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RESEARCH ARTICLE

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CLASSIFICATION ASPECTS OF SERRATED FORMATIONS OF THE LARGE INTESTINE.

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Abstract

Serrated formations of the colon in modern classification are divided into three main categories: hyperplastic polyp (HP), sessile serrated adenoma/polyp (SSA/P), traditional serrated adenoma (TSA). Despite the existing difficulties, pathologists are encouraged to make every effort to identify SSA/P, since their potential malignancy is higher than that of HP. This work is devoted to the study of morphological characteristics of HP and SSA/P to clarify the possibilities of differential diagnostics and compare their potential malignancy based on the immunohistochemical profile (cdx2, beta-catenin, p53, ki67, claudin-1, -3, -4, CD44, Musashi-1). The results we obtained indicate the vague nature of the morphological differences between HP and SSA. The immunohistochemical profile does not differ between HP and SSA/P either. The nature of expression of most markers studied, indicates a lower potential malignancy of HP and SSA/P compared to TSA. The relatively high incidence of abnormal CDX2 expression suggests a significant role of alternative ways of HP and SSA/P malignization. TSA is fundamentally different from HP and SSA/P in immunophenotype, which may indicate both a higher potential malignancy of TSA and different ways of carcinogenesis of these types of polyps, despite their unification into a general group of “serrated formations”.

Introduction:-

Until recently, most polyps of the large intestine belonged to one of the two groups: hyperplastic or dysplastic formations (tubular, tubulovillous, villous adenomas). In 1984, S.J. Urbanski *et al.* described a polyp of mixed morphology: a serrated crypt architecture, as in a hyperplastic polyp, in combination with dysplasia, like in an adenoma (Urbanski et al., 1984). In 2003, E.E. Torlakovic *et al.* (Torlakovic et al., 2003) revised the morphology of hyperplastic polyps and isolated a group of formations whose characteristic feature was a pronounced dilation of the basal portions and a horizontal growth of the crypts along the mucosal muscular plate (sessile serrated adenoma/polyp). In 2003, N.S. Goldstein et al (Goldstein et al., 2003) described a number of cases of colon cancer whose precursors were formations similar in structure to hyperplastic polyps, without signs of epithelial dysplasia, which served as a reason for including serrated formations in the WHO classification in the group of premalignant

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lesions of the colon in 2010 (Snover D, 2010) and for an active search for markers reflecting their potential malignancy.
Serrated formations of the large intestine in modern classification (Snover D, 2010) are divided into three main categories: hyperplastic polyp (HP), sessile serrated adenoma/polyp (SSA/P) and traditional serrated adenoma (TSA). In general, the type of a serrated formation is determined by its cytological and architectural features. One of the key problems in the differential diagnostics of serrated formations of the large intestine is a differential diagnosis between HP and SSA/P. Despite the existing difficulties, pathologists are encouraged to make every effort to identify SSA/P (Rex et al., 2012), since their potential malignancy is higher than that of HP, and in a number of studies it is similar to the potential malignancy of classical adenomas (East et al., 2008; Kawasaki et al., 2016; Salaria et al., 2012) .

The current hypothesis about the difference in the potential malignancy and different frequency of malignization of serrated formations prompts a search for additional criteria for distinguishing different morphologic types of polyps from each other. An active search for prognostically significant factors continues – immunohistochemical profiles of the expression of tight junction protein markers, adhesion molecules, tumor stem cell markers, proliferative activity markers, and others (Baker et al., 2015; Bettington et al., 2015; Caruso et al., 2014; Chai et al., 2013).

Claudins is a family of transmembrane proteins belonging to tight junction proteins. Tight junctions of malignant cells often contain structural and functional anomalies, manifested in a changed expression level of various types of claudins (Soler et al., 1999). Possibly, a change in the level or nature of the reaction of tight junction proteins can be also detected in serrated formations of the large intestine and will serve as one of the markers of started malignant transformation.

Cancer stem cells (CSC) are discussed as a source of colorectal cancer, they are capable of self-maintenance and multipotent differentiation and can be formed from mutant stem cells or by dedifferentiation of crypt epithelial cells (Fanali et al., 2014). Musashi-1 marker (Msi-1) is considered by some authors as a CSC marker in colon and stomach cancer (Kuang et al., 2013; Li et al., 2011). CD44 protein, which is an adhesion molecule, is also considered as a CSC marker. A change in the CD44 level is associated with a poor prognosis, an increase in the number of metastases in patients with colorectal cancer (Dallas et al., 2012). Possibly a change in the level or nature of the reaction of these markers can be also detected in serrated formations of the large intestine with greater potential malignancy.

Beta-catenin is a transcription factor that participates in signal transduction along the Wnt-signaling pathway, which controls the expression of key developmental genes. Nuclear accumulation of beta-catenin is possible not only when the receptor is activated to Wnt, but also due to an inactivating mutation in the APC gene or appearance of mutant beta-catenin, which can lead to tumor transformation of the colon epithelium (MacDonald et al., 2009). The described properties of beta-catenin suggest that the level of its nuclear expression determines potential malignancy.

CDX2 is a nuclear transcription factor that controls the expression of a variety of intestinal specific genes responsible for proliferation and differentiation of cells (Li and Folpe, 2004). It is assumed that CDX2 is also a tumor suppressor gene that is involved in the beta-catenin/Wnt-signaling pathway in colorectal cancer (Liu et al., 2012). CDX2 binds to beta-catenin and prevents its binding to DNA, which, in turn, leads to inhibition of the expression of Wnt-signaling pathway controlled genes (Guo et al., 2010). Decreased CDX2 expression in a tumor is associated with more aggressive morphological characteristics and a worse prognosis (Dalerba et al., 2016). In a study of colon cancer, there was found an association between the decreased CDX2 expression in the tumor and the presence of BRAF mutation, CIMP-high status and MMR-deficiency, based on which it can be assumed that a loss of CDX2 expression plays a certain role in the serrated way of carcinogenesis (Baba et al., 2009).

P53 is a transcription factor inducing cell cycle arrest, senescence and apoptosis under cellular stress. P53 regulates a large number of diverse downstream genes to exert regulative function in multiple signaling processes. Dysregulation of p53 tumor suppressor gene is one of the most frequent events contributing to the transformation of colorectal cancer, which makes it possible to use the p53 expression level as a marker of potential malignancy (Li et al., 2015).

This work is devoted to the study of morphological characteristics of HP and SSA/P in order to clarify the possibilities of differential diagnostics, as well as to study and compare their potential malignancy based on the immunohistochemical profile.

Materials and methods:-

Different studies use different morphological criteria for HP and SSA/P (Rex et al., 2012; Snover D, 2010; Torlakovic et al., 2003) . Thus, according to the recommendations of the American Gastroenterology Association, the presence of one crypt with dilated basal portions is sufficient for verification of SSA, and according to the WHO classification, a minimum of three (or two neighboring crypts) is needed to confirm the diagnosis. In this connection, morphological characteristics of a combined group of cytoplasmic serrated lesions (CSL) were analyzed in this study. For this purpose, 466 CSL (HP and SSA/P) were selected by continuous sampling method from the archival material in the course of revision of mucosal resection preparations, endoscopic polypectomies, and colon biopsies. The inclusion criteria were presence of cytoplasmic serration, longitudinal orientation of the sample in the preparation, presence of mucosa in the muscular plate preparations. Formations with severe dysplasia and/or malignization foci were excluded from the study.

In the course of the work the following morphological criteria were evaluated:

- presence and rate of basal dilation of the crypts (doubtful, significant, horizontal growth);
- spread of the serration (the upper half of the crypt, the entire crypt extension);
- presence of atrophy;
- presence of mitoses;
- presence of low grade dysplasia;
- presence of epithelial eosinophilic changes;
- crypt gemmation;
- crypt branching;
- inverted growth.

When selecting samples for immunohistochemical determinations, based on WHO recommendations (Snover D, 2010) and updated 2012 recommendations (Rex et al., 2012) the following was adopted as HP criteria:

- presence of cytoplasmic serration;
- absence of dilation and/or horizontal growth of the basal portions of the crypts along the mucosal muscular plate;
- absence of dysplasia foci;
- absence of foci of epithelial eosinophilic changes.

Based on WHO recommendations (Snover D, 2010) and updated 2012 recommendations (Rex et al., 2012) the following was adopted as SSA/P criteria:

- presence of cytoplasmic serration;
- presence of one or more of the following criteria:

- significant dilation of the basal portions of one or more crypts;
- horizontal growth of the basal portions of one or more crypts along the mucosal muscular plate;
- focal epithelial eosinophilic changes;
- presence of dysplasia foci.

43 HPs and 49 SSA/Ps were selected. Antibodies to proliferative activity marker Ki-67, transcription factors (beta-catenin, cdx-2, p53), tight junction proteins (claudin-1, claudin-3, claudin-4), and markers of stem tumor cells (musashi-1 and CD44) were used (Table 1). As a comparison group, 32 TSAs were selected, because the modern classification includes them into the serrated formations group.

Ki-67 was evaluated according to the conventional scheme by counting the number of stained nuclei per 100 nuclei of the preparation, the average result was expressed as a percentage. Distribution of the Ki67 reaction was evaluated separately for crypt portions divided into thirds (lower, middle and upper).

The claudins intensity and distribution were considered in the semiquan-titative assessment of the staining pattern. The intensity of staining was subjectively graded as weak, 1; moderate, 2; or intense, 3. The distribution of staining in the tumor cells was graded as focal (<10%), 1; regional (11%-50%), 2; or diffuse (>50%), 3. Intensity and

distribution were given equal weight in our analysis as a multiplicative index, obtained by multiplying the intensity by the distribution for a total score (Sheehan et al., 2007).)

Intensity of the Msi-1 reaction was evaluated separately in the cytoplasm as negative, weak, moderate, or pronounced and in the nucleus (negative, very weak, weak, moderate, vivid or very vivid).
A separate semi-quantitative evaluation of the membrane and cytoplasmic CD44 reaction was performed: the reaction intensity was evaluated (negative, weak, moderate, or pronounced) and the distribution of the mark along the crypt portions divided into thirds (lower, middle and upper).

For beta-catenin marker, the presence and intensity of the nuclear reaction, the proportion and nature of the distribution of stained cells was evaluated.

When evaluating CDX2 expression, the staining intensity (weak, moderate or pronounced) and percentage of stained nuclei was evaluated in each case. Staining of 90% or more of the nuclei was taken as the normal level of expression. Marked nuclear staining of 100% cells was registered in all available for evaluation fragments of the intact mucous membrane adjacent to the studied formations.

In assessing the p53 reaction, the intensity of staining (negative, weak, moderate or pronounced), the percentage of stained nuclei, as well as the predominance of stained nuclei in the upper or lower half of the formation was taken into account. We assumed the intensity of staining score 2 and 3, staining of 5% or more nuclei as a significant reaction.

Statistical data processing was carried out using PASW Statistics 22 software. The confidence level (p) was assumed to be 0.05.

Results:-

Morphological characteristics
When dividing the CSLs based on the rate of dilation of the basal portions of the crypts, 5 groups were formed:

- CSLs without dilation of the basal portions of the crypts – 56.2% (n = 262);
- CSLs with doubtful dilation of the basal portions of the crypts – 14.6% (n = 68);
- CSLs with dilation of the basal portions of one crypt – 5.8% (n = 27);
- CSLs with dilation of the basal portions of two and more crypts – 13.1% (n = 61);
- CSLs with horizontal growth of the basal portions of one or more crypts – 10.3% (n = 48);

Significant differences were found between CSLs of different types by a number of morphological characters (dilation of the serration area, presence of atrophy, mitoses, low grade dysplasia, eosinophilic changes in the epithelium, crypt gemmation, crypt branching, inverted growth) (Table 2). Our analysis of morphological characteristics of CSL made it possible to reveal that the limit of significant differences between the described subgroups exists at different levels.
1. Expansion of the serration area outwards the upper half of the crypts in CSLs without dilation of the basal portions was significantly less frequent than in the other groups; among the CSLs with horizontal growth of the basal portions of the crypts, expansion of the serration area was significantly more frequent than in all the other groups (Figure 1). Based on the frequency of detection of an enlarged serration area, CSLs with doubtful dilation of the basal portions of the crypts and CSLs with a significant dilation of 1 or 2 or more crypts can be combined into one group.

2. Atrophy was significantly less frequently observed among CSLs without dilation of the basal portions of the crypts than among CSLs with doubtful dilation of the basal portions of the crypts, CSL with dilation of 2 or more crypts, and CSL with horizontal growth of the basal portions of the crypts. CSL with the presence of doubtful and significant dilation of the basal portions of the crypts and CSL with horizontal growth of the basal portions of the crypts can be combined into one group.

3. According to the presence of mitoses, CSL without dilation of the basal portions of the crypts (61.5%) significantly differed from CSL with dilation of the basal portions of one crypt (37.0%) and CSL with dilation of the basal portions of two crypts (42.4%). CSL with the presence of doubtful and significant dilation of the basal portions

of the crypts and CSL with horizontal growth of the basal portions of the crypts can be combined into one group (Figure 2).

4. Eosinophilic changes in the epithelium were observed significantly more frequently in CSLs with horizontal growth of the basal portions of the crypts than among CSLs without dilation of the basal portions of the crypts, with doubtful dilation of the basal portions of the crypts, and with dilation of the basal portions of two and more crypts; also significantly differed from each other CSLs without dilation of the basal portions of the crypts and CSLs with dilation of the basal portions of one crypt (Figure 3). Based on the obtained results, CSL can be divided into CSL with horizontal growth of the basal portions of the crypts and the rest of CSLs.

5. Most groups differed by the presence of branching. No significant differences were observed between CSL without dilation of the basal portions of the crypts and CSL with dilation of one crypt; CSL with doubtful dilation of the basal portions of the crypts and with dilation of the basal portions of two and more crypts. Nominally, based on the frequency of detecting crypt branching, CSL without dilation of the basal portions of the crypts, CSL with doubtful dilation and with significant dilation of the basal portions of one crypt can be combined into one group, and CSL with dilation of the basal portions of two and more crypts and with horizontal growth of the basal portions of the crypts – into the other (Figure 4).

6. By the size, CSLs without dilation of the basal portions of the crypts and CSLs with doubtful dilation of the basal portions are significantly smaller than formations of the remaining groups, while CSLs with horizontal growth of the basal portions of the crypts are larger (Figure 5). The diagram shows that it is not possible to combine CSLs into several groups based on their size, there is a gradual increase in the size of CSL, co-directional with the manifestation of dilation of the basal crypt portions.

In terms of the morphological characters examined in this study, the differential limit runs at different levels, and it is possible to form groups that include several previously identified subgroups of CSL, which may indicate an existence of a common biological continuum in the HP – SSA/P range. In this case, it is natural to consider HP and SSA/P not as two separate types of pre-tumor lesions, but as different stages of a continuous development process of CSL. At the same time, in the course of the formation evolution, along with the increase in size, there occurs a gradual expansion of the serration area, an increase in the frequency of crypt branching, an increase in the number of crypts with dilation of the basal portions and the dilation intensity, an increase of the proportion of formations with signs of atrophy.

Immunohistochemical profile

Proliferative activity marker: Ki67

In HP, the proliferative activity index ranged from 3% to 45% (mean 24.1%), and two types of mark distribution were observed (Table 3):

- reaction in the basal portions of the polyp (lower third of the crypts) – was observed in 69.8%;
- reaction in the basal portions with proliferation to the middle third of the polyp (Figure 6a) – was observed in 30.2%.

In SSA/P, the proliferative activity index ranged from 10% to 40% (mean 22.1%). Only one variant of mark distribution was observed: in the basal portions of the polyp, that is, in the lower third of the crypts (100%), without proliferation to the middle third (Figure 6b).

According to the literature, SSA/P is characterized by an offset of the proliferative area from the "usual" basal portions of the crypts, by the formation of an ectopic proliferation focus, which causes the formation of L- and T-shaped crypts (Langner, 2015), and "dentation" is formed in HP and SSA due to the fact that the ripening zones are located on both side of the proliferative area (Moussata et al., 2015). In our study, it was not possible to detect an offset of the proliferation area in HP and SSA/P by Ki67 expression (Table 3).

The nature of Ki67 reaction in TSA was fundamentally different (Table 3). The level of Ki67 in TSA was higher than in HP and SSA: the mean was 39.7%, and the mark distribution along the height of the crypt was different:

- uniformly diffuse throughout the polyp in 78.1%;
- mainly in the superficial portions of the adenoma (the upper third of the crypts) – in 23.5%.

Tight junction protein markers: claudin-1; -3; -4.
The levels of claudin-1 and claudin-3 in HP (mean values of 2.38 and 2.38, respectively) and in SSA/P (mean values of 3.63 and 3.21, respectively) were approximately the same (Table 4). In most cases, the reaction with claudin-1, claudin-3 and claudin-4 in cancer and polyps of the large intestine had membrane localization, as in the intact mucosa of the large intestine. In 15.4% HP and 26.3%, in addition to the membrane reaction, a cytoplasmic reaction was also observed. Also in 26.3%, against the background of membrane and cytoplasmic reactions, a paradoxical nuclear reaction with a characteristic reaction mosaicism was revealed. In TSA, the level of all claudins was statistically higher ($p<0.05$).

Markers of tumor stem cells: CD44 and Musashi-1
In all observations in HP and SSA, the CD44 reaction had a membrane nature, which is a typical localization for this marker (Figure 6c), and was detected in the basal portions of the crypts (the lower third) (Figure 6d). In 23.3% of HP and only in 12.2% of SSA, the reaction was also detected in the middle portions. The intensity of the reaction decreased towards the lumen of the intestine, but did not reach the latter.

It was not possible to reveal statistically significant differences between HP and SSA ($p>0.05$) by Ki67 and CD44 markers. These results do not seem to contradict the current classification, since the source of SSA is not defined. According to some reports, SSA often develops from microvesicular HP (Bettington et al., 2013; Szylberg et al., 2015), but there is an opinion that SSA develops *de novo*. Possibly, this controversy is associated with the conflicting criteria for determining SSA (Bordacahar et al., 2015). Depending on the criteria, both the total number of SSA and their origin vary. CD44 expression was characterized by exactly the same patterns of polyp distribution, as it was with Ki67, although CD44 does not reflect proliferative activity. Such co-expression can be explained by the participation of CD44 molecules in the transduction of specific signals via the cytoskeleton components, which stimulates an increase in the mitotic activity of the cells.

In all TSAs, the CD44 reaction was observed in the upper and middle thirds. In a number of cases (65.6%), the reaction was also detected in the lower third. It is noteworthy that even if the reaction was observed diffusely throughout the adenoma (*i.e.*, in the upper, middle, and lower thirds of the crypt), a gradient was there in 52.9%: the reaction was significantly more pronounced in the apical portions of the crypts facing the lumen than in the basal ones, which indicated the proliferation of the reaction "from the top downward" for TSA, in contrast to HP and SSA.

The Msi-1 marker in all observations of HP and SSA/P had a nuclear localization. Statistical difference between the groups could not be identified. In the TSA group, the reaction was mixed (cytoplasmic + nuclear) in 59.0%, in the remaining 35.0% – it was nuclear. Reaction distribution and the level of Msi-1 was significantly higher in TSAs than in HPs and SSA/Ps ($p<0.05$) (Table 5).

Components of Wnt-signaling pathway: Beta-catenin and CDX2
Nuclear expression of beta-catenin was never detected in the HP and SSA/P groups (Figure 7a). TSA statistically significantly differed from HP and SSA/P: nuclear expression of beta-catenin was observed in 15.6% of cases, of which 12.5% showed a pronounced reaction in 10% of cells in the form of small groups of stained nuclei in the upper portion of the crypts, in 3.1% – moderate diffuse staining of 50% of nuclei throughout the crypts (Figure 7b).

The results of our evaluation of beta-catenin expression suggest a possible malignization of TSA via the Wnt-signaling pathway. Absence of beta-catenin nuclear expression among HP and SSA/P indicates a lower potential malignancy and makes malignization via the Wnt-signaling pathway unlikely.

Impaired CDX2 expression was observed in 46.5% of HPs and 42.8% of SSA/Ps and was manifested in most cases by a focal decrease in the number of CDX2-positive nuclei and/or staining intensity against diffuse staining of 90-100% of moderate or pronounced intensity nuclei (Table 6). However, there was a diffuse disruption of CDX2 expression in 2.3% of HPs and in 10.2% of SSA/Ps and there was no CDX2 expression in 2% of SSA/Ps. Diffuse moderate or pronounced CDX2 nuclear expression was detected in all TSAs, at the same time the reaction was less pronounced than in the adjacent fragments of intact mucosa.

The revealed CDX2 protein expression anomalies in HP and SSA/P may be indicative of involvement of alternative activation mechanisms of developmental genes controlled by Wnt-signaling pathway. At the same time, a weaker

expression of CDX2 in a part of HPs and SSA/Ps may indicate less differentiation of the epithelium in serrated formations compared to TSA and normal mucosa, which can be considered as one of the possible mechanisms of CSL malignization.

Despite the absence of statistically significant differences between HP and SSA/P both in the frequency of CDX2 expression impairment and in its nature, there is a tendency for more pronounced impairment of CDX2 expression in SSA/P. HP and SSA/P are probably stages in the development of the same formation, in which a gradual accumulation of "breakdowns" of the genetic apparatus takes place. One of the biological markers of this process is probably decrease and subsequent loss of CDX2 expression. Whether CDX2 is involved directly in the malignant transformation of HP and SSA/P or it is only a marker of the ongoing molecular changes, as well as at what stage CDX2 expression is disrupted, remains unclear at the present time.

Cellular stress marker: p53
No expression of p53 was detected in 4,7% HPs, 10,2% SSA/Ps and 6,2% TSAs. Expression of weak intensity was observed in 51,2% HPs, 38,8 % SSA/Ps and 18,8% TSAs. Expression of moderate or strong intensity in less than 5% of cells was detected in 18,6% HPs and 20.4% SSA/Ps.

The aberrant expression of p53 was detected in 25,5% HPs, 30.6% SSA/Ps and 75,0% TSAs (Table 7). In all HPs and SSA/Ps p53 positive cells (aberrant expression) were located in lower part of the crypts (Figure 7c). Superficial pattern of distribution was detected in 63.6% TSAs with aberrant expression. Diffuse pattern of distribution was detected in 36.4% TSAs with aberrant expression (Figure 7d). Pattern of distribution was similar between HPs and SSA/Ps ($p<0,05$). Medium amount of p53 positive cells (moderate or strong intensity) was 6.6% in HPs, 6.0% in SSA/Ps and 25.4% in TSAs. There were no statistical difference in the amount of p53 positive cells between HPs and SSA/Ps ($p>0,05$). TSAs were significantly different from HPs and SSA/Ps in all the criteria studied.

Based on the p53 expression level, it can be assumed that both HP and SSA/P have a very low potential malignancy and do not differ from each other by this criterion. Dysfunction and overexpression of p53 appear in the late stages of carcinogenesis and, as a rule, are associated with the appearance of severe dysplasia. We believe that the revealed level of expression of p53-positive nuclei is not a sign of the onset of malignant transformation but a "basic" level of detection of wild-type p53 whose active functioning in the basal portions of HP and SSA/P (the area of maximum mitotic activity and consequently most dangerous in terms of possible mutations) seems to be biologically justified. The expression level of p53 in TSA is significantly higher than in HP and SSA/P and it probably reflects a higher potential malignancy of TSA. The nature of p53-positive nuclei distribution in TSA is fundamentally different from that found in HP and SSA/P, which suggests biological diversity of TSA and CSL.

Conspicuousness is the fundamental difference between TSA and HP/SSA by all the markers studied ($p<0.01$). Despite the fact that the modern official classification ascribes TSA to "serrated" polyps, it should be remembered that initially this group was isolated by T.A. Longacre and C.M. Fenoglio-Preiser (1990) by the simultaneous featuring of both traditional adenoma signs (AT and ATV) and hyperplastic polyp ones (HP) (Longacre and Fenoglio-Preiser, 1990). In addition, the main classification attribute based on which the group of "serrated" polyps were identified – so-called "dentation" of the crypt lumen – but the nature of "dentation" of the crypt lumen in TSA, on the one hand, and in HP and SSA, on the other hand, is fundamentally different. Thus, HP and SSA are characterized by "dentation" of cytoplasmic type whose formation is associated with swelling of cellular cytoplasm into the crypt lumen in the shape of cogs (the lumen of longitudinally oriented crypts is saw-shaped, that of transversely oriented ones is stellate). The "dentation" of the crypt lumen in TSA is due to the pathological crypt branching with the formation of so-called "ectopic crypts" located perpendicular to the main axis of the previous crypt, forming a special type of histoarchitectonics, different from the normal mucosal structure.

Conclusion:-

The results we obtained indicate the vague nature of the morphological differences between HP and SSA. The continuity and unevenness of the morphological characteristics in the HP-SSA/P range complicates pathologic anatomic diagnostics and criteria for isolating each subtype separately. The immunohistochemical profile (estimated by tight junction proteins – claudin-1, -3, -4, transcription factors – beta-catenin, cdx2, p53, tumor stem cells – CD44, Msi-1, and proliferative activity marker Ki-67) does not differ between HP and SSA/P either. The nature of expression of most markers studied, with the exception of CDX2, indicates a lower potential malignancy of HP and SSA/P compared to TSA. The relatively high incidence of abnormal CDX2 expression suggests a significant role of

alternative ways of HP and SSA/P malignization. TSA is fundamentally different from HP and SSA/P in immunophenotype, which may indicate both a higher potential malignancy of TSA and different ways of carcinogenesis of these types of polyps, despite their unification into a general group of "serrated formations".

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Table 1:- Information about used antibodies

	GROUP	PROTEIN	ANTIBODY`S CLONE	DILUTION	PRODUCER
1	Marker of proliferative activity	Ki67	MIB1	RTU	Dako (USA)
2	Tight junction proteins	Claudin-1	Polyclonal	RTU	Thermo (UK)
3		Claudin-3	Polyclonal	RTU	Thermo (UK)
4		Claudin-4	Polyclonal	RTU	Thermo (UK)
5	Cancer stem cells` markers	Musashi-1	69-Q	1:100	Santa Cruz (USA)
6		CD44	DF 1485	1:25	Dako (USA)
7	Transcription factors	Beta-catenin	β-Catenin-1	RTU	Dako (USA)
8		Cdx-2	DAK-CDX2	RTU	Dako (USA)
9		P53	DO-7	RTU	Dako (USA)

Table 2:- Comparison of CSLs with dilation of the basal portions of the crypts of different rate

Characteristic	Presence	CSL					Total
		without dilation of the basal portions of the crypts	doubtful dilation of the basal portions of the crypts	dilation of the basal portions of one crypt	dilation of the basal portions of two and more crypts	horizontal growth of the basal portions of the crypts	
		n (%)	n (%)n	n (%)n	n (%)n	n (%)n	n (%)n
Dilation of the serration area	Yes	63 (24,0%)	41 (60,3%)	18 (66,7%)	44 (72,1%)	44 (91,7%)	210 (45,1%)
	No	199 (76,0%)	27 (39,7%)	9 (33,3%)	17 (27,9%)	4 (8,3%)	256 (54,9%)
Presence of atrophy	Yes	72 (27,5%)	29 (42,6%)	11 (40,7%)	34 (55,7%)	23 (47,9%)	169 (36,3%)
	No	190 (72,5%)	39 (57,4%)	16 (59,3%)	27 (44,3%)	25 (52,1%)	297 (63,7%)
Mitoses	Yes	161 (61,5%)	37 (54,4%)	10 (37,0%)	26 (42,6%)	25 (52,1%)	259 (55,6%)
	No	101 (38,5%)	31 (45,6%)	17 (63,0%)	35 (57,4%)	23 (47,9%)	207 (44,4%)
Low grade dysplasia	Очаговая	15 (5,7%)	0 (0%)	2 (7,4%)	6 (9,8%)	5 (10,4%)	28 (6,0%)
	No	247 (94,3%)	68 (100%)	25 (92,6%)	55 (90,2%)	43 (89,6%)	438 (94,0%)
Eosinophilic changes in the epithelium	Очаговые	1 (0,4%)	0 (0%)	2 (7,4%)	1 (1,6%)	7 (14,6%)	11 (2,4%)
	No	261 (99,6%)	68 (100%)	25 (92,6%)	60 (98,4%)	41 (85,4%)	455 (97,6%)
Crypt gemmation	Yes	8 (3,1%)	2 (2,9%)	0 (0%)	5 (8,2%)	3 (6,3%)	18 (3,9%)
	No	254 (96,9%)	66 (97,1%)	27 (100%)	56 (91,8%)	45 (93,8%)	448 (96,1%)
Crypt	Yes	14	13	2	20	17	66

branching		(5,3%)	(19,1%)	(7,4%)	(32,8%)	(35,4%)	(14,2%)
	No	248 (94,7%)	55 (80,9%)	25 (92,6%)	41 (67,2%)	31 (64,6%)	400 (85,8%)
Inverted growth	Yes	11 (4,2%)	4 (5,9%)	0 (0%)	7 (11,5%)	5 (10,4%)	27 (5,8%)
	No	251 (95,8%)	64 (94,1%)	27 (100%)	54 (88,5%)	43 (89,6%)	439 (94,2%)
Total		**262 (56,3%)**	**68 (14,6%)**	**27 (5,8%)**	**61 (13,1%)**	**47 (10,1%)**	**466 (100%)**
Average size, mm		5,3 ± 0,3	5,4 ± 0,5	6,2 ± 0,9	7,8 ± 0,9	8,6 ± 0,9	

Table 3:- Localization of Ki67 and CD44 markers in the study groups

Localization (part of crypt)	**HP**		**SSA**		**TSA**	
	CD44	**Ki67**	**CD44**	**Ki67**	**CD44**	**Ki67**
Upper third	0	0	0	0	0	2 (6,3%)
Upper and middle thirds	0	0	0	0	11 (34,4%)	5 (15,6%)
All crypt	0	0	0	0	21 (65,6%)	25 (78,1%)
Basal third	33 (76,7%)	30 (69,8%)	43 (78,8%)	49 (100%)	0	0
Basal and middle thirds	10 (23,3%)	13 (30,2%)	6 (12,2%)	0	0	0
TOTAL	**43**		**49**		**32**	

Table 4:- Level of claudins` reaction in the study groups

Intensity (score)	Claudin-1			Claudin-3			Claudin-4		
	HP	**SSA**	**TSA**	**HP**	**SSA**	**TSA**	**HP**	**SSA**	**TSA**
Negative (0 score)	7,69%	0%	0%	15,38%	0%	0%	0%	0%	0%
Weak (1-3 score)	69,23%	21,05%	11,76%	53,85%	47,37%	29,41%	0%	5,26%	0%
Moderate (4-6 score)	23,08%	63,16%	47,06%	30,77%	52,63%	52,94%	46,15%	36,85%	11,76%
Strong (7-9 score)	0%	0%	41,18%	0%	0%	17,65%	53,85%	57,89%	88,24%
Mean value (score)	**2,38**	**3,63**	**6,17**	**2,38**	**3,21**	**5,29**	**7,62**	**7,26**	**8,65**

Table 5:- Localization of Msi-1 reaction

	Cytoplasmic				Nuclear					
	negative	weak	moderate	strong	negative	very weak	weak	moderate	strong	very strong
HP	100,0%	0,0%	0,0%	0,0%	23,3%	16,3%	7,0%%	46,4%	7,0%	0,0%
SSA	100,0%	0,0%	0,0%	0,0%	6,1%	20,4%	20,4%	26,5%	16,3%	10,3%
TSA	40,6%	46,8%	12,6%	0,0%	6,3%	12,6%	31,3%	37,2%	12,6%	0,0%

Table 6:- Expression of CDX2

	Normal level of expression	Decreased expression			Total
		Quantity	Intensity	Quantity and intensity	
HP	23 53,5%	1 2,3%	13 30,2%	6 14,0%	43 100%
SSA/P	28 57,2%	5 10,2%	8 16,3%	8 16,3%	49 100%

TSA	32 100%	0	0	0	32 100%

Table 7:- Expression of p53

	Negative	Weak	Moderate or strong		Total
			Less than 5%	5% or more	
HP	2 4,7%	22 51,2%	8 18,6%	11 25,5%	43 100%
SSA/P	5 10,2%	19 38,8%	10 20,4%	15 30,6%	49 100%
TSA	2 6,2%	6 18,8%	0	24 75,0%	32 100%

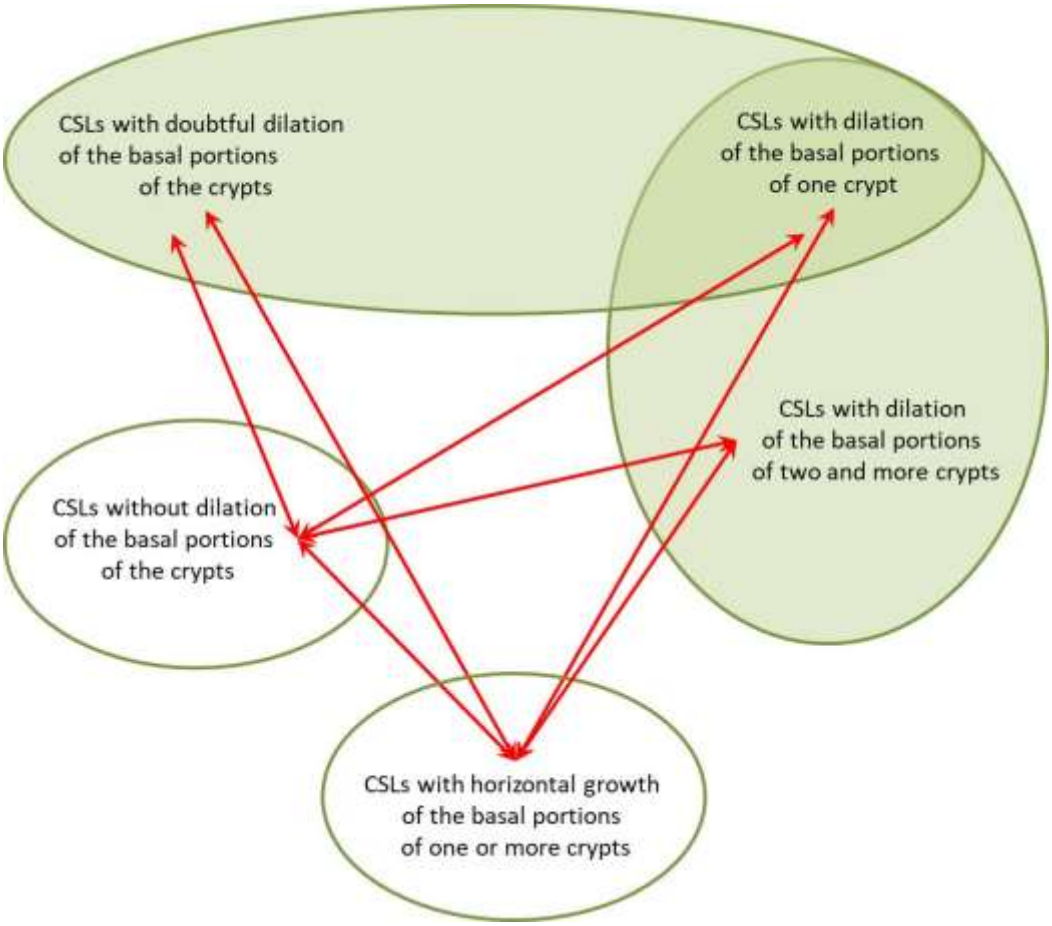

Figure 1:- Expansion of the serration area outwards the upper half of the crypts. Red arrows indicate the presence of statistically significant differences ($p<0.05$), green lines indicate conditional groups whose formation is possible based on the analyzed morphological character.

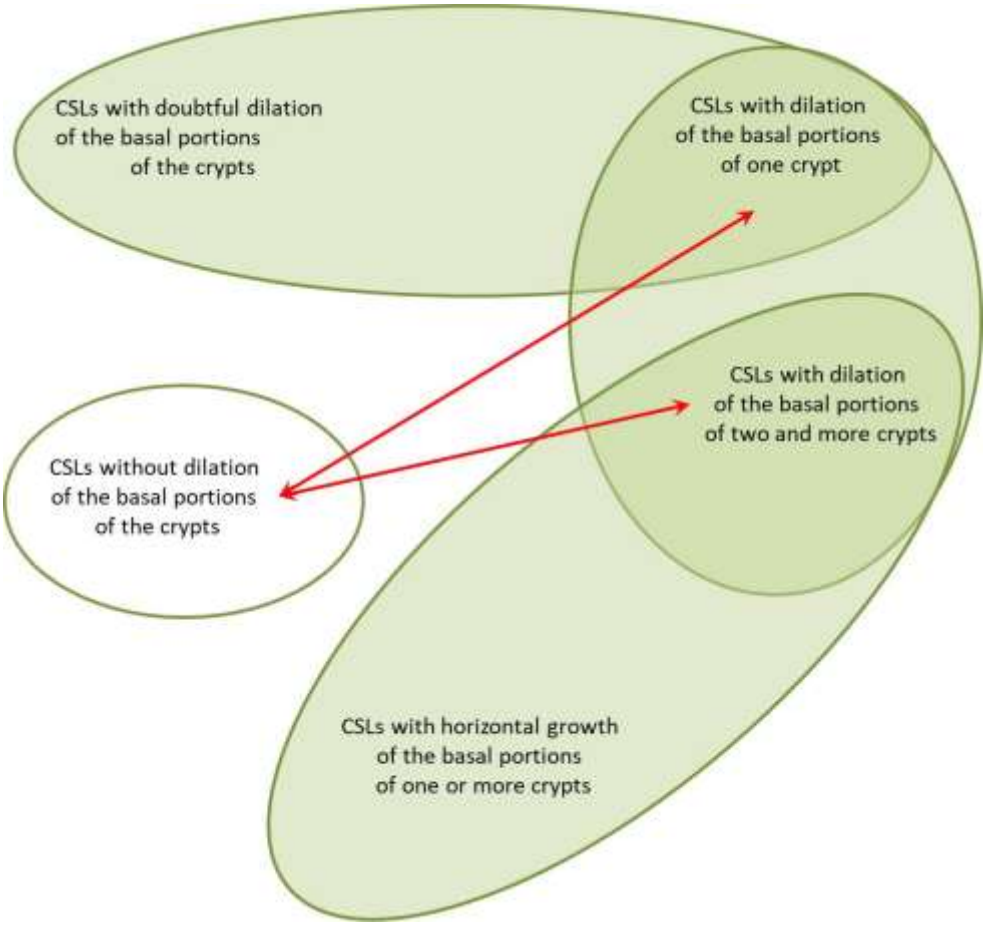

Figure 2:- Presenc of mitoses. Red arrows indicate the presence of statistically significant differences ($p<0.05$), green lines indicate conditional groups whose formation is possible based on the analyzed morphological character.

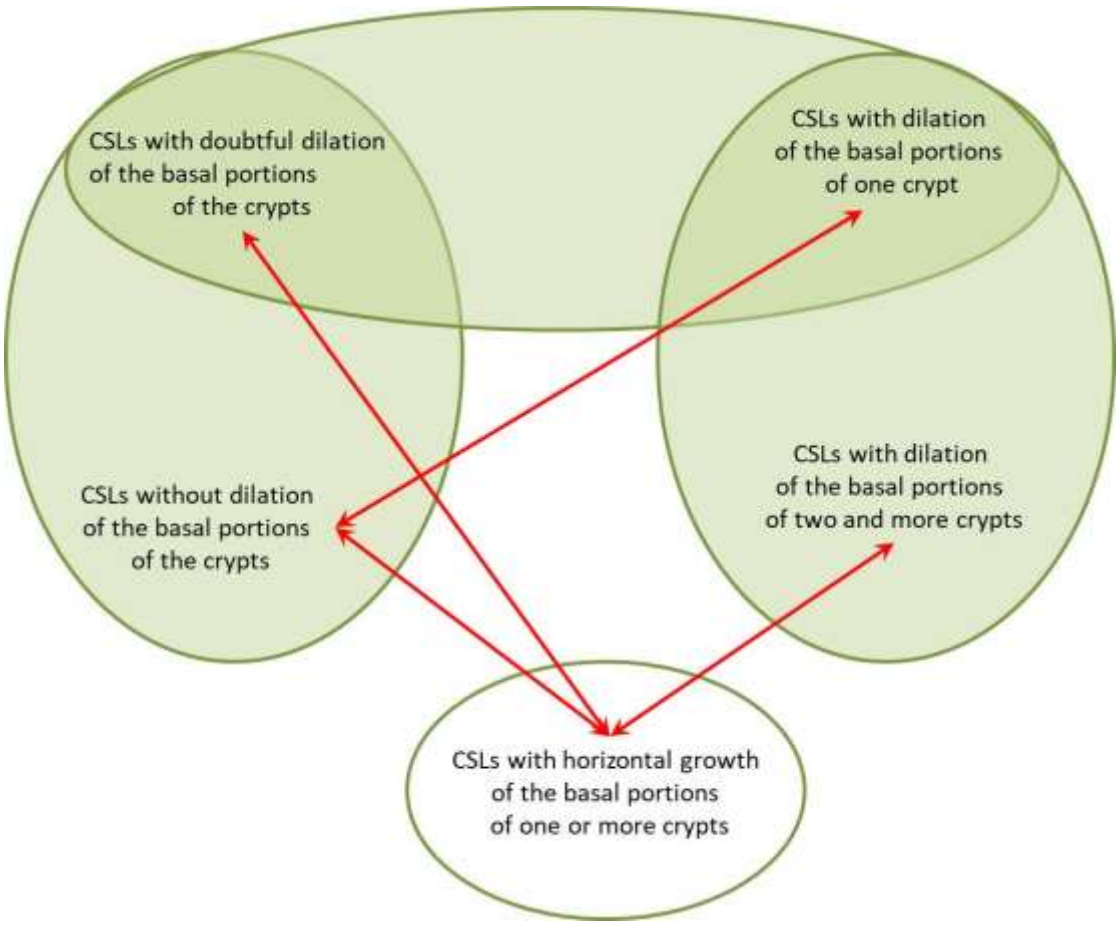

Figure 3:- Presence of epithelial eosinophilic changes. Red arrows indicate the presence of statistically significant differences ($p<0.05$), green lines indicate conditional groups whose formation is possible based on the analyzed morphological character.

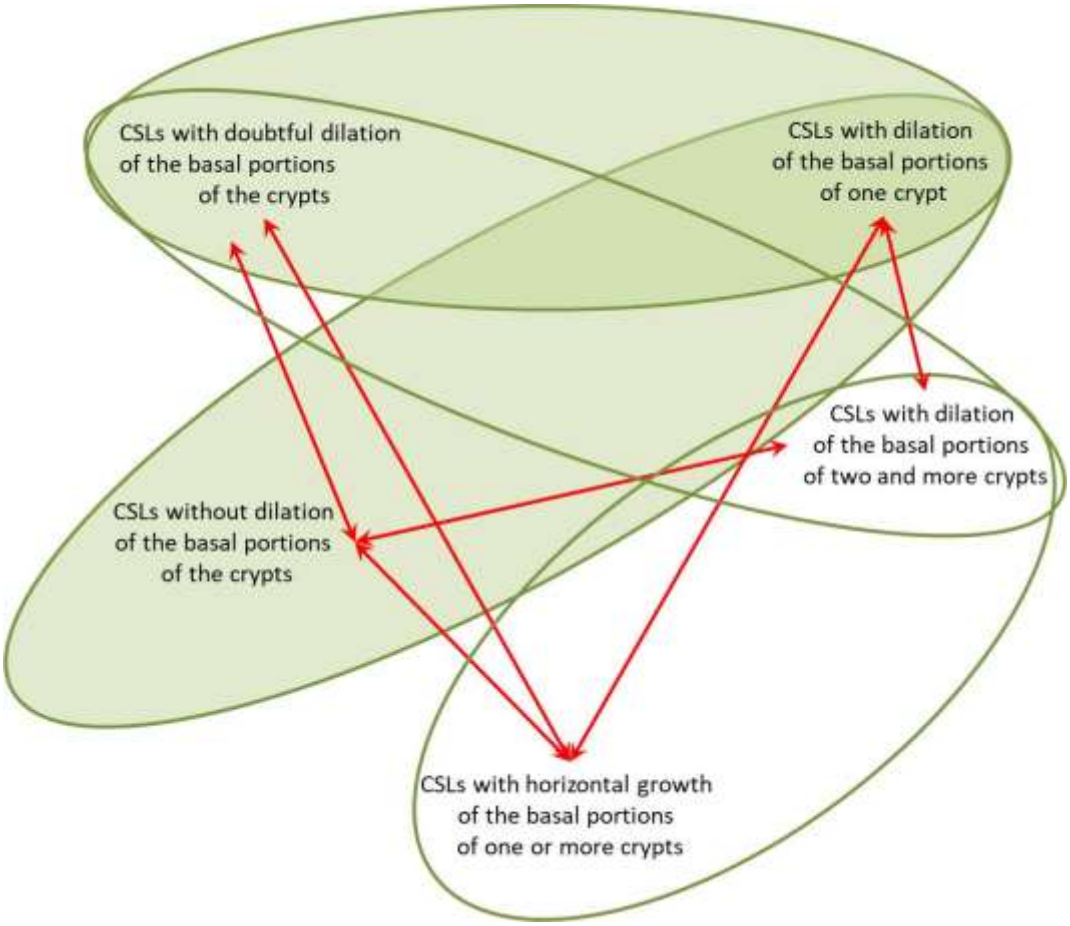

Figure 4:- Presence of branching. Red arrows indicate the presence of statistically significant differences ($p<0.05$), green lines indicate conditional groups whose formation is possible based on the analyzed morphological character.

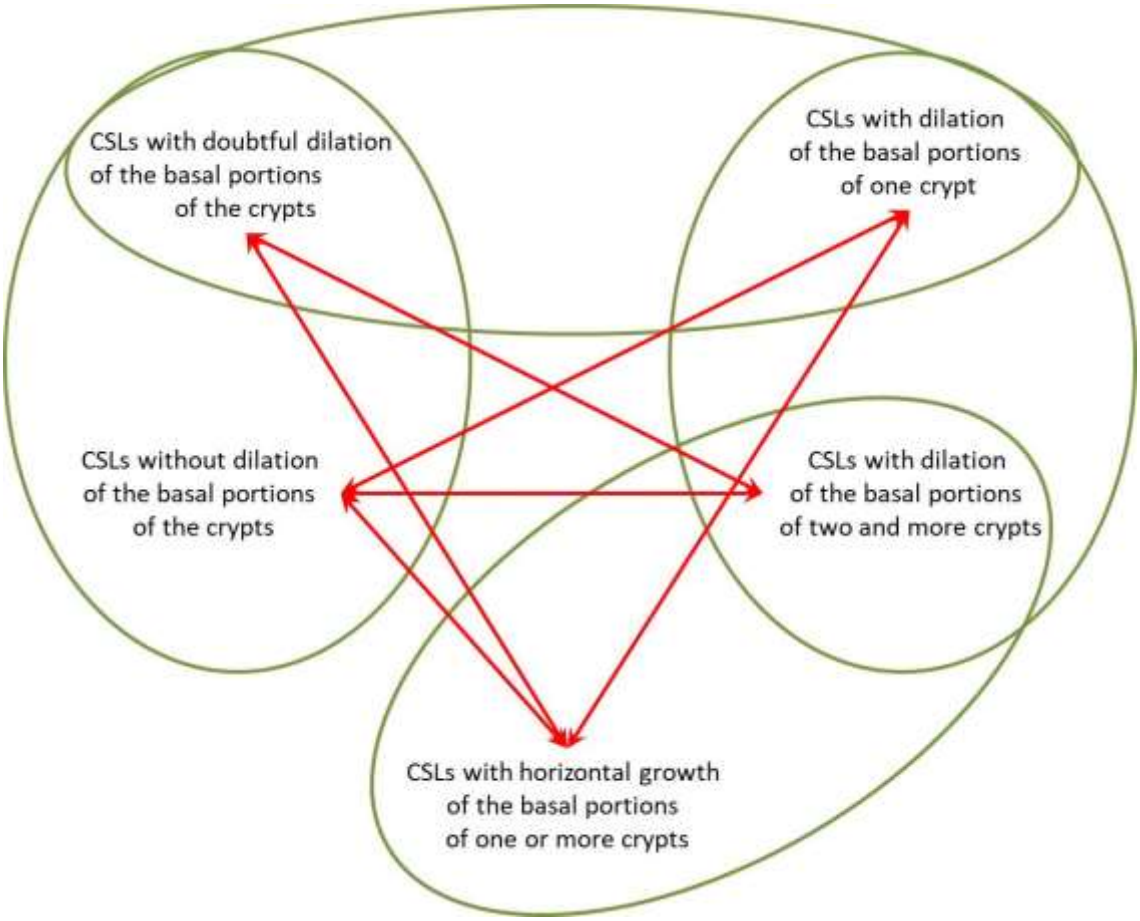

Figure 5:- CSL size. Red arrows indicate the presence of statistically significant differences ($p<0.05$), green lines indicate conditional groups whose formation is possible based on the analyzed morphological character.

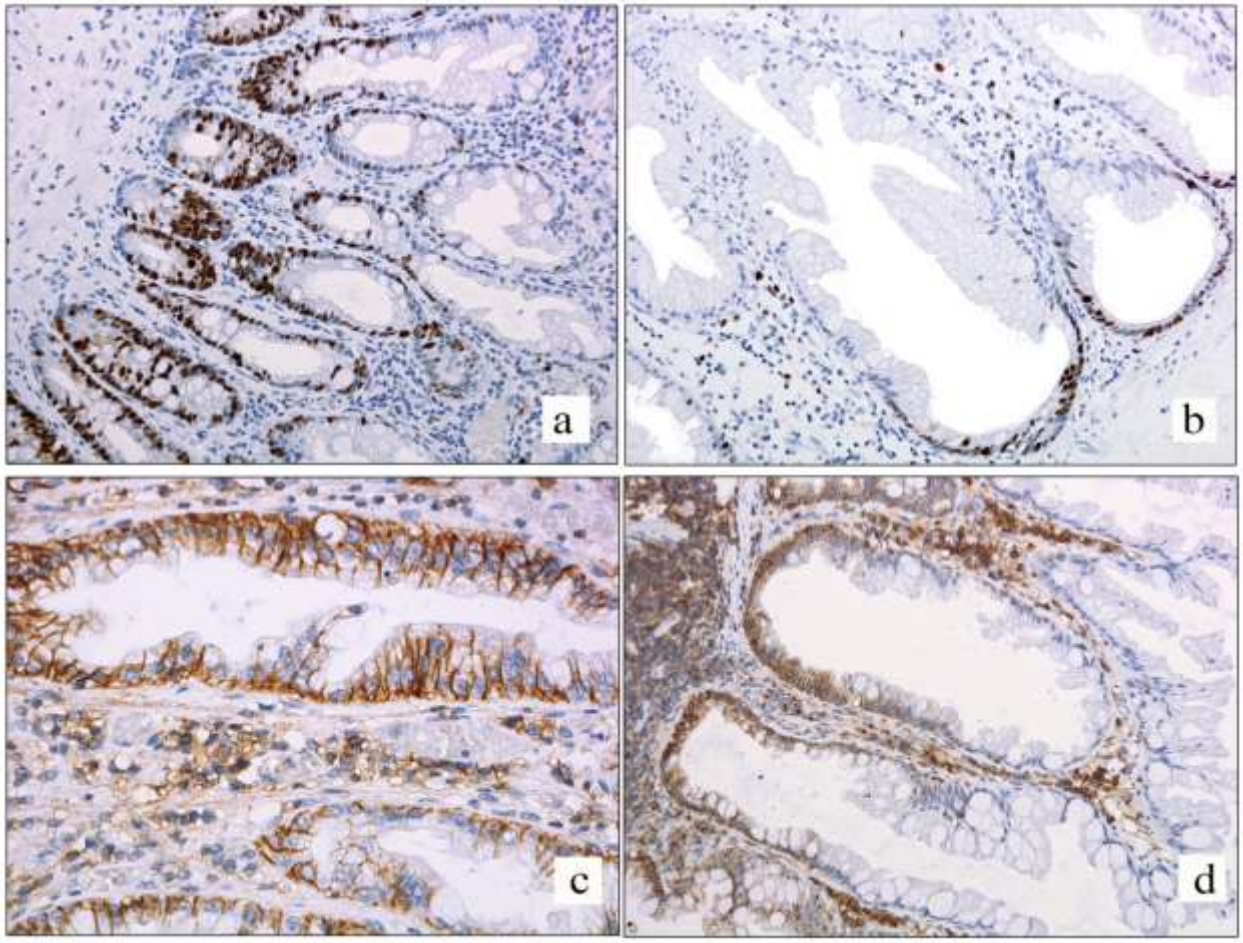

Figure 6:- Nuclear reaction Ki67 and CD44 in serrated formations of the large intestine. *a* - HP, Ki67 reaction in the basal portions with proliferation to the middle third, obj. x10; *b* – SSA/P, nuclear reaction Ki67 in the basal portions of the crypts with dilation along the muscular plate, obj. x40; *c* - membrane reaction CD44 in crypts with a "serrated lumen", obj. x40; *d* - membrane reaction CD44 in SSA/P in the basal portions and middle third of the crypt; there is no reaction in the upper third of the crypt, obj. x20.

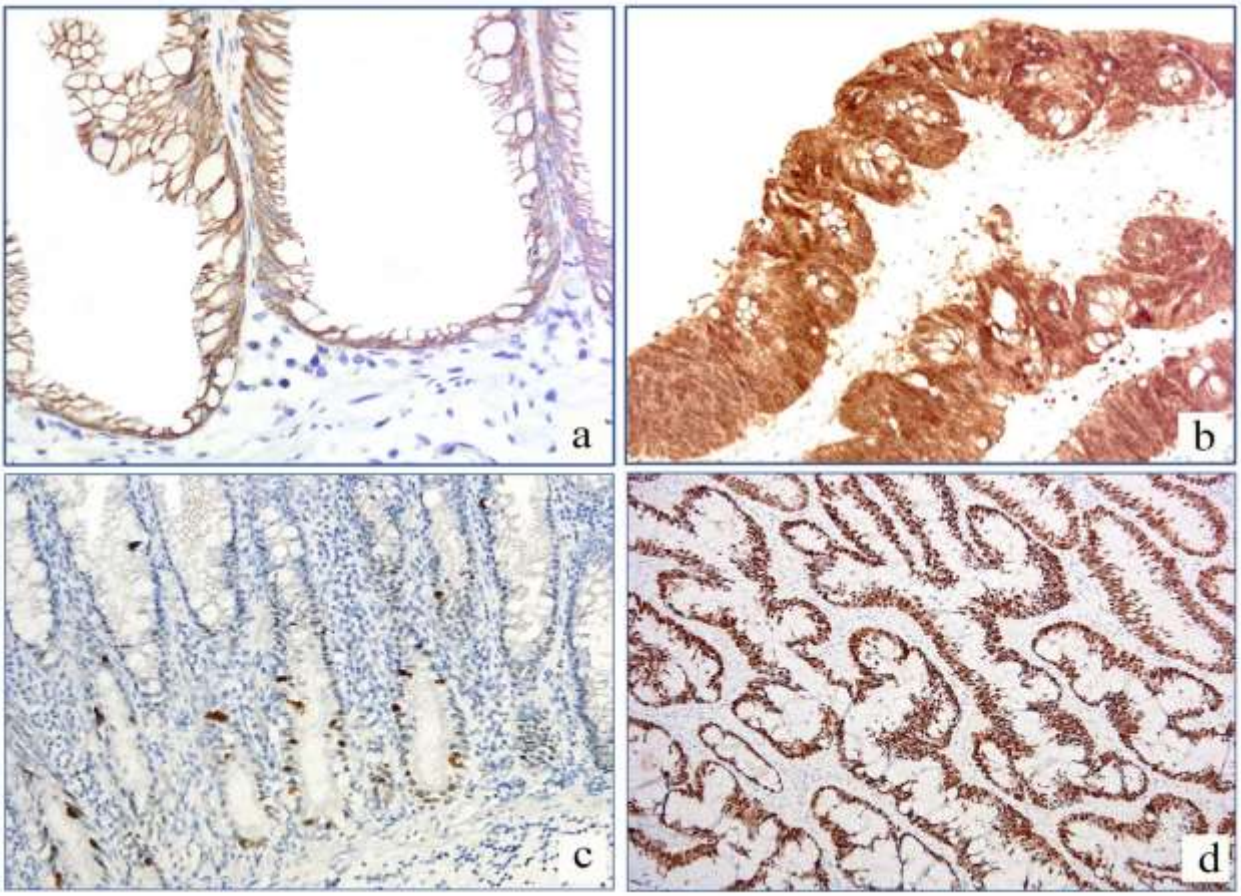

Figure 7:- Reaction beta-catenin and p53 in serrated formations of the large intestine. A – SSA/P: no nuclear beta-catenin expression (x40); B – TSA: diffuse nuclear staining with beta-catenin, moderate intensity (x40). C – HP: aberrant expression of p53, positive cells in the low half of the crypts (x20); D – TSA: aberrant expression of p53, positive cells throughout the crypts (x10).

Literature:-

1. Baba, Y., Nosho, K., Shima, K., Freed, E., Irahara, N., Philips, J., Meyerhardt, J.A., Hornick, J.L., Shivdasani, R.A., Fuchs, C.S., *et al.* (2009). Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. Clinical cancer research : an official journal of the American Association for Cancer Research *15*, 4665-4673.
2. Baker, A.M., Graham, T.A., Elia, G., Wright, N.A., and Rodriguez-Justo, M. (2015). Characterization of LGR5 stem cells in colorectal adenomas and carcinomas. Scientific reports *5*, 8654.
3. Bettington, M., Walker, N., Clouston, A., Brown, I., Leggett, B., and Whitehall, V. (2013). The serrated pathway to colorectal carcinoma: current concepts and challenges. Histopathology *62*, 367-386.
4. Bettington, M.L., Walker, N.I., Rosty, C., Brown, I.S., Clouston, A.D., McKeone, D.M., Pearson, S.A., Klein, K., Leggett, B.A., and Whitehall, V. (2015). A clinicopathological and molecular analysis of 200 traditional serrated adenomas. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc *28*, 414-427.
5. Bordacahar, B., Barret, M., Terris, B., Dhooge, M., Dreanic, J., Prat, F., Coriat, R., and Chaussade, S. (2015). Sessile serrated adenoma: from identification to resection. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver *47*, 95-102.
6. Caruso, M., Fung, K.Y., Moore, J., Brierley, G.V., Cosgrove, L.J., Thomas, M., Cheetham, G., Brook, E., Fraser, L.M., Tin, T., *et al.* (2014). Claudin-1 Expression Is Elevated in Colorectal Cancer Precursor Lesions Harboring the BRAF V600E Mutation. Translational oncology *7*, 456-463.

7. Chai, N., Zhang, W., Wang, Y., Zhou, Z., Zhang, Y., Liu, H., Wan, J., Qin, J., Wang, S., Wang, Y., *et al.* (2013). [Lgr5 and CD44 expressions in different types of intestinal polyps and colorectal cancer]. Nan fang yi ke da xue xue bao = Journal of Southern Medical University *33*, 972-976.
8. Dalerba, P., Sahoo, D., Paik, S., Guo, X., Yothers, G., Song, N., Wilcox-Fogel, N., Forgo, E., Rajendran, P.S., Miranda, S.P., *et al.* (2016). CDX2 as a Prognostic Biomarker in Stage II and Stage III Colon Cancer. The New England journal of medicine *374*, 211-222.
9. Dallas, M.R., Liu, G., Chen, W.C., Thomas, S.N., Wirtz, D., Huso, D.L., and Konstantopoulos, K. (2012). Divergent roles of CD44 and carcinoembryonic antigen in colon cancer metastasis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *26*, 2648-2656.
10. East, J.E., Saunders, B.P., and Jass, J.R. (2008). Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management. Gastroenterology clinics of North America *37*, 25-46, v.
11. Fanali, C., Lucchetti, D., Farina, M., Corbi, M., Cufino, V., Cittadini, A., and Sgambato, A. (2014). Cancer stem cells in colorectal cancer from pathogenesis to therapy: controversies and perspectives. World journal of gastroenterology : WJG *20*, 923-942.
12. Goldstein, N.S., Bhanot, P., Odish, E., and Hunter, S. (2003). Hyperplastic-like colon polyps that preceded microsatellite-unstable adenocarcinomas. American journal of clinical pathology *119*, 778-796.
13. Guo, R.J., Funakoshi, S., Lee, H.H., Kong, J., and Lynch, J.P. (2010). The intestine-specific transcription factor Cdx2 inhibits beta-catenin/TCF transcriptional activity by disrupting the beta-catenin-TCF protein complex. Carcinogenesis *31*, 159-166.
14. Kawasaki, K., Kurahara, K., Yanai, S., Oshiro, Y., Yao, T., Kobayashi, H., Nakamura, S., Fuchigami, T., Sugai, T., and Matsumoto, T. (2016). Colonoscopic features and malignant potential of sessile serrated adenomas: comparison with other serrated lesions and conventional adenomas. Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland *18*, 795-802.
15. Kuang, R.G., Kuang, Y., Luo, Q.F., Zhou, C.J., Ji, R., and Wang, J.W. (2013). Expression and significance of Musashi-1 in gastric cancer and precancerous lesions. World journal of gastroenterology : WJG *19*, 6637-6644.
16. Langner, C. (2015). Serrated and non-serrated precursor lesions of colorectal cancer. Digestive diseases (Basel, Switzerland) *33*, 28-37.
17. Li, D., Peng, X., Yan, D., Tang, H., Huang, F., Yang, Y., and Peng, Z. (2011). Msi-1 is a predictor of survival and a novel therapeutic target in colon cancer. Annals of surgical oncology *18*, 2074-2083.
18. Li, M.K., and Folpe, A.L. (2004). CDX-2, a new marker for adenocarcinoma of gastrointestinal origin. Advances in anatomic pathology *11*, 101-105.
19. Li, X.L., Zhou, J., Chen, Z.R., and Chng, W.J. (2015). P53 mutations in colorectal cancer - molecular pathogenesis and pharmacological reactivation. World journal of gastroenterology : WJG *21*, 84-93.
20. Liu, X., Zhang, X., Zhan, Q., Brock, M.V., Herman, J.G., and Guo, M. (2012). CDX2 serves as a Wnt signaling inhibitor and is frequently methylated in lung cancer. Cancer biology & therapy *13*, 1152-1157.
21. Longacre, T.A., and Fenoglio-Preiser, C.M. (1990). Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. The American journal of surgical pathology *14*, 524-537.
22. MacDonald, B.T., Tamai, K., and He, X. (2009). Wnt/beta-catenin signaling: components, mechanisms, and diseases. Developmental cell *17*, 9-26.
23. Moussata, D., Boschetti, G., Chauvenet, M., Stroeymeyt, K., Nancey, S., Berger, F., Lecomte, T., and Flourie, B. (2015). Endoscopic and histologic characteristics of serrated lesions. World journal of gastroenterology : WJG *21*, 2896-2904.
24. Rex, D.K., Ahnen, D.J., Baron, J.A., Batts, K.P., Burke, C.A., Burt, R.W., Goldblum, J.R., Guillem, J.G., Kahi, C.J., Kalady, M.F., *et al.* (2012). Serrated lesions of the colorectum: review and recommendations from an expert panel. The American journal of gastroenterology *107*, 1315-1329; quiz 1314, 1330.
25. Salaria, S.N., Streppel, M.M., Lee, L.A., Iacobuzio-Donahue, C.A., and Montgomery, E.A. (2012). Sessile serrated adenomas: high-risk lesions? Human pathology *43*, 1808-1814.
26. Sheehan, G.M., Kallakury, B.V., Sheehan, C.E., Fisher, H.A., Kaufman, R.P., Jr., and Ross, J.S. (2007). Loss of claudins-1 and -7 and expression of claudins-3 and -4 correlate with prognostic variables in prostatic adenocarcinomas. Human pathology *38*, 564-569.
27. Snover D, A.D., Burt RW, Odze RD et al. (2010). Serrated polyps of the colon and rectum and serrated ("hyperplastic") polyposis. In WHO Classification of Tumours Pathology and genetics Tumours of the digestive system, C.F. Bozman FT, Hruban RH, et al., ed. (Lyon: IARC Press), pp. 160-165.
28. Soler, A.P., Miller, R.D., Laughlin, K.V., Carp, N.Z., Klurfeld, D.M., and Mullin, J.M. (1999). Increased tight junctional permeability is associated with the development of colon cancer. Carcinogenesis *20*, 1425-1431.

29. Szylberg, L., Janiczek, M., Popiel, A., and Marszalek, A. (2015). Serrated polyps and their alternative pathway to the colorectal cancer: a systematic review. Gastroenterology research and practice *2015*, 573814.
30. Torlakovic, E., Skovlund, E., Snover, D.C., Torlakovic, G., and Nesland, J.M. (2003). Morphologic reappraisal of serrated colorectal polyps. The American journal of surgical pathology *27*, 65-81.
31. Urbanski, S.J., Kossakowska, A.E., Marcon, N., and Bruce, W.R. (1984). Mixed hyperplastic adenomatous polyps--an underdiagnosed entity. Report of a case of adenocarcinoma arising within a mixed hyperplastic adenomatous polyp. The American journal of surgical pathology *8*, 551-556.