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RESEARCH ARTICLE

CLINICOPATHOLOGICAL SIGNIFICANCE OF FASCIN1 AND B-CATENIN EXPRESSION IN COLORECTAL ADENOCARCINOMA; AN IMMUNOHISTOCHEMICAL STUDY"

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Key words:-
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Abstract

Background: Colorectal cancer (CRC) is the fourth most common human malignancies worldwide. Fascin1 is an actin bundling protein along the entire length of filopodia and its diminution leads to reduction in the number of filopodia. Beta-catenin (β-catenin) is one of the catenin protein family, involved in the regulation of adhesion between the cells and gene transcription. It is one of the cadherin protein complexes acting as intracellular signal transducer in the Wnt signaling pathway**.**

Objective: we aimed in this study to investigate the immunohistochemical expression of FascinI and **β**- catenine incancer colon and correlate their expression with other clinicopathologic features.

Material and methods: 45cases of colorectal adenocarcinoma and 30 cases of adenomatous polyp were collected from Pathology Department. Faculty of Medicine. Zagazig University between January 2013 to December 2016, using immunohistochemical antibodies to Fascin 1 and β- catenine.

Results: positive Fascin1 expression was observed in 31.1%) and 40% of CRC and colorectal adenomatous polyp respectively. Positive **β**-catenineimmunohistochemicalstaining (IHC) where 35.6% and 40% CRC and colorectal adenomatous polyp.

The expression of cytoplasmic **β** - catenin protein in CRCwas significantly (p=0.0001) higher than in the adenomatous polyp.

Both markers are significantly correlated with tumor grade (p<<0.001). No significant association between (IHC) staining was found between both markers expression and N stage of tumor. Expressions of both of them were significantly positively correlated with each other (p<0.001).

Conclusion: Fascin 1 has known roles in cell morphology and migration and may represent a potential novel marker or therapeutic target for patients with colorectal cancer.Fascin1is regulated by**β** - catenine. IHC staining of β-catenin is considered a useful marker to predict the prognosis in colorectal cancer (CRC). Keywords: Fascin1; Bcatenin; colorectal cancer; immunohistochemistry; prognosis

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Introduction:-

Colorectal cancer (CRC) is the fourth most common human malignancies worldwide**[1].**CRC remains the second main cause of cancer related mortality. The 5-year survival rate for CRC exceeds 50% depends on the stage of the disease**[2].** Recently molecular markers are considered essential factors that affect CRC patient prognosis.Fascin1 is an actin bundling protein along the entire length of filopodia and its diminution leads to reduction in the number of filopodia**[7].** Fascin1 promotes cell migration, neuronal tissue normally express the Fascin1 and is not expressed in normal epithelial cell. Invasion of cancer cells occurs through presence of sensory organelles called filopodia hustling of actin filaments. Many researches noticed that Fascin 1 significantly accelerate cell migration in transfilter assays **[8, 9].** **F**ascin1 expression was recognized in many types of malignant cells, including colonic carcinoma **[8, 10, 11].**

Beta-catenin (β-catenin) is one of the catenin protein families, involved in the regulation of adhesion between the cells and gene transcription. It is one of the cadherin protein complex acting as intracellular signal transducer in the Wnt signaling pathway **[3].**Different type of malignancy can affect by the degree of β-catenin expression and the rearrangement of its genes [**4**]**.**B-catenin expression in CRC outcome is tarnished. The prognostic importance of different forms of β-catenin expression either cytoplasmic, or nuclear accumulation of it may be an autonomous marker of poor prediction **[5,6].**

So we aimed in this study to investigate the expression of Fascin 1 and β- catenine in colorectal carcinoma and their correlation with other clinicopathological features.

Material and Methods:-

This is a prospective cohort study, where 75 cases of colorectal masses were admitted to general surgery hospital oncology unit, faculty of medicine Zagazig University, in the period between January 2013 to December 2016. 45cases of them that were diagnosed as colorectal adenocarcinoma and were managed by right hemicolectomy, left hemicolectomy, transverse colectomy sigmoidectomy and proctocolectomy with radical excision of the mesocolon and mesorectum, 30 cases of them were diagnosed as adenomatous polyp and excisional biopsy was done to all cases. Samples of all cases were sent to Pathology Department. Faculty of Medicine Zagazig University where they were processed and subjected to routine hematoxylin and eosin staining, diagnosed and carcinoma cases were graded and staged.

In patients submitted to surgery for CRC, tissue samples were obtained from the tumor and from adjacent non-neoplastic colorectal mucosa.

Immunohistochemical staining:-

We used streptavidine-biotin technique **[12].**Paraffin-embedded blocks have been cut in to Four-micron thick sections, deparaffinisation was done in series of xylene and rehydratation was done in descending grades of alcohol, for blooking endogenous peroxidase activity the section were placed in 0.5% hydrogen peroxide in methanol for 10 min microwave antigen retrieval.

Primary mouse monoclonal antibody directed against Fascin1 (DakoCytomation, Carpenteria, CA, USA used at a 1:50dilution) and rabbit polyclonal primary anti-β- -catenin antibody (Santa Cruz Biotechnology, Inc.,Santa Cruz, CA, USA used at dilution1:100) and by employing diaminobenzidine (DAB) as the chromogen. Were added for 30 minutes at room temperature. Secondary antibody was addedto sections for 30 minutes. After incubation, the reaction product was seenby diaminobenzidine. At the end, the sections were counterstained with Mayer's hematoxylin.Negative controls had primary antibody replaced by buffer.

Immunohistochemical evaluation of both markers:-

For Fascin1 expression

Fascin 1-positive cells were graded as follows: **<** 5percentage, 0; 5-25%, 1 **+** ; 25-75%, 2 **+** ; and **>** 75%, 3 **+** . Staining of **>** 5% of cancer cells was recorded as positive immunoreactivity*) **[13].***

Forβ- **catenine expression**

β- catenine positive expression it was graded using a range of 0–3 as follows: 0, No staining; 1, weak staining; 2, moderate staining; and 3, strong staining. **[14].**Membrane staining, four categories were used (+++, ++, +, **-[15].**The

cytoplasmic staining was also graded into 4categories: (0) Negative, no detectable staining, (1) Weak, (2) Moderae, (3) intense staining, intense. The nuclear staining index (NI) was also graded to into four categories (+++, ++, +, -): all forms of expression ranged from (0) Negative (1) Weak (2) Moderate (3) Heavy staining. The extent of staining was graded on a scale as follows: 0, ≤5%; 1, 6–25%; 2, 26–50%; 3, 51–75%; or 4, 76–100% according to the percentage of the section that has positive staining. The intensity and extent scores were multiplied to generate the immunoreactivity score (IS; range, 0–12) for each case**[16].** High cytoplasmic and nuclear β-catenin expression grades were defined as >50% reactivity of the tumor cells **[17].**

Statistical Analysis:-
Continuous variables were expressed as the mean ± SD & median (range), and the categorical variables were expressed as a number(percentage). Continuous variables were checked for normality by using Shapiro-Wilk test. Independent samples Student's t-test was used to compare between two groups of normally distributed variables while Mann Whitney U test was used for non-normally distributed variables. Percent of categorical variables were compared using the Pearson's Chi-square test or Fisher's exact test when was appropriate. Trend of change in distribution of relative frequencies between ordinal data were compared using Chi-square test for trend. Correlation between immunohistochemical markers was analyzed usingSperman correlation. A p-value <0.05 was considered significant. All statistics were performed using SPSS.

Results:-
Clinico-pathological results:-
For patients operated with CRC, 25 were females and 20 were males. The median age was 55(43-80) years and 60%of the CRC was in the colon in while 40% of cases wasin the rectum.

Regarding adenomatous polyp, 15 patients were male and 15 were female. The median age was 60.7±3.4 years (29 to 88 years). 46 .7% of polyp present in the left colon and 43.3% in the right colon. table 1

Immunohistochemical results (table 2,3 and 4):-
Fascin1 was stained in the cytoplasm of cancer cells (Figure 1,2,3,4and 5). B- catenine expression may be membrance, cytoplasmic or nuclear. Figure 6,7,8,9,10,11and12).

In this study,Fascin1 was negative in normal mucosa but only detected in infiltrating stromal cells, in the extracellular matrix, fibroblasts and blood vessel. β-catenin staining was commonlyseen at cell-cell junction sitesin normal colon epithelium, whereas. In the more differentiated adenocarcinoma, β-catenin staining was observedin the membrane and in the cytoplasm with weak or no nuclear staining. On the contrary, the invasive front of tumors exhibiting cytoplasmic and nuclear β-catenin localization. Fascin1 expression was high in sheets of invading tumor cells.

- Positive Fascin1 expression was observed in 31.1%and 40% of colorectal cancer and colorectal adenomatous polyp respectively. Positiveβ- catenineIHC staining where 35.6% and 40%colorectal cancer and colorectal adenomatous polyp.
- Positive Fascin 1 expression is significantly correlated with L.N state (p=0.0497)
- Both markers are significantly correlated with tumor grade (p<0.001).
- There is statistically significant difference between cytoplasmic beta-catenin expression andadenomatous polyp (p=0.0001).
- No significant association between IHC staining of both markers andstage of tumor.
- Expressions of both markers were significantly positively correlated with each other (p=0.0001)

Table (1):- Comparison between colorectal cancer group and polyp group.

	Caner group (N=45)			Adenomatous Polyp group (N=30)		p-value
	No.	(%)		No.	(%)	
Age (years)						
Mean ± SD	57.33	±8.78		58.78	±4.32	0.312•
Median (Range)	55	(43-80)		60	(47-66)	

36-45 years	3	(6.7%)		0	(0%)	0.2931
46-60 years	28	(62.2%)			22(73.3%)	
> 60 years	14	(31.1%)		8	(26.6%)	
Sex						
Male	20	(44.4%)		15	(50%)	0.6366
Female	25	(55.6%)		15	(50%)	
Location						
Rt colon	14	(31.1%)		13	(43.3%)	0.0018
Transverse colon	5	(11.1%)		0	(0%)	
Lt colon	8	(17.8%)		14	(46.7%)	
Rectum	18	(40%)		3	(10%)	
Number						
Single	39	(86.7%)		27	(90%)	0.6634
Multiple	6	(13.3%)		3	(10%)	
Size (cm)						
Mean ± SD	74.17	±18.52		8.78	±6.30	<0.001•
Median (Range)	75	(30-120)		7	(4-40)	
<10 mm	0	(0%)		22	(73.3%)	<0.001
10-50 mm	6	(13.3%)		8	(26.7%)	
>50 mm	39	(86.7%)		0	(0%)	
Histopathological type						
Tubular adenoma	0	(0%)		10	(33.3%)	<0.001
Tubullovillous adenoma	0	(0%)		18	(60%)	
Villous adenoma	0	(0%)		2	(6.66%)	
Adenocarcinoma	45	(100%)		0	(0%)	
Grade						
Low grade dysplasia	0	(0%)		26	(86.7%)	<0.001
High grade dysplasia	0	(0%)		4	(13.3%)	
Grade 1	31	(68.9%)		0	(0%)	
Grade 2	9	(20%)		0	(0%)	
Grade 3	5	(11.1%)		0	(0%)	
Cytoplasmic Fascin1						
Negative	31	(68.8%)		18	(60%)	0.4281
Positive	14	(31.1%)		12	(40%)	
β- catenin						
Negative	29	(64.4%)		18	(60%)	0.6967
Positive	16	(35.6%)		12	(40%)	
Nuclear β- catenin						
Positive	22	(48.9%)		11	(36.66%)	
Negative	23	(51.1%)		19	(63.33%)	0.2962
Cytoplasmic β- catenin						
Positive	12	(26.7%)		22	(73.3%)	0.0001
Negative	33	(73.3%)		8	(26.7%)	
Membranous β- catenin						
Positive	12	(26.7%)		11	(36.7%)	0.3575
Negative	33	(73.3%)		19	(63.3.%)	

Categorical variables were expressed as number(percentage).

Continuous variables were expressed as mean ± SD & median (range).

• Mann Whitney U test;§Chi-square test.

$p<0.05$ is significant.

Table (2):- Relation between clinicopathological featuresand immunohistochemicalmarkers in 45 patients with colorectal carcinoma.

Characteristics	All		Cytoplasmic Fascin 1				p-value	β- catenin				p-value
			Negative (N=31)		Positive (N=14)			Negative (N=29)		Positive (N=16)		
	(N=45)											
	No.	(%)	No.	(%)	No.	(%)		No.	(%)	No.	(%)	
Age (years)												
Mean ± SD	57.33	±8.78	55.81	±7.71	58.78	±9.63	0.262*	54.36	±5.44	58.29	±9.48	0.099*
Median (Range)	55	(43-80)	54.50	(44-76)	60	(43-80)		53	(45-62)	55.50	(43-80)	
36-45 years	3	(6.7%)	1	(3.22%)	2	(14.2%)	<0.001	2	(6.89%)	1	(6.25%)	.9965
46-60 years	28	(62.2%)	26	(83.8%)	2	(14.2%)		18	(62.1%)	10	(62.5%)	
> 60 years	14	(31.1%)	4	(21.9%)	10	(42.9%)		9	(31.1%)	5	(31.3%)	
Sex												
Male	20	(44.4%)	12	(38.7%)	8	(57.2%)	0.2493	10	(34.4%)	10	(62.5%)	0.0702
Female	25	(55.6%)	19	(61.3%)	6	(42.8%)		19	(65.5%)	6	(37.5%)	
Location												
Rt colon	14	(31.1%)	10	(32.2%)	4	(28.5%)	0.1082	8	(27.5%)	6	(37.5%)	0.0588
Transverse colon	5	(11.1%)	5	(16.1.%)	0	(0%)		1	(3.22%)	4	(25%)	
Lt colon	8	(17.8%)	3	(9.67%)	5	(35.7%)		5	(17.2%)	3	(18.75%)	
Rectum	18	(40%)	13	(41.9%)	5	(35.7%)		15	(51.7%)	3	(18.75%)	
Number												
Single	39	(86.7%)	29	(93.5%)	10	(42.9%)	0.0433	28	(96.6%)	11	(68.7%)	0.0086
Multiple	6	(13.3%)	2	(6.45%)	4	(28.5%)		1	(3.22%)	5	(31.3%)	
Gross pattern												
Ulcerative	23	(50%)	14	(45.1%)	9	(64.3%)	0.3310	17	(58.6%)	6	(37.5 %)	0.0209
Fungating	9	(20.5%)	6	(19.3%)	3	(21.4%)			7(24.1%)	2	(12.5%)	
Annular	13	(29.5%)	11	(35.4%)	2	(14.2%)		4	(13.7%)	9	(56.25%)	
Size (cm)												
Mean ± SD	74.17	±18.52	74.90	±19.15	73.47	±18.30	0.799*	75	±22.47	73.91	±17.44	0.868*
Median (Range)	75	(30-120)	75	(40-120)	75	(30-100)		70	(40-120)	77.50	(30-100)	
10-50 mm	7	(13.3%)	5	(16.1%)	2	(14.2%)	0.8745	3	(10.3%)	4	(25%)	0.1941
>50 mm	38	(86.7%)	26	(83.8%)	12	(85.7%)		26	(89.6%)	12	(75%)	
Grade												
Grade 1	27	(68.9%	25	(80.6%)	2	(14.3%	0.0001	26	(89.6%)	1	(6.25%)	<0.00

		)						)										1
Grade 2	9	(20%)		4	(12.8%)		5	(35.7%)				1	(3.22%)		8	(50%)		
Grade 3	9	(11.1%)		2	(6.45%)		7	(50%)				2	(6.89%)		7	(43.75%)		
T																		
T1	2	(4.4%)		1	(3.22%)		1	(7.14%)		0.3095		1	(3.22%)		1	(6.25%)		0.4466
T2	3	(6.7%)		1	(3.22%)		2	(14.2%)				1	(3.22%)		2	(12.5%)		
T3	40	(88.9%)		29	(93.5%)		11	(78.5%)				27	(93.1%)		13	(81.25%)		
N																		
N0	11	(24.4%)		10	(91%)		1	(10%)		0.0497		9	(81.8%)		2	(18.2%)		0.2866
N1	34	(75.6%)		20	(58.8%)		14	(41.2%)				22	(64.7%)		12	35.3%)		
Stage																		
Stage I	2	(4.4%)		1	(3.22%)		1	(7.14%)		0.3095		1	(3.22%)		1	(6.25%)		0.9076
Stage II	3	(6.7%)		1	(3.22%)		2	(14.2%)				2	(6.89%)		1	(6.25)		
Stage III	40	(88.9%)		29	(93.5%)		11	(78.5%)				26	(89.6%)		14	(87.5%)		
Cytoplasmic Fascin1																		
Negative	31	(68.8%)										26	(89.6%)		5	(31%)		0.0001
Positive	14	(31.1%)										3	(10.3%)		11	(68.1%)		
β- catenin																		
Negative	29	(64.4%)		26	(89.6%)		3	(10.3%)		0.0001								
Positive	16	(35.6%		5	(31.3%		11	(68.1%)										

- Categorical variables were expressed as number(percentage), continuous variables were expressed as mean ± SD & median (range).
- * Independent samples Student's t-test;§Chi-square test; ‡ Chi-square test for trend; $p<0.05$ is significant.

Table (3):- Relation between clinicopathologicalfeaturesandimmunohistochemical markers in 45 patients with colorectal carcinoma.

Characteristics	All (N=45)			Nuclear β catenin				p-value		Cytoplasmic β-catenin				p-value		Membranous β catenin				p-value
				Negative (N=23)			Positive (N=22)			positive (N=12)			negative (N=33)			positive (N=12)			negative (N=33)		
	No.	(%)		No.	(%)		No.	(%)			No.	(%)		No.	(%)		No.	(%)		No.	(%)	
Age (years)																						
Mean ± SD	57.33	±8.78		57.34	±9.37		57.31	±8.32	0.991*		53.58	±6.61		58.69	±9.15	0.084*		53.58	±6.61		58.69	±9.15	
Median	55	(43-		55	(44-		8.3	(43-			52.	(44-		56	(43-			52.	(44-		56	(43-	

(Range)		80)			80)		2	75)			50	65)			80)			50	65)			80)	
36-45 years	3	(6.7 %)		2	(66.7 %)		1	(33.3 %)	0.856 §		2	(66.7 %)		1	(33.3 %)	0.165 §		2	(66.7 %)		1	(33.3 %)	0.165 §
46-60 years	28	(62.2 %)		14	(50%)		14	(50%)			8	(28.6 %)		20	(71.4 %)			8	(28.6 %)		20	(71.4 %)	
> 60 years	14	(31.1 %)		7	(50%)		7	(50%)			2	(14.3 %)		12	(85.7 %)			2	(14.3 %)		12	(85.7 %)	
Sex																							
Male	20	(44.4 %)		10	(50%)		10	(50%)	0.894 §		5	(25%)		15	(75%)	0.821 §		5	(25%)		15	(75%)	0.821 §
Female	25	(55.6 %)		13	(52%)		12	(48%)			7	(28%)		18	(72%)			7	(28%)		18	(72%)	
Location																							
Rt colon	14	(31.1 %)		7	(50%)		7	(50%)	0.667 §		3	(21.4 %)		11	(78.6 %)	0.177 §		3	(21.4 %)		11	(78.6 %)	0.177 §
Transverse colon	5	(11.1 %)		2	(40%)		3	(60%)			2	(40%)		3	(60%)			2	(40%)		3	(60%)	
Lt colon	8	(17.8 %)		3	(37.5 %)		5	(62.5 %)			0	(0%)		8	(100 %)			0	(0%)		8	(100 %)	
Rectum	18	(40%)		11	(61.1 %)		7	(38.9 %)			7	(38.9 %)		11	(61.1 %)			7	(38.9 %)		11	(61.1 %)	
Number																							
Single	39	(86.7 %)		20	(51.3 %)		19	(48.7 %)	1.000 §		11	(28.2 %)		28	(71.8 %)	1.000 §		11	(28.2 %)		28	(71.8 %)	1.000 §
Multiple	6	(13.3 %)		3	(50%)		3	(50%)			1	(16.7 %)		5	(83.3 %)			1	(16.7 %)		5	(83.3 %)	
Gross pattern																							
Ulcerative	22	(50%)		14	(63.6 %)		8	(36.4 %)	0.308 §		7	(31.8 %)		15	(68.2 %)	0.794 §		7	(31.8 %)		15	(68.2 %)	0.794 §
Fungating	9	(20.5 %)		4	(44.4 %)		5	(55.6 %)			2	(22.2 %)		7	(77.8 %)			2	(22.2 %)		7	(77.8 %)	
Annular	13	(29.5 %)		5	(38.5 %)		8	(61.5 %)			3	(23.1 %)		10	(76.9 %)			3	(23.1 %)		10	(76.9 %)	
Size (cm)																							
Mean ± SD	74.17	±18.52		79.34	±15.61		68.77	±20.09	0.055 *		79.58	±18.64		72.21	±18.36	0.242 *		79.58	±18.64		72.21	±18.36	0.242 *
Median (Range)	75	(30-120)		80	(55-120)		70	(30-100)			80	(55-120)		70	(30-100)			80	(55-120)		70	(30-100)	
10-50 mm	9	(13.3 %)		1	(16.7 %)		5	(83.3 %)	0.096 §		0	(0%)		6	(100 %)	0.171 §		0	(0%)		6	(100 %)	0.171 §
>50 mm	39	(86.7 %)		22	(56.4 %)		17	(43.6 %)			12	(30.8 %)		27	(69.2 %)			12	(30.8 %)		27	(69.2 %)	
Grade																							
Grade 1	31	(68.9 %)		16	(51.6 %)		15	(48.4 %)	0.759 ‡		9	(29%)		22	(71%)	0.974 ‡		9	(29%)		22	(71%)	0.974 ‡
Grade 2	9	(20%)		5	(55.6 %)		4	(44.4 %)			1	(11.1 %)		8	(88.9 %)			1	(11.1 %)		8	(88.9 %)	
Grade 3	5	(11.1 %)		2	(40%)		3	(60%)			2	(40%)		3	(60%)			2	(40%)		3	(60%)	
T																							
T1	2	(4.4 %)		1	(50%)		1	(50%)	0.791 ‡		0	(0%)		2	(100 %)	0.925 ‡		0	(0%)		2	(100 %)	0.925 ‡
T2	3	(6.7		2	(66.7		1	(33.3			2	(66.7		1	(33.3			2	(66.7		1	(33.3	

		%)			%)			%)				%)			%)				%)			%)	
T3	40	(88.9 %)		20	(50%)		20	(50%)			10	(25%)		30	(75%)			10	(25%)		30	(75%)	
N																							
N0	6	(13.3 %)		4	(66.7 %)		2	(33.3 %)	0.665 §		3	(50%)		3	(50%)	0.319 §		3	(50%)		3	(50%)	0.319 §
N1	39	(86.7 %)		19	(48.7 %)		20	(51.3 %)			9	(23.1 %)		30	(76.9 %)			9	(23.1 %)		30	(76.9 %)	
Stage																							
Stage I	2	(4.4 %)		1	(50%)		1	(50%)	0.791 ‡		0	(0%)		2	(100 %)	0.925 ‡		0	(0%)		2	(100 %)	0.925 ‡
Stage II	3	(6.7 %)		2	(66.7 %)		1	(33.3 %)			2	(66.7 %)		1	(33.3 %)			2	(66.7 %)		1	(33.3 %)	
Stage III	40	(88.9 %)		20	(50%)		20	(50%)			10	(25%)		30	(75%)			10	(25%)		30	(75%)	
Nuclear β catenin																							
Positive	22	(48.9 %)									0	(0%)		22	(100 %)			0	(0%)		22	(100 %)	
Negative	23	(51.1 %)									12	(52.2 %)		11	(47.8 %)	<0.00 1§		12	(52.2 %)		11	(47.8 %)	<0.00 1§
Cytoplasmi cβcatenin																							
Positive	12	(26.7 %)		12	(100 %)		0	(0%)	<0.00 1§									12	(100 %)		0	(0%)	<0.00 1§
Negative	33	(73.3 %)		11	(33.3 %)		22	(66.7 %)										0	(0%)		33	(100 %)	
Membrano us β catenin																							
Positive	12	(26.7 %)		12	(100 %)		0	(0%)	<0.00 1§		12	(100 %)		0	(0%)	<0.00 1§							
Negative	33	(73.3 %)		11	(33.3 %)		22	(66.7 %)			0	(0%)		33	(100 %)								

- Categorical variables were expressed as number(percentage), continuous variables were expressed as mean ± SD & median (range).
- * Independent samples Student's t-test;‡ Chi-square test for trend; §Chi-square test; p<0.05 is significant.

Table (4):- correlation between Fascin1 andβ-catenine expression in colorectal cancer

β- catenin * Fascin 1Crosstabulation						
Count						
		Fascin1				Total
		.00	1.00	2.00	3.00	
βcatenin	0.00	25	3	1	0	29
	1.00	4	3	2	0	9
	2.00	2	2	0	2	6
	3.00	0	1	0	0	1
Total		31	9	3	2	45

- Sperman correlation .528 P value<0.001.

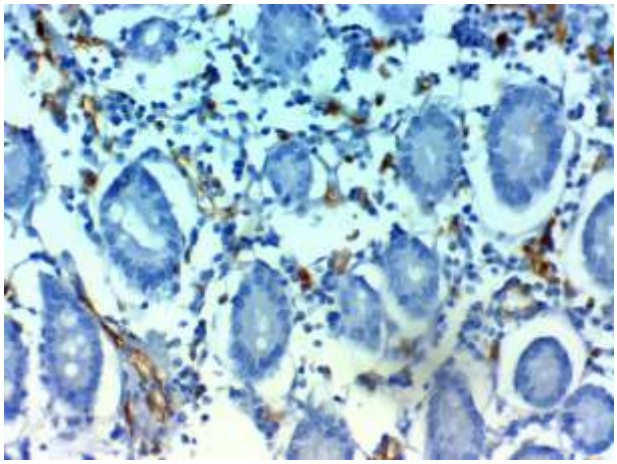

Fig 1 a

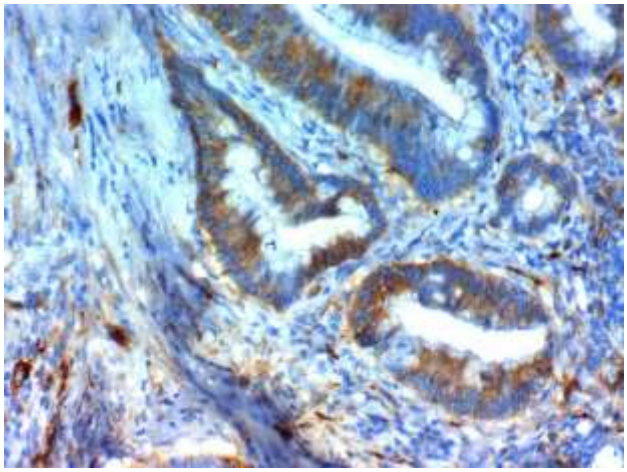

Fig 1b

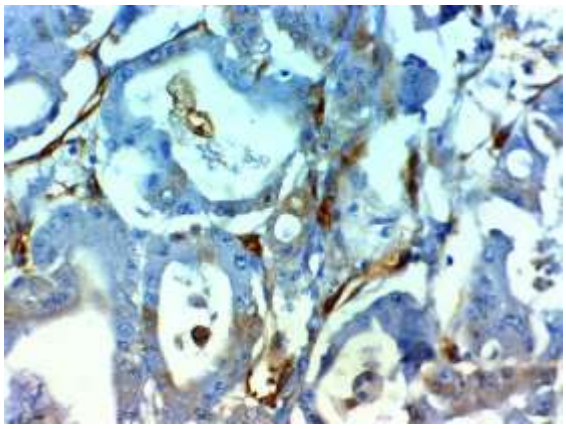

Fig 1 c

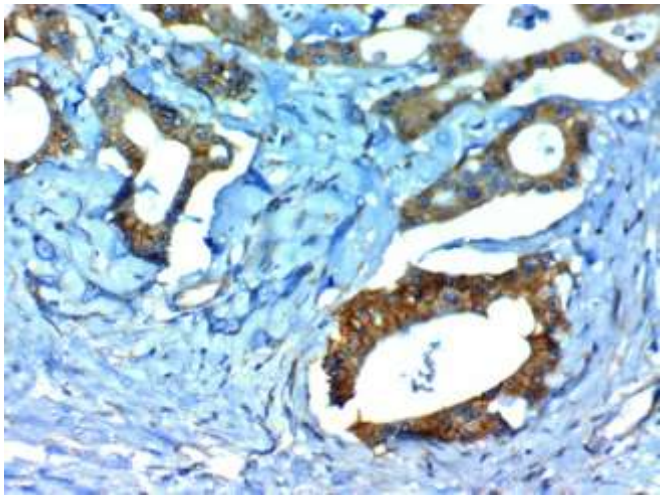

Fig 1 d

Fig. 1: fascin 1 in cancer colon and adenomatous polyps

a -Negative expression of fascin 1 in normal mucosa (ABC, DAB x400).

b - Positive sever cytoplasmic expression(+3) of Fascin 1 in colonic adenomatous polyp (ABC, DABx400).

c- Positive moderate cytoplasmic expression (+2) of Fascin1 in cancer colon (ABC, DABx400).

d- Positive severe cytoplasmic expression(+3) of Fascin 1 1in invasive front of tumor (ABC, DABx400).

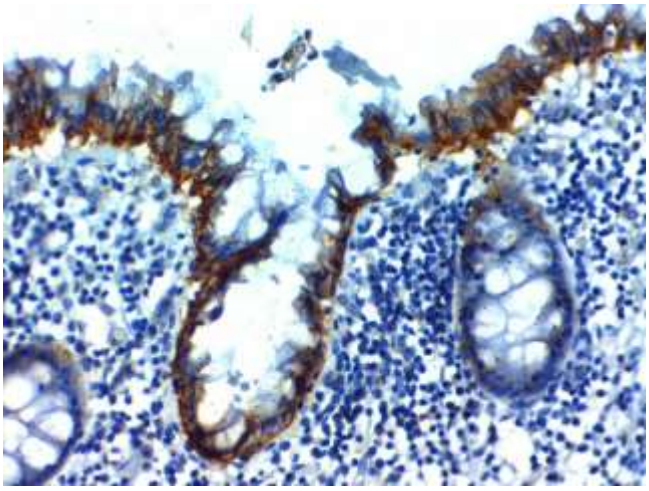

Fig 2 a

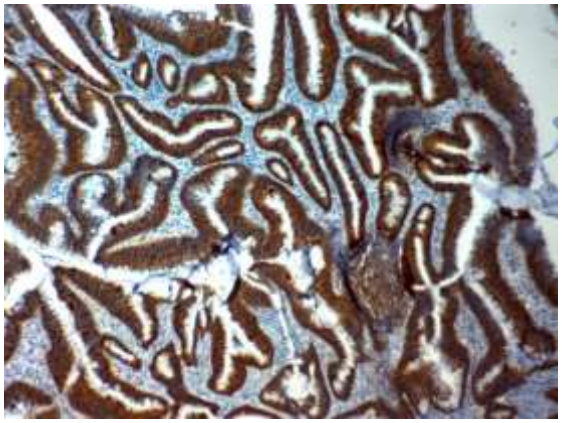

Fig 2 b

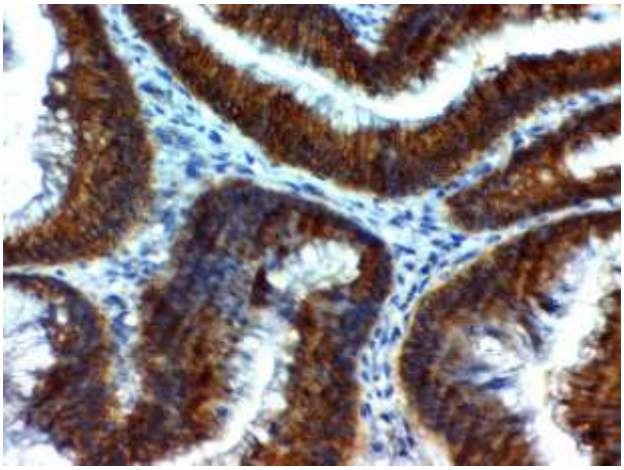

Fig 2 c

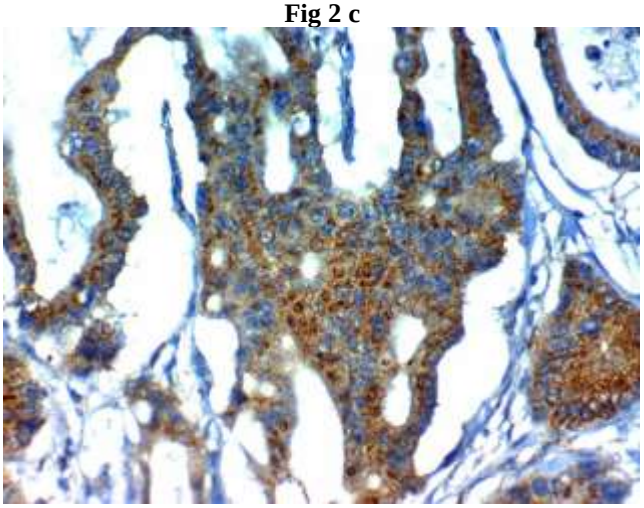

Fig 2 d

Fig. 2: β- catenine in cancer colon

a-Strong positive membranous expression of β- catenine in normal mucosa (ABC, DABx400).

b. - Colonic adenomatous polyp showing strong cytoplasmic and membranous immunostaining of β- catenine (ABC, DABx100

c- - High power field of previous image (ABC, DABx400).

d- Aberrant high nuclear and cytplasmic expression of β- catenine in addition to memberance expression in cancer colon (ABC, DABx 400).

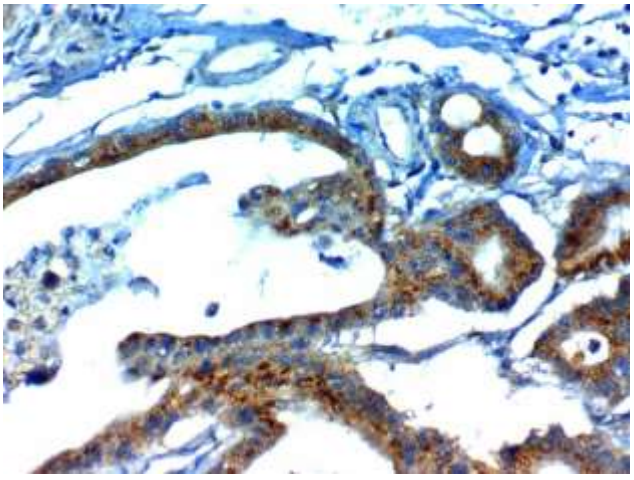

Fig 3 a

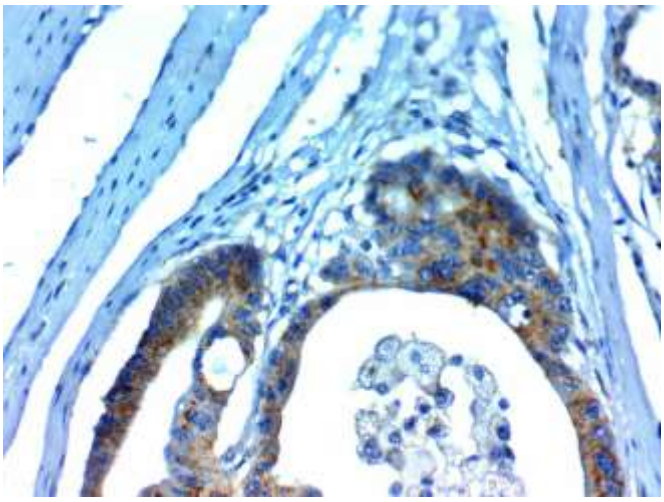

Fig 3 b

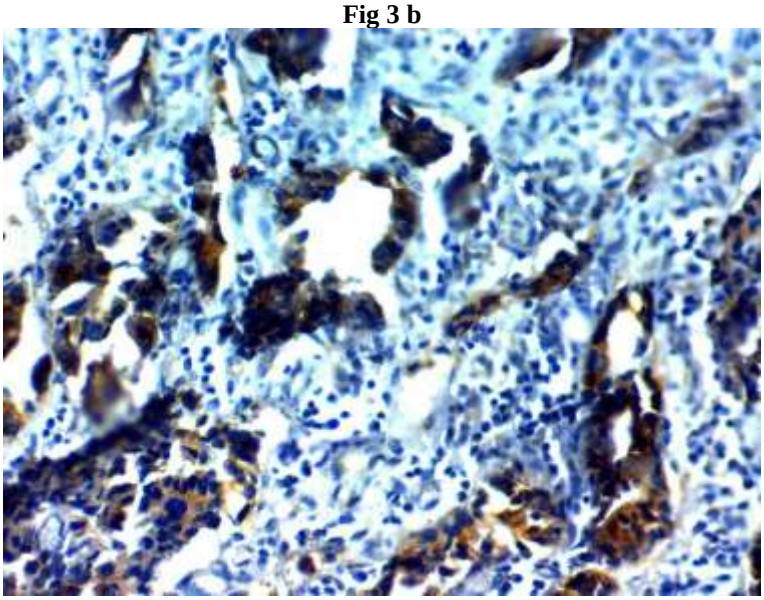

Fig 3 c

Fig3: β- catenine in cancer colon

a. - Positive nuclear and cytoplasmic expression of β- catenine in cancer colon (ABC, DAB x400).

b- -Positive expression of β- catenine in invasive front of tumor (ABC, DABx400)

c- Positive cytoplasmic expression of β- catenine in moderately differentiated adenocarcinoma(ABC, DABx400).

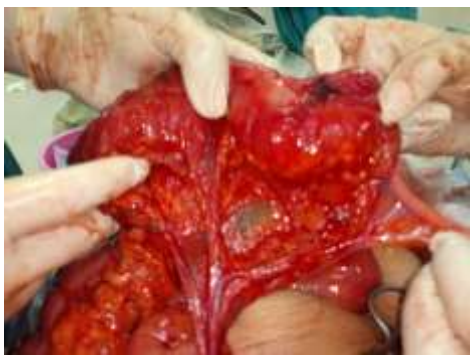

Fig 4 A

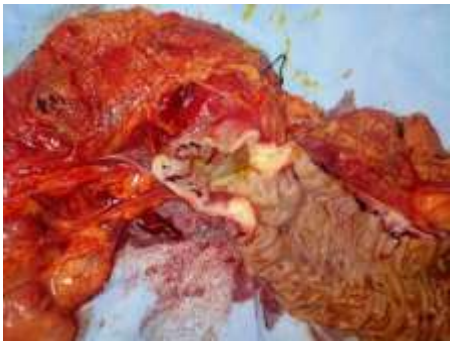

Fig 4 B

Fig 4:- gross description of CRC

Fig 4 A; transverse colectomy was done for fungating colon adenocarcinoma in the transverse colon

Fig 4 B; proctocolectomy done for annular CRC

Discussion:-

Fascin 1 binds beta-catenin, a molecule the Wnt signaling pathway.Fascin1expression was high in several types of transformed epithelial cell lines and in several solidtumors**[8].**

Fascin 1expression in malignant colonic cells increased their migration and invasion in cell culture and caused cell propagation and metastasis in vivo. on the contrary, the inhibition of fascinactivity by interfering RNA decreased cell invasion**[33].**

In thisstudy, no immunohistochemical staining of Fascin 1was detected in the normal colonic epithelial cells adjacent to the tumor, in accordance with previous studies by.**Jawhari and Hashimoto[8,18].**

We detectFascin 1 positive expression in 40% of colorectal adenomatous polyp. The results are different from that of**Hashimoto[19]** who found Fascin 1expression in 16% of adenomas. The difference may be due to different sample size (107 cases in their studied group).

In this study,no significant difference was found between Fascin 1 expression in adenomas and carcinoma,in contrast,**Tasi and Hashimoto[20,10]**found that higher Fascin 1expression were significantly associated with high grade dysplasia in adenoma of the colon.

This study also revealed that Fascin 1 staining 31.1% of colorectal adenocarcinomas. The results are close to that of**Ozerhan and Hashimoto. 2006 and Hashimoto 2011[21,10,19]**who detect Fascin1 expression in 35.3%, 26% and26%) of their studied cases respectively however ,**Puppa and Jeong[11,13]**,found that Fascin 1 was detected in 71% of and 79.7% of their studied cases respectively.

In the current study, no significant differencewas identified between Fascin 1 expression and gender but associated with age the results similar to **Hashimoto 2006[10]** .However, in a study by **puppa[11]**, Fascin 1 correlated significantly with the female sex.

We found Fascin 1 expression was associated withhigh grade of the tumors(p=0.0001), similar to**Ozerhan[21]**.and**VignjevicAlso Tsai [7,20]** who found that higher Fascin 1immunostaining were significantly associated with high-grade of colonic carcinomas.

In this work,no association between Fascin 1 expression and the tumor stage of colorectal adenocarcinoma but there is association with L.N state (p= 0.0497). Results parallel to**Hashimoto 2006[10]** but **Tsai [20]andPuppa [11]**found Fascin1 was associated with highTNM stage. This difference may be due to small number of cases.

This study showed strong Fascin 1 at the invasive front this was also explained by***Jung[22]***.Migratingcells commonly concentrated at the invading border of tumors. Thus, tumors having a large portion of Fascin1-positive cells might have a high potential for invasive manners.

Jawhari andShonukan[8,9], suggesting that Fascin 1 can enhance the directional motility of cells. Also high expression of fascin in colonic epithelial or its diminution in esophageal carcinoma cells correlated, respectively, with increased or decreased cell proliferation in culture.***Jawhariand Xie*** **[8,23]**

Fascin 1 expression is associated with high stage in breast carcinomas,Fascin1 associated with metastatic cancer lung, **Minn[24]**.Fascin 1 expression also with an aggressive type of colorectal adenocarcinomas.

β-catenin signaling pathway plays arole in the carcinogenesis of colorectal carcinoma. Nuclear accumulation of β-catenin is an important step in colorectal tumorogenesis**,Wongand chung [25,26]. Wong [27]**noticed that the expression of nuclear β-catenin increased during the development of carcinoma. There is also evidence that carcinoma in situ CRCs are frequently associated with high nuclear β-catenin expression **[28].**

The current study showed β-catenin in normal colonic epithelium, neoplastic cells establishedashift from a membranous expression to a more widespread distribution (membranous, cytoplasmic, and nuclear) in malignant lesions. This is in agree with previous studies by **Mikami and Horkko [29,30]**describing β-catenin expression in cancer cells with this type of altered pattern.

In this study no nuclearβ- catenineexpression in normal mucosa this is in concordance with studies by**Roca [31]and Ougolkov [5].**

In our study There is statistically significant difference between cytoplasmic β- -catenin expression and adenomatous polyp (p=0.0001). **Wong [27]**alsonoticed no nuclear β-catenin accumulation in normal tissues, whereas it was seen in 8% of polyps, 92% of adenomas, and 100% of carcinomas.

In our studyatypical cytoplasmic and nuclear β-catenin wasseen in26.7% and 48.9% of cases of carcinoma. the results go with**Hashimoto [19],**who observed nuclear expression in 48% of the cancer. Also similar to**Stanczak[32]** who detected unusual cytoplasmic and nuclear β-catenin in 51.5% (34/66) and 31.8% (21/66) of patients, respectively

Results parallel to**Wong[27]**who observed no nuclear β-catenin accumulation in normal tissues, whereas it was present in 8% of polyps, 92% of adenomas, and 100% of carcinomas.

Expression of nuclear β catenin amplified significantly during the progression from normal to carcinoma. **Wong[25]** In the current study significant relation was found between cytoplasmic and membrance expression of β- catenine (p value<0.001) and between cytoplasmic and nuclear expression of β- catenine (p value<0.001).

In this study, In the inner more differentiated regions of tumors, β-catenin staining was detected in the membrane and in the cytoplasm. In the invasive front of tumors,we noticed cytoplasmic and nuclear β-catenin localization and was associated with strong Fascin1 expression. Fascin 1 expression was high in sheets of invading tumor cells. Results similar to **Vignjevic[7],** and this explained by β-catenin-TCF signaling is involved in the regulation of *fascin1* gene transcriptio**n** in human colorectal carcinoma

In our study highly significant correlation between both markers, was found this is explained by Fascin 1has been established that its actin-binding properties are regulated by adhesion receptors and receptor tyrosine kinases similar to studies by.***Cohan andJawhariand Ross [33,8,34].***

Fascin1 in cancer colon and cultures of colonic cancer cells correlates with the presence of β-catenin in the nuclei of cells, indicative of its activity in β-catenin-TCF signaling. **Vignjevic[7]**and they concluded that Fascin 1 is a new target of β- -catenin-TCF signaling. They projected that transient up-regulation of Fascin1 in colorectal cancer promotes the achievement of migratory and invasive phenotypes that lead to metastasis.

Summary:-

Positive Fascin1 expression is significantly correlated with L. N state (p=0.0497). Both markers are significantly correlated with tumor grade (p<<0.001). Both markers are significantly correlated with each others.

Conclusion:-

Fascin1 has important roles in cell morphology and migration and may represent a potential new marker or therapeutic target for patients with colorectal cancer. Fascin 1is regulated by β- catenine. IHC staining of β-catenin is considered to be ahelpful marker to predict the prognosis in CRC. We recommended more, further studies to investigate the prognostic significance of β-catenin.

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