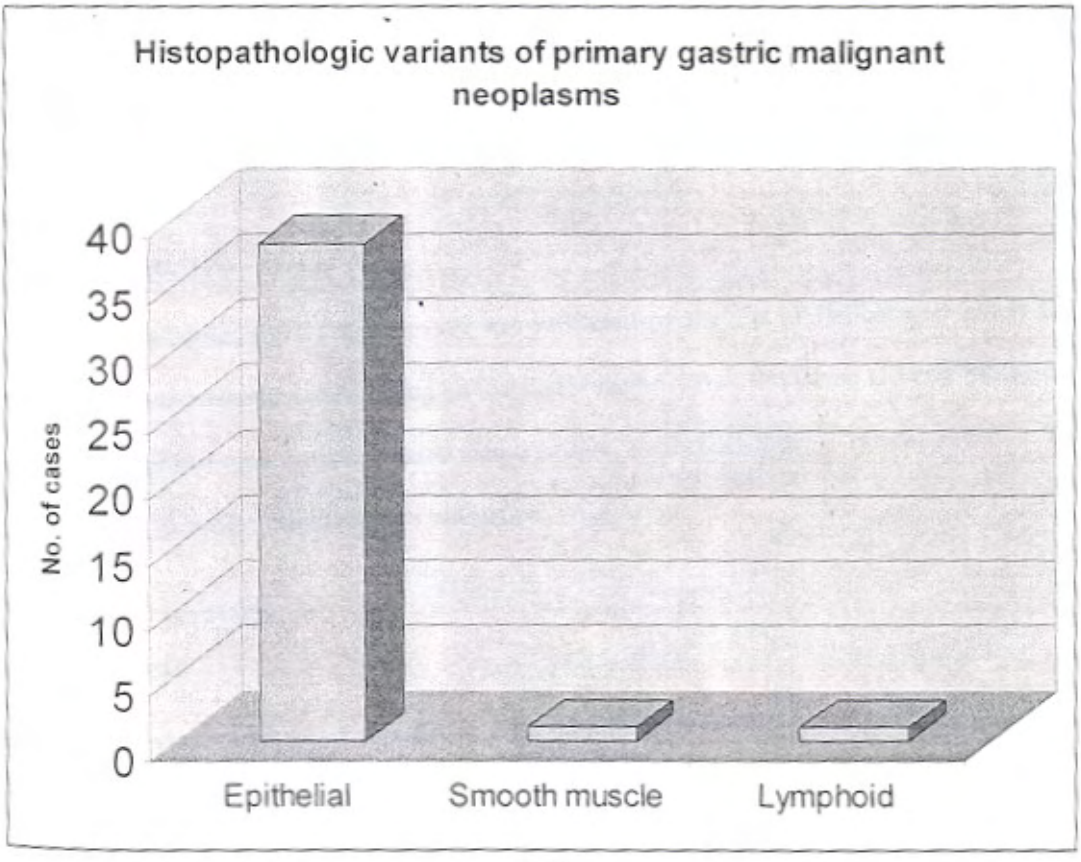


Fig.1

Table No. 2

Morphological variants of Gastric Adenocarcinoma

Histopathologic types	No.of cases	%
Tubular	28	73.69
Signet-ring cell	6	15.79
Papillary	2	5.26
Mucinous	2	5.26
Total	38	100.00

Among 38 gastric adenocarcinomas ,28(73.69%) cases were of tubular type ; while cases with signet- ring cell type, papillary type & mucinous type were 6 (15.79%); 2 (5.26%) & 2(5.26%) respectively.

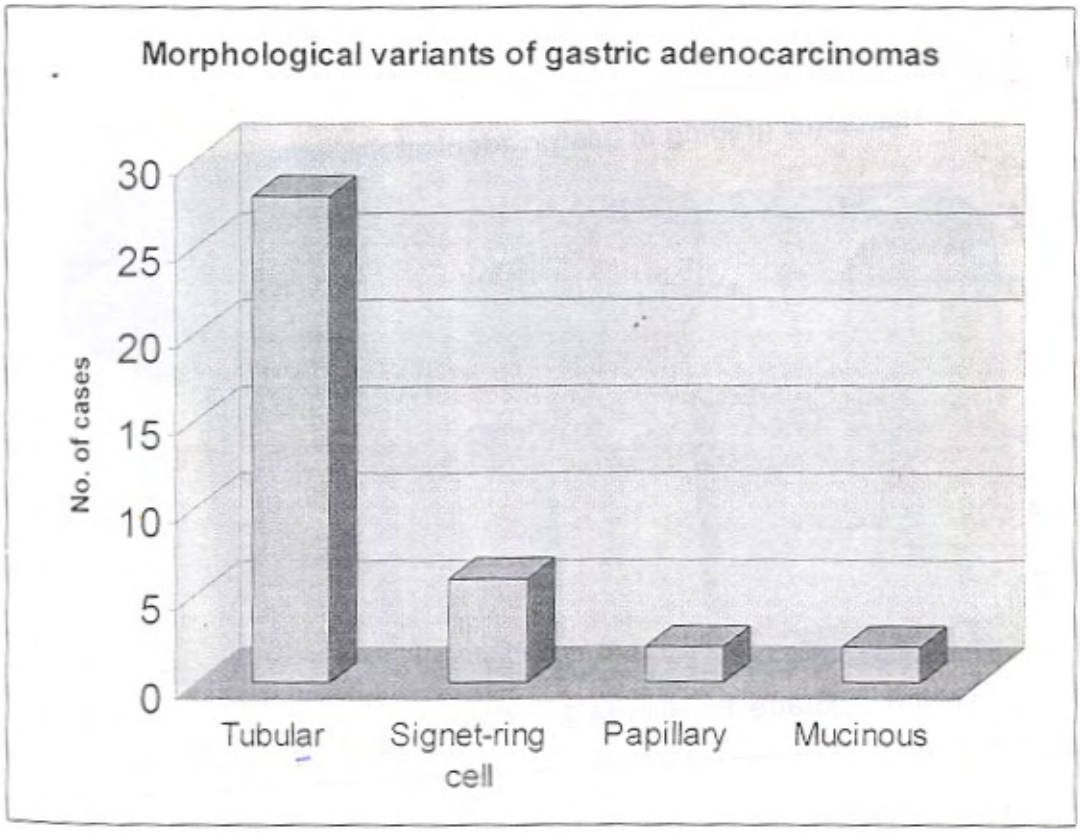


Fig.2

Table No.3

Histologic Grading of Gastric Adenocarcinomas

Morphological variants	Grading				
	Grade X	Grade 1	Grade 2	Grade 3	Grade 4
Tubular	0 (0.00%)	6 (15.79%)	8 (21.05%)	6 (15.79%)	8 (21.05%)
Signet-ring cell	0 (0.00%)	0 (0.00%)	0(0.00%)	6 (15.79%)	0 (0.00%)
Papillary	0 (0.00%)	1 (2.63%)	1 (2.63%)	0 (0.00%)	0 (0.00%)
Mucinous	0 (0.00%)	0 (0.00%)	2 (5.27%)	0 (0.00%)	0 (0.00%)
Total	0 (0.00%)	7 (18.42%)	11 (28.95%)	12 (31.58%)	8 (21.05%)

Most of the gastric adenocarcinomas belonged to grade 3 (31.58%)& grade 2(28.95%). Among tubular adenocarcinomas 6 cases (15.79%) belonged to grade 1; 8 cases (21.05%) to grade 2 ;6 cases (15.79%) to grade 3 and 8 cases (21.05%) to grade 4. All signet-ring cell carcinomas belonged to grade 3; and all mucinous adenocarcinomas to grade 2. Among papillary adenocarcinomas 1 case (2.63%) belonged to grade 1 and another (2.63%) to grade 2

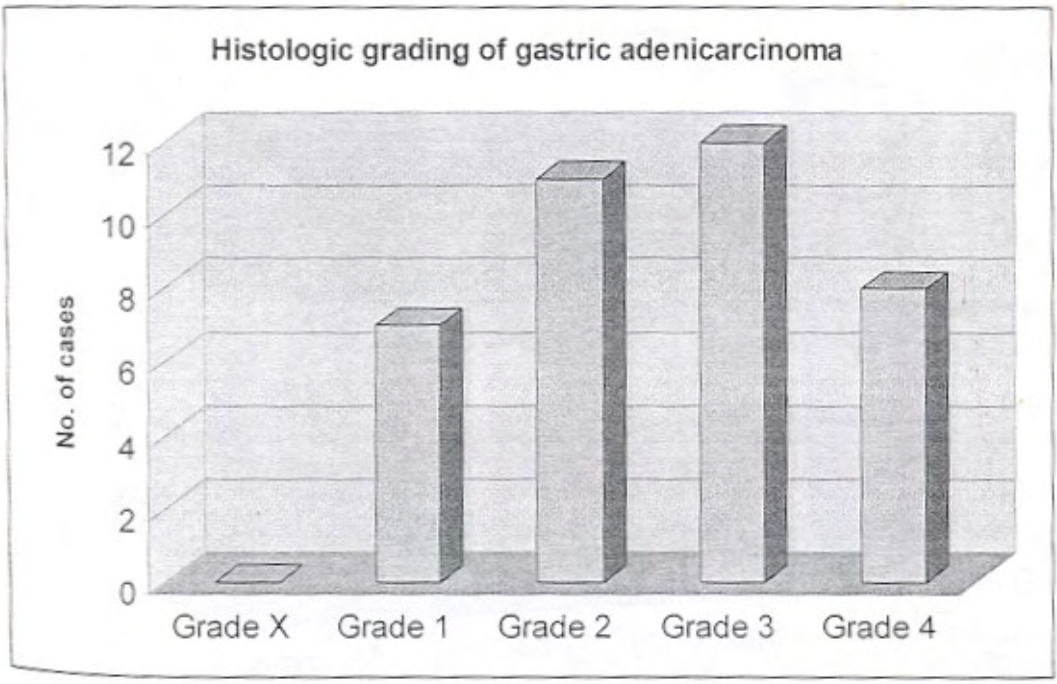


Fig.3

Table No. 4

Type of Specimens

Type	No.of specimens
Endoscopic gastric biopsy	21 (52.5%)
Gastrectomy specimens	19 (47.5%)
Total	40 (100%)

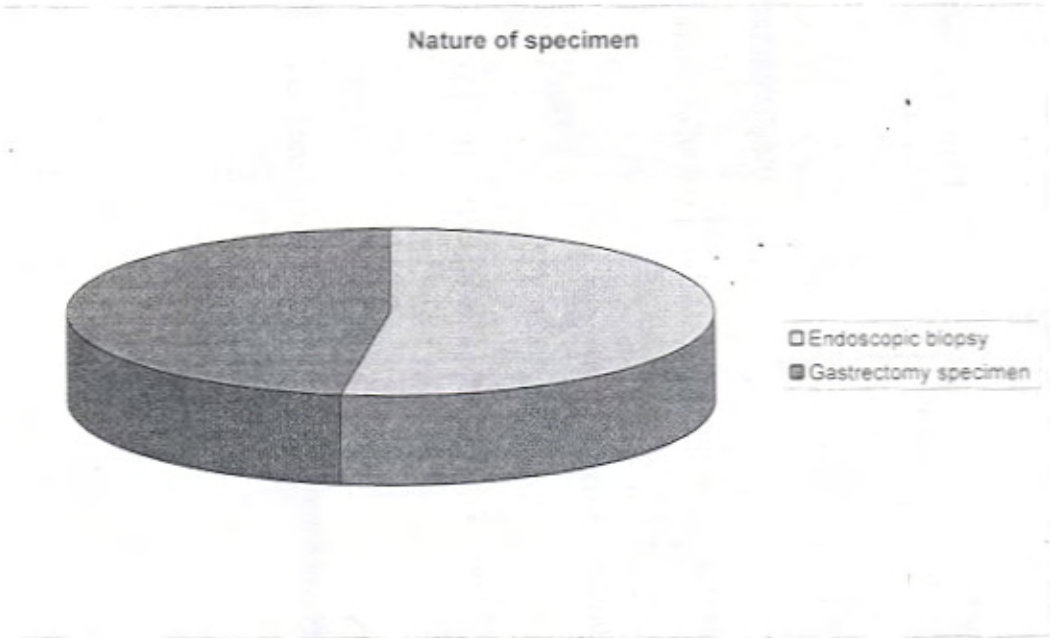


Fig. 4

Table No. 5

Gross Features of Primary Malignant Gastric Neoplasms (Gastrectomy Specimens)

Histologic Type	Site of the tumor				Type of growth				Size (greatest dimension)		
	Antrum	Pylorus	Corpus	Cardia	Ulcera-tive	Infiltra-tive	Diff. Infiltrative	Exophy-tic	<3 cm	3-5 cm	>5 cm
Adenocarcinoma	13 (68.43%)	2 (10.53%)	1 (5.26%)	1 (5.26%)	16 (84.22 %)	1 (5.26%)	0 (0.00%)	0 (0.00%)	4 (22.22%)	4 (22.22%)	9 (50.00%)
Leiomyosarcoma	1 (5.26%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (5.26%)	0 (0.00%)	-	-	-
Non-Hodgkin's Lymphoma	1 (5.26%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (5.26%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (5.56%)
Total	15 (78.95%)	2 (10.53%)	1 (5.26%)	1 (5.26%)	17 (89.48 %)	1 (5.26%)	1 (5.26%)	0 (0.00%)	4 (22.22%)	4 (22.22%)	10 (55.56%)

The size of tumor mass in case of leiomyosarcoma is not mentioned as it has a diffuse growth.

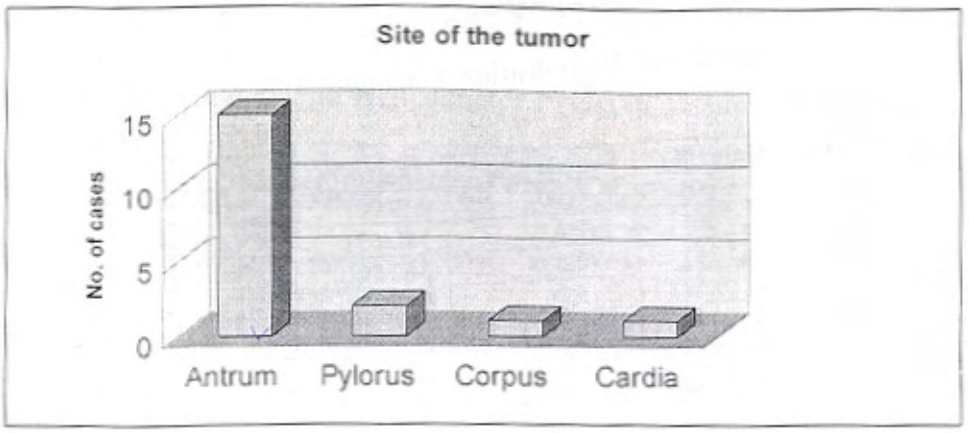


Fig.5

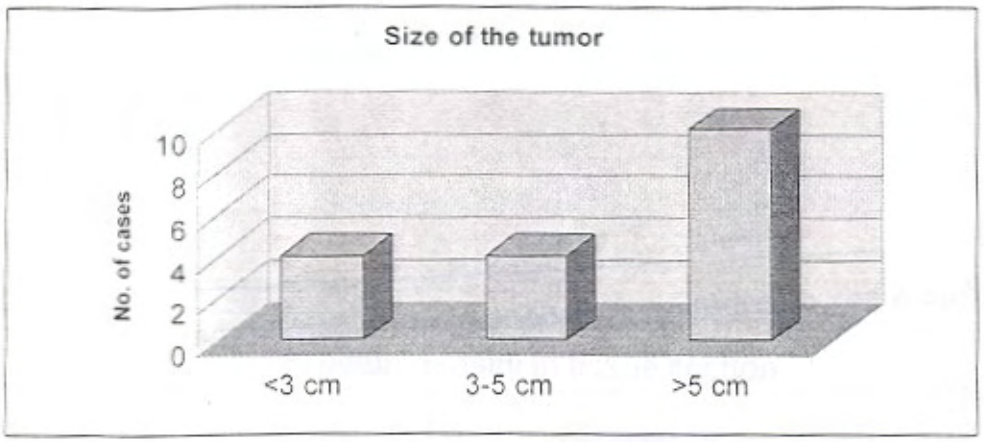


Fig.6

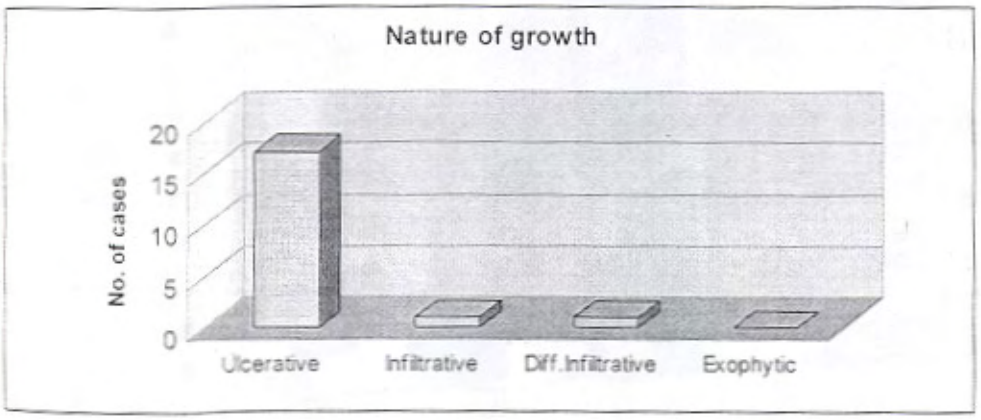


Fig. 7

Table No.6

Age & Sex Distribution(Carcinoma)

Age (Years)	Male (%)	Female (%)	Total (%)
0-20	0 (0.00)	0 (0.00)	0 (0.00)
21-30	0(0.00)	1 (2.63)	1 (2.63)
31-40	2 (5.26)	1 (2.63)	3 (7.89)
41-50	4 (10.52)	2 (5.26)	6 (15.78)
51-60	11 (28.95)	5 (13.17)	16 (42.12)
>60	11 (28.95)	1 (2.63)	12 (31.58)
Total	28 (73.68)	10 (26.32)	38 (100.00)

Primary gastric adenocarcinomas were more common in male (73.68%)
Most of the tumors (73.7% cases) were found after the age of 50 years with significant P value (P value =0.000005).

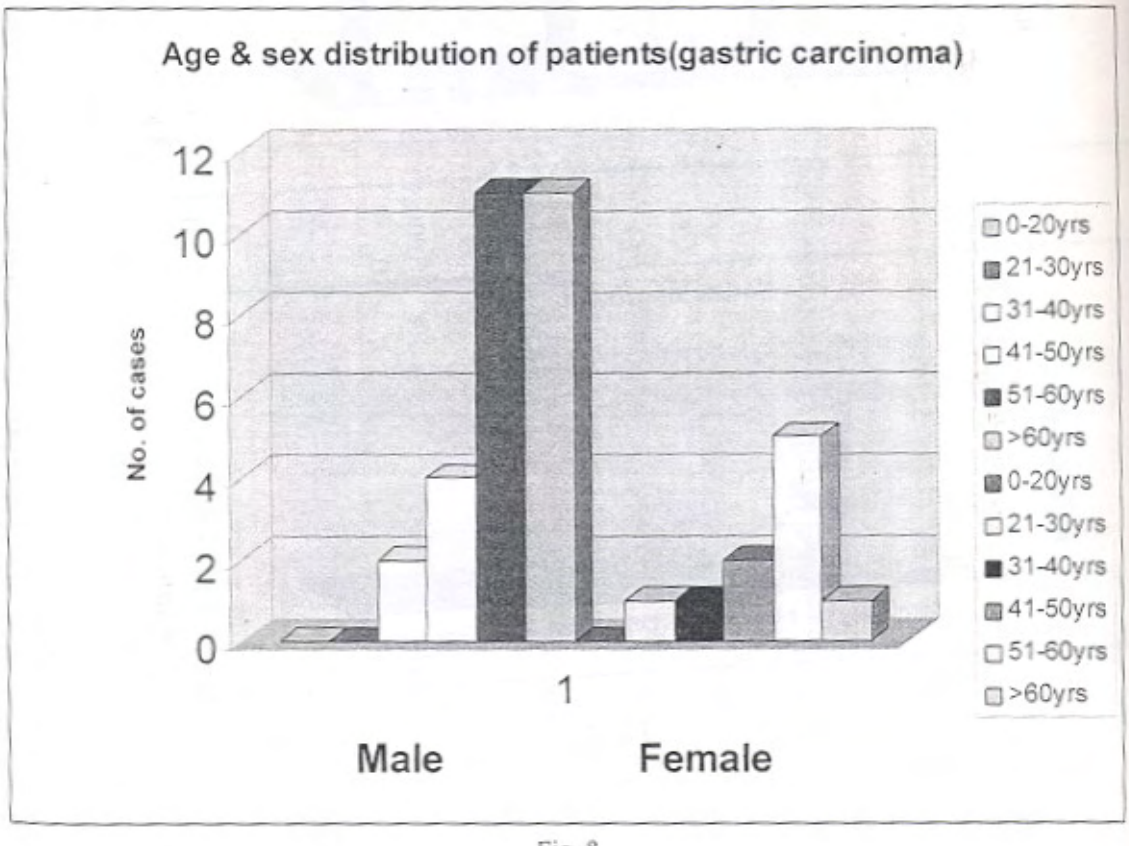


Fig. 8

Table no. 7

Gastric Malignancy and Helicobacter Pylori in Tissue Section (Giemsa Staining)

Hispathologic variants	No. of cases	Helicobacter pylori density						
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6
Adenocarcinoma	38 (95.0%)	8 (20.0%)	3 (7.5%)	8 (20.0%)	12 (30.0%)	6 (15.0%)	1 (2.5%)	0 (0.0%)
Leiomyosarcoma	1 (2.5%)	1 (2.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Non-Hodgkin's lymphoma	1 (2.5%)	0 (0.0%)	0 (0.0%)	1 (2.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	40 (100%)	9 (22.5%)	3 (7.5%)	9 (22.5%)	12 (30.0%)	6 (15.0%)	1 (2.5%)	0 (0.0%)

Among 40 gastric malignancies ,Helicobacter pylori was seen in 31 cases (77.5%).
H. pylori was found in a case of Non-Hodgkin's lymphoma and not found in a case of leiomyosarcoma.

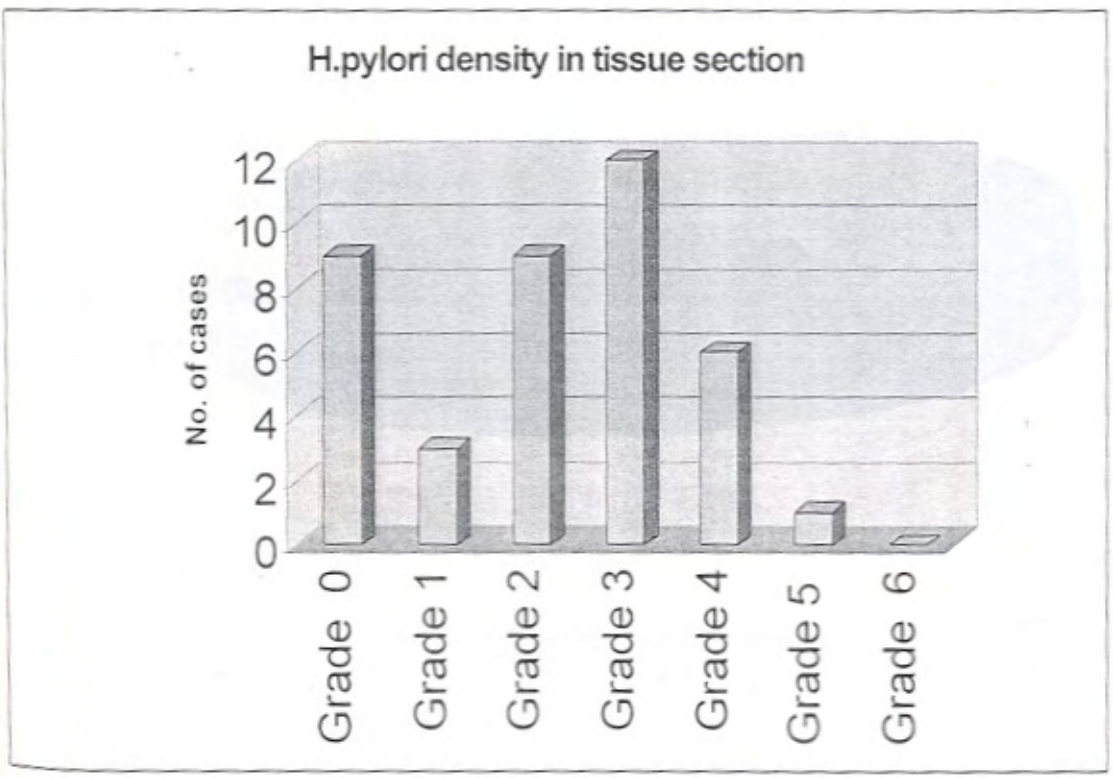


Fig. 9

Table No. 8

Gastric Adenocarcinoma and H.pylori in Tissue Section (Giemsa Staining)

Histopathologic Types	Helicobacter Pylori density						
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Grade 6
Tubular	5 (13.16 %)	3 (7.90%)	4 (10.53%)	10(26.31%)	5 (13.16%)	1 (2.63 %)	0 (0.00 %)
Signet -ring cell	0 (0.00 %)	0 (0.00%)	4 (10.53%)	2 (5.26 %)	0 (0.00%)	0 (0.00 %)	0 (0.00%)
Papillary	1 (2.63 %)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (2.63%)	0 (0.00 %)	0 (0.00%)
Mucinous	2 (5.26 %)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00 %)
Total	8 (21.05%)	3 (7.90%)	8 (21.06%)	12(31.57%)	6 (15.79%)	1 (2.63%)	0 (0.00 %)

Among 38 gastric adenocarcinomas; H. pylori was seen in 30 cases (78.95%).

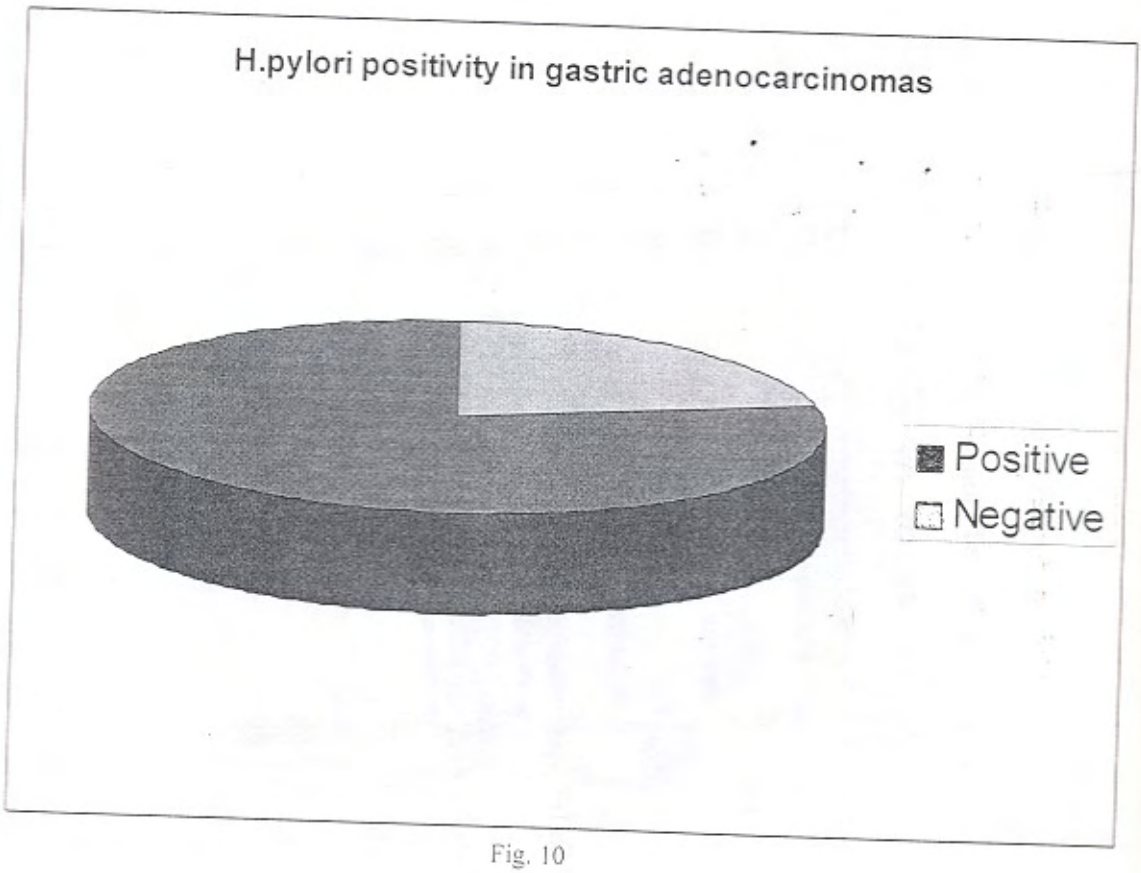


Fig. 10

Table no.9

Gastric Malignancy & Seroprevalence of Helicobacter pylori

Histopathologic variants	No. of cases	Seropositive	Seronegative
Adenocarcinoma	38 (95.0%)	29 (72.5%)	9 (22.5%)
Leiomyosarcoma	1 (2.5%)	0 (0.0%)	1 (2.5%)
Non-Hodgkin's lymphoma	1 (2.5%)	1 (2.5%)	0 (0.0%)
Total	40 (100%)	30 (75.0%)	10 (25.0%)

Among 40 gastric malignancies, H. pylori seropositivity was seen in 30 cases (75.0%).

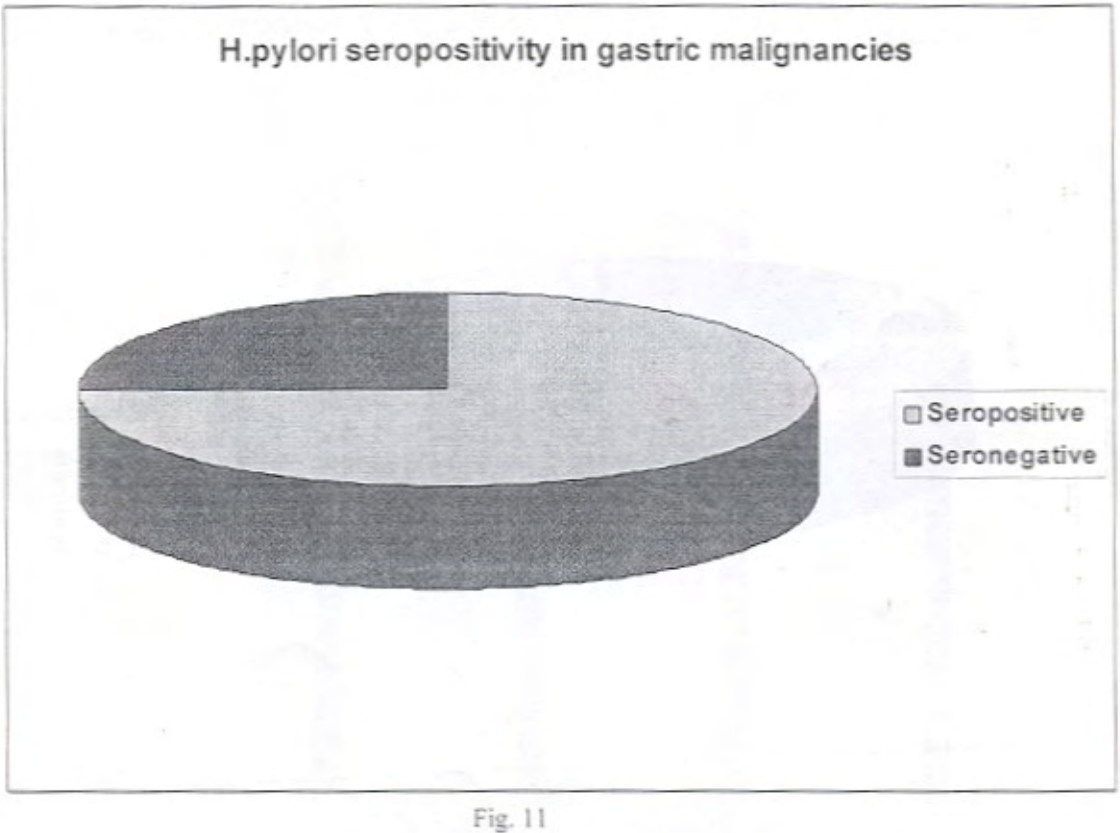


Fig. 11

Table No. 10

Gastric Adenocarcinoma and Helicobacter pylori seroprevalence

Histopathologic types	No. of cases	Seropositive	Seronegative
Tubular	28(73.69%)	21(72.40%)	7(77.78%)
Signet-ring cell	6(15.79%)	6(20.70%)	0(0.00%)
Papillary	2(5.26%)	1(3.45%)	1(11.11%)
Mucinous	2(5.26%)	1(3.45%)	1(11.11%)
Total	38(100%)	29(100%)	9(100%)

Among 38 gastric adenocarcinomas, H.pylori seropositivity was seen in 29 cases (76.31%).

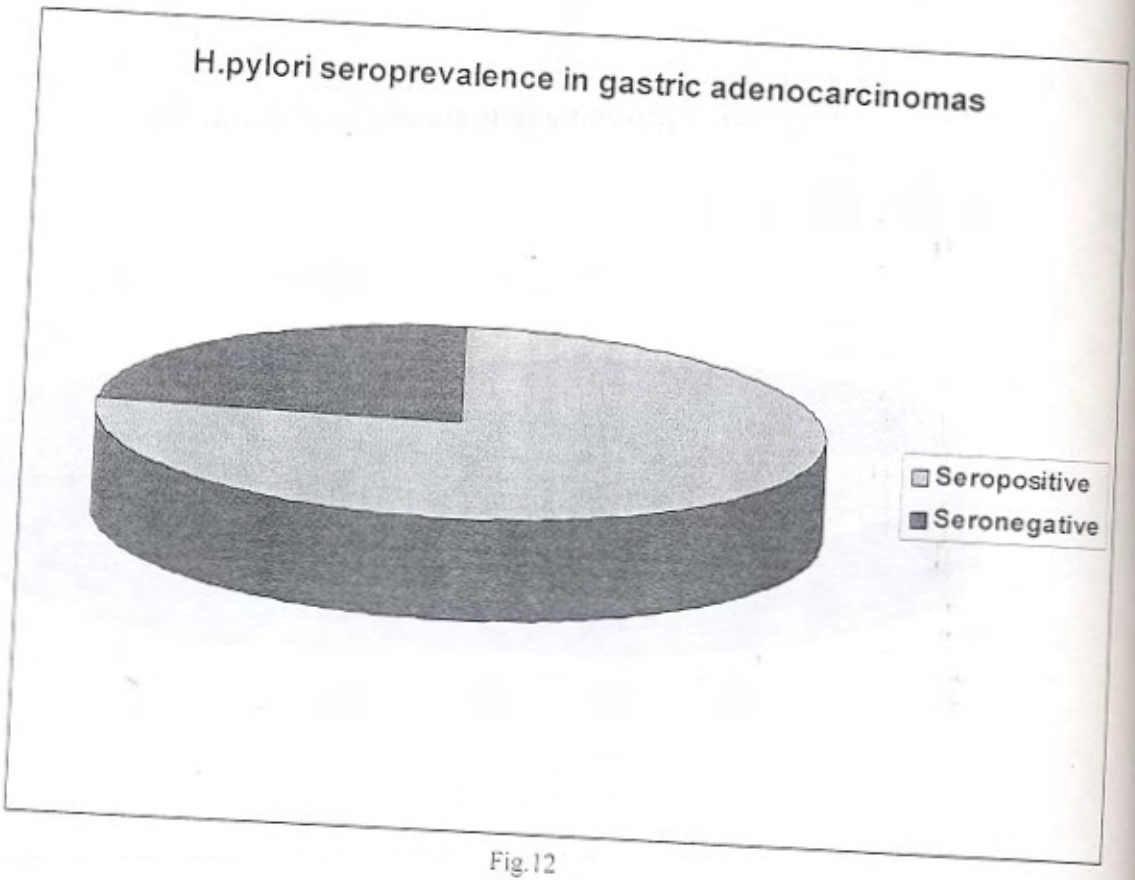


Fig.12

Table No. 11

Comparision of Serological test Taking Histological Test as Standard Method to Diagnose Helicobacter Pylori

	Serological test	Mean of association at 95% confidence interval
Sensitivity	86.7	68.4; 95.6
Specificity	50.0	20.1; 79.9
Positive predictive value	83.9	65.5; 93.9
Negative predictive value	55.6	22.7; 84.7

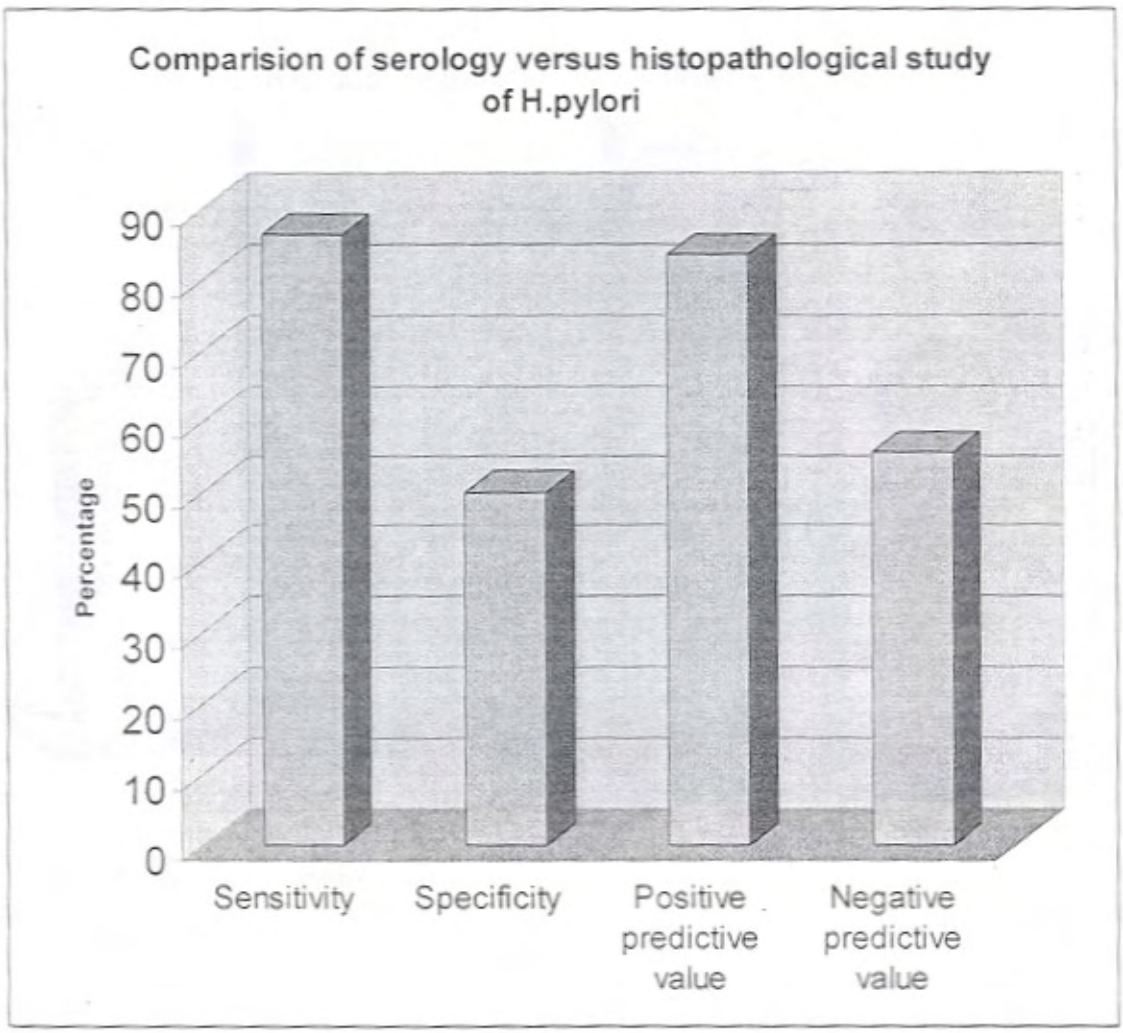


Fig.13

Table No. 12

Comparision of Histological Method Taking Serological Method as Standard Method to Diagnose Helicobacter Pylori

	Serological test	Mean of association at 95% confidence interval
Sensitivity	83.9	65.5; 93.9
Specificity	55.6	22.7; 84.7
Positive predictive value	86.7	68.4; 95.8
Negative predictive value	50.0	20.1; 79.9

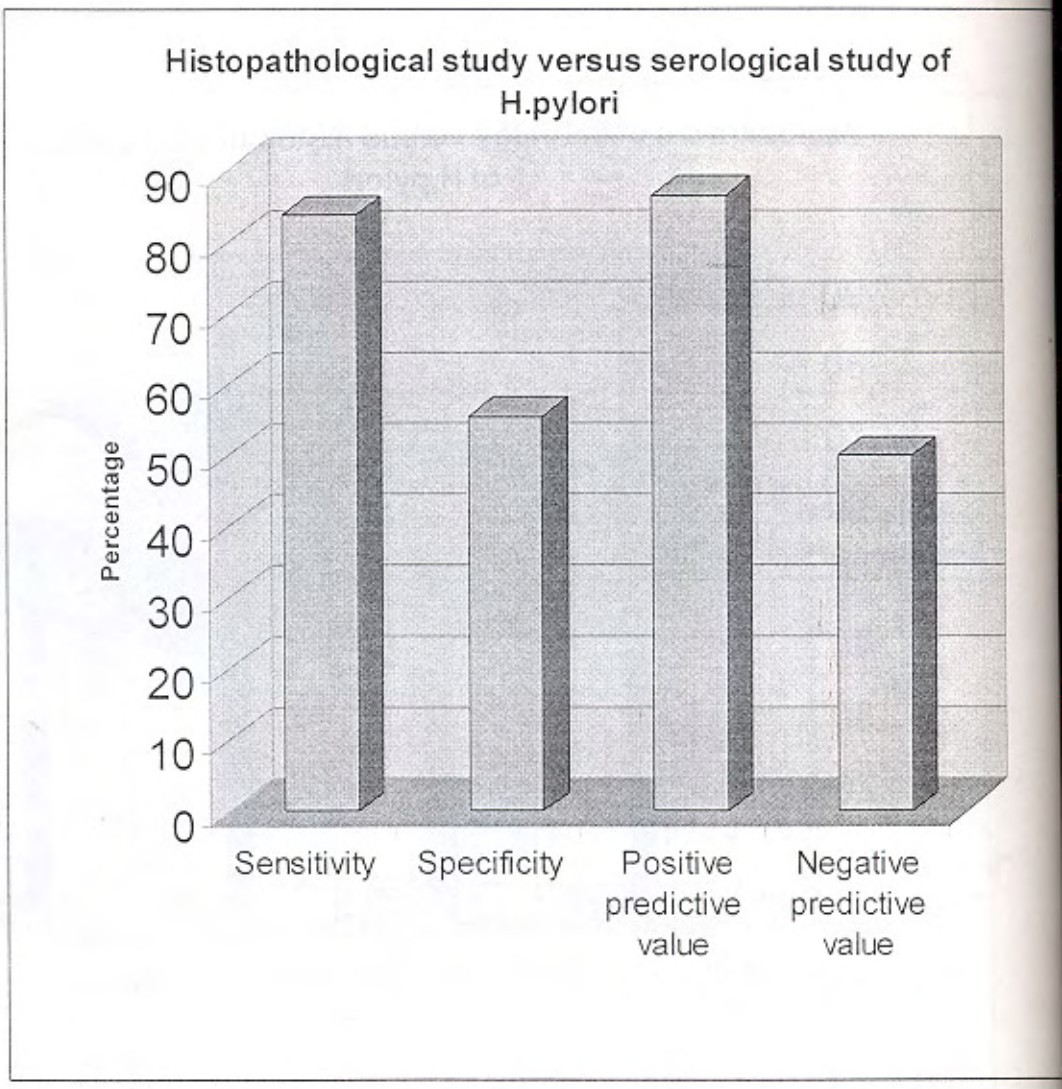


Fig. 14

Table No. 13

Gastric carcinoma and Blood grouping

Blood group	No. of cases(%)
O	14(36.84)
A	14(36.84)
B	8(21.05)
AB	2(5.27)
Total	38(100)

Blood group A was found in 14 (36.84%);and blood group O was also found in 14(36.84%) cases out of 38 cases.

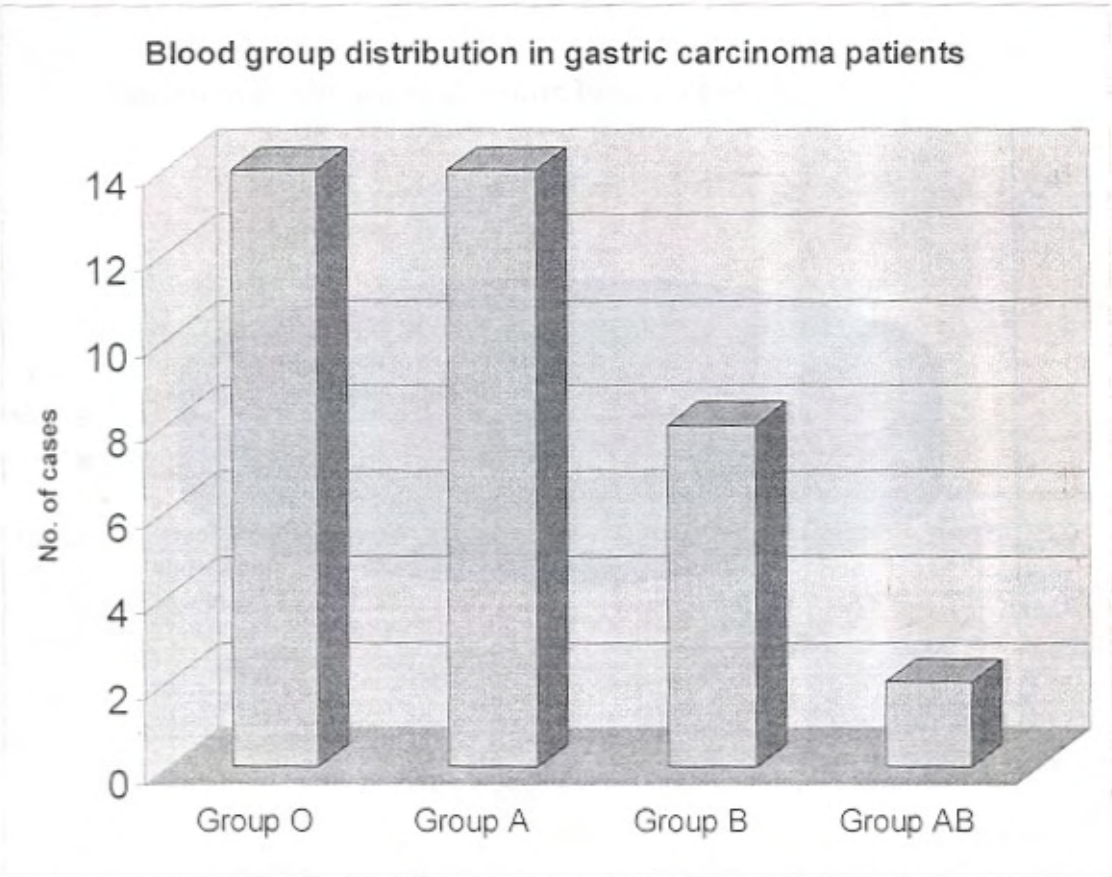


Fig. 15

Table No. 14

Gastric carcinoma and Hemoglobin Level

Hemoglobin level	No. of cases(%)
<4 gm/dl	0 (0.00)
4-8 gm/dl	6 (15.79)
8-12 gm/dl	20 (52.63)
>12 gm/dl	12 (31.58)
Total	38 (100.00)

Most of the patients (52.63%) were mildly anaemic ; while 15.79% cases were moderately anaemic and 31.58% cases were non-anaemic.

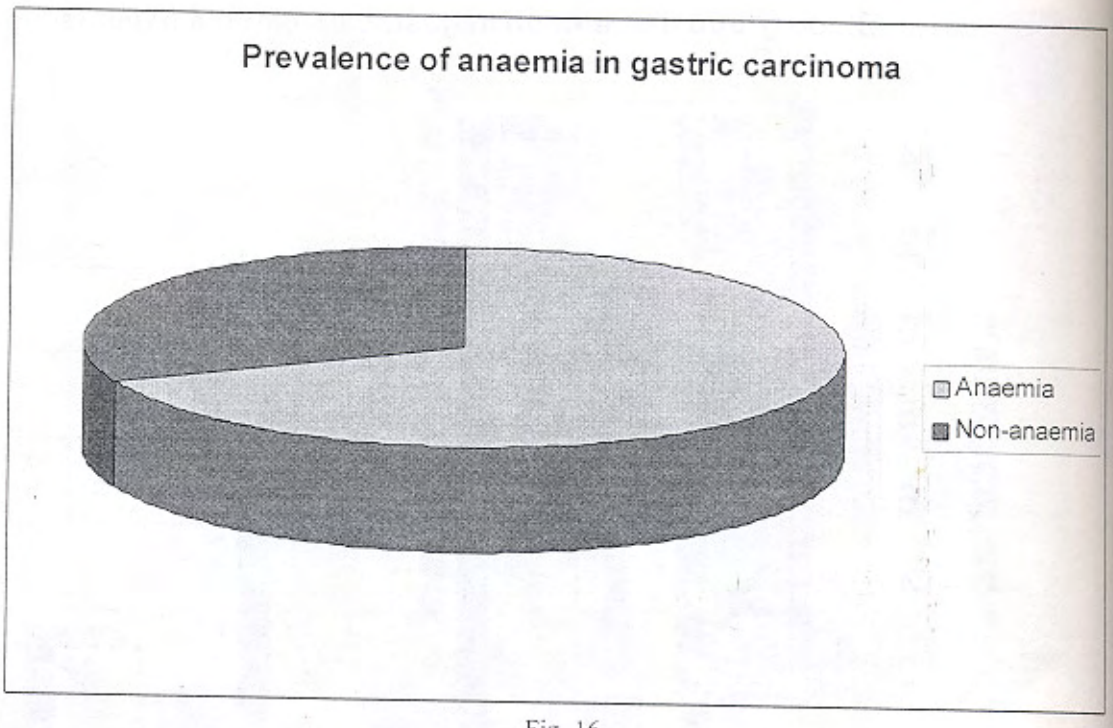


Fig. 16

Table No.15

Relation of Symptoms with Malignant Gastric Neoplasms

Histopathologic variants	Symptoms					
	Abdominal discomfort	Abdominal pain	Vomiting	Hemate-mesis	Melaena	Loss of weight
Adenocarcinoma	36 (90.0%)	36 (90.0%)	24 (60.0%)	6 (15.0%)	10 (25.0%)	26 (65.0%)
Leiomyosarcoma	1 (2.5%)	1 (2.5%)	1 (2.5%)	0 (0.0%)	0 (0.0%)	1 (2.5%)
Non-Hodgkin's lymphoma	1 (2.5%)	1 (2.5%)	1 (2.5%)	0 (0.0%)	0 (0.0%)	1 (2.5%)
Total	38 (95.0%)	38 (95.0%)	26 (65.0%)	6 (15.0%)	10 (25.0%)	28 (70.0%)

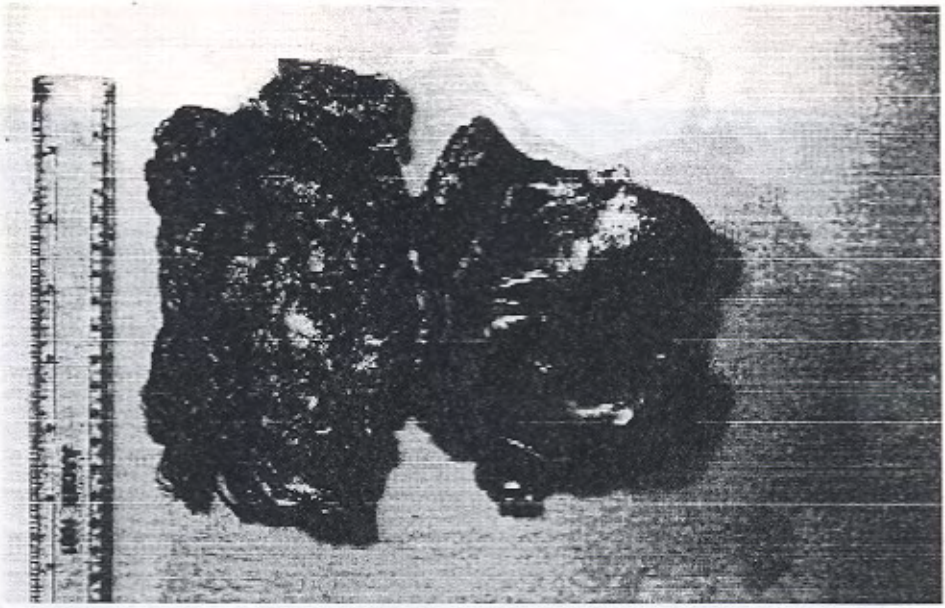
The most common complaints were abdominal pain (95% cases) & abdominal discomfort (95% cases); followed by loss of weight (70% cases) &vomiting (65% cases). Hematemesis & melaena were found in 15% & 25% cases respectively.

Table No. 16

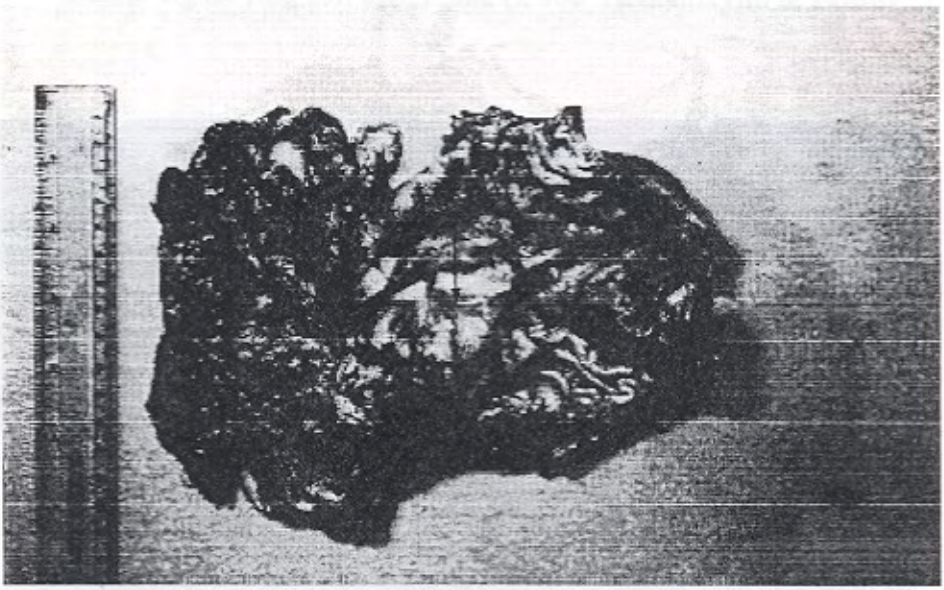
Relation of Symptoms with Gastric Adenocarcinomas

Histopathologic types	Symptoms					
	Abdominal discomfort	Abdominal pain	Vomiting	Hemate-mesis	Melaena	Loss of weight
Tubular	2 (68.42%)	26(68.42%)	18(47.37%)	5 (13.16%)	6 (15.79%)	20(52.63%)
Signet-ring cell	6 (15.79%)	6 (15.79%)	3 (7.90%)	1 (2.63%)	3 (7.90%)	3 (7.90%)
Papillary	2 (5.26%)	2 (5.26%)	2 (5.26%)	0 (0.00%)	0 (0.00%)	2 (5.26%)
Mucinous	2 (5.26%)	2 (5.26%)	1 (2.63%)	0 (0.00%)	1 (2.63%)	1 (2.63%)
Total	36(94.73%)	36(94.73%)	24(63.16%)	6 (15.79%)	10(26.32%)	26(68.42%)

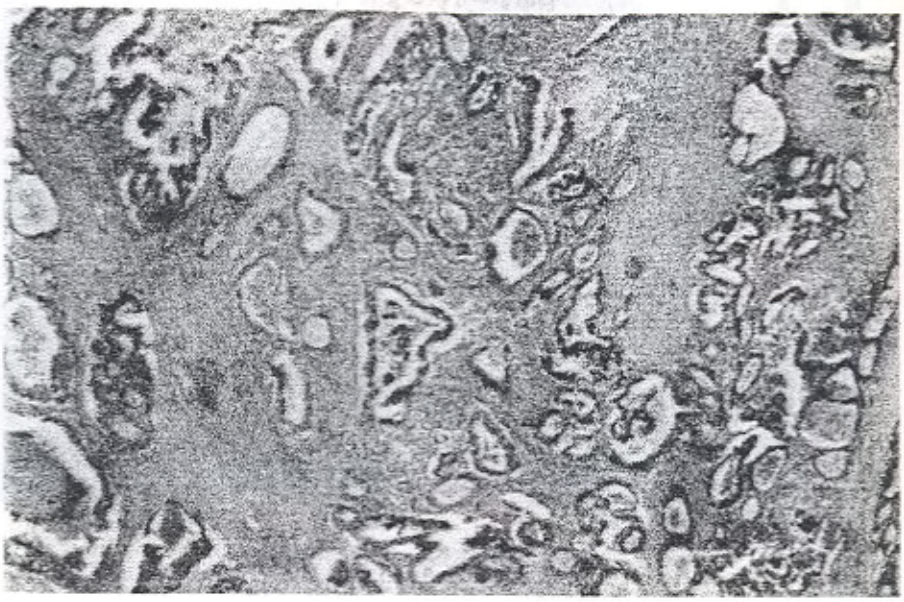
Abdominal pain (94.73%) & abdominal discomfort (94.73%) were the most common complaints in patients with gastric adenocarcinomas.



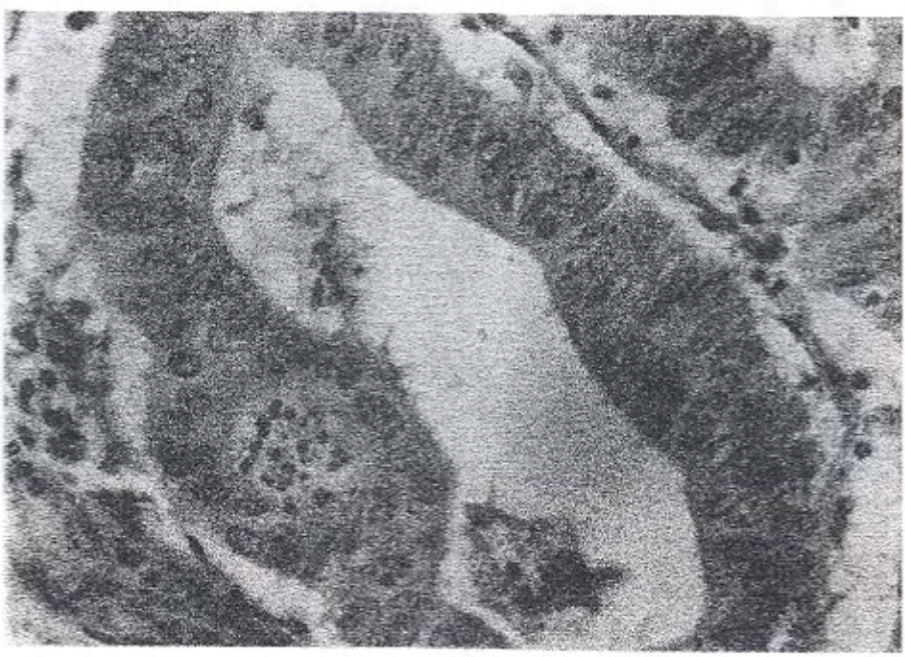
Stomach with greater omentum



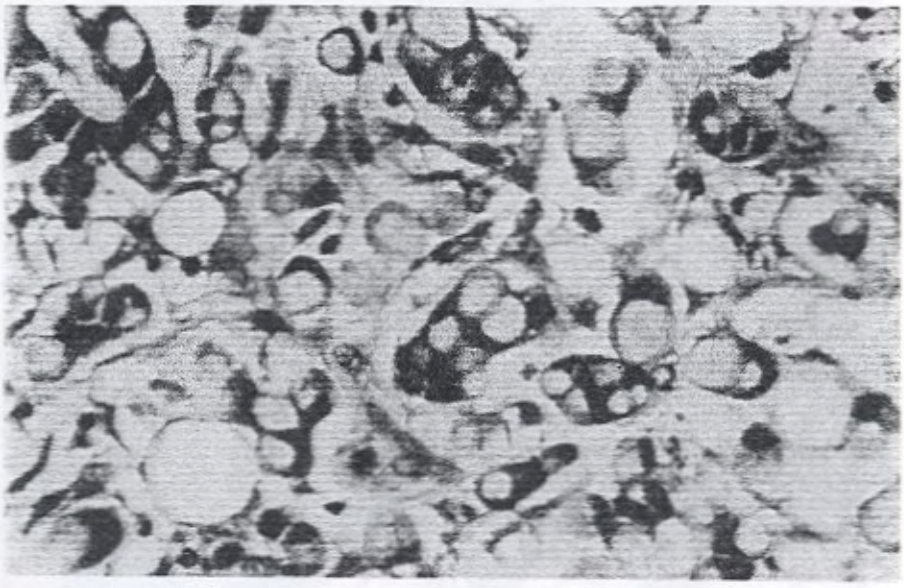
Malignant ulcer in a stomach cut open



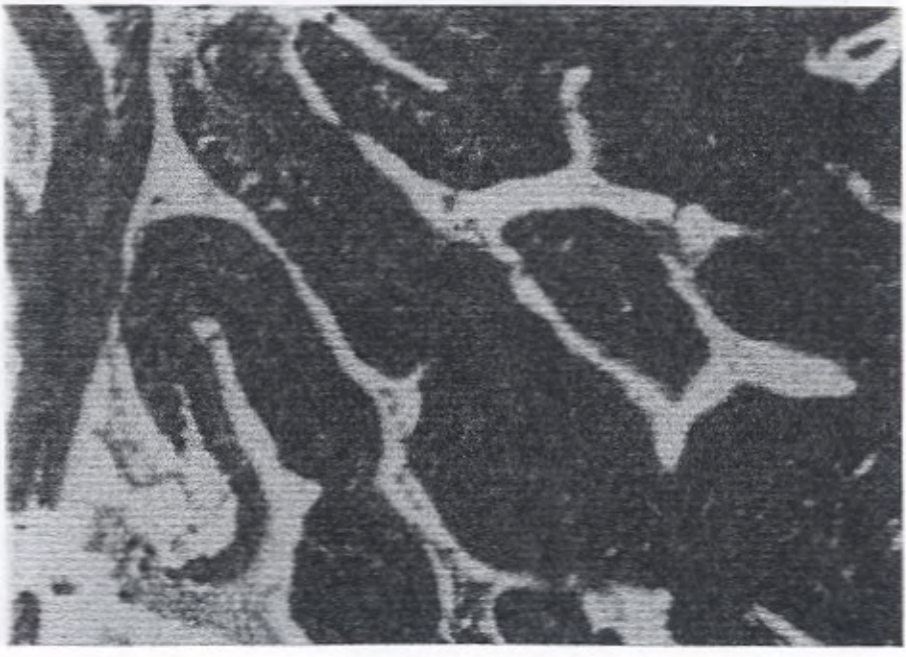
Gastric tubular adenocarcinoma (x 100 Hematoxylin & Eosin)



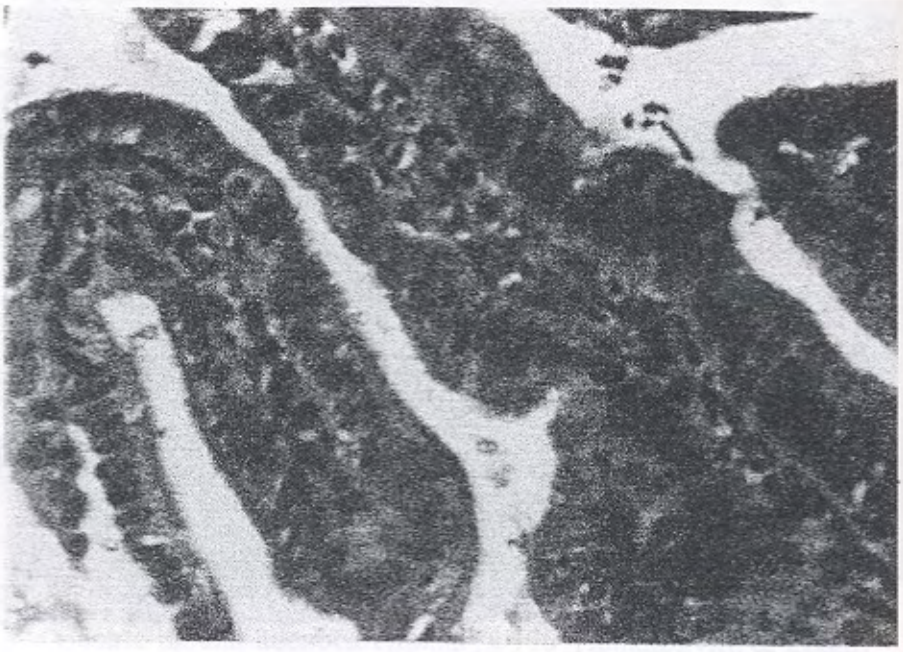
Gastric tubular adenocarcinoma (x400 Hematoxylin & Eosin)



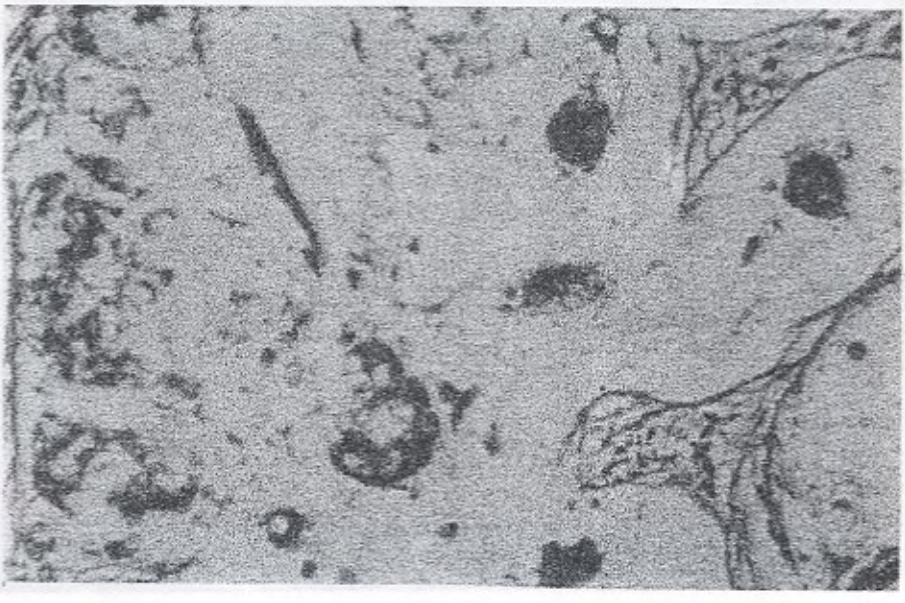
Gastric signet-ring cell carcinoma (x 400 Hematoxylin & Eosin)



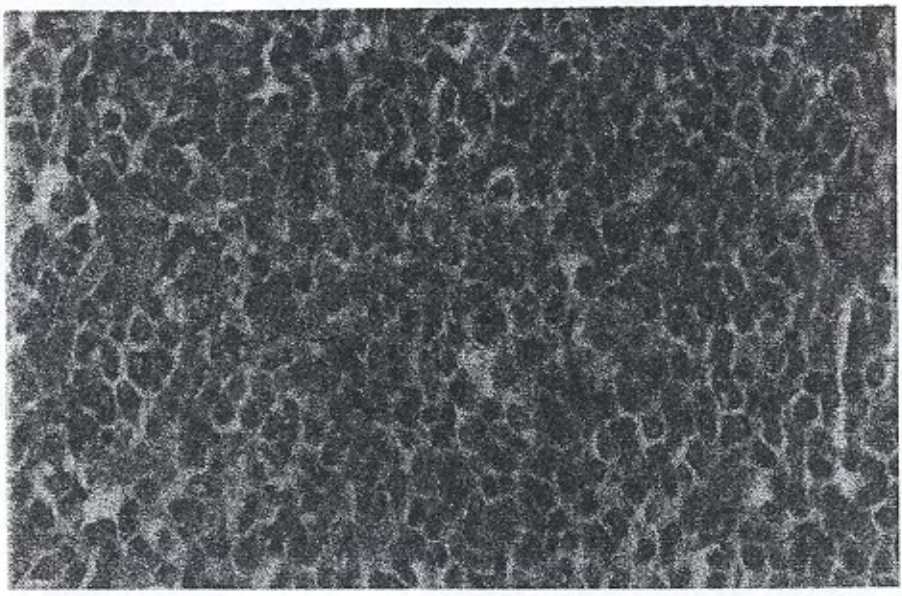
Gastric papillary adenocarcinoma (x 100 Hematoxylin & Eosin)



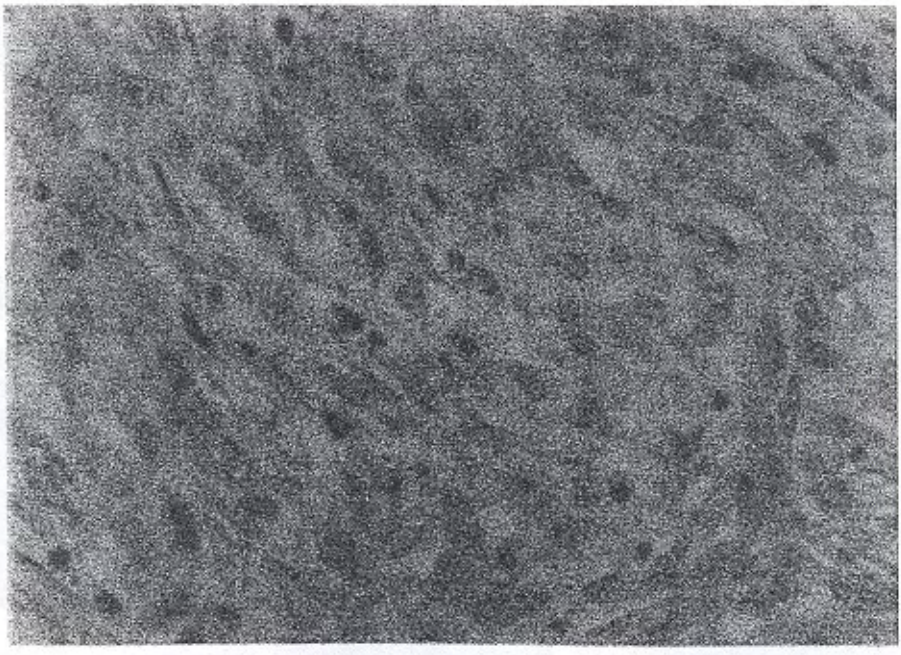
Gastric papillary adenocarcinoma (x 400 Hematoxylin & Eosin)



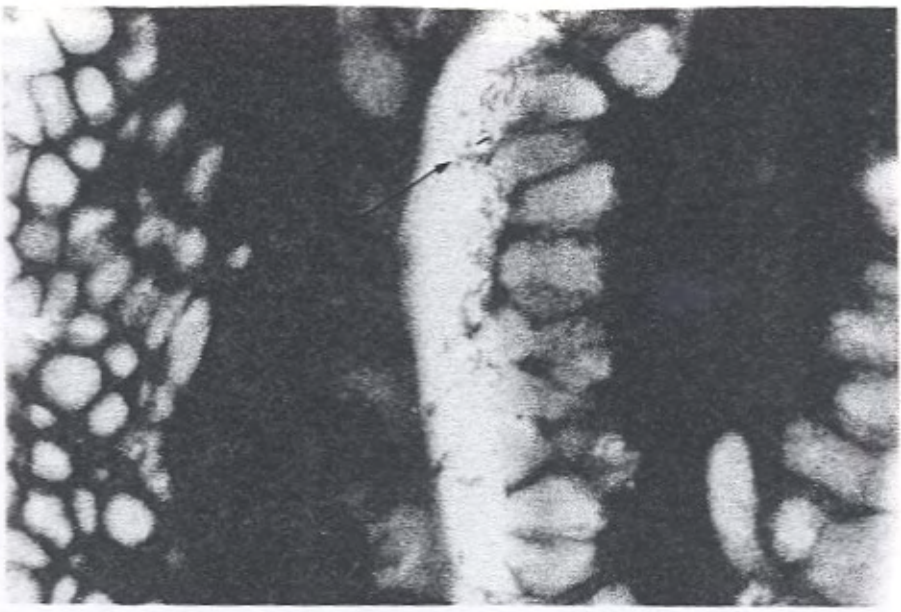
Gastric mucinous adenocarcinoma (x 200 Hematoxylin & Eosin)



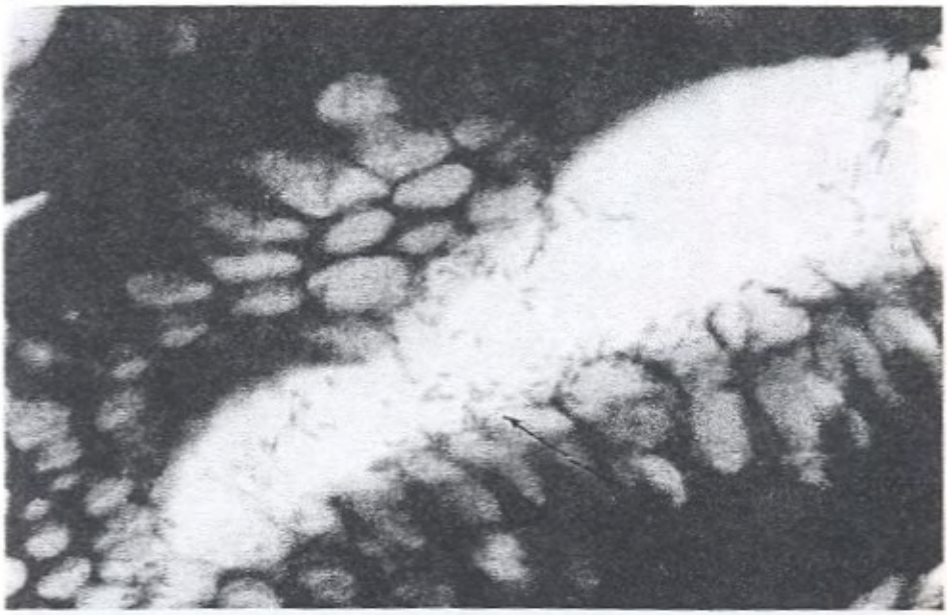
Gastric MALToma (x 400 Hematoxylin & Eosin)



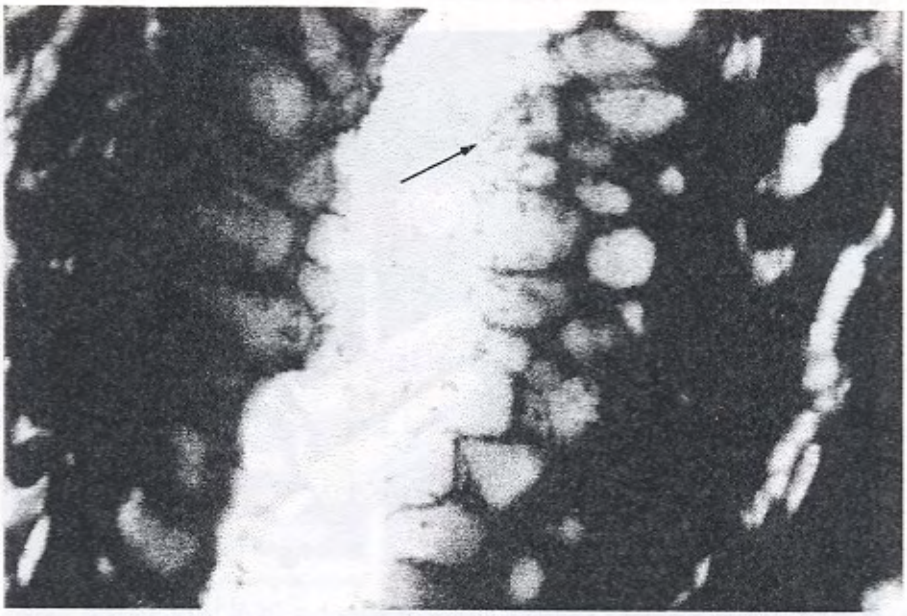
Gastric leiomyosarcoma (x 400 Hematoxylin & Eosin)



Gastric glands showing Helicobacter pylori (x 400 Giemsa stain)

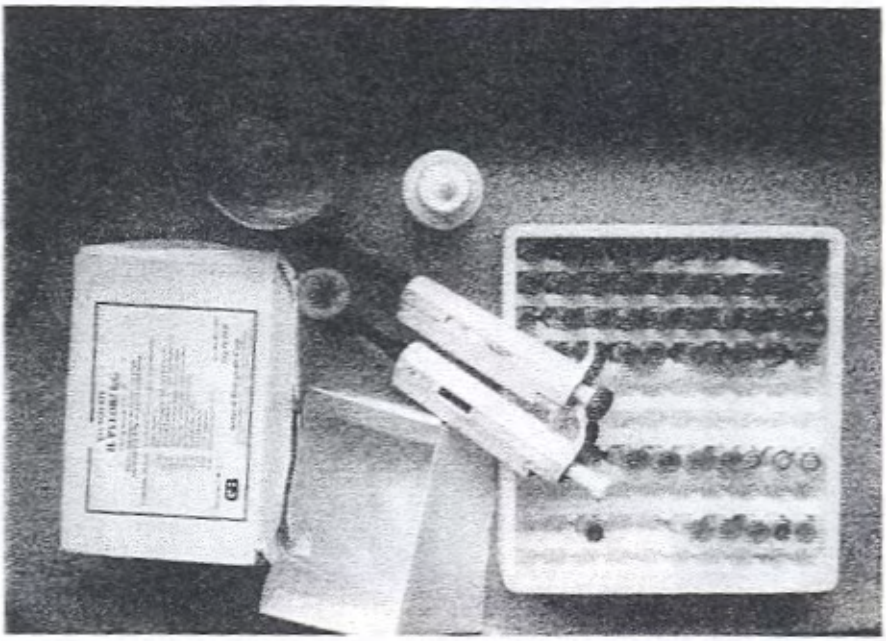


Gastric glands showing Helicobacter pylori (x 1000 Giemsa stain)

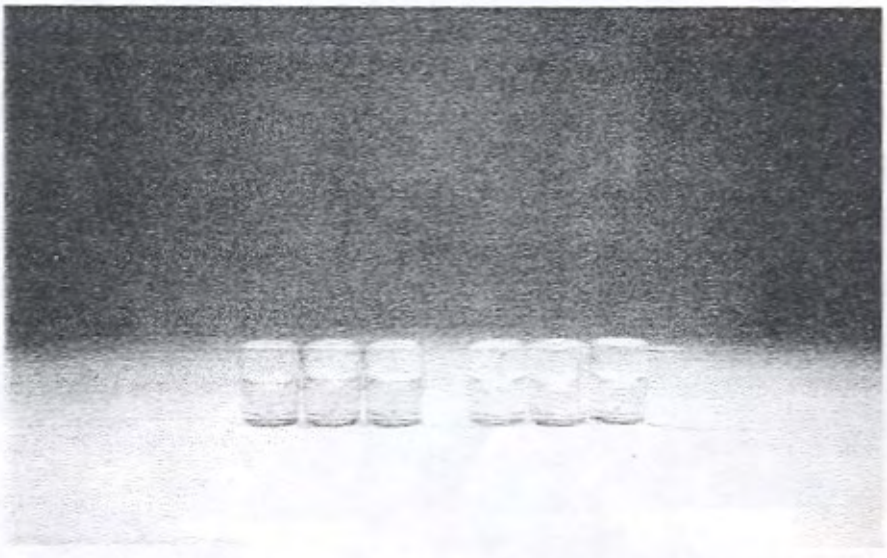


Gastric mucosal surface showing Helicobacter pylori (x 400 Giemsa stain)

Gastric mucosal surface showing Helicobacter pylori
(x 1000 Giemsa stain)



Serological kit and sera of the patients



Microwells detecting antibodies against H. pylori showing 3 positive sera (left) & 3 negative sera (right)

Discussion

Gastric cancer is one of the common malignant tumors and commonest malignant tumors in Nepal[7]. It is one of the 'captains of the men of death.'

In this study, 40 cases of gastric malignancies were taken. A correlative study of 38 gastric carcinomas was done including the age, sex, symptoms, anaemia, blood group, gross features and H. pylori infection.
Out of 40 cases, there were 38 (95%) cases of adenocarcinoma, 1 (2.5%) case of non-Hodgkin's lymphoma and 1 (2.5%) case of leiomyosarcoma. Worldwide incidence has shown that carcinoma is the most common malignant tumor (90% to 95%), following in order of frequency lymphoma (4%), carcinoids (3%) and malignant spindle cell tumors (2%) [4].

Gastric cancer is considered to be a disease primarily affecting the middle aged and the elderly; indeed its peak incidence occurs in patients older than 50 yrs[83]. Gastric carcinoma patients younger than 40 yrs are rare (approx 5%)[84].

In this study, Age of patients with gastric carcinoma ranged from 26 to 87 yrs with mean age of 56.9 yrs. Patients with > 50 yrs. of age were 32 (84.2%) and < 50 yrs of age were 6 (15.8%). In a study of Shrestha & Dali et al. [7] gastric cancer patients < 50 yrs of age comprised 42.5% and > 50 yrs of age 57.5%. Manohar et al. [6] found that gastric cancer patients below the age of 50 yrs were 39.14% and above the age of 50 yrs were 61.6%. Scott A Hundahl et al. [81] found mean age of 68.3 yrs in gastric cancer patients during the year 1992 - 93; while Charles P Theuer et al. [82] found the mean age of 67 yrs. In study of Chung-Ying Wu et al.[85], the mean age of patients with non-mucinous gastric carcinoma was 63.0 yrs with a range of 13-90 yrs.

Age specific incidence rates of gastric adenocarcinoma in different population is compared with this study as follows[82].

Age (yrs)	Asians (Per100,000)	Blacks (Per100,000)	Latinos (Per 100,000)	whites (Per 100,000)	This stud
00-19	0.2	0.0	0.0	0.0	0.0
20-39	2.1	1.5	1.3	0.4	7.9
40-59	13.4	11.6	11.9	5.0	50.0
60-79	67.8	39.8	50.2	28.5	39.5
80 and above	164.9	112.2	140.6	53.8	2.6

In the study of Charles P Theuer et al.[82], mean age of patients with gastric carcinoma below the age of 40 yrs (<40 yrs) was 33 years and above the age of 40 yrs was 69 yrs; whereas Yoshihiko M et al[86] in Japan found that the mean age of patients younger than 40 yrs was 38.8 yrs and older than 70 yrs was 74.8 yrs.

Gastric carcinoma is more common in male with a M:F ratio of 1:1 in young and 2:1 in 60-70 yrs of age[87]. In this study, 73.7% patients were male and 26.3% were female. Out of patients aged below 40 yrs; 66.7% patients were female & 33.3% patients were male i.e. with a M:F ratio of 1:2. Among patients aged above 40 yrs; 77.1% cases were male and 22.9% were female i.e. with a M:F ratio of 3.36:1.

Lai IR et al.[88] also found female predominance in young gastric carcinoma patients with a M:F ratio of 1:1.36. Yoshihiko M et al.[86] found 51.1% male and 48.9% female patients in patients younger than 40 yrs; whereas 69.4% male & 30.6% female patients in patients older than 70 yrs of age. In the study of Scott A. Hundahl et al.[81] during the year 1992-93, male patients of gastric carcinoma constituted 61.4% & female 38.6%. Chun-Ying Wu et al.[85] found that mucinous carcinomas were more common in male (86%) than in female (14%). In a study of Charles P Theuer et al.[82], among gastric cancer patients younger the age of 40 yrs, 63% were male & 37% were female; whereas among patients older than 40 yrs of age, 62% were male & 38% were female. In a study of Manohar et al.[6]; there were 62.5% male patients and 37.5% female patients

of gastric cancer; whereas in a study of Shrestha & Dali et al.[7], there were 61.5% male and 38.5% female patients of gastric carcinoma.

Gastric cancer patients may be asymptomatic or have symptoms like gastric distention, anorexia, abdominal pain, hematemesis, melaena, vomiting etc.

In this study, abdominal discomfort & pain were found in 94.73% cases; vomiting in 63.16% cases; hematemesis, melaena & loss of weight in 15.79%; 26.32% & 68.42% cases respectively.
In one series, anorexia & weight loss were found in 95% cases; hematemesis in less than 5% cases and abdominal mass in 50% cases[89]. Lai IR et al.[88] found that epigastralgia was the most common complain (65.4%). In a study of Adachi Y et al.[90] epigastric pain was found in 64% gastric cancer patients younger than 40 yrs of age. The incidence of asymptomatic gastric cancer increased gradually and has amounted to 30% of the total resected cases in recent years[91].

Anaemia may be present in gastric cancer patients, usually owing to chronic blood loss in the form of hematemesis & melaena.

In this study, 67.5% patients were anaemic; most of them were only mildly anaemic. In most of studies[8] [92] anaemia was detected in 40% to 50% of patients with gastric cancer. The anaemia is usually microcytic, but it may be megaloblastic or mixed in type.

In 1953, Aird made the observation that blood group A was more frequent in patients with gastric cancer[28]. This was later confirmed by other studies. The signification of this association remains uncertain.

In this study, blood group A was found in 37.5% cases. There is approximately 20% increased liability of people of blood group A to have the disease compared with people of other blood groups[10]. Klaamas K et al.[93] found that the proportion of Cag A strong responders was higher at the first stage of gastric cancer in only blood group O and A individuals as compared with related controls.

Gastric carcinoma may grossly present as exophytic, ulcerating, infiltrative, diffusely infiltrative, expansile or annular growth.

In this study, ulcerative growth was seen in 89.48% cases, infiltrative growth in 5.26% cases and diffusely infiltrative in 5.26% cases. The growth was located at antrum in 78.95% cases; at pylorus in 10.53%; on corpus in 5.26% cases and on cardia in 5.26% cases. Yoshihiko M et al.[86] found infiltrative growth in 47.1% patients younger than 40 yrs of age and in 33.7% patients older than 70 yrs of age. In a study of Hiroshi Isozaki et al.[94] found protruding growth in 7.7% cases; depressed or excavated growth in 55.4% cases. Adachi Y et al.[90] found depressed type of growth in early state in 100% cases. In a study of Charles P Theuer et al.[82], in gastric cancer patients younger than 40 yrs of age, tumor was located at antrum in 17% cases, in cardia in 16% cases and in body in 66% cases; whereas in patients older than 40 yrs of age, at antrum in 22% cases, in cardia 27% cases and in body in 52% cases.

Helicobacter pylori has been documented to be associated with gastric cancer.

In this study, H. pylori seropositivity (IgG) was found in 76.31% cases of gastric adenocarcinomas; whereas H. pylori was detected in Giemsa stained tissue sections in 78.95% cases of gastric adenocarcinomas. In one study, Thapa[109] found H.pylori seropositivity in 74% patients, presented with dyspepsia. In his study, H.pylori was detected in Giemsa stained tissue sections in 74% gastric biopsy specimens taken from these patients.

Wu MS et al.[95] found H. pylori seropositivity (Ig G) in 65.6% cases of gastric carcinoma. In a study of Komoto et al.[96] H. pylori seroprevalence was prevalent in 93% patients with gastric carcinoma and it was more prevalent in non cardia tumors. In another study, Shimoyama et al.[97] found cag A seropositivity in 60% gastric cancer patients. In the city of Malmo, Sweden, the overall seropositivity prevalence in gastric cancer cases was 82%[98]. In a study of patients with gastric carcinoma younger than 40 yrs, cag A positivity was found in 50.5% cases and H. pylori was histologically detected in 70.5% cases[84]. In resected specimens,

H. pylori was detected only in 27.5% cases with adenocarcinoma using haematoxylin-eosin stain in a study of Hung et al[99].

Thus, Helicobacter pylori is strongly associated with gastric carcinogenesis.

Conclusion

Primary gastric malignancy is common malignant condition. It is most common malignant tumor in our country. Among these tumors, adenocarcinoma is the commonest one; followed by non-Hodgkin's lymphoma and malignant smooth muscle tumor. These tumors are more common in male and after the age of 50 yrs. Among adenocarcinomas, tubular type predominates; followed by signet-ring cell type. Grossly most of the tumors are located at antrum; they have ulcerating growth. Abdominal discomfort & abdominal pain are the commonest complaints; followed by loss of weight & vomiting.

Blood group A and O are the frequent blood groups found in patients with gastric malignancy. Majority of patients are anaemic.

Helicobacter pylori is detected in 78.95% cases by Giemsa staining; in 76.31% cases by serological test. Both Giemsa staining and serology are positive in 65.79% cases. So, it is concluded that H. pylori infection is strongly associated with development of gastric carcinoma and detection of H. pylori in tissue section by Giemsa staining is more sensitive, feasible & economical method to diagnose H. pylori infection in our context.

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PROFORMA

Case No: ..... Date: .....

Name: ..... Age: ..... Sex: .....

Address: .....

Occupation: Service ( ) Agriculture ( ) Business ( ) Others ( )

Marital status: Married ( ) Unmarried ( )

Ward (if admitted): ..... Bed No: ..... IP No: .....

OPD No: .....

Clinical History

Abdominal discomfort: Yes ( ) No ( )

Pain abdomen:

Site: Epigastric ( ) Umbilical ( ) Right hypochondrial ( ) Left hypochondrial ( ) Others ( )

Periodicity: Periodic ( ) Continuous ( )

Relation to meal: After ( ) Before ( )

Anorexia: Yes ( ) No ( )

Nausea: Yes ( ) No ( )

Vomiting: Yes ( ) No ( )

Haematemesis: Yes ( ) No ( )

Melaena: Yes ( ) No ( )

Loss of weight: Yes ( ) No ( )

Clinical Examination

Pallor: Yes ( ) No ( )

Cachexia: Yes ( ) No ( )

Abdominal lump:	Yes ( )	No ( )
Epigastric tenderness:	Yes ( )	No ( )
Hepatomegaly:	Yes ( )	No ( )
Splenomegaly:	Yes ( )	No ( )
Others:	Yes ( )	No ( )

Haematological Laboratory Investigations

Hb ..... gm/dl Blood group .....

Histopathological Report of Gastric Biopsy Histopathology No. S .....

Gross Examination:

Received specimen labelled as gastric biopsy containing SINGLE/ MULTIPLE pieces of ..... coloured SOFT/ FIRM tissues. Necrosis: Yes ( ) No ( )

Microscopic Examination:

A) Histologic type:

Adenocarcinoma: Papillary ( ) Mucinous ( ) Tubular ( ) Signet-ring cell ( )

Lymphoma: Hodgkin's ( ) Non-Hodgkin's ( )

Carcinoid tumors: ( )

Leiomyosarcoma: ( )

Others: .....

B) Histologic grade (for adenocarcinoma)

Grade X ( ) Grade 1 ( ) Grade 2 ( ) Grade 3 ( ) Grade 4 ( )

Impression: .....

Histopathological Report of Gastrectomy Specimen

Histopathology No. S.....

Gross Examination:

Type of gastrectomy: Partial ( ) Subtotal ( ) Total ( )

Site of tumor: Pylorus ( ) Antrum ( ) Corpus ( ) Cardia ( ) Fundus ( ) Whole Stomach ( ) Lesser curvature ( ) Greater curvature ( )

Growth of tumor: Exophytic ( ) Infiltrative ( ) Diffusely infiltrative ( ) Expansile ( ) Ulcerative ( ) Annular ( )

Size of tumor: .....

Surface of tumor: Regular ( ) Irregular ( ) Smooth ( ) Rough ( )

Mucosa of stomach: Normal ( ) Hypertrophied ( ) Atrophied ( )

Depth of invasion: Into mucosa or submucosa ( ) Into muscularis propria or subserosa ( ) To other organ or structure ( )

Lymph node: Multiple ( ) Single ( )

Microscopic Examination:

A) Histologic type:

Adenocarcinoma: Papillary ( ) Mucinous ( ) Tubular ( ) Signet-ring cell ( )

Lymphoma: Hodgkin's ( ) Non-Hodgkin's ( )

Carcinoid tumors: ( )

Leiomyosarcoma: ( )

Others: .....

B) Histologic grade (for adenocarcinoma)

Grade X ( ) Grade 1 ( ) Grade 2 ( ) Grade 3 ( ) Grade 4 ( )

C) Lymph node: Free ( ) Involved ( )

Impression: .....

Investigations for Helicobacter pylori

1. Giemsa stain: 0 ( ) 1+ ( ) 2+ ( ) 3+ ( ) 4+ ( ) 5+ ( ) 6+ ( )
2. Serological test: Positive ( ) Negative ( )