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Original Research

Features of Childhood Colorectal Carcinomas and Frequency of K-ras Mutations

Ayşe Büyükcam[1], Canan Akyüz[2], Diclehan Orhan[3], Bilgehan Yalçın[2], Ali Varan[2], Münevver Büyükpamukçu[2], Tezer Kutluk[2]

[1]Department of Pediatrics, Hacettepe University School of Medicine, Ankara, Turkey

[2]Department of Pediatric Oncology, Hacettepe University School of Medicine, Ankara, Turkey

[3]Department of Pediatric Pathology, Hacettepe University School of Medicine, Ankara, Turkey

ABSTRACT

Objective: Colorectal carcinoma (CRC) is extremely rare in childhood and has a poor prognosis in young patients. The tumorigenesis of CRC in children and adolescents is still unclear and probably evolves through different stages. There are not enough studies about the rarity of K-ras mutations with childhood CRC. This study aimed to investigate the features and outcomes of childhood CRC as well as examine the frequency of K-ras mutations in CRC among children and adolescents.

Methods: The clinical and pathologic features, prognostic factors, and outcomes of CRC in 28 children and adolescents (ages 10 to 17 years) referred to the Pediatric Oncology Department of Hacettepe University Children's Hospital between 1974 and 2010 were reviewed for this study. Paraffin-embedded tissues of 18 patients were available and these tissues were analyzed by using the "pyrosequencing" method to detect K-ras mutations.

Results: The median age of patients was 14 years and the male/female ratio was 2.5/1. At presentation, the most common symptoms were abdominal pain (57%) and weight loss (43%). The time between symptoms and diagnosis was 4 months. The most common sites of involvement were the rectum (43%) and sigmoid colon (25%). Mucinous adenocarcinoma was the most common histiotype (71%). At presentation, 89% of patients had metastatic disease, especially to the peritoneal surface (39%). Overall survival rates at 3 and 5 years were 10%. Distant stage (p=0.045), incomplete resection, and macroscopic tumor (p=0.000) were poor prognostic outcomes. A K-ras mutation was identified in three of the 18 patients (17%). The most common mutation of the patients was GGT→GAT at codon 12.

Conclusion: Childhood colorectal carcinomas occur in a shorter time than in adults, with different histiotypes and more likely different steps. It seems that K-ras mutation plays a role in this different biology of pediatric CRC. However, further studies are essential to investigate and understand the biology of childhood CRC.

Keywords: Childhood colorectal carcinoma, K-ras mutation

INTRODUCTION

Colorectal carcinoma (CRC) is extremely rare in the pediatric age. It accounts for less than 1% of all cancer cases in children younger than 20 years. The incidence is approximately one case per million in this age group (1, 2). In addition, a recent study by Ferrari et al. (3) has shown that the incidence of epithelial tumor of the colon is 0.3/100.000. Although CRC has a good prognosis in adults when diagnosed early and treated by multidisciplinary approach, it has a poor prognosis in children because of the rarity of the tumor and its high potential for dissemination (4). Further, the pathobiology of pediatric and adult CRC may differ (5). The biology of CRC in adults is well known. In contrast, the tumorigenesis of childhood CRC, which necessarily occurs over a shorter period, is still unclear and most likely evolves through different stages (6).

K-ras is a proto-oncogene located on chromosome 12p12.1, encodes the plasma membrane-bound Guanosine Triphosphate (GTP) binding protein that is a key regulatory component of numerous signal transduction pathways, and is activated by point mutations that occur at the critical hot-spot coding sequences (7, 8). Point mutations in codons 12,13, and 61 in the K-ras gene result in amino acid alterations in the $p12^{(ras)}$ protein and activation of the oncogenic potential (9). However the biology of childhood carcinoma is unclear and the role of K-ras mutations is not known very well in CRC of children and adolescents. The current study aimed to investigate features and outcomes of CRC as well as examine the frequency of K-ras mutations in colorectal among children and adolescents.

METHODS

Patients and Clinical Data

Twenty-eight children and adolescents (aged 10–17 years) who had CRC diagnosed and referred to the Pediatric Oncology Department of Hacettepe University Children's Hospital between

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Corresponding Author: Ayşe Büyükcam **E-mail:** dr.aysebaktir@gmail.com

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Table 1. Demographic characteristics of the 28 patients with colorectal carcinoma

		n	%
Sex	Female	8	26.8
	Male	20	71.4
Age	Median=14 (10–17)		
Time between symptoms and diagnosis	Median=4.1 (2.0–6.2)		
Cancer History of relatives		6	21.4
Anemia at diagnosis		17	60.7
Most common symptoms	Abdominal pain	16	57.1
	Weight loss	12	42.8
Location	Rectosigmoid	19	67.8
	other sites	9	32.2
Stage (Modified Dukes*)	A	–	0
	B	3	10.7
	C	18	64.3
	D	7	25
Histology	Mucinous Adenocarcinoma	20	71.4
	Signet– ring cell Adenocarcinoma	4	14.2
	Adenocarcinoma	4	14.2
Metastatic disease at diagnosis		25	89.2
Most common metastatic site	Peritoneal surface	11	39.3
Chemotherapy		26	92.8
Radiotherapy		6	21.4
Overall survival of 3 and 5 years		3	10.7

*Modified Dukes' Classification of Colorectal Carcinoma
A: Lesion confined to the bowel wall
B: Direct extension to serosal fat without lymph node involvement
C: Lymph node involvement
D: Distant metastases (may include extranodal intra–abdominal tumor, lung, brain, bones, etc.)

1974 and 2010 were retrospectively reviewed for this study. Patient data were reviewed for age, sex, presenting symptoms, other chronic medical diseases, second malignancy, familial cancer history, consanguinity, diagnostic procedures, clinical characteristics, hemoglobin levels, body mass indexes, histological type, stage of disease according to the Modified Dukes Staging (10), treatment methods, the interval between CRC diagnosis and recurrence or progression, prognostic factors, frequency of K-ras mutation, and mutation analyses. This study was approved by the institutional review board of the Hacettepe University Faculty of Medicine, and written informed consent was obtained from the patients.

Main Points:

- Colorectal carcinoma (CRC) is extremely rare in childhood and has a poor prognosis in young patients.
- CRCs occurs in a shorter time than in adults, with a different histology and more likely with different stages.
- It seems that K-ras mutations play a role in the different biology of pediatric CRC.

Tumor Tissue Preparation and K-ras Sequencing

Paraffin-embedded tissues of 18 patients were available. Mutations on 12th, 13th, and 61st codons of the K-ras gene were analyzed in colorectal carcinoma sample tissues by using the 'pyrosequencing' method. Study was composed of two analyses, which were performed using the PyroMark K-ras kit. The mutations were searched on the 12th and 13th codons in the first analyses and on the 61st codon in the second analyses. "QIAamp DNA

FFPE Tissue Kit" was used for DNA isolation from the paraffin-embedded tissues, obtained from 10 micron thickness samples that represent the tumor. DNA quantity was 10–20 ng/µl in a sample. Then, K-ras polymerase chain reaction (PCR) protocol was applied. Amplification was done using the "Thermal Cycler 9700" device. K-ras studies were done using the "PyroMark Q24 MDx" device through the "pyrosequencing" method (11, 12).

Statistical Analysis

All data were analyzed using the SPSS 17.0 (SPSS Inc.; Chicago, IL, USA) for Windows package program. Continuous variables that are normally distributed were expressed as mean ± standard deviation, and those that were not normally distributed as median (min-max). Categorical data were expressed as percentages. Normal distribution of continuous data was determined by a histogram and the "Kolmogorov Smirnov Test." The significance of the difference between the normally distributed data was analyzed using the "One Sample t-test"; the significance of the difference between the data that were not normally distributed was analyzed using the "Mann Whitney U-test." The difference between pathologic types was determined by "Kruskal–Wallis Test." Estimation of the duration of survival was done by the "Kaplan–Meier" method. "Log Rank Test" was used in determining the difference of survival duration between groups. The p values less than 0.05 was accepted as significant.

RESULTS

Of the 28 patients, 8(28.6%) were females and 20(71.4%) were males. The male/female ratio was 2.5/1. The median age of patients was 14 years at diagnosis (range 10 to 17 years). The other features are shown in Table 1. All patients had more than one symptom at presentation. The time between symptoms and diagnosis was 4.1 months (range: 2–6.2 months). The predominant symptoms were abdominal pain (n=16,57.1%) and the second was weight loss (n=12,42.8%), followed by abdominal distention (n=9), vomiting (n=9), constipation (n=8), loss-of-appetite (n=7), weakness (n=6), diarrhea (n=5), hematochezia (n=5), melena (n=3), fever (n=3), intestinal obstruction (n=2), and dysuria (n=2).

Five patients had a relevant medical history, including Bloom's syndrome (n=1), chronic glomerulonephritis (n=1) and guatr, hamartomatosis polyposis coli and hypertrophic osteoarthropathy (n=1), non-familial polyposis coli (n=1), and nephrolithiasis (n=1). Regarding familial cancer history, patients had no family cancer history (n=22), family members had a history of colon cancer (n=2) and non-colonic cancer (n=2), some patients has an undetailed cancer history (n=1), and one patient's family cancer history was unclear. In addition, patients had no secondary malignancy. Further, the degree of consanguinity in the parents was evaluated. Eighteen parents of patients had no consanguinity, followed by first-degree relatives (n=7), second degree relatives (n=2), and unknown degree relatives (n=1). Seventeen (60.7%) of 28 patients with hemoglobin data were anemic at presentation, with hemoglobin values of less than 8 g/dL (5.6–7.9 g/dL) (n=2), 9.6 g/dL (n=1), and between 10.1 g/dL and the lower limit of the normal value for age and sex (n=14). The documentation of the fecal occult test results was poor.

Body mass indexes (BMI) were calculated from the charts of 18 patients at diagnosis. The BMI of 16 patients was less than 20, one patient between 20 and 25, and one patient between 25 and 30. Five of the patients with a BMI of less than 20 (31.2 %) had a secondary disease but the other 11 (69%) did not have. Of the 28 patients who had a pathologic diagnosis, the charts of 25 patients reported that the diagnostic evaluation was performed with more than one procedure. The main initial procedure was barium enema (n=13), endoscopy (n=10), abdominal computed tomography (n=9), abdominal ultrasonography (USG) (n=13), and exploratory laparatomy (n=6).

The primary site of the tumor was the rectum in 12 patients, sigmoid colon in 7, descending colon in 2, splenic flexura in 1, transverse colon in 4, hepatic flexura in 1, and cecum in 2 patients. The ascending colon location did not exist. Histopathological findings included mucinous adenocarcinoma in 20 (71.4%), single-ring cell carcinoma in 4 (14.2%), and adenocarcinoma in 4 (14.2%) patients. The other pathologic types did not exist. According to the colon localization, the most common histopathologic type was the mucinous adenocarcinoma in the recto sigmoid and other sites.

Of the 28 patient, only 3 (10.7%) had a localized disease, while the others (n=25, 89.2%) had a metastatic disease. The extent of disease was determined using the Modified Dukes Staging. Although stage A did not exist, stage B was observed in 3 patients (10.7%), stage C in 18 (64.3%), and stage D in 7 (25%). The most common site of metastatic disease was the peritoneal surface (n=11, 39.3%), followed by the close lymph nodes (n=10, 35.7%), distant lymph nodes (n=2, 7.1%), omentum (n=6, 21.4%), mesenterium (n = 7, 25%), lung (n=2, 7.1%), liver (n=5, 17.9), kidney (n=1, 3.6%), bladder (n=1, 3.6%), and stomach (n=1, 3.6%).

Surgical procedures for diagnosis or treatment were biopsy (n=9), colon resection (n=20), colostomy (n=19), expiratory laparotomy (n=7), and anastomosis (n=3). Complete resection (R0) was not preferred for any of the patients. Seventeen patients (60.7%) had incomplete resection and microscopic tumor (R1) and 11 (39.3%) had incomplete resection and macroscopic tumor.

The other treatment procedures were chemotherapy and radiotherapy. Of 28 patients, 26 received chemotherapy, while the other two patients after diagnosis went to another medical center for treatment. The treatment and radiotherapy information were incomplete. However, 13 (46%) patients were diagnosed on or before the year 1990. The treatment choices were as follows: 5-FU, lomustine (CCNU), dacarbazine (DTIC), adriamycin, and mitomycin C. On the other hand, 15 (53%) patients were diagnosed after 1990, and received chemotherapy consisting of 5-FU, levamizole, adriamycin, mitomycin C, irinotecan, bevacizumab, oxaplatin, and interferon. Only six patients (21%) received radiotherapy. There were three known long-term survivors in our study, who received a treatment consisting of 5-FU, lomustine, irinotecan, and oxaplatin, and are still alive.

Paraffin-embedded tissues of 18 patients were available and these tissues were analyzed using the 'pyrosequencing' method for detecting K-ras mutations. K-ras mutation was identified in three of the 18 patients (16.6%). The most common mutation of the pa-

tients was GGT→GAT at codon 12. The patients with K-ras mutations were 13, 16, and 10 years old and the male/female ratio was 2/1. The most common location was sigmoid and the most common histiotype was mucinous adenocarcinoma. Stages were C, B, and D (Modified Dukes), respectively. No one had other illnesses. Survival times were 25, 14.5, and 10 months, respectively.

The twenty-eight patients were evaluated for the survival analysis. Event-free survival (EFS) was evaluated only in 23 of 28 patients as 5 patients did not receive all the treatments in our medical center. Overall survival and EFS rates at 3 and 5 years were 10% and 17%, respectively. Distant stage (p=0.045), incomplete resection, and macroscopic tumor (p=0.000) were poor prognostic outcomes.

DISCUSSION

CRC is primarily a disease of adulthood, and is very rare in children and adolescents (13). There is limited data on childhood CRC in the literature (14). Young colorectal cancer patients have a distinct group of malignancies with various clinical and pathobiological features (6, 5). To our knowledge, the current study is one of the rare CRC case series in children and adolescents, which demonstrates the relationship K-ras mutations and childhood CRC (15).

As was previously noted, the male predominance in childhood CRC is in contrast to the situation for adult patients. Sex differences in adult CRC have been associated with biological, behavioral, and environmental factors; however, the data on gender differences in childhood CRC are limited (16). In a study of 11,071 primary CRC cases diagnosed at the ages of 15–39, Teng et al. found that male gender was associated with an increased risk of death (17). People in their 30s or 40s with positive family histories exhibited a higher relative risk compared to their age-matched peers, as well as older people with the same positive family histories (18-20). The evidence for an elevated risk of bowel cancer in children and adolescents with a family history of the disease is unclear (21). The predisposing genetic diseases, polyposis syndromes, and inflammatory bowel diseases associated with pediatric CRC include familial adenomatous polyposis, Lynch syndrome (hereditary non-polyposis colorectal cancer [HNPCC]), and ulcerative colitis (22, 14). In some series, 10%–30% of reported childhood CRCs had predisposing factors (23, 24, 5); however, most childhood cases occur without known predisposing syndromes (21, 22).

The presenting features of pediatric and adult CRC such as the interval between symptoms and diagnosis, pathological findings, stage, and prognosis are similar, even as observed in this study. The symptoms of childhood CRC can vary from abdominal pain, nausea and vomiting, abdominal distention, changes in bowel habits, and weight loss to bloody stool or rectal bleeding. Patients usually have more than one symptom (25). In accordance with the findings of previous studies, abdominal pain was the most predominant presenting symptom (26). Compared to adults, CRC rarely occurs in children; thus, there is a low index of suspicion. The limited experience with this disease in children often results in delayed diagnosis (27, 14).

A review of the literature suggests that the duration of the symptoms of childhood CRC varies widely, and the median interval is short. Acute presentation is more frequent in young patients (28). The duration of symptoms in our series was 15 days–12 months, with an average of 1.5 months despite the presence of advanced disease in most of the cases. The short duration could confirm the aggressiveness of the tumor biology and the rapid tumor growth besides a delayed diagnosis or both of them (29, 24, 30). Similar to the findings of previous studies, the most common stage in accordance with the modified Duke criteria was stage D, followed by stage C, and the most common sites of metastatic disease in our study were the lymph nodes, peritoneal surfaces, and liver (31). Children and adolescents contributed to the advanced disease at presentation (29, 22, 28, 24). Important CRC studies in which childhood was defined as ages lower than 21 have demonstrated that left colon cancers are more predominant. The differences in presentation, histology, undifferentiation, and outcome are likely to reflect the distinctive features in the tumor biology of pediatric and adult CRCs. This suggests that the disease progresses through different stages in these populations (5, 24). CRC in adults is usually linked to preexisting adenomas. It develops over approximately 10 years through a well-known multistep process. In contrast, premalignant adenomas are rarely seen in relation to the presence of sporadic CRC in children, and the tumorigenesis of childhood CRC occurs over a shorter period (21, 26, 24). The incidence of mucinous adenocarcinoma is higher in children and adolescents (44%–91.7%) in comparison to adults (5%–15%) (32, 29). In the largest cohort of pediatric CRC, the signet-ring cell carcinoma accounted for 15.4% of the CRCs in patients under 21 years old; the incidence for adults was 0.01%–2.6% (33, 22). Mucinous CRC has distinctive clinical and molecular features from non-mucinous CRC (34). In a study of the molecular features of early-age onset (age ≤ 30) CRC, Khan et al. found that microsatellite instability (MSI) was more prevalent in early-onset CRC. Unlike CRC in adults, CRC in children is not tightly linked to MLH1/PMS2 loss, and it has never been associated with BRAFV600E mutations. The MSS/BRAFV600E genotype had a poor prognosis and was more prevalent in early-age CRC (9% vs. 3%) (15).

The K-ras gene is an oncogene, the natural form of which is a proto-oncogene or wild-type oncogene, which is involved in the regulation of cellular responses (35, 36). The K-ras protein is responsible for the transduction of mitogenic signals from the epidermal growth factor receptor (EGFR) on the cell surface to the cell nucleus (37). This short gene sequence is susceptible to point mutations, and GTP activity is lost with mutation (37). K-ras mutations occur during the progression and metastasis of CRCs. In addition, it might be present in other cancers, such as lung adenocarcinomas and pancreatic ductal adenocarcinoma (38-40). K-ras mutations have been found in 30%–50% of colorectal CRCs in adults, and the most frequent point mutation has been found in codons 12, 13, and 61 (35, 41). K-ras mutations have a strong effect on the growth of colonic polyps and early cancers (42); however, the K-ras mutation is not sufficient alone to influence the progression malignant transformation. The associations of the K-ras mutations with other mutations, such as APC, are also important (38, 35). The K-ras mutation is

used in CRC as a useful marker for predicting tumor responsiveness to anti-EGFR antibody therapy, as its key role in EGFR signaling (36, 43). It is also a negative predictor of the response to anti-EGFR therapy (41). The value of the K-ras mutation as a prognostic factor after CRC diagnosis in adults is controversial. It would appear that the K-ras mutation is a useful marker for CRC treatment rather than a prognostic factor (36). The data on K-ras mutations in early-onset CRC are heterogeneous and limited. Previous studies have reported that K-ras gene mutations occur at rates of 4%–27% in early-onset CRC (44-47), as well as a significantly higher proportion (54%), with mean age over 30 years, as reported by Watson et al. (48). Khan et al. reported the rate of K-ras mutations in individuals diagnosed with early age (≤ 30 years) onset CRC as 28% (15). The mean age was not under 18 years in these previous studies; consequently, these results might not reflect the rates of K-ras mutations in childhood CRC. The treatment options for childhood CRC are similar to those for adults (21).

Modified adult CRC protocols are utilized initially because of a lack of standardized protocols for CRC treatment of children (22). Surgery is the mainstay of treatment for CRC, and complete resection is a good prognostic factor (49, 28, 21). However, the complete resection rate is reportedly less than optimal in children because most of the patients present with advanced disease at diagnosis (14). Poorer outcomes might be also associated with the delayed diagnosis and low index of suspicion attributed to the rarity of childhood CRC, nonspecific symptomatology that mimics several common infections, and functional gastrointestinal disorders in childhood, with a shorter time between symptoms and diagnosis. The unfavorable histologic variant presentation with the distant stage and the inapplicability of adult screening tests in children are also possible factors in the poor outcomes (2, 50, 51).

CONCLUSION

Childhood CRCs have a poor prognosis even with new therapies by the reason of the delayed diagnosis, nonspecific symptomalogy, lack of feasibility to use adult screening tests in children, distant stage at presentation, and unfavorable histologic variants. CRCs occurs in a shorter time than in adults, with a different histology and more likely with different stages. It is significant that three of 18 (16.6%) patients showed K-ras mutations at an early age. It seems that K-ras mutations play a role in the different biology of pediatric CRC. K-ras mutation's prognostic significance could not be portrayed due to the small number of patients. Further studies are necessary with larger series of patients to investigate and understand the biology of childhood CRC and the relevance of the K-ras mutations on the prognosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Hacettepe University Faculty of Medicine (FON 11/34-32).

Informed Consent: Informed consent was obtained from the patients who participated in this study.

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REFERENCES

1. Pappo AS, Radriguez-Galindo C, Furman WL. Management of Infrequent cancers of childhood In: Pizzo PA, Poplack DG, editors. Principles and Practice of Pediatric Oncology. 6th ed. Philadelphia: Lipincott Williams & Wilkins; 2010. p. 1109-12.
2. Ferrari A, Rognone A, Casanova M, Zaffignani E, Piva L, Collini P, et al. Colorectal carcinoma in children and adolescents: the experience of the Istituto Nazionale Tumori of Milan, Italy. Pediatr Blood Cancer 2008; 50: 588-93. [Crossref]
3. Ferrari A, Brecht IB, Gatta G, Schneider DT, Orbach D, Cecchetto G, et al. Defining and listing very rare cancers of paediatric age: consensus of the Joint Action on Rare Cancers in cooperation with the European Cooperative Study Group for Pediatric Rare Tumors. Eur J Cancer 2019; 110: 120-6. [Crossref]
4. Kravarusic D, Feigin E, Dlugy E, Steinberg R, Baazov A, Erez I, et al. Colorectal carcinoma in childhood: a retrospective multicenter study. J Pediatr Gastroenterol Nutr 2007; 44: 209-11. [Crossref]
5. Hill DA, Furman WL, Billups CA, Riedley SE, Cain AM, Rao BN, et al. Colorectal carcinoma in childhood and adolescence: a clinicopathologic review. J Clin Oncol 2007; 25: 5808-14. [Crossref]
6. Durno C, Aronson M, Bapat B, Cohen Z, Gallinger S. Family history and molecular features of children, adolescents, and young adults with colorectal carcinoma. Gut 2005; 54: 1146-50. [Crossref]
7. Palmirotta R, Savonarola A, Ludovici G, Marchis MLD, Covello R, Ettorre GM, et al. Concurrent mutation in exons 1 and 2 of the K-ras oncogene in colorectal cancer. Folia Histochem Cytobiol 2011; 49: 729-33. [Crossref]
8. Einspahr JG, Martinez ME, Jiang R, Hsu CH, Rashid A, Bhattacharrya AK, et al. Associations of Ki-ras proto-oncogene mutation and p53 gene overexpression in sporadic colorectal adenomas with demographic and clinicopathologic characteristics. Cancer Epidemiol Biomarkers Prev 2006; 15: 1443-50. [Crossref]
9. Kressner U, Bjorheim J, Westring S, Wahlberg SS, Påhlman L, Glimelius B, et al. Ki-ras mutations and prognosis in colorectal cancer. Eur J Cancer 1998; 34: 518-21. [Crossref]
10. Sherlock P, Lipkin M, Winawer SJ. Predisposing factors in carcinoma of the colon. Adv Intern Med 1975; 20: 121-50.
11. Poehlmann A, Kuester D, Meyer F, Lippert H, Roessner A, Schneider-Stock R. K-ras mutation detection in colorectal cancer using the Pyrosequencing technique. Pathol Res Pract 2007; 203: 489-97. [Crossref]
12. Gao J, Li YY, Sun PN, Shen L. Comparative analysis of dideoxy sequencing, the KRAS StripAssay and pyrosequencing for detection of KRAS mutation. World J Gastroenterol 2010; 16: 4858-64. [Crossref]
13. Saab R, Furman WL. Epidemiology and management options for colorectal cancer in children. Paediatr drugs. 2008; 10: 177-92. [Crossref]

14. Kim G, Baik SH, Lee KY, Hur H, Min BS, Lyu CJ, et al. Colon carcinoma in childhood: review of the literature with four case reports. Int J Colorectal Dis 2013; 28: 157-64. [Crossref]
15. Khan SA, Morris M, Idrees K, Gimbel MI, Rosenberg S, Zeng Z, et al. Colorectal cancer in the very young: a comparative study of tumor markers, pathology and survival in early onset and adult onset patients. J Pediatr Surg 2016; 51: 1812-7. [Crossref]
16. Chen TA, Kang HY, Chang HC, Lin WC, Chao TM, Horng JT. Gender differences in colorectal cancer during the past 20 years in Taiwan. Int J Colorectal Dis 2012; 27: 345-53. [Crossref]
17. Teng A, Lee DY, Cai J, Patel SS, Bilchik AJ, Goldfarb MR. Patterns and outcomes of colorectal cancer in adolescents and young adults. J Surg Res 2016; 205: 19-27. [Crossref]
18. Henrikson NB, Webber EM, Goddard KA, Scrol A, Piper M, Williams MS, et al. Family history and the natural history of colorectal cancer: systematic review. Genet Med 2015; 17: 702-12. [Crossref]
19. Taylor DP, Burt RW, Williams MS, Haug PJ, Cannon-Albright LA. Population-based family history-specific risks for colorectal cancer: a constellation approach. Gastroenterology 2010; 138: 877-85. [Crossref]
20. Sandhu MS, Luben R, Khaw KT. Prevalence and family history of colorectal cancer: implications for screening. J Med Screen 2001; 8: 69-72. [Crossref]
21. Blumer SL, Anupindi SA, Adamson PC, Lin H, Price AP, Markowitz RI, et al. Sporadic adenocarcinoma of the colon in children: case series and review of the literature. J Pediatr Hematol Oncol 2012; 34: e137-41. [Crossref]
22. Poles GC, Clark DE, Mayo SW, Beierle EA, Goldfarb M, Gow KW, et al. Colorectal carcinoma in pediatric patients: A comparison with adult tumors, treatment and outcomes from the National Cancer Database. J Pediatr Surg 2016; 51: 1061-6. [Crossref]
23. Salas-Valverde S, Lizano A, Gamboa Y, Vega S, Barrantes M, Santamaría S, et al. Colon carcinoma in children and adolescents: prognostic factors and outcome-a review of 11 cases. Pediatr Surg Int 2009; 25: 1073-6. [Crossref]
24. Sultan I, Rodriguez-Galindo C, El-Taani H, Pastore G, Casanova M, Gallino G, et al. Distinct features of colorectal cancer in children and adolescents: a population-based study of 159 cases. Cancer 2010; 116: 758-65. [Crossref]
25. Lamego CM, Torloni H. Colorectal adenocarcinoma in childhood and adolescent. Report of 11 cases and review of the literature. Pediatr radiology 1989; 19: 504-8. [Crossref]
26. Goldberg J, Furman WL. Management of colorectal carcinoma in children and young adults. J Pediatr Hematol Oncol 2012; 34 Suppl 2: S76-9. [Crossref]
27. Singer G, Hoellwarth ME. Colorectal carcinomas in children: an institutional experience. Pediatr Surg Int 2012; 28: 591-5. [Crossref]
28. Kaplan MA, Isikdogan A, Gumus M, Arslan UY, Geredeli C, Ozdemir N, et al. Childhood, adolescents, and young adults (</=25 y) colorectal cancer: study of Anatolian Society of Medical Oncology. J Pediatr Hematol Oncol 2013; 35: 83-9. [Crossref]
29. Weber ML, Schneider DT, Offenmuller S, Kaatsch P, Einsiedel HG, Benesch M, et al. Pediatric Colorectal Carcinoma is Associated With Excellent Outcome in the Context of Cancer Predisposition Syndromes. Pediatr Blood Cancer 2016; 63: 611-7. [Crossref]
30. LaQuaglia MP, Heller G, Filippa DA, Karasakalides A, Vlamis V, Wollner N, et al. Prognostic factors and outcome in patients 21 years and under with colorectal carcinoma. J Pediatr Surg 1992; 27: 1085-9. [Crossref]
31. Koh KJ, Lin LH, Huang SH, Wong JU. CARE--pediatric colon adenocarcinoma: a case report and literature review comparing differences in clinical features between children and adult patients. Medicine 2015; 94: e503. [Crossref]
32. Rao BN, Pratt CB, Fleming ID, Dilawari RA, Green AA, Austin BA, et al. Colon carcinoma in children and adolescents. A review of 30 cases. Cancer 1985; 55: 1322-6. [Crossref]
33. Anthony T, George R, Rodriguez-Bigas M, Petrelli NJ. Primary signet-ring cell carcinoma of the colon and rectum. Ann Surg Oncol 1996; 3: 344-8. [Crossref]
34. Kim HS, Kang SH, Park CH, Yang WI, Jeung HC, Chung HC, et al. Genome-wide molecular characterization of mucinous colorectal adenocarcinoma using cDNA microarray analysis. Oncol Rep 2011; 25: 717-27. doi:10.3892/or.2010.1126. [Crossref]
35. Raskov H, Pommergaard HC, Burcharth J, Rosenberg J. Colorectal carcinogenesis--update and perspectives. World J Gastroenterol 2014; 20: 18151-64. [Crossref]
36. Inoue Y, Saigusa S, Iwata T, Okugawa Y, Toiyama Y, Tanaka K, et al. The prognostic value of KRAS mutations in patients with colorectal cancer. Oncol Rep 2012; 28: 1579-84. [Crossref]
37. Dobre M, Comanescu M, Arsene D, Iosif C, Bussolati G. K-ras gene mutation status in colorectal cancer: comparative analysis of pyrosequencing and PCR-RFLP. Rom J Morphol Embryol 2013; 54: 567-74.
38. Moon BS, Jeong WJ, Park J, Kim TI, Min DS, Choi K. Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/beta-catenin signaling. J Natl Cancer Inst 2014; 106: djt373. doi:10.1093/jnci/djt373. [Crossref]
39. Guibert N, Ilie M, Long E, Hofman V, Bouhlel L, Brest P, et al. KRAS Mutations in Lung Adenocarcinoma: Molecular and Epidemiological Characteristics, Methods for Detection, and Therapeutic Strategy Perspectives. Curr Mol Med 2015; 15: 418-32. [Crossref]
40. Bryant KL, Mancias JD, Kimmelman AC, Der CJ. KRAS: feeding pancreatic cancer proliferation. Trends Biochem Sci 2014; 39: 91-100. [Crossref]
41. de Macedo MP, de Lima LG, Begnami MD, de Melo FM, Andrade LDB, Lisboa BCG, et al. KRAS insertions in colorectal cancer: what do we know about unusual KRAS mutations? Exp Mol Pathol 2014; 96: 257-60. [Crossref]
42. Li W, Zhi W, Zou S, Qiu T, Ling Y, Shan L, et al. Distinct Clinicopathological Patterns of Mismatch Repair Status in Colorectal Cancer Stratified by KRAS Mutations. PloS one 2015; 10: e0128202. [Crossref]
43. Markman B, Javier Ramos F, Capdevila J, Tabernero J. EGFR and KRAS in colorectal cancer. Advances in clinical chemistry. 2010; 51: 71-119. [Crossref]
44. Yantiss RK, Goodarzi M, Zhou XK, Rennert H, Pirog EC, Banner BF, et al. Clinical, pathologic, and molecular features of early-onset colorectal carcinoma. Am J Surg Pathol 2009; 33: 572-82. [Crossref]
45. Goel A, Nagasaka T, Spiegel J, Meyer R, Lichliter WE, Boland CR. Low frequency of Lynch syndrome among young patients with non-familial colorectal cancer. Clin Gastroenterol Hepatol 2010; 8: 966-71. [Crossref]
46. Chang DT, Pai RK, Rybicki LA, Dimaio MA, Limaye M, Jayachandran P, et al. Clinicopathologic and molecular features of sporadic early-onset colorectal adenocarcinoma: an adenocarcinoma with frequent signet ring cell differentiation, rectal and sigmoid involvement, and adverse morphologic features. Mod Pathol 2012; 25: 1128-39. [Crossref]
47. Alsop K, Mead L, Smith LD, Royce SG, Tesoriero AA, Young JP, et al. Low somatic K-ras mutation frequency in colorectal cancer diagnosed under the age of 45 years. Eur J Cancer 2006; 42: 1357-61. [Crossref]
48. Watson R, Liu TC, Ruzinova MB. High frequency of KRAS mutation in early onset colorectal adenocarcinoma: implications for pathogenesis. Hum Pathol 2016; 56: 163-70. [Crossref]
49. Karnak I, Ciftci AO, Senocak ME, Buyukpamukcu N. Colorectal carcinoma in children. Journal of pediatric surgery. 1999; 34: 1499-504. [Crossref]
50. Chattopadhyay S, Gupta P, Aich RK, Deb AR. Colorectal carcinoma in a ten-year-old girl: a case report. J Cancer Res Ther 2012; 8: 120-2. [Crossref]
51. Angel CA, Pratt CB, Rao BN, Schell MJ, Parham DM, Lobe TE, et al. Carcinoembryonic antigen and carbohydrate 19-9 antigen as markers for colorectal carcinoma in children and adolescents. Cancer 1992; 69: 1487-91. [Crossref]