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IMMUNOHISTOCHEMICAL EXPRESSION OF STEM CELL MARKERS CD44, CD166 AND ALDH1A1 IN NON-NEOPLASTIC POLYPS OF THE COLON IN SAMPLE OF IRAQI PATIENTS

Nawal M Abdullah[*], Mustafa M Ibraheem[@] & Basim Sh Ahmed[#]

[*]MB,ChB, MSc, Assist. Prof., Department of Anatomy, College of Medicine, University of Basrah. [@]MB,ChB, MSc, PhD, Assist. Prof., Department of Anatomy, College of Medicine, University of Al-Mustansiryah. [#]MB,ChB, FICMS, Assist. Prof. Department of Pathology, College of Medicine, University of Al-Mustansiryah, IRAQ.

Abstract

The aim of this retrospective study on tissue specimens of benign colorectal polyps is to evaluate the significance of IHC markers CD44, CD 166 and ALDH1A1 expression in non-neoplastic (Juvenile and Hamartomatous) polyps and their association with different clinicopathological parameters & to compare their expression in benign polyps with that of normal colonic tissue .

Seventy cases enrolled in the study including benign polyps (juvenile & hamartomous polyps) & benign looking colonic tissue obtained from archive of histopathology unit in Gastroenterology and Hepatology hospital in Baghdad Medical City and histopathology unit in Al-Sadder Teaching Hospital in Basrah city for the period Sept. 2015 to Dec. 2016. Four micrometer sections were obtained from formalin fixed paraffin-embedded blocks treated IHC with CD44, CD166 and ALDH1A1 tumor markers.

Majority of polyps were juvenile, no difference regarding gender distribution. The common location was rectum/rectosigmoidal region. CD166, CD44 and ALDH1A1 showed high expression regarding age, gender and location in benign tumors when compared with healthy looking tissue.

In conclusion, colorectal polyps are critical clinical entity and many of them are a precursors to malignant diseases so colonoscopy is essential screening test, polypectomy and follow up is mandatory for patients with colorectal polyps even if they are benign.

Introduction

Colorectal polyps are usually common in the general population with incidence rate variation according to different geography, e.g. The incidence rate is 30% in Western countries, 10% in Asian countries and 15% in African countries[1-3]. The incidence increased with age with variation in the size and number[4,5].Colorectal polyps are classified into; *neoplastic polyp* (adenomas) forming two thirds of all colon polyps, often benign but have potential malignant changes, *non–neoplastic* as hyperplastic, juvenile, inflammatory & hamartomatous are benign polyps & have no malignant potential, *malignant polyps* which are cancerous lesions[6].

The hamartomatous polyps are abnormal mixture of normal tissues[7] that contains both stromal and epithelial components, most often solitary, occur sporadically with the rare autosomal dominant juvenile polyposis syndrome[8]. The juvenile polyps(congenital polyp, retention polyp, juvenile adenoma) are the most frequent colorectal tumor in children occurring in 2% of pediatric population and may detected as solitary

juvenile polyp in adult male[9] commonly found at rectosigmoidal colon[10]. Juvenile polyp is neither a neoplasm nor a premalignant condition[11] and may have substantial risk of recurrence or undergo mutated changes. Beside Intraepithelial dysplasia is unusual in sporadic juvenile polyps and could result from inactivation of APC/beta-catenin pathway analogous to the genetic of adenoma formation[12].

Cancer stem cells characterized by self-renewal and generating progenitor cells, they are responsible for cancers originating from epithelium including colorectal carcinoma[13], they are similar to normal adult stem cells found in different tissues such as colonic epithelium and are believed to originate from the normal stem cells or progenitors undergo mutations. These cells are recognized by specific surface epitopes and expressed by surface markers like CD44 which is a cell surface glycoprotein involved in cell adhesion, migration and malignant progression[14]. CD166 is a cell adhesive molecule associated with adenoma-carcinoma development[15] and ALDH1A1 is a detoxifying enzyme involved with early differentiation of stem cells[16]. Several studies demonstrate the role of these markers as predictor of malignant transformation in colon polyps and adenoma[17,18]. A study by Goodman found a diagnostic sensitivity of detecting adenomas was 69%, while the specificity of hyperplastic polyps was 86%[19]. Expression pattern of these markers regarding percentage and intensity of staining is variable depending on the type of marker and the accuracy of biopsy[20].

The aim of this study is to evaluate the significance of CD44, CD166 and ALDH1A1 expression as IHC markers in early detection of abnormal changes in non-neoplastic (juvenile and hamartomatous) polyps of the colon, and to correlate the expression with the different clinico-pathological parameters including age, gender and location of polyp in comparison with normal colonic tissue

Material and Methods

Tissue specimens: This retrospective study included 34 specimens of non-neoplastic tumor of the colon divided into 11 hamartomatous polyps and 23 juvenile polyps with control group including 36 cases of healthy looking normal tissue. Samples were collected from colonoscopies and operative resections of the colon and provided in form of formalin fixed paraffin–embedded blocks from Gastroenterology and Hepatology hospital in Baghdad medical city and histopathology unit in AL-Sadder teaching hospital-Basrah during the period from Sept. 2015 to Dec. 2016. The Clinical data retrieved from patient records included age, gender and tumor location. Patients were divided into three age groups(<40, 40-60 & >60years). Ethical approval was obtained from Baghdad medical city and Basrah teaching hospital. Patients with inflammatory bowel disease, colorectal carcinoma, or genetic syndromes associated with polyposis were excluded from the study.

Histopathological evaluation and IHC analysis: For each specimen a Haematoxylene and Eosin (H&E) stained section was made from formalin fixed paraffin–embedded tissue blocks to confirm the histological type[21], another 3 sections of 4 micrometer thickness each were put on positively charged slides and stained IHC with anti-CD44, anti-CD166 and ALDH1A1 markers. An immune peroxidase method was used with a streptavidin biotinylated horseradish peroxidase complex (Abcam). To determine the site of CD44, CD166, and ALDH1A1, sections were dewaxed in xylene and rehydrated with graded alcohol, retrieved for 20 minutes then washed with phosphate buffer saline before application of primary antibody (anti-CD 44 clone f10-44-2

dil1:200Abcam: anti-CD166 clone 8E12C7dil1:300Abcam:LDH1A1neuronal marker dil1:300) for 30 minutes. Sections were subsequently incubated with secondary antibody for 10 minutes followed by HRP streptavidin for 10 min, and then DAB were used as a chromogen followed by slight haematoxyline counterstaining. A negative control slides were done by omitting the primary antibody. A positive control from colorectal adenocarcinoma patients were treated with anti-CD44, anti-CD166 and ALDH1A1 and included in each run of staining protocol. A membranous staining was determined in CD44 and CD166 whereas for ALDH1A1 cytoplasmic immune reactivity was evaluated.

All slides of both tested and control group were scored semi-quantitatively by calculating the proportion of positive stem cells over the total number of cells (% of the positive stem cells) and differences in expression pattern were described namely, whether increased or decreased (high, low, loss), the intensity of staining was separately graded as follows: 0=no staining, 1=faint or weak, 2=moderate, 3=strong.

Statistical analysis was performed by using SPSS software V22 and Chi-square test used to show expression CD44 and CD 166, ALDH1A1 in polyps and normal control. P value <0.05 was considered as statistically significant

Results

According to age distribution the healthy looking tissue was the most common finding in age group 40-60yrs. Retention polyps in group <40yrs and hamartomatous polyps in group >60yrs. P value <0.01 as shown in Table I.

Table I: Age groups and type of tissue

		Age group level		
		<40	40-60	>60
Type of Biopsy	Control = Normal tissue	42.1%	64.7%**	30.0%
	Retention (juvenile) polyp	50%**	11.8%	16.7%
	Hamartamatous polyp	7.9%	23.5%	53.3%**

**= P value < 0.01 (high statistical significance).

Gender has no significant effect on disease prevalence in either benign tumor or control group. Findings were closely distributed among males & females, except hamartomatous polyp with lower frequency in males. P value <0.01 as shown in Table II.

Table II: Effect of gender on biopsy type

Control		Gender	
		Female	Male
Type of Biopsy	Normal tissue (HN)	53.3%	45.9%
	Retention polyp	40%	29.7%
	Hamartamatous polyp	6.7%**	24.3%

**=P value <0.01 (High statistical significance).

Distribution of biopsies according to their site within the colon: Results show normal tissue findings were mostly found in the right colon. Retention polyps were more frequent in the rectal-rectosigmoidal region and hamartomatous polyps were most frequently seen in left colon. P value <0.05 as shown in Table III.

Table III: Effect of site on biopsy type.

		Site of Biopsy		
		Right Colon	Left Colon	Rectum/ Rectosigmoid
Type of Biopsy	Normal tissue	78.6%*	55.6%	27.3%
	Retention polyp	17.9%	11.1%	51.5%*
	Hamartomatous polyp	3.6%	33.3%*	21.2%

*= P value < 0.05 (statistically significant).

General expression of markers according to sample type negative (N) or positive (P): Most positive results with CD166 found in benign tumor(BT) (79.4%), and most negative in healthy looking normal (HN) (88.8%). CD 44 show less positivity in HN than BT (16.7%) and (38.2%) respectively while ALDH1A1show less positivity in HN (13.9%) compared with (38.2%) in BT as demonstrated in Table IV.

Table IV: Effect of marker expression on BT (General)

Type of marker		BT		Control = Healthy looking		P value
		Count	Column N %	Count	Column N %	
CD44	N	21	61.8%	30	83.3%	0.0054
	P	13	38.2%	6	16.7%	
ALDH1-A1	N	21	61.8%	31	86.1%	0.00028
	P	13	38.2%	5	13.9%	
CD166	N	7	20.6%	32	88.8%	0.0037
	P	27	79.4%	4	11.1%	

Effect of age group level on marker expression in BT: CD166 stained positive at higher rates of statistical significance in all age groups with normal tissue or hamartamoatous polyps. The same was found for CD166 only in age group <40 years with retention (juvenile) polyps. Age group 40-60 yrs totally stained negative for CD44 giving a high significance P= <0.001% as shown in Table V.

Table V: Effect of age group levels on marker expression in BT

		Marker	Percentage of positive staining biopsies according to age group		
			<40	40-60	>60
Type of Biopsy	Normal tissue	CD44	11.8%	25%	14.3%
		ALDH1-A1	11.8%	16.7%	14.3%
		CD166	58.8%*	41.7%*	57.1%*
	Retention polyp	CD44	42.1%	0%**	50%
		ALDH1-A1	36.8%	50%	50%
		CD166	84.2%*	50%	50%
	Hamartamatous polyp	CD44	66.7%	25%	25%
		ALDH1-A1	33.3%	50%	25%
		CD166	100%*	100%*	50%*

Effect of gender on marker expression: CD166 stained positively equal among Male/Female in all types. CD44 stained

positive at higher rates in M with hamartoma as shown in Table VI.

Table VI: Effect of gender on marker expression:

Type of Biopsy	Marker	% of positive staining biopsies according to sex	
		Female	Male
Normal tissue	CD44	10.5%	23.5%
	ALDH1-A1	10.5%	17.6%
	CD166	52.6%	52.9%
Retention polyp	CD44	41.7%	36.4%
	ALDH1-A1	25%	54.5%
	CD166	75%	81.8%
Hamartomatous polyp	CD44	0%	44.4%**
	ALDH1-A1	100%**	22.2%
	CD166	100%	77.8%

Effect of Biopsy site on marker expression: Only CD166 showed significant positive staining frequency in the right colon & rectal/ recto sigmoid regions of patients with retention polyps, compared to CD44 and ALDH1A1 as demonstrated in Table VII.

Table VII: Effect of site on marker expression.

Control		Marker	% of + ve staining biopsies according to site		
			Rt colon	Lt colon	Rectum (RS)
Biopsy Type	Normal tissue	CD44	22.7%	20%	0%
		ALDH1A1	13.6%	20%	11.1%
		Cd166	45.5%	80.0%	55.6%
	Retention polyp	Cd44	0%	100%	47.1%
		ALDH1-A1	0%	0%	52.9%
		CD166	20%**	100%	94.1%**
	Hamartomatous	CD44	0%	33.3%	42.9%
		ALDH1A1	0%	0%	57.1%
		CD166	100%	66.7%	85.7%

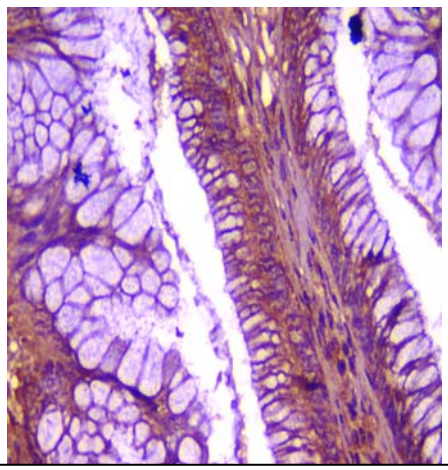

Fig. 1: Microphotograph shows positive (moderate) Immunohistochemical expression of CD44 in benign Tumor (Hamartomous polyp), 40x.

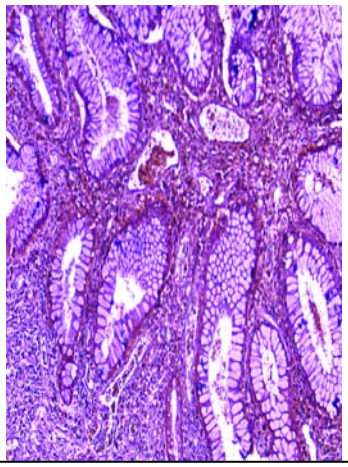

Fig.2; Microphotograph shows negative IHC Expression of CD44 in benign tumor (Hamartomous polyp),40x.

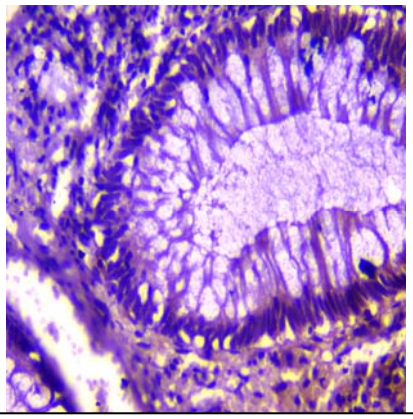

Fig.3: Microphotograph shows negative IHC Staining of CD166 in apparently healthy colonic tissue, 40X

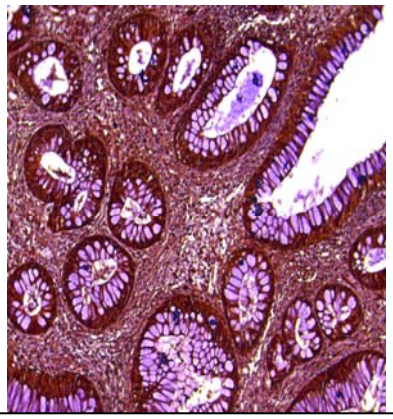

Fig.4: Microphotograph shows positive (strong) immunohistochemical Staining of CD166 in benign tumor (retention polyp),40x.

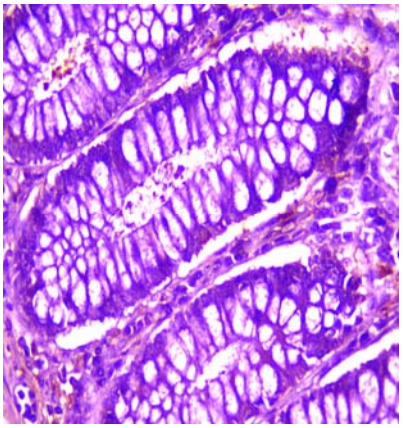

Fig.5:Microphotograph shows negative immuno-histochemical expression of ALDH1A1 in apparently healthy colonic tissue,40X.

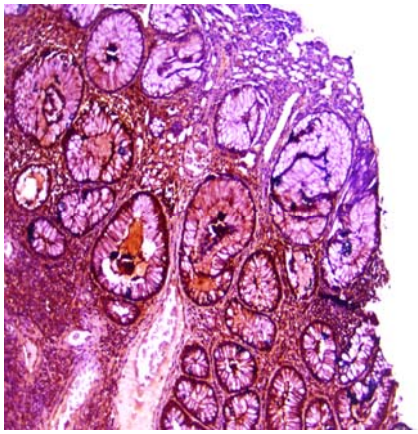

Fig.6: Microphotograph shows positive immunohistochemical expression of ALDH1A1 in benign tumor(retention polyp),40x.

Discussion

Colorectal polyps are common findings on colonoscopies with predominance of epithelial polyps (adenoma) that have potential for malignant transformation (adenoma-carcinoma sequence). It is a slow process allow time for interruption by endoscopy which made resection of polyps possible[4,22]. Non-epithelial polyps includes; inflammatory, hamartomatous and juvenile[23-25]. A definite cause of juvenile polyp is unknown but there are many theories including retained secretions, congenital, inflammatory, allergic, neoplastic and hamartomatous causes[26-28]. Current study shows that most benign tumors were found in patients age ranged from 4-68 years (mean age=28 years), this disagree with Mirzaie *et al* 2012 in which the age level is higher ranged from 16-81yrs with mean age=58.4yrs[29]. Wang *et al* 2009 noticed a younger age level with mean age=6.8yrs[30], and this difference may be due to inclusion of more peadiatric cases and or a large sample size or may be due to geographical variability. In addition it was found that juvenile polyps are most frequent type of benign polyps mainly below 10 years of age, while hamartomatous polyps show slightly increase in age incidence. Recently there is a steady decline in incidence during childhood which may be due to auto-amputation and or regression of polyps which may be caused by existence for along time with possibility of ischemic changes. Similar polyps were

recorded in adults also and this correspond with other studies and frequency of juvenile polyps are similar to the results of El-Shabrawi *et al* 2011[31].

Differences regarding gender distribution in the current study did not reach statistical significance except it is slightly lower in males than females with hamartomatous polyps, and this is not in agreement with previous studies in which male predominance was noticed[9,24,26,28,29]. Females seems to be more protective from malignant potentials factors and post-menopausal women with hormonal therapy found to have lower rates of frequency than women of the same age who are not on such therapy[32]. McCashland *et al* 2001 found that men have greater risk of developing polyps more than women and tendency increase with age especially in those above 69 years of age[33].

Results also demonstrate that juvenile polyps were most commonly situated in the rectum/rectosigmoidal region which correspond with other studies[29,30]. Furthermore, hamartomous polyps found to be located in the left colon. The right-sided lesions can be indicative of increased risk of recurrence or may exhibit a malignant potential[34], which may occur through multiple mutations affecting the DNA-mismatch-repair pathways. This study shows that most juvenile and hamartomatous polyps are found in the distal colon and rectum (Left side) which disagreed with Kumar 2010 who found right sided polyps are more frequent than left sided in older patients in addition, women are more liable to develop polyps on the right side than men[35].

According to immunohistochemical results of the present study, it was found that CD166 expressed in all types of lesions including the apparently healthy colonic tissue, juvenile & hamartomous polyps, in all age groups with predominance of patients>60 years in healthy looking tissue & hamartomous. CD166 expression was more in patient below 40 years in juvenile polyp. These findings may be due to the putative and aggressive behavior of CD166 toward colon stem cell changes. Kumar 2010 found that CD44 is totally negative <40 years, while our results showed that expression is found to be low below 40 years of age in apparently healthy colonic tissue and may be explained in that CD44 is not expressed by all replicating cells and may be related to some other aspects of cell activation or proliferation in non-neoplastic tissues. ALDH1A1 show more positivity in older age groups which may explain its effect as a detoxifying enzyme is more with increasing age. Results also determine that CD44 have high positivity in male in hamartomatous polyps. CD166 was found to be high in the right colon and rectum/rectosigmoidal region in juvenile polyps obviously.

Differences in marker expression between benign tumors and healthy looking normal may be explained by the fact that cells forming the non-neoplastic polyps grow more slowly and have a longer lifespan than nearby normal mucosal cells[36]. The variation in expression pattern may help to diagnose which polyps are more liable to mutant changes assisted by long period history of polyp formation. For colorectal carcinoma with long asymptomatic history and the existing of treatable precancerous lesions made a routine population wide screening is essential . Colonoscopy is considered the method of choice for this purpose. Randomized clinical trials and several cohort studies have shown that colonoscopic polypectomy reduces the incidence by 76-90% as compared with a general population registry[37].

In conclusion, our current observation is that cancer stem cell markers such as CD166, CD44 and ALDH1A1 are co-localized more in benign tumor glands than healthy looking normal which support the idea that increase the number of stem like cells hyperproliferation may predispose colonic mucosa to subsequent transformation. Colon polyps are important clinical entity, and many are precursors to

malignant diseases so it is recommended that colonoscopy is inevitable as routine screening test. Polypectomy is essential and continued surveillance is indicated in patients with colorectal polyps whether they are benign or malignant.

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