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CYTOMORPHOLOGY OF GASTROINTESTINAL CANCERS OF SOUTH INDIAN COHORT - CASE STUDIES REPORT

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ABSTRACT

The significant features of Adenocarcinomatous cancers of gastro colo-rectal regions include the presence of a cloud of peripheral glycoprotein viz., mucins, growth factors and expression of cell surface specific (CSSA) antigens. The appearance of a similar type of cancer in the different regions of gastro-intestinal- rectal continuum with differential cellular architecture in the present study infers the monotyphic cancer expansion driven by factors mentioned per se. The metabolic antero-posterior axis/gradient of the gastro-intestinal tract might have favored the transits of the shed CSS antigens, growth factors and the mucin expression in succession. Understanding of this antero-posterior gradient of cancer cells phenotypic expression is of diagnostic value in tumor therapy of colorectal cancers since ablation of the malignant potions and chemotherapy would not suffice, in case of promoting factors/ antigens dispersion from proximal region to the distal region of the digestive continuum. The present study on the histopathological findings of the gastro-intestinal cancers in the patients reveal that they have been subjected to numerous risk inducing factors before the manifestation. The epidemiology data reveal the following risk factors in these cancer patients namely, consanguineous marriage, tobacco chewing, smoking, pesticide usage, taking spicy pickle, alcohol, betel nut chewing, occupation, physical work nature, marital status, family history, eating habits, micro nutrients deficiency, co morbid conditions etc., Reducing the exposure to risk factors can prevent the development of gastro intestinal cancers. Cytomorphological tests and early assessment of cancer symptoms confer assured survival.

INTRODUCTION

The gastro-intestinal cancer profiles include the following types of pathological descriptions.

Peptic Ulcers

Peptic ulcers are caused by superficial erosion of the gastric mucosa due to breach of normal productive mechanisms afforded by the mucus and to rapid turnover of epithelial cells. This may be caused by low gastric acidity, back diffusion of H+ ion into the mucosa and by the augmenting effect of certain drugs like aspirin, corticosteroids and other cyto toxic drugs. Mucosal gastric erosion becomes an ulcer ultimately when it penetrates into the sub mucosa. Ulcers have been reported in various parts of elementary tract viz., stomach, duodenum, esophagus, meckel's diverticular or adjacent areas of ileum, jejunum.

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The various prognostic and causative factors presumed to cause the ulcer include heredity, smoking, stress, polygenic predisposition etc [1].

Acute Stomach Ulcer

This represents an early phase in its formation. The whole thickness of the mucosa in this becomes necrotic and the muscularis mucosae become breached. Nuclear debris is scattered throughout the necrotic tissue and some fragments of epithelium are visible [2].

Chronic Peptic (Stomach) Ulcer

In this the sub serosa becomes affected. The ulcer pus penetrated through almost the whole thickness of the stomach wall and its flow becomes necrotic. In the subserosa granulation of the tissues and connective tissue with plenty of implementary cells are seen. In this ulcer the mucous coat may be destroyed. Dense fibrous tissue and necrotic debris are seen in the floor. Healthy granulation tissue is present in the floor. Gastric epithelium is only hyper plastic but not malignant [3].

Primary Scirrous Carcinoma:

In this, the tumor shows a diffuse appearance. The region shows a scirrous plaque. The muscle fibres are separated by connective tissues throughout which remain scattered as small dark staining cells. These are undifferentiated carcinoma cells. Sometimes the malignant cells also can be identified by staining the mucin in the cytoplasm. The connective tissue represents here the tumor stroma which is extensive in appearance [4].

Primary Adeno Carcinoma of Stomach:

"Adeno" generally means a gland present in the epithelial layer. Hence cancer in the epithelial tissue especially in a gland tissue is called the Adeno carcinoma. This type of tumor formed a large ulcer extensively invaded by tumor cells which contain deeply basophilic mucin which may obscure the cell nuclei. The cells are large and pleomorphic and they form imperfect acini. In some the malignant cells are arranged in cords as irregular acini. These cells secrete mucin over which they remain afloat. The tumor cells have large basophilic nuclei. The serosal cells are unusually prominent. In the subserosa lie many mucin laden tumor cells. The neoplastic cells contain so much mucin in their cytoplasm that the nucleus has been pushed to the side to produce a "signet ring" shape. The cells are metastatic and can spread to peritoneal cavity and to liver [5].

Adeno Carcinoma of Stomach:

The malignant cells in adeno carcinoma are pleomorphic. The variation in shape and size being most evident in the nuclei and nuclear cytoplasmic ratio. The nuclei may be moderately hyper chromic or normo chromic or hypo chromic. Nucleoli often vary in size, and are eosinophilic in a hypo chromic nucleus. The cytoplasm is intensely basophilic and vascularisation is not a diagnostic feature but rarely a mucous vacuole may be seen [6].

Primary Gastric Lymphoma:

This type may show the symptoms and appearance of a benign ulcer. Lymphocytes and plasma cells occurring in this are to be confirmed through immune histology, as similar cells may be seen in a normal gastro intestinal brush smear or in the chronic gastritis [7].

Adeno Carcinoma of the Colon:

The majority of the primary colon carcinomas occur in the descending part. The tumors are adenocarcinomas and remain similar cytologically to gastric carcinomas. The malignant cells may be moderately differentiated with more cytoplasm but the limiting cell membrane is less well defined. The nuclei and nucleoli may be larger. In poorly differentiated tumors the inter cytoplasmic membranes may be less distinct and the cytoplasm forms a syncytium. Aniso nucleosis is a marked feature. The nucleoli are prominent and variable in number and shape. Peri nucleolar condensation of chromatin around the nucleoli may be characteristic [8].

Meta Static Colon Carcinomas:

It is a mucoid type carcinoma. Large amount of extra cellular mucin in which the tumor cells are embedded indentifies the above. The cells are moderately differentiated and contain few intra cellular mucus vacuoles [9].

Meta Static Squamous Cell Carcinoma:

It is a kind of cancer occurs in the squamous cell lining of the digestive tract. When the tumor becomes invasive it penetrates to the adjacent part and developed its metastatic stage [9].

Gastrointestinal Stromal Tumor (GIST):

This type of cancer is very rare in nature. It arises from interstitial cells of Cajal that regulate intestinal contractions [9].

Gastrointestinal Leiomyosarcoma:

The occurrence of this cancer is very uncommon. It rarely spreads to the lymph nodes. It starts and spreads from smooth muscle cells in muscularis mucosa or propria of the stomach lining [9].

Gastrointestinal Carcinoid:

It rarely occurs in the fundus. Initially it is present the mucosa in the gastric body and fundus. The chances of metastatisizing are limited [8].

Gastrointestinal Lymphoma:

This type of cancer occurs very rarely. Normally it evolves in the stomach, but it can start in any part of gastrointestinal tract [9].

Leiomyosarcomas:

This type of colon cancer occurs in the smooth muscle of the colon. It accounts for less than two percent of colorectal cancers and the metastatic potential is high [7].

Lymphomas:

The colorectal lymphomas are rare and are more likely to start in the rectum than in the colon. But the lymphomas that start from any part of the body are more likely to spread to the colon than to the rectum. The Non-Hodgkins lymphoma accounts for about 0.5 percent of all colorectal cancers and it has many forms [8].

Melanomas:

It is also very rare. Like lymphoma, it also starts from any part of the body and spreads to the colon or rectum. Melanomas account for less than two percent of colorectal cancers [9].

Neuroendocrine Tumors:

This type of tumors is divided into two main categories: aggressive and indolent. Large cell and small cell neuroendocrine tumors are considered as aggressive, while carcinoid tumors are considered as indolent [9].

Mucinous Carcinoma:

The term "mucinous" means that something which has lot of mucus. Carcinomas that are comprised of at least 60% mucus are referred to as mucinous. The presence of mucus allows cancer cells to spread faster. As a result, mucinous carcinomas are considered as more aggressive than regular carcinomas and are harder to successfully treat. Mucinous carcinomas account for about 10-15% of all adenocarcinomas [9].

MATERIALS AND METHODS

HISTOPATHOLOGICAL STUDIES

(I) Light Microscopy

The gastro intestinal cancer tissues were collected for histological studies after clearance from the Institutional Ethical Committees in the Cancer Hospitals (Chennai, India). Tissues were separated and immediately fixed in aqueous Bouin's fluid (saturated solution of Picric acid-75ml, Formaldehyde-20 ml and Acetic acid-50 ml) for a period of 24 hours. After fixing, they were washed over night in running water to remove the fixative. To remove the water content present in the tissues, dehydration was carried out by transferring them to a series of gradually increasing percentages of alcohol in water. The materials were then cleaned in xylol and embedded in wax (melting point 52^0C). Sections were cut at a7µ thickness and stained with haematoxylin and eosin [10].

(II) Transmission Electron Microscopy

Gastro intestinal cancer tissue was isolated in as close as possible to in vivo condition prior to placing it into the fixation medium. The sample was fixed in 3% glutaraldehyde and washed in buffer. This double fixation gives stability during dehydration embedding and during electron bombardment. Further it also provides staining contrast decrease distortion, fix fine cellular ultra structure, suitable for cancer tissue. Then the sample is dehydrated by ascending series graded alcohol (50% to 100%) and clearing by propylene oxide. After it gets dehydrated it is infiltrated by propylene oxide and epoxy resin. Once it is infiltrated, it is embedded in siligonised rubber mold with epoxy resin. Embedded mold kept in incubator at 600 C for 48 hrs, and allowed it to cool down till the blocks are ready for sectioning. The sample is cut through ultra microtome (Leica Ultra cut UCT) to a thickness of 1 micron with glass knife and stained toludine blue. Before cutting it into section light microscopic examination is extremely useful when sections are needed to give a general idea of the orientation of the tissue and for marking the areas of interest in the blocks to cut ultra thin sections which further trimming of the blocks for ultra microtomy. An ultra thin section, of the size of below 100nm is cut through ultra microtome with a diamond knife (DDiatome). Ultra thin sections are taken on copper grid and stained (Double metallic) uranyl acetate and Reynold's solution (sodium citrate + Lead nitrate) which gives contrast. Sections transmitted in Electron Microscope (Philips 2010 by Netherland).

RESULTS AND DISCUSSION

The various histological types of gastro-intestinal cancers include the mucinous carcinoma, squamous cell carcinomas, lymphomas, sarcomas, malignant melanomas and carcinoid tumors. In recent years the histopathological identification has been made to find out the degree of tumor penetration into the bowel wall. In the present study besides light microscopic details, electron microscopic ultra structural details are also illustrated to delineate the functional morphology of the tumor at the organelles level inside the cells [10].

In order to define the various histopathological changes observed in the present study, an appraisal of the previous information's seems to be necessary. The control picture of stomach and colorectal regions is deduced from literature so as to focus the malignant changes contrastingly [11]. The slides prepared from the cancer patient samples are described for histo pathological changes. All photomicrographs of the gastro intestinal cancer patients samples revealed by the light microscopy the type of malignancy as the adenocarcinoma. Adeno carcinoma is the dominating form of colorectal cancers in

the present study undertaken and account for more than 95%. The following diagnostic characteristic have been identified in light microscopy (Fig.1-3).

1) Disruption of the glandular and columnar organization of the tissue architecture is characteristic in most of the samples.
2) Non cohesive disassociated cell mass.
3) Darkly stained cells revealing the hyper chromatic nature of the nuclei.
4) Loss of distinguishing morphological features of cells revealing both monomorphic and or pleomorphic structures of the cells.
5) The nucleus occupies the whole portion of cell volume with scanty cytoplasm revealing higher nuclear / cytoplasmic ratio.

Though the above cytomorphological features are discernible in light microscopy, the ultra structural changes in the malignant cells have been deduced more clearly from the electron microscopic preparations of the gastro-intestinal cancer tissues of patients.

Carcinomatous characteristics of stomach and colorectal cancers:

1) Reduced cytoplasm is one the notable characteristics of malignant cells
2) The tissues solved dissociated cells.
3) The malignant nature of the cells is indicated by the nuclei which practically fill the cells are variable in size and have disorganized chromatin patterns.
4) In poorly differentiated Adeno carcinoma, loss of distinguishing morphological features is obvious.
5) In much depleted colon carcinoma the cells are packed together in a tight cluster.
6) The malignant nuclei are larger, variable and have hyper chromatic chromatin clumps.
7) Nuclear cytoplasmic ratio is high fairly.
8) Nuclei may be pleomorphic.
9) Free floating tumor cells may have microvilli. Pseudo cilia may be seen in gastric carcinoma.

Electron Microscopic Ultra Structure:

Ultra structural studies on gastro intestinal carcinoma are meager in literature. However similar studies on other carcinoma cells reveal that these cells basically are characterized by prominent organelles. The cell to cell variations are not uncommon and such variations infer the different functional statuses of neoplastic cells. In the present study the electron micrographic structures of gastro intestinal tissue supports the previous hypothesis that malignant cells show different of functional phase. The nuclear morphological features and their variations, the mitochondrial shape variegations, presence of filamentous structures resembling the membrane fenestrate and fatty acid inclusions etc., were revealed in the present study. In cancer cells generally both increase in the number of mitochondria as well as reduction of their numbers has been reported in studies. Electron microscopic observations have shown that cancer cells often contains smaller mitochondria than normal cells. Some may be normal in size but with longer diameter due to luminal swelling. Very occasionally cancer cells contain elongated giant mitochondria. In the present study the electron micrographs prepared from the gastro intestinal cancer tissue revealed both the elongated and swollen mitochondria and also the degenerating mitochondrial structures. The above observations reveal that the cells are under respiratory stress. It is an indication of development of malignancy [12].

In the present study, Fig.(a) shows the goblet cells, mast cells surrounded by cluster of cancer cells. In the Fig.(b), a single cell is focused with granulated nucleus with a clear round contour. Inside the cell, the cell organelles like mitochondria, endoplasmic reticulum, and fat droplets are visible. In the Fig.(c), a goblet cell is focused in between the cancer cells which have micro villi and huge amount of mitochondria. The Fig.(d) shows the cellular inclusion in the inter cellular space. The irregular, huge and granulated nuclei with concentrated fat droplets. The Fig.(e) exhibits a cancer cell with microvilli on its one side, and with completely obliterated, irregular, and highly granulated nucleus having nucleoli pushed to a corner with a clear contour. It is surrounded by large amount of swollen mitochondria. The Fig.(f) concentrating the microvilli arises from the epithelium of a cancerous cell and the cellular matrix in the inter cellular space. The Fig.(g) shows the visible endoplasmic reticulum dispersed in the whole cancer cell. The nucleus and mitochondria getting ejected from the cell occupies the inter cellular space. The Fig.(h) lucidly picturizes the ejected nucleus, endoplasmic reticulum along with the vacuoles and fat bodies. The Fig.(i) reveals huge amount of swollen mitochondria burst out from the cell and occupies the inter cellular space. The Fig.(j) clearly shows the cell membrane disrupted and released the cyto plasmic material including mitochondria, endoplasmic reticulum and vacuole in the inter cellular space. The Fig.(k) focused a cancer cell with a large irregular, highly granulated, chromatin packed with its nucleolus pushed to a corner surrounded by many mitochondria are seen. This slide exhibits the inner change of a cancer cell. Fig.(l) which gives a bird eye view of the group of cancerous cells with its legible, irregular shaped nuclei, in between present the mitochondria. Fig.(m) shows the inter cellular space containing the vacuole intruded by the micro villi of the surrounding cancerous cells. Fig.(n) shows the cell inclusion of mitochondria dispersed with vacuole. The vacuole is elongated and mitochondria are swollen. Fig. (o) reveals the presence of epithelial layer of cells with elongated cells present in the lumen. Fig.(p) focused a single, large, irregular in shape, but with a clear contour, densely granulated nucleus surrounded by enormous mitochondria. Fig.(q) shows the vacuoles in enormous amount inside the cell [13 & 14].

Figure 1. Adenocarcinoma in gastric region Hyper plasia of malignant cells Fatty inclusions

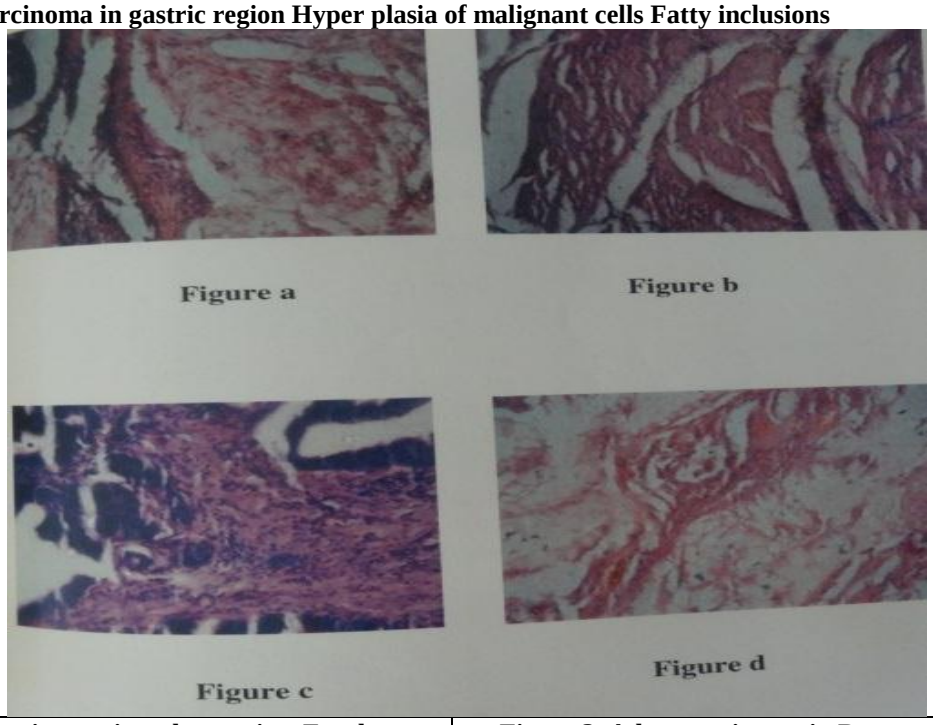


Figure 2. Adenocarcinoma in colon region Focal areas showing vesicular hyper chromatic moderate pleomorphic nuclei

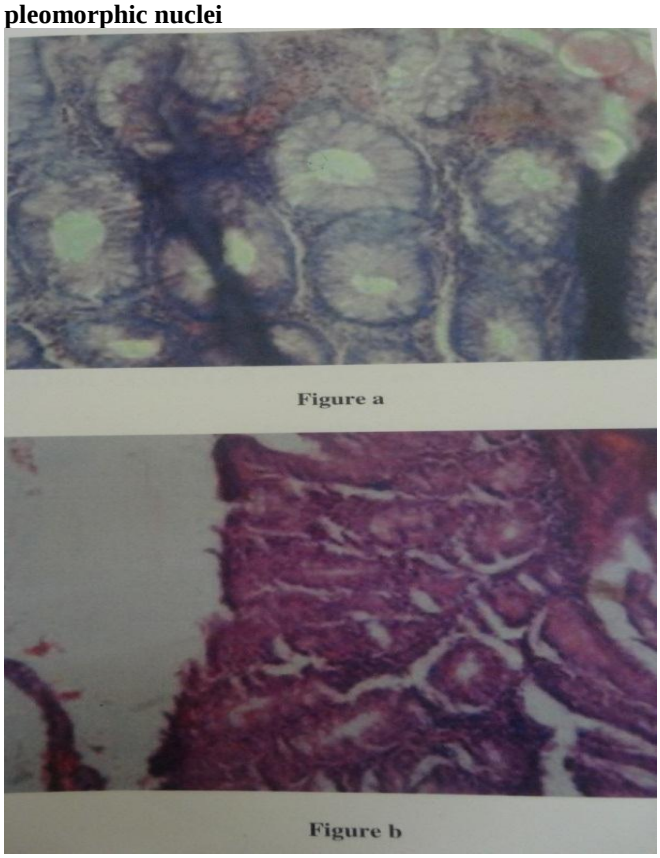


Figure 3. Adenocarcinoma in Rectum Clusters of Cell

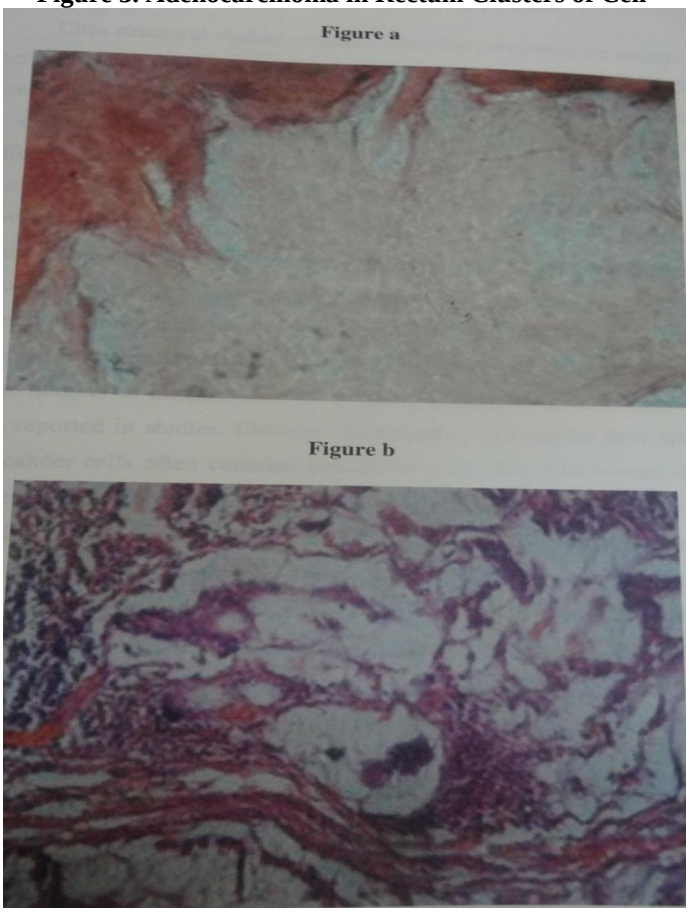
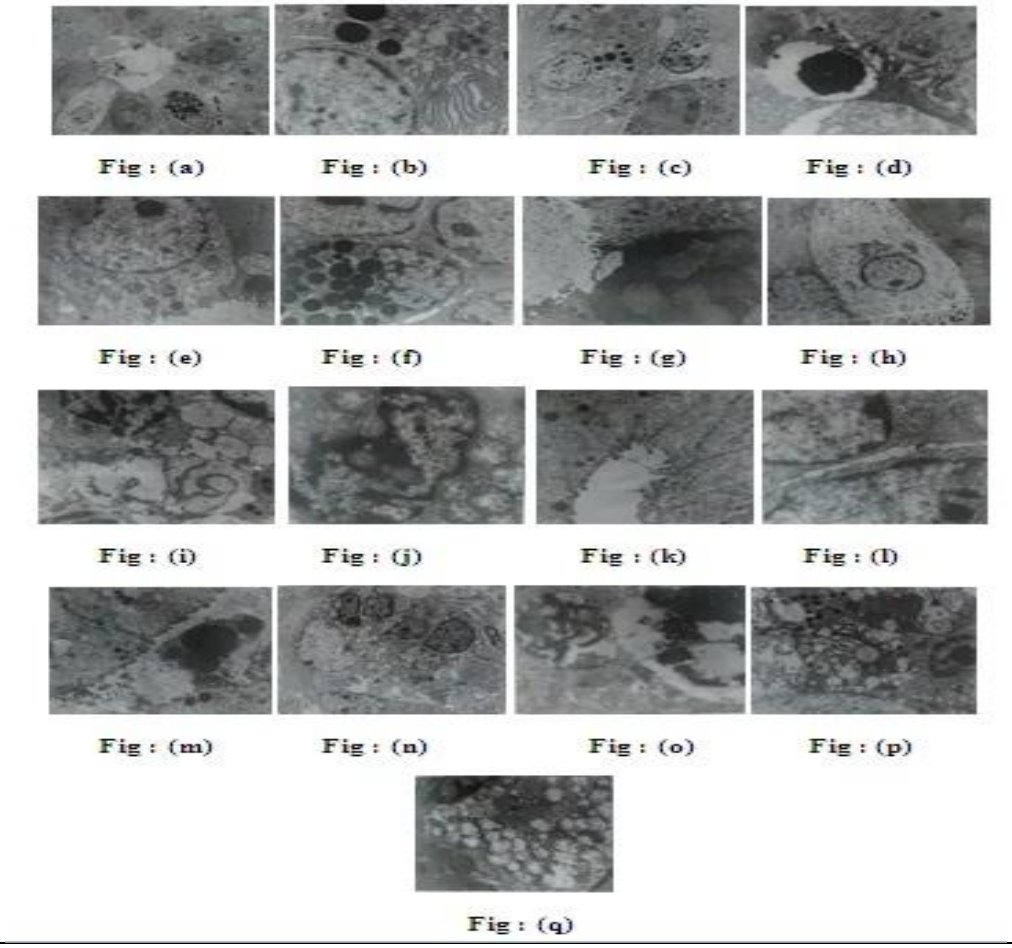


Figure 4. Electron Micrographs of Gastro Intestinal Cancer



CONCLUSION

The type of gastro-intestinal cancer incidence has a correlation to ethnicity of the populations. Among the Asiatic population, the gastro-intestinal cancers show the descending order of incidence in the Asian countries viz., Japan> Chinese>Indians>Malays. High incidence of gastric cancers has also been reported in far East, Eastern Europe, Britain and Russia [15-18]. Our histopathological findings point out the predominance of Adeno carcinoma in all the gastro-intestinal regions of south Indian haplo groups (Dravidan Race). As early diagnosis of gastro-intestinal cancer guarantees assured survival of patients, for individuals with dyspeptic symptoms, the histo pathological diagnosis seems to be pertinent.

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