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The role of CDX2, MUC5AC, and p53 in the evaluation of the progression of serrated lesions toward colorectal carcinoma

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Abstract

Background: Serrated colorectal lesions represent a potential precursor of 15–30% of colorectal carcinoma, although the exact mechanisms are not fully understood. The serrated pathway of colorectal carcinogenesis may be correlated with gastric-type metaplasia of the colon and modified expression of caudal type homeobox 2 (CDX2) and local mucins (MUCs). *Patients, Materials and Methods*: We performed a retrospective study of patients with resected polyps during 2014–2021. The prevalence of serrated lesions, risk factors associated with malignant polyps, and the role of gastric-type metaplasia associated with serrated pathway of carcinogenesis (CDX2, MUC5AC) and of p53 immunostaining in serrated lesions with dysplasia and carcinoma were assessed. *Results*: Five hundred twenty two (522) patients had 1199 polyps removed. We noted a 12.8% prevalence of sessile serrated adenoma/polyps (SSA/P) and traditional serrated adenomas (TSA); 17.4% had hyperplastic polyps. The malignancy rate of resected polyps was higher in tubulovillous adenoma (TVA) and villous adenoma (12.3–20%) than in SSA/P (8.3%) and TSA (4.8%). In TSA, no significant associations between malignancy and age, gender, size, location, or endoscopic appearance were noted. In SSA/P, malignant polyps were much larger, especially for the right side lesions, but the macroscopic type was not correlated with malignancy risk. Immunohistochemistry (IHC) was performed in 26 polyps with dysplasia or carcinoma; all cases had CDX2 immunostaining in the tumor cell's nucleus, with an average percentage of 96.5%. MUC5AC immunostaining was identified in the tumor cells' cytoplasm, with an average percentage of 31.81%; the intensity reaction was variable (average score 4.5). The percentage, intensity, and score for CDX2 IHC were similar for TSA and TVA and were lower for SSA/P with low-grade dysplasia (LGD), while for SSA/P with high-grade dysplasia (HGD) or carcinoma *in situ* (CIS), the percentage, intensity, and score were similar to TSA and TVA. p53 immunostaining was also positive in all cases, with an average percentage of 50.95% in the tumor cells' nucleus. p53 and MUC5AC had increased percentage, intensity, and scores from LGD to HGD in SSA/P and decreased mean percentage, intensity, and scores in TSA and TVA. *Conclusions*: The risk of malignancy in serrated lesions seems lower than in conventional adenomas, however, sessile serrated lesions can be involved in the appearance of interval cancers because of flat and pale macroscopic aspects. Gastric-type intestinal metaplasia may be associated with the serrated pathway, but more studies are needed to clarify mechanisms associated with serrated carcinogenesis.

Keywords: malignant polyp, serrated lesion, CDX2, MUC5AC, p53.

Introduction

Colorectal cancer (CRC) represents the most frequent gut malignancy and also a major cause of mortality [1]; the disease appears by progressive accumulation of genetic and epigenetic alterations [2]. Most CRCs arise from a conventional adenoma, but recent studies highlighted an alternative way called the "serrated polyp neoplasia pathway", which can occur in 15–30% of CRCs [1, 3–7] with serrated polyps as intermediary lesions. The progress from serrated lesions to carcinoma is faster compared to conventional adenoma [8], and sessile serrated adenoma/polyps (SSA/P) is considered the main culprit for "interval cancers" during screening colonoscopies [1, 4, 9–11].

In 2010, the *World Health Organization* (WHO) classified serrated lesions [5, 8, 9, 12] in SSA/P, traditional serrated adenomas (TSA), and hyperplastic polyps (HP) [2, 8, 13]. HP are the most frequent form of serrated lesions (70–95%) [1, 9], have three forms [microvesicular hyperplastic polyps (MVHP), goblet cell-rich hyperplastic polyps (GCHP) and mucin (MUC)-poor hyperplastic polyps (MPHP)] [10], and are usually small, with pale or mucosa-like color, left side location [5, 9], and star-shaped crypts (Kudo II) [5]. HP usually does not progress to carcinoma [10, 13], although rare cases of CRC may originate from large HP or patients with multiple HP or HP associated with other serrated lesions [12]. SSA/P are the second most frequent serrated lesions (5–25%) [1, 9, 10], have a sessile or flat appearance [14], with pale color, fuzzy pattern, and poorly delineated limits, frequently covered with yellow mucus [5, 9, 11, 15], with average size above 5 mm (50% above 10 mm in size) [9], are located to the right colon in more than 80% of

cases [5]; they may have high-grade dysplasia (HGD) in 10–13% [5] and can progress to carcinoma [5, 9, 10, 16]. TSA is the least frequently serrated lesion [1, 5, 9, 16], has a mixed appearance with both conventional tubular adenoma and sessile serrated polyps (SSP), and has the possibility of ectopic crypt formation [6, 13]; areas of dysplasia have been noted in a subset of TSA [6, 9]. Macroscopically, TSA is often above 10 mm, protrusive (0–Ip) [9], exophytic, with an erythematous surface and unspecific Kudo pattern (III–L, III–H, IV–S, II, IIIs) [5]. Advanced TSA has dysplasia diagnosed as an abrupt transition from traditional TSA to cytological features suggestive of dysplasia [6], and 11% of TSA may contain intramucosal carcinoma [9].

The traditional adenoma–carcinoma sequence (in conventional adenomas) requires a Wnt route with biallelic inactivation of the adenomatous polyposis coli (*APC*) gene, followed by an accumulation of mutations in other oncogenic genes, mainly in Kirsten rat sarcoma virus (*KRAS*) and *p53* [1, 13]. By contrast, in serrated pathway, a mechanism of epigenetic methylation of CpG islands located inside the promoter region of tumoral suppressor genes is involved [10], appeared as a consequence of a gene silencing of the MutL homolog 1 (*MLH1*) mismatch repair gene, facilitated by the presence of CpG island methylator phenotype (CIMP) [2, 17, 18], and main carcinogenic signals are activated within RAS/RAF/mitogen-activated protein kinase (MAPK) pathway, with predominant B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) or *KRAS* mutations [1, 2, 6, 7, 17, 19], predominantly in proximal colon [2]. *BRAF* mutation is seen in 70–80% of SSA/P and 70% of proximal HP or MVHP [12], with increased promoter methylation (leading to SSP) and silencing of *MLH1* (CIMP high) leading to SSP with dysplasia; later a Wnt route activation and p16 silencing can generate a *BRAF* mutant microsatellite instability (MSI) carcinoma. The possibility of rapid growth was noted [16, 18], especially after the appearance of dysplasia [18]. *KRAS* mutations are encountered in 29–46% of TSA [14, 20], and in 50% of HP from the distal colon and rectum, mainly in the GCHP [12]; in the KRAS route, TSA can appear initially, and carcinoma can develop by Wnt activation and *p53* mutation and microsatellite stability (MSS) [6]. It is suggested that HP, TSA, and SSA/P represent a morphological continuum within the same neoplastic pattern [3, 12], HP being a precursor of SSA/P (MVHP) or TSA (GCHP) [8].

Caudal type homeobox 2 (*CDX2*) is a homeobox gene with a role in embryonic development and regulation of the dynamic homeostasis of the gut [9, 21, 21–24]. In animal studies, *CDX2* mutation in intestinal epithelium induces gastric-type imperfect metaplasia with no progression toward carcinoma [21, 24], while a combined *CDX2* inactivation and $BRAF^{V600E}$ mutation promoted serrated benign and invasive tumors [12]. Loss of *CDX2* was associated in studies with unfavorable prognosis (advanced stage, poor differentiation, vascular invasion, *BRAF* mutations, and CIMP-positive phenotype) [14]. MUC5AC is a gastric-type MUC normally expressed, like MUC6, in the foveolar epithelium and also in deep antro-pyloric glands; it is absent in normal colonic mucosa [17, 25, 26]. A higher expression of MUC5AC was noted in HP and SSA/P [1, 26] together with MUC2 and MUC6 [26]; because of gastric possible origin, an overexpression of MUC5AC can be related to the inactivation of *CDX2* gene in serrated pathway neoplasia. The regulation of gastric-type MUC5AC was noted mostly in proximally located colon carcinoma [25] and in patients with MSI-high (MSI-H) or CIMP-positive CRC, and a correlation between MUC5AC, MUC6, absent/reduced CDX2 expression, and the presence of CIMP-positive and *BRAF* mutation was noted [27].

p53 is involved in most cases of CRC originating from conventional adenomas [28], but the loss of tumor protein p53 (*TP53*) function with nuclear staining for p53 is also seen in many advanced TSA [6]. The activation of a second event in the serrated pathway can induce the appearance of MSS serrated carcinomas [14, 17]. Although in HP and SSA/P usually there is no aberrant staining, in a study of 12 cases of SSA/P with dysplasia, six (50%) have nuclear p53 accumulation [29], and in another series of 24 TSA with dysplasia, six (25%) have nuclear p53 accumulation only in dysplastic cells [30]. p53 staining is increased in all serrated lesions [31], although the positivity was lower than in traditional adenomas [7].

Aim

The study aimed to estimate the prevalence of serrated lesions in patients with colon polyps, to assess risk factors associated with serrated lesions and with malignant polyps (size, macroscopic type, location, pathological type of the polyp, age, gender), and to evaluate the role of gastric-type metaplasia associated with serrated pathway of carcinogenesis (CDX2, MUC5AC) and of p53 immunostaining in serrated lesions with dysplasia and carcinoma.

Patients, Materials and Methods

We performed a retrospective study of colorectal polyps resected in the Department of Gastroenterology, Emergency County Hospital, Craiova and Research Center of Gastroenterology and Hepatology, University of Medicine and Pharmacy of Craiova, Romania, during eight years (2014–2021). Informed consent was obtained for every patient before polypectomy. Polyps were removed by simple polypectomy or endoscopic mucosal resection, especially in sessile large polyps or lateral spreading lesions. En-bloc resection was performed, when possible, although piecemeal resection was necessary in most large lesions. The macroscopic description noted Paris type (sessile or Paris 0–Is, pedunculated or Paris 0–Ip, semi-pedunculated or Paris 0–Isp, flat or lateral spreading lesions) [32]. All resected polyps were analyzed by three experienced pathologists (CG, MF, and MG) using the pathological classification of polyps, including the *WHO* 2010 Classification of serrated lesions [33]. In this study, we used the 2010 *WHO* Classification of serous polyps, instead of the 2019 one, to analyze possible significant differences that could support the changes introduced in the current classification. Pathological results described the type of polyp (adenoma, serrated or hyperplastic lesion, carcinoma), adenoma subtype (tubular, tubulovillous, villous), the presence and the grade of dysplasia (LGD or HGD), and the presence of carcinoma; revised Vienna Classification [24] was used for the presence of dysplasia and carcinoma. In 26 resected polyps, immunohistochemical analysis was performed; the panel of antibodies used is shown in Table 1.

Table 1 – ***The antibodies used in the study***

Antibody	Host, clone, manufacturer	Dilution	Pretreatment	External positive control
CDX2	Rabbit anti-human, CDX2, Thermo Fisher Scientific	1:250	Microwaving in EDTA-based buffer, pH 8.0	Pancreas
MUC5AC	Rabbit anti-human, MUC5AC, Thermo Fisher Scientific	1:500	Microwaving in EDTA-based buffer, pH 8.0	Gastric mucosa
p53	Rabbit anti-human, p53, Thermo Fisher Scientific	1:500	Microwaving in citrate buffer, pH 6.0	Tonsil

CDX2: Caudal type homeobox 2; EDTA: Ethylenediaminetetraacetic acid; MUC5AC: Mucin 5AC.

We used the amplification system Labeled Streptavidin–Biotin 2 (LSAB2)–Horseradish peroxidase (HRP) (DAKO, Redox, Bucharest, Romania, code K0675), and signal visualization with 3.3'-Diaminobenzidine (DAB) tetrahydrochloride (Dako, code 3467) and we reported the data as average values ± standard deviation (SD); a Nikon Eclipse E600 microscope with Lucia 5 imaging software was used for image acquisition. We categorized the number of marked cells as 0 (absent), 1 (<10% marked cells), 2 (10–25% marked cells), 3 (26–50% marked cells), and 4 (>50% marked cells), and the intensity of the marked cells as 0 (absent), 1 (poor), 2 (moderate), and 3 (strong). The resulting scores were considered low (1 to 4) and high (6 to 12).

Statistical analysis was made using IBM Statistical Package for the Social Sciences (SPSS) Statistics Faculty Packs, odds ratio (OR) calculation for estimation of risk, unpaired Student's *t*-test for continuous parameters, and χ^2 (*chi*-squared) contingency table for categorical parameters.

Results

Prevalence of serrated lesions

From 2014 to 2021, in both Clinics, a total of 470 patients had a total of 1199 polyps that were resected during colonoscopy; 104 patients had two types of polyps, and 18 cases of juvenile polyps, hamartomas, fibroid, or inflammatory polyps in 14 patients were also recorded (Table 2). Eight hundred and eighteen were conventional adenomas (Figure 1), 154 lesions were serrated polyps, of which 96 were SSA/P (Figures 2 and 3), and 58 were TSA (Figures 4 and 5); 209 were HP (Figure 6). Clinical and pathological characteristics (sex, age, size, location, macroscopic type) are illustrated in Table 3.

Large HP are considered similar to sessile serrated lesions by some authors, especially in the right colon. In our study, six HP from the right colon (13% of right colon polyps) had a diameter of 10 mm or above, and 23 of 163 (14.1%) left or rectum colon HP had a diameter equal to or above 10 mm.

Table 2 – ***Type of resected polyps in selected group***

Type	Patients, *n*	Polyps, *n* (%)
Serrated (SSA/P, TSA)	90	154 (12.8)
Hyperplastic	72	209 (17.5)
Classical adenoma	346	818 (68.2)
Other	14	18 (1.5)
Total		*1199 (100)*

n: Number; SSA/P: Sessile serrated adenoma/polyp; TSA: Traditional serrated adenoma.

Table 3 – ***Clinical and pathological types of serrated lesions***

Parameter	HP	SSA/P	TSA	Traditional adenoma
M/F ratio	48/24	32/22	25/11	215/131
Age, median (range) [years]	59.6 (35–85)	64.6 (33–79)	65.4 (37–82)	63.5 (33–87)
Size, median (range) [mm]	6.3 (2–20)	11.97 (3–50)	28.05 (3–90)	10.17 (2–80)
▪ Right lesions	6.1	8.9	10.1	7.8
▪ Left + rectum lesions	6.3	14.9	32.3	11.5
▪ >10 mm	6/46 R, 15/163 L	17/47 R, 29/49 L	5/11 R, 42/47 L	61/266 R, 249/552 L
Location, *n* (%)				
▪ Right colon	46 (22)	47 (49)	11 (19)	266 (32.5)
▪ Left colon (excluding rectum)	112 (53.6)	30 (31.2)	22 (37.9)	439 (53.7)
▪ Rectum	51 (24.4)	19 (19.8)	25 (43.1)	113 (13.8)
Endoscopic appearance, *n* (%)				
▪ Paris Ip+Isp	9 (4.3)	22 (22.9)	16 (27.6)	249 (30.5)
▪ Paris Is	185 (88.5)	58 (60.4)	16 (27.6)	495 (60.6)
▪ Flat polyp	15 (7.2)	9 (9.4)	7 (12.1)	54 (6.6)
▪ LST	0 (0)	7 (7.3)	19 (32.8)	19 (2.3)

F: Female; HP: Hyperplastic polyp; L: Left; LST: Lateral spreading tumor; M: Male; *n*: Number; R: Right; SSA/P: Sessile serrated adenoma/polyp; TSA: Traditional serrated adenoma.

The presence of dysplasia and carcinoma

A significant percentage of patients with polyps may have concomitant dysplasia (LGD or HGD) or even carcinoma. We analyzed all polyps, including traditional adenomas and serrated lesions (SSA/P, TSA, and HP) for the presence and grade of dysplasia and also for carcinoma presence (Table 4).

The analysis of data showed that concomitant carcinoma in resected polyps was more frequent in tubulovillous adenoma (TVA) and villous traditional adenoma (12.3–20%), while in SSA/P was 8.3% and in TSA was 4.8%. In tubular adenoma, the frequency of carcinoma was low (1%). The frequency of HGD was high in TSA (87.8%) and traditional adenoma (66.4%), and moderate in SSA/P (46.9%).

We analyzed risk factors for carcinoma presence in patients with TSA, SSA/P, and traditional adenoma (Tables 5–7). In TSA, no significant associations with age, gender, size, location, and endoscopic appearance were noted. In SSA/P, malignant polyps were much larger, especially for the right side lesions, and polyps above 10 mm diameter were more often malignant; macroscopic appearance (Paris type) was not correlated with malignancy risk. In conventional adenomas, the malignancy risk was greater in larger lesions and lesions above 10 mm regardless of the location, but right-sided conventional adenomas were more often benign [OR 2.78, 95% confidence interval (CI) 1.39 to 5.54, p=0.0038]. Pedunculated and semi-pedunculated types and lateral spreading types of conventional adenomas also had a higher risk for malignancy (OR 5.89, 95% CI 3.39 to 10.24, p<0.0001, and OR 5.90, 95% CI 2.16 to 16.09, p=0.0005, respectively).

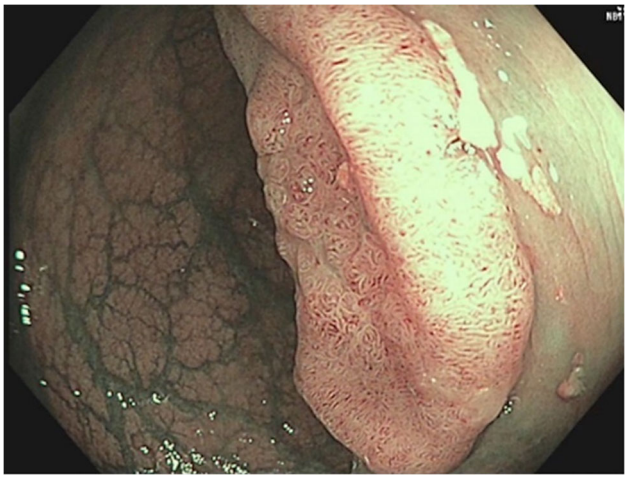

Figure 1 – ***Conventional adenoma with a lateral spreading appearance: endoscopic aspect.***

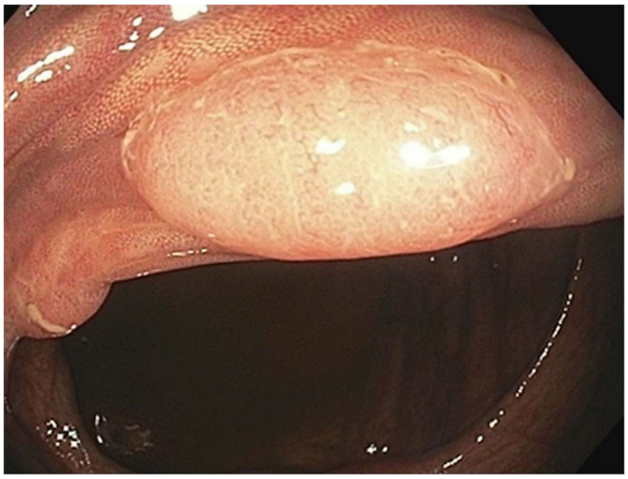

Figure 2 – ***Sessile serrated lesion in the right colon: endoscopic aspect.***

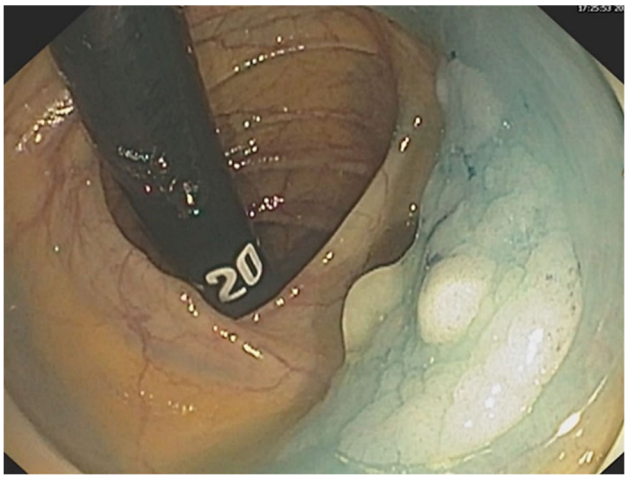

Figure 3 – ***Sessile serrated adenoma/polyp-flat lesion in the right colon: endoscopic aspect.***

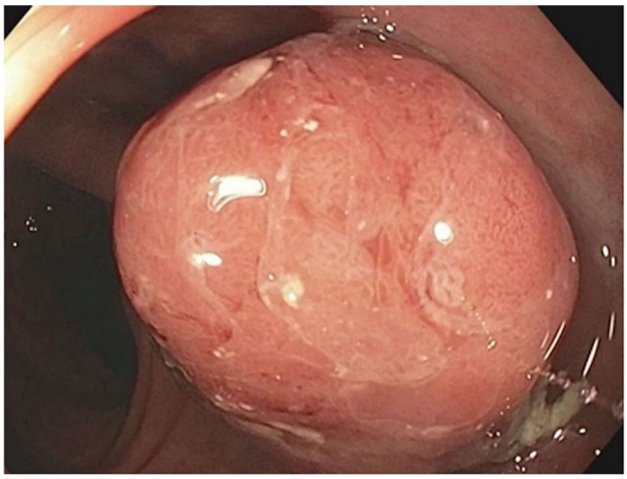

Figure 4 – ***Pedunculated traditional serrated adenoma Paris 0–Ip: endoscopic aspect.***

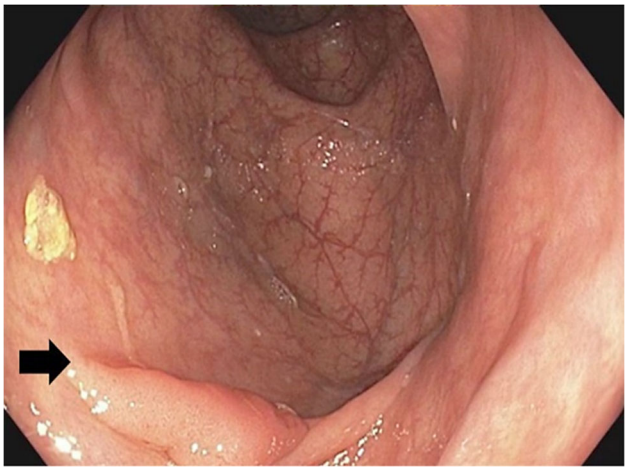

Figure 5 – ***Sessile (Paris 0–Is) traditional serrated adenoma: endoscopic aspect.***

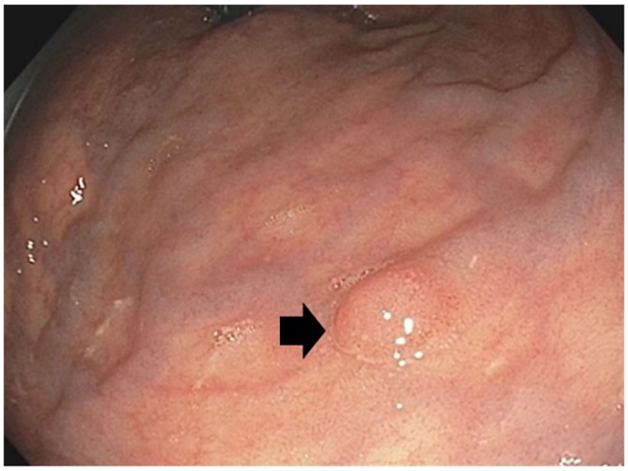

Figure 6 – ***Small hyperplastic polyp: endoscopic aspect.***

Table 4 – ***Frequency of dysplasia and coexistent carcinoma in resected polyps***

Polyp type	Polyps, *n*	Dysplasia, *n* (%)	LGD, *n* (%)	HGD, *n* (%)	Carcinoma, *n* (%)
SSA/P	96	4 (4.2)	47 (49)	45 (46.9)	8 (8.3)
TSA	58	6 (10.3)	8 (13.8)	44 (75.9)	11 (4.8)
Hyperplastic	209	115 (55)	31 (14.8)	63 (30.1)	0 (0)
Traditional adenoma	818	32 (3.9)	243 (29.7)	543 (66.4)	64 (7.8)
Tubular	190	19 (10)	112 (58.9)	59 (31.1)	2 (1)
▪ Tubulovillous	237	0 (0)	29 (12.2)	208 (87.8)	39 (16.5)
▪ Villous	15	0 (0)	3 (20)	12 (80)	3 (20)
▪ Adenomatous	376	13 (3.5)	99 (26.3)	264 (70.2)	19 (12.3)

HGD: High-grade dysplasia; LGD: Low-grade dysplasia; *n*: Number; SSA/P: Sessile serrated adenoma/polyp; TSA: Traditional serrated adenoma.

Table 5 – ***Risk factors for colorectal carcinoma (TSA)***

Parameter	Malignant polyps	Benign polyps	*p*-value
M/F ratio (% M)	8/1 (88.9)	19/9 (67.9)	0.2406
Age, median ± SD (range) [years]	66.2±7.5 (57–80)	65.1±10.8 (37–82)	0.7822
Size, median ± SD (range) [mm]	34.5±24 (10–90)	26.7±22.3 (3–90)	0.3259
▪ Right lesions	–	10.1±8 (4–30)	–
▪ Left lesions	23±15.7 (10–50)	22.6±16 (3–50)	0.9600
▪ Rectum lesions	46±26.8 (25–90)	39.4±25.3 (10–90)	0.6083
▪ >10 mm, *n* (%)	10 (100)	37 (77.1)	0.2101
Location, *n* (%)			0.3098
▪ Right colon	0 (0)	11 (22.9)	
▪ Left colon (excluding rectum)	5 (50)	17 (35.4)	
▪ Rectum	5 (50)	20 (41.7)	
Endoscopic appearance, *n* (%)			0.0946
▪ Paris Ip+Isp	4 (40)	12 (25)	
▪ Paris Is	1 (10)	15 (31.3)	
▪ Flat polyp	0 (0)	7 (14.6)	
▪ LST	5 (50)	14 (29.2)	
Dysplasia, *n* (%)			0.2723
▪ LGD	0 (0)	8 (70.8)	
▪ HGD	10 (100)	34 (16.7)	

F: Female; HGD: High-grade dysplasia; LGD: Low-grade dysplasia; LST: Lateral spreading tumor; M: Male; *n*: Number; SD: Standard deviation; TSA: Traditional serrated adenoma.

Table 6 – ***Risk factors for colorectal carcinoma (SSA/P)***

Parameter	Malignant polyps	Benign polyps	*p*-value
M/F ratio (% M)	5/2 (71.4)	26/18 (59.1)	0.5381
Age, median ± SD (range) [years]	69.1±5.8 (63–79)	63.9±10 (33–79)	0.1845
Size, median ± SD (range) [mm]	21.6±10.6 (10–40)	11.2± 9.5 (3–50)	*0.0068*
▪ Right lesions	17.5±10.6 (10–25)	8.6±4.3 (3–20)	*0.0090*
▪ Left lesions	16.6±8.3 (7–30)	12.4±11.5 (4–50)	0.4420
▪ Rectum lesions	40±0 (40–40)	16.5±13.5 (3–50)	*<0.0001*
▪ >10 mm, *n* (%)	7 (100)	39 (43.8)	*0.0454*
Location, *n* (%)			0.3403
▪ Right colon	2 (28.6)	45 (50.6)	
▪ Left colon (excluding rectum)	4 (43.1)	26 (29.2)	
▪ Rectum	1 (14.3)	18 (20.2)	
Endoscopic appearance, *n* (%)			0.5039
▪ Paris Ip+Isp	2 (28.6)	20 (22.5)	
▪ Paris Is	4 (57.1)	54 (60.7)	
▪ Flat polyp	0 (0)	9 (10.1)	
▪ LST	1 (14.3)	6 (6.7)	
Dysplasia, *n* (%)			*0.0481*
▪ LGD	0 (0)	47 (52.8)	
▪ HGD	7 (100)	38 (42.7)	

F: Female; HGD: High-grade dysplasia; LGD: Low-grade dysplasia; LST: Lateral spreading tumor; M: Male; *n*: Number; SD: Standard deviation; SSA/P: Sessile serrated adenoma/polyp.

Table 7 – ***Risk factors for colorectal carcinoma (conventional adenoma)***

Parameter	Malignant polyps	Benign polyps	*p*-value
M/F ratio (% M)	40/22 (64.5)	175/108 (61.8)	0.6310
Age, median ± SD (range) [years]	62.8±9.5 (38–87)	63.7±10 (33–86)	0.5508
Size, median ± SD (range) [mm]	21.6±11.5 (5–60)	9.2±7.7 (2–80)	*<0.0001*
▪ Right lesions	15.8±11.4 (7–45)	7.4±5.5 (3–40)	*<0.0001*
▪ Left lesions	22.1±10.7 (5–50)	9.9±7 (2–40)	*<0.0001*
▪ Rectum lesions	24.1±12.9 (12–60)	11±12.9 (3–80)	*0.0004*
▪ >10 mm, *n* (%)	60 (93.8)	250 (33.2)	
Location, *n* (%)			*<0.0001*
▪ Right colon	10 (15.6)	256 (34)	
▪ Left colon (excluding rectum)	39 (60.9)	400 (53.1)	
▪ Rectum	15 (23.4)	98 (13)	
Endoscopic appearance, *n* (%)			*<0.0001*
▪ Paris Ip+Isp	44 (68.8)	205 (27.2)	
▪ Paris Is	12 (18.8)	484 (64.2)	
▪ Flat polyp	2 (3.1)	52 (6.9)	
▪ LST	6 (9.4)	13 (1.7)	
Dysplasia, *n* (%)			*<0.0001*
▪ LGD	1 (1.6)	242 (32.1)	
▪ HGD	63 (98.4)	480 (63.7)	

F: Female; HGD: High-grade dysplasia; LGD: Low-grade dysplasia; LST: Lateral spreading tumor; M: Male; *n*: Number; SD: Standard deviation.

Immunohistochemistry (CDX2, MUC5AC, p53)

All cases had CDX2 immunostaining located in the tumor cell's nucleus, with an average percentage of 96.5%.

A strong CDX2 intensity reaction was noted (Figure 7, A–D). MUC5AC immunostaining was identified in the tumor cells' cytoplasm, with an average percentage of 31.81%; the intensity reaction of MUC5AC was variable, with an average score of 4.5 (Figure 8, A–D). p53 immunostaining was also positive in all cases, with an average percentage of 50.95% in the tumor cells' nucleus. p53 intensity reaction was variable, with an average score of 4.04 (Figure 9, A–D). The percentage, intensity, and score for CDX2 immunohistochemistry were similar for TSA and TVA and were lower for SSA/P with low-grade dysplasia (LGD), whereas for SSA/P with HGD or carcinoma *in situ* (CIS) the percentage, intensity, and score were similar to TSA and TVA (Figure 7C). In SSA/P, both p53 and MUC5AC had increased percentage, intensity, and scores from LGD to HGD, while in TSA and TVA, lower mean percentage, intensity, and scores were noted during progressions from LGD to HGD. In patients with CIS, MUC5AC was similar to LGD in both TSA and TVA and lower than in HGD (Table 8).

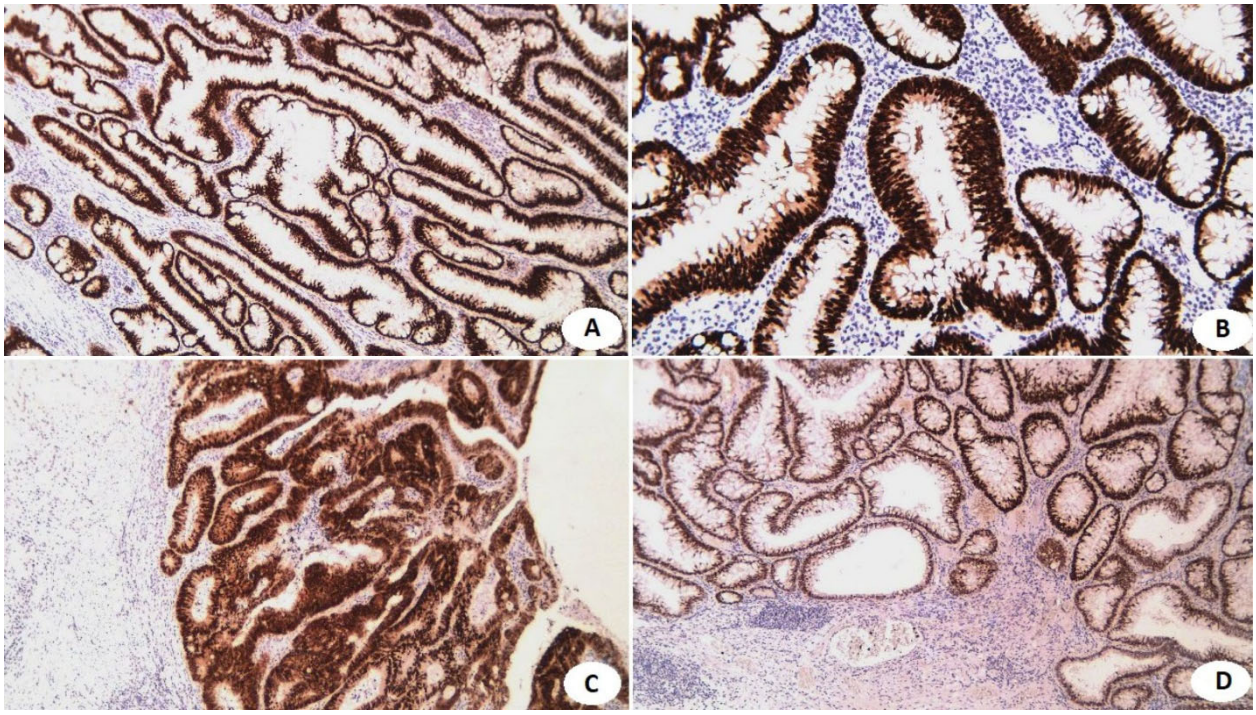

Figure 7 – ***CDX2 immunostaining: (A) Traditional serrated adenoma, 100×; (B) Tubular adenoma, 200×; (C) Carcinoma in situ, 100×; (D) Sessile serrated lesion, 100×. CDX2: Caudal type homeobox 2.***

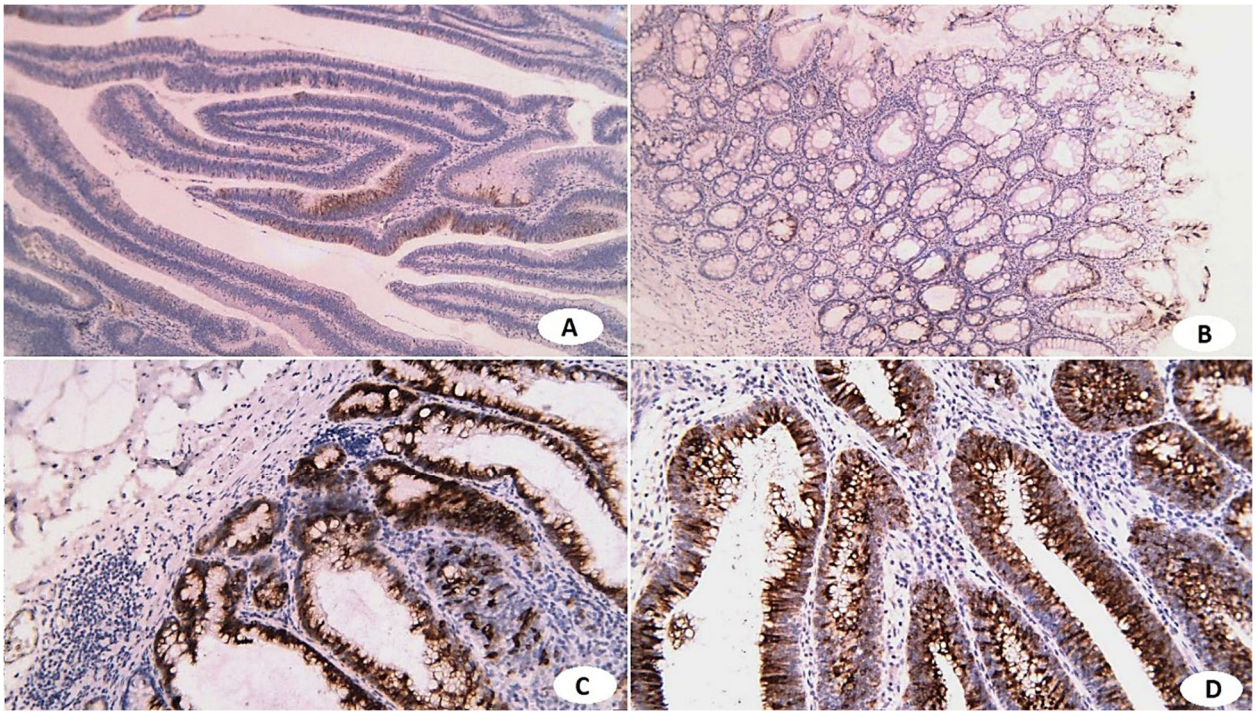

Figure 8 – ***MUC5AC immunostaining: (A) Tubulovillous adenoma, 100×; (B) Hyperplastic polyp, 100×; (C) Sessile serrated lesion, 100×; (D) Tubular adenoma, 100×. MUC5AC: Mucin 5AC.***

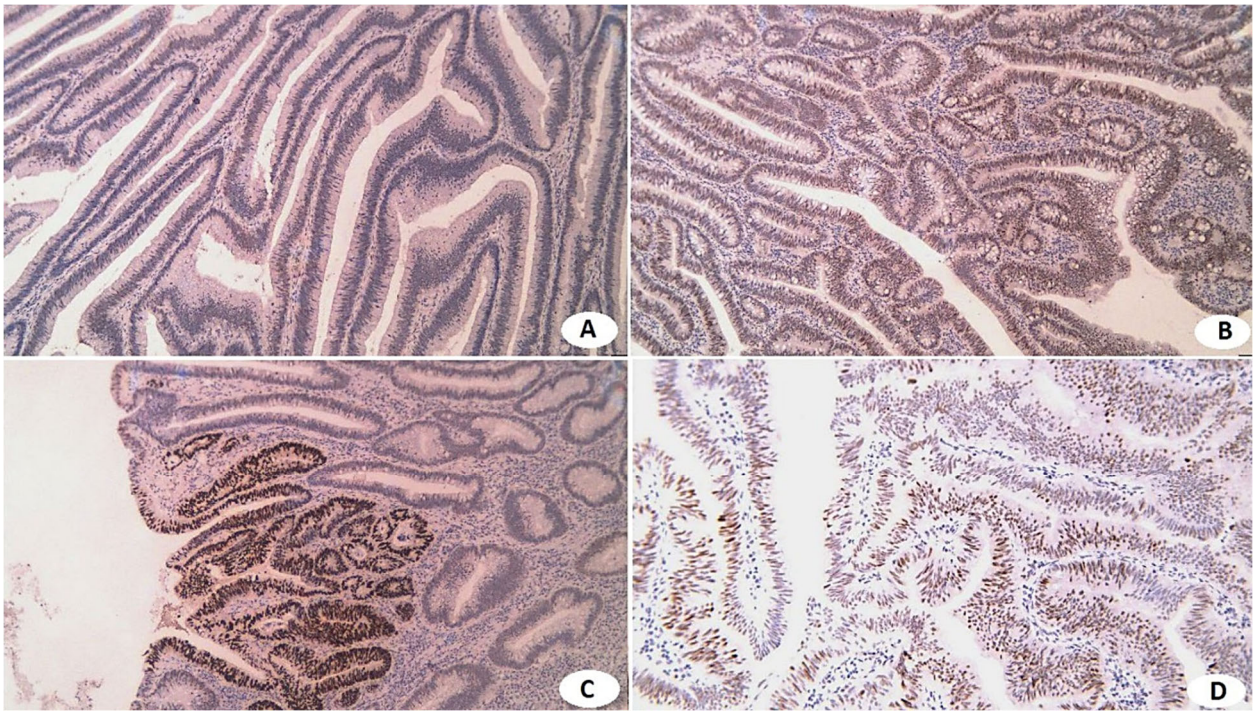

Figure 9 – ***p53 immunostaining: (A) Tubulovillous adenoma, 100×; (B) Sessile serrated lesion, 100×; (C) Carcinoma in situ, 100×; (D) Tubular adenoma, 100×.***

Table 8 – ***Immunohistochemistry (CDX2, MUC5AC, p53)***

Parameter	CDX2			MUC5AC			p53		
	%	*Intensity*	*Score*	*%*	*Intensity*	*Score*	*%*	*Intensity*	*Score*
TSA (all)	99.08	3	12	28.92	2.31	4.31	60.54	1.38	4.54
▪ LGD	96.66	3	12	18.33	2	2.67	58.33	1.67	5.67
▪ HGD	100	3	12	44.2	2.8	7	54.4	1.2	3.6
▪ CIS	100	3	12	20	2	2.6	68	1.4	4.8
SSA/P (all)	93.75	2.63	10.5	42.5	1.88	4.88	43.88	1.38	3.63
▪ LGD	92.5	2.5	10	33.33	1.5	3.67	33.5	1.33	3.17
▪ HGD	97.5	3	12	70	3	8.5	75	1.5	5
▪ CIS	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TVA (all)	92.5	3	12	19	2.25	4.5	35.5	1.5	3.75
▪ LGD	100	3	12	32.5	3	7.5	57.5	1.5	5
▪ HGD	90	3	12	1	1	1	2	1	1
▪ CIS	80	3	12	10	2	2	25	2	4
HP	100	3	12	35	2	4	45	1	2
LGD (all)	95	2.73	10.91	29.09	1.91	6.63	44.64	1.45	4.18
HGD (all)	97.88	3	12	45.25	2.63	6.63	53	1.22	3.63
CIS (all)	96.67	3	12	18.33	2	2.5	60.83	1.5	4.67
All polyps	96.46	2.88	11.54	31.81	2.15	4.5	50.96	1.38	4.04

CDX2: Caudal type homeobox 2; CIS: Carcinoma *in situ*; HGD: High-grade dysplasia; HP: Hyperplastic polyp; LGD: Low-grade dysplasia; MUC5AC: Mucin 5AC; N/A: Not applicable (available); SSA/P: Sessile serrated adenoma/polyp; TSA: Traditional serrated adenoma; TVA: Tubulovillous adenoma.

Discussions

The frequency of sessile serrated lesions and TSA was 12.8%, and for hyperplastic lesions it was 17.4%, similar to the literature data. Some studies have noted a prevalence between 0.6–13% for SSP [8, 15], with SSP and HP more frequently discovered than TSA [8, 15, 34], One large study including 9191 patients has shown a prevalence of 0.53% for serrated polyps, 41.2% being HP, 7.2% SSA/P and 51.6% TSA [35], while in another large study of 4989 patients serrated lesions represent 5.6% of total number of polyps, with 3.7% having proximal serrated polyps and 0.4% having large serrated polyps [11]. In autopsy reports, the prevalence of serrated polyps in the general population has been estimated to be between 13% and 40%, much higher than in clinical studies [23]. It is worth noting that the reclassification of polyps as serrated lesions may be encountered in 25% of cases in studies performed after 2010 [36].

In our study, male gender predominance was noted in all types of polyps. Gender predisposition in serrated lesions is a controversial issue [16]; some studies found a male predisposition [15, 35, 37, 38], while others did not show any predisposition [39–41]. A large study of 179 000

patients with polyps and 2416 SSA/P found that 54% of patients with SSA/P were females (OR 1.21, 95% CI 1.11–1.32) [42], and female/male sex ratio increases with age; similar data were provided by some studies [43, 44], while another study showed that female sex has no significant association with SSA/P [4, 40]; 50.9% of patients with TSA were women and there was a trend toward the risk of dysplasia in a study (although statistical significance was not reached) [42]. In a study of 163 serrated lesions, the male/female ratio was 4/18 SSA/P with dysplasia/carcinoma [34]. The mean age was slightly lower (59.6 years) in HP (with no statistical significance) than in other serrated lesions (64.6 in SSP and 65.4 years in TSA) or traditional adenoma (63.5 years), similar to literature data [6, 8, 15, 16, 34, 42, 45, 46].

Regarding macroscopic appearance, HP were usually small (only 10% being 10 mm or larger), were more frequently located to the left colon and rectum (78%), and with sessile aspect (88.5%), while TSA were larger (mean diameter 10 mm in the right colon and 32.1 mm in the left colon and rectum), were located in almost 50% of cases in the right colon and had a higher proportion of lateral spreading pattern (32.8%). Sessile serrated lesions and traditional adenomas had a similar mean diameter (8.9 mm for the right location and 14.9 mm for the left location *versus* 7.8 mm and 11.5 mm, respectively) and were more often sessile (60.4% and 60.6%); sessile serrated lesions were more often located to the right colon (49% *versus* 32.5%). Data regarding location, macroscopic type, and size were similar to literature data [6, 15, 34, 45].

In our study, traditional TVA and villous adenomas had a higher risk for malignancy than serrated lesions (SSA/P and TSA). In the literature, the potential of serrated lesions to progress toward carcinoma was considered lower (3.2%) compared to conventional adenomas (9.3%), and fewer cases of serrated lesions had intraepithelial neoplasia (IEN) compared to conventional adenomas (4.8% serrated lesions, including 6.2% with IEN) [8]; female sex and diameter of serrated lesion >10 mm are considered risk factors. 5.3% of serrated lesions and 2.2% of conventional adenomas can be complicated by carcinoma [8]; approximately 5% of SSA/P develop into carcinoma during 20-year intervals [8]. Because of its small diameter and difficult-to-detect feature, SSA/P is considered the main factor for interval carcinoma [15]. In a monocentric study of over 15 000 patients with resected polyps, only 1.3% of patients with serrated lesions had concomitant CRC [15]. In another study of 5347 patients with polyps, 258 had serrated lesions, and the presence of invasive carcinoma/high-grade intraepithelial neoplasia (HIN) was noted in 16 (6.2%) cases, seven cases being TSA, six SSA/P, and three HP [8].

Several studies have correlated the serrated pathway of CRC with gastric-type metaplasia [12, 24–27, 47, 48] and also with a modified expression of CDX2 and local MUCs (most often MUC2, MUC5AC, and MUC6) [49–51]. CDX2 inhibits the proliferation of colon cancer cells by stopping the transition from G0/G1 phase of the cell cycle to S phase and acting as a negative mediator of Wnt signaling in colon cancer cells [22, 52, 53]. CDX2 was identified as a protein capable of binding to the MUC2 promoter and initiating transcription [24]. Several mechanisms were proposed to explain reduced expression of CDX2: an epigenetic silencing of the CDX2 promoter [26], downstream effectors of RAF/MAPK activity [26], trans-acting pathway of repression for CDX2 with diminished expression and a possible role for phosphatidylinositol 3-kinase (PI3K) activity and lipid phosphatase and tensin homolog (PTEN) overexpression [54]. In our study, the CDX2 expression seems lower only in the case of SSA/P associated with LGD and higher in most SSA/P and TSA; this last finding was similar to conventional adenoma. Studies in mice have shown that metaplastic-type depleted CDX2 areas do not become tumorigenic but stimulate tumorigenesis in adjacent areas, which suggests a tumoral suppressor effect of the *CDX2* homeobox gene [54], and this may be an explanation for the high expression of CDX2 in preneoplastic lesions in our study. In human CRC, the reduction of CDX2 is inversely related to tumor grade and stage, lymphatic metastasis, and dismal prognosis [22]. A strong loss of CDX2 appears correlated with poor survival [21] and is encountered predominantly in right-sided, poorly differentiated colon carcinomas [54].

MUC expression (especially MUC2, MUC5AC, and MUC6) can be related to serrated carcinogenesis pathways [55–58]. In our study, MUC5AC immunostaining was identified in the tumor cells' cytoplasm, with an average percentage of 31.81%; a higher percentage was noted in serrated lesions (42.5% in SSA/P, 35% in HP, and 28.9% in TSA) than in TVA (19%). In a study of colorectal serrated lesions (with and without cytological dysplasia or carcinoma) by molecular pattern [12], MUC5AC expression was present in all groups (52.4–92.3%) with higher expression in group 1 [92.3% – *BRAF* mutation, high-methylation epigenotype (HME), MSI] and group 2 (87.0% – *BRAF* mutation, HME, MSS). The appearance of MUC5AC in tissues that normally do not express MUC5AC is a marker of neoplastic progression [25]. In another study of four SSA/P with dysplasia and four SSA/P with carcinoma, seven polyps expressed MUC5AC and MUC6, and all polyps expressed MUC2 [17]. The possible explanations for enhanced MUC expression include a methylation of the CpG island promoter [24], the activation of the epidermal growth factor receptor (EGFR)–RAS–RAF signal transduction pathway [24], and the regulation by transcriptional factors, such as Sp1, AA-1, and CDX2.

In our study, p53 immunostaining was noted in all types of polyps (60.5% in TSA, 43.9% in SSA/P, 35.5% in TVA, and 45% in HP). Proteins encoded by *TP53* can induce apoptosis, cellular cycle abnormalities, senescence, alterations in deoxyribonucleic acid (DNA) repair, or metabolic changes induced by cellular stress [17]. In a study, p53 expression was seen in HP in the lower part of the crypts, while in SSA/P and TSA, p53 expression was both increased and extended to the upper half of the crypts, with a disorganized distribution in TSA [30]. Another study found positivity for p53 in 33% of HP, 40% of serrated adenomas, 62% of tubular adenomas, and 77% in TVA, while positivity percentage increased to 77% in dysplastic polyps and 75% in polyps with carcinoma [7]. In our study, the positivity for p53 has increased from LGD (44.6%) to HGD (53%) and CIS (60.8%).

Conclusions

In our study, sessile serrated lesions and TSA represent 12.8%, and hyperplastic lesions 17.4% of all resected polyps.

The risk of malignancy was higher in villous adenoma and TVA as compared to sessile serrated lesions and TSA; no significant associations with age, gender, size, location, and endoscopic appearance were noted in TSA, while in SSA/P, malignant polyps were much larger, especially for the right-side lesions. In conventional adenomas, the malignancy risk was greater in larger lesions, in lesions above 10 mm, in pedunculated and semi-pedunculated types, and in lateral spreading types.

CDX2 immunostaining was identified in the tumor cell's nucleus in all cases, with slightly lower expression only in SSA/P with LGD. MUC5AC immunostaining was identified in the tumor cells' cytoplasm, with an average percentage of 31.81%; a higher percentage was noted in serrated lesions than in TVA. p53 immunostaining was noted in all types of polyps. In SSA/P, both p53 and MUC5AC had increased percentage, intensity, and scores from LGD to HGD, while in TSA and TVA, lower mean percentage, intensity, and scores were noted during progressions from LGD to HGD. In patients with CIS, MUC5AC was similar to LGD in TSA and TVA and lower than in HGD.

Conflict of interests

The authors declare no conflict of interests.

Authors' contribution

Sevastiţa Iordache and Sergiu Marian Cazacu equally contributed to this article.

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