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Evaluation of B-catenin Expression with Stromal Mast Cells Concentration in Tubular Colorectal Adenomatous Polyps by using Immunohistochemical Method

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A B S T R A C T

Currently, one of the most important issues that have attracted the attention of specialists at different medical fields is proper treatment as well as assessment of disease behavior and prognosis. One of the latest methods for assessing the prognostic characteristics of tumors is to study the reactions of inflammatory cells of the patient's body to these lesions. In these methods, the number of inflammatory cells (such as Natural Killer Cells, polymorphonuclears, eosinophils, and mast cells) in the involved tissue or peripheral blood is calculated and based on the resulting number the tumor behavior is predicted. The aim of this study was to examine the relationship between the expression of the beta-catenin gene and concentration of stroma mast cells in colorectal Adenomatous polyps using the Immunohistochemical method. In a descriptive-analytical study that was carried out in Tabriz on patients with colorectal Adenomatous polyps, the relationship between the expression of the beta-catenin gene and concentration of stroma mast cells was studied using the Immunohistochemical method in colorectal Adenomatous polyps. The mean level of CD117 positive mast cells% in patients with tubular adenoma lesion and severe dysplasia was significantly lower than patients with mild and moderate dysplasia. The mean level of nuclear and cytoplasm beta-catenin% in patients with tubular adenoma lesions and mild dysplasia was significantly lower than patients with moderate and severe dysplasia.

Introduction

Currently, one of the most important concerns of specialists from different medical disciplines is proper treatment, assessment of patient's behavior and prognosis. In the current methods for clinical assessment, the prognostic characteristics of diseases along with all of the clinical and histopathological findings about diseases are examined (1).

However, in the case of tumors these assessments call for identification of the place of tumor and full removal of the lesion. It also involves histopathological examination and final pathological diagnosis. In addition, surgeons have always wondered about the upper boundary of lesions and whether it is necessary to apply aggressive treatments to the lesion. They also wonder whether tumors involve micro-metastasis and if yes they wonder about the chances of micro-metastasis. They also are concerned about the survival rate of patients and whether the chances of recurrence are high or very low. Such concerns have resulted in plenty of studies aimed at finding new and simple solutions to the examination of patients (1-2).

One of the newest methods for assessing the prognostic characteristics of tumors is to study the reaction of inflammatory cells of the patient's body to this lesion. In these methods, the number of inflammatory cells such as natural-killer-cells, polymorphonuclears, eosinophils, and mast cells in the diseased tissue or in the peripheral blood is calculated and the behavior of the tumor is predicted based on the results (3-5).

Analysis of the relationship of tissue mast cells in the invasive tumor margin with patient's survival and prognosis is one of the

mentioned methods. This method has yielded promising results so far (6).

If the relationship between the concentration of mast cells and dysplasia is significant, it can be said that inflammation plays a major role in increased dysplasia. Moreover, considering the presence of mast cells in many tumors and their ability to secrete different substances, it could be said that mast cells play an important role in the development of many tumors. According to the above discussion, when mast cells are related to dysplasia it is possible to use medicines targeting mast cells. This highlights the need for further studies of the number of mast cells in different tumors (6). Polyps contain various tissues. For example, Adenomatous polyps have hyperplastic tissues while inflammatory polyps mostly cause large intestine cancer. In order to diagnose the type of polyps and to distinguish them from cancer, it is necessary to conduct colonoscopy and biopsy. Most non-tubular polyps are malignant. Therefore, it is possible to prevent cancer by timely diagnosis and removal of these polyps (7-9). From the medical point of view, colon and rectum polyps shall be removed through surgery or colonoscopy and shall be exposed to pathological examinations. In inflammations, the number mast cells increases and the increase can play a significant role in the development of polyps (10).

Metastasis is controlled and regulated by a series of genetic agents such as intercellular adhesion molecules. Deterioration of cell-to-cell connections and cell-to-matrix connections is necessary for the onset of metastasis. Several families of adhesion molecules such as Cadherins, Integrins, Catenins, etc. are known. The function of a certain type of Cadherins known as the E-Cadherin is dependent on Catenin cells

which are composed of the following three components: α , β and γ (11-12). The mutual interaction between E-Cadherin and B-Catenin provides a mechanism in which the inactivation of E-Cadherin can cut the growth signal and cause tumorigenesis. In addition to its role in regulation of cellular adhesion, B-Catenin acts as a transcription cofactor in this path and the increase in free B-Catenin levels in humans is associated with tumorigenesis (11-13).

Considering the different results discussed in this section, the objective of this study was to examine the relationship between the expression of the B-Catenin gene and concentration of stroma mast cells in tubular Adenomatous colorectal polyps using the Immunohistochemical method.

The aim of this study was to examine the relationship between the expression of the B-Catenin gene and concentration of stroma mast cells in tubular Adenomatous colorectal polyps using the Immunohistochemical method.

Materials and Methods

In a descriptive-analytical study that was carried out in the pathology department of Tabriz Medical Sciences University on patients with Adenomatous colorectal polyps, the relationship between the expression of B-Catenin gene and concentration of stroma mast cells in tubular Adenomatous colorectal polyps was studied using the Immunohistochemical method.

The study population included the samples of all patients who were pathologically diagnosed with tubular Adenomatous colorectal polyps. The samples had been archived in the pathology ward. A total of 30 paraffin blocks associated with samples with tubular Adenomatous colorectal polyps blocks collected within 6 months in the

pathology ward were studied. 4-micron slices of the blocks were prepared and stained in an immunohistological process using the Envision method as described in the following phases.

1. Deparaffinization phase: Samples were kept for one hour in an oven with a temperature of 60°C .
2. Rehydration and clarification phase: It is done using 96% Xylenol, 100% Alcohol, and distilled water
3. Inactivation of androgens peroxidase using 3% hydrogen peroxide solution and methanol
4. Washing with TBS buffer
5. Incubation of slides with Beta-Catenin-specific primary antibody and CD117 (DAKO, made in Denmark) at room temperature for one hour
6. Washing with TBS buffer solution
7. Adding the Envision solution
8. Washing with TBS solution
9. Adding DAB substrate
10. Counterstaining using Gills Hematoxyline

The prepared slides were examined using a light microscope. In a highly-magnified field (400) all of the cells were counted. The number of stained cells was counted based on tonality and the tonality percentages were calculated. The tonality of cells was determined based on the following ranks: mild (1), moderate (2) and strong (3). Next, the percentage of positive cells was determined based on the sum of tonality percentage and tonality potential. As a result, one figure in the 1 to 8 interval was obtained for each sample and the final score of each sample was calculated.

Ethical Considerations

Since this study was carried out on samples archived in the pathology ward of the aforementioned hospital and the results of

the study had no effect on the treatment and follow up of patients, only informed consent of the patients or their relatives was obtained by making phone calls to use the patient information and archived slides. It is worth mentioning that all of the information in the files of patients will remain fully confidential.

Possible Restrictions and Problems and Methods for Reducing Them

The possibility of restrictions on preparation of kits: It was difficult to obtain the slides and prepare them for the histochemical studies due to the existing economic problems. Seemingly, it was decided to use the aid of the university's authorities and the private sector to solve this problem.

Results and Discussion

In this research, 30 patients with Adenomatous colorectal polyps were selected and examined using the immunohistological method. The results are mentioned in the following.

One of the newest methods of assessing the prognostic characteristics of tumors is to examine the reaction of inflammatory cells in the patient's body to this lesion. In these methods, inflammatory cells such as the natural-killer-cells, polymorphonuclears, eosinophils, and mast cells are counted in the diseased tissue or the peripheral blood (10).

Numerous studies have confirmed the peripheral dissemination of these cells around many human tumors (11-13). Research results indicated that the concentration of mast cells in inflammatory infiltrations around tumors influences the development of a uniform medium for carcinogens, dissemination and metastasis.

The role of MC mediators in the growth and dissemination of malignancy can be studied through different methods such as suppression of the immune system, increased angiogenesis, destruction of cells external matrixes, and increased mitosis (13). The increase in the number of these cells in neoplasms in the skin, esophagus, prostate and mouth is proved (11-16). In our study, the mean CD117 positive mast cells% in patients with tubular adenoma was $25.33 \pm 15.97\%$.

In 2004, a comparison was made between inflammatory tissues and a reduction in mast cells of the diseases tissues was observed (17). Gunham also referred to the significant increase in the number of these cells in diseased tissues as compared to healthy tissues (18). Some studies also examined the effects of medicines designed for inhibiting the release of granules (degranulation) of mast cells such as lodoxamide ethyl and cromoglycate de sodium (19-21).

Hernandez assessed cellular populations in the progressive and still phases of an inflammatory disease and reported a significant increase in mast cells in the progressive sites. This finding reflects the importance of these cells in the advancement of diseases (22).

The findings by Gemmell showed a reduction in the number positive tryptase mast cells in the inflammatory tissues as compared to healthy tissues (12). In some studies references are made to the increase in the number of mast cells in inflammatory tissues as compared to healthy tissues. Some studies also showed a reduction in the number of these cells in inflammatory tissues (15-16). The numbers of mast cells in AIDS patients with inflammatory and health tissues did not differ significantly in the study by Naesse (14).

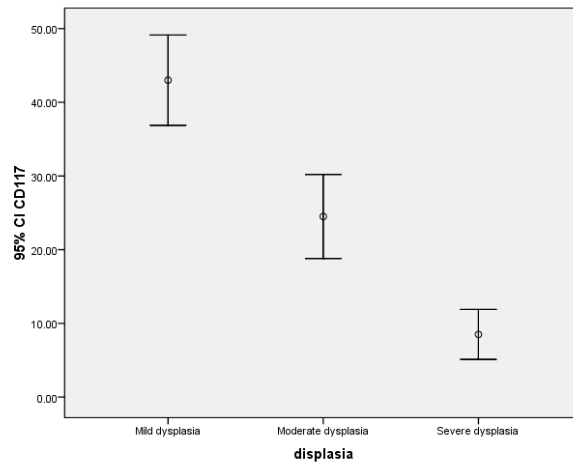


Chart.1 distribution of CD117 in patients with tubular Adenomatous colorectal polyps

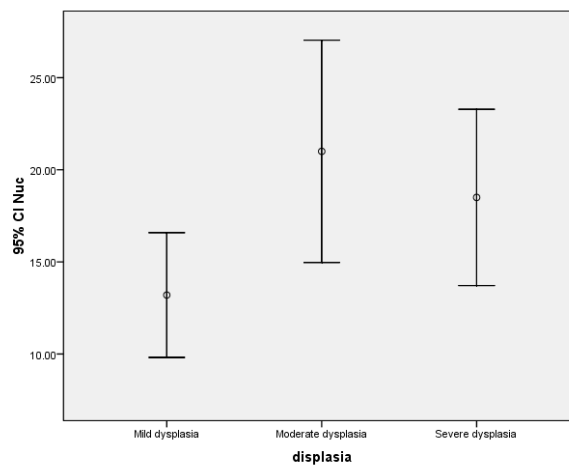


Chart.2 distribution of CD117 in patients with tubular Adenomatous colorectal polyps

In our study, the mean Nuclear and cytoplasmic Beta-catenin% in patients with tubular adenoma lesions was $25.33 \pm 15.97\%$. The cycle of transformation of adenomatous polyps to malignant cancer is among the highly important concerns in patient assessment and follows up of patients following polypectomy. Considering the endoscopic and colonoscopic treatments of polyps by modern medical sciences and lower use of segmental resection in surgical procedures,

prediction and probability assessment of malignancy through colonoscopic assessments is highly important. Polyp size and its villosity histopathology are among the very important factors in determining the malignancy potential. In this study, in addition to assessing the relationship of the aforementioned factors with malignancy, special attention was paid to the site of involvement and its relationship with malignancy and dysplasia.

In recent years, several genes have been introduced to be related to prognosis and survival of patients through their expression. Some of these genes include the genes generating P53, P16, E-Cadherin, and B-Catenin. The expression of these genes may be related to prognosis, probability of metastasis of tumors to lymphatic nodes and distant metastasis, patient survival and success of treatment (23). Abnormal expression and function of E-Cadherin and B-Catenin have been reported in the case of several cancers (24).

The colorectal cancer is one of the most common cancers of the digestive system. In women, colorectal is the second most common cause of cancer-induced death after breast cancer. However, colorectal cancer is the third most common cause of cancer-induced death in men after lung carcinoma and prostate (27). Many scientific sources and experts believe that most colorectal cancers originate from benign Adenomatous polyps (28). Hence, the cycle of transformation of an Adenomatous polyp into a cancer is among the very important topics in cancer diagnosis and selection of the polyp removal method. In the past, the malignancy or benignity of polyps did not matter before surgery because they used to carry out segmental resection on both malignant and benign polyps (28). However, a revolution has occurred in the treatment of Adenomatous polyps. Therefore, techniques such as endoscopic mucous resection, photodynamic treatments, and laparoscopic colectomy are developed. The proper use of these techniques depends on the accurate diagnosis of the probability of hidden aggressive malignancy in Adenomatous polyps by the endoscopists. Moreover, today some surgeons are interested in using less aggressive or non-aggressive laparoscopic techniques for the diagnosis and treatment of Adenomatous polyps (29).

Therefore, determining the malignancy potential of a polyp is considerably important in determining the treatment for the polyp. Colonoscopic resection is questioned when the malignancy potential is high, the existence of aggressive cancer is confirmed, and there is a potential for the spread of the cancer to the surrounding lymph nodes (30). Hence, the question is "What are the chances of determining malignancy potential prior to histological diagnosis?" Two important factors, namely polyp size and its histology, are considered important indicators of malignancy in the transformation of the polyp into cancer. In fact, with an increase in the poly villosity and size its malignancy declines (30). However, perhaps it is possible to estimate the potential of malignancy and development of aggressive cancer in the future during colonoscopy.

The presence or absence of aggressive cancer and malignancy is among the most important factors determining the treatment for colon adenoma. That is to say, if neoplastic changes are limited to high grade dysplasia, endoscopic treatment limits endoscopy (31). But if the aggressive cancer center is found in the polyp, the probability of invasion of the surrounding lymph nodes restricts the removal of polyps through colonoscopy. In such cases, surgical treatment and resection are suitable (31). In this regard, the question is that "Are the measurements recorded by colonoscopists adequately reliable?" (30).

In the research by Fennerty et al., which was conducted using colon poly endoscopic models, a significant difference was observed between the polyp sizes recorded in two examination sessions (30). In another study, the sensitivity and detection success of endoscopy were estimated to vary between 60 and 80% (31). However, the

most precise predictions of malignancy of lesions are resulted from colonoscopic examinations (32).

In 2000, the American Association of Gastroenterology and the American Association of Endoscopy determined the transformation from rectosigmoidoscopy to colonoscopy in the distal colon in the course of Adenomatous polyp diseases especially in small polyps (<1cm) using the criteria and information specific to each participant (33). Hence, the effect of different factors on dysplasia and malignancy differs by society because the transformation of Adenomatous polyps into carcinoma is influenced by genetic and environmental factors (34).

Various studies of the American and African races have resulted in unexpected results. That is to say, the potential of malignancy increases as the involvement advances toward the right colon (35). In Taiwan, in an article the distribution of Adenomatous polyp's malignancy was reported to be higher in the right colon in contrast to other articles (36). The studies of different races have also been disputable in this country (36).

The mean CD117 positive mast cells% in patients with tubular adenoma lesions and severe dysplasia was significantly lower than patients with mild and moderate dysplasia ($P<0.001$). The mean Nuclear and cytoplasmic Beta-catenin% in patients with tubular adenoma lesions with mild dysplasia was also significantly lower than patients with moderate and severe dysplasia ($P=0.047$).

The mean CD117 positive mast cells% in patients with tubulovillous adenomatous lesions and severe dysplasia was significantly lower than patients with mild and moderate dysplasia ($P<0.001$). The

mean Nuclear and cytoplasmic Beta-catenin% in patients with tubulovillous adenomatous lesions and mild dysplasia was significantly lower than patients with mild and moderate dysplasia ($P<0.001$).

The mean Nuclear and cytoplasmic Beta-catenin% in patients with villous adenoma lesions and mild dysplasia was significantly lower than patients with mild and severe dysplasia ($P=0.003$). The mean CD117 positive mast cells% in patients with villous adenoma lesions and severe dysplasia was significantly lower than patients with mild and moderate dysplasia ($P<0.001$).

Conclusion

The mean CD117 positive mast cells% in patients with tubular adenoma lesions and severe dysplasia was significantly lower than patients with mild and moderate dysplasia ($P<0.001$). The mean Nuclear and cytoplasmic Beta-catenin% in patients with tubular adenoma lesions with mild dysplasia was also significantly lower than patients with moderate and severe dysplasia ($P=0.047$).

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