RESEARCH PAPER | Biology | Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Assessment the Immunohistochemical Expression of Galectin-3 in Iraqi Patients with Colorectal Carcinoma.

KEYWORDS: Galectin-3, colorectal cancer ,Immunohistochemical detection.

Ban Jasim Mohamad	Khawla Hoori Zghair	Faeza Aftan Zghair
Department of Biology, College of Science, University of Baghdad, Baghdad Iraq.	Department of Biology, College of Science, University of Baghdad, Baghdad Iraq.	Ibn-Sina College of Medicine, Al-Iraqia University, Baghdad Iraq.

ABSTRACT *In the current study hundred ten of Iraqi patients with colorectal tumors were studied to evaluate the expression of Galectin-3 using Tissue microarray-Immunohistochemisty (TMA-IHC) technique. Of them, Ninety cases had colorectal carcinoma, and twenty had benign tumors. A group of twenty cases of non-specific colitis and other twenty colonic biopsies with no significant pathology were also studied. Results revealed that Galectin3 expression was positive in 66.7% of malignant cases. With significant correlation (P=0.04) with tumor grade, compared with adenoma cases in which 10% of them showed positive expression of galectin-3.*

Introduction:

Among the various types of cancers, colorectal cancer (CRC) is a serious and common health problem worldwide. This cancer is the third most common visceral malignancy with nearly 1.4 million new cases diagnosed in 2012 (Ferlay *et al.,* 2013). Despite advances in surgical techniques and therapeutic interventions during the past few decades, CRC remains a major health problem worldwide due to therapy resistance (Akhter *et al.,* 20-8; Kirana *et al.,* 2012). Studies aiming at optimizing the diagnostic process and treatment of this disease are increasing, which has probably caused CRC to be one of the most-studied and best characterized processes of tumorigenesis. Through more biological knowledge of tumorigenesis in CRC, more emphasis on early detection and development of new and improved treatment regimens (Jemal *et al.,* 2011; Galizia *et al.,* 2012). Because CRC is a major cause of cancer related deaths worldwide, a lot of research has been focused on the discovery and development of biomarkers to improve the diagnostic process and to predict treatment outcomes. Up till now only a few biomarkers are recommended by expert panels (Marlies *et al.,* 2013). Discovery of additional prognostic markers might permit the development of guidelines for better management of CRC in order to improve overall survival. Although many biomarkers have been described, only a select few have provided prognostic data. Among those markers, Galectin-3(Gal3), an endogenous β-galactoside-binding protein, that is expressed in a wide range of normal, inflammatory cells and neoplastic tissues and exhibits a variety of biological and pathological functions, including effects on RNA processing, cell growth, differentiation, cellular adhesion, growth regulation, intracellular signaling, cell to cell interaction and exchanges between cells and the extracellular matrix, apoptosis, immune response, malignant transformation, angiogenesis, invasion and metastasis, and cancer drug resistance (Dumic *et al.,* 2006; Fukumori *et al.,* 2007). Changes in Gal3 expression and subcellular and intercellular localizations are commonly seen in cancer and precancerous conditions (Anna *et al.,* 2011). However, there are contradictory findings regarding the over or under expression of Gal3 in human CRC (Endo *et al.,* 2005; Shi *et al.,* 2007; Wu *et al.,* 2013). Higher Gal3 expression in colon cancer patients is associated with increased risk of metastasis and has been suggested to be a useful prognostic marker (Arfaoui-Toumi *et al.,* 2010). The involvement of Gal3 in the invasiveness of colon cancer cells remains to be determined (Wu *et al.,* 2014). In contrast, a decrease in Gal3 expression levels has also been found in CRC (Lee *et al.,* 2013). The aim of this study is to assess the expression of Gal3 in colorectal tissue samples, inflammatory and neoplastic, from Iraqi patients using TMA-IHC technique, and its relation with various clinicopathologic variables.

Materials & Methods:

150 cases of colonic biopsies were collected. Among these, 90 cases colorectal cancer, 20 cases benign lesions, 20 cases non-specific colitis and 20 cases reveal no significant pathology. Clinical information regarding patient's age, tumor size, grade, and pathological stage was obtained from the available histological reports. Hematoxylin and Eosin (H&E) stained sections were re-examined by two pathologists. All the preparations for tissue microarray (TMA) and immunohistochemistry (IHC) were performed in Pathology Unit - Southern General Hospital (SGH), University of Glasgow, United Kingdom.

Construction of tissue microarrays (TMAs)

Tissue cores of 0.6 mm in size were obtained from three paraffin-blocks in this cohort. Five tumor tissue cores (0.6mm in diameter) were taken from each paraffin block with Beecher automated tissue arrayer (Beecher Instruments, Sun Prairie, Wisconsin, USA). The cores were placed in a new recipient paraffin block that ultimately contained 325 tissue cores. The information of all TMAs cases were put in data sheet which called TMA map. This TMA map consists of a simple Excel sheet, which served as a guideline to blocks arrangement and sequence in which they arrayed. Thus the TMA map was contained the exact location for each core in TMAs slide or block and another map (TMA code map) contained the code number for each core to each block. TMA block was cut at a thickness of 5µm on a microtome cutter (Leica RM2235). Sections were then placed on Salinized coated slides, (DAKO,UK) and heated at 58°C for 24 hours.

TMA- Immunohistochemistry:

IHC was applied on TMA sections. Staining were carried out for cathepsin D Sections were deparaffinized in xylene and rehydrated in decreasing concentration of ethanol (100%, 90%, 80%, 70%). Antigen was retrieved using

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

citrate buffer (prepared by dissolving 2.1 gm of citric acid powder into 1 litre of dH2O, adjust pH to 6) for 10min in pressure cooker. Slides were incubated in peroxidase – blocking solution (Dako, ready- to- use) for 20 minutes. Non-specific binding of antibodies was blocked by the addition of 2.5% normal horse serum, from (ImmPRESS™ ,Vector , USA).

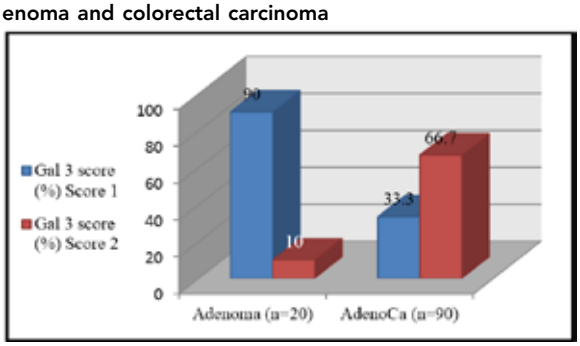
Primary antibodies were diluted (1:200) using antibody diluant (ready-to-use, Code No. (ab64211) abcam, Cambridge, UK), and incubated for 1hour at room temperature. Mouse monoclonal antibodies kit from (Abcam, Cambridge, UK) was used to detect primary antibodies. Secondary antibodies (Anti Mouse peroxidase , Cat. No. MP-7402,_ImmPRESS™ Vector, USA) was applied to the slides and incubated for 30 minutes at room temperature in a humidified chamber. The colorimetric detection of reaction was achieved by the Diaminobenzidine (DAB) Peroxidase Substrate method. Then, sections were counterstained with haematoxylin, dehydrated, and mounted. Tissue microarray slides scanned by using of digital image scanning analysis computer,(NDP, U10074-01, UK).

Scoring system of IHC in TMAs:
Gal3 expression was detected by the presence of dark brown precipitate in the cytoplasm and\or nucleus (Thiago *et al.*, 2014). The expression was scored as follow; score 1 (negative or weak) for no staining or when less than 50% of tumor cells were stained, and score 2 (moderate or strong) when ≥50% of tumor cells were stained as described by (Arfaoui-Toumi *et al.*, 2010 and Thiago *et al.*, 2014)

Statistical analyses
Statistical analysis was carried out using (SPSS V. 20). The association between Gal3 and patient clinico-pathological features was assessed by chi -square test and Fisher exact test when the Chi square test was not fit. Statistical tests were approved by assuming a null hypothesis of no difference between variables, a probability was considered statistically significant when P values ≤ 0.05.

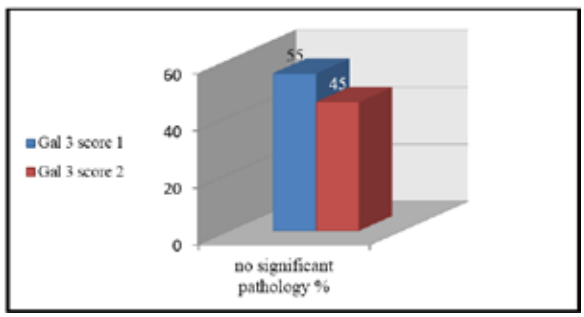
Results & Discussion:
Gal3 positive expression was observed as a dark brown cytoplasmic precipitate. In this study, 90% of total adenoma group showed negative to weak expression (score 1) when stained with Gal3, and only 10% showed moderate to strong expression (score2) of cytoplasmic Gal3. In comparison, there were 33.3% of the total malignant cases within score 1(negative or weak), whereas there were 66.7% of score 2 (moderate or strong) (Figures 1&3).

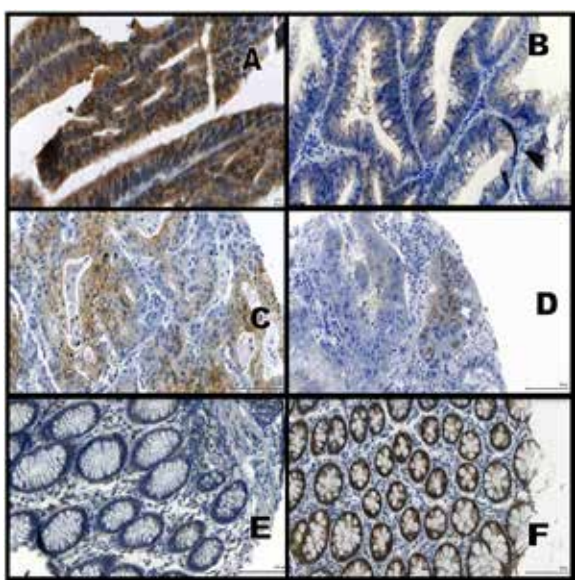
Figure (1) Expression and scoring of Gal3 in colonic adenoma and colorectal carcinoma



In colitis group, all 20 cases (100%) showed negative expression when stained with Gal3, in comparison with sections of colonic biopsies with no significant pathology group in which there were55% of the cases within score 1 and 45% within score 2 (Figures 2 & 3).

Figure (2) Expression and scoring of Gal3 in cases of colonic biopsies with no significant pathology





Figure(3) Immunohistochemical assessment of Galectin 3.(A: score(2) villous adenoma showing moderate to strong cytoplasmic Galectin3 expression, (Hematoxylin & DAB, 20X) B: score(1) tubular adenoma showing negative to weak cytoplasmic Galectin3 expression, (Hematoxylin & DAB, 40X) C: score(1) poorly differentiated adenocarcinoma showing weak cytoplasmic Galectin3 expression (Hematoxylin & DAB, 40X) D: mucinous carcinoma showing moderate to strong cytoplasmic Galectin3 expression (Hematoxylin & DAB, 40X) E: score(1) colitis tissue showing negative cytoplasmic Galectin3 expression, (Hematoxylin & DAB, 20X) F: score(2) normal colon showing strong cytoplasmic Galectin3 expression, (Hematoxylin & DAB, 20X).

Association of Galectin3 expression with clinicopathological features:
A- Association of Galectin 3 expression with age:
Results showed no significant correlation between Gal 3 expression and age in all study groups (Table 1).

Table (1) Association of Galectin 3 expression with age.

Groups	Age (years)	No. of cases n %	Score 1 n %	Score 2 n %	Fisher exact test	*P value

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Carcinoma	≤ 55	43 47.7	15 16.6	28 31.1	0.2	1.00
	> 55	47 52.3	17 18.9	30 33.3		
adenoma	≤ 55	14 70	12 60	2 10	0.5	1.00
	> 55	6 30	6 30	0 0		
Colitis	≤ 55	16 80	16 80	0 0	1.00	1.00
	> 55	4 20	4 20	0 0		
No significant pathology	≤ 55	17 85	9 45	8 40	0.4	1.00
	> 55	3 15	2 10	1 5		

(*Pβ 0.05)

B- Association of Gal 3 expression with gender :
Results showed no significant differences in the expression of Gal3 between male and female in the study groups (Table 2).

Table (2) Association of Gal 3 expression with gender

Groups	Gender	No. of cases n %	Score 1 n %	Score 2 n %	Fisher exact test	*P value
Carcinoma	Male	53 58.8	18 20	35 38.8	0.2	0.823
	Female	37 41.2	14 15.6	23 25.6		
Adenoma	Male	18 90	13 65	5 25	0.6	1.00
	Female	2 10	2 10	--- ---		
Colitis	Male	12 60	12 60	--- ---	1	1.00
	Female	8 40	8 40	--- ---		
No significant pathology	Male	14 70	7 35	7 35	0.3	0.642
	Female	6 30	4 20	2 10		

(*Pβ 0.05)

C-Association of Gal 3 expression with tumor site:
There was no significant correlation between Gal 3 expression and tumor site in adenoma and carcinoma groups (Table 3).

Table (3) Association of Gal 3 expression with tumor site.

Groups	Tumor site	No. of cases n %	Score 1 n %	Score 2 n %	Fisher exact test	P value
Carcinoma	Rt. Colon	28 31.2	6 6.6	22 24.6	2.7	0.062
	Lt. Colon	62 68.8	26 28.9	36 40.0		
Adenoma	Rt. Colon	4 20	4 20	--- ---	0.2	0.474
	Lt. Colon	16 80	14 70	2 10		

(*Pβ 0.05)

D-Association of Gal 3 expression with tumor type, stage & grade:
This study revealed that there was a significant differences (P=0.04) in the expression of Gal 3 with grade, but not with stage (Table 4).The highest expression of Gal 3was in poorly differentiated comprising (30\36) of cases whereas the moderately differentiated cases showed the lowest proportion of Gal3 expression (11\25)of cases .

Table (4) Association of Gal 3 expression with tumor stage & grade.

Carcinoma group		No. of cases n	% %	Score 1 n	% %	Score 2 n	% %	Fisher exact test	P value
Histological type	Adenocarcinoma	81	90	31	34.5	50	55.5	8.7	0.150
	Mucinous ca.	9	10	1	1.1	8	8.9		
Histological grade	Well diff.	29	32.3	12	13.5	17	18.8	1.9	0.04 *
	Moderately diff.	25	27.7	14	15.5	11	12.2		
	Poorly diff.	36	40	6	6.6	30	33.4		
Dukes stage	A	15	16.6	6	6.6	9	10	1.1	0.865
	B 1	15	16.5	8	8.8	7	7.7		
	B 2	19	21.1	7	7.7	12	13.4		
	C 1	21	23.3	6	6.6	15	16.7		
	C 2	20	22.2	5	5.5	15	16.7		

(*P< 0.05)

(*P< 0.05)

Early diagnosis of CRC, successful surgical treatment, better knowledge of its clinicopathological prognostic factors and response to adjuvant therapy have participated to improved outcome in affected patients. Therefore, identification of molecular markers progression associated with carcinogenesis, tumor growth, invasion and metastasis has been critical to develop potential therapeutic intervention (Doger *et al.,* 2006).

Gal-3 is a β-galactoside binding, small molecular weight protein (about 30,000Da) was described as a versatile multifunctional protein involved in multiple biological processes, including cell growth, cell cycle progression, cell migration, cell adherence, proliferation, differentiation, RNA processing, negative regulation of apoptotic mechanisms and malignant transformation (Hittelet et al., 2003; Ibrahim *et al.,* 2015). Some researches hypothesized that there is a prognostic value of Gal-3 expression in colorectal cancer as a marker of progression & metastatic potential (Bresalier *et al.,* 1998; Nakamura *et al.,* 1999 ; Nangia-Makker *et al.,* 2002; Nagy *et al.,* 2003; Ibrahim *et al.,*2015). However, opposite results were reported in others, regarding the prognostic value of Gal-3 expression in colon cancer as the pattern of immunohistochemical Gal- 3expression in human colorectal cancer was a matter of debate because some investigators found increasing Gal-3 levels in colorectal cancer progression, whereas others did not (Lahm *et al.,* 2001; Tsuboi *et al.,* 2007).

The current study demonstrated moderate to strong expression of Gal-3 in (66.7%)of malignant cases whereas negative to weak expression comprised the remaining (33.3%), compared to cases of colonic biopsies with no significant pathology in which there were (45%)of moderate to strong expression versus(55%) were negative expression. The increased Gal -3 expression in CRC cases in the current study agrees with findings of many studies, (Lee *et al.,* 1991, Ohannesian *et al.* , 1994, Schoeppner *et al.* 1995, Sanjuan *et al.* 1997, Endo *et al.* 2005, Tsuboi *et al.* 2007 and Arfaoui-Toumi *et al.* 2010), all agreed that Gal-3 expression increases in colorectal cancer. For instance; Endo *et al.* (2005) reported (65%) of malignant cases were strong positive for Gal-3 expression, as well as Sanjuán *et al.* (1997) who mentioned that the cytoplasmic expression of Gal3 in carcinoma cases was (64%), and Ibrahim *et al.* (2015) stated that Gal 3 expression was moderate to strong positive in (68.4%) of total colorectal carcinoma studied cases. While, Povegliano *et al.* (2011) recorded that the immunoexpression of Gal-3 was moderate or strong in (42%) of the colorectal tumors.

Previous studies have revealed that Gal-3 overexpression

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

is correlated with increased metastatic potential in cancer (Tsuboi *et al*. 2007). Bresalier *et al*. (1998) reported that Gal-3 expression was correlated with colon cancer metastasis, Arfaoui-Toumi *et al*. (2010) investigated the involvement of Gal-3 in colorectal cancer development by immunohistochemical analysis and concluded that, Gal-3 played an important role in colorectal cancer progression concerning the non mucinous carcinoma and could be used as a prognostic factor to predict poor outcome of patients. They showed a strong and diffuse positive staining of Gal 3 in both adjacent and distanced normal mucosa, in well differentiated adenocarcinoma and in metastasis. However, they noted a progressive decrease of Gal 3 staining according to the decreasing degree of tumoral differentiation. They also observed a loss of Gal3 in adenocarcinoma with mucinous component< 50%, where the positive staining was limited only to the well differentiated areas of tumor. Wu *et al*. (2007) explored the correlation between the expressions of Gal-3 and lymph node metastasis of colon cancer and concluded that Gal-3 expression was higher in tumors with lymph node metastasis than the tumors without metastasis and it might serve as a prognostic indicator for colon cancer patients . Huang *et al*. (2008) observed the expression of Gal-3 in the liver metastasis of colon cancer in mice and found that the expression of Gal-3 was significantly increased in the liver metastasis of colon cancer. However, the role played by Gal-3 in CRC biology is still controversial, Yoshii *et al*. (2002) thought that the anti-apoptotic activity of Gal-3 was regulated by its phosphorylation. While John *et al*. (2003) explained that Gal-3 provides tumor cells with anti-apoptotic and antianoikis activities, which are thought to be critical for anchorage-independent cell survival in the circulation that takes place during dissemination. Endo *et al*. (2005) hypothesized that Gal-3 modulates malignant behavior through interaction with its ligand mucin-2 (MUC2) which plays an important role in colon cancer metastasis and progression. And Ahmed *et al*. (2011) summarized the crucial roles of Gal-3 anticancer activities through its cytoplasmic anti-apoptotic properties, angiogenesis promoting, involving in homotypic aggregation, tumor –endothelial cell interactions required for metastasis which is mediated by endothelium associated Gal3 and cancer cell associated TF-disaccharide (TFD), and the tumor cells secreted Gal-3 induces apoptosis of cancer infiltrating T cells, thus, to promote immune escape and tumor progression.

In contrast, the expression of Gal-3 in cases of no significant pathology was less than that of carcinoma cases, and this goes in concordance with Legendre *et al*.(2003) who confirmed that the level of expression of Gal-3 was significantly higher in epithelial tumor tissues when compared with normal epithelial specimens, Nakamura *et al*.(1999) observed that normal mucosa of patients were strongly positive for Gal-3 in (31.6%)of specimens , but the staining in these tissues was still significantly less than in the lesions of the cancer. The presence of Gal-3 in tumor free colonic tissues support that the Gal-3 is normally developed and expressed in various cells and tissues, and displays multiple related functions(Ahmed *et al*., 2011;Tuβçe, 2012). Barrow *et al*. (2011) concluded that Gal-3 is expressed in the human colon and rectum and its expression show significant changes during colorectal cancer development and metastasis. And these changes in Gal-3 expression correlate with alterations in cancer cell growth, apoptosis, cell–cell and cell–matrix interactions and angiogenesis.

Other finding in this work, that the expression of Gal-3 in colonic adenoma was (90%) negative to weak compared to only (10%) of moderate to strong positive expression, which were slightly lower than what was reported by Sanjuán *et al*. (1997) who stated that cytoplasmic expression of Gal3 was down-regulated in adenomas (16%) compared to carcinoma(64%) and normal mucosa (100%). Fathi (2013) found that Gal-3 was expressed in (33.3%, 5out of 15) of adenomatous cases compared to (91.4%, 32/35) of cases of colorectal adenocarcinoma. And James *et al*. (2008) stated in their study that Gal-3 expression in adenomatous tissues was significantly lower than that of high grade dysplasia and early invasive cancer (p=0.008), which is most agreement with our results. Otherwise, our results disagree with Greco *et al*. (2004) who found that Gal-3 expression was found significantly higher on the surface of cells from adenoma samples with respect to the corresponding healthy mucosal cells.

In colitis group, all cases showed negative or weak expression of Gal-3 and this agree with Arfaoui-Toumi *et al*.(2010) and Müller *et al*. (2006), they concluded that Gal-3 was significantly downregulated in inflamed biopsies from inflammatory bowel disease patients, recommending that downregulation of epithelial Gal-3 in the inflamed mucosa reflects a normal immunological consequence, whereas under non-inflammatory conditions, its constitutive expression may help to prevent inappropriate immune responses against commensal bacteria or food compounds. Therefore, Gal-3 may prove valuable for manipulating disease activity. Mathieu *et al*. (2008) observed in their evaluation of Gal-3 expression in acute and chronic colitis in Balb-C mice, that chronic colitis was associated with decreased expression of Gal-3 whereas acute colitis showed increased expression suggesting an inversely correlation with Gal-3 expression in stages of colonic colitis.

In the current study no statistically significant relationship could be seen between Gal-3 expression and the age (P value =1.00), this goes in agreement to what was demonstrated by Endo *et al*. (2005), Tsuboi *et al*. (2007) and Ibrahim *et al*. (2015), that insignificant statistical relationship could be found between Gal-3 expression and age (P value = non-significant , P value = 0.93 and P value =0.323) respectively.

Also, no statistically significant relationship could be detected between Gal-3 expression and gender in cancer group (P value =0.823) in this study, and this goes in harmony with what was reported by Endo *et al*. (2005), Tsuboi *et al*. (2007) and Ibrahim *et al*. (2015) that insignificant statistical relationship could be observed between Gal 3 expression and sex (P value = insignificant, P value =0.15 and P value=0.875) respectively.

As well, insignificant relationship was detected between Gal-3 expression and the tumor site (P value=0.062), and this matched to what was reported by Endo *et al*. (2005) that no significant relationship (P value = nonsignificant) could be found between Gal-3 expression and tumor site, but our result was on the contrary with the results obtained by Ibrahim *et al*.(2015) who detected a significant relationship between Gal-3 expression and the tumor site (P value=0.038)where Gal-3 expression positive cases were predominantly in rectum, considering that the rectum was the predominating site in their study population.

Other hand, although (8\9) cases of mucinous carcinoma shows moderate to strong expression of Gal-3, no statistically significant differences was detected in Gal-3 expres-

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

sion between adenocarcinoma and mucinous carcinonma (P value=0.15). This is reverse the results noted by Endo *et al.* (2005) who detected significant correlation (P value = 0.0037) between Gal-3 expression and tumor type as well as Ibrahim *et al.*(2015)who detected significant relationship where (89.8%) of conventional adenocarcinoma cases were Gal-3 positive while (63.6%) of mucinous carcinoma cases were Gal-3 negative (P value <0.001). Arfaoui-Toumi *et al.* (2010) observed a loss of Gal-3 in adenocarcinoma with mucinous component< 50%, where the positive staining was limited only to the well differentiated areas of tumor.

The current study revealed no significant differences in the expression of Gal-3 among Dukes' stages (P= 0.805). This result was in agreement with reports of *Nagy et al.* (2003) and Tsuboi *et al.* (2007), that no significant variation was observed in Gal-3 expression when the Dukes' stages increased from A to C. In contrast, our findings weren't coinciding with Endo *et al.* (2005) regarding the relationship between modified Dukes' stage and Gal-3 expression among their studied cases, since there were significant differences (P value=0.0004) as well as Ibrahim *et al.* (2015) who found a statistically significant relationship between Gal-3 expression and modified Dukes' stage where (98%) of positive Gal-3 cases where modified Dukes' stage B&C (49%) each, (P value=0.018). Additionally, James *et al.*(2008) mentioned that Gal-3 expression varied according to Dukes' stages and they detected significant differences (P=0.016) among different stages.

A statistically significant relationship (P value=0.04) was detected between Gal-3 expression and the histological grade, where the largest expression of Gal-3 was in poorly differentiated cases (30\36), than in well differentiated (17\29) and the lowest was in moderately differentiated cases(11\25). The results reveal that there was an increase in Gal-3 expression in relation to the increasing degree of tumor differentiation which indicate poor outcome. These results disagree with Tsuboi *et al.*(2007) who observed no significant differences (P=0.2) in Gal-3 expression in tumor surface among tumor grades, but the results agree with Endo *et al.*(2005)and Dawson *et al.*(2013)who observed significant differences in Gal-3 expression among tumor grades (P=0.0037 & P value=0.002)respectively. Previous studies had controversial findings, Arfaoui-Toumi *et al.*(2010) observed that there was a progressive decrease in Gal-3 expression in relation to decreasing degree of tumor differentiation, while Ibrahim *et al.*(2015) detected significant correlation(P value=0.002) between Gal-3 expression and the histological grade where the expression increased with decreasing the degree of tumor differentiation.

Acknowledgment:
Special thanks and gratitude to **Dr. James J. Going, Section Head of Academic Pathology & Medical Genetics/ University of Glasgow**, **United Kingdom(UK),** for his expert supervision in the accomplishment of the practical part of this project. And a lot of thanks should be expressed to **Mr. Rod Ferrier, Lab Manager** of **Pathology Unit, Southern general hospital, University of Glasgow, UK,** for providing the laboratories, materials and equipments, and to **Miss. Alexandra Bell, Pathology Unit, Southern general hospital, University of Glasgow, UK,** for her assistance in achieving the Tissue Microarray blocks.

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

REFERENCE

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F.(2013). Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. GLOBOCAN 2012 v1.0. | 2. Akhter M, Inoue M, Kurahashi N, Iwasaki M, Sasazuki S et al. (2008) Dietary soy and isoflavone intake and risk of colorectal cancer in the Japan public health center-based prospective study. Cancer Epidemiol Biomarkers Prev. 2008 Aug;17(8):2128-35 | 3. Chandra Kirana, Hongjun Shi, Emma Laing, Kylie Hood, Rose Miller, Peter Bethwaite, John Keating, T. William Jordan, Mark Hayes, and Richard Stubbs(2012).Cathepsin D Expression in Colorectal Cancer: From Proteomic Discovery through Validation Using Western Blotting, Immunohistochemistry, and Tissue Microarrays. International Journal of Proteomics,Volume2012, ArticleID245819, 10pages. | 4. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D.(2011). Global cancer statistics. CA Cancer J Clin., 61(2):69-90. | 5. Galizia G , Gemei M, Del Vecchio L, Zamboli A, Di Noto R, Mirabelli P, Salvatore F, Castellano P, Orditura M, De Vita F, Pinto M, Pignatelli C, Lieto E.(2012). Combined CD133/CD44 expression as a prognostic indicator of disease-free survival in patients with colorectal cancer. Arch Surg.147(1):18-24 | 6. Marlies S. Reimers, Eliane C.M. Zeestraten, Peter J.K. Kuppen, Gerrit Jan Liefers and Cornelis J.H. van de Velde(2013). Biomarkers in precision therapy in colorectal cancer. Gastroenterology. Rep.,(Oxf) 1(3):166-183. | 7. Dumic J, Dabelic S, Flögel M (2006) Galectin-3: an open-ended story. Biochim Biophys Acta.1760(4):616-35. | 8. Fukumori T, Kanayama HO, Raz A (2007) The role of galectin-3 in cancer drug resistance. Drug Resist., 10: 101-108. | 9. Anna U. Newlaczyl, Lu-Gang Yu (2011) Galectin-3 – A jack-of-all-trades in cancer Cancer Letters. Vol. 313 (2): Pages 123-128. | 10. Endo K , Kohnoe S, Tsujita E, Watanabe A, Nakashima H, Baba H, Maehara Y.. (2005) Galectin-3 expression is a potent prognostic marker in colorectal cancer. Anticancer Res.25(4):3117-21. | 11. Shi Y , He B, Kuchenbecker KM, You L, Xu Z, Mikami I, Yagui-Beltran A, Clement G, Lin YC, Okamoto J, Bravo DT, Jablons DM.(2007). Inhibition of Wnt-2 and galectin-3 synergistically destabilizes beta-catenin and induces apoptosis in human colorectal cancer cells. Int J Cancer, 121: 1175-1181. | 12. Wu KL , Huang EY, Jhu EW, Huang YH, Su WH, Chuang PC, Yang KD.(2013). Overexpression of galectin-3 enhances migration of colon cancer cells related to activation of the K-Ras-Raf-Erk1/2 pathway. J Gastroenterol.,48(3):350-9. | 13. Arfaoui-Toumi A, Kria-Ben Mahmoud L, Ben Hmida M, Khalfallah MT, Regaya-Mzabi S, Bouraoui S.(2010). Implication of the Galectin-3 in colorectal cancer development. Bull Cancer; 97: E1–E8. | 14. Yung-Kuo Lee, Tsung-Hsien Lin, Chuan-Fa Chang, and Yu-Li Lo(2013). Galectin-3 Silencing Inhibits Epirubicin-Induced ATP Binding Cassette Transporters and Activates the Mitochondrial Apoptosis Pathway via β-Catenin/GSK-3β Modulation in Colorectal Carcinoma PLoS One.,8(11): e82478. | 15. Thiago Simao Gomes, Celina Tizuko Fujiyama Oshima, Nora Manoukian Forones, Flavio De Oliveira Lima and Daniel Araki Ribeiro.(2014). Expression of galectin3 in gastric adenocarcinoma.Indian J. Med. Res., 140(1):69-76. | 16. Doger, K., I. Meteoglu and P. Tuncyurek, 2006. Dose the EGFR & VEGF expression predicts the prognosis in colon cancer. Eur. Surg. Res; 38: 540-544. | 17. Hittelet, A., H. Legendre, N. Nagy, Y. Bronckart, J.C. Pector, I. Salmon and P. Yeaton,(2003). Upregulation of galectins-1 and -3 in human colon cancer and their role in regulating cell migration Int J Cancer, 103: 370-379. | 18. Badawia B. Ibrahim, Dina O. Helmy, Samar A. El.Sheikh and Rasha R. Mostafa (2015).Immunohistochemical Expression of Galectin-3 in Colorectal Carcinoma Middle-East Journal of Scientific Research 23 (4): 580-591. | 19. Bresalier, R.S., N. Mazurek, L.R. Sternberg, J.C. Byrd, C.K. Yunker, P. Nangia-Makker and A. Raz, 1998. Metastasis of human colon cancer is altered by modifying expression of the betagalactoside-binding protein galectin 3 Gastroenterology; 115: 287-296. | 20. Nakamura, M., H. Inufusa, T. Adachi, M. Aga, M. Kurimoto and Y. Nakatani, 1999. Involvement of galectin-3 expression in colorectal cancer progression and metastasis. Int J Oncol; 15: 143-148. | 21. Nangia-Makker, P., V. Hogan, Y. Honjo, S. Baccarini, L. Tait, R. Bresalier and A. Raz, 2002. Inhibition of human cancer cell growth and metastasis in nude mice by oral intake of modified citrus pectin J Natl Cancer Inst; 94: 1854-1862. | 22. Nagy, N., H. Legendre, O. Engels, S. André, H. Kaltner, K. Wasano, Y. Zick, J.C. Pector, C. Decaestecker, H.J. Gabius, I. Salmon and R. Kiss, 2003. Refined prognostic evaluation in colon carcinoma using immunohistochemical galectin finger-printing. Cancer, 97: 1849-1858. | 23. Lahm, H., S. Andre, A. Hoeflich, J.R. Fischer, B. Sordat and H. Kaltner, 2001.Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. J Cancer Res Clin Oncol; 127: 375-86. | 24. Tsuboi, K., T. Shomura, N. Masuda, M. Ide, S. Tutumi, S. Yamaguchi, T. Asao and H. Kuwano, 2007. Galectin-3 Expression in Colorectal Cancer: Relation to Invasion and Metastasis Anticancer research; 27: 2289-2296. | 25. Lee, E.C., H.J. Woo, C.A. Korzelius, G.D. Steele and Jr, A.M. Mercurio, 1991. Carbohydrate-binding protein 35 is the major cell-surface laminin-binding protein in colon carcinoma. Arch Surg, 126: 1498-502. | 26. Ohannesian, D.W., D. Lotan and R. Lotan, 1994. Concomitant increases in galectin-1 and its glycoconjugate ligands (carcinoembryonic antigen, lamp-1 and lamp-2) in cultured human colon carcinoma cells by sodium butyrate. Cancer Res, 54: 5992-6000. | 27. Schoeppner, H.L., A. Raz, S.B. Ho and R.S. Bresalier, 1995. Expression of an endogenous galactose binding lectin correlates with neoplastic progression in the colon Cancer, 75: 2818-2826. | 28. Sanjuan, X., P.L. Fernandez, A. Castells, V. Castronovo, F. van den Brule, F.T. Liu and A. Cardesa, 1997. Differential expression of galectin 3 and galectin 1 in colorectal cancer progression Gastroenterology, 113: 1906-1915. | 29. Zaia Povegliano L, Oshima CT, de Oliveira Lima F, Andrade Scherholz PL, Manoukian Forones N (2011) Immunoexpression of galectin-3 in colorectal cancer and its relationship with survival. J Gastrointest Cancer 42: 217-221. | 30. Wu ZH, Gan L.(2007). Association of galectin-3 and E-cadherin expressions with lymph node metastasis of colon cancer. Nan Fang Yi Ke Da Xue Xue Bao, 27:1731-1733. | 31. Huang ZL, Liu HY.(2008). Expression of galectin-3 in liver metastasis of colon cancer and the inhibitory effect of modified citrus pectin Nan Fang Yi Ke Da Xue Xue Bao, 28:1358-1361. | 32. Yoshii, T., T. Fukumori, Y. Honjo, H. Inohara, H. R. C. Kim, and A. Raz.(2002). Galectin 3 phosphorelation is required for its antiapoptotic function and cell cycle arrest. J. Biol. Chem. 277:6852-6857. | 33. John, C.M., H. Leffler, B. Kahl-Knutsson, I. Svensson and G.A. Jarvis, 2003. Truncated galectin-3 inhibits tumor growth and metastasis in orthotropic nude mouse model of human breast cancer Clin Cancer Res; 9: 2374-2383. | 34. Hafiz Ahmed, Prasun Guha, Engin Kaptan and Gargi Bandyopadhyaya. (2011). Galectin 3: A potential target for cancer prevention.Trends in Carbohydrate Research. Vol.3(2):13-22 | 35. Hugues Legendre, Christine Decaestecker, Nathalie Nagy, Alain Hendlisz, Max-Peter Schüring,Isabelle Salmon,Hans-Joachim Gabius,Jean-Claude Pector,and Robert Kiss(2003). Prognostic Values of Galectin-3 and the Macrophage Migration Inhibitory Factor (MIF) in Human Colorectal Cancers. Mod Pathol 2003;16(5):491–504. | 36. Çay, Tuβçe (2012). Immunohistochemical Expression of Galectin-3 in Cancer: A Review of the Literature. Turk Patoloji Derg. 1 10 (1): 1–10. | 37. Hannah Barrow, Jonathan M. Rhodes and Lu-Gang Yu.(2011). The role of galectins in colorectal cancer progression J. Cancer: 129, 1–8. | 38. Anan Fathi (2013).Immunohistochemical expression of Galectin-3 and c-erbB-2 in colorectal adenoma and adenocarcinoma Egyptian Journal of Pathology 33(1):114-119. | 39. James C, Byrd and Robert S, Bresalier.(2008). Galectin -3 in the progression and metastasis of colorectal neoplasia. John Wiley & Sons, Inc. Anderson Cancer Centre, USA. | 40. Greco C , Vona R, Cosimelli M, Matarrese P, Straface E, Scordati P, Giannarelli D, Casale V, Assisi D, Mottolese M, Moles A, Malorni W.(2004). Cell surface overexpression of galectin-3 and the presence of its ligand 90k in the blood plasma as determinants in colon neoplastic lesions. Glycobiology, 14: 783-792. | 41. Müller S, Schaffer T, Flogerzi B, Fleetwood A, Weimann R, Schoepfer AM, Seibold F. (2006). Galectin-3 modulates T cell activity and is reduced in the inflamed intestinal epithelium in IBD. Inflamm Bowel Dis , 12:588-597. | 42. Anne Mathieu, Nathalie Nagy, Christine Decaestecker, Liesbeth Ferdinande, Klaas V andenbroucke, Pieter Rottiers, Claude A Cuvelier, Isabelle Salmon and Pieter Demetter.(2008). Expression of galectin 1, 3 and 4 varies with strain and type of experimental colitis in mice. Inc J Exp Pathol, 89(6):438-446. | 43. Dawson, H., S. André, E. Karamitopoulou, I. Zlobec and H.J. Gabius, 2013. The growing galectin network in colon cancer and clinical relevance of cytoplasmic galectin-3 reactivity. Anticancer Res. 2013 Aug; 33(8): 3053-9.