



MUCIN HISTOCHEMISTRY IN BENIGN AND MALIGNANT LESIONS OF STOMACH –AN INSTITUTION BASED STUDY.

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INTRODUCTION

Mucins are high molecular weight glycoproteins that are synthesized, stored and secreted by the epithelial mucosal cells of GIT. The gastric mucosa can be divided into three histological areas like superficial, neck and deep zones. The superficial zone composed of single layer of mucin secreting columnar cells. The neck zone, largely of immature stem cells mixed with some mucous neck cells. The deep zone is composed of glands, the bases of which lie close to or in muscularis mucosae[1]. Mucins of the stomach are large glycoproteins with attached side chain of sugar residue, N acetyl derivative of hexosamine and terminal sialic acid group. They perform significant functions like selective impermeability to hydrochloric acids and bile salts, antibacterial effects, lubricating the luminal contents, regulate epithelial dehydration, prevent tissue edema, anti-metastatic and immunological phenomenon. Understanding the mechanisms involved in the biosynthesis and degradation of mucins along with the analysis of genes coding for the mucin proteins may give insight into the factors that lead to the development of various pathological conditions of the stomach[2,3]. Mucins are classified into neutral and acidic mucins; the latter include sulpho and sialomucins.[4]. In the GIT, both neutral and acidic mucin are present at specific locations and their production is increased or reversed in various diseases and malignancies of GIT [5, 6].

There are two broad categories of mucins: **Acid mucins** which carry a negative charge on the mucin molecules and can be classified as either simple (carboxyl group added) or complex (sulfuric acid group added). They are found widely throughout the gastrointestinal and respiratory tracts. **Neutral mucins** which lack acid groups and carry no charge. They are found in the epithelium of the stomach and the Brunner's glands of the duodenum. They protect the lining of the duodenum from stomach acid content, help enable absorption by providing an alkaline environment for digestive enzymes, and lubricate intestinal walls and prostate epithelium[7]. Several staining techniques can be used in the clinical histology to demonstrate mucin [8]. Alcian Blue is used to demonstrate acid mucins and combined with PAS staining procedure to demonstrate both acid and neutral mucin; Alcian Blue will stain acidic mucin blue and PAS stains neutral

mucin magenta color. PAS Stains glycogen as well as mucin, but tissue can be predigested with diastase to remove glycogen [9].

Periodic Acid Schiff's (PAS) Stain This technique is based on the reactivity of free aldehyde groups, the initial step of PAS technique is the oxidation of Hydroxyl groups attached to adjacent carbon atoms[10]. Glycoprotein, mucin particularly neutral mucin is demonstrated by PAS. Neutral mucin stains Magenta coloured and Nucleus Stains Blue colour[11]. 2) PAS with Diastase This technique refers to the use PAS stain in combination with Diastase, which is an enzyme that digests the Glycogen. The whole purpose of this stain is to differentiate Glycogen from other PAS positive tissues. PAS with Diastase stains light Pink colour[12]. 3) Alcian Blue Alcian Blue is a group of water soluble polyvalent basic dye containing phthalocyanine ring with copper in its centre. basic. Copper imparts blue color to mucin. Alcian Blue 8GX is the recommended dye for histological techniques[13].

The various types of mucins and their staining reactions include,

Neutral Mucin: PAS Positive .

Acidic Mucin(sialomucin): PAS and Alcian Blue Positive at pH 2.5 .

Acidic Mucin(sulfomucin): Alcian Blue positive at pH 1.0

AIMS AND OBJECTIVES OF STUDY

The main aims of this study was,

1. To study the pattern of Mucin staining in gastric biopsies and resected specimens.
2. To find out the difference in Mucin distribution in Benign and Malignant lesions of stomach.

MATERIALS AND METHODS

The specific procedures which were used to identify mucin distribution and pattern in Normal and Malignant Stomach include following steps..

FIXATION: Specimens were primarily fixed in 10% Neutral Buffered Formalin.

The time of fixation was 24 hours for resected specimens and 4-6 hours for small gastric biopsies.

GROSSING: All the specimens were grossed by a pathologist. The sections were properly labeled and marked in microcassettes.

TISSUE PROCESSING: Sections were placed in 10% Formalin for 3 Hours then Dehydrated in Alcohol for 6 Hours Followed by Xylene for 2 Hours and Infiltrated With Paraffin wax for 3 Hours.

EMBEDDING: i).Sections were embedded properly with Tissue Tek System at specific temperatures of different plates. ii).Sections were placed in wax filled Tissue moulds facing downwards and capsule rings were attached. Iii).Kept for cooling. Iv).Tissue blocks were submitted for Microtomy.

SECTION CUTTING: I). Tissue Blocks were trimmed(Rough cutting). Ii).Kept for cooling effect for 15-20 minutes. Iii).Tissue Blocks were subjected to proper cutting. Iv).Tissue Sections were taken on Albumin coated slides.

STAINING :

- A. **HEMATOXYLIN and EOSIN Stain:** Sections were stained with routine H and E stain. It is most widely used histological stain simple to use and demonstrates different tissue structures clearly. Hematoxylin stained the cell nuclei Blue-Black while as Eosin stained cell cytoplasm with pink shades.
- B. **SPECIAL STAINS:** The various special stains were used to stain the different types of mucin structure of normal and malignant stomach .
1. **PAS STAIN (Periodic acid schiff's):** The OH group of carbohydrate oxidized to CHO group by periodic acid and reacted with schiff's reagent ,magenta coloured was formed. Neutral mucin was stained.
 2. **PAS WITH DIASTASE:** PAS stain was used in combination with diastase, which is an enzyme that digests the Glycogen. This stain PAS-D was used for differentiating Glycogen from PAS-positive mucins, mucin stained light pink colour.
 3. **ALCIAN BLUE stain:** Water soluble polyvalent basic dye made of copper containing phthalocyanine ring linked to 4 isothiuronium groups through thioether bonds. This stain was used at 2.5 pH and 1.0 pH levels. Acidic mucin stained blue. The simple acidic epithelial mucin (sialomucin) was reactive at pH 2.5. The complex epithelial mucin (sulfomucin) was reactive at pH 1.0.
 4. **COMBINED ALCIAN BLUE-PAS Stain:** The neutral mucin was differentiated from acidic mucin by using combined Alcian Blue-PAS stain. Mixture of two mucins stained purple, due to positive reaction of both Alcian Blue and Schiff's reagent.

RESULTS:

A total of 45 cases were enrolled during the entire study period. The specimen received included gastric biopsies (30 cases) and gastrectomies (15).

TYPE OF SPECIMEN	NO. OF CASES	PERCENTAGE
Biopsy	30	66.67
Gastrectomy Specimen	15	33.33
Grand total	45	100

In our study 57.8% were males and 42.2 % were females and the male:female ratio was 1.36. The age range was from 20-80 years and the mean age was 55.3 years shown in below table,

AGE GROUP	NO OF CASES	PERCENTAGE	MIN. AGE	MAX. AGE	AVG. AGE	SD
20 - 40 YEARS	9	20	25	40	32.33	4.72
40 - 60 YEARS	18	40	43	60	52.44	5.72
60 - 80 YEARS	18	40	62	80	69.78	5.58
Grand Total	45	100	25	80	55.36	15.02

Total no. of cases were 45, of which females were 18 (40%) and males were 27 (60%) as shown in the table.

GENDER	NO. OF CASES	PERCENTAGE
FEMALE	18	40
MALE	27	60
GRAND TOTAL	45	100

The diagnosis made in our study was chronic gastritis ((51.11%), Adenocarcinomas(40%) and hyperplastic polyps (8.89%) as shown in the table.

DIAGNOSIS	NO OF CASES	PERCENTAGE
Adenocarcinoma	18	40
Chronic Gastritis	23	51.11
Hyperplastic Polyp	4	8.89
Grand Total	45	100

The patients age ranged between 25 years and 80 years with a mean of 55.36, shown in the table below.

DIAGNOSIS WITH AGE GROUP	NO OF CASES	PERCENTAGE	MIN AGE	MAX AGE	AVG AGE	SD
Adenocarcinoma	18	40	28	80	57.33	15.03
Gastritis	23	51.11	25	80	53.74	16.05
Hyperplastic Polyp	4	8.89	45	66	55.75	9.88
Grand Total	45	100	25	80	55.36	15.02

Out of 18(40%) Adenocarcinomas cases,11(24.44%) were males and 7(15.56%) were females. Total cases Chronic Gastritis were 23(51.11%) of which 15 (33.33%) were males and 8(17.78%)were females. 4(8.89%) were hyperplastic polyps in which females outnumbered the males, 3 (6.67%) were females and 1(2.22%)was male, shown in table below.

DIAGNOSIS * GENDER	NO. OF CASES	PERCENTAGE
Adenocarcinoma	18	40.00
FEMALE	7	15.56
MALE	11	24.44
Gastritis	23	51.11
FEMALE	8	17.78
MALE	15	33.33
Hyperplastic Polyp	4	8.89
FEMALE	3	6.67
MALE	1	2.22
Grand Total	45	100

All the 45 cases were PAS positive, shown in table below.

PAS STAIN	NO. OF CASES	PERCENTAGE
+++	45	100
Grand Total	45	100

Out of 45 cases, all cases were positive for PAS with DIASTASE as shown in table below.

PAS WITH DIASTASE	NO. OF CASES	PERCENTAGE
+++	45	100
Grand Total	45	100

Out of 45 cases, 24 cases showed weak or variable staining for combined Alcian Blue-PAS (+/-). 19 cases showed moderate staining for combined Alcian Blue-PAS (++), and 2 cases revealed Strong staining reaction for combined Alcian Blue –PAS stain, shown in table below.

COMBINED PAS-ALCIAN BLUE	NO. OF CASES	PERCENTAGE
+/-	24	53.33%
++	19	42.22%
+++	2	4.44%
Grand Total	45	100.00%

Out of 45 cases, 35 case were weakly or variably stained for Alcian Blue reaction(+/-).8 were moderate stained for Alcian Blue(++), and only 2 showed strong staining reaction for Alcian Blue(+++),shown in table below.

ALCIAN BLUE	NO. OF CASES	PERCENTAGE
+/-	35	77.78
++	8	17.78
+++	2	4.44
Grand Total	45	100

The total no. of Adenocarcinoma cases were 18(40%), all of these cases were positive (++++) for PAS stain. The 23(51.11%) cases of gastritis were also positive for PAS stain (++++) as were the cases of hyperplastic polyp 4(8.89%).

DIAGNOSIS * PAS STAIN	NO. OF CASES	PERCENTAGE
Adenocarcinoma	18	40.00%
+++	18	40.00%
Gastritis	23	51.11%
+++	23	51.11%
Hyperplastic Polyp	4	8.89%
+++	4	8.89%
Grand Total	45	100.00%

The staining pattern of our cases with PAS with Diastase is shown in the below table:

DIAGNOSIS * PAS WITH DIASTASE	NO. OF CASES	PERCENTAGE
Adenocarcinoma	18	40.00%
+++	18	40.00%
Gastritis	23	51.11%
+++	23	51.11%
Hyperplastic Polyp	4	8.89%
+++	4	8.89%
Grand Total	45	100.00%

Combined PAS Alcian Blue staining pattern is shown in the below table:

DIAGNOSIS WITH COMBINED PAS ALCIAN BLUE	NO. OF CASES	PERCENTAGE
Adenocarcinoma	18	40.00%
+/-	1	2.22%
++	15	33.33%
+++	2	4.44%
Gastritis	23	51.11%
+/-	23	51.11%
Hyperplastic Polyp	4	8.89%
++	4	8.89%
Grand Total	45	100.00%

The staining pattern for Alcian Blue is shown in the table:

DIAGNOSIS * ALCIAN BLUE	NO. OF CASES	PERCENTAGE
Adenocarcinoma	18	40.00%
+/-	8	17.78%
++	8	17.78%
+++	2	4.44%
Gastritis	23	51.11%
+/-	23	51.11%
Hyperplastic Polyp	4	8.89%
+/-	4	8.89%
Grand Total	45	100.00%

Overall Staining pattern in our cases is shown in table below,

DIAGNOSIS * TYPE OF SPECIMEN	PAS STAIN	PAS STAIN WITH DIASTASE	COMBINED PAS- ALCIAN BLUE	ACIAN BLUE
Adenocarcinoma	+++ (18)	+++ (18)	+++ (18)	+++ (18)
Gastritis	+++ (23)	+++ (23)	+/- (23)	+/- (23)
Hyperplastic Polyp	+++ (4)	+++ (4)	++ (4)	+/- (4)
Grand Total	45	45	45	45

- : Negative staining ± : Weak or variable staining + : Slight staining ++ : Moderate staining +++ : Strong staining.

DISCUSSION

The study was carried out in the department of Pathology Sher-i-Kashmir Institute of Medical Sciences, SKIMS, Srinagar from DEC.2021-NOV.2022.

Total number of cases in our study were 45 with age range of 20 – 80 years in both the males and females. The mean age group (55.3 years) was almost similar to the studies conducted by **Ajay S, et al** [4].

In our study the most common diagnosis was chronic gastritis followed by adenocarcinoma which was comparable with the study conducted by Krishnappa, et al[14].

Among 45 patients 26 were males (57.8%) and 19 were females (42.2%). The male;female ratio in our study was 1.36 which was slightly less than the study conducted by **Silva et al[6]** and **Ajay S, et al,[4]** however there was still a male preponderance.

Gastritis leads to loss of parietal mass and is usually associated with intestinal metaplasia and the presence of goblet cells show positivity for acidic mucins, as it carries increased risk of Adenocarcinoma. In our Study 23 cases of gastritis were PAS positive which were in contrast to findings of **Prathima, et al[15]** and comparable to the study conducted by **Ajay S, et al [4]**.

According to our study, it clearly suggests that gastritis as well as adenocarcinoma are more common in males than females, which was comparable to the studies conducted by **Ajay S. Wani, Shilpi Sahu[4]**.

Our study showed that all cases of gastritis on special staining were positive for PAS neutral mucin) with a small percentage (8.7%) positive for Alcian Blue (acidic mucin) and our results were comparable with the study conducted by **Silva S, Filipe MI, et al[6]**.

In our study gastric polyp showed predominance of neutral mucins than acidic mucins which correlated with the study conducted by **Prathima S, et al[15]**.

Most of the cases of adenocarcinoma were positive for PAS as well as Alcian Blue. Only 2 cases of Poorly differentiated adenocarcinoma showed PAS positivity and were negative for Alcian blue. Our results were same as the study conducted by **Minematsu, H., Saito, et al[16]**.

CONCLUSION

In the current study we concluded that mucin stains play an important role in the diagnosis of various benign and malignant lesions of the stomach. The pattern of mucin stains done with PAS, PAS with Diastase, Alcian Blue and Combined PAS- Alcian Blue were helpful in diagnosis and categorisation of various lesions. These stains added beneficially to the histopathological findings on routine H and E stains, besides being easy to perform and cost-effective.

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