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Research article

Comparable study of expression of CD44, CD166 and ALDH1A1 markers in normal tissue adjacent to mucinous and non-mucinous adenocolorectal carcinoma in sample of Iraqi patients

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Abstract

Background: Colorectal cancer is one of the commonest malignant tumors among men and women worldwide with large morbidity and mortality 90% of colorectal carcinoma are adenocarcinoma while mucinous type comprising >20% of all colorectal cancers . Development process is slowly with late diagnosis, therefore early detection and screening is of vital importance. CD44, CD166 and ALDH1A1 are stem cell markers with different expressions depending on type of tumor and relation to clinical parameters.

Objective: To highlight expression of CD44,CD166 and ALDH1A1 in normal tissue adjacent to mucinous and non-mucinous colorectal adenocarcinoma and correlate with clinic- pathological parameters in a group of Iraqi patients.

Methods: A retrospective study of 70 cases with normal tissue adjacent to colorectal carcinoma obtained from two hospitals from 2015 to 2016, divided into two groups. Paraffin blocks were IHC treated with CD44, CD166 and ALDH1A1 markers to compare the expression pattern of these stem cell markers.

Results: The study revealed that 15.7 % were mucinous CRC with mean age 59yrs and equal M / F ratio and common site is rectum and recto sigmoidal region .CD44 CD166 and ALDH1A1 markers had different expression pattern among mucinous and non-mucinous CRC.

Conclusion: The normal tissue adjacent to CRC had different marker expression properties depending on type of the tumor.

Key Words: CRC, CD44, CD166 and ALDH1A! Markers and IHC

Introduction:

Colorectal carcinoma is the 4th most common cause of cancer death worldwide[1). The incidence in Iraq had increased abruptly in the last decade, It encompasses 5.3% of all cancers in 2011 according to National Cancer Registry Center [2] which is still less than developed countries 6-13% and 17-51% in industrialized nations [3).Nearly 90% of colorectal cancers (CRC) are adenocarcinomas [4] .Mucinous adenocarcinoma is a s a histological subtype of colorectal adenocarcinoma, which account for 10-20 % of all CRC [5.6.7.). .It contains cancer cells that yield ≥ 50% mucin components of tumor volume [8] and differs from non-mucinous adenocarcinoma in regard to its clinico pathological features, distinctive genetic outlines and pathogenic background.

The incidence of mucinous carcinoma in western population ranges from 9.6-25.4% [9, 10.11.12.13] while in Asian population it ranges from 3.9-11.7 % [14.15, 16, 17] .In Iraq registry for mucinous type is nil.

Cancer stem cells or as called tumor initiating cells or are small subset of cells within a solid tumor with a stem cell like characteristics of low proliferative rates ,increased self-renewal capacity, ability to differentiate into active proliferating tumor cells and resistance to chemotherapy or radiation [18, 19). Several CRC research had suggested a hopeful biomarkers [20), which provided a prognostic data for CRC such as CD44, ALDH1A and CD166, these biomarkers are expressed in many solid organ epithelial malignancies including colon and rectum. [21.22.23]

CD44 is a cell surface glycoprotein involved in cell adhesion; facilitate tumor cell migration and malignant progression [24] ALDH1A1 is a detoxifying enzyme responsible for oxidation of intracellular aldehydes, early differentiation of stem cells and resistance to chemotherapy. While CD166 involved in neuronal extension, cell adhesion and embryonic angiogenesis [25] .Several studies were done on expression of these markers in the tumor tissue and normal tissue but no such studies were done on normal tissue adjacent to cancer region (NAC) of mucinous and non-mucinous colorectal carcinoma.

Aim: To evaluate the expression of CD44, CD166 and ALDH1A1 in normal tissue adjacent to mucinous and non-mucinous colorectal adenocarcinoma and correlate with clinicopathological parameters.

Material and Methods:

A total of 70 tissue biopsies were taken from normal tissue adjacent to colorectal carcinoma (≥ 5 cm) from colectomy specimens of patients attending two hospitals

(Gastroenterology center and Oncology hospital at Baghdad Medical City and Al-Sadder teaching hospital at Basrah city) during the period 2015-2016 .

Clinical data regarding age, gender, site of tumor, grade, differentiation and lymph node involvement were obtained from pathological reports of the patients .Ethical approval was obtained from ethics committee at Baghdad medical city and Al-Sadder teaching hospital /Basrah.

Patients divided into two groups Group 1 of 59 specimens of normal tissue adjacent to non-mucinous adenocarcinoma (NANMC) with mean age 54 years, and Group 2 of 11 specimens of normal tissue adjacent to mucinous colorectal carcinoma (NAMC) with mean age 59 yrs. Patients were divided into three age group levels (<40yrs,40-60 and >60 yrs.).

Other tumors like undifferentiated tumors and signet cell carcinoma were excluded from this study, in addition to those patients on chemotherapy or radiotherapy.

Formalin fixed paraffin embedded blocks were achieved and serial sections of 4µm thickness was obtained. One section stained with routine haematoxylene - eosin stain for pathological classification of the CRC in agreement to the classification of tumors by the World Health Organization (WHO) (28).Another three paraffin sections were IHC treated with anti-CD44 clone(f10-44-2) dil 1/200 ABCAM; anti-CD166 Clone (8E12C7) dil 1/300 ABCAM and ALDH1A1 neural marker dil 1/300 ABCAM ,respectively for determination of colonic cancer stem cells expression. Procedure was done as mentioned by the manufactured kit protocol.

Each marker was examined at high power and scored quantitatively by evaluating the proportion of positive cells and the intensity of positively stained cells [26,27] .The percentage of positive cells was calculated as the following 0 = < 10 %cell, +1= 10-24% , +2 = 25-49%, +3 = 50-74%.,+ 4 = 75-100% while intensity was graded as the following: 0 = no staining.1=weak.2=moderate ,3= strong .4= severe.

Statistical analysis:

It was performed by using the SPSS package version 18 for window , Chi-square test.& Frequency distribution . P value < 0.05 regarded as significant.

Results:

1. Patients characteristics:

A. Age distribution

Group 1 represent normal tissue adjacent to non-mucinous **adenocarcinoma** (NANMC) it was significantly higher at age group 40-60 (97.5%)& >60yrs(78.6%) respectively, while G2 represent normal tissue adjacent to **mucinous** carcinoma(NAMC), it was common at younger age group <40 (75%).A high statistical significance is found between the two, P value = < 0.01 as in Table1.Fig 1

Table 1: Effect of age group on biopsy finding

Normal adjacent to cancer(NAC)		Age group (years)		
		<40	40-60	>60
Type of biopsy	ANMC	25%	97.5%**	78.6%**
	NAMC	75%**	2.5%	21.4%

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

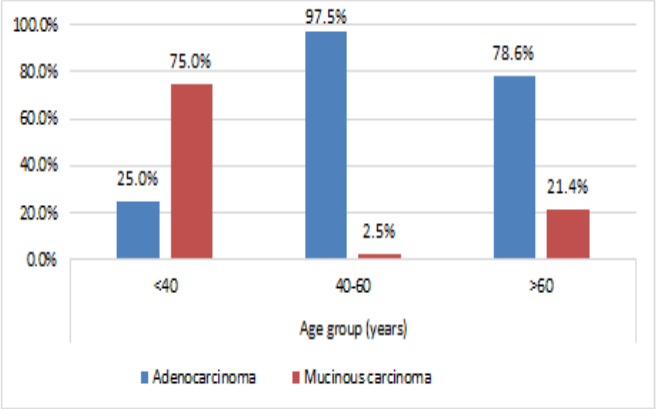

Figure 1: Age group distribution and biopsy type

B: Gender and biopsy type

Sex had no significant effect in G1 &G2, Findings were closely distributed among males & females as in Table 2 &Fig 2

Table 2: Effect of gender on biopsy type.

NAC		sex	
		Female	Male
Type of Biopsy	NANMC	84.6%	87.9%
	NAMC	15.4%	12.1%

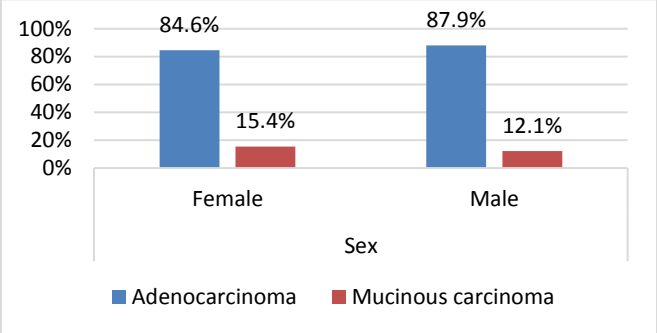

Fig 2: Effect of gender on type of biopsy

C: Location of the tumor.

No significant difference regarding site of biopsy in G1&G2 as shown in Table 3and Fig 3.

Table 3: Site of biopsy and type

NAC		Site of Biopsy		
		Right Colon	Left Colon	Rectum/Rectosigmoid
Type	NANMC	79.3%	90.9%	96.7%
	NAMC	20.7%	9.1%	3.3%

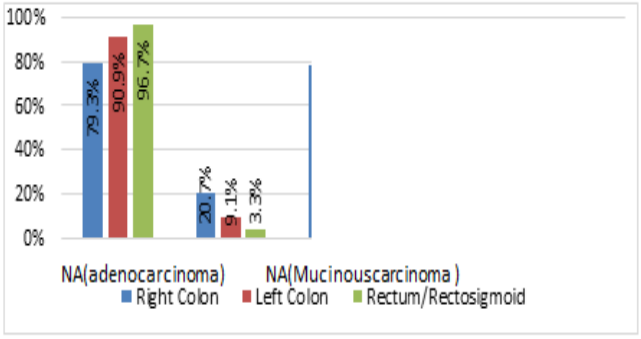

Fig 3: Site and biopsy and type

2. Immunohistochemical study of CD44, CD166 and ALDH1A1 marker expression and patients characteristics

A. Age level distribution and marker expression

In NANMC, the CD44, CD166 and ALDH1A1 are equally positive at all age groups. In NAMC, the CD44 & CD166 stained equally positive at all age groups but ALDH1A1 was negative at age <40 years. (P value = 0.019).as in Table 4, Fig 4

Table 4: Age group levels and marker expression

NNAC		Marker	Percentage of positive staining biopsies according to age group		
			<40	40-60	➢ 60
T Type	NANMC	CD44	100%	78.4%	95.5%
		ALDH1-A1	100%	62.2%	77.3%
		CD166	100%	100%	100%
	NAMC	CD44	100%	100%	100%
		ALDH1-A1	0%*	50%	100%
		CD166	100%	100%	100%
P value	0.019				

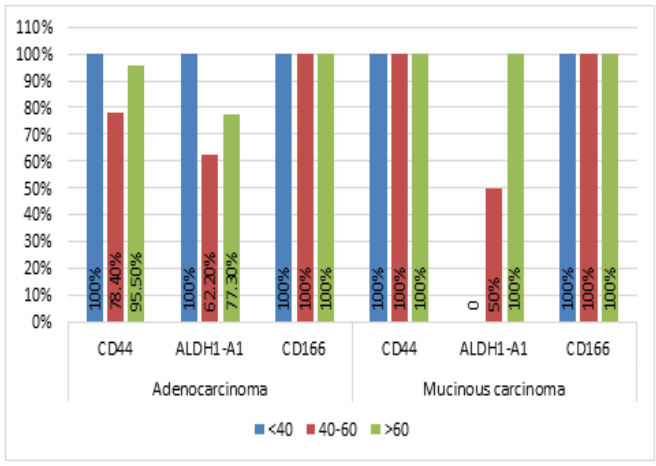

Figure 4: age group level and marker expression.

B. Gender distribution and marker expression:

CD166 and CD44 stained positive among males and females of both groups. ALDH1A1 stained positive at significantly lower frequencies in females in both groups as shown in Table 5 and Fig 5

Table 5: Effect of sex on marker expression

Type of Biopsy	Marker	Percentage of positive staining biopsies according to sex	
		Female	Male
NANMC	CD44	83.9%	86.2%
	ALDH1-A1	54.8%**	82.8%
	CD166	100%	100%
NAMC	CD44	100%	100%
	ALDH1-A1	33.3%**	100%
	CD166	100%	100%

.P v= <0.05

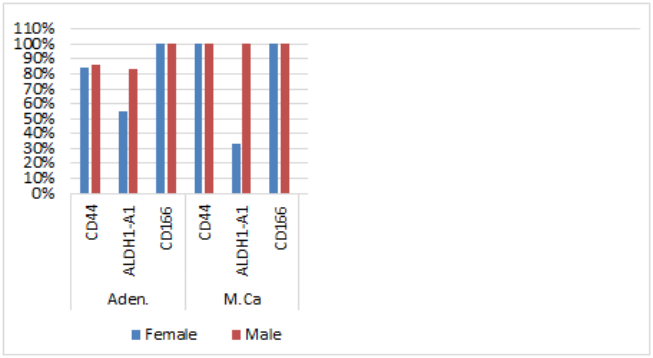

Fig 5: Effect of sex on marker expression

C: Location of the tumor and marker expression:

Only ALDH1A1 showed significantly reduced positive staining in the left colon in patients with NANMC. In NAMC, all markers stained positive with similar rates at all sites. As in Table 6 and Fig 6.

Table 6: Site of tumor and marker expression

NAC		Marker	P Percentage of positive staining biopsies according to site of biopsy		
			Right colon	Left Coon	Rectum (RS)
Type of Biopsy	NANMC	CD44	77.3%	90%	89.3%
		ALDH1-A1	63.6%	40%**	82.1%
		CD166	100%	100%	100%
	NAMC	CD44	100%	100%	100%
		ALDH1-A1	57.1%	100%	50%
		CD166	100%	100%	100%

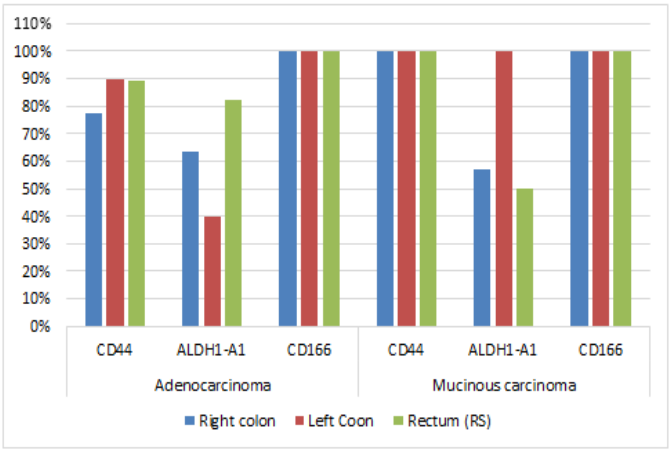

Fig 6: site of tumor and marker expression

3. Effect of differentiation of CRC and marker expression

CD44, CD166 and ALDH1A1had highest expression (100%) in poorly differentiated tumors. In moderately differentiated tumors, the ALDH1A1 showed reduced expression (P<0.05). In well-differentiated tumors, CD166 showed increased positive expression, as shown in table 7.

Table 7: Differentiation pattern and marker expression

Marker	Percentage of positive staining biopsies according to differentiation of adenocarcinoma		
	Poor	Moderate	Well
CD44	100%	85.2%	66.7%
ALDH1-A1	100%	66.7%*	66.7%
CD166	100%	100%	100%*

4. Effect of tumor grade and marker expression in colorectal carcinoma.

CD44 had the greatest staining reactions in grade 1 followed by grade 2. ALDH1A1 showed increasing staining reaction at grade 2& grade 3. CD166 had positive staining reaction in about two thirds of cases of grade 0, but positive in all cases of other grades, as in Table 8 and Fig7. High statistical analysis was detected as shown in Fig 7.

Table 8: Grade of the tumor and marker expression

% of marker positive samples	Tumor grade				P-value
	0	1	2	3	
CD44	27.1%	100.0%	85.7%	75.0%	0.0044
ALDH1-A1	25.7%	.0%	67.9%	75.0%	0.0024
CD166	65.7%	100.0%	100.0%	100.0%	0.0025

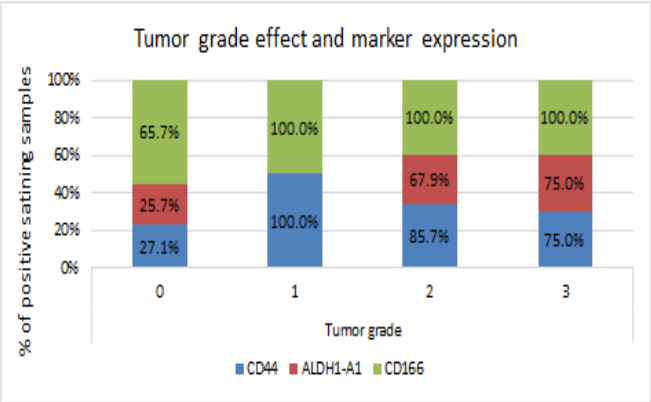

Fig 7: Grade of the tumor and marker expression

5. Effect of Lymph node involvement and marker expression

Results showed lymph node involvement in MC is less than NMC.CD44 expression in NMC is (++) and in MC is less expressed (+). While ALDH1A1 stained (+++)at NMC and(+) at MC.CD166 showed positivity at both NMC and MC with more staining at NMC ++++.

Table 9: Lymph node involvement and marker expression:

Lymph node involvment		NMC		MC	
		N	P	N	P
		N %	N %	N%	N %
CD44+ cells %	Negative	21.1%	4.5%	.0%	.0%
	+	52.6%	31.8%	42.9%	66.7%
	++	21.1%	59.1%	57.1%	33.3%
	+++	5.3%	4.5%	.0%	.0%
	>+3	.0%	.0%	.0%	.0%
P value		**0.02***		0.40	
ALDH1-A1+ cells %	Negative	47.4%	4.5%	57.1%	.0%
	+	10.5%	13.6%	14.3%	66.7%
	++	15.8%	27.3%	.0%	.0%
	+++	26.3%	54.5%	28.6%	33.3%
	>+3	.0%	.0%	.0%	.0%
P value		0.07		0.16	
CD166+ cells %	Negative	.0%	.0%	.0%	.0%
	+	5.3%	.0%	14.3%	.0%
	++	23.7%	13.6%	.0%	.0%
	+++	31.6%	59.1%	42.9%	66.7%
	>+3	39.5%	27.3%	42.9%	33.3%
P value		0.17		o.70	

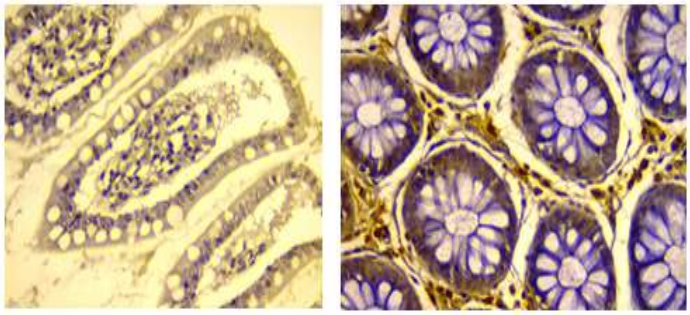

Fig 8: IHC of anti-CD44 positive 40 xs
A: NAMC **B: NANMC**

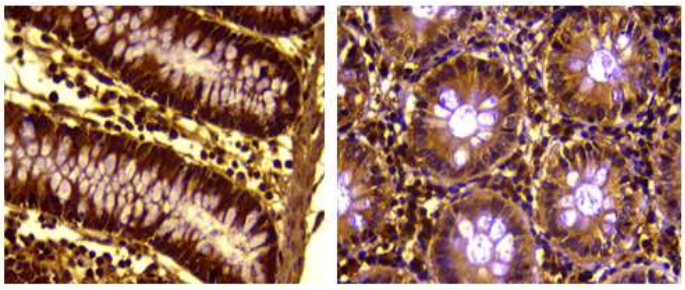

Fig 9: IHC of ALDH1A1 Positive 40 xs
A: NAMC **B: NANMC**

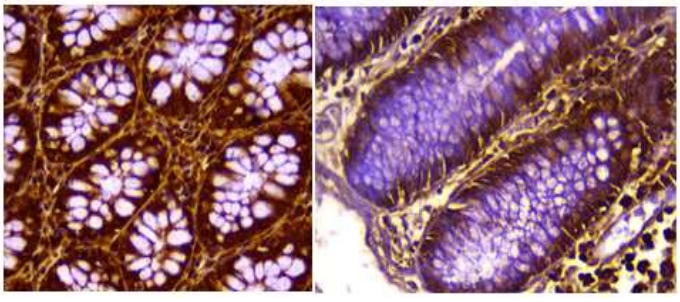

FIG 10: IHC of anti-CD166 Positive 40 xs
A: NAMC **B: NANMC**

Discussion:

Mucinous adenocarcinoma represents 10 - 15% of all colorectal cancers with mucin content of at least 50% of tumor volume [28] ,it has a bad prognosis when compared with non-mucinous adenocarcinoma [7,11 ,29] ,the reason is not apparent but it may be due to difficulty in obtaining complete resection at surgery [30] or tendency for earlier spread to L.N [31] or late diagnosis when the disease reaches late stages [32] .

This study focused on normal tissue adjacent to CRC and results showed that normal tissue adjacent to MC (NAMC) accounts for 15.7% of all colorectal cancers with mean age of 59yrs.No significant difference between male and female in both NANMC and NAMC were detected and these findings are nearly similar to other studies conducted on CRC in Iraq and abroad [30. 32.33).

Results showed also the most common site of the tumor was mainly at R/RS area in both NAMC & NANMC, this agree with many previous studies like [30, 34, 35 & 36).

Expression of CD44, CD166 and ALDH1A1 in normal tissue adjacent to colorectal carcinoma (NAC) may be called (cancer field area) is not studied widely and articles of concern are either few or nil.

In this study the marker expression was calculated as percentage (number of cells stained positive) and intensity of staining (weak. moderate and severe). In NANMC we found that CD44,CD166 and ALDH1A1 markers have positive expression at all age groups while in NAMC ,ALDH1A1showed no expression below 40yrs.CD166 showed high expression in both NANMC and NAMC, the strong expression of CD166 is pathologically correlated with the aggressiveness which is not only noticed in CRC but in other types of tumors [37).These findings may differ from Safa *et al* [8).who found the expression of CD166 in mucinous type was significantly lower than non- mucinous type of CRC.

Expression of CD44,CD166 and ALDH1A1 were N.S regarding M/F ratio except for ALDH1A1 which showed lower female rate in both groups and this agree with Glasgow *et al* [28). Who showed male predominance. Furthermore CD44, CD166 and ALDH1A1 showed a common site for the tumor at R/RS in NANMC and NAMC except for ALDH1A1which show non-significant elevation at Lt Colon.

Regarding differentiation of the tumor most of our patients had histopathological reports of moderate differentiation often at young age, which indicate that carcinoma of the colon and rectum is more malignant and invasive in young patients and this is also reported in other studies [7,8] .

Current study showed CD44, CD166 and ALDH1A1 markers were highly expressed in poorly differentiated colon cancer. while ALDH1A1 showed reduced expression in moderately differentiated carcinoma and CD166 showed high expression in poor , moderate and well differentiated carcinoma indicating the invasive behavior of this marker .Talib *et al* & Rahman *et al* [38,39] found that percentage of well differentiated , moderate and poorly differentiated carcinoma was nearly the same. While McCoy and Parks [40] found that well differentiated carcinoma was most common (41.39%), moderately differentiated was less common (22.9%) and poorly differentiated was (35.48%). This study demonstrated that CD44 had greatest staining reactions in grade 1 followed by grade 2 which reinforce the role of CD44 in early cancer initiation and cancer progression.ALDH1A1 showed increasing staining reaction at grade 2&3. While CD166 had positive staining reaction in about two thirds of cases of grade 0 but in all cases of all other grades. High statistical analysis was detected with p value < 0.05.Current study showed that lymph node involvement in MC is less than NMC. CD44 expression in NMC is (++) and in MC is less expressed (+) While ALDH1A1 stained (+++) at NMC and (+) at MC.CD166 showed positivity at both NMC and MC with more staining at NMC ++++. These results may explain that each marker had its specific criteria for staining depending on its histochemical properties and function.

Relation between marker expression and grade of the tumor are agreed with Dangho *et al*[41]who noticed an increased expression associated with high grades of CRC (G2&G3), in contrast to Lugli *et al*[42] who found a relationship between lack of expression of CD44 & CD166 and invasiveness of colorectal tumor .He noticed the lack of expression of CD166 and CD44 markers were accompanied with a higher pathologic T stage, lymph node metastasis, and worse survival. Moreover Weichert *et al*[37] found no considerable relationship among expression of CD166 marker and tumor grade, stage of illness and involvement of lymph nodes. Tachezy *et al* [43] showed a reversed significant relationship between CD166 marker expression rate and tumor grade with no significant relationship between marker expression and the rest of clinical and histopathological characteristics of tumor, this discrepancy need more studies to confirm the differences , further studies on a larger number of patients may provide important additional information for prognostic relevance of these molecules in colorectal cancer patients.

Conclusion: NANMC and NAMC should be further studied because it convey wide range of different expression of markers related to colorectal carcinoma and IHC study may help in early diagnosis and detection of cancer with more attention to increased rate at younger age groups .To minimize recurrence we aimed that surgical treatment is to provide adequate clear margins ensuring removal of whole tumor burden.

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