The Ki-67 immunohistochemical expression of colorectal polyps

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Abstract

Background and objective: The chosen study mainly investigates the importance of immunohistochemical expression of Ki-67 in Colorectal polyps which are considered intraepithelial neoplasms and can be found in diverse dimensions from small polyps to large ones. During the examination process, dysplasia was observed in all polyps examined in the Ki-67 proliferation index. The study will clarify also their relationship to the size, dysplasia, and location of the polyps. Malignancy of the polyps with high and low dysplasia is far more expected. The idea of the development of colorectal cancer from previously existing adenomas has been widely spread based on epidemiological, clinical, postmortem and molecular biological studies. The significance of cell proliferation in the change of adenomatous polyps into colorectal cancer is incontestable, which is considered an axiom. The Ki-67 protein is another immunohistochemical marker applied for the identification of proliferative cells. So that there is a significant relationship between the type of adenoma and the Ki-67 expression, and three of the polyps in the Ki-67 expression were considered dysplasia.

Methods: Between the period of 2011 and 2016, 100 patients, (out of 40 were women and 60 of them were men) with endoscopic polypectomy were retrospectively analyzed at the Endoscopy Department of the Central Customs Hospital. Immuno-histochemically Ki-67 expressive polyps were performed in eight patients involved in the study group. The Ki-67 response was conducted via the monoclonal reflection bodies. (Leica Bond-Maxautomated immune strainer apparatus) The percentage of positively charged cells was recorded as Ki-67 labeling index (Ki-67-LI) and each painted nuclear was considered positive.

Results: The retrospective study included 100 paraffin blocks of polypectomized specimens. According to the histological examination of Hematoxylin and Eosin stained (H&E) preparations, there were 45 tubes, 23 tubulovillous, 16 inflammatory, 13 hyperplastic, 2 serrated and one polyp in the villous form were detected. The mid-aged patients were 56.78 ± 1.64. Most of the patients around 38 patients in this study constituted 50 to 69 age groups and five patients were included between 40 to 49 age groups. The gender distribution of the cases was determined in 60 of males, 40 of females. Dysplasia was observed in all polyps examined in Ki-67 proliferation index. There is a significant relationship between the type of adenoma and the Ki-67 expression, and three of the polyps in the Ki-67 expression were considered dysplasia.

Conclusions: The study revealed that the Ki-67 immunohistochemical expression is significantly related to the size and the degree of dysplasia in colorectal adenomas, but does not have a significant relationship with the sex, age, and the type colorectal adenomas.

Key words*: colorectal adenoma, colorectal polyps, colon polyps, dysplasia, Ki-67 expression,*

Introduction

Colorectal polyps are intraepithelial neoplasms growing through from the wall of the colon and rectum to its origin can be found in different sizes ranging from small polyps to large ones. Generally, colon polyps are more founded pathology and commonly seen over 50 aged patients and are not only the malignant pathology but more the precursor of malignant neoplasms, where the polyps have a high likelihood of cancer. Cancer normally develops in around 5% of adenomatous polyps. (1,2)

Colorectal adenomas are more commonly observed in the developed Western states. Therefore, nutritional factors, environment, inflammatory diseases of the gastrointestinal tract, ulcerative colitis, and Crohn's disease play an essential role in the formation of colorectal polyps. (3,4) Thus, the reduction of proliferation and apoptosis in colon cells increases the risk of carcinogenesis within the background of inflammatory diseases. Colorectal adenomas are mainly accompanied by epithelial dysplasia and as the main cause of malignant neoplasms, are "sneak" lesions of colon (intestinal) cancer, which plays a major role in the spread of colorectal polyps, and the formation of malignant neoplasms. (5,6) The adenomatous polyps are the crucially important cause of colorectal cancer. (7) Because of the fact that possibility of being cancer is higher in patients with adenomatous polyps.

Colon cancers encompass 30% to 50% of adenomas. The process of colorectal adenomas' transition to cancer is considered to be whether "seated" tumor, particularly "unformed" crypts located in the left hemi-colon, "toothed" tumor including crypts until basal layer or polyp-similar tumors consisted of ectopic crypts. (8.9,10)

The formation of ectopic crypts results in dysplasia. Abnormal differentiation of crypts in the basal layer leads to the development of colorectal cancer. (10) S that the colorectal polyps are considered in different sizes, ranging from small to large-sized polyps, even to pedunculated ones. Histologically, polyp cancers include normal epithelium, adenomatous tissue, atypia, and invasive invasion. So that the bigger polyps have a higher possibility of being cancer.

Polyps less than (<) 1 cm in size constitute 1% likely to be malignant, slightly greater than (>) 1 cm, around 1 to 2 cm of those have a 10% chance of becoming malignant, but those are 2 cm or greater (>) have a 40% chance of transforming into malignancy. However, in villous adenomas, polyps less than (<) 1 cm and 1 to 2 cm have a 10%, and more than 2 cm have a 53% chance of becoming malignant. So that these derivatives can be repeated and the risk of transition to the neoplastic process can be variable depending on their characteristics. (multiplicity, dimensions, histological structure, and dysplasia) It has been considered that 15% of all derivatives with a size of more than 1 cm are likely to be transmitted to malignant neoplasms within 10 years. (2,10)

The significance of cell proliferation in the change of adenomatous polyps into colorectal cancer is incontestable, which is considered an axiom. The Ki-67 protein is another immunohistochemical marker applied for the identification of proliferative cells. It is expressed in all phases of the cellular cycle, except the G0 phase. Thus, Ki-67 is both a nuclear and a nucleolus protein. (10) Contrary to many other cell cycle-associated proteins like PCNA (Proliferating Cell Nuclear Antigen), the Ki-67 antigen is consistently absent in inactive cells and is not detectable during the DNA reparation processes. Consequently, the presence of Ki-67 antigen is strictly related to the cell cycle, which is restrained to the nucleus by signifying an important role of this structure in the maintenance or regulation of the cell cycle. (11)

The monoclonal antibody Ki-67 has the particular feature of recognizing. In this case, Ki-67 is the key marker of cellular proliferation sensitivity, participating in cell proliferation as both a nuclear and a nucleolus protein. (3) Ki-67 proteins are present in all active phases of the cell cycle (G1, S, G2, and mitosis) but are not found in inactivated cells, and not in the process of DNT reparation. Thus, the Ki-67 antigen is seriously associated with the cell chain and is restricted to the nucleus showing that this structure plays a significant role in maintaining and/or regulating the cell cycle. A number of studies have shown that the clinical evaluation of the Ki-67 immuno-histochemical expression is important and there is no relationship between dysplasia and immunoreactivity of the Ki-67 expression. (5,6)

The study was conducted in order to assess the importance of the Ki-67 expression as an immunohistochemical marker for the early detection of the malignant changes in various colorectal adenomas. Hence, the chosen research is also aimed at evaluating the relationship between this marker and the different clinical-pathological parameters. (Including dysplasia of adenomas, location, type, size of the polyps). The aim of the study is to analyze the Ki-67 expression in the colorectal polyps and to clarify their relationship to the size, dysplasia, and location of the polyps.

Methods

Between the period of 2011 and 2016, 100 patients, (out of 40 were women and 60 of them were men) with endoscopic polypectomy were retrospectively analyzed at the Endoscopy Department of the Central Customs Hospital. The colonoscopy examination was performed in patients with complaints of gastrointestinal disorders, bleeding, bloody mucosal excretion, constipation, and at the same time, more than 45 aged people applied for screening purposes. The day before, the intestinal bowel preparation was done, and the cardiology consultation was requested for those with heart problems. The polyps detected during the investigation were interrupted depending on their sizes. So that the small polyps of less than 0.5 cm were taken out through the forceps biopsy and the large polyps were taken into the squeezed circle and then were eliminated. The tissue preparation was examined in the Central Customs Hospital Patomorphology Department. As a result, sections were taken from the paraffin embedded tissues by using microtome. All polyps detected amid the examination were sent to the pathological investigation. Pursuant to the pathohistologic

examination, there were 45 tubes, 23 tubulovillous, 16 inflammatory, 13 hyperplastic, 2 serrated and one polyp in the villous form were detected.Initially, all polyps were evaluated with Hematoxylin and Eosin stain. (H&E stain) At this time, the injured drugs were removed out from the investigation group.

Immunohistochemically Ki-67 expressive polyps were performed in eight patients involved in the study group. The Ki-67 response was carried out via the monoclonal reflection bodies. (Leica Bond-Maxautomatedimmunostainerapparatus) The percentage of positively charged cells was recorded as the Ki-67 labeling index (Ki-67-LI). As a result, each painted nuclear was considered positive.

Results

This retrospective study included 100 paraffin blocks of polypectomized specimens. According to the histological examination of Hematoxylin and Eosin stained (H&E) preparations, there were 45 tubes, 23 tubulovillous, 16 inflammatory, 13 hyperplastic, 2 serrated and one polyp in the villous form were detected. The middle age of the patients was 56.78 ± 1.64. Most of the patients around 38 patients in this study constituted50 to 69 age group and 5 patients were included between 40 and 49 age group. The gender distribution of the cases was determined in 60 of males, 40 of females

Generally, the proportion of the detection of colorectal adenomas in men and women is about 1.5:1. As for the localization of colorectal adenomas, the distal region of the colon was superior to the proximal region. The average size of the colorectal adenomas was 1.27 cm ± 0.11, which ranged from 0.3 to 3.2 cm. The average size of the villous adenomas was 1.14 cm. The size of the tubular adenomas ranged from 0.8 to 3 cm, with an average of 1.38 cm. Tubulovillous adenomas range from 0.3 to 3.2 cm, with an average of 1.4 cm.
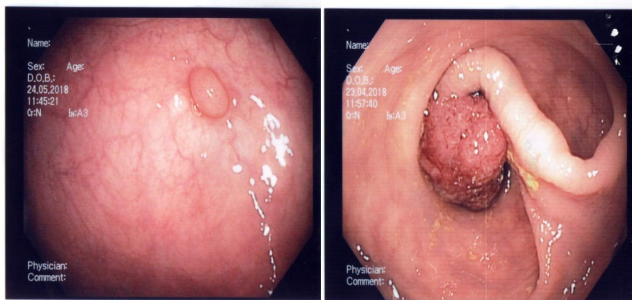

Figure 1. The presence of colorectal polyps during their colonoscopic examination

When the cases were inspected in terms of having or not having dysplasia, it was detected that dysplasia was present in 42 cases and dysplasia was absent in 58 cases. When 42 cases with dysplasia were followed, 26 cases constituting 61.9 % of it proceeded to the malignancy over time. 16 cases estimating 38.1 % of it did not develop the malignancy. Malignancy of the polyps with high and low dysplasia is far more expected. Dysplasia has been detected in large dimensional polyps, which overlaps with the literature data. In this manner, in the chosen study, there is 32.5% of dysplasia in polyps less than 1 cm, and polyps 1 to 3 cm have 70.58% of dysplasia, and 100% of dysplasia is observed in 3 cm large polyps. Dysplasia was detected 26 (32.5%) of 80 patients, which have smaller polyps of 1 cm. We found dysplasia in 12 out of 17 polyps (70.58%) between 1 to 3 cm in size, while we found dysplasia in all 3 polyps (100%) larger than 3 cm.

However, 13 of the smaller polyps with 1 cm diameter are hyperplastic polyps and none of these hyperplastic polyps has dysplasia. Serrated polyps at a size smaller than 1 cm did not have dysplasia. All of the 15 inflammatory polyps were smaller than 1 cm in size, and none was showing the signs of dysplasia. Dysplasia was observed in all polyps examined in the Ki-67 proliferation index. There is a significant relationship between the type of adenoma and the Ki-67 expression, and three of the polyps in the Ki-67 expression were considered dysplasia (Figure 2)
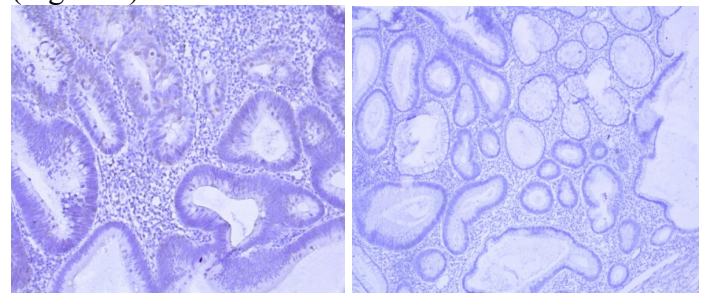

Figure 2. Ki-67 expression in colorectal adenomas.

Discussion

The colorectal carcinomas are the second most common carcinomas in women and the third most common carcinomas in males. So that 90% of colorectal carcinoma cases are adenocarcinoma. (13) The colorectal carcinomas are a multifactorial disease, the majority of which constituting 80 % are sporadic, while the rest of them have an identifiable genetic or ancestral history. It has been shown that the colorectal carcinoma develops from the existing

colorectal polyps, especially a few years after adenomas. The conversion of adenomatous polyps to carcinoma is the widely investigated process and is commonly known as the adenomas-carcinoma sequence. The different features such as size, the number of adenomas, histological type, and grade of dysplasia are the key predictors of the malignant potential determination. (14) The concept of the formation of colorectal cancer from previously existing adenomas has been widely spread based on epidemiological, clinical, postmortem and molecular biological studies. (10) Although this is not a major epidemiological survey, it has generally shown that colorectal cancer was detected in men aged between 50 and 69 (72.34%) constitutes 59.57% of them. Malignancy is detected with the various features such as size, number of adenomas, histological type, and grade of dysplasia, which are the prognosticators of potential malignancy.(14) Tubulovillous changes in polyps have been reported to be associated with malignancy.(15)

In our study, we also observed malignancy over time in tubular adenomas (20%), tubulovillous adenomas (96.57%), and villous adenomas (100%). However, it was worth mentioning that malignancy did not grow in the hyperplastic polyps and the inflammatory polyps. According to the scientific research conducted by Khatibzadeh, Andrei, and Alexander in the Russian Federation, taking into account the pathological parameters, tubulovillous adenomas were dominant in this study and subsequently found to have villous adenomas. (7,8)

As the size of polyps increases, the histopathological changes have also turn out to be heavier. (15) It is known that the large polyps are associated with dysplasia. (23) However, the size is not a certain decisive measure to define it. (15) In our study, we also found 32.5% of the dysplasia in polyps smaller than 1 cm. We observed that 70.58% dysplasia in 1 to 3 cm polyps and 100% of dysplasia in polyps larger than 3 cm. As the size of polyps increases, the histopathological changes have also become heavier. (15) The correlation dysplasia was not observed in the polyps of Ki-67. While evaluating the relationship between the Ki-67 and the immunohistochemical expression of the polyps, it has been shown that there was no significant correlation between age, sex, and type of adenoma. (9)

There was no significant relationship between the Ki-67 expression and the dimensions of the polyps. Thus, 5 polyps having expression are smaller than 1 cm in size, and 3 polyps are 1 to 3 cm. Although there is a general positive correlation between Ki-67 expression of colorectal polyps, it has not been considered statistically significant. The chosen research agrees with the study of Tocantins de Sousa et al,2012 claiming that the expression of Ki-67 was higher in adenomas as well as in adenomas with high-grade dysplasia.(11) The study revealed that the Ki-67 immunohistochemical expression was significantly correlated with the size and degree of dysplasia in colorectal adenomas, but it was found that Ki-67 did not have a significant relationship with the sex, age, and the type colorectal adenomas.Thus, Ki-67 immunohistochemical expression can be considered the part of routine pathological assessments with other conventional prognostic factors in patients with dysplastic colorectal adenomas.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of Central Customs Hospital at which the examination process were conducted and with with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The patients who underwent endoscopic polypectomy at the Endoscopy Unit of Central Customs Hospital was examined. Patients with intestinal habitual disorders, bleeding, bloody mucus, constipation for colonoscopy was selected. In addition, patients who have completed the age of 45 and who have applied for screening purposes will be taken to work. Removed polyps were assessed by Tattooing and Hematoxylin Eosin (HE) staining procedure and immunehistochemical expression of Ki-67 at the Department of Pathology of Central Customs Hospital.

Authorship

The two aforementioned authors contributed equally to the study.

References

1. Andrey B, Alexander D. "Peculiarities of Vascular Component of Communicative Systems in Rectal Adenomas". International Journal of Collaborative Research on Internal Medicine & Public Health 2009 (1):12-21.

2. Atkin WS, Saunders BP, British Society for Gastroenterology. "Association of Coloproctology for Great Britain and Ireland Surveillance guidelines after removal of colorectal adenomatous polyps". Gut 2002. Oct; 51(5)(Suppl 5):V6-V9 10.1136/gut.51.suppl 5.v6.

3. Citarda F, Tomaselli G, Capocaccia R, Barcherini S, Crespi M; Italian Multicentre Study Group, Gut. "Efficacy in standard clinical practice of colonoscopic polypectomy in reducing colorectal cancer incidence", 2001 Jun;48 (6):812-5.

4. Hahn WC, Weinberg RA. "Modelling the molecular circuitry of cancer". Nat. Rev Cancer 2002 May;2(5):331-341.

5. Khatibzadeh N, Ziaee SA, Rahbar N, Molanie S, Arefian L, Fanaie SA, "The indirect role of site distribution in high-grade dysplasia in adenomatous colorectal polyps", 2005 Oct-Dec;1(4):204.

6. Scholzen T, Gerdes J. "The Ki-67 protein: from the known and the unknown". J Cell Physiol 2000 Mar;182(3):311-322.

7. Su Y, Shrubsole MJ, Ness RM, Cai Q, Kataoka N, Washington K, Zheng W. "Immunohistochemical expressions of Ki-67, cyclin D1, beta-catenin, cyclooxygenase-2, and epidermal growth factor receptor in human colorectal adenoma: a validation study of tissue microarrays", Cancer Epidemiol Biomarkers Prev. 2006 Sep; 15(9):1719-26.

8. Vernillo R, Lorenzi B, Banducci T, Minacci C, Vindigni C, Lucenti Fei A, et al. "Immunohistochemical expression of p53 and Ki-67 in colorectal adenoma and prediction of malignancy and development of new polyps". Int J Biol Markers 2008 Apr-Jun;23(2):89-95.

9. Walysson Alves Tocantins de Sousa, LusmarVeras Rodrigues, Raimundo Gerônimo da Silva Jr, Fernando Lopes, "Immunohistochemical evaluation of p53 and Ki-67 proteins in colorectal adenomas". Vieira Department of Surgery, Federal University of Ceará, Fortaleza, Brazil Arq. Gastroenterol. vol.49 no.1 São Paulo Jan. /Mar. 2012

10. Winawer SJ, Zauber AG, Fletcher RH, Stillman JS, O'Brien MJ, Levin B, et al; US Multi-Society Task Force on Colorectal Cancer; American Cancer Society. "Guidelines for colonoscopy surveillance after polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society". Gastroenterology 2006 May;130(6):1872-1885.

11. Tocantins de Sousa W, Rodrigues L, Gerônimo da Silva R, Vieira F, "Immunohistochemical evaluation of p53 and Ki-67 proteins in colorectal adenomas". Brazil protocol. Gastroenterol. vol.49 no.1 São Paulo Jan/Mar. 2012. 593.