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HISTOCHEMICAL DETECTION OF MUCIN IN CANCER COLON AND ITS RELATION TO HISTOLOGICAL GRADE

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ABSTRACT

Background: Colon cancer is one of the major reasons of morbidity and growing mortality rate around the world. Colon cancer is the third most popular cancer in the world with about one million new cases of Colon cancer identified every year. Mucin production by the tumor cells has an influence on the prognosis and therapeutic management. **The aim of this study** was to assess the mucin content in the tumors histochemically and to associate the mucin content with the histological grading of the Tumors. **Subjects and methods :**Retrospective study was conducted on 30 cases of diagnosed colorectal carcinoma, by collecting important clinical and gross examination data from patient's files, doing histopathological examination and histochemistry using Periodic Acid Schiff's (PAS). **Results:** The study showed the peak incidence of colorectal carcinoma was above the age of 50 years (73%) and the patients were predominantly males (60%). The most predominant histological type of colorectal carcinoma in our study was adenocarcinoma (30 cases) from which 6 cases (20%) have mucin content more than 50% and categorized as mucinous adenocarcinoma. The common detected grade was the intermediate grade (58.3%), and the common site was the rectum (40%). From 30 studded cases, mucin positivity was seen in 24 cases (80%) and negative in 6 cases (20%), out of these 24 cases the marked mucin stain were seen in the mucinous carcinoma and high grade non mucinous carcinoma, the moderate mucin stain were detected mainly ingrade 3 carcinoma (64.3%), the mild mucin stain detected in some cases of grade 2 carcinoma (35.7%) while all cases (6 cases) of grade 1 were negative for mucin. **Conclusion:** Mucin positivity was prominent in intermediate and high grade tumor cases and absent in low grade tumors**.** So the use of both histopathological tumor grade and histochemical mucin detection can be valuable prognostic indicators for patients of colorectal carcinoma outcomes.

KEYWORDS: Colorectal Carcinoma, Histochemistry, Mucin, PAS Stain.

INTRODUCTION

Colorectal cancer (CRC) is a serious health problem in the Western world, and has the third highest among the tumor which affect the inhabitants of developed and developing countries. Around the world there are approximately one million new cases per year, affecting 550,000 men and 470,000 women, which reaffirm the importance of this pathology as a public health problem. CRC is the 3rd most common cancer in men and the second most common cancer in women **(Arveloet al, 2015).**

With regard to incidence of CRC, countries with the uppermost incidence rates include Australia, New Zealand, Canada, the United States, and part of Europe. The countries with the lowermost risk include China, India, and parts of Africa and South America. These geographic differences seem to be attributable to differences in dietary and environmental exposures that are imposed upon a background of genetically determined susceptibility **(Alsanea et al, 2015)**.

CRC incidence rates are higher in men by approximately 30% than in women. Reasons for the gender disparity are not fully understood, but partly reflect differences in exposures to risk factors (e.g., cigarette smoking) and sex hormones, as well as complex interactions between these influences **(Ahmad et al, 2017).**

Colorectal cancer risk factors are divided into two groups, those that can be altered and those that cannot. The no changeable risk factors are these factors that cannot be taken under control. These include age, sex, genetics (personal or family history of CRC), chronic colon diseases such as ulcerative colitis, inflammatory bowel disease or Crohn'sdisease. Changeable risk

factors: These are behavioral factors that can be changed to help reduce the risk of CRC. It is reported that more than half of all cancers are linked to risky health behaviors. Changeable factors is like smoking, alcohol consumption, obesity, excessive consumption of red meat, physical inactivity. **(koc,esin and ardic,2016).**

Adenocarcinomas account for the vast majority of colorectal carcinomas, about 90%. It is graded as well, moderately and poorly differentiated depending on the regularity of the glandular architecture as per the WHO classification. Mucin production varies from minimal to abundant (that is 50% or more of the tumor area having mucin content) these cases are categorized as mucinous adenocarcinoma. About 10% of colorectal carcinomas are mucinous type. Mucins are membrane-associated or secretory highmolecular-weight glycoproteins expressed by epithelial tissues and characterised by variable nucleotide tandem repeat subdomains that provide sites for O-glycosylation and play a role in normal physiological processes and in the neoplastic progression of colorectal cancer..Mucin production by the Tumor cells has an influence on the Prognosis and Therapeutic management. **(Lugli et al, 2007; Li et al, 2012).**

It is still not clear if patients with mucinous carcinomas have poorer prognoses than those with non-mucinous adenocarcinomas, or if the excessive mucin production worsens the prognosis **(Li et al, 2012; Lugli et al, 2007). So the aim of this study was** to assess the mucin content in the tumors histochemically and to correlate the mucin content with thehistological gradings of the Tumors.

Subjects and methods

Retrospective study including thirty cases of formalin fixed paraffin –embedded tissue sections diagnosed as colorectal carcinoma. For each case age, gender, tumor site and histologic type were retrieved from the archives of histopathology laboratory, histopathological examination and histochemical staining using Periodic Acid Schiff's (PAS) was done.

Ethical clearance: was taken from the Institutional ethical committee before starting the study.

Representative sections were taken and fixed in 10% buffered formalin for histopathological examination. Sections were stained with the routine haematoxylin and eosin stain using standard protocol. The histological diagnosis was ascertained regarding the type, grade and the amount of mucin content, also special staining with PAS to assess the presence of mucin.

Microscopic examination

After staining, each H&E and PAS stained section carefully examined under microscope to confirm the diagnosis of cancer colon and to detect histological type and histological grades and to detect mucin content.

The interpretation of PAS staining was as follows

Mucins were stained Magenta color with blue nuclei. The colorectal carcinoma cases were divided into the three groups on the basis of mucin content in the whole section. The cases with less than 20% mucin content (+ ve) were categorized as mild, those cases with 20-50% mucin content (++ ve) as moderate and those with more than 50% mucin content (+++ ve) as marked.

RESULTS

A total of 30 cases of histologically confirmed colorectal carcinomas. 22 patients (73%) above 50 years old and 8 patients (27%) were below 50 years old as shown in figure 1, and the patients were predominantly males18 cases (60%) as shown in figure 2. Regarding site 12 cases (40%) were from the rectum followed by sigmoid and transverse colon 6 cases (20%) from each, ascending colon 4 cases (13.3%) and 2 cases (6.7%) from descending colon as shown in figure 3.

Histological finding (types and grading)

All studded cases were histologically diagnosed as adenocarcinoma, and 6 cases of them showed mucin content more than 50% and categorized as mucinous carcinoma. So the studded cases divided into non mucinous adenocarcinoma 24 cases and mucinous adenocarcinoma 6 cases. The grading (differentiation) of non mucinous adenocarcinoma was done according WHO guidelines and showed predominance of grade II carcinoma (moderately differentiated) as more than 50% of examined sections showed gland formation and less 50% solid sheets of malignant cell in 14 cases (58.3%), then grade I carcinoma (well differentiated) as all the examined section showed well-formed malignant glands in 6 cases (25%) and grade III carcinoma (poorly differentiated) as most tumor sections showed solid sheets of malignant cell and less than 50% gland formation in 4 cases (16.7%).

PAS stain finding

Regarding mucin content (PAS positivity), out of 30 studded cases, mucin positivity was seen in 24 cases (80%) and absent in 6 cases (20%). The marked mucin positivity both intracellular and extracellular was seen in all cases of mucinous carcinomas (6 cases) and intracellular in all cases of grade III non mucinous carcinoma (4 cases). The moderate mucin positivity was observed in 9cases of grade II carcinoma, while the mild mucin positivity seen in only 5 cases of grade II carcinoma. The negative mucin staining was observed in grade 1 carcinoma cases.

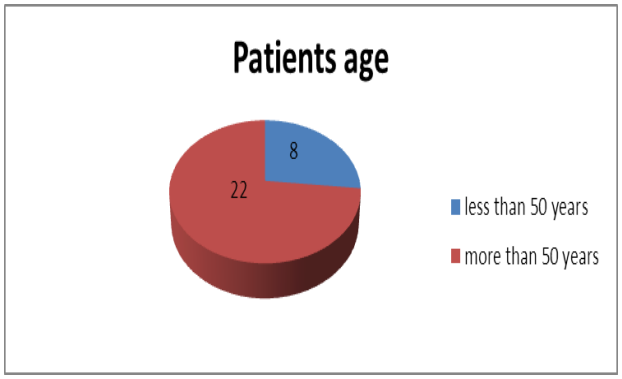

Figure. 1: Frequency of patient's age under study.

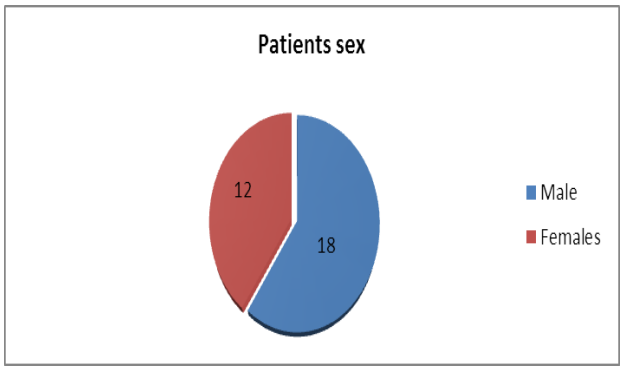

Figure. 2: Frequency of patient's sex.

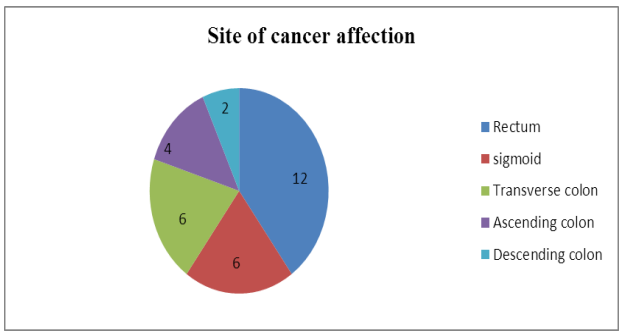

Figure. 3: Site distribution in studded cases.

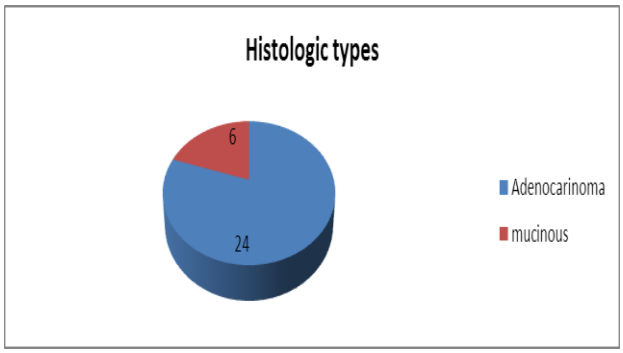

Figure. 4: Frequency of histologic types in patients under study.

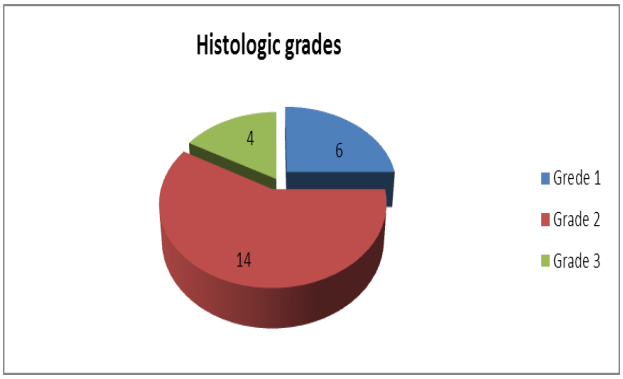

Figure. 5: Frequency of histologic grades of patients under study.

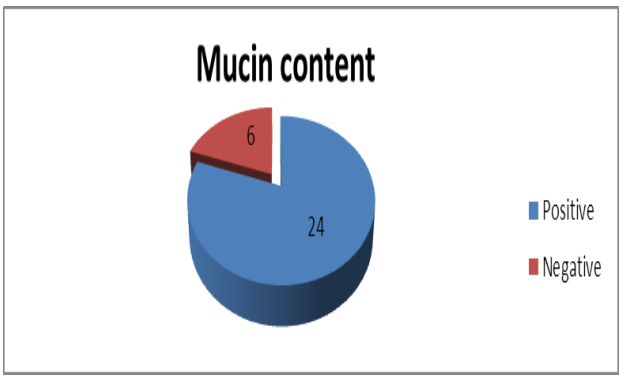

Figure. 6: Frequency of mucin content in studded cases.

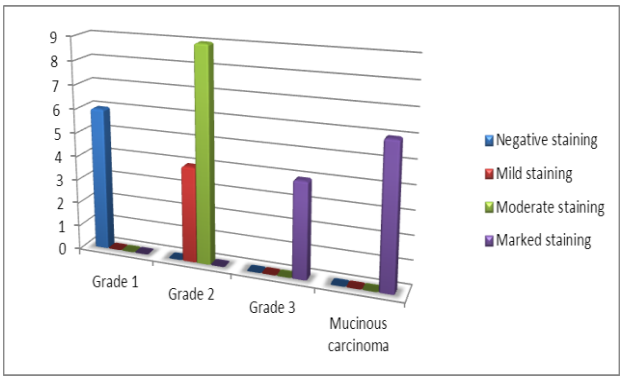

Figure. 7: Frequency of mucin content according to tumor grades.

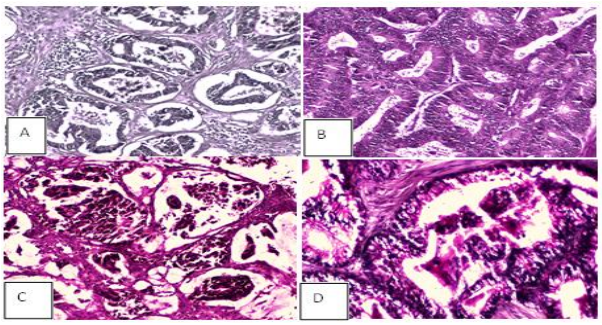

Figure. 8: Microscopic examination of PAS stained sections. (A) negative staining in grade I carcinoma, (B) moderate staining in grade 2 and (C&D) marked staining in mucinous and poorly differentiated carcinoma sections.

DISCUSSION

Colorectal cancer (CRC) is diagnosed in more than 1.3 million people worldwide, annually, with the number steadily increasing. Currently globally, this cancer is the third most common cancer in men and second most common in women**.(Kasprzak et al , 2018**). Amongst the gastrointestinal malignancy, Colorectal Carcinomas are the commonest variety. Many factors are responsible for its increased incidence; recent trends have shown that its incidence is even increasing in the developing countries. (**Borgohain et al, 2017**). In the present study the commonest age group for Colorectal Carcinomas was above 50 years which was consistent with the findings of **Arvelo et al, 2015 and Alharbi, 2017,** in their study the age group was 50-65 years as shown in figure 1. Out of 30 cases, 18 patients were males and 12 were females hence the ratio 1.5:1, colorectal carcinomas were predominantly seen in males in the studies done by **Ahmad et al, 2017 and Borgohain et al, 2017** as shown in figure 2**.** Most of the cases were detected in the rectum in our study as shown in figure 3, this finding was consistent with the findings of **Qing *et al.;* in 2003 and Neil & Oliver, 2004**. In our study the commonest histological type was adenocarcinoma and amongst which mucinous adenocarcinoma accounted for 20% and was similar to results shown by **Danquah et al, 2017 and Borgohain et al, 2017**. Also consistent with others previous studies done by **Laishram et al,** that have shown that mucinous adenocarcinoma comprises about 15% of colorectal carcinomas and occurs most commonly in the rectum as shown in figure 4.

Certain pathologic features have been considered important prognostic determinants in adenocarcinoma of colon and rectum, including differentiation (grading) as high grade tumor (poorly and undifferentiated tumor) usually indicating bad patient prognosis and outcomes. As regard grading of our cases, the predominant grade was grade II (intermediate grade tumor) accounting for (58.4%)which agreed with result of **Ahmad et al, 2017 and Ueno et al , 2012** as shown in figure 5.

The other pathologic prognostic feature is the Mucin production by the tumor. Mucins are complex carbohydrates secreted by epithelial cells, and thought to play an important role in protecting the intestine from chemical or physical injury. Mucin glycoprotein has been implicated in the pathogenesis of epithelial cell malignancies **(Boland and Goel 2010**) as shown in figure 6.Alteration of expression pattern of mucins has been described in carcinomas as well as in their precursor's lesions. On the basis of the mucin content, the marked mucin positivity was found in mucinous carcinoma and grade III non mucinous carcinoma. The moderate positivity was found mainly in (64.3%) of grade II moderately differentiated carcinoma while mild mucin positivity was detected in (35.7%) of grade II carcinoma. All cases of grade 1 were negative to mucin staining, which was consistent with the finding of **Danquah et al., 2017,** and **Nikumbhet al., 2012** as shown in figure 7. A positive result will lead to a more thorough examination of the patient and increase the chance of an early diagnosis of cancer(**Hadi et al, 2009**).

CONCLUSIONS

This study concludes that: the occurrence of colorectal carcinoma is more common in male sex than female and in older than younger age groups. The adenocarcinoma is the main histological variant of colorectal cancer and most patients represented at moderate grade tumors. Finally mucin positivity was prominent in high grade and intermediate tumor cases and absent in low grade tumors. So the histochemical detection of mucin in cases of colorectal carcinoma may be used as valuable and cost effective test for assessing and prediction of the prognosis of this cancer.

RECOMMENDATIONS

We support the importance of the use of mucin histochemical study as a valuable and cost-effective tool for assessing the prognosis of colorectal carcinoma specially the intermediate grades of non-mucinous type and can be taken as an routine diagnostic tool in histopathology.

More studies should be undertaken in the future to establish the role of histochemical detection of mucin as a prognostic indicator and correlate it to clinical outcome of the patients by post study follow up of the patients

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