The Prognostic Importance of Cancer Stem Cells in Colorectal Polyps

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Background: This purpose aims to investigate the usefulness of CD133, a stem cell marker, for the prognosis of colon polyps. This study aimed to assess the adenomatous polyps that have an essential role in the development of colorectal cancer. The risk of colorectal carcinogenesis can be reduced by polypectomy and close medical supervision of the patients with adenomatous polyps. The prominence of stem cells in carcinoma development is also a recognized verdict. It must be noted that stem cell evaluation in adenomatous polyps may provide information about carcinoma development.
Method: Previously pathologically assessed colorectal polyps in 60 males and 40 females at Azerbaijan Medical University were reevaluated at the Pathology Department under the Meram Medical Faculty. Hematoxylin-eosin stained preparations were examined, and cases with and without dysplasia were determined. The image analysis program re-examined the preparations, and the same image analysis system automatically counted CD133 positive stained cells in the unit area. At the end of the follow-up period after polypectomy, the cases of malignancy were detected.
Results: The relationship between CD133 expression of dysplasia and malignancy was statistically compared. During the investigation, the statistically significant relationship between CD133 expression and dysplasia, as well as malignancy development, was observed in this study.
Conclusion: During the examination, the statistical significance of CD133 expression was detected in cases with dysplasia and malignancy. The investigation of CD133 expression in colorectal polyps is crucial in determining the presence of dysplasia and malignancy development, particularly in obtaining prognostic data in colorectal polyps.

Keywords: adenomatous polyps; CD133; colorectal polyps; dysplasia; malignancy

Introduction

The colorectal polyps are intraepithelial lesions, which can be of different sizes. These lesions are mainly observed in patients over 50 years of age [1, 2]. Colorectal polyps are benign large intestine tumors and a precursor of malignancy. Dysplasia can also be seen in colorectal adenomas. Adenomas showing dysplasia are the predecessors of malignant tumors. The growth of colorectal adenomas in the colon signals the progression of the malignant tumor. The number of polyps, histological structure, and grade of dysplasia are essential amid the transition to malignancy; the malignancy rate of adenomatous polyps larger than 1 cm has been reported as 15% within ten years [3].

Many studies show that stem cells are effective against the development of malignancy [4–6]. In order to identify the cancer stem cells accurately, it is necessary to find the correct determinant. Therefore, immunofluorescent staining of tissue arrays, cell culture, the growth conditions, immunefluorescent staining of cells, flow cytometer, and Western blot analysis are used for this purpose [7]. CD133 is a sensitive marker for colorectal adenomas and colorectal carcinomas, a potential tumor-initiating cell (TIC) indicator, and transmembrane glycoprotein secreted in the apical portion of the cell surface, which is released from human hematopoietic primitive cells and epithelial stem cells. CD133 has been associated with several single-organ tumor-initiating cells such as breast, prostate, liver, pancreas, lung, brain, and colon [8]. It has also been reported that CD113 may be a marker for predicting aggressive behavior and poor prognosis in colorectal cancer (CRC) patients [9]. As the stem cell marker, CD133 was also associated with the histological grade and size in colorectal adenomas [10].

This study's main objective is to determine the relationship between dysplasia in colorectal polyps and the risk of developing malignancy by examining the CD133 expression in colorectal polyps. It has been shown that CD133 promotes colon cancer progression, activating the protein kinase B signaling pathway. However, this activation mechanism is

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still unclear [10]. The literature has reported that the CD133 expression is high in polyps that subsequently develop cancer [11].

Materials and Methods

Search Strategy

In this study, the polyp's endoscopic biopsy material was examined in correlation to the patient's following at the Endoscopy Unit of the Central Customs Hospital in Baku, Azerbaijan. The patients having bowel habit disorders, bleeding, bloody mucus, and constipation were selected for colonoscopy examination. The patients at the age of 45 who applied for screening were also included in the study. The intestinal bowel preparation was conducted on the day before the examination. In the case of polyps finding, these patients underwent polypectomy. The procedure was performed according to the size of the polyp. The forceps biopsy was performed if the polyp was less than 0.5 cm in size. During the process, the larger polyps were pulled with a clamping ring.

The tissue preparation was completed in the Central Customs Hospital Patomorphology Department. The sections were taken from the paraffin-embedded tissues using a microtome, put on the slide, and stained with hematoxylin-eosin (HE). The HE-stained slides were re-evaluated immunohistochemically at the Department of Pathology under Meram Medical School. As a result of the examination, the two groups: "with" and "without" dysplasia—were performed. The group "without dysplasia" included cases of dysplasia absence as well as cases of mild-grade dysplasia. The "dysplasia" group also included cases of severe dysplasia findings. Then, new 0.5-micron sections were made from the previously paraffin-embedded tissues using a microtome. The tissue sections were taken on the slide and were immunohistochemically stained with CD133 (CD133 Polyclonal Antibody, Protein Tek, Catalog Number: 18470-1-AP) using an automatic staining machine (Fig. 1).

After the staining, the preparations were covered with lamellae. The preparation was stained with CD133 and examined with a Nikon Eclipse E400 light microscope. The same fields were selected as possible in each CD133 stained slide. These areas were photographed with a Nikon Coolpix 5000 camera attached to a Nikon Eclipse E400 light microscope at the same microscopic magnification. Nikon micrometer microscope slides were also photographed at the same microscopic magnification. All photographs were transferred to a PC environment and analyzed using the Clemex Vision Lite 3.5 Image Analysis Program (3.5, Clemex Vision Lite, Longueuil, Canada). The image analysis process started with length calibration, for which a Nikon micrometer slide was used (Fig. 2). Then, 319,066.2 μm^2 areas in all cases of photographs were determined using Clemex Vision Lite 3.5 Image Analysis program. CD133 positive stained cells in the 319,066.2 μm^2 areas were marked and automatically counted by the same Image Analysis Program. The damaged cells were not evaluated during this procedure. While counting, the evaluator did not know the nature of the cases.

We chose the CD133 expression because of its well-known properties as a stem cell biomarker [12]. All patients were followed up clinically in the medical procedure. The biopsy was performed in patients with suspected malignancy. A pathologic examination was conducted from these biopsies, and patients with malignancy were selected. The CD133 values in previous biopsies of patients with malignancy were found. These values were compared with the CD133 values of patients whose malignancy did not develop. It was investigated whether CD133 values could give an idea about the development of malignancy in advance. During the procedure, whether the cases had dysplasia or not, the relationship between CD133 and the number of positive cells, sex, malignancy, size, and histological type of polyps were also evaluated by using SPSS 20.0 (IBM Corp., Armonk, NY, USA).

The relationship between dysplasia and malignancy development was found statistically significant by the McNemar test ($p < 0.000$). The agreement was found when the Kappa value was evaluated statistically (Kappa = 0.653, $p < 0.000$). The ethics committee's approval for this study was obtained from The Medical Service Department of the Customs Committee of the Republic of Azerbaijan (2017/053).

Results

During the medical examination, the gender distribution of the cases was 60 males and 40 females. The histopathological evaluation revealed tubular adenoma in 45 cases, tubulovillous adenoma in 23 cases, inflammatory polyp in 16 cases, hyperplastic polyp in 13 cases, serrated polyp in 2 cases, and villous adenoma in one case (Fig. 3). In the cases examined, we found a number of polyps ranging from one to three.

During the initial HE study, dysplasia was detected in 42 cases, and dysplasia was absent in 58 cases. When these 42 cases with dysplasia were followed, 26 (61.9%) cases progressed to malignancy over six months. Malignancy did not develop in the rest 16 cases (38.1%). We did not find malignancy in 58 dysplasia absence cases over the following period (Fig. 4).

In dysplastic cases, the number of CD133 positive cells in the unit area was calculated as 33.60 ± 15.22. This value was 3.47 ± 3.62 in non-dysplasia cases. When assessed by the Mann-Whitney U test, the median, first, and second quartiles of the instances with dysplasia were found to be 31.50 (24.00–39.50).

In dysplasia cases, the median, first, and second quartiles were calculated as 3.00 (25–5). Accordingly, there was a

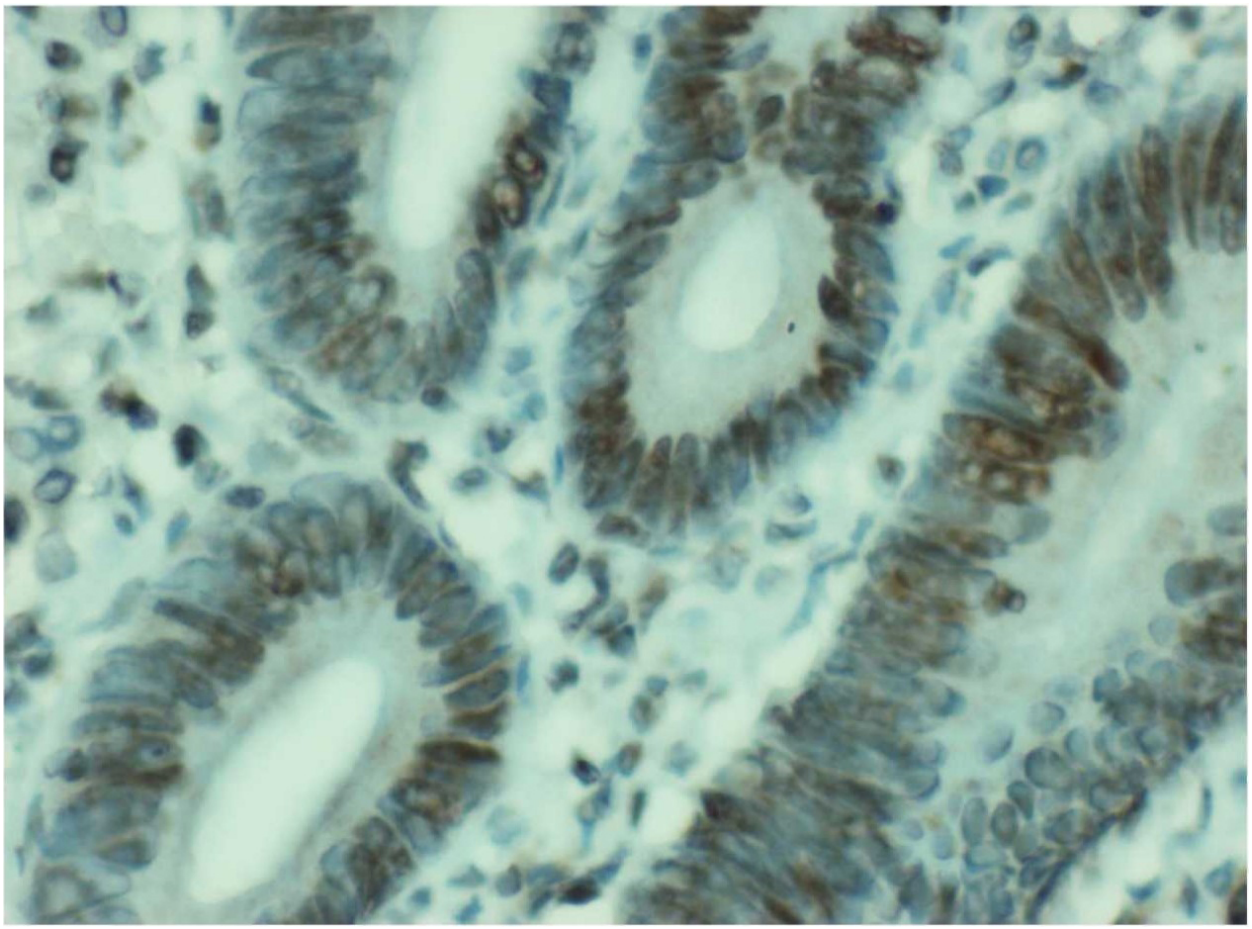

Fig. 1. Positively stained cells are labeled in the image analysis system.

statistically significant difference between the numbers of the CD133 positive staining cells in cases with and without dysplasia ($p < 0.000$). There was a high degree of correlation between the presence and absence of dysplasia with the CD133 staining (Eta = 0.827).

When the receiver operating characteristic (ROC) curve was plotted, the area under the curve was found to be 0.997 (Fig. 5). The cut-off value of the CD133 was suggested to be 10 according to the ROC curve. Subsequently, in cases of developing malignancy, the number of cells stained with the CD133 in the unit area was 36.08 ± 17.63. The cells stained positive with the CD133 were calculated as 9.11 ± 12.06 in the cases without malignancy.

When assessed by the Mann-Whitney U test, the median, first, and second quartiles of malignant developing cases were found to be 36.08 (24.00–49.50). On the other hand, in non-malignant cases, the median, first, and second quartiles were 5.00 (1–9.75). According to this, there was a statistically significant difference between the CD133 positive cell counts in malignant developing cases and malignancy-free cases ($p < 0.000$). There was a high correlation between malignancy development, the absence of malignancy, and the CD133 staining (Eta = 0.658). At the end of the examination, the CD133 expression level was analyzed according to the polyp's size, where three groups were divided: small polyps (up to 1cm), middle polyps (1–3 cm), and large polyps (over 3 cm) (Fig. 6).

In 80 small polyps, the CD133 expression was detected as 12.40 ± 15.12, in 17 middle polyps as 28.41 ± 21.83, and 3 large polyps as 45.67 ± 13.80. So, we observed that the CD133 expression in the unit area increased with the polyp size. Dysplasia was detected via the microscopic examination at 26 (32.5%) out of 80 patients shaving small polyps, at 12 (70.58%) out of 17 middle polyps, and in all (100%) of three large polyps (Fig. 4). However, several histologic cell types did not present dysplasia in small-size polyps. The 13 polyps were hyperplastic, one serrated, and 15 were an inflammatory type.

The patients were kept in a clinical follow-up for four years. The biopsy was performed on patients with the suspicion of malignancy in the clinical follow-up. The diagnosis of malignancy was made pathologically. During the clinical investigation, 12 small polyps with malignancy, 7 tubular adenomas, 5 tubulovillous adenomas, 11 middle-size polyps with malignancy, 2 tubular adenomas, and 9 tubule villous adenomas were detected. Two of the three large polyps with malignancy were tubular adenomas, and one was a tubule villous adenoma.

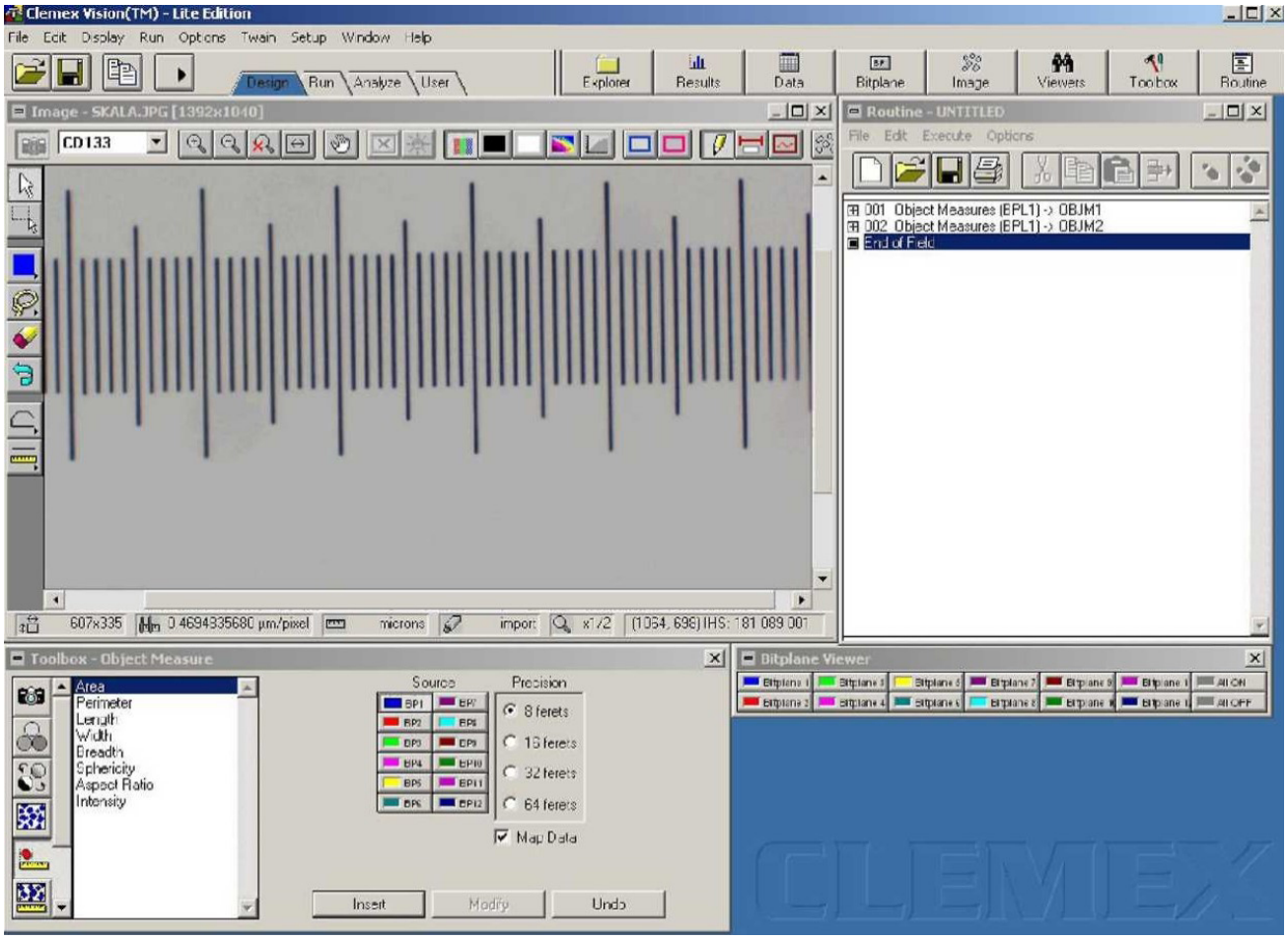

Fig. 2. The length calibration was performed in Clemex Vision Lite 3.5 Image Analysis System using Nixon.

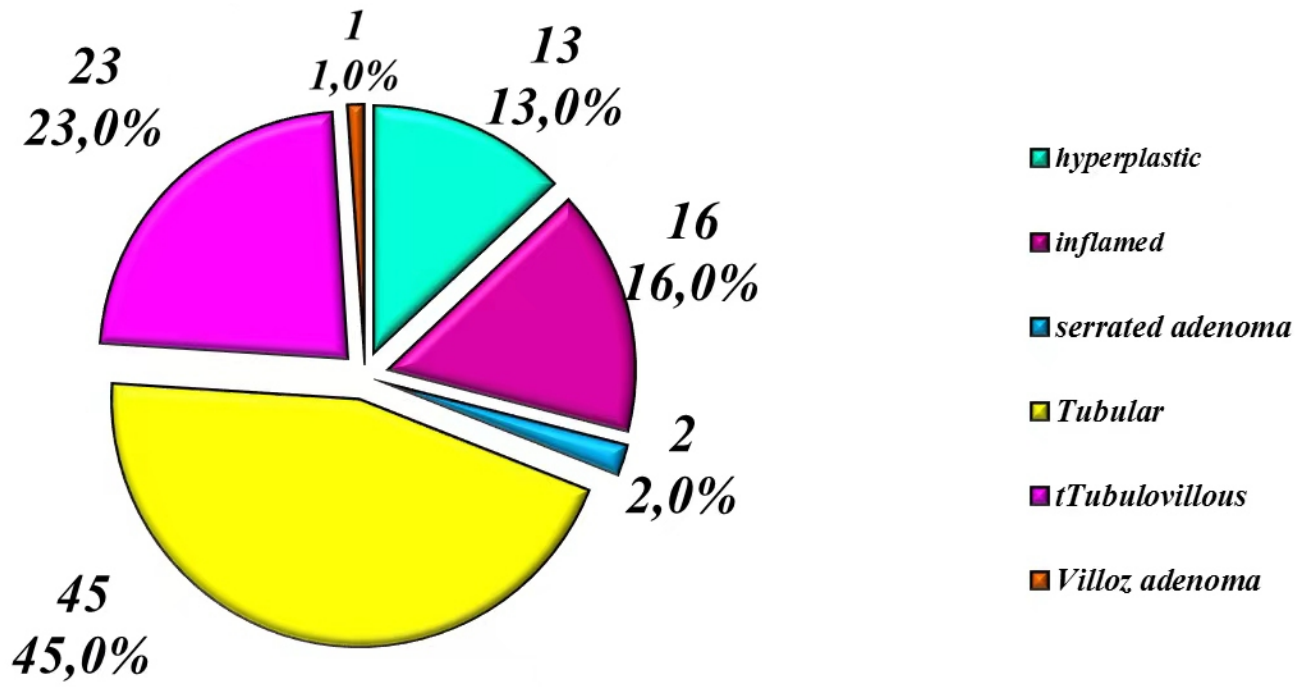

Fig. 3. Diagram pathohistological description of polyps.

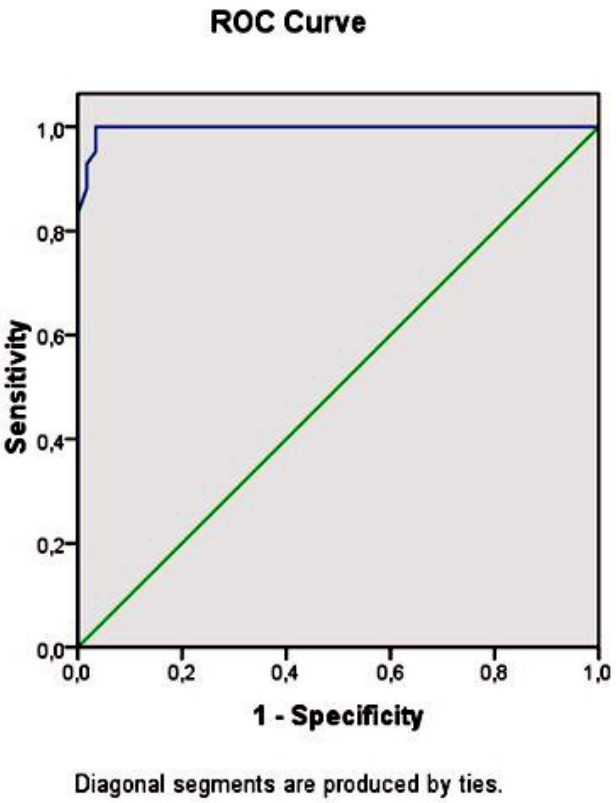

Fig. 4. The receiver operating characteristic (ROC) curve is drawn from positive CD133 values in the unit area of the cases with and without dysplasia. Integral indicator of specificity and sensitivity.

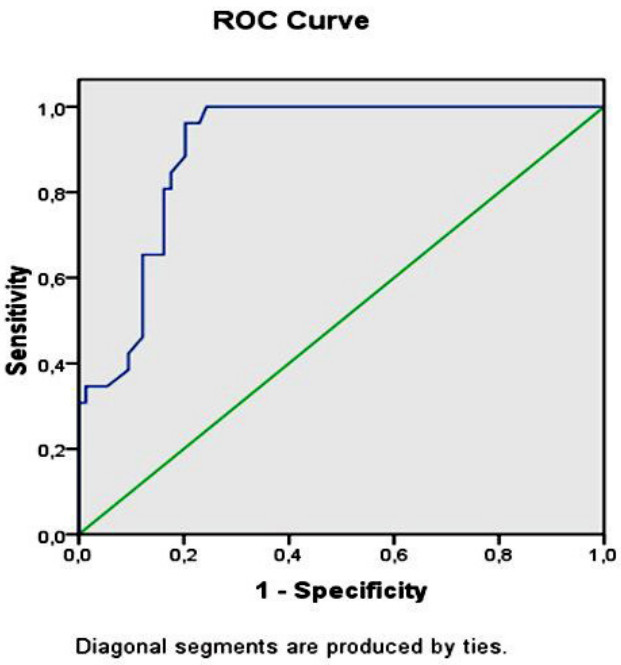

Fig. 5. The ROC curves are drawn from the point of CD133 values of the cases specifying developed and not developed malignant in the unit area. Integral indicator of specificity and sensitivity.

As for adenomas, dysplasia changes were detected in 22 (48.89%) out of 45 tubular adenoma in 18 (78.26%) out of 23 tubule villous, and in 1 (100%) of villous ones. No dysplasia was seen in any of the 13 hyperplastic polyps. In addition, dysplasia was detected in one out of 16 inflammatory polyps (6.25%) and one of 2 (50%) serrated polyps. The presence of dysplasia was determined during the first microscopic examination. No new biopsy was performed during the clinical follow-up.

The biopsies of patients suspected of malignancy were evaluated pathologically during the four-year clinical observation period. Malignancy developed in nine out (20%) of 45 tubular adenomas, in 16 (69.57%) out of 23 tubule villous adenomas, and in one (100%) villous adenoma. Thirteen hyperplastic polyps, 16 inflammatory polyps, and 2 serrated polyps did not develop malignancy during these follow-up periods (Fig. 5).

We found the following results when we examined the values of CD133 according to the histological types of the polyps. The number of cancer stem cells in tubular polyps was 15.8 $\pm$ 2.5, in tubulovillous polyps constituted 30.3 $\pm$ 4.0, in villous polyp (n = 1) was 56.0, in hyperplastic polyps with estimated 2.8 $\pm$ 0.8, in inflammatory polyps was 4.7 $\pm$ 2.1, and in serrated adenomas was 17.5 $\pm$ 14.5 ($p < 0.001$). The CD133 value was 28.86 $\pm$ 15.84 in patients with the tubular adenoma showing dysplasia. Nine of the cases with the tubular adenoma developed malignancy during four years of medical follow-up control. The CD133 value of these cases was determined as 28.78 $\pm$ 5.66.

Discussion

Colorectal carcinomas are the second most common in women and the third most common in males, and 90% of colorectal carcinoma cases are adenocarcinomas [13]. The conversion of adenomatous polyps into carcinoma is a widely investigated process commonly known as the adenoma-carcinoma sequence. The various features such as size, number of adenomas, histological type, and grade of dysplasia are predictors of malignant potential determination [14].

Tubule villous changes in polyps have been reported to be associated with malignancy [15]. During the course of the examination, the malignancy was observed over time for tubular adenomas (20%), tubulovillous adenomas (96.57%), and villous adenomas (100%). However, it was noteworthy that malignancy did not develop in hyperplastic polyps and inflammatory polyps. These findings are consistent with literature data.

There is substantial evidence supporting the idea that stem cells can play an essential role in the development of colorectal cancer. Stem cells can induce tumor formation and progression. It is observed that CD133 plays a crucial role in determining stem cells [16–18]. One of the most significant markers of tumor stem cells is CD133, which promotes colon cancer progression by activating the protein kinase B

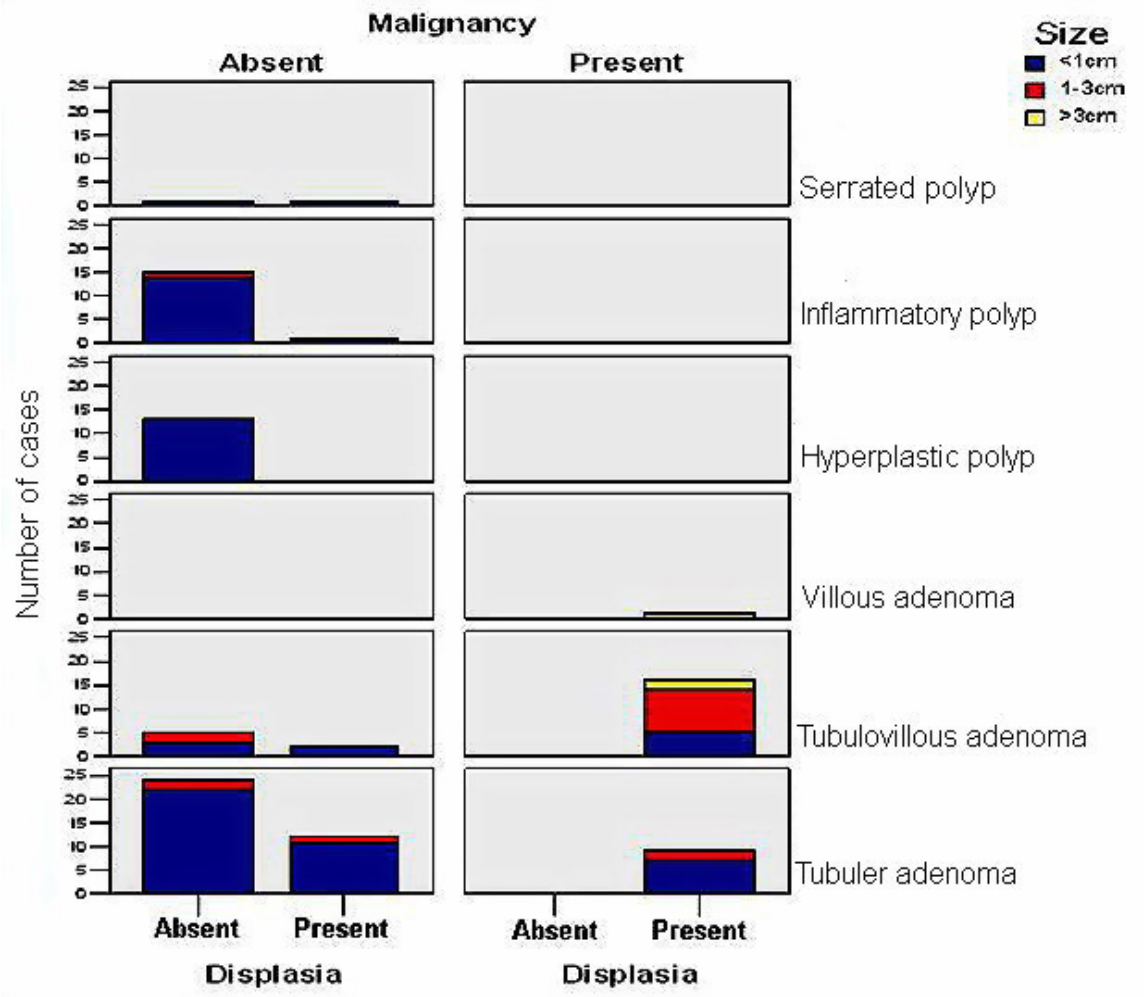

Fig. 6. The cases' distribution in size, dysplasia, malignancy, and histological type is seen. Cancer stem cells in colorectal polyps is directly correlated with the histological type and size of the polyps.

signaling pathway. However, this activation mechanism is still unclear [10]. The CD133 was chosen as the stem cell marker because of its proper properties for the purpose of our study.

Clinical trials have shown that cancer stem cells may be associated with recurrence, metastasis, and poor survival in solid tumors [19]. The main goal of this study was to investigate the staining characteristics of CD133, a stem cell marker, and to answer whether the colorectal polyps would become malignant. It is already known that colorectal polyps are precursors of colorectal cancers. According to the report in the literature, CD133 expressions are high in polyps that subsequently develop cancer [11]. Simultaneously, CD133 expression increases in colorectal carcinomas with liver metastasis [20]. The information shows that CD133 may be a messenger in the development of colorectal carcinoma. In addition, the excess of CD133 expression may also be indicative of the aggressive behavior of carcinoma. CD133 expression (36.08 $\pm$ 17.63) in patients who developed malignancy in the selected 319,066.2 μm^2 areas in our study was considerably higher than the value (9.11 $\pm$ 12.06) in patients who did not develop a malignancy. This value was much higher ($p < 0.000$) than the value of the non-malignancy cases (9.11 $\pm$ 12.06). According to the ROC curve, the cut-off value of the CD133-positive stained cells in the unit area for malignancy can be suggested as 10. However, it is a value found in the unit field in our work. Multilevel studies are needed to detect this value as more susceptible.

As the size of polyps increases, the histopathological changes have also become heavier. It is known that large polyps are mainly associated with dysplasia [21]. However, the size is not a definite decisive measure while detecting dysplasia [16]. In our study, we also found 32.5% dysplasia in polyps smaller than 1 cm, and 70.58% dysplasia in 1 to 3 cm polyps and 100% in polyps larger than 3 centimeters. Furthermore, as the tumor sizes increased, we detected an increase in the number of cells stained positive with the CD133 in the unit area. If CD133 values are related to the development of dysplasia and malignancy, the prognosis is also expected to worsen as the size increases [3]. Polyps with low and high-grade dysplasia are expected to develop

adenocarcinoma [22]. The risk of malignancy in polyps depends on the histological structure, size, and grade of dysplasia [2, 4].

In the study, we found that none of the 58 patients without dysplasia developed malignancy later. In addition, malignancy developed in 26 out of 42 cases with dysplasia. The development of malignancy overlaps with this knowledge. If we consider the relevance of dysplasia and malignancy, in dysplasia cases, it is expected that cells are positive with the CD133 in the unit area and will be statistically higher than those without dysplasia. Studies in the literature suggest that the CD133 expression increases in dysplasia cases [17, 23]. According to the ROC curve, the cut-off value of cells stained positive for the CD133 in the unit area for dysplasia may be suggested as 10.

We also observed that malignancy did not develop in 58 cases without dysplasia. This finding was also noteworthy. We found that 61.9% of the patients with dysplasia developed malignancy in our follow-ups. This finding is not surprising either. This shows compatibility with our literature and classical knowledge [24–26]. The statistically significant difference between the CD133 expression in dysplasia and non-dysplasia cases also allows us to evaluate dysplasia with the CD133 expression values.

It has been reported that 5% of all adenomatous polyps turn into cancer. Adenomatous polyps are present in the background in 30 to 50% of bowel cancer. Nutritional factors, environmental factors, inflammatory diseases of the gastrointestinal tract, ulcerative colitis, and Crohn's disease also play a significant role in the formation of colorectal polyps. Inflammatory diseases increase the risk of carcinoma genesis by stimulating the proliferation of intestinal epithelial cells and suppressing apoptosis [1, 2, 27].

In the study, it was found that the malignancy develops later in 20% of tubular adenomas, 69.57% of tubule villous adenomas, and 100% of villous adenomas. During the clinical examination, some of our patients in this group had bleeding, bloody mucus, constipation, and bowel habit disorder. In the study, malignancy was not observed in the hyperplastic polyp, serrated polyp, and inflammatory polyps. During the process, we did not observe the symptoms in our patients. The CD133 expression was also low in this group of polyps. As seen in previous studies, there is a relationship between CD133 expression and colorectal carcinoma development [11, 16, 17]. During the investigation, the statistically significant relationship between CD133 expression and dysplasia, as well as malignancy development, was observed in this study.

Conclusion

The identification of cancer stem cells with CD133 might be a prognostic marker in colorectal polyps. As seen in the present study, there is a relationship between CD133 expression and colorectal carcinoma development. In addition, the excess of CD133 expression may also be indicative of the aggressive behavior of carcinoma. We thought that the expression of CD133 could be used to determine polyps' behavior and observe treatment response. However, it is also clear that there is a need for more extensive work in this regard.

Availability of Data and Materials

The author will supply the relevant data in response to reasonable requests.

Author Contributions

AS, MCA, NB: made substantial contributions to conception and design. AS: Planning of publication, surgical procedures. MCA: Planning of the publication, writing of the article. NB: Planning of publication. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of Central Customs Hospital at which the examination process was conducted and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The ethics committee's approval for this study was obtained from The Medical Service Department of the Customs Committee of the Republic of Azerbaijan (2017/053) Informed patient consent was obtained for this study.

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Conflict of Interest

The authors declare no conflict of interest.

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