Introduction

Colorectal cancer is a common malignant tumor and is the fourth leading cause of cancer death worldwide. In Saudi Arabia, the incidence of all cancers has steadily increased, and colorectal cancer is now ranked as the second most common cancer in Saudi Arabia the most common cancer among males and the third most common among females. Colorectal cancer incidence is increasing among patients > 50 years old, and a significant proportion of colorectal cancer cases are identified with stage II disease.

The judgment to use adjuvant treatment for cases after restorative surgical removal for stage II colorectal cancer is difficult, and it has been proposed that this should be decided on a case-by-case basis. The long-term survival rate of colon cancer patients is 55–58%. Early diagnosis and prompt treatment are believed to improve survival in patients with colon cancer. Therefore, the detection of colon cancer using an accurate histopathological method is important. DNA content of colorectal carcinoma appears to play an important role in indicating the biologic aggressiveness of the disease. Many studies highlighted the role of tumor DNA content and its prognostic significance in patients with colorectal carcinoma. Deoxyribonucleic acid detection using Feulgen reaction may provide valuable prognostic information in cancer, therefore, to start of appropriate treatment, more specific detection methods of colon cancer are needed. There is anticipation, however, that these decisions could be made more rationally if more solid data were available as dis-
Materials and Methods

Study design: This retrospective observational descriptive hospital-based study was conducted at the College of Applied Medical Science, Clinical Laboratory Sciences Department and King Abdul Aziz specialized hospital, during the period between December 2018 to February 2019.

Study population: Paraffin sections from 50 patients previously diagnosed with colon cancer were included in this study. Specimens with mucosal autolysis or those from patients who had undergone chemotherapy or radiotherapy were excluded.

Tissue preparation and staining methods

The tissues were cut into 4-μm thick sections and mounted on microscope slides. All sections were incubated in an oven at 60°C for 1 h, then in three changes of xylene for 2 minutes each to remove the wax and were treated with three changes of absolute ethyl alcohol each for 2 minutes, then rehydrated through gradual ethyl alcohol and placed in water. For all cases, two-section were treated with three changes of absolute ethyl alcohol each for 2 minutes, then rehydrated through gradual ethyl alcohol and placed in water. For all cases, two-section were

The other paraffin sections were used for DNA demonstration using the Feulgen reaction. Sections were placed in normal hydrochloric acid (N - HCL) at room temperature for 1 minute, then treated with preheated (N - HCL) at 60°C for 10 minutes. Again, sections were rinsed in N - HCL at room temperature for 1 min. After that, sections were transferred to Schiff’s reagent for 45 minutes, then rinsed in three concentrations of bisulfite solution for 2 minutes each, then rinsed in water and counterstained in 1% light green for 1 minute. Finally, sections were dehydrated in alcohol, cleared in xylene, and mounted in distyrene a plasticizer and xylene (DPX). Twenty fields were examined for each section by expert pathologist blinded to the tissue identity and grades for intensity of staining was as follows: levels of staining assessed were low staining that is compatible to control slide, medium staining, and intense staining. All sections were stained in the same batch to eliminate inter batch variation.

Ethical concerns: Ethical approval was obtained from the research ethics committee of the College of Applied Medical Sciences at Al-Taif University.

Data analysis: Data analysis was performed using SPSS version 21. Qualitative data were expressed as numbers and percentages, and a Chi-squared test was done to assess the relationship between variables. Quantitative data were expressed as mean and standard deviation (mean ± SD). A p-value of less than 0.05 was considered significant.

Results

The age of the participants ranged from 22 to 100 years, and the mean age was 60.66 ± 16.41 years. Twenty-six patients (52%) were male, 48% were between 61 and 100 years old, 42% were between 41 and 60 years old. Most of the specimens (82%) were from the colon, and 18% were from the rectum.

Regarding the clinical presentation of the disease, most patients (n = 39, 78%) presented with tumor mass, four (8%) had obstruction bowel disease, three (6%) presented with symptoms of ulcerative colitis, and four (8%) complained of bleeding. The histopathological diagnosis showed that 41 (82%) were adenocarcinoma cases, and nine (18%) were benign tumors. The adenocarcinoma grading was as follows: 28 (56%) moderate differentiated, nine (18%) low differentiated, three (6%) high differentiated, and one case (2%) poorly differentiated adenocarcinoma.

The staining intensity of the Feulgen reaction showed that 46% of tissue sections had intense staining, 22% had medium staining, and 14% had results compatible to control staining. (Figs. 1 and 2).

There was a non-significant association between cancer grade and age group, and the most frequent cases were moderate differentiation among cases between 61 and 100 years old (n = 15, 53.5%). Regarding the association between tumor grades and gender, there was no significant association, and the moderate differentiation was the most common histopathology result occurring among both sexes (Table 1).

No significant association was found between grade and result of the Feulgen reaction, and the most common moderate differentiation occurred in intense staining.
Fig. 1  Staining results of Feulgen reaction

Hematoxylin and eosin X10 adenocarcinoma of the colon

Hematoxylin and eosin X20 adenocarcinoma of the colon

Feulgen reaction colon cancer intense staining X10

Feulgen reaction colon cancer moderate staining X40

Feulgen reaction colon cancer intense staining X20

Feulgen reaction colon cancer intense staining X10

Fig. 2  Illustration and slides pictures
Table 1 Demographical Characteristics and grads of colorectal tumors.

| Age group    | Benign N (%) | Low differentiated N (%) | Moderate differentiated N (%) | High differentiated N (%) | Poorly differentiated N (%) | Total | p-value |
|--------------|---------------|--------------------------|-------------------------------|----------------------------|-----------------------------|-------|---------|
| 20-40 Years  | 4 (44.5%)     | 1 (11.2%)                | 4 (14.4%)                    | 0 (0%)                    | 0 (0%)                      | 9     | 0.817   |
| 41-60 Years  | 5 (55.5%)     | 4 (44.4%)                | 9 (32.1%)                    | 1 (33.3%)                 | 1 (100%)                    | 20    | 0.408   |
| 61-100 Years | 0 (0%)        | 4 (44.4%)                | 15 (53.5%)                   | 2 (66.7%)                 | 0 (0%)                      | 21    | 0.425   |

Gender

| Gender | Benign N (%) | Low differentiated N (%) | Moderate differentiated N (%) | High differentiated N (%) | Poorly differentiated N (%) | Total | p-value |
|--------|--------------|--------------------------|-------------------------------|----------------------------|-----------------------------|-------|---------|
| Male   | 3 (33.3%)    | 5 (55.5%)                | 13 (46.4%)                   | 2 (66.7%)                 | 0 (0%)                      | 23    | 0.668   |
| Female | 6 (66.7%)    | 4 (44.5%)                | 15 (53.6%)                   | 1 (33.3%)                 | 1 (100%)                    | 27    | 0.668   |

Total 9 (100%) 9 (100%) 28 (100%) 3 (100%) 1 (100%) 50 (100%) 

Table 2 The relationship between Feulgen reaction and colorectal tumor grade.

| Feulgen reaction | Benign N (%) | Low differentiated N (%) | Moderate differentiated N (%) | High differentiated N (%) | Poorly differentiated N (%) | Total | P-value |
|------------------|--------------|--------------------------|-------------------------------|----------------------------|-----------------------------|-------|---------|
| 0                | 2 (28.6%)    | 0 (0%)                   | 4 (57.1%)                    | 0 (0%)                    | 1 (14.3%)                   | 7     | 0.284   |
| 1                | 1 (11.1%)    | 3 (33.3%)                | 5 (55.6%)                    | 0 (0%)                    | 0 (0%)                      | 9     | 0.187   |
| 2                | 1 (9.1%)     | 2 (18.2%)                | 8 (72.7%)                    | 0 (0%)                    | 0 (0%)                      | 11    | 0.229   |
| 3                | 5 (21.7%)    | 4 (17.4%)                | 11 (47.8%)                   | 3 (13.1%)                 | 0 (0%)                      | 23    | 0.462   |

N.B.: The tissue identity and scored for intensity and location of staining as fellows: level of staining assessed as 0 = Comparable to control that is 1 = low staining, 2 = medium staining, and 3 = intense staining.

Discussion

This study found that more than 80% of the study groups were between 40 to 100 years old. The frequency of colon cancer increases among those older than 50 years, and continues to increase with increasing age. Our data support these findings: 40% of the colon cancer patients included in our study was between 40 to 60 years old, and 42% were > 61 years old, the cases of more than 61 years old reported with an increased incidence of high grades colon cancer.

Age was a risk factor for colon neoplasia and cancer, and older age is the most important predictor for the prevalence of colon adenocarcinoma. Moderately differentiated adenocarcinoma is more likely frequent in females (n = 15, 53.6%) than males (n = 13, 46.4%), but this did not reach statistical significance. Furthermore, we detected a tendency of an increase in the incidence of various grades of colon cancer (from low grades to poorly differentiated adenocarcinoma) among females (n = 27, 54%) than males (n = 23, 46%). Numerous studies have supported this finding and have additionally stated that patients with colon cancer are elderly and more frequently female; moreover, they have more advanced tumor stages, and often poorly differentiated adenocarcinoma.

Nuclear DNA analysis is a generally recognized and accessible method for assessment of proliferative activity correlated with increasing histological tumor grade. Abnormal DNA content can be one of the characteristics in neoplasia and associated with a high occurrence in malignancy. Deoxyribonucleic acid content, as a predictive aspect of colon cancer, is extremely controversial. Numerous studies have proposed that DNA ploidy is an independent predictive factor, but others have stated that DNA content is not related to clinical consequence. Some of these discrepant observations might be explained by the different methods used for nuclear DNA demonstration. In this study, we focused on different grades of colon cancer; particular emphasis was placed on the Feulgen reaction for DNA, which can provide important information and the staining intensity increases in high-grade colon cancer compared to low-grade colon cancer and hyperplasia.

Here, we report that the staining intensity of the Feulgen reaction in colorectal cancer could be used to semi-quantitatively evaluate the DNA content of colon cancer cells. Although the DNA content can contribute to the prognosis of colon cancer, our data did not show a significant correlation between staining intensity and the different grades of colon cancer. This finding is likely because of the sample size of this study.

Conclusion

DNA demonstration using the Feulgen reaction is a simple and successfully estimated in this study. However, the correlations between DNA staining and several clinicopathological factors were not statistically significant. We recommend that DNA staining should be combined with routine histopathological examination for better understanding of patients’ clinical malignant potential of...
the colorectal cancer.

Limitations:
The present study had a small sample size. Future work, including a greater number of patients, is now is recommended.

Acknowledgments:
Authors gratefully to the laboratory staff at the pathology department at King Abdul-Aziz specialized hospital at Taif provinces for their great helping in collecting the samples.

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