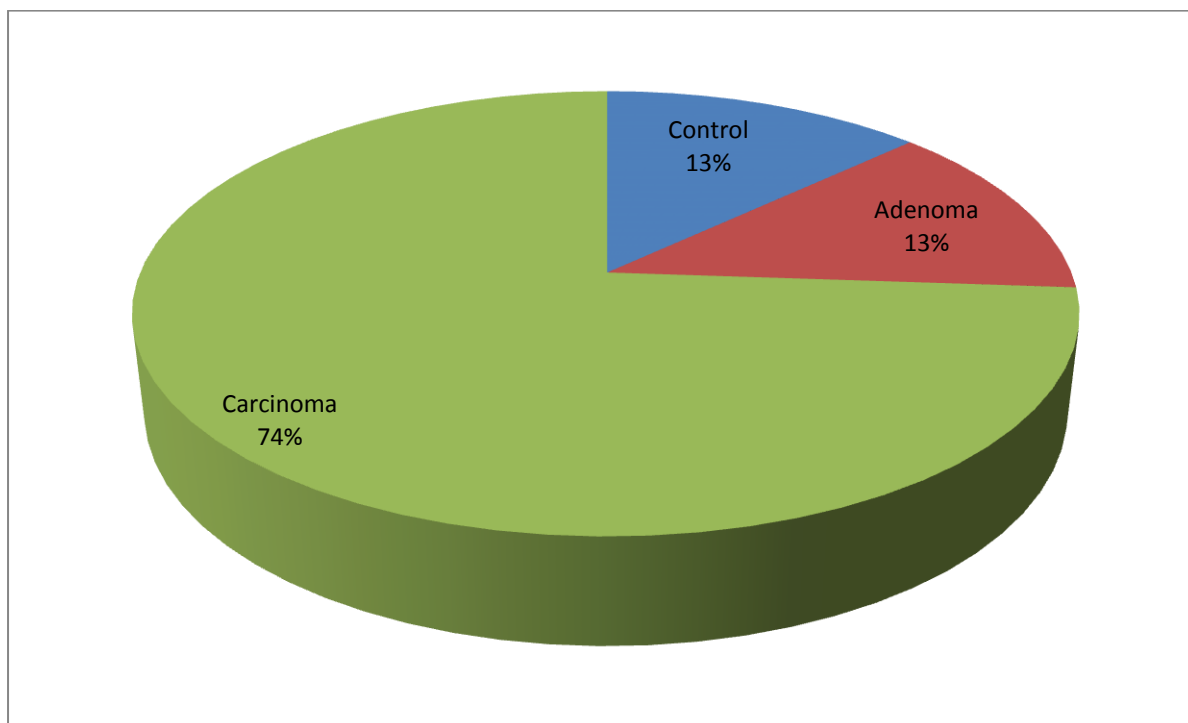


Results

This is a retrospective study included 46 cases of colorectal lesions. Six cases (13%) of apparently normal colonic mucosa were taken as control (figure 11). Histopathological examination of cases revealed six cases(13%) of colonic adenoma (figure 12) and 34cases (74%) of colorectal carcinoma,20 cases (58.8%) of them were Cribriform adenocarcinoma (figures 13, 14& 15) ,8 cases(23.5%) were mucoid carcinoma(figures 16, 17 & 18) and 6 cases (17.7%) were signet ring carcinoma (figures 19&20).

Table (8) :histopathological classification of all studied cases.

Type of lesion	No of cases
Control	6 (13%)
Adenoma	6 (13%)
Total No .of Carcinoma	34 (74%)
Cribriform adenocarcinoma	20
Mucoid carcinoma	8
Signet ring carcinoma	6
Total	46(100%)



Graph (1) :histopathological classification of all studied cases.

Clinicopathological results

1-Age distribution in the examined cases:

The age of all examined cases ranged between 21- 80 years with the mean age 49 years.

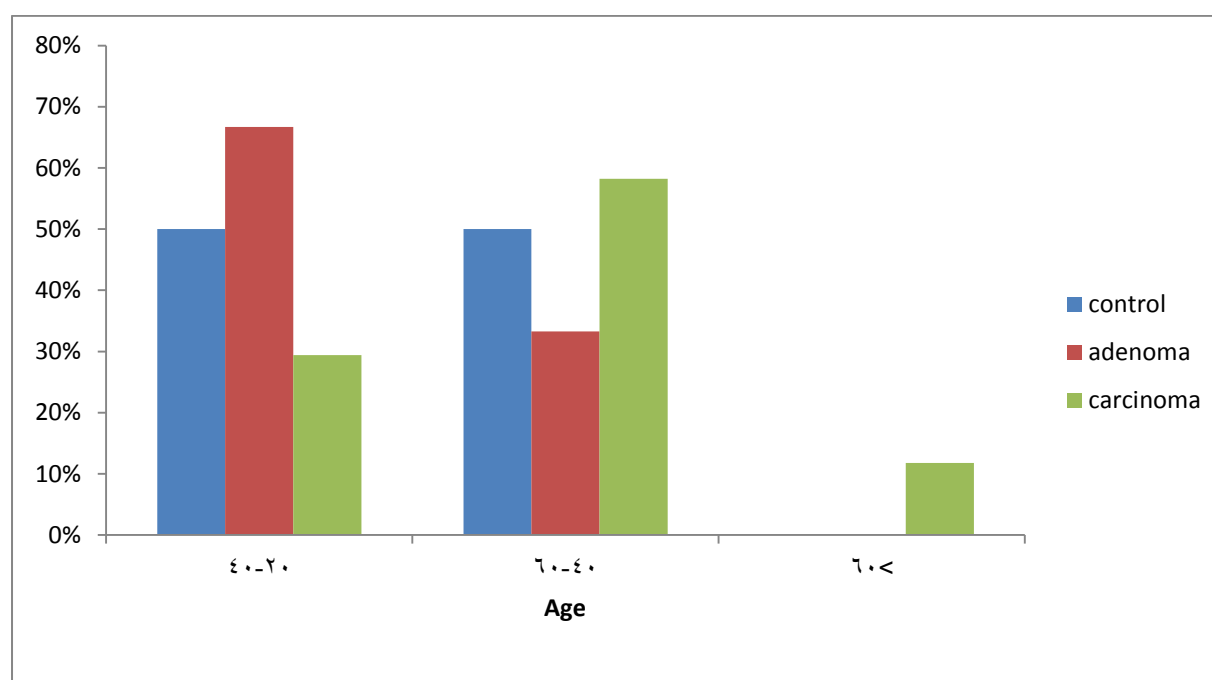
In six control cases age ranged from 20 to 60 years with the mean age (42.5 years). Three cases(50%) aged between 20 to 40 and 3 cases (50%) aged between 41 to 60 years .

In cases of colonic adenoma age ranged from 30 to 48 years with the mean age (38.33 years). Four cases(66.7%) aged between 30 to 40 and 2 cases (33.3%) were older than 40.

In cases of colorectal carcinoma age ranged between 21- 80 years with the mean age 49. Ten cases(29.4%) aged between 20-40 years ,20 cases(58.8%) ranged between 41-60 years and 4 cases(11.8%) aged above 60 years.

Table (9): Age distribution in the examined cases

	No of cases	Age in years			Mean
		20-40	41-60	>60	
Control	6	3 (50%)	3(50%)	0	42.5
Adenoma	6	4(66.7%)	2 (33.3%)	0	38.33
Carcinoma	34	10(29.4%)	20(58.8%)	4(11.8%)	49
Total	46	17	25	4	



Graph: (2) :Age distribution in the examined cases.

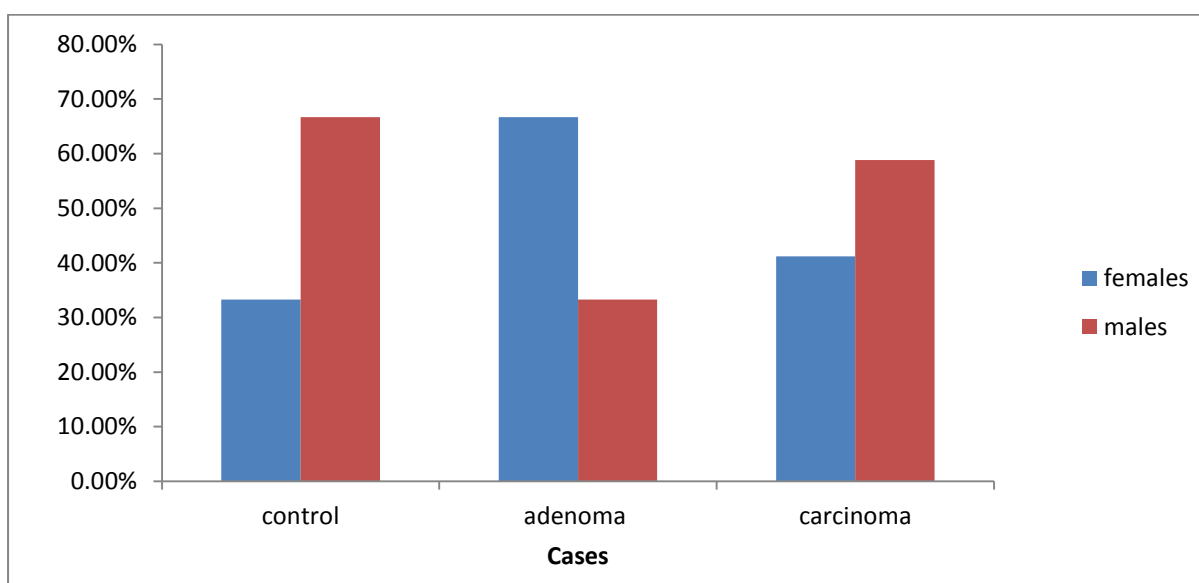
2-Gender distribution in the examined cases

In six control cases, 2 cases(33.3%) were females and 4 cases(66.7%) were males.

Out of 6 cases of adenoma, 4 cases (66.7%) were females and 2 cases (33.3%) were males. Out of 34 cases of colorectal carcinoma, 14 cases(41.2%) were females and 20 cases(58.8%) were males.

Table (10): Gender distribution in the examined cases.

Cases	No of cases	sex	
		females	Males
Control	6	2(33.3%)	4(66.7%)
Adenoma	6	4 (66.7%)	2(33.3%)
carcinoma	34	14(41.2%)	20(58.8%)
Total	40	18	22



Graph (3): Gender distribution in the examined cases.

Histopathological results

In colorectal carcinoma:

Among the examined 34 cases of colorectal carcinoma ,20 of them (58.8%) were cribriform adenocarcinoma , 8 of them (23.5%) were mucoid carcinoma and 6 cases (17.7%) were signet ring carcinoma.

All malignant cases were graded into 21 cases (61.7%) of moderately differentiated carcinoma (grade II) (in which 18 cases were cribriform adenocarcinoma ,3 case were mucoid carcinoma) and 13 cases (38.3%) were poorly differentiated (grade III) (2 cases were cribriform adenocarcinoma ,5 cases were mucoid carcinoma and 6 cases were signet ring carcinoma) . No cases were graded as grade I carcinoma .

Regarding the depth of tumor invasion ,2 cases (5.9%) were T1, 3 cases (8.8%) was T2 ,9 cases (26.5%) were T3 and 20 cases(58.8%) were T4

Lymph node metastasis was detected in 25 cases (73.5%)

Distant metastasis was detected in 19 cases (55.9%)

According to TNM staging system, 9 cases (26.5%)were stage II ,6 cases (17.6%) were stage III and 19(55.9%) cases were stage IV.

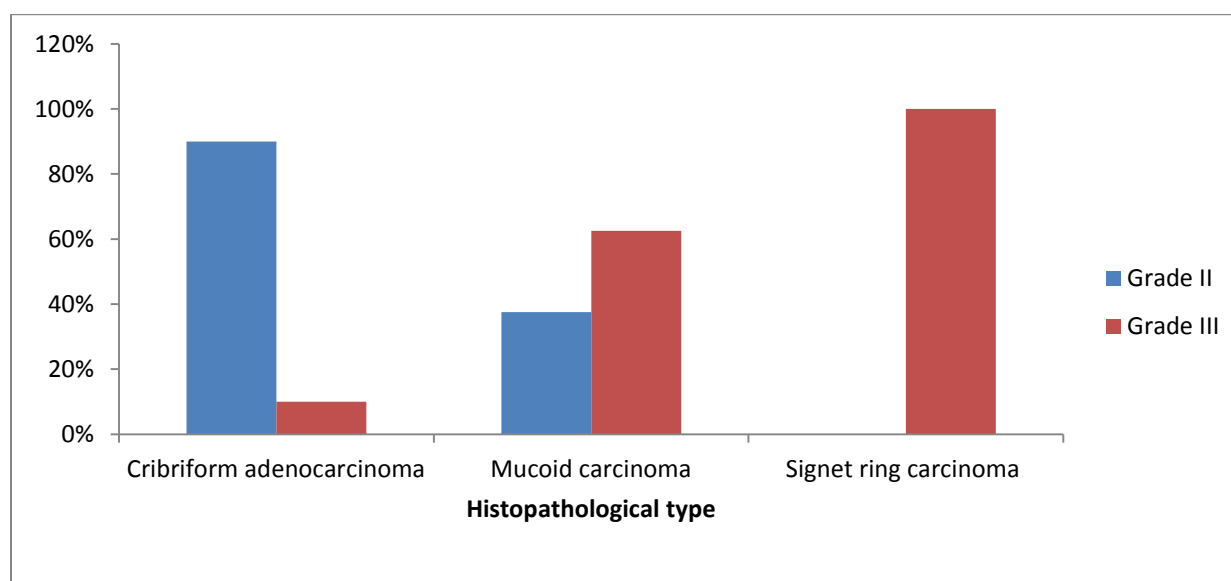
Relation between histopathological type & the grade of CRC

Out of 20 cases of cribriform adenocarcinoma , 18 cases (90%) were grade II ,2 cases (10%) were grade III.Out of 8 cases of mucoid carcinoma 3 cases (37.5%) were grade II and 5 cases(62.5%) were grade III .All cases of signet ring carcinoma (100%) were grade III.

There was a positive statistical correlation between histological type and the grade of CRC (p value <0.01).

Table (11): Relation between histopathological type & the grade of CRC.

Histopathological type	No of cases	Grade II	Grade III	P value
Cribriform adenocarcinoma	20	18(90%)	2(10%)	<0.01
Mucoid carcinoma	8	3(37.5%)	5(62.5%)	
Signet ring carcinoma	6	0	6(100%)	
Total	34	21	13	

**Graph (4): Relation between the histopathological type & the grade of CRC.**

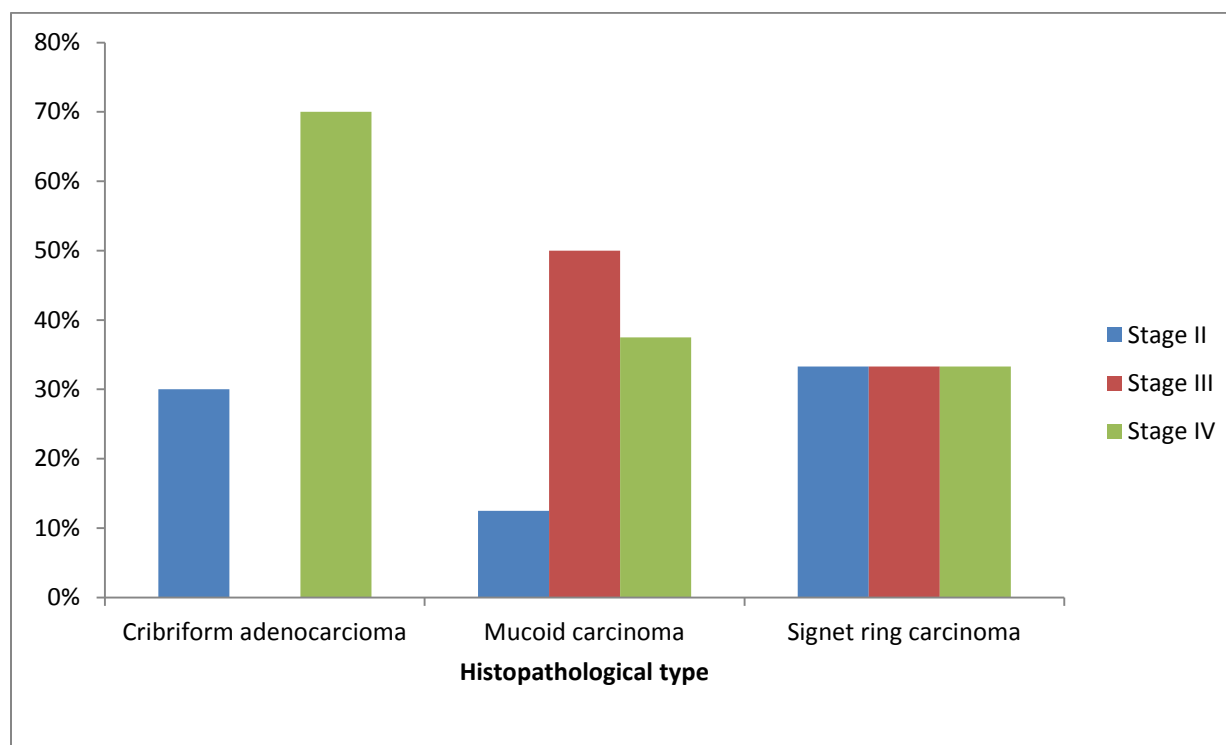
Relation between the histopathological tumor type & the stage of CRC.

Out of 20 cases of cribriform adenocarcinoma , 6 cases (30%) were stage II ,14 cases (70%) were stage IV. Out of 8 cases of mucoid carcinoma, 1 case(12.5%) was stage II ,4 cases (50%) were stage III and 3cases(37.5%) were stage IV. Out of 6 cases of signet ring carcinoma, 2 case(33.3%) was stage II ,2 cases(33.3%) were stage III and 2 cases(33.3%) were stage IV.

There was no significant correlation between the histopathological tumor type and the stage of CRC (p value >0.05).

Table (12): Relation between the histopathological tumor type & the stage of CRC.

Histopathological type	No of cases	Stage II	Stage III	Stage IV	P value
Cribriform adenocarcinoma	20	6(30%)	0	14(70%)	>0.05
Mucoid carcinoma	8	1(12.5%)	4(50%)	3(37.5%)	
Signet ring carcinoma	6	2(33.3%)	2(33.3%)	2(33.3%)	
Total	34	9	6	19	



Graph (5) Relation between the histopathological tumor type & the stage of CRC.

Relation between the grade & the stage of CRC

Out of 21 cases of grade II, 6 cases (28.6%) were stage II, 2 cases (9.5%) were stage III and 13 cases (61.9%) were stage IV. Out of 13 cases of grade III, 3 cases (23.1%) was stage II, 4 cases (30.8%) were stage III and 6 cases (46.2%) were stage IV.

There was no positive statistical correlation between the grade & the stage of CRC (p value > 0.05).

Table (13): Relation between the grade and the stage of CRC.

Tumor grade	No of cases	Stage II	Stage III	Stage IV	P value
Grade II	21	6(28.6%)	2(9.5%)	13(61.9%)	>0.05
Grade III	13	3(23 %)	4(30.8%)	6(46.2%)	
Total	34	9	6	19	

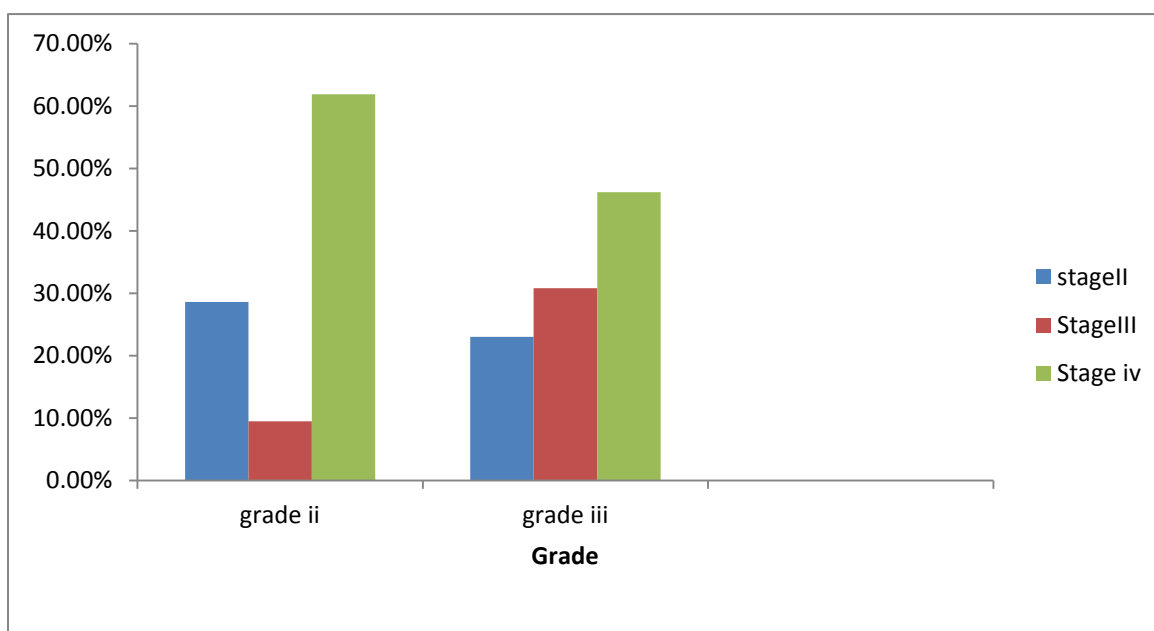
**Graph (6): Relation between the grade & the stage of CRC.**

Figure (11) :Normal colonic mucosa taken as control

Figure (12) :Adenoma of the colon

Figure (13): Grade II cribriform adenocarcinoma of colon composed of well formed glands in which nuclei are pleomorphic in size and shape and hyperchromatic (H&Ex100)

Figure (14): Grade II cribriform adenocarcinoma of colon composed of well formed glands in which nuclei are pleomorphic in size and shape and hyperchromatic (H&Ex200)

Figure (15:) Grade III cribriform adenocarcinoma of colon showing solid sheets of malignant cells with absent of glandular pattern(H&Ex200)

Figure (16:) Muroid carcinoma of the colon formed of irregular clusters of cells floating in pools of mucin (H&Ex200)

Figure (17): Muroid carcinoma of the colon formed of irregular clusters of cells floating in pools of mucin (H&Ex200)

Figure (18): Muroid carcinoma of the colon (H&Ex200)

Figure (19): Signet ring carcinoma of the colon, intracellular mucin accumulation displacing the nucleus giving signet ring appearance (H&Ex200)

Figure (20): Signet ring carcinoma of the colon intracellular mucin accumulation displacing the nucleus giving signet ring appearance (H&Ex400)

Immunohistochemical staining results:

Lgr5 was detected as cytoplasmic brown granules with different expression pattern in normal colonic mucosa, adenoma and carcinoma.

Lgr5 expression in control cases:

In normal control colonic crypts ,scattered ,rare single crypt epithelial cells were lgr5 immunoreactive which were deemed negative because of the paucity of immunopositive cells according to the formentioned scoring criteria figure (21)

Figure (21) : Normal colonic mucosa showing scattered Lgr5 expression (Streptavidin -biotin x400)

LGR5 expression in examined cases:

In colonic adenoma:

Among 6 cases of adenoma ,3 cases (50%) showed intensity of expression (1)(pale brown)and the other 3cases(50%) showed intensity of expression (2) (brown)

All cases of adenoma (100%) showed extent of staining (1))(<50%) .

All cases (100%) of adenoma showed score group (1) of lgr5 expression ,figure (22)

Figure (22) Adenoma showing intensity of Lgr5 expression (1) (Streptavidin - biotin x400)

In malignant cases:

Out of 34 cases of malignancy, 5 cases(14.7%) showed intensity (1) (pale brown), 4 cases were cribriform adenocarcinoma and 1 case was signet ring carcinoma. Nine cases (26.4%) showed intensity (2) (brown), 2 cases were cribriform adenocarcinoma, 4 cases were mucoid carcinoma and 3 cases were signet ring carcinoma. Twenty cases (58.8%) showed intensity (3) (dark brown), 14 cases were cribriform adenocarcinoma, 4 cases were mucoid carcinoma and 2 cases were signet ring carcinoma.

Out of 34 cases of malignancy, 8 cases (23.5%) showed extent of expression (1), 3 cases were cribriform adenocarcinoma, 3 cases were mucoid carcinoma and 2 cases were signet ring carcinoma. Twenty six cases (76.5%) showed extent (2), 17 cases were cribriform adenocarcinoma, 5 cases were mucoid carcinoma and 4 cases were signet ring carcinoma.

Out of 34 cases of malignancy, 8 cases (23.5%) showed score group (1), 4 cases were cribriform adenocarcinoma, 1 case was mucoid carcinoma and 3 cases were signet ring carcinoma. Eight cases (23.5%) showed score group (2), 2 cases were cribriform adenocarcinoma, 5 cases were mucoid carcinoma and 1 case was signet ring carcinoma. Eighteen cases (53%) showed score group (3), 14 cases were cribriform adenocarcinoma, 2 cases were mucoid carcinoma and 2 cases were signet ring carcinoma figures (23),(24),(25),(26), (27),(28),(29),(29),(30)& (31).

Figure (23) : Cribriform adenocarcinoma of colon showing intensity of Lgr5 expression (1) (streptavidin -biotin x400)

Figure (24) : Cribriform adenocarcinoma of colon showing intensity of Lgr5 expression (2) (streptavidin -biotin x400)

Figure (25): Cribriform adenocarcinoma of colon showing intensity of lgr5 expression (3) (streptavidin -biotin x400)

Figure (26) : Muroid carcinoma of colon showing intensity of Lgr5 expression (1) (streptavidin -biotin x400)

Figure (27) : Muroid carcinoma of colon showing intensity of Lgr5 expression (2)
(streptavidin -biotin x400)

Figure (28) : Muroid carcinoma of colon showing intensity of Lgr5 expression
(3) (streptavidin -biotin x400)

Figure (29) :Signet ring carcinoma of colon showing intensity of Lgr5 expression (1) (streptavidin -biotin x400)

Figure (30 : Signet ring carcinoma of colon showing intensity of Lgr5 expression (2) (streptavidin -biotin x400)

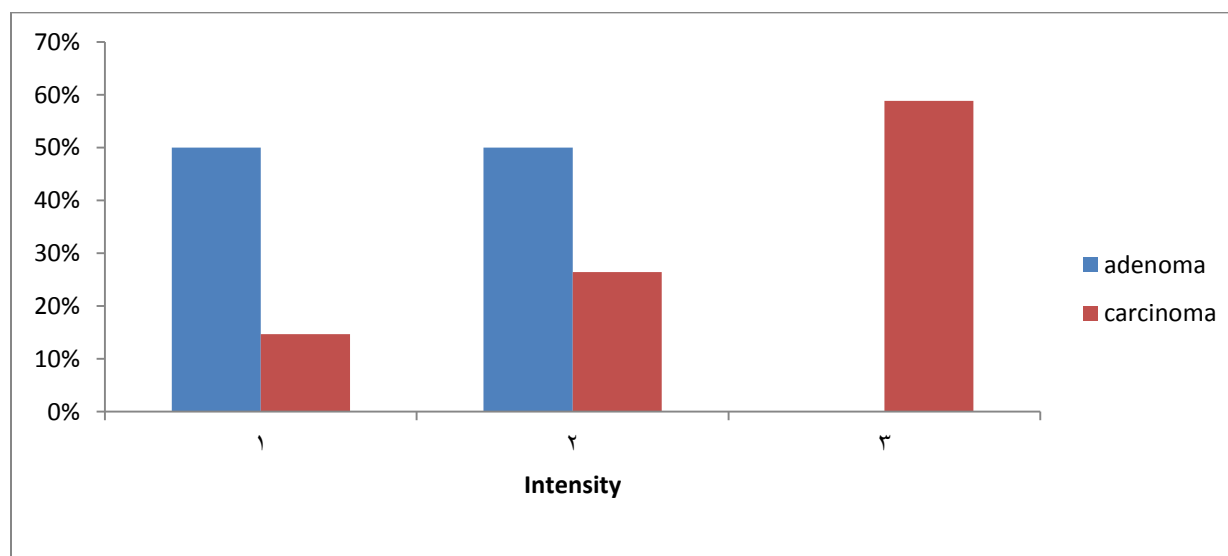
Figure (31): Signet carcinoma of colon showing intensity of Lgr5 expression (3)
(streptavidin -biotin x400)

Relation between intensity of lgr5 expression & the type of lesions in all examined cases.

There was a significant statistical correlation between intensity of lgr5 expression and the type of the lesion as the intensity increases with progression from adenoma to carcinoma (p value <0.05).

Table (15): Relation between intensity of lgr5 expression & the type of lesions in all examined cases.

Type of lesion	Intensity			P value
	1	2	3	
Adenoma	3(50%)	3(50%)	0	<0.05
Carcinoma	5(14.8%)	9(26.4%)	20(58.8%)	
Total	8	12	20	



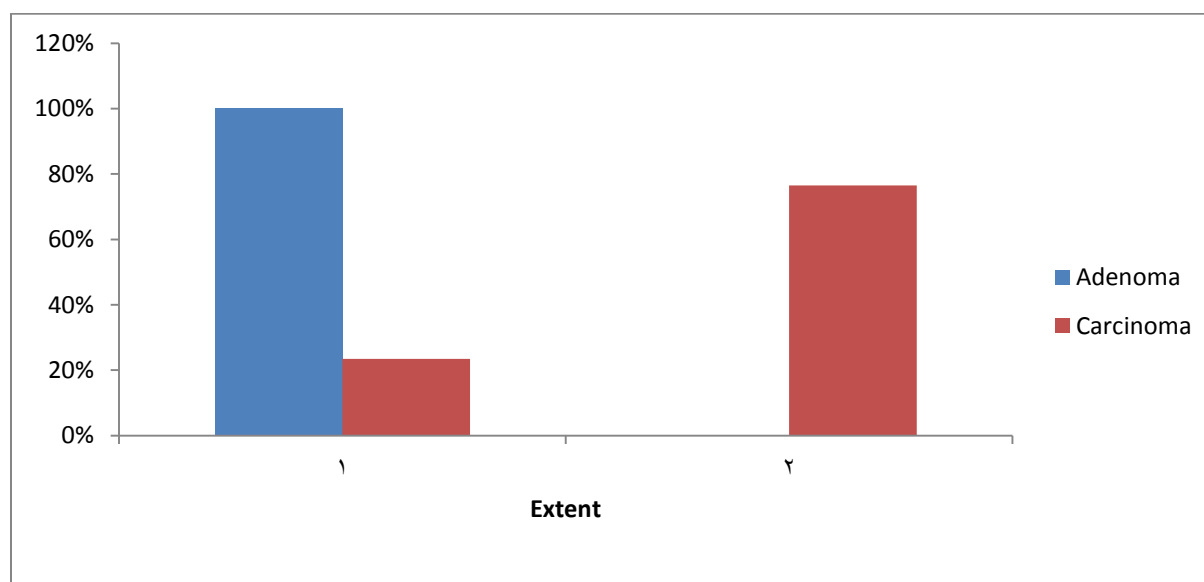
Graph (7): Relation between intensity of lgr5 expression & the type of lesions in all examined cases.

Relation between the extent of lgr5 expression & the type of lesion in all examined cases

There was a significant statistical correlation between the extent of lgr5 expression and the type of the lesion as the intensity increases with the lesion progression from adenoma to carcinoma (p value <0.01)

Table (16): Relation between the extent of lgr5 expression & the type of lesion in examined cases.

Type of lesion	No of cases	extent		P value
		1(<50%)	2(>50%)	
Adenoma	6	6(100%)	0	<0.01
Carcinoma	34	8(23.5%)	26(76.5%)	
total	40	14	26	



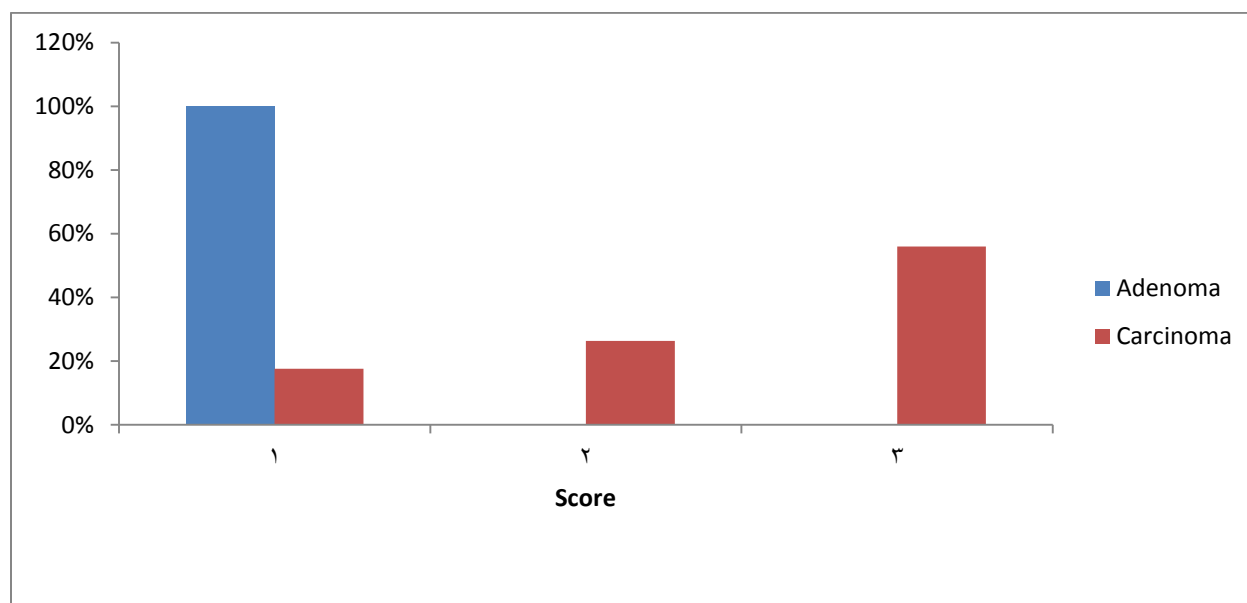
Graph (8): Relation between the extent of lgr5 expression and the type of lesion in examined cases.

Relation between the score of lgr5 expression & type of lesion in examined cases

There was a significant statistical correlation between score of lgr5 expression and the type of the lesion as the score of expression increases with progression from adenoma to carcinoma (p value <0.01).

Table (17): Relation between the score of lgr5 expression and type of lesion in examined cases

Type of lesion	No of cases	score			P value
		1	2	3	
Adenoma	6	6(100%)	0	0	<0.01
Carcinoma	34	8(23.5%)	8(23.5%)	18(53%)	
Total	40	14	8	18	



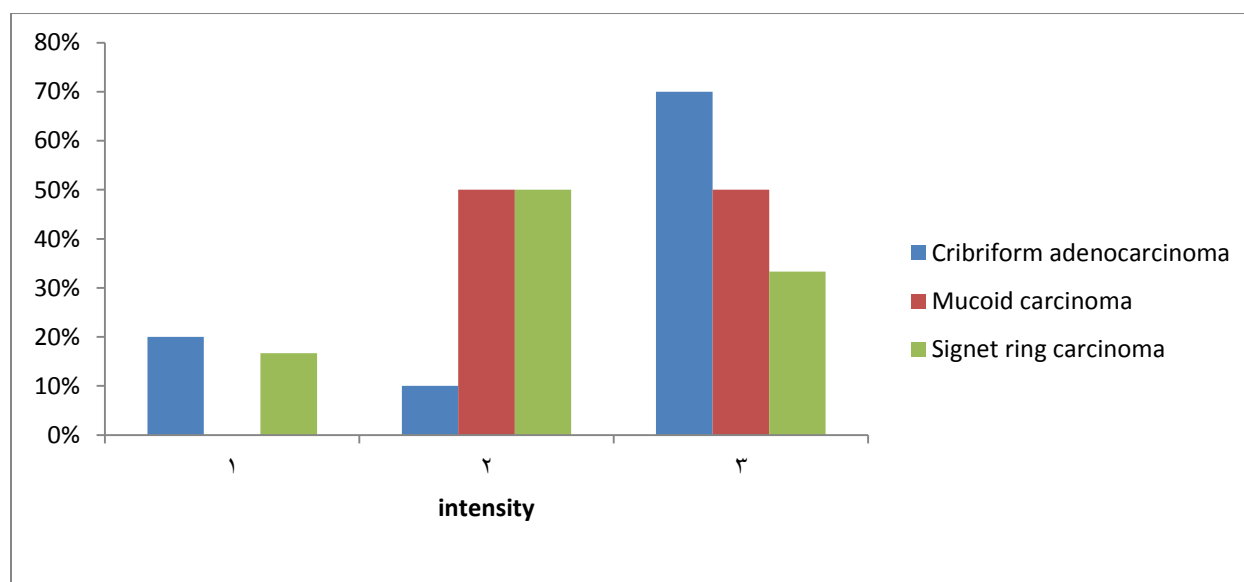
Graph (9): Relation between the score of lgr5 expression and type of lesion in examined cases.s

Relation between intensity of lgr5 expression & histopathological type of carcinoma.

There was no significant statistical correlation between lgr5 intensity and the histopathological type of carcinoma (p value>0.05).

Table (18): Relation between the intensity of lgr5 expression & the histopathological type of CRC.

Tumor type	No of cases	intensity			P value
		1	2	3	
Cribriform adenocarcinoma	20	4(20%)	2(10%)	14(70%)	>0.05
Mucoid carcinoma	8	0	4(50%)	4(50%)	
Signet ring carcinoma	6	1(16.7%)	3(50%)	2(33.3%)	
Total	34	5	9	20	



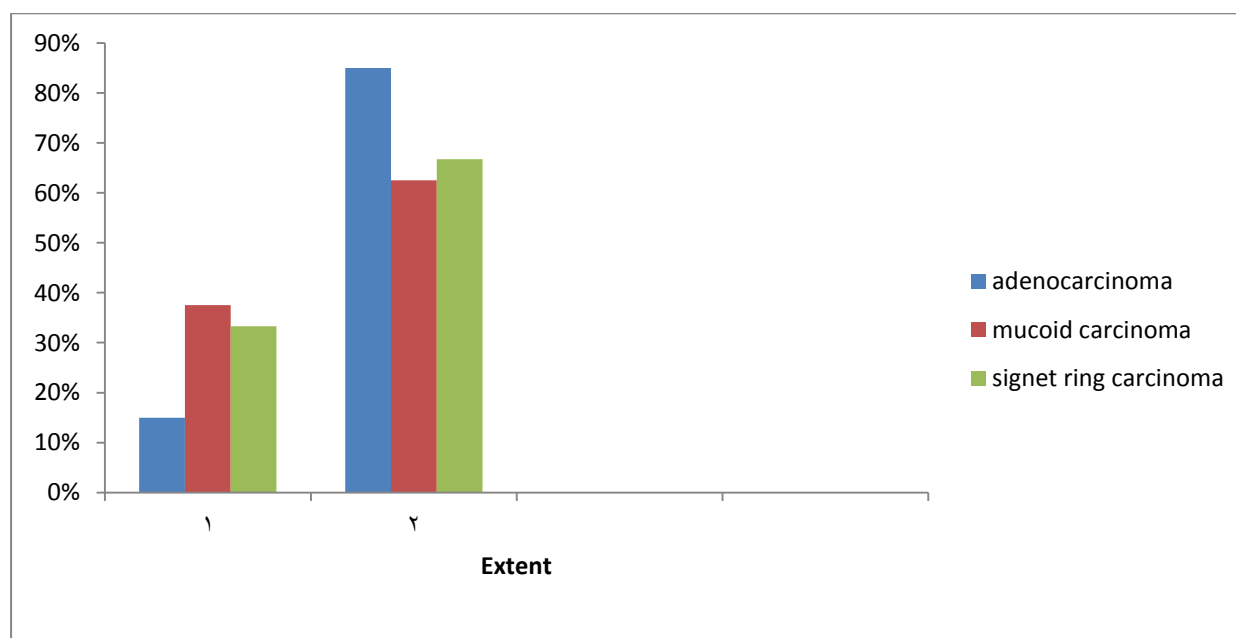
Graph (10) :Relation between the intensity of lgr5 expression & the histopathological type of CRC.

Relation between the extent of lgr5 expression & the histopathological type of carcinoma

There was no significant statistical correlation between lgr5 extent and the histopathological type of CRC (p value >0.05).

Table(19): Relation between the extent of lgr5 expression & the histopathological type of malignant tumors.

Tumor type	Extent			P value
	No of cases	1(<50%)	2(>50%)	
Cribriform adenocarcinoma	20	3(15%)	17(85%)	>0.05
Mucoid carcinoma	8	3(37.5%)	5(62.5%)	
Signet ring carcinoma	6	2(33.3%)	4(66.7%)	
total	34	8	26	



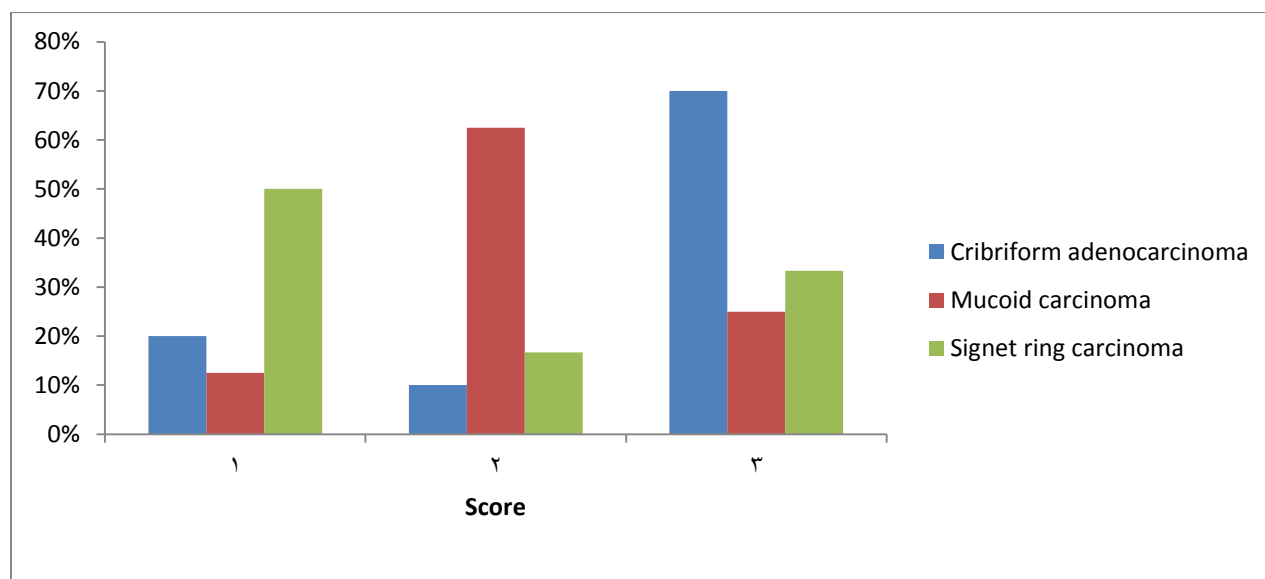
Graph (11): Relation between the extent of lgr5 expression & the histopathological type of carcinoma.

Relation between score of lgr5 expression & the histopathological type of carcinoma.

There was no significant statistical correlation between lgr5 score & the histopathological type of carcinoma (p value > 0.05).

Table (20): Relation between score of lgr5 expression & the histopathological type of carcinoma.

Tumor type	No of cases	Score			P value
		1	2	3	
Cribriform adenocarcinoma	20	4(20%)	2(10%)	14(70%)	> 0.05
Mucoid carcinoma	8	1(12.5%)	5(62.5%)	2(25%)	
Signet ring carcinoma	6	3 (50%)	1(16.7%)	2(33.3%)	
total	34	8	8	18	



Graph (12): Relation between score of lgr5 expression & the histopathological type of carcinoma.

Relation between intensity of lgr5 expression & the grade of CRC:

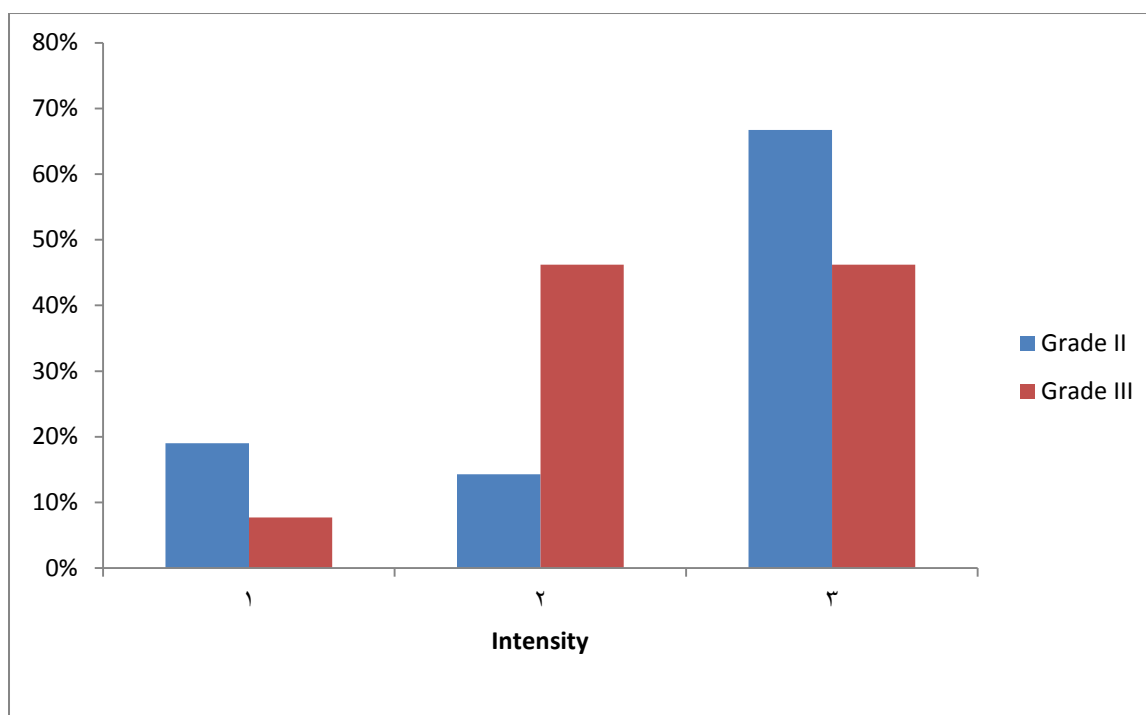
Out of 21 grade II carcinoma , 4 cases (19%) showed intensity of expression (1) , 3 cases (14.3%) showed intensity(2) and 14 cases(66.7%) showed intensity(3)

While in 13 cases of grade III ,1 case (7.6%) showed intensity of expression (1), 6 cases (46.2%) showed intensity(2) , 6 cases (46.2%) showed intensity(3) .

There is no statistical correlation between lgr5 intensity and the grade of carcinoma (p value >0.05).

Table (21): Relation between intensity of lgr5 expression & the grade of CRC.

Tumor grade	No.of cases	intensity			P value
		1	2	3	>0.05
Grade II	21	4(19%)	3(14.3%)	14(66.7%)	
Grade III	13	1(7.6%)	6(46.2%)	6(46.2%)	
Total	34	5	9	20	



Graph (13) :Relation between intensity of lgr5 expression & the grade of CRC.

Relation between the extent of lgr5 expression & the grade of CRC.

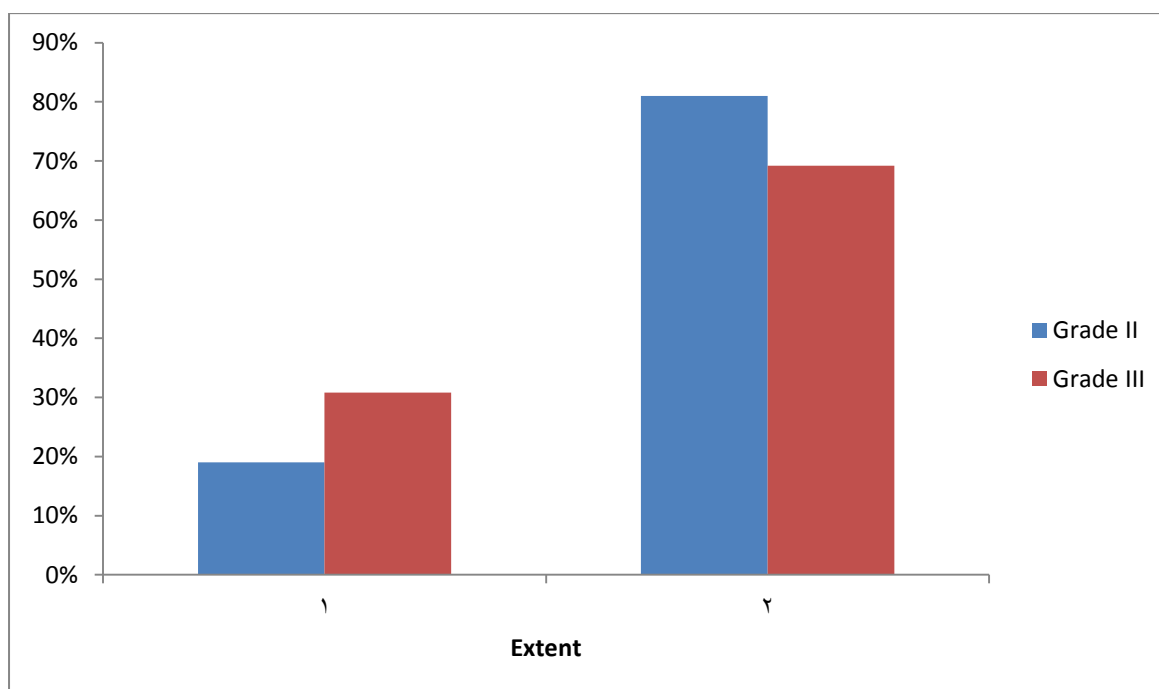
Out of 21 grade II carcinoma ,4 cases (19%) showed extent of expression (1),17 cases (81%) showed extent (2)

While in 13 cases of grade III, 4 cases (30.8%) showed extent of expression (1), 9 cases (69.2%) showed extent (2).

There was no statistical correlation between extent of lgr5 expression & the grade of carcinoma (p value >0.05).

Table (22): Relation between the extent of lgr5 expression & the grade of CRC.

Tumor type	No of cases	Extent		P value
		1(<50%)	2(>50%)	
Grade II	21	4(19%)	17(81%)	>0.05
Grade III	13	4(30.8%)	9(69.2%)	
Total	34	8	26	



Graph(14): Relation between the extent of lgr5 expression & the grade of CRC.

Relation between the score of lgr5 expression & the grade of CRC:

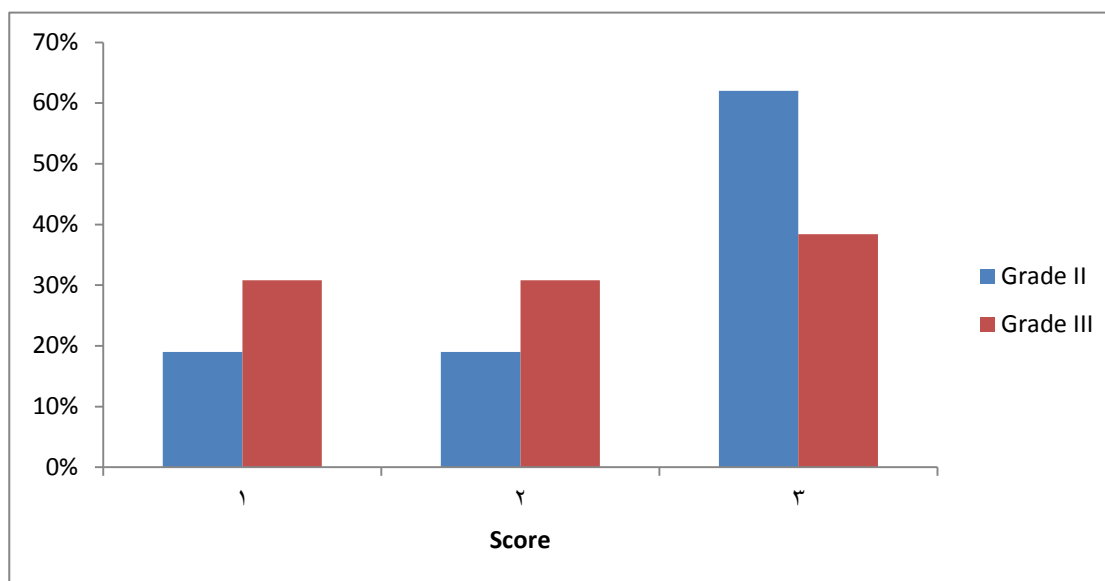
Out of 21 cases of grade II, 4 cases (19%) showed score group (1) ,4 cases(19%) showed score group (2) and 13 cases(62%) showed score group (3).

Out of 13 cases of grade III, 4 cases(30.8%) showed score group (1),4 cases(30.8%) showed score group (2) and 5 cases(38.5%) showed score group (3) .

There is no statistical correlation between score of lgr5 expression and the grade of CRC (pvalue >0.05)

Table (23): Relation between score of lgr5 expression &the grade of CRC.

Tumor grade	No.of cases	Score			P value
		1	2	3	
Grade II	21	4(19%)	4(19%)	13(62%)	>0.05
Grade III	13	4(30.8%)	4(30.8%)	5(38.4%)	
Total	34	8	8	18	



Graph (15): Relation between score of lgr5 expression & the grade of CRC.

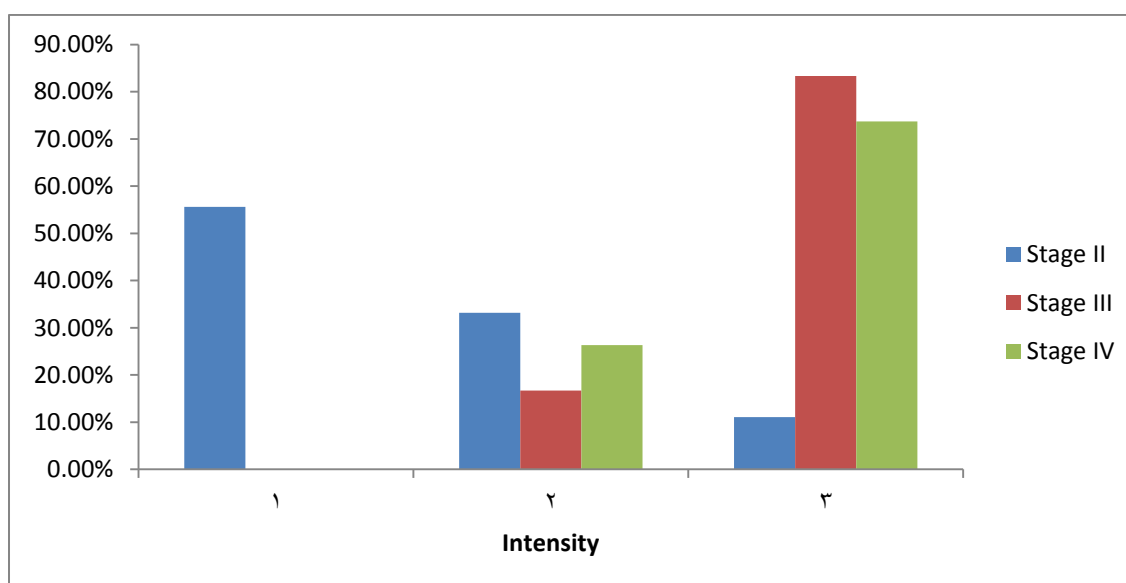
Relation between intensity of lgr5 expression and the stage of CRC

In stage II, 5 cases (55.6%) showed intensity of lgr5 (1), 3 cases (33.3%) showed intensity of lgr5(2) and 1 case (11.1%) showed intensity of lgr5 (3), in stage III, 1 case (16.7%) showed intensity of lgr5 (2), 5 cases (83.3%) showed intensity of lgr5 (3) and in stage IV, 5 cases (26.3%) cases showed intensity of lgr5 (2) and 14 cases (73.7%) showed intensity of lgr5 (3).

A significant statistical correlation was found between intensity of lgr5 expression and the stage of CRC including depth of tumor invasion, lymph node metastasis and distant metastasis (p value <0.01).

Table(24):Relation between intensity of lgr5 expression and the stage of CRC

Stage	No of cases	Intensity			P value
		1	2	3	
II	9	5(55.6%)	3(33.3%)	1(11.1%)	<0.01
III	6	0	1(16.7%)	5(83.3%)	
IV	19	0	5(26.3%)	14(73.7%)	
total	34	5	9	20	



Graph (16) : Relation between intensity of lgr5 expression and the stage of CRC.

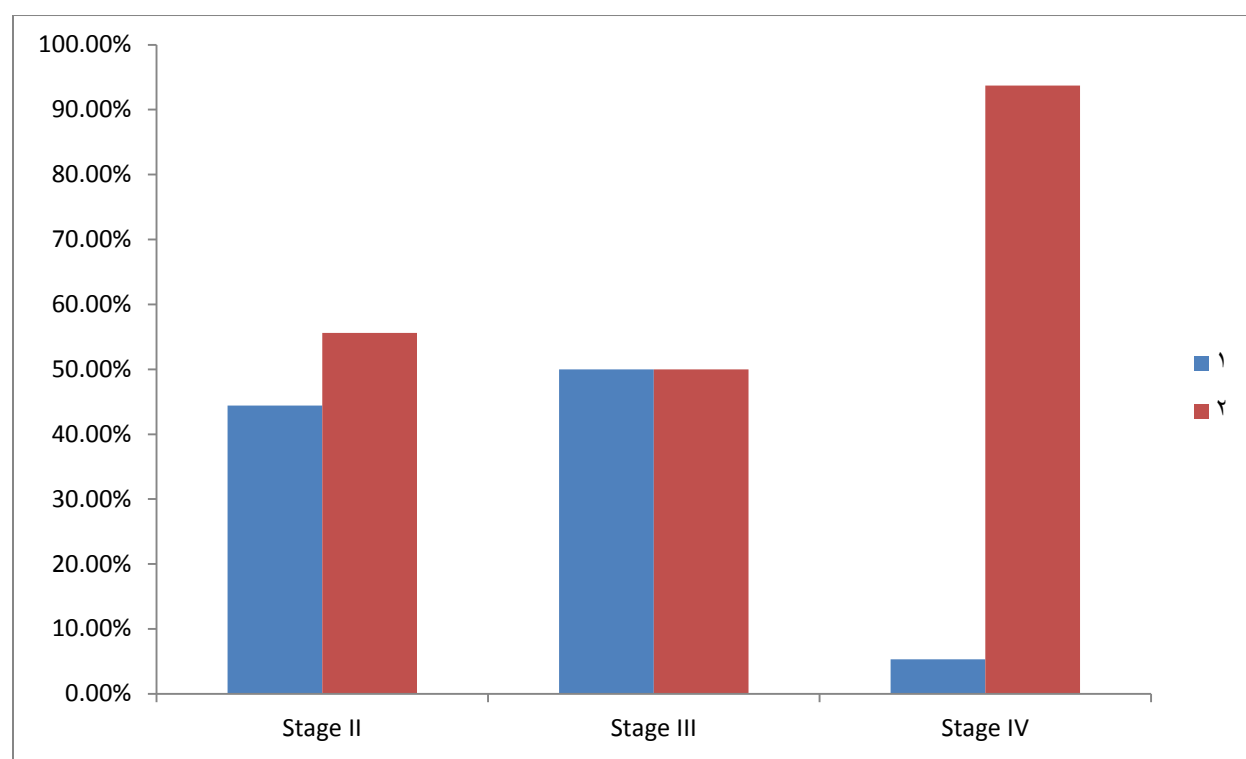
Relation between extent of lgr5 expression and the stage of CRC

In stage II, 4 cases (44.4%) showed extent of lgr5 (1) , 5 cases (55.6%) showed extent of lgr5(2) ,in stage III 3 cases (50%) showed extent of lgr5 (1) ,3 cases (50%) showed extent of lgr5 (2) and in stage IV, 1 cases (5.3%) cases showed extent of lgr5 (1) and 18 cases (94.7%) showed extent of lgr5 (2)

A significant correlation was found between extent of lgr5 expression and the stage of CRC including depth of tumor invasion ,lymph node metastasis and distant metastasis (p value <0.05) .

Table (25): Relation between extent of lgr5 expression and the stage of CRC

Stage	No of cases	Extent		P value
		1	2	
II	9	4(44.4%)	5(55.6%)	<0.05
III	6	3(50%)	3(50%)	
IV	19	1(5.3%)	18(94.7%)	
total	34	8	26	



Graph (17): Relation between extent of lgr5 expression and the stage of CRC.

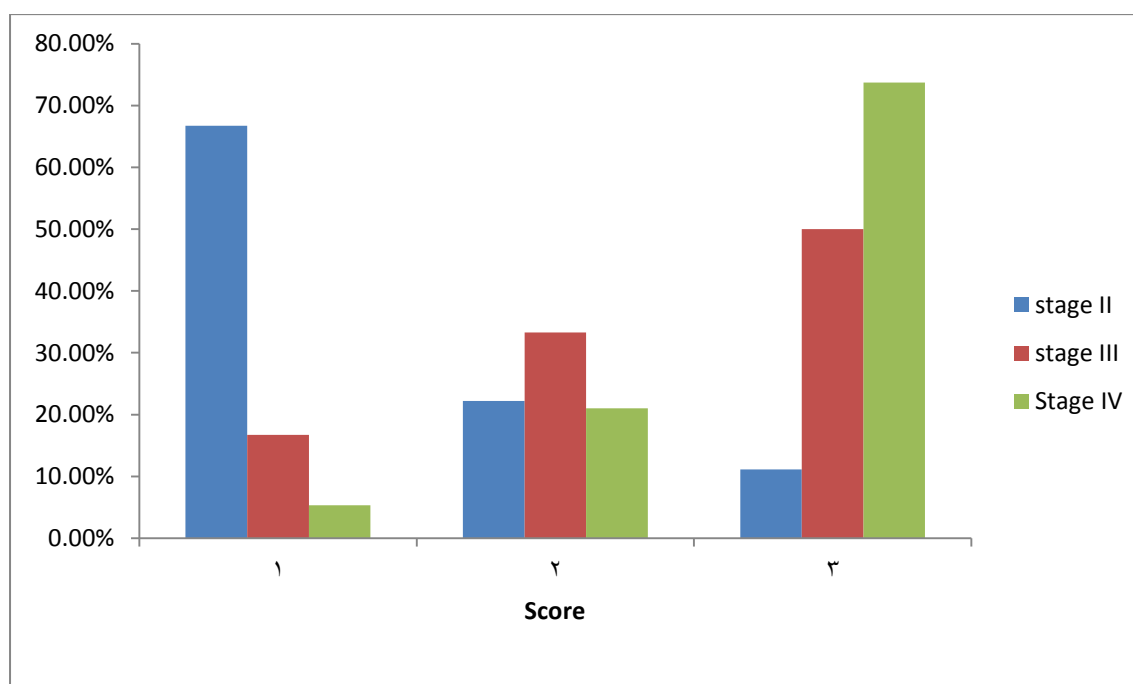
Relation between score lgr5 expression and the stage of CRC

In stage II, 6 cases (66.7%) showed score of lgr5 (1) , 2 cases (22.2%) showed score of lgr5(2) and 1 case (11.1%) showed score of lgr5 (3) ,in stage III, 1 case (16.7%) showed score of lgr5 (1), 2cases(33.3%) showed score of lgr5 (2) and 3 cases (50%) showed score of lgr5 (3). In stage IV, 1 case (5.3%) showed score of lgr5 (1) , 4 cases(21%) showed score of lgr5 (2) and 14 cases (73.7%) showed score of lgr5 (3)

A significant statistical correlation was found between score of lgr5 expression and the stage of CRC including depth of tumor invasion ,lymph node metastasis and distant metastasis (p value <0.05).

Table(26): Relation between score lgr5 expression and the stage of CRC

Stage	No of cases	score			P value
		1	2	3	
II	9	6(66.7%)	2(22.2%)	1(11.1%)	<0.05
III	6	1(16.7%)	2(33.3%)	3(50%)	
IV	19	1(5.3%)	4(21%)	14(73.7%)	
total	34	8	8	18	

**Graph(18):Relation between score lgr5 expression and the stage of CRC**