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Detection of Gastric Carcinoma by Endoscopic Imprint Cytology

Hossain, Md. Mokter

University of Rajshahi

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THESIS SUBMITTED FOR THE DEGREE
OF
DOCTOR OF PHILOSOPHY
IN THE
INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI, RAJSHAHI-6205
BANGLADESH

BY

MD. MOKTER HOSSAIN
MBBS, MCPS (CLINICAL PATH) M. PHIL (PATH)
MCPS (FAMILY MEDICINE)

SEPTEMBER, 2013

Pathology Laboratory
Khulna Medical College
Khulna, Bangladesh

Molecular Biology Laboratory
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Rajshahi- 6205, Bangladesh

PhD Thesis

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CERTIFICATE

We do there by certify that **Md. Mokter Hossain**, Assistant Professor, Pathology Department, Khulna Medical College, Khulna, Bangladesh is the sole author of the dissertation entitled **"DETECTION OF GASTRIC CARCINOMA BY ENDOSCOPIC IMPRINT CYTOLOGY"**. This dissertation or part thereof has not been previously submitted for the award of any degree, diploma or associate ship of any other similar title.

We are forwarding this dissertation to be examined for the degree of Doctor of Philosophy (Clinical Pathology) in the Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh. Md. Mokter Hossain has fulfilled all the requirements according to the rules of the University for Submission of a dissertation for the PhD (Clinical Pathology) degree.

Principal Supervisor

Dr. Parvez Hassan, Ph.D
Professor
Institute of Biological Sciences
University of Rajshahi
Rajshahi, Bangladesh.

Co-Supervisor

Dr. Md. Abu Sayeed
Professor,
Department of Pathology
Khulna Medical College (ex)
Noakhali Medical College
Bangladesh.

DECLARATION

I, hereby, declare that, the research work submitted as a dissertation entitled **"DETECTION OF GASTRIC CARCINOMA BY ENDOSCOPIC IMPRINT CYTOLOGY"** submitted to the institute of Biological sciences, University of Rajshahi, Rajshahi, Bangladesh for the degree of doctor of philosophy is the result of my own investigation and was carried out under the supervision of Dr. Parvez Hassan, Ph.D, Professor, Institute of Biological Sciences, University of Rajshahi and Dr. Md. Abu Sayeed, M. Phil, Professor, Department of Pathology, Khulna Medical College, Khulna (ex) and now Noakhali Medical College, Bangladesh.

I, further, declare that this dissertation or part thereof has not been the basis for the award of any degree, diploma or associate ship of any other similar title.

(Md. Mokter Hossain)

Signature of the Candidate

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DEDICATED TO

My parents I owe my life and basic learning

My teachers for their teaching and advices

My wife for her great loses and sacrifices

My children, my inheritance and future

My brothers and sisters for their continuous support and inspiration

My relatives for their love and affections

My neighbors and villagers for whom I am a social being

My colleagues and fellows I feel always

Philosophers and social reformers for whom I feel life

And mankind whom I interacted in different spheres of life.

ABSTRACT

Background:

This study was undertaken with the view to determine the Sensitivity, Specificity and agreement between two methods of the stained gastric and esophageal imprint cytology smears and stained gastric and esophageal endoscopic specimen biopsy for detection of gastric and esophageal carcinoma.

Methods:

Imprint smears of endoscopic gastric and esophageal biopsies from 272 patients were stained by Papanicolaou staining (Pap's stain) in the endoscopy suit and examined for carcinoma, providing results within 10 minutes. The same biopsy was processed and stained with H & E, and reviewed by pathologists.

Results:

Two hundreds and seventy two male and female patients were studied in between 2008 to 2011. Esophageal lesions were 83 cases (30.51%) and gastric lesion were 189 cases (69.48%) cases. One hundred and twenty two (122 *i.e.* 64.55%) cases of gastric carcinomas were detected by histopathology, whereas, Imprint cytology detected 124 (65.60%) cases.

Conclusion:

Gastric and esophageal imprint smears stained with Papanicolaou (Pap's stain) method is a rapid and cost-effective method in addition to histopathology for detecting gastric and esophageal carcinoma in patients undergoing upper gastro-intestinal endoscopy and biopsy. It does not require an additional biopsy.

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CHAPTER-1

INTRODUCTION

1.0. INTRODUCTION

The pathogenesis of gastric carcinoma is closely related to environmental factors. Its incidence has markedly decreased in recent years in some countries such as the United States and England. But it remains inordinately high in others, such as Japan, Chile and Italy (Correa 1991). Most patients are over 50 years of age (Dupont *et al.* 1978) but cases in younger individuals and even children have been recorded (Tso *et al.* 1987).

Practically all gastric carcinomas arise from the generative or basal cells of the foveolae (Taki and Kuwabara 1981). In most instances on a background of chronic atrophic gastritis with intestinal metaplasia and proceeded by various stages of dysplasia, CIS and superficial carcinoma (Hattori 1986).

Gastric carcinoma is accompanied by hypochlorhydria in 85% to 90% of the cases, and it has been shown that hypochlorhydria may precede gastric cancer by several years. It has been postulated that high intra-gastric pH promotes the growth of bacteria that reduce dietary nitrate to nitrite and then convert dietary amines, in the presence of this nitrate, into carcinogenic N-nitroso compounds (Hall 1986).

The co-existence of chronic atrophic gastritis and carcinoma is common, but the etiopathogenic link between the two and the relative risk for malignancy in the former condition remain controversial (Corrca 1992).

The same could be said for pernicious anaemia; the rate of carcinoma development, although statistically increased, is not high enough to justify surveillance in asymptomatic patients (Schafer *et al.* 1985).

Lately, Helicobacter Pylori has been implicated as a possible etiologic factor in gastric carcinoma through its role in the development of chronic gastritis (Clarkson and West 1993).

Radiologic examination of the stomach will demonstrate the lesion in most cases, but in about 10% it will be impossible to determine whether it is benign or malignant (Rosai 1996.).

In countries with a high incidence of gastric carcinoma, particularly Japan, the increased use of mass screening, endoscopy, cytology and biopsy has resulted in the identification of a large percentage (up to a third) of early cases, with a corresponding increase in survival rates (Rosai 1996).

With the introduction of the flexible fiberoptic gastroscope, the results of direct-vision gastric biopsy and brush cytology have improved dramatically (Tatsuta *et al.* 1989).

Recently it is observed all over the world that cytology plays a very important role in the initial diagnosis of tissue pathology and in many parts of the world, biopsy for histopathology are being replaced by fine needle aspiration cytology. However, fine needle aspiration cytology of gastric tissue will be very difficult, as in many cases, there is no lump. Therefore, it may be assumed that if smears are prepared of endoscopic biopsy material of gastric tissue for cytological examination, it may help in the diagnosis of gastric carcinoma.

Histopathology of endoscopic gastric tissue may not give any confirmative result in some cases as only part of the biopsy material sectioned and examined. Therefore it appears that if cytological examination of the biopsy material is also performed the possibility of detecting gastric carcinoma if present may be further increased. With this view in mind the present study was under taken.

Imprint and smears of the endoscopic gastric biopsy is a very safe procedure and is highly effective in the diagnosis of suspected cases of gastric carcinoma.

Therefore if the combination of the gastric biopsy, imprint or smears from endoscopic biopsy and visual endoscopic finding's are employed confirmatory diagnosis in most of the cases can be achieved. Then it could be of immense help to both the physician and the patients.

CHAPTER-2

RATIONALE, HYPOTHESIS, AIMS AND OBJECTIVES

2. RATIONALE, AIMS AND OBJECTIVES.

2.1. RATIONALE:

Gastric cancer is one of the most common malignancies of the gastrointestinal tract. In the evaluation of these lesions, the diagnostic value of cytology in addition to biopsy remains controversial. There is wide variability in the reported diagnostic accuracy rates for biopsy and cytology in gastric lesions (Geramizadeh *et al.* 2002). Determined efforts in Japan, where the disease is common, have led to remarkable improvements in diagnostic methods (Young and Hughes 1980). Today, various cytologic techniques like brush cytology, crush preparation, touch smear cytology etc. are commonly used along with routine endoscopic biopsy (Batra *et al.* 2008).

There are a few studies in the literature on the role of touch smear cytology in gastric cancer (Kochhar *et al.*1990, Gupta *et al.* 1984). On the basis of available data, there is no general consensus as to whether cytology should be done routinely, only in selected instances or not at all. However, the use of touch smear as simple, cheap and rapidly available cytologic technique in under-resourced countries is an added asset to the biopsy (Geramizadeh *et al.* 2002, Batra *et al.* 2008).

No such study to find out the link between thyroid function and dyslipidemia has yet been done in Bangladesh. The present prospective study was undertaken with the view to evaluate this association in the context of Bangladesh with particular emphasis to the population from the Northern part along the belt of the river Brahmaputra and the river Jamuna. The observations (information) and results

emerging from the study are expected to be useful in the treatment as well as in the prevention of atherosclerotic cardiovascular diseases.

The aim of the present study was to evaluate the utility and accuracy of touch smear cytology in clinically suspected cases of gastric malignancy with a subsequent correlation with histopathology.

2.2. RESEARCH HYPOTHESIS:

Imprint cytology from endoscopic biopsy to detect gastric carcinoma.

Detection of gastric carcinoma can be made by endoscopic imprint cytology.

2.3. AIMS AND OBJECTIVES:

- The aim of the study was to find out an efficient method to diagnose gastric carcinoma
- Compare the efficacy of diagnostic methods including endoscopic imprint cytology and biopsy.

CHAPTER-3

REVIEW OF LITERATURES

3.0. REVIEW OF LITERATURES

3.1. Anatomical aspect of the gastrointestinal tract:

The gastrointestinal (GI) tract is a hollow tube extending from the oral cavity to the anus that consists of anatomically distinct segments, including the esophagus, stomach, small intestine, colon, rectum, and anus (Pathological Basis of diseases 8th ed. 2010).

The stomach is divided grossly into the following regions: Cardiac, fundus, corpus or body, pyloric antrum and pylorus.

These show some correspondence to (but should not be equated with) the three major microscopic types of gastric mucosa; cardiac, fundic and pyloric (antral), which exhibit transitional areas in between (Rosai 1996).

The normal stomach is lined by a complex mucosa. This consists of surface epithelium, below which are the foveolae (pits) and the more deeply located gastric glands (Stacey 2004).

All of the gastric glands have two major components: Foveola (crypt, pit) and secretary portion (adenomere) (Rosai 1996).

The foveolae represent the most important area for the genesis of gastric carcinoma, in particular the layer of generative cells located at their base (Rosai 1996). The epithelium lining the surface and foveolae are similar throughout the stomach and consist of a single layer of columner cells, all of which contain mucus in the superficial cytoplasm.

The other two components of the mucosa besides lining epithelium are the lamina propria and the muscularis mucosa. The latter is formed of an inner circular and an outer longitudinal layer; it is continuous with thin fascicles of smooth muscle that go up inside the lamina propria to reach beneath the surface epithelium (Rosai 1996).

Mucosa, the innermost layer of stomach and the other layers of the stomach are the same as for the rest of the gastro-intestinal tract (i.e from inner to outer: Submucosa, muscularis externa (Propria), and serosa) (Rosai 1996).

The submucosa consists of loose connective tissue with numerous elastic fibers; it contain plexuses of arteries, veins, lymph vessels, and Meissner's nerve plexus. (Rosai 1996).

The musculoris externa is composed of three layers; outer longitudinal, inner circular, and inner most oblique (Rosai 1996). The Auerbach (Myenteric) plexus is located between the circular and longitudinal layers of the muscularis externa (Rosai 1996).

The blood supply of the stomach originates from the celiac axis, hepatic artery, and splenic artery. Lymphoid tissue is sparse in the normal stomach.

3.2. Endoscope:

An endoscope is a device with a light attached that is used to look inside a body cavity or organ. The scope is inserted through a natural opening, such as the mouth during a bronchoscopy, the retum for a sigmoidoscopy, or the vagina for a cystocopy.

- A medical procedure using any type of endoscope is called endoscopy.

What is upper gastrointestinal (GI) endoscopy?

Upper GI endoscopy is a procedure that uses a lighted, flexible endoscope to see inside the upper GI tract – includes the esohagus, stomach, and duodenum

- The first part of the small intestine.

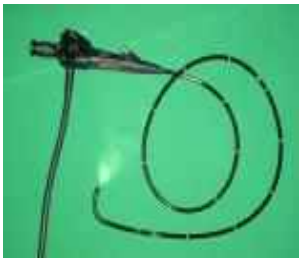

Fig.1. An Endoscope.

What problems can upper GI endoscopy detect?

Upper GI endoscopy can detect

- Ulcers

- abnormal growths

- Precancerous conditions

- Bowel obstructions

- Inflammation

- Hiatus hernia

When is upper GI endoscopy used?

Upper GI endoscopy can be used to determine the cause of

- Abdominal pain

- Nausea

- Vomiting

- Swallowing difficulties

- gastric reflux

- Unexplanied weight loss

- Anaemia

- Bleeding in the upper GI tract uses of upper endoscopy

Uses of upper endocopy:

Upper GI endoscopy can be used to remove stuck objects, including food, and to treat conditions food, and to treat conditions such as bleeding ulcers. It can also be used to biopsy tissue in the upper GI tract. During a biopsy, a small piece of tissue is removed for later examination with a microscope.

How to prepare for upper GI endoscopy?

The upper GI tract must be empty before upper GI endoscopy. Generally, no eating or drinking is allowed for 4 to 8 hours before the procedure. Smoking and chewing gum are also prohibited during this time.

- Patients should tell their doctor about all health conditions they have __ especially heart and lung problems, diabetes, and allergies ___ and all medications they are taking. Patients may be asked to temporarily stop taking medications that affect blood clotting or interact with sedatives, which are often given during upper GI endoscopy.

Medications and vitamins that may be restricted before and after upper GI endoscopy include:

- Non steroidal anti-inflammatory drugs such as aspirin, Iburpfen, and naproxen.

- Blood thinners

- Diabetes medications

- Antidepressants

- Dietary supplements.

Driving is not permitted for 12 to 24 hours after upper GI endoscopy to allow sedatives time to completely wear off. Before the appointment, patients should make plans for a ride home.

How is upper GI endoscopy performed?

- Upper GI endoscopy is conducted at a hospital or outpatient centre.

Patients may receive a local, liquid anesthetic that is gargled or sprayed on the back of the throat. The anesthetic numbs the throat and calms the gag reflex. An intravenous (IV) needle is placed in a vein in the arm if a sedative will be given. Sedatives help patients stay relaxed and comfortable while patients are sedated, the doctor and medical staff monitor vital signs.

- During the procedure, patients lie on their back or side on an examination table. An endoscope is carefully fed down the esophagus and into the stomach and duodenum. A small camera mounted on the endoscope transits a video image to a

devio monitor, allowing close examination of the intestinal liming. Air is pumped through the endoscope to inflate the stomach and duodenum, making them easier to see. Special tools that slide through the endoscope allow the doctor to perform biopsies, stop bleeding, and remove abnormal growths.

Recovery from upper GI endoscopy?

After upper GI endoscopy, patients are moved to a recovery room where they wait about on hour for the sedative to wear off. During this time, patients may feel bloated or mauseated. They may also have a sore throat, which can stay for a day or two. Patients will likely feel tired and should plan to rest for the remainder of the day. Unless otherwise directed, patients may immediately resume their normal diet and medications.

Some results from upper GI endoscopy are available immediately after the procedure. The doctor will often share results with the patient after the sedative has worn off. Biopsy results are usually ready in a few days.

- What are the risks associated with upper GI endoscopy?

Risks associated with upper GI endoscopy include:

- Abnormal reaction to sedatives

- Bleeding from biopsy

- Accidental puncture of the upper GI tract

Patients who experience any of the following rare symptoms after upper GI endoscopy should contact their doctor immediately:

- Swallowing difficulties

- Throat, chest and abdominal pain that worsens

- Vomiting

- Bloody or very dark stool

- Fever

3.3. Prognostic factors of Carcinoma of Stomach and classification of gastric carcinoma

3.3.1. Prognostic factors of carcinoma of Stomach:

The overall prognosis of gastric cancer is poor, with an average of only 10% to 15% 5 year survival, even in Patients who receive a "Curative" resection. Adverse prognostic factors include: age older than 70 years, tumour location (Proximal is worse than distal), venous/lymphatic invasion, Carcino-embryonic antigen (CEA) more than 10 ng/ml and CA 19-9 more than 37 g/ml (Taki and Kuwabara 1981). Independent prognostic significance has not been demonstrated for gross tumour configuration and tumour size. Survival is highest in individuals with intestinal type Carcinoma because those with these tumors generally present earlier with less advanced disease. When matched stage for stage, there is no difference in Survival between the tumor types (Hattori 1986). There modes of tumor spread may occur; through lymphatics, through the bloodstream, or though the transperitoneal route. Lymphatic metastases to lymph nodes along the greater and lesser curvatures are present in more than 70% of resection specimens. Later, there is spread to porta hepatis and para aortic nodes.

Occasionally, there may be early spread to the thoracic duct, to a left supraclavicular node (The node of Virchow). Spread through the blood stream results initially in liver metastases. Later, the lungs and other distant sites are affected. Spread transperitoneally may involve any intra-abdominal site, but particularly the pelvis, the ovary being a favored location.

Krukenberg tumour is ovarian metastases of a signet-ring Carcinoma, which in most cases originates in the stomach. The most powerful determinant of prognosis is the pathologic stage. The TNM system (Hall *et al.* 1986) is widely used and it is recommended that staging data be included in all surgical pathology reports of resection specimen. In the American joint committee on Cancer's 6^{th} editions, published in 2002, a number of changes have been introduced to the TNM staging. The tumor grade has been added to enable coding for well differentiated (grade 1), moderately differentiated (grade 2), and poorly differentiated (Grade 3) neoplasm. The T1 and T2 lesions have 60^{th} been subdivided in (a) and (b) to allow for a more precise coding of depth of invasion.

Lymphatic invasion is coded as LO-no invasion, or L1- invasion present. Venous invasion is coded as Vo-No invasion.

V1-microscopic invasion, or V2-macroscopic invasion. Micrometastases in lymphnodes are defined as collections of cells no greater than 0.2 mm in greatest dimension. If micrometastases are present as a isolated finding the N stage remains zero "0" but the designation (IT) is added [i.e PNO (IT)]. Similarly, if metastatic spread is detected by molecular methods, the designation PNO (molt) can be recorded.

DNA Ploidy studies now appear to be of limited value in the diagnosis and assessment of gastric Cancer (Taki and Kuwabara 1981).

Overall 50% to 75%of tumors are aneuploid, although early Carcinomas may be diploid. Tumours of the gastric Cardia and distally located intestinal Carcinomas are more likely to be aneuploid than are diffuse Carcinomas. Anauploidy also has been reported in gastric dysplasia and gastritis.

3.3.2. Carcinoma of Stomach:

Classification and Histopathology:

The vast majority of gastric cancers are adeno carcinomas.

There is no entirely satisfactory histologic sub classification of gastric Carcinoma. The world health organization (WHO) classification lists adenocarcinoma (Intestinal and diffuse types), Papillary, tubular, mucinous (greater than 50% mucinous component), Signet-ring (greater than 50% signet component), adenosquamous, squamous, small cell, undifferentiated, and others. Adenocarcinoma may be graded into well differentiated (greater than 95% of tumour composed of glands), moderately differentiated (50%-95% composed of glands), and poorly differentiated (49% or less composed of glands). This system is simple and reproducible, but may be difficult to apply because of morphologic heterogeneity present in many neoplasms.

The classification system developed by Carneiro and colleagues recognized four histologic types of adenocarcinoma; glandular, solid, isolated, and mixed cell.

Mixed cell carcinomas account for 39% of all tumours and carry a significantly worse prognosis.

The classification system of Lauren (1965) who recognized three histologic types – intestinal, diffuse, and mixed – is now the most widely used. However, these types should be further subdivided by location, into those occurring at the Cardia and those present in the remainder of the stomach.

- Neoplasms that straddle the gastro-esophageal junction may have arisen either in the Cardia or in Barrett esophagus. By convention, if more than 50% involves the stomach it is classified as gastric. If the tumour is located equally above and below the anatomic gastro esophageal junction, it is designated as junctional. Intestinal adenocarcinoma of the stomach closely resembles a colon cancer. Grossly, it tends to be nodular, polypoid, or ulcerated and is well demarcated. Histologically, it is characterized predominantly by a well formed glandular pattern, which may have solid, or papillary areas. The individual cells are columner or cuboidal, with a basally located nucleus. Cells with intracytoplasmic mucin are uncommon, although moderate quantities of mucin are present within gland lumens.

It contrast, diffuse carcinoma is more likely to have a plaque like surface component and an ill-defined , widely infiltrating growth, which is composed of individual cells or small groups and cords of cells. Between these is a fibrous or mucoid stroma. Many cells of diffuse – carcinoma contain mucin droplets, sometimes producing a signet-ring configuration. Most cases of linitis plastica (lather bottle stomach) will be classified as diffuse carcinomas. Mucinous carcinoma can be intestinal or diffuse, depending on their gross and microscopic

configuration. As with all schemes, Lauren's classification is not perfect; about 16% of cases will be unclassifiable or of mixed type. Some authors have tended to equate the terms intestinal and diffuse with well differentiated and poorly differentiated. However, this is misleading, as some poorly differentiated carcinomas may be sharply circumscribed and intestinal in type. Diffuse carcinoma is usually poorly differentiated with regard to gland formation, but some may have low grade nuclear features.

Medullary carcinoma of the stomach has been defined as one in which more than 50% of the tumour consists of poorly differentiated adenocarcinoma with no fibers stroma. These neoplasms tend to occur in the proximal one-third of the stomach, have a pushing margin; and show frequent lymphatic invasion with hepatic metastases (Stacey 2004).

Table 1. TNM Staging of gastric Carcinoma.

T 1s	----------	Carcinoma in situ
T1a	----------	Tumor invades lamina propria
T1b	----------	Tumor invades submucosa
T2a	----------	Tumorinvades muscularis propria
T2b	----------	Tumor invades subserosa
T3	----------	Tumor penetrates visceral peritoneum

Table 1. (contd.) TNM Staging of gastric Carcinoma.

T4	----------	Tumor invades adjacent structures
No	----------	No regional nodes involved
N1	----------	Tumor involves 1 to 6 regional nodes
N2	----------	Tumor involves 7 to 15 regional nodes
Mo	----------	No distant metastases
M1	----------	Distant metastases present

Source: American joint committee on cancer. AJCC Cancer staging manual, 6th ed. 2002

3.4. Gross appearance, Biopsy, and cytologic diagnosis:

Intestinal carcinomas may show a variety of gross appearances and can be described as superficial, polypoid, fungating, or ulcerated. Diffuse cancers are generally infiltrative in type. These appearances carry no prognostic significance independent of stage. Ulcerated Carcinomas may be distinguished from gastric peptic ulcer by being bigger, more irregular, and having a heaped-up or rolled edge. Any endoscopically suggestive lesion in the stomach should have multiple biopsies taken for full pathologic evaluation. Eight is not an unreasonable number of biopsies, and these should be taken from the edge of an ulcer, than the base; otherwise, only necrotic debris may be obtained.

Special problems may arise in some cases of diffuse carcinoma, because occasionally, the intra-mucosal component may be small in comparison to an

extensive sub-mucosal and mural involvement. In this clinical situation, the biopsies must be examined carefully, as commonly the only tumour present is in the form of inconspicuous "histiocyte" like cells in the lamina propria or superficial sub-mucosa. On closer examination, the "histiocytes" turn out to be tumour cells, often with a signet-ring appearance. Signet-ring cells may have nuclei that, on first examination, appear deceptively bland. If a diagnostic problem is encountered, it is recommended that, first, the presence of cytoplasmic mucus be confirmed by an Alcian blue with PAS stain, and second, that the nuclei of the signet-ring cells be compared with the nuclei of typical lamina, propria histiocytes. The nuclei of signet ring cells will be much larger. If doubts persist, an immuno stain for pan keratin or CEA will serve to distinguish histocytes from epithelial cancer cells.

With adequate biopsy material, the diagnostic accuracy of gastric biopsies performed for a suspicion of cancer is 83%. Brush cytology of these lesions, when used by itself, is 85% accurate; however, when used in conjunction with biopsy a combined accuracy of 96% is achieved (Schafer *et al.*1985, Clarkson and West 1993). Normal gastric surface epithelial cells appear in clumps in brush cytology specimens. The clumps are regular with a honey comb appearance, and the individual cells have rounded nuclei containing an even distribution of chromatin.

Occasional individual cells can be seen to be columnar (tapered at one end, squat at the other). The cytology of inflamed gastric mucosa is similar, but the cells become more cuboidal and have reduced amount of cytoplasm. They also have

mildly enlarged nuclei, with prominent single ordouble nucleoli. Cells showing intestinal metaplasia can be confused with signet-ring cancer. They have a globle of cytoplasmic mucus displacing (and sometimes indenting) the nucleus, but the nuclei themselves are bland and show inflammatory features only. Carcinoma cells are usually present singly or in small irregular groups. They are large with distorted hyperchromatic nuclei that contain multiple or giant nucleoli.

Immuno-histochemical stains show that overall, over 95% of gastric adeno-carcinomas are positive for AE1/AE3, pan keratin, and CAM 5.2, 60% to 90% are positive for epithelial membrane antigen, CK 20, CA 19-9, CK7 and polyclonal CEA, 40% are positive for P^{53}, less than 25% are positive for bcl-2, S100, and prostate specific antigen (PSA), and close to 0% are positive for chromogranin, synaptophysin, and melan A.

3.5. Gastric Carcinoma with lymphoid stroma:

Attention has been drawn to a subset Carcinomas that are poorly differentiated and have a prominent lymphocytic stromal infiltrate (Rosai 1996). These tumours are more common in men that in women and may have a better prognosis than the usual type of gastric cancer. Many tumors bear a striking resemblance to the so called lymphoepithelimoa of the upper respiratory tract. In 75% to 85%of cases Epstein-Barr virus (EBV) RNA can be demonstrated by *in situ* hybridization techniques. However, at the present time it is not clear what role, if any, the virus has in Carcinogenesis (Rosai 1996).

3.6. Pathogenesis of gastric carcinoma:

In possible pathogenic path way, proteins involved with cell cycle or apoptosis process emerge as a candidate for having a crucial development of neoplasms. Some of these, like c-Myc,p53 and the apoptotic family members such as Bcl-2 deserve attention for playing a key function in the cell proliferation and the cell fate, besides they already involvement in the tumorigenesis process in a variety of tumours, including gastric cancer (Bertram 2000, Pelengaris *et al.* 2002,Calcagno *et al.* 2006).

H. pylori infection alone, presented Bcl-2 expression. Although some authors have shown an increased Bcl-2 expression in *H. pylori* – positive cases, in the group (Hp+/EBV-) the Bcl-2 expression was very low (Konturek *et al.* 1999, Jorge *et al.* 2003, Choi *et al.* 2003,Yang *et al*. 2003, Zhan *et al.* 2003).

Although it was not statistically significant, studies suggest a possible correlation between *H. pylori* and Bcl-2 expression, unlike with EBV. A few *in vitro* studies have shown that EBV facilitates the tumor development by inducing the expression of the c-Myc protein (Niller *et al.* 2004,Yang *et al.*2004). In a study, the expression of c-Myc was higher in the groups without EBV infection indicating a relationship of c-Myc gastric carcinogenesis but not with EBV infection (Nardone *et al.* 2001). Additionally, some studies point to the involvement of *H. pylori* with c-Myc (Pelengaris *et al.* 2002, Calcagno *et al.* 2006). The biological significance between *H. pylori* and c-Myc is still not understood. A study from Zang *et al.* (2004) has linked the c-Myc expression with cell proliferation while a study from Yang *et al.* (Bertram 2000) on a gastric

carcinoma cell line has pointed to a relationship with apoptosis. On the other hand, Chapel *et al.* (2000) have shown a decrease of c-Myc expression after *H. pylori* eradication, but not in its mRNA level, showing the complexity of this process. Therefore the c-Myc expression may be influenced by the presence of *H. pylori*, but it is also triggered in a *H. pylori* independent way. Nevertheless, it seems that the presence of the EBV may also play a Role since the frequency of c-Myc protein was the lowest the groups and this may be a disturbing factor when its biologic significance is considered.

The mutation of the p53 gene is among the major alteration of the multi-step process of gastric carcinogenesis, while it has also been reported in pre-malignant lesions of the stomach, such as chronic gastritis, intestinal metaplasia and dyspepsia. Kodama *et al.* (2007) have suggested an accumulation of wild-type p53, especially in the *H. pylori* infected mucosa probably due to the *H. pylori*-induced DNA damage. The present study shows the highest frequency of p53mutation in the groups with *H. pylori* infection, especially among the cagA+ cases, corroborating previous studies (Shibata *et al.* 2002, Brown *et al.* 2002, Parkin *et al.* 2004). The high percentage of p53 mutation in EBV-associated and EBV – negative gastric carcinomas was observed in the study which was also demonstrated in other studies, demonstrating that the p53 mutation is a relevant alteration in gastric carcinogenesis independent of the infection (Szkaradkiewicz *et al.* 2006, Shibata *et al.* 2002). Finally, the study showed the high prevalence of *H. pylori* infection in gastric carcinomas in Ceara state. The groups indicate that there is a different pathway according to the presence of infectious agents, with female

being predominant in the group without infection. *H. pylorus seems* to influence the expression of Bcl-2, since this protein was observed only in the group infected exclusively by this microorganism. The Bax and c-Myc expression was present in all groups. However, the highest expression in the EBV-negative. It was suggested that EBV may inhibit their expression. The mutation of the p53 groups possibly indicating that it was not only a consequence of the infectious agent.

3.7. EUS-Guided FNA of Intramural (Sub mucosal) GI Lesions:

A sub mucosal lesion of the GI tract is a bulge within the GI lumen that is covered by normal overlying mucosa. Such a lesion can be due to extrinsic compression from an adjacent organ (e.g., splenic artery) or a lesion (e.g., pancreatic pseudocyst) or an intramural GI lesion arising from the layers of the GI tract (e.g., muscularis mucosa, submucosa, muscularis propria) deeper to the superficial mucosa.

Endosonography is the most accurate imaging modality to differentiate intramural GI lesions from extrinsic compression of the GI tract. Although some lesions such as lipomas (echogenic lesions arising from the sub-mucosa) have characteristic endosonography features, and a GIST may be likely when a hypoechoic lesion arising form the muscularis propria is seen, there is some overlap and uncertainly surrounding the histological diagnosis of many lesions (Rosch *et al.*1991, Rosch *et al.*1995, Boyce *et al.*1991, Caletti *et al.*1989, Yasuda *et al.* 1989,Yoshikane *et al.*1993). Most sub-mucosal masses create a visible bulge during endoscopy, and a

transendoscopic FNA may further assist in the etiological diagnosis of these lesions (Benya *et al.*1993, Wegener *et al.*1995).

Developments in real-time EUS-guided FNA allows cytological diagnosis of these abnormalities with greater precision (ensuring that the needle is within the lesion rather than superficial to it) and perhaps improved safety (by avoiding a large submucosal vessel) (Haranda *et al.* 1996).

The sensitivity of EUS-guided FNA cytology for sub-mucosal tumors in some initial studies was 60%-64%. The diagnostic role of aspiration cytology can be enhanced by performing cytology can be enhanced by performing immuno-histochemical stains on aspirates for tumor-specific makers such as CD 117 and CD 34, which are crucial for arriving at a conclusive diagnosis of sub-mucosal stromal neolasms known to have at least a malignant potential such as GIST (Li *et al.* 2001, Stelow *et al.* 2003) with diagnostic accuracies as high as 91% and 100% having been reported for EUS-guided FNA.

However, the differentiation between a benign and malignant sub-mucosal tumor may often be difficult via cytopathology alone. Alternatively, the development of larger-bore needles and devices that provide a thicker tissue core and a histological rather than a cytological diagnosis may help increase the diagnostic accuracy of EUS-guided FNA in sub-mucosal lesion (Caletti *et al.*1991, Haranda *et al.*1996).

If a small sub-mucosal lesion (<1-15 cm) is encountered, another approach in these cases is to perform endosonography. If the lesion is limited to the deep mucosa or the sub-mucosa (the first three endosonography layers), a snare excision of the

entire lesion can be performed after injection of Saline and subsequent elevation of the lesion, providing a diagnosis as well as removal of the mass. Economic analysis of patients with unclear GI wall compression on endoscopy (sub-mucosal lesions) reveals that endosonography is clearly more cost-effective than CT as the next diagnostic method (Allgayer 1995).

CHAPTER-4

MATERIALS AND METHODS

4.0. MATERIALS AND METHODS

4.1. Type, place and period of study:

The present study was a prospective one carried out in the Department of Pathology, Khulna Medical College, Khulna, Bangladesh from September 2008 to August 2011. This study was carried out in different Private Clinics in Khulna City, Bangladesh with the collaboration of the department of Pathology in Khulna Medical College, Khulna, Bangladesh. The aim of the study was to find out an efficient method to diagnose gastric carcinoma and compare the efficacy of diagnostic methods including endoscopic imprint cytology and biopsy.

4.2. Study population:

A total of 274 cases were included in this study on the basis of clinical signs and symptoms. Among 274 cases, 01case was gastric polyp and another 01 case GIST (Gastrointestinal stromal tumour). After deducting 02 cases, 272 cases were included in this study

4.3. Data recording:

All necessary and relevant data regarding patients will be recorded methodically and meticulously as far as possible in a data sheet. All cases will be numbered chronologically. Data sheets and same numbers will be used for slides of cytology & histopathology.

4.4. Collection of specimen:

Patients attending in different specialized private clinic in Khulna City, Bangladesh especially Khulna Surgical and Medical Hospital, Khulna who was suspected cases of gastric carcinoma by clinical presentation, included the study. Endoscopic biopsy was carried out in all patients and biopsy material sent to the laboratory immediately with normal saline. A smear was prepared immediately from the specimen and rest of the specimen was kept in formalin for histopathological examination.

4.5. Staining:

Imprint and biopsy material was stained by H & E staining and Papanicoloauo's (PAP) stain.

4.6. Examination of smears:

Cytopathological and histopathological examination was done in the department of Pathology by expert Cyto and Histopathologist.

CHAPTER-5

RESULTS AND OBSERVATIONS

5.0. OBSERVATIONS AND RESULTS

The present study was a prospective one carried out in the Department of Pathology, Khulna Medical College, Khulna, Bangladesh from September 2008 to August 2011. A total of 274 cases were included in this study on the basis of clinical signs and symptoms. Among 274 cases, 01case was gastric polyp and another 01 case GIST (Gastrointestinal stromal tumour). After deducting 02 cases, 272 cases were included in this study.

5.1. Case distribution of the study:

Endoscopy was done in every cases and biopsy was taken for imprint and smear cytology and histopathological examination. As shown in Table 2, on endoscopic examination, it was found that 189 cases were related to gastric lesion while 83 cases were related to esophageal lesion.

Table 2. Case distribution in 272 cases (n = 272).

	Total cases	**Percentages**
Esophageal lesion	83	30.51
Gastric lesion	189	69.48

Among 83 cases esophageal carcinoma were detected in 65 patents and 18 cases were of esophagitis. And again among 65 cases of esophageal carcinoma 44 subjects were male and 21 were female. The Male, Female ratio was calculated to be 2.09:1

5.2. Sex-wise distribution of esophageal carcinoma of the study population:

The sex-wise distribution of sixty five (65) cases of esophageal carcinoma of the present study has been presented in Table 3.

Table 3. Sex-wise distribution of esophageal carcinoma of the study population (n = 65).

Sex	Total cases	Percentages
Male	44	67.69%
Female	21	32.30%

5.3. The age-wise and sex-wise distribution of Esophageal Carcinoma of the study population

The age-wise and sex-wise distribution of Esophageal Carcinoma of the current study has been shown in Table 4. As shown in the Table, among total 65 cases of Esophageal Carcinoma, most of the patients were in between 50 to 79 years of age, with a peak age of 60-69 years.

Table 4. The age distribution of Esophageal Carcinoma (n= 65).

	20-29		30-39		40-49		50-59		60-69		70-79		80-89		Total	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
M	02	3.07	02	3.07	05	7.69	11	16.92	11	16.92	09	13.84	04	6.15	44	71.82
F	0	0	01	1.53	03	4.61	04	6.15	07	10.76	04	6.15	01	1.53	21	24.12
Grand Total = 65 cases																

5.4. The age-wise and sex-wise distribution of esophagitis of the study population

The age and sex-wise distribution of esophagitis of the present study has been represented in Table 5. Out of 18 cases of esophagitis, 13 cases were in male and 05 cases female making the male, female ratio 2.6:1.

Table 5. The age and sex distribution of 18 cases of Esophagitis (n = 18).

	20-29		30-39		40-49		50-59		60-69		70-79		80-89	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
M	0	0	0	0	02	11.11	01	5.55	04	22.22	06	33.33	13	72.21
F	0	0	0	0	02	11.11	0	0	0	0	03	16.66	05	27.77
Total	0	0	0	0	04	22.22	01	5.55	04	22.22	08	49.99	18	99.98

5.5. The age-wise and sex-wise distribution of gastritis of the study

The sex–wise distribution of 65 case of gastritis of the current study has been demonstrated in Table 6. As shown in Table, Among 189 cases of gastric lesion, 65 cases were gastritis. Of the 65 cases, 38 cases were detected in male and 27 in female and the male, female ratio was 1.40:1 (Table 6).

Table 6. The male - female distribution of 65 cases of gastritis patients (n = 65).

	Total cases	**Percentages**
Male	38	58.46
Female	27	41.53

The age and sex-wise distribution of gastritis:

The age and sex-wise distribution of gastritis of the current study has been represented in Table 7. Among 65 cases of gastritis, most of the cases lies between 40-79 years with peak 60-69 years.

Table 7. Age and sex wise distribution of gastritis (n = 65 cases).

	20-29		30-39		40-49		50-59		60-69		70-79		80-89	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
M	01	1.53	01	1.53	09	13.84	12	18.46	08	12.30	07	10.76	0	0
F	04	6.15	02	3.06	07	10.76	04	6.15	03	4.61	06	9.23	01	1.53
Total	5	7.68	03	4.59	16	24.60	16	24.61	11	16.91	13	19.99	01	1.53

Of the study population, out of 189 gastric cases, 124 were diagnosed as gastric carcinoma, of which 90 were male and 34 cases were female. And the male and female ratio was 2.64:1.

The sex-wise incidence of gastric carcinoma of the study has been shown in Table 8. Among 124 cases of gastric carcinoma, most of the cases lied in between 40-79 years of ages both in male and females with peak in 60-69 years of ages (Table 9.)

Table 8. Sex-wise incidence of gastric Carcinoma (n = 124).

	Total cases	**Percentages**
Male	90	72.58%
Female	34	27.41%

Table 9. The age-wise prevalence of 124 cases of gastric carcinoma

	20-29		30-39		40-49		50-59		60-69		70-79		80-89		Total	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
M	05	4.03	02	1.61	18	14.51	13	10.48	29	23.38	17	13.70	06	4.83	90	72.90
F	01	0.80	04	3.22	09	7.25	07	5.64	08	6.45	04	3.22	01	0.80	34	27.38
Grand Total 124 100																

5.6. Results of Imprint cytology of gastric tissue:

Among 189 cases of gastric lesion, imprint cytology confirmed the presence of carcinoma of stomach in 124 (65.60%) patients and gastritis (non carcinoma) in 65 cases (34.39%) as shown in Table 10. The presence or absence of carcinoma in gastric tissue smear has been shown Fig. 2 (colour plate) and Fig 3, respectively. The normal endoscopic view of gastric tissue has been shown in Fig. 4 (colour plate).The imprint cytology of gastric tissue showing carcinoma has been presented in Fig.5.

Table. 10. Gastric smear Cytology in 189 cases.

Cytological diagnosis	Number	Percentage
Carcinoma	124	65.60%
Gastritis	65	34.39%

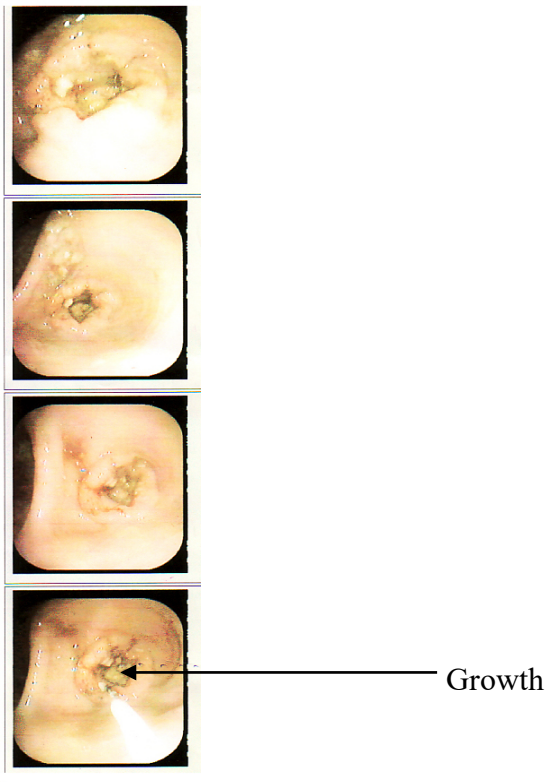

Fig.2. Endoscopic finding's of Stomach: Growth at Pyloric antrum (Presence of Cancer).

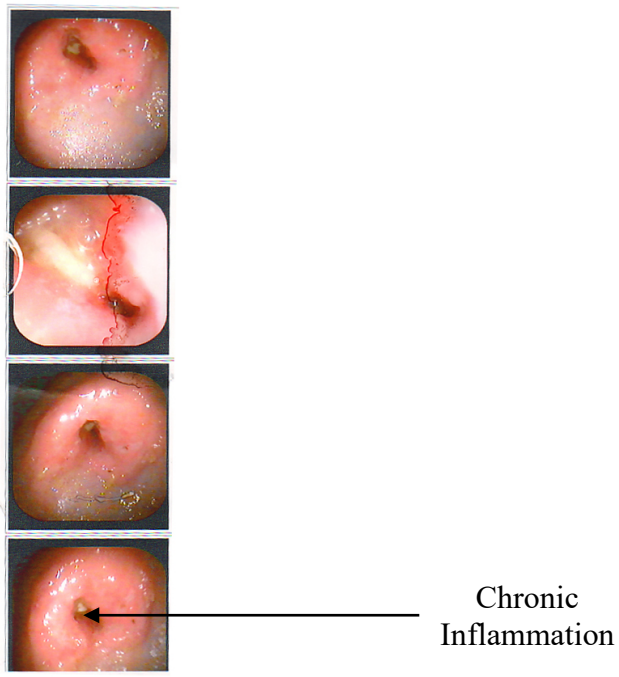

Fig. 3. Endoscopic finding's of Stomach: Pyloric Stenosis due to chronic Inflammation (absence of cancer).

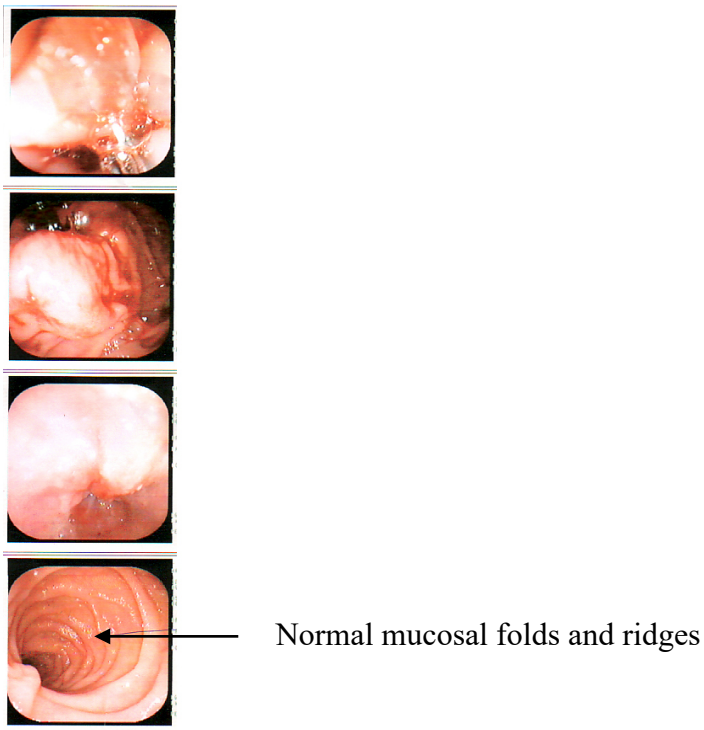

Fig.4. Normal Endoscopic finding's of Stomach.

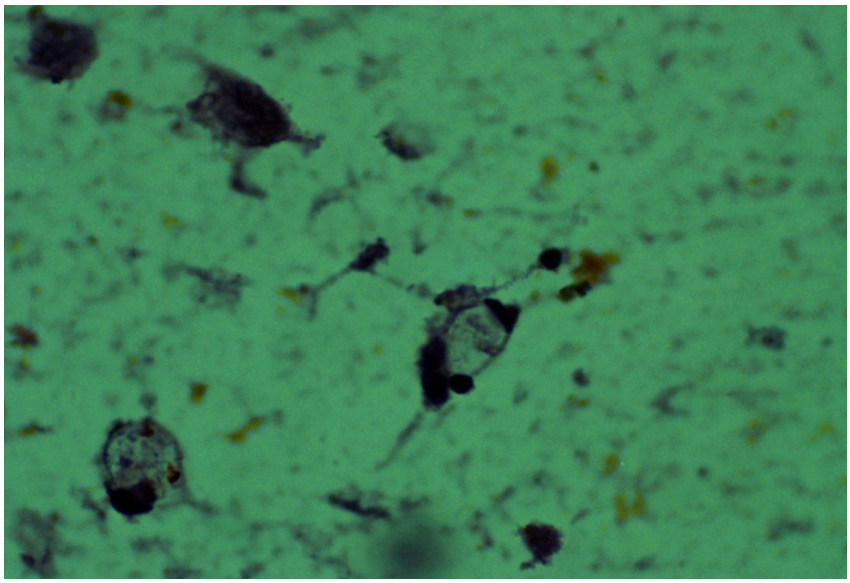

Fig.5. Imprint cytology of endoscopic biopsy showing adeno-carcinoma of stomach (Case no. 4).

5.7. Results of Histopathology of gastric tissue:

One hundred and eighty nine (189) cases of gastric tissue histopathology confirmed 122 cases (64.55%) of gastric carcinoma and 67 (35.44%) cases of gastritis (non carcinoma). The findings are shown in Table 11 and Fig.6. (Color plates)

Table 11. Histopathology of gastric tissue.

Histopathological diagnosis	Number	Percentage
Carcinoma	122	64.55%
Gastritis	67	35.44%

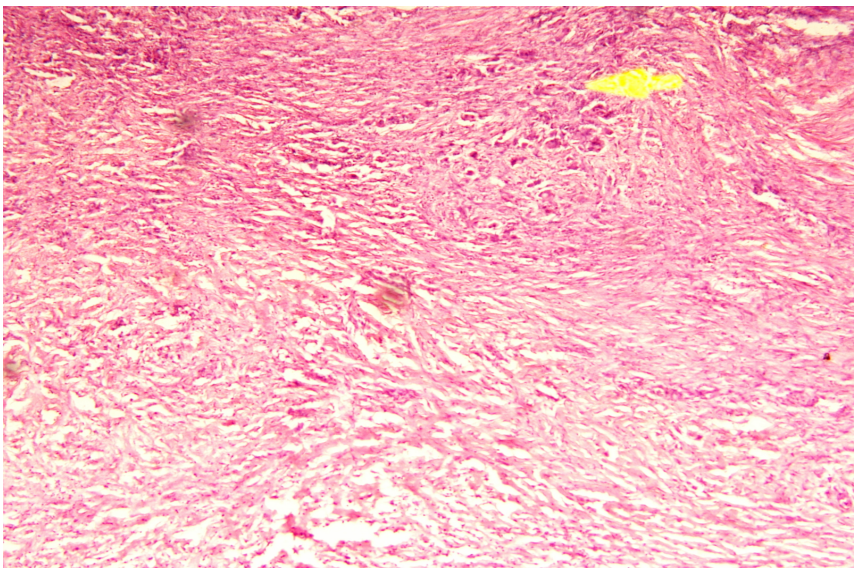

Fig. 6. Histopathology of endoscopic gastric biopsy. Indicator (yellow) indicating adenocarcinoma (case no. 4).

5.8. Comparison of Cytology and histopathology of suspected cases of gastric Carcinoma:

The results of the comparison of cytology and histopathology of 183 suspected cases of gastric carcinoma of the present study showed that by imprint cytology 124 cases of gastric carcinoma were detected (Table 12). Whereas, histopathology confirmed presence of gastric carcinoma in 122 patients. Two cases of gastric carcinoma could not be detected by histopathology due to inadequate sampling of endoscopic biopsy.

The presence of carcinoma in gastric tissue both by imprint cytology and histopathology has been shown Fig.7 and Fig. 8 (colour plate), respectively. Photograph of Imprint cytology of endoscopic gastric biopsy showing normal gastric tissue has been shown in Fig. 9. Photograph of histopathologyof endoscopic gastric biopsy showing normal gastric tissue has been shown in Fig 10.

Table 12. Comparison of cytology and histopathology of gastric tissue to detect gastric carcinoma (n=183).

	Gastric tissue smear/imprint Cytology		Gastric tissue histopathology	
	N	%	N	%
Carcinoma	124	65.60%	122	64.55%
Non-Carcinoma	59	34.39%	61	35.44%

From the above observation it may be concluded that if all the two methods along with endoscopic findings are applied we can diagnose gastric carcinoma in more cases confidently.

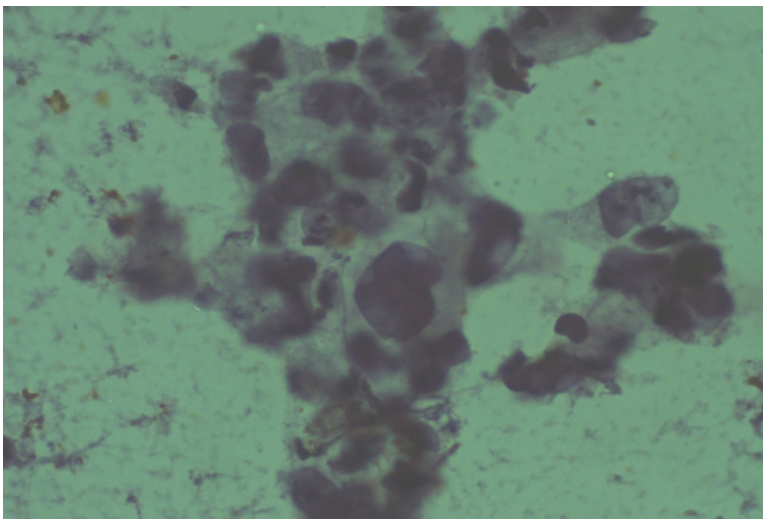

Fig.7. Photographic Imprint cytology of endoscopic gastric biopsy showing clusters and singly arranged malignant cell (Case No.18).

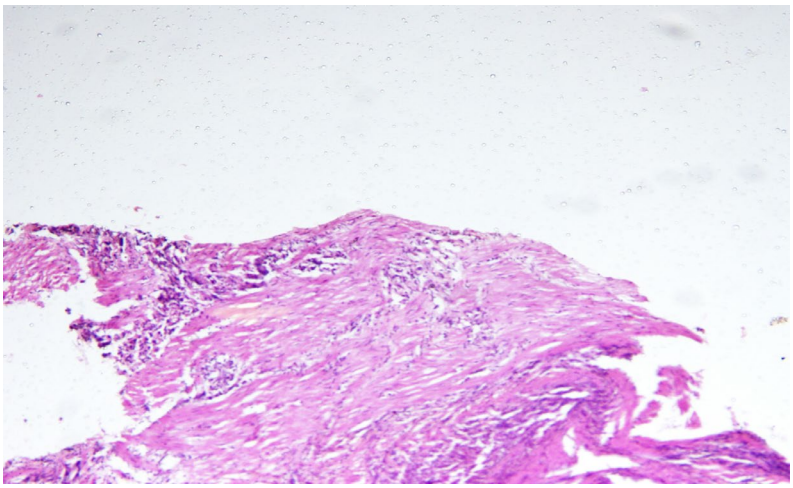

Fig. 8. Photograph showing Histopathology of endoscopic gastric biopsy showing adeno-carcinoma of stomach (Case no. 18).

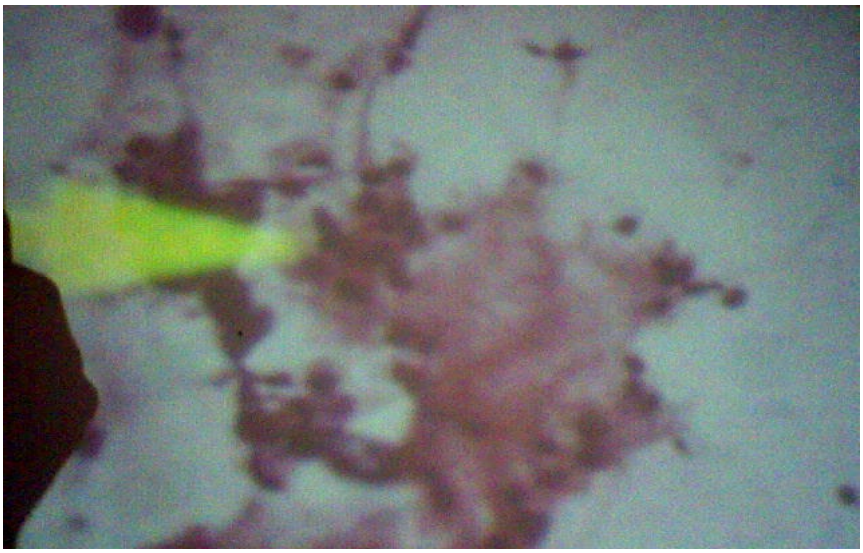

Fig. 9. Photograph of Imprint cytology of endoscopic gastric biopsy showing normal gastric tissue.

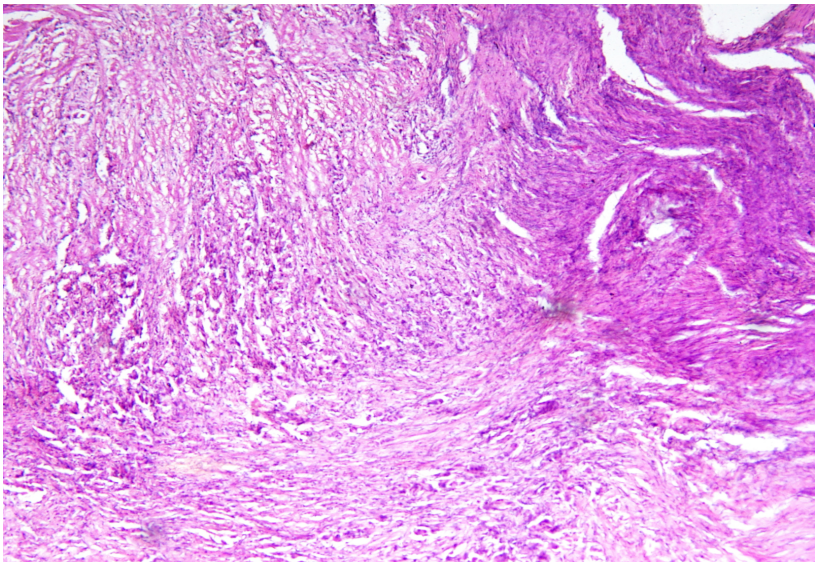

Fig. 10. Photograph of Histopathology of endoscopic gastric biopsy showing normal gastric tissue.

CHAPTER-6

DISCUSSION

6.0. DISCUSSION

The idea of making diagnosis of gastric cancer by detecting cancer cells or tissues from gastric cavity has already been reported since late in 19th century by Rosenbach (1882) and in the early 20th by Marini (1909). But the difficulties in identifying of free single cancer cell and in collecting the cancer tissue directly from inside the stomach rather provided further investigations into the field from making marked progress until around 1940.

Even since the cytological diagnostics of uterine cancer was established by Papanicolaou and Traut). Since 1963, manufacture of fiber gastroscope has made a rapid progress also in Japan by Machida Endoscope Co. Ltd and Olympus Optical Industry Co. Ltd

In 1964 Machida Endoscope Co. Ltd. further developed fiber gastroscope for biopsy under direct observation (type-B) and in 1965, fiber gastroscope for cytology by lavage under direct observation (type-k), which made it possible to collect tissues or cells from the lesion in the stomach.

From 1966, these instruments were devised to be able to make movement part of the distal part of the scope to raise up or lower down and also to rotate towards left and right with simple handle manipulation (New type –B, and -C) by Machida Endoscope Co. Ltd.

The aim of the present study was to find out whether gastric tissue imprint and/or smear cytology can aid in the diagnosis of gastric cancer and to compare it with conventional method of endoscopic gastric biopsy. This is the first study of its kind in Bangladesh. Two hundred seventy four cases were selected for the study.

As per the findings of Dupont *et al.* (1978) most patients of gastric cancer are over year 50 years of age. But cases in younger individuals and even children were reported by Tso *et al.* (1987).

In the present study, the age of the cases ranged from 20 years to 89 years, in which most of the cases were in between 40-70 years of ages both in male and females with peak in 60-69 years of ages. The above finding of ours shows good agreement with the above studies.

Any portion of the gastro intestinal tract may be affected by malignancy. The highest incidence of malignancy is in the oesophagus, stomach and colorectal regions. In fact esophagogastric and colorectal malignancies are almost the commonest cancers in humans.

Our study is related to lesion located in esophagus and stomach. Among 274 cases, oesophageal lesion is 85 cases (31.02%) and gastric lesion is 189 cases (68.97%).

Since early cancer is a microscopic cellular entity, the gastric smear is one microscopic cellular technique that can be used to advance in the recognition of incipient disease. In as much as the surface cells of a malignant lesion are practically the same in both early and advanced disease, gastric cytology may play an increasing role in the interception of early malignant disease (Frederick *et al.* 2008)

There are discrepancies in reports of the accuracy of the diagnosis of gastric Cancer by biopsy and brush or Imprint cytology. Biopsy has been used as a routine examination method for the diagnosis of gastric cancer, but its diagnostic accuracy

is not as high as it was considered previously. Many factors influence diagnostic accuracy. The positive rate of biopsy is low in Cancer of the gastric region and in infiltrative, ulcerative, and recurrent carcinomas. When the cancerous tissue is covered by normal mucosa or necrotic tissue, the biopsy may be too superficial. The anatomical location of the cancer may prevent multiple biopsies being taken. Stenosis of the cardiac or antral canal may also restrict access of the tumour. The distribution of the carcinomatous tissue may not be uniform and the scope for biopsy a quite limited. It is to be accepted that brush or imprint cytology examination would provide additional information on these circumstances (Shanghai gastro intestinal endoscopy co-operative group).

The cytological biopsy that is taken with the gastric tube is dependent upon the proper preservation of the individual cells being processed for study. Because diseased gastric cells have an inherent tendency to exfoliate, mechanical methods can be employed to collect the necessary abnormal cells from the stomach.

In this way, gastric cytology may be helpful in differentiating the benign from the malignant gastric lesion with a substantial degree of accuracy.

The single most accurate method was the biopsy touch/imprint smear. Furthermore, it was the only cytological technique which gave the correct malignant diagnosis. In the correct malignant diagnosis in two of these patients this increase in sensitivity due to the use of touch/imprint smears as a procedure complementary to biopsy is similar to the findings of Japanese cytologists (Jennifer and Helena 1980).

This techniques is especially useful in the examination of necrotic material, when malignancy can sometimes be recognized at a “cellular level” even though the specimen is inadequate for histologic diagnosis (Jennifer and Helena 1980)

Diagnostic accuracies for cytology ranging from 85.7% to 96.0% have been reported by Kasugai and Kobayashi (1974).

Using various methods is of endoscopic cell collection, (Prolla *et al.*1977) is achieved an overall accuracy of 84.7% in a series of 183 patients with cancer of the stomach and oesophagus with 82.3% accuracy in 69 cases of adenocarcinoma of the stomach (Jennifer and Helena 1980).

Since sensitivity may be defined as the ability of the test to give a positive finding when the individual screened has the discuses or abnormality under investigation 10 and specificity may be defined as “The ability of the test to give a negative finding when the individual does not have the disease or abnormality under investigation.

The results of the study showed that the accuracy achieved by the cytologic programme was higher than that of routine endoscopic histology.

This higher level of sensitivity was obtained without loss of specificity; as no false positive or “Suspicious” catology reports had been issued.

The superiority of cytology was demonstrated in both sections of the study, but the advantage was more marked in the “clinical” section (Jennifer and Helena 1980).

The knowledge of clinical findings and especially whether peptic ulceration was present or not- was found helpful in interpretation of cytology. Our study also showed that cytologic programmed is higher (65.60%) than that of routine

endoscopic histology (64.55%).Our clinical findings adjunct with gross endoscopic finding's also helped in interpretation of cytology and histopathological diagnosis.

The accuracy of cytology for the 124 gastric malignant cases in the complete trial was 65.60%, which was 1.05% higher than the histology results (Table 12 page 35 in Results chapter 5 of this dissertation).

From the above observation it can be concluded that if all the technique or methods are applied, maximum number of cases can be detected. It can be assumed that in early lesion, gastric cancer may not be detected by histopathological examination. So, Cytopathological analysis may be helpful for early detection of gastric carcinoma. Gastric tissue biopsy touch/imprint smear Cytology preparation is simple, easy, less cost effective, less time consuming and suitable for our country.

CHAPTER-7

CONCLUSION AND RECOMMENDATIONS

7.0. SUMMARY AND CONCLUSION

Cytopathology of gastro intestinal (GI) tract lesions can be used successfully to diagnose neoplastic and non neoplastic Conditions. Specially when combined with endoscopic finding's and endoscopic biopsies. Cytologic evaluation is widely accepted as a cost-effective method that allows rapid interpretation and triaging of material. Successful Cytologic examination of the GI tract is highly dependent on the Skill of the endoscopist, Specimen preparation, the expertise of the pathologist, and the recognition of the limitation of cytology.

From this study, we form same opinion as Prolla *et al.* (1977) that meticulous care in technique is needed. Adequate sampling and a variety of cell collection and processing methods are required to obtain optimal Sensitivity and specificity. Examination of the necessary specimens may sometimes be both time-consuming and difficult, but despite these reservations we feel that cytology is a valuable aid to the diagnosis of malignancy in the Stomach and duodenum and deserves to be more widely practiced as an adjunct to endoscopy (Jennifer A Young *et al.*,1980)

Further Investigations : Recommend :

To differentiate different type of Carcinoma of GIT cy Differentiation of GIST from other primary spindle Cell tumours has important therapeutic implications; and Immuno histochemical (CD $_{117}$, CD $_{34}$, Smooth muscle actin, muscle specific actin, S-100 protein) Stains are useful for the differential diagnosis.

CHAPTER-8

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8. REFERENCES

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CHAPTER-9

APPENDICES

9. APPENDICES

APPENDIX-I

9.1:1. TISSUE PROCESSING AND STAINING PROCEDURES

1. Fixation, Gross and Tissue processing

Specimens were collected and kept in 10% formalin for fixation. In the Department of Pathology, Khulna Medical College (KMC), Khulna, the specimens were meticulously examined and two to three or more representative blocks of 3-5 mm thickness were taken from the specimen and cell block specimen were embedded as whole in 10% formalin. Following overnight fixation in 10% formalin, the tissue blocks were gradually dehydrated in ascending concentration of ethyl alcohol. For Carnoy's fixation method, following overnight fixation in Carnoy's fixation solution, the tissue blocks were gradually dehydrated in absolute alcohol I and absolute alcohol II (It was followed in the Department of Pathology, KMC). Then the blocks were cleared in xylene, impregnated in paraffin and then embedded with proper orientation in melted paraffin. For making blocks and sectioning, the following steps were followed according to routine paraffin section method.

a. Fixation: In 10% formalin ..... overnight

b. Dehydration:

70% alcohol	1 hour
80% alcohol	1 hour
90% alcohol	1 hour
Absolute alcohol	1 hour
Absolute alcohol	1 hour or overnight
Acetone	30 minutes or 1 hour.

(For Carnoy's fixative method:

a. Fixation: In Carnoy's fixative ..... overnight

b. Dehydration:

Absolute alcohol I ..... 1 hour

Absolute alcohol II ..... 1 hour

C. Clearing: Xylene (or touline) I ..... 1 hour

Xylene (or touline) II ..... 1 hour

d. Paraffin impregnation: A paraffin bath with a temperature of 50^0-60^0C was used. Melting point of the paraffin was 58^0 to 62^0 C. The tissues were treated in paraffin for 1 hour or 2 hour.

e. Paraffin embedding: Metallic moulds were used for embedding the tissue in melted paraffin. The moulds were first lubricated with liquid paraffin, Melted paraffin was poured into it. The tissue was carefully embedded in proper plane at the bottom of the mould. The melted paraffin was then allowed to harden at room temperature properly to mount on a block holder. Then the blocks were kept in an ice chamber of a refrigerator for sometimes before cutting sections.

f. Microtomy or section cutting: Each block of tissue on the holder was fitted in the microtome machine. A properly sharpened microtome knife was used for cutting sections. A water bath with a temperature of 45^0C-50^0C was used for floatation of the sections. Sections were cut at 3-5 micron thickness. Ribbons of section were selected

and placed on the water bath. The sections were then taken on glass slides, which were previously smeared with egg albumin. The slides were kept in slanting position for sometime to drain off the excess water and allowed to dry at room temperature. They were then ready for staining.

2. Staining methods/procedure:

All the slides for histopathological examination were stained by routine haematoxylin and eosin method (H & E).

9.1.2. STEPS OF HAEMATOXYLIN AND EOSIN STAINING

a. Deparaffinization:

i) Slides were kept in paraffin oven at 60^0C for 15 minutes.

ii) Xylene I ..... 05 minutes

iii) Xylene II ..... 05 minutes

b. Rehydration:

i) Absolute alcohol I ..... 03 minutes

ii) Absolute alcohol II ..... 03 minutes

iii) 90% alcohol ..... 02 minutes

iv) 80% alcohol ..... 02 minutes

v) 70% alcohol ..... 02 minutes

vi) 50% alcohol ..... 02 minutes

vii) Running tap water ..... 8-10 minutes

c. Staining with haematoxylin and eosin:

i) Harris's haematoxylin ..... 02 minutes

ii) 1% acid alcohol for differentiation ..... 2-3 quick dips

iii) Running tap water for bluish until the sections become pale blue (or dipped in ammonium water the sections)

iv) Counterstain with 1% watery

solution of eosin ..... 2-3 minutes

d. Dehydration:

i) 50% alcohol ..... 02 minutes

ii) 70% alcohol ..... 02 minutes

iii) 80% alcohol ..... 02 minutes

iv) 90% alcohol ..... 02 minutes

v) Absolute alcohol I ..... 02 minutes

vi) Absolute alcohol II ..... 02 minutes

Then blotting and cleaning of the slides with cotton gauze.

e) Clearing: i) Xylene I ..... 05 minutes

ii) Xylene II ..... 05 minutes

f) Mounting: After clearing the slides were mounted with DPX using cover slip of variable sizes according to the number and total length of the fragment in a particular slide.

g) Results (Staining character):

Cell nuclei ..... Blue

Cytoplasm ..... Pink

Collagen fibers ..... Pink

Reticulin fibers ..... Pink

RBC ..... Bright red

WBC ..... Nuclei blue and cytoplasm vary with cell type

9.1:3. STAINING PROCEDURE OF SMEARS BY PAPANICOLAOU'S METHOD:

Appropriately identified with diamond marking pencil the smeared slides from the fixative were selected and following steps were followed:

1. Smeared slides kept in 95% ethyl alcohol ..... 30 minutes (at least)

2. 80% ethyl alcohol .....5dips (8-10 sec)

70% ethyl alcohol ..... 5dips (8-10 sec)

50% ethyl alcohol ..... 5dips (8-10 sec)

Distilled water ..... 5dips (8-10 sec)

3. Harris's haematoxylin ..... 6 minutes

   (without acetic acid)

4. Distilled water ..... 5dips (8-10 sec)

5. 0.5% aqueous solution of HCl ..... 3-5 dips

6. Running tap water ..... 8 minutes

7. 70% ethyl alcohol .....5dips (8-10 sec)

   80% ethyl alcohol .....5dips (8-10 sec)

   95% ethyl alcohol .....5dips (8-10 sec)

8. Orange G-6 (OG-6) ..... 1 minute

9. 95% ethyl alcohol .....5dips (8-10 sec)

   95% ethyl alcohol .....5dips (8-10 sec)

10. EA-65 ..... 1 minute

11. 95% ethyl alcohol .....5dips (8-10 sec)

95% ethyl alcohol ..... 5dips (8-10 sec)

Absolute alcohol ..... 5dips (8-10 sec)

Absolute alcohol ..... 5dips (8-10 sec)

12. Xylene ..... 5dips (8-10 sec)

Xylene ..... 5dips (8-10 sec)

Xylene ..... 5dips (8-10 sec)

13. Mounted in DPX using cover slip (22 x 22 mm)

14. Staining characteristics: Papanicolaou's stain gives sharp nuclear staining, transparency of cytoplasm and good differential coloring of acidophilic and basophilic cells. This method stains:

Nuclei ..... Blue

Acidophilic cells ..... Red to orange

Basophilic cells ..... Blue to green

Erythrocytes ..... Orange to red

9.1:4. PREPARATION OF STAINS AND CHEMICALS:

1. Haematoxylin and Eosin stains:

a) Harris's haematoxylin: Ingredients:

Haematoxylin crystals ..... 5.0 gm

95% alcohol .....50 ml

Ammonium or potassium alum 100gm

Distilled water ..... 950 ml

Mercuric oxide (red) ..... 2.5 gm

Glacial acetic acid ..... 40 ml

Procedure: Haematoxylin crystals were dissolved in 95% alcohol. Ammonium or potassium alum was dissolved in distilled water by heating. Two solutions were then mixed and brought to boil rapidly and continued for one minute with frequent stirring, then it was removed from flame and mercuric oxide was added slowly. It was then reheated until the solution became dark purple. The mixture was then allowed to cool rapidly in cold water. The stain was ready for use after cooling and kept at room temperature. Two to 4 ml of glacial acetic acid was added per 100 ml of solution to enhance precision of nuclear stain. It was filtered before use.

b) Acid alcohol: Ingredients:

Hydrochloric acid (pure) ..... 1 ml

70% ethyl alcohol ..... 99 ml

c) Eosin solution (stock): Ingredients:

Eosin (water soluble) powder ..... 1 gm

Distilled water ..... 100 ml

(This solution is called 1% Eosin stock solution)

d) Eosin solution (working)

Stock Eosin solution ..... 25 ml

70% alcohol ..... 75 ml

Procedure: Eosin was dissolved in distilled water and mixed thoroughly. It was stored at room temperature and filtered before use.

2. Papanicolaou's stain: Reagent preparation:

a) Harris's haematoxylin (only for Papanicolaou's stain)

Ingredients:

Haematoxylin (dark crystals) ..... 8 gm

95% ethyl alcohol ..... 80 ml

Aliminum ammonium sulfate ..... 160 ml

Distilled water ..... 1600 ml

Mercuric oxide ..... 6 gm

Procedure: Aluminum ammonium sulfate was dissolved in distilled water by heating. Haematoxylin crystals were dissolved in 95% ethyl alcohol and then mixed sulfate solution. This mixture was boiled to 95^0C. The flame was removed and the mercuric oxide was added slowly by stirring the mixture. Then the mixture was allowed to cool into a cold water bath. The mixture was then filtered. This is the stock Harris's haematoxylin solution. For use, the required amount of solution was diluted with an equal amount of distilled water and filtered again.

b) Orange G-6 (OG-6) solution: Ingredients:

Orange G crystals 10gm

Distilled water ..... 100 ml

95% ethyl alcohol ..... 1000 ml

Phosphotungstic acid ..... 0.15 gm

Procedure:

10% aqueous solution was peppered by dissolving 10 gm orange G in 100 l distilled water. The solution was shaken and allowed to stand for a week. This is stock solution no. 1. 50 ml of stock solution no 1 was made upto 1000 ml with 95% ethyl alcohol. This is stock solution no 2. Final solution for 1000 ml stain was prepared by adding 0.15 gm of phosphotungstic acid to stock solution No. 2 and thoroghly mixed. The solution was stored in dark brown stoppered bottles. It was filtered just before use.

c) Eosin-Azure 65 (EA-65): Ingredients:

Eosin Y ..... 10 gm

Bismarck brown Y ..... 10 gm

Light green SF (Yellow) ..... 10 gm

Distilled water ..... 300 ml

95% ethyl alcohol ..... 4 gm

Saturated lithium carbonate solution in distilled water .. 20 drops

Procedure: At first 10% solution of each of the stain was prepared separately by dissolving 10 gm of Eosin Y in 100 ml of distilled water, 10gm of Bismarck brown Y

in 100 ml of distilled water, and 10gm of light green SF yellowish in 100 ml of distilled water. Then 50 ml of Eosin Y stock solution No. 1, 10 ml of Bismarck brown Y stock solution No 1 and 9 ml of Light green SF stock solution No 1 were mixed. 95% ethyl alcohol was then mixed to make volume upto 2000 ml. Then 4 gm phosphotungstic acid and 20 drops of saturated lithium carbonate solution were added to the mixture and shaken. The solution was stored in dark brown stoppered bottle. In was filtered before use.

d) Aqueous solution of HCl.

Ingredient:

Hydrochloric acid ..... 0.5 ml

Distilled water ..... 99.5 ml

It was mixed thoroughly.

PROFORMA

Detection of gastric Carcinoma by endoscopic imprint and smear Cytology.

1. Identification: .....

2. Clinical Presentation:

i) Pain in abdomen.....	yes/no	Duration
ii) H/O vomiting.....	yes/no	Duration
iii) Difficulties in diglutation.....	yes/no	Duration
iv) Weight loss	yes/no	Duration

Other: For Ascities/ Enlarged lymph nodes/ other Swelling in the body.

3. Physical examination:

.....
.....

4. Routine laboratory examination: .....

5. Special investigations:

X-ray:
Ba-meal X-ray:
Ultrasonography:
Endoscopic examination with findings:

6. Clinical diagnosis:

7. Gastric tissue Cytopathology of endoscopic biopsy: (Imprint and Smear Cytology)

Specimen :
Lab No :
Date :
M/E :
Dx :

8. Histopathology (Gastric tissue):

Lab No :
Gross :
M/E :
Dx :

9. Follow up of the patient:

Date: **Signature of Prof.**