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Original Research Paper

Pathology

A STUDY OF MUCIN EXPRESSION PATTERN IN GASTRIC ADENOCARCINOMA: AN AMBIDIRECTIONAL OBSERVATIONAL STUDY

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ABSTRACT **Objective:** This study investigates mucin expression patterns in gastric adenocarcinoma (GAC), examining neutral, sialomucins, and sulfomucins using histochemistry. We aim to correlate histological grading and molecular classification with mucin histochemistry offering potential diagnostic and prognostic markers. These findings contribute to understanding the complex interplay between mucin alterations, molecular pathways, and clinical outcomes in GAC. **Materials and Methods:** This observational study, conducted from March 2021 to August 2022, included 50 cases of gastric adenocarcinoma. It utilized both prospective and retrospective approaches to gather specimens and employed histochemical staining techniques to assess mucin changes. Data analysis was performed using SPSS 20.0, focusing on mucin expression patterns across different histopathological variants. **Results:** Our findings revealed age and sex distribution trends, distinct tumor histological patterns, and significant peri-neural and lymphovascular invasion. In diffuse gastric adenocarcinoma, mucin staining patterns indicate increased mucin production and a shift toward neutral mucin. Conversely, intestinal-type gastric adenocarcinoma exhibits strong positivity for intestinal-type mucin components and sialomucins, with significant sulphomucin presence. **Conclusion:** In our study, we explored various clinicopathological aspects of gastric adenocarcinoma (GAC) and delved into mucin staining patterns. These results contribute to a deeper understanding of GAC characteristics with potential clinical implications.

KEYWORDS : "Gastric Mucins" ; "Gastric Cancer"; "Adenocarcinoma" ; "Histopathology"

INTRODUCTION

Gastric adenocarcinoma (GAC) represents approximately 95% of all stomach malignancies and continues to pose a substantial global health burden (1). While its incidence has stabilized, variations exist between endemic and non-endemic regions (2). Unlike some cancers with well-defined genetic pathways, gastric cancer exhibits histopathological and molecular heterogeneity, limiting comprehensive characterization (10).

GAC exhibits significant intra- and inter-patient heterogeneity, complicating treatment responses. Despite advancements in endoscopic, surgical, and systemic treatments and an emphasis on multidisciplinary care, global 5-year survival rates remain around 25-30%(1).

Mucins are complex carbohydrates secreted by gastrointestinal epithelial and glandular tissues. Mucins serve various functions, including lubrication and protection against acids, and can contain immunoglobulins, lactoferrin, and lysosomes, impacting bacterial growth(4).

Mucosubstances are categorized into neutral and acidic mucins, further divided into weakly acidic (carboxylated or sialomucins) and strongly acidic (sulphomucins)(4). Identifying mucin types can aid in assessing neoplastic changes within tissues, such as detecting acid or sulphomucins in gastric mucosa, which may indicate intestinal metaplasia associated with gastric carcinoma (4).

Our study aims to explore the pathological variations in gastric adenocarcinomas and their associated mucin expression patterns, including neutral mucin, sialomucins, and sulfomucins (5). Histochemical mucin staining techniques were employed for this purpose (5).

MATERIALS AND METHODS

Study Setting:

This observational study was conducted in collaboration with the Department of Pathology and the Department of Surgery at a tertiary care medical college and hospital serving a diverse population from a very densely populated area.

Study Period:

The study was conducted from March 2021 to August 2022.

Population Definition:

The study population consisted of all specimens from previously provisionally diagnosed cases of gastric adenocarcinoma. These cases were referred for complete histopathological evaluation for staging and treatment purposes in the Department of Pathology.

Study Design:

Both prospective and retrospective approaches were employed in the Pathology Department. Resected specimens of gastric adenocarcinoma were provided by the Department of Surgery for histopathological diagnosis. Additionally, paraffin-embedded blocks from previously diagnosed cases of gastric adenocarcinoma were retrieved from the Pathology Department. Sections from normal-looking gastric mucosa, distal to the resected specimens, were utilized as normal controls, while sections from tumour tissues of gastric adenocarcinoma were used for testing.

Study Variables:
- Dependent Variables: Mucin histochemical changes in gastric adenocarcinomas.
- Independent Variables: Age, sex, tumour location, duration of illness, tumour size, depth of penetration, lymph node and distant metastasis status etc. and other relevant clinical parameters.

Inclusion Criteria:
- Newly diagnosed cases of gastric adenocarcinoma.
- Blocks and specimens retrieved from the Department of Pathology in previous cases of gastric adenocarcinoma with detailed clinical history and reports.

Exclusion Criteria:
- Inadequately resected specimens(margin positive).
- Lack of detailed clinical history.
- Presence of malignant tumours other than pure adenocarcinomas.
- Improperly fixed specimens or poorly preserved blocks.

Sample Size:
A total of 50 cases of gastric adenocarcinoma, each case inclusive of both test specimens (from the tumour) and uninvolved distal resected mucosa as control tissue, were included in the study.

Sampling Design:
This study followed a cross-sectional observational design.

Control Group:
Normal gastric mucosa from resected margins, distal to the tumour and at least 5 cm away, served as the control.

Data Collection Methods:
- Histopathological diagnosis was conducted in the Department of Pathology.
- Mucin histochemistry studies were performed using sections from paraffin blocks.

Laboratory Investigation:
- Specimens were obtained from the Department of Surgery, cleaned, photographed, and fixed in 10% buffered formalin.
- Routine processing and sectioning were carried out, and histochemical staining methods were applied as necessary.

Histopathological Techniques:
The collected tissues were fixed in a preservation solution containing 10% formal saline, 2% calcium acetate, and a small amount of phosphotungstic acid. Paraffin-embedded blocks were prepared through standard histopathological techniques, and sections were cut at 5-6 microns.

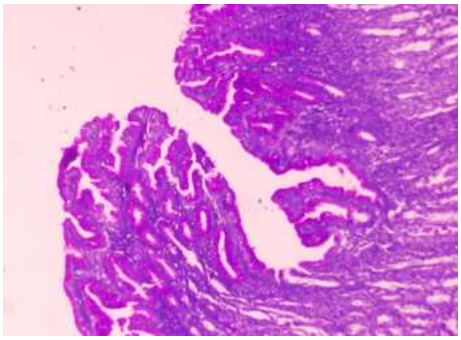

Figure. 1: PAS stain of normal (control) gastric mucosa. (x400)

Hematoxylin and Eosin staining was performed on these sections.

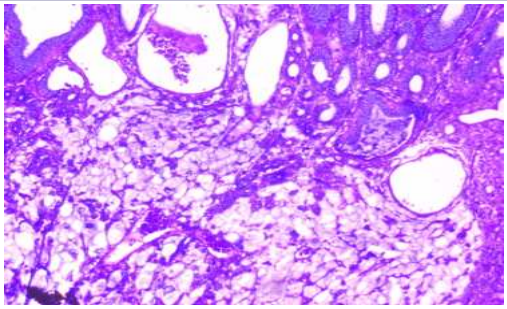

Figure 2: AF-AB stain of diffuse GAC showing weakly stained areas as only neutral mucin is present(x400)

Histochemical Stains (14,15,16):
Several histochemical staining techniques were employed to characterize different mucosubstances:

1. P.A.S. (Periodic acid Schiff):
This stain detects all carbohydrates, including mucosubstances, rendering mucosubstances P.A.S. positive**.** PAS with Diastase confirms mucin as the target substance **(Figure 1)**

2. Alcian Blue(AB):
This stain can operate at various pH levels and is capable of staining both carboxylated and sulphomucins.

3. Combined AB-PAS:
This staining procedure differentiates various mucin types, highlighting neutral mucins in magenta, carboxylated mucins in blue, and sulphated mucins in purple.

4. Combined AF-AB (Aldehyde Fuchsin-Alcian Blue):
This staining technique aids in the distinction between carboxylated and sulphated mucins, with carboxylated mucin staining in blue and sulphated mucin staining in purple**.(Figure 2)**

5. HIDA (High Iron Diamine):
This technique stains sulphomucins brown to black, and Alcian Blue was used as a background stain.

Grading System:
The staining results were graded based on color intensity, ranging from 0 (negative) to 3 (strong positive reaction).(14,15,16)
- +++: Strong positive reaction (3).
- ++: Moderate reaction (2).
- +: Weak reaction (1).
- -: Negative (0).

Data Analysis:
Statistical analysis was performed using SPSS version 20.0. The chi-squared test was used to compare nominal categorical data between groups. A p-value less than 0.05 was considered statistically significant.

Parameter Evaluation:
Various clinical, pathological, and mucin-related parameters were evaluated, including mucin expression patterns across different histopathological variants of gastric adeno carcinomas.

Data Collection Schedule:
Data was manually collected, compiled, and tabulated for subsequent analysis.

RESULTS :

I. Patient Demographics and Tumour Characteristics
In this section, we present the key findings related to patient demographics and tumour characteristics from our study.

Patient Demographics:
Our study comprised 50 patients, The majority of patients (60%) fell within the 50-60 years age group. Other 40% were within 60-70 year age group. Among the patients(n=50), 56% were male, and 44% were female.

Chief Complaints:
Patients presented with various chief complaints, The most common chief complaint was bleeding per rectum (42%), followed by obstruction (20%) and pain (18%).

Histological Types:
Histological analysis revealed of the patients, 46% exhibited diffuse lesions, while 54% showed intestinal lesions.

Growth Patterns:
Tumour growth patterns were categorized. The most frequently observed growth pattern was ulceroproliferative (52%).

Tumour Characteristics:
We assessed several tumour characteristics as follows: Perineural invasion was present in 16% of cases Lymphovascular invasion was present in 14% of cases Serosa involvement was present in 20% of cases. Necrosis was present in 54% of cases.

These findings lay the foundation for further analyses and clinical implications, in this geographical location.

II. Mucin Staining Patterns in Diffuse Gastric Adenocarcinoma (GAC) (Table 1)
This section presents the results of mucin staining patterns in patients with diffuse gastric adenocarcinoma (GAC).

PAS staining of normal gastric mucosa showed a predominance of moderate to strong reactivity. In cancer areas, strong reactions were significantly more prevalent (78%) than moderate reactions (21%), as indicated by a significant p-value of <0.001.

PAS-D staining showed that 91% of cases in cancer areas exhibited strong reactions, while 8% had moderate reactions. In comparison, control areas had 52% strong reactions, 26% moderate reactions, and 21% weak reactions. This finding was statistically significant (p-value < 0.001), indicating increased mucin production in diffuse-type gastric adenocarcinoma.

The analysis of combined Alcian blue with PAS at pH 2.5 showed that 39% of cases from cancer areas exhibited moderate reactions (++) compared to 60% from control areas. In cancer areas, 60% showed strong reactions (+++), while only 13% of control areas showed strong reactivity. However, this comparison was statistically insignificant (p-value > 0.001), suggesting decreased acidic mucin expression in diffuse-type gastric adenocarcinoma and a predominance of neutral mucin.

AF-AB Sialo staining revealed that 91% of cases in cancer areas showed moderate reactions, while only 9% from control areas displayed such reactions. In control areas, 90% exhibited weak reactions compared to 10% in cancer areas. The statistical analysis showed insignificance (p-value > 0.001), indicating that diffuse carcinoma doesn't express sialomucins.

AF-AB Sulpho staining showed that 21% of cases in cancer areas exhibited moderate reactions, while 78% in control areas displayed weak reactions. The statistical analysis showed insignificance (p-value > 0.001), indicating that diffuse carcinoma doesn't express sulphomucins.

In cancer areas, 60% of cases exhibited weak reactions, while 13% of control areas showed moderate reactions. Strong positivity was not observed in cancer areas but was present in control areas. The p-value was statistically insignificant (p-value > 0.001), indicating reduced or absent expression of sulphomucins in diffuse-type gastric adenocarcinoma.

Table I: Mucin Expression Patterns in Diffuse Gastric adenocarcinomas

Staining	Cancer Area (+)	Cancer Area (++)	Cancer Area (+++)	P value	Control Mucosa (+)	Control Mucosa (++)	Control Mucosa (+++)
PAS Staining	0	5	18	<0.001	0	10	13
PAS-D Staining	0	2	21	<0.001	5	6	12
AB-PAS Staining	0	9	14	0.351	6	14	3
AF-AB Sialo Staining	2	21	0	0.068	20	3	0
AF-AB Sulpho Staining	18	5	0	0.099	18	10	5
HIDA Sulpho Staining	20	3	0	0.106	3	9	11

(PAS - Periodic acid Schiff reagent, PAS-D- - Periodic acid Schiff reagent with diastase
AB - Alcian blue AF-AB – Aldehyde fuschin- Alcian blue HIDA – High iron diamine)

Table II: Mucin Expression Patterns in Intestinal Type Gastric adenocarcinomas

Staining	Cancer Area (+)	Cancer Area (++)	Cancer Area (+++)	P value	Control Mucosa (+)	Control Mucosa (++)	Control Mucosa (+++)
PAS Staining	0	7	20	<0.001	1	14	12
PAS-D Staining	0	4	23	<0.001	4	12	11
AB-PAS Staining	1	16	10	<0.001	9	10	8
AF-AB Sialo Staining	0	15	12	<0.001	24	3	0
AF-AB Sulpho Staining	0	14	13	<0.001	11	9	2
HIDA Sulpho Staining	5	5	17	<0.001	2	13	12

(PAS - Periodic acid Schiff reagent, PAS-D- - Periodic acid Schiff reagent with diastase AB - Alcian blue AF-AB – Aldehyde fuschin- Alcian blue HIDA – High iron diamine)

III. Mucin Staining Patterns in Intestinal Type Gastric Adenocarcinoma (GAC)(Table II)

PAS staining of normal gastric mucosa predominantly displayed moderate to strong reactivity. In cancer areas, strong reactions were significantly more prevalent (75%) than moderate reactions (25%), as indicated by a p-value of <0.001.

PAS-D staining showed that 85% of cases in cancer areas exhibited strong reactions, while 17% had moderate reactions. In contrast, control areas had 52% strong reactions, 26% moderate reactions, and 17% weak reactions. This finding was statistically significant (p-value < 0.001) and confirms the mucinous origin of the secretion in intestinal-type gastric adenocarcinoma.

Analysis of AB-PAS staining at pH 2.5 revealed that cancer areas had a higher proportion of strong positivity (38%) compared to moderate (32%) and weak (6%) positivity. In control areas, strong positivity was lower (17%), with a higher percentage of moderate (32%) and weak (29%) positivity. The statistical analysis showed significance (p-value < 0.001), indicating the presence of intestinal-type mucin in intestinal-type gastric adenocarcinoma.

AF-AB Sialo staining demonstrated that 55% of cases in cancer areas exhibited moderate reactions, while 44% showed strong reactions. In contrast, control mucosa displayed 85% weak reactions and 11% moderate reactions. This analysis showed a statistically significant value (p-value <0.001), confirming the presence of sialomucins as a component of intestinal-type mucin in intestinal-type gastric adenocarcinoma.

AF-AB Sulpho staining in cancer areas showed that 51% of cases exhibited moderate reactions, while 48% displayed strong reactions. In contrast, control mucosa had 33% weak reactions, 18% moderate reactions, and 7% strong reactions. The statistical analysis demonstrated a significant value (p-value <0.001), indicating the presence of sulphomucins as a component of intestinal-type mucin in intestinal-type gastric adenocarcinoma.

In cancer areas, 62% of cases exhibited strong HIDA Sulpho Staining reactions, 18% had moderate reactions, and 18% showed weak reactions. Control areas displayed 44% strong positivity, 48% moderate positivity, and 7% weak positivity. The statistical analysis revealed significance (p-value <0.001), confirming the presence of sulphomucins in intestinal-type gastric adenocarcinoma.

These findings provide insights into the mucin staining patterns in intestinal-type gastric adenocarcinoma, contributing to our understanding of mucin production and its implications for tumour characteristics.

Gastric adenocarcinoma (GAC) is a complex and clinically significant malignancy that demands in-depth investigations to unravel its diverse clinicopathological characteristics. In this study, we analysed resected specimens of gastric adenocarcinoma and revisited paraffin blocks from previously diagnosed cases to gain insights into this formidable disease.

Demographics and Histopathology:

Our study revealed an intriguing age distribution among GAC patients. A substantial majority, comprising 60% of our cohort, fell within the age group of 56-60 years, with the remaining 40% aged between 60-70 years. The parallel gender distribution trends reaffirm the slight male predilection observed in GAC incidence, a pattern noted across multiple investigations. (11,12) Histologically, our study delineated two primary patterns in GAC patients. Our percentage distribution of patients harmonize with the observations made by in recent study in 2022(13), as well as older studies with larger cohort.(11,12)

Histopathology and Histochemistry:

For shedding light on the intricacies of mucin patterns within different histological subtypes of gastric adenocarcinomas we evaluated expression of mucin in diffuse gastric adenocarcinoma (GAC). The PAS stain analysis corroborating the notion of mucin preservation in this subtype is well documented in the literature(11-13)

However, it's noteworthy that this study solely relied on PAS-D stain for distinguishing between neutral and acidic mucin. Fumiaki Toki. (18) conducted a similar investigation, assessing the mucin phenotypes in advanced gastric adenocarcinoma (AGA) through immunohistochemical staining. Their findings demonstrated that gastric-type-related mucin phenotypes were highly expressed across various histological subtypes of AGA, further supporting the observed mucin patterns in the diffuse GAC subtype.

Transitioning to the AB-PAS stain at pH 2.5, Intriguingly, this comparison yielded a statistically insignificant p-value (>0.001), suggesting a reduced or diminished presence of acidic mucin expression in diffuse GAC and implying that this subtype predominantly expresses neutral mucin. aligning with the outcomes of other research[13].

Conversely, in intestinal GAC, the statistical analysis yielded a significant p-value (<0.001), confirming the presence of sialomucins as a constituent of intestinal-type mucin in diffuse GAC. Notably, this corresponds with research conducted by Elisabetta Cavalcanti.(11), which classified mucin phenotypes into foveolar and intestinal categories.

Lastly, the HIDA stain analysis revealed further insights. Strikingly, strong positivity (+++) was entirely absent in cancer areas but present in control regions. This discrepancy resulted in a statistically insignificant p-value (>0.001), suggesting a reduced or absent expression of sulphomucins in diffuse GAC. Conversely, in intestinal GAC, cancerous areas predominantly displayed strong positivity (+++). Importantly, the statistical analysis revealed a significant p-value (<0.001), affirming the presence of sulphomucins in this subtype. These findings concur with the research conducted by Ajay . (17), reporting a decrease in the number of gastric glands expressing neutral mucins and highlighting the association between long-standing chronic gastritis, intestinal metaplasia, and increased risk of gastric adenocarcinoma development. This underscores the complexity of mucin expression patterns in gastric adenocarcinomas, involving both neutral and acidic mucins and their potential implications in tumour origin.

In conclusion, our study of gastric adenocarcinoma (GAC) has shed light on its complex histopathological features and mucin staining patterns. The identification of distinct staining profiles between diffuse and intestinal GAC provides valuable diagnostic insights, allowing for a more precise characterization of tumour types.. Ultimately, this research represents a step toward enhancing the diagnostic and therapeutic precision in managing gastric adenocarcinoma.

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