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ROLE OF SERUM GROWTH DIFFERENTIATION FACTOR 15 IN DISCRIMINATION OF METASTASIS IN COLORECTAL CANCER PATIENTS

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ABSTRACT

Background: The rising incidence and mortality of colorectal Cancer (CRC) around the world, makes it as a major public health concern. Human growth differentiation factor 15 (GDF-15) is elevated in many cancer patients and is associated with tumor pathogenesis and progression. The aim of the present study was to find the best diagnosis of metastatic CRC and to compare serum levels of GDF-15 in patients with non-metastatic CRC and those with metastatic CRC.

Patients and methods: The study included 60 subjects and was carried out at Internal Medicine Department, Faculty of Medicine, Zagazig University. All subjects of this study were subjected to the following full history taking, clinical examination and laboratory investigations.

Results: Cirrhotic liver (10%) but others had normal liver by imaging (56.7%), there were 12 patient had enlarged spleen (40%) and 11 patients had IPPF (36.7%). There were four patients with lymph node metastasis (13.3%). Two patients had peritoneal metastasis (6.7%). There were statistically significant differences between colorectal carcinoma and metastatic colorectal carcinoma groups regarding imaging of the liver, splenomegaly. There were 14 patients with resected primary tumor (46.7%).Twenty five patients presented by malignant features in colonoscopy (83.3%) and 23 patients had polyps (76.7%).There were no statistically significant differences between colorectal carcinomaand metastatic colorectal carcinoma groups regarding all colonoscopy findings. Twenty eight patients had adenocarcinoma type (93.3 %) and only two had mucinous type (6.7%). There was statistically significant difference between colorectal carcinoma group and metastatic colorectal carcinoma group regarding staging as P< 0.001. There were no statistically significant differences the two groups regarding other histopathological examination**.** on comparing GDF-15 with other parameters in non-metastatic colorectal carcinoma group there were direct significant correlation between GDF-15 and CEA, CA 19-9 and ESR with P= 0.007, 0.022 and0.033 respectively. In metastatic colorectal carcinoma group, on comparing GDF-15 with other parameters there were direct significant correlation between GDF-15 and(total bilirubin and CEA) with P=0.011 and 0.048 respectively.

Conclusion: High Growth differentiation factor 15 (GDF-15) can act as a valuable independent biomarker for screening CRC in comparison with CEA and other tumor biomarkers. Furthermore, an elevated GDF-15 in a cutoff value can identify CRC metastasis especially to the liver metastasis.

Keywords: GDF-15; Colorectal Cancer; CEA

INTRODUCTION

The majority of colorectal Cancer (CRC) occurs in developed countries. But over the past decades; Arnold et al. have witnessed a rapid increase of its incidence in countries of lower human development index. Indeed, studies have reported an increasing incidence of CRC in many medium-to-high human development index countries in Asia, South America, and Eastern Europe **(1).**

Mutations in specific genes can lead to the onset of colorectal cancer, as happens in other types of cancer. Those mutations can appear in oncogenes, tumor suppressor genes and genes related to DNA repair mechanisms.Depending on the origin of the mutation, colorectal carcinomas can be classified as sporadic, inherited and familial **(2).**

Tumor staging is by far the most important prognostic predictor of clinical outcome for patients with colorectal carcinoma. Histologic examination of surgically resected specimens serves an irreplaceable role in determining the depth of tumor invasion (T) and the extent of nodal metastasis (N). The histologic determination of T1 (tumor invades submucosa), T2 (tumor invades muscularispropria) and T3 (tumor invades through the muscularispropria into pericolorectal tissues) is usually straightforward when using the AJCC TNM staging system **(3).**

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Clinical manifestations of CRC depend on the location of the lesion. Both right and left colon lesions occasionally cause hematochezia, but more often bleeding is occult, causing anemia and fatigue. Rectal lesions cause hematochezia, bleeding and tenesmus. Up to 30% of patients with colorectal carcinoma are primarily diagnosed in an acute stage with sub/obstructing symptoms **(4).**

Most of the guidelines endorsed by the World Health Organization divide CRC screening tools into two main categories: those capable of detecting both adenomatous polyps and cancer and those screening (fecal occult blood test, immunohistochemical stool test and fecal DNA test **(5).**

Serum tumor biomarkers may serve not only for auxiliary diagnosis of CRC, but also as tools for estimating survival and prognosis.Notably, commonly used tumor markers for the diagnosis and assessment of patients with CRC are carcinoembryonic antigen (CEA), cancer antigen (CA) 19-9, CA125 and CA242**(6).**

Serum carcinoembryonic antigen (CEA) is the most widely used tumor marker in patients with colorectal cancer. Pretreatment CEA levels of5 ng/ml have been associated with decreased 5-year survival.After curative resection of colorectal cancer, the CEA level usually fallsand normalizes within 4–6 weeks. Persistent elevation ofCEA level after surgery could indicate incomplete resection or occult metastatic disease **(7).**

Carbohydrate antigen 19-9 (CA 19-9) is an antigen defined by monoclonal antibody binding to CA 19-9, the tumor surface marker, Sialyl-Lewis A.Serum CA 19-9 is known to be elevated in subjects with various gastrointestinal cancers, such as pancreatic, gastric, hepatic, and biliary tract carcinomas, and it has also been used as a tumor marker of CRC in clinical practice, usually accompanied by CEA (**8).**

Human growth differentiation factor 15 (GDF-15), also known as MIC-1, is a divergent member of the TGF-β1 superfamily of proteins. Serum GDF-15 is elevated in many cancer patients and is associated with tumor pathogenesis, progression, and invasion **(9)**. In vitro, the neutralizing anti-growth differentiation factor 15 (GDF-15) antibody could be utilized to reverse the differentiated macrophages induced migration and invasion of colorectal cancer cells. This reflects the involvement of GDF-15 in inflammation-induced invasion in CRC **(10).**

Therefore, the current study aimed to find is the best diagnosis of metastatic CRC and to compare serum levels of GDF-15 in patients with non-metastatic CRC and those with metastatic CRC.

PATIENTS AND METHODS

The study included 60 subjects and was carried out at Internal Medicine Department (gastroenterology and endoscopy unit), Faculty of Medicine, Zagazig University, and the technical part was performed at Clinical Pathology Department, Faculty of Medicine, Zagagzig University.

Approval of the study design was obtained from the Institutional Review Board (IRB) unit, Faculty of Medicine, Zagazig University. Written informed consents were taken from the participants before sample collection.

A total number of 60 subjects were included in this study. The included subjects were divided in two groups as follow: Group I: Included 30 patients diagnosed with colorectal cancer and subdivided into two groups: I(A): 15 patients of colorectal cancer without metastasis; I(B): 15 patients of colorectal cancer with metastasis. Group II: Included 30 healthy volunteers as control group. They didn`t have any acute or chronic diseases.

Inclusion and exclusion criteria for CRC groups:

Patients diagnosed with colorectal carcinoma aged above 18 years old of both sexes. While, patient with other primary malignant and patient with inflammatory bowel disease were excluded.

Technique:

All subjects of this study were subjected to the following full history taking and complete clinical examination including thorough physical examination was done to assess manifestations and presence of distant metastasis.

Laboratory investigations:

Blood samples were collected and placed in a plain tube. The samples were left to coagulate for 30 min then were centrifuged (at 3000 r.p.m for 15 minutes) for obtaining serum samples for liver and kidney functions and tumor markers analysis.

Complete blood count (CBC): by automated cell counter “Sysmex XS” (Sysmex Corporation, Japan). Liver function test: serum bilirubin (total and direct), serum albumin, serum alanine transferase and aspartate transferase measured by kinetic method. Kidney function test: serum creatinine and serum urea. Coagulation Profile. Erythrocyte sedimentation rate (ESR). Qualitative C- Reactice Protein (CRP).

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Measurement of serum GDF-15 level:

GDF15 was measured using the GDF15 Direct enzyme linked immunosorbent assay Kit (Shino-Test Corporation, Kanagawa, Japan) by following the manufacturer's instructions.The Chroma of color and the concentration of the human substance GDF15 of sample were positively correlated.

Colonoscopy and histopathological examination for biopsy from the tumor were performed for the studied patients with CRC.

Radiological investigation including CT scan or MRI for primary colorectal cancer and sites of distant metastasis (e.g. liver, bone or lung metastasis).. Whole body PET scan if needed. Diagnosis of distant metastasis is well established according to diagnostic imaging criteria.

Statistical analysis:

All data were analyzed using Statistical Package for the Social Sciences version 17.0 (SPSS,Chicago,IL). Continuous quantitative variables were expressed as the mean ± SD & median (range). Shapiro- Wilk test, Student's t-test, Chi square test, One Way ANOVA test, Kruskal- Wallis H test, Levene's test, A post - Hoc test were used.Spearman's rank correlation coefficient was calculated to assess the relationship between various study variables, (+) sign indicate direct correlation & (-) sign indicate inverse correlation also values near to 1 indicate a strong-correlation while values near 0 indicate a weak- correlation coefficient.Receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff values of CRC markers. The optimal cutoff point was established at a point of maximum accuracy. All tests were two-sided, p-value < 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS) and p-value ≥ 0.05 was considered statistically nonsignificant (NS).

RESULTS

The present study included 60 subjects participated in the current study, and they were assigned into a control group (n=30),non-metastatic CRC group (n=15)and metastatic CRC group (n=15). The study comprised 35 males (58.3%) and 25 females (41.7%), with a mean age of 61±9 years. Twenty-six participants were from urban areas (43.3%) and 34 from rural areas (56.7%). Thirteen participants had a suspiciousoccupational exposure (21.7%), and 27 of them were smoker (45%).Mean BMI of all participants was 31±6. There were no statistically significant differences among the three studied groups regarding all recorded socio demographic data **(Table 1)**.

There were statistically significant differences among the three studied groups regarding hemoglobin, platelets, total bilirubin, AST, ALT, CEA, CA 19-9, ESR, CRP and GDF -15 as P<0.001, 0.005, <0.001, 0.011, 0.02,<0.001, <0.001, <0.001, <0.001, <0.001 respectively. There were no statistically significant differences among the three studied groups regarding other laboratory values **(Table 2)**.

There were statistically significant differences between metastatic colorectal cancer and colorectal cancer groups regarding total bilirubin, AST, ALT and GDF-15 as P=0.001, 0.023, 0.041 and 0.004 respectively. There were no statistically significant differences between colorectal cancer and metastatic colorectal cancer groups regarding other laboratory values. There were statistically significant differences between colorectal cancer and control groups regarding hemoglobin, platelets, CEA, CA 19-9, ESR, CRP and GDF-15 as P<0.001, 0.013,<0.001,<0.001,<0.001, 0.001 and<0.001 respectively. There were statistically significant differences between colorectal cancer and control groups regarding other laboratory values. There werestatistically significant differences between metastatic colorectal cancer and control groups regarding hemoglobin ,platelets,total Bilirubin, aspartate transaminase,alanine transaminase, CEA, CA 19-9,ESR, CRP and GDF-15 as P<0.001, 0.004, <0.001, 0.003, 0.006, <0.001, <0.001, <0.001, <0.001 and <0.001 respectively. There were no statistically significant differences between metastatic colorectal cancer and control groups regarding other laboratory values **(Table 3)**.

There were 10 patients had focal lesion in liver (33.3%), three patients with cirrhotic liver (10%) but others had normal liver by imaging (56.7%), there were 12 patient had enlarged spleen (40%) and 11 patients had IPPF (36.7%). There were four patients with lymph node metastasis (13.3%). Two patients had peritoneal metastasis (6.7%). There were visualized mass by imaging in four patients (13.3%). By CT chest, there were four patient with pleural effusion (13.33%), two patients had focal pulmonary lesions (6.7%) and three patients had both focal lesion and pleural effusion (10%). Only one patient had brain metastasis (3.3%) and other with bone metastasis (3.3%).There were statistically significant differences between colorectal carcinoma and metastatic colorectal carcinoma groups regarding imaging of the liver, splenomegaly, IPFF, lymph node metastasis and hepatic metastasis as P<0.001, <0.001, <0.001, 0.032 and< 0.001 respectively. There were no statistically significant

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differences between colorectal carcinomaand metastatic colorectal carcinoma groups regarding other imaging data in ultrasound and CT **(Table 4)**.

There were 14 patients with resected primary tumor (46.7%).Twenty five patients presented by malignant features in colonoscopy (83.3%) and 23 patients had polyps (76.7%). Site of colorectal mass was rectum in eight patients (26.7%), left colon in 16 patients (53.3%) and right colon in six patients (20%).There were no statistically significant differences between colorectal carcinomaand metastatic colorectal carcinoma groups regarding all colonoscopy findings **(Table 5)**.

There were 4 patients with stage I (13.33%), 11 patients with stage II (36.66%) and 15 patients with stage IV (50%). According the degree of differentiation, there were eight patients had poorly differentiated carcinoma (26.7%), 22 patients had moderate to well differentiated carcinoma (73.3%). Twenty eight patients had adenocarcinoma type (93.3 %) and only two had mucinous type (6.7%). There was statistically significant difference between colorectal carcinoma group and metastatic colorectal carcinoma group regarding staging as P< 0.001. There were no statistically significant differences the two groups regarding other histopathological examination **(Table 6)**.

on comparing GDF-15 with other parameters in non-metastatic colorectal carcinoma group there were direct significant correlation between GDF-15 and CEA, CA 19-9 and ESR with P= 0.007, 0.022 and0.033 respectively. In metastatic colorectal carcinoma group, on comparing GDF-15 with other parameters there were direct significant correlation between GDF-15 and(total bilirubin and CEA) with P=0.011 and 0.048 respectively **(Table 7)**.

GDF-15 at level > 2.1 ng/ml had sensitivity to diagnose Colorectal carcinoma (93.4%) with the 95% confidence interval (77.9-99.2), specificity (93.2%) with the 95% confidence interval (77.9-99.1), positive predictive value (93.1%) with the 95% confidence interval (78.5-98.1), negative predictive value (93.3%) with the 95% confidence interval (78.5-98.2) and at area under the ROC curve(0.89) with the 95% confidence interval (0.788-0.959) P was<0.001**(Table 8 &Figure1)**.

Table 1: Comparison of socio-demographic data among the studied groups

		Total N=60		Group						Test1	P1	Test2	P2
				CRC N=15		Met CRC N=15		Control N=30					
		N	%	N	%	N	%	N	%				
Personal History													
Age		61±9		61±8		63±9		60±9		0.7	0.51	0.61	0.532
Sex	Female	25	41.7%	5	33.3%	7	46.7%	13	43.3%	0.6	0.734	0.556	0.456
	Male	35	58.3%	10	66.7%	8	53.3%	17	56.7%				
Residence	urban	26	43.3%	5	33.3%	5	33.3%	16	53.3%	2.4	0.295	10.00	>0.999
	Rural	34	56.7%	10	66.7%	10	66.7%	14	46.7%				
occupation exposure	No	47	78.3%	13	86.7%	13	86.7%	21	70.0%	2.5	0.293	10.00	>0.999
	Yes	13	21.7%	2	13.3%	2	13.3%	9	30.0%				
BMI		31±6		31±6		31±6		31±7		0.0	0.995	0.01	0.998
Smoking	No	33	55.0%	8	53.3%	9	60.0%	16	53.3%	0.2	0.904	0.136	0.713
	Yes	27	45.0%	7	46.7%	6	40.0%	14	46.7%				

CRC: colorectal carcinoma, Met CRC: metastatic colorectal carcinoma and BMI: body mass index.

Laboratory Investigation	Total N=60	Group			Test	Sig
		CRC N=15	Met CRC N=15	Control N=30		
Hemoglobin	10.6±2.0	9.5±1.5	9.0±1.4	11.9±1.4	27.2	**<0.001**
Platelets	219±82	191±79	181±77	252±73	5.8	0.005
White blood cells	6.6 (3.9-15.0)	7.0 (3.9-14.0)	6.0 (3.9-15.0)	6.5 (4.0-12.0)	0.4	0.698

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Total Protein	6.7 (5.0-7.9)	7.0 (5.4-7.9)	6.5 (5.0-7.4)	6.6 (5.1-7.6)	2.5	0.094
Albumin	3.3±0.5	3.5±0.6	3.2±0.6	3.4±0.4	1.7	0.2
Total Bilirubin	0.9 (0.3-20.0)	0.8 (0.4-2.9)	2.8 (0.3-20.0)	0.9 (0.3-3.4)	9.4	**<0.001**
Aspartate transaminase	25 (13-264)	24 (13-82)	31 (14-264)	25 (13-56)	4.9	0.011
Alanine transaminase	21 (10-231)	21 (10-67)	22 (11-231)	20 (11-43)	4.2	0.02
Creatnine	1.1 (0.4-7.6)	1.0 (0.5-7.2)	1.3 (0.6-7.6)	1.0 (0.4-7.2)	1.4	0.247
Urea	29 (13-193)	35 (13-193)	32 (16-180)	25 (16-180)	0.9	0.418
CEA (ug /l)	19.0 (1.9-390.0)	299.0 (5.0-390.0)	273.0 (2.5-361.0)	3.5 (1.9-102.0)	47.6	**<0.001**
CA 19-9 (KIU/L)	53 (19-1350)	902 (37-1255)	834 (34-1350)	32 (19-301)	48.6	**<0.001**
ESR	48 (3-130)	106 (69-130)	103 (79-120)	11 (3-26)	574.2	**<0.001**
CRP	10 (3-110)	19 (9-70)	12 (3-110)	6 (3-12)	10.4	**<0.001**
GDF-15 (ng / ml)	2.9 (0.2-14.2)	7.2 (3.6-10.3)	8.9 (4.2-14.2)	1.7 (0.2-2.1)	74.6	**<0.001**

Table 2 : Comparison of basal Laboratory values among the studied groups

CRC: colorectal carcinoma,Met CRC: metastatic colorectal carcinoma, CEA:carcino-embryonic antigen, CA 19-9: carbohydrate antigen 19-9, ESR: erythrocyte sedimentation rate, CRP: C- reactive protein and GDF-15: Growth differentiation factor15.

Table 3: Post-hoc test using LSD and Dunn's Multiple Comparison, to indicate which groups were significantly different from each other

	Met CRC Vs. CRC	***CRC Vs. Control***	***Met CRC Vs. Control***
Hemoglobin	0.291	**<0.001**	**<0.001**
Platelets	0.719	0.013	0.004
Total bilirubin.	0.001	0.958	**<0.001**
Aspartate transaminase	0.023	0.725	0.003
Alanine transaminase	0.041	0.672	0.006
CEA (ug /l)	0.453	**<0.001**	**<0.001**
CA 19-9 (KIU/L)	0.826	**<0.001**	**<0.001**
ESR	0.767	**<0.001**	**<0.001**
CRP	0.67	0.001	**<0.001**
GDF-15 (ng / ml)	0.004	**<0.001**	**<0.001**

CRC: colorectal carcinoma, Met CRC: metastatic colorectal carcinoma, CEA: carcino-embryonic antigen, CA 19-9: carbohydrate antigen 19-9, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein and GDF-15: Growth differentiation factor 15.

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Table 4: Comparison of Ultrasonographic and CT data between CRC and metastatic CRC groups

		Total N=30		Group				X^2 Test	Sig
				CRC N=15		Met CRC N=15			
		N	%	N	%	N	%		
Abdominal ultra-sound and CT									
Liver	Normal	17	56.7%	12	80.0%	5	33.3%	38.7	**<0.001**
	cirrhotic	3	10.0%	3	20.0%	0	0.0%		
	Focal Lesion	10	33.3%	0	0.0%	10	66.7%		
Spleen	Normal	18	60.0%	13	86.7%	5	33.3%	24.5	**<0.001**
	Enlarged	12	40.0%	2	13.3%	10	66.7%		
IPFF	No	19	63.3%	13	86.7%	6	40.0%	20.6	**<0.001**
	Yes	11	36.7%	2	13.3%	9	60.0%		
Lymph Node metastasis	No	26	86.7%	15	100.0%	11	73.3%	4.6	**0.032**
	Yes	4	13.3%	0	0.0%	4	26.7%		
peritoneal metastasis	No	28	93.3%	15	100.0%	13	86.7%	2.1	0.143
	Yes	2	6.7%	0	0.0%	2	13.3%		
Visualized mass	No	26	86.7%	14	93.3%	12	80.0%	1.2	0.283
	Yes	4	13.3%	1	6.7%	3	20.0%		
Hepatic Metastasis	Normal	20	66.7%	15	100.0%	5	33.3%	15.0	**<0.001**
	Focal Lesion	10	33.3%	0	0.0%	10	66.7%		
CT Chest	No abnormalities	21	70.0%	12	80.0%	9	60.0%	6.4	0.093
	effusion	4	13.3%	3	20.0%	1	6.7%		
	focal lesion	2	6.7%	0	0.0%	2	13.3%		
	focal lesion+ effusion	3	10.0%	0	0.0%	3	20.0%		
CT Brain (focal lesion)	No	29	96.7%	15	100.0%	14	93.3%	1.0	0.309
	Yes	1	3.3%	0	0.0%	1	6.7%		
Bone Metastasis.	No	29	96.7%	15	100.0%	14	93.3%	1.0	0.309
	Yes	1	3.3%	0	0.0%	1	6.7%		

CRC: colorectal cancer, Met CRC: metastatic colorectal cancer, IPFF: intra peritoneal free fluid and CT: computerized tomography.

Table 5: Comparison of endoscopic Findings betweenCRC and metastatic CRC groups

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Colonoscopy finding		*Total N=30*		*CRC*				X^2 *Test*	*Sig*
				CRC N=15		Met CRC N=15			
		N	%	N	%	N	%		
Primary tumor	Resected	14	46.7%	5	33.3%	9	60.0%	2.1	0.143
	Present	16	53.3%	10	66.7%	6	40.0%		
Malignant Features	No	5	16.7%	3	20.0%	2	13.3%	0.2	0.624
	Yes	25	83.3%	12	80.0%	13	86.7%		
Polyps	No	7	23.3%	3	20.0%	4	26.7%	0.2	0.666
	Yes	23	76.7%	12	80.0%	11	73.3%		
Site	Rectum	8	26.7%	4	26.7%	4	26.7%	0.01	>0.999
	Left Colon	16	53.3%	8	53.3%	8	53.3%		
	Right Colon	6	20.0%	3	20.0%	3	20.0%		

CRC: colorectal carcinoma and Met CRC: metastatic colorectal carcinoma.

Table 6: Comparison of Histopathological examination between CRC and metastatic CRC groups

Histopathological examination		*Total N=30*		*CRC*				X^2 *Test*	*Sig*
				CRC N=15		Met CRC N=15			
		N	%	N	%	N	%		
Stage	I	4	13.33%	4	26.7%	0	0.0%	30.0	<0.001
	II	11	36.66%	11	73.3%	0	0.0%		
	III	0	0.0%	0	0.0%	0	0.0%		
	IV	15	50.0%	0	0.0%	15	100.0%		
Degree of differentiation	Poorly	8	26.7%	4	26.7%	4	26.7%	0.0	>0.999
	Well/Moderate	22	73.3%	11	73.3%	11	73.3%		
Type	Mucinous	2	6.7%	1	6.7%	1	6.7%	0.0	>0.999
	adenocarcinoma	28	93.3%	14	93.3%	14	93.3%		

CRC: colorectal carcinoma and Met CRC: metastatic colorectal carcinoma.

Table 7: Correlations between serum GDF-15 ng/ml level and certain studied parameters within each group of CRC

Spearman's rho	*GDF-15 (ng / ml)*			
	CRC N=15		Met CRC N=15	
	Correlation Coefficient	Sig. (2-tailed)	Correlation Coefficient	Sig. (2-tailed)
Age	-0.052	0.854	0.343	0.211
Body mass index	0.224	0.423	0.206	0.462
Hemoglobin	0.413	0.126	-0.501	0.057
White blood cells	0.282	0.308	-0.222	0.426
Platelets	0.382	0.159	-0.190	0.498

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Total Protein	0.301	0.276	-0.211	0.45
Albumin	0.380	0.162	-0.227	0.415
Total bilirubin	-0.323	0.241	0.632	0.011
Aspartate transaminase	-0.121	0.666	0.061	0.829
Alanine transaminase	-0.172	0.541	0.154	0.584
Creatnine	0.018	0.95	-0.243	0.383
Urea	-0.063	0.825	-0.234	0.401
CEA (ug /l)	0.668	0.007	0.518	0.048
CA 19-9 (KIU/L)	0.586	0.022	0.461	0.084
ESR	0.552	0.033	0.404	0.135
CRP	0.350	0.201	-0.061	0.83

GDF-15: growth differentiation factor 15, CRC: colorectal carcinoma, Met CRC: metastatic colorectal carcinoma, CEA: carcino-embryonic antigen, CA 19-9: carbohydrate antigen 19-9, ESR: erythrocyte sedimentation rate and CRP: C-reactive protein.

Table 8: The validity of serum GDF-15 ng/ml levelwith areaundertheROCcurve(AUC) as a diagnostic marker for CRC

Cut-off	*Sensitivity % 95% CI*	*Specificity % 95% CI*	*PPV 95% CI*	*NPV 95% CI*	*AUC 95% CI*	*Z*	*P*
>2.1	93.4 77.9 - 99.2	93.2 77.9 - 99.1	93.1 78.5 - 98.1	93.3 78.5 - 98.2	0.89 0.788 - 0.959	7.6	<0.001

CI: 95% confidence interval,Positive predictive value (PPV), negative predictive value (NPV) and Area under the ROC curve (AUC)

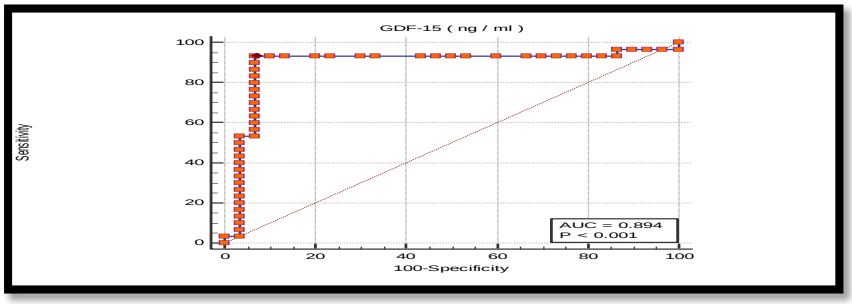

Figure (1): The validity of serum GDF-15 ng/ml levelwith area under the ROC curve (AUC) as a diagnostic marker for CRC

DISCUSSION:

CRC is the on the third rank among the top five common cancers and the second in terms of mortality **(11)**. In Egypt, it is the 7th commonly diagnosed malignancy representing 3% and 3.47% in females and males respectively (**12)**. A biomarker is a biological molecule measured in body fluids and it is a marker for physiological or pathological condition such as cancer. It may be a protein, an antibody, a nucleic acid, a peptide, or a lipid. It can be used for early diagnosis and prognosis of cancer **(13).** Due to the highly heterogeneous nature of CRC, a single tumor marker is unlikely to represent an accurate diagnostic standard with sufficient sensitivity or specificity for all cases, So, to date, the search for novel biomarkers is essential in CRC research (**14)**.

Our study is a case control study conducted at faculty of medicine, Zagazig university, and included 60 participants classified into twogroups as the following: Group I included 30 patients subdivided into two subgroups: I(A) included 15 non-metastatic CRC patients, I(B) included 15 metastatic CRC patients and Group II included 30 healthy subjects as a control group. According to socio-demographic data (Age, sex, residency, occupation exposure, BMI and smoking), there were no statistically significant differences among the three studied groups. This result was consistent with a

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previous study by **Mehta et al., (15)** who had similar classification of CRC patients and control subjects.

Our results showed that,metastatic CRC group had higher activities of liver enzymes (ALT and AST) and Bilirubin but they had lower levels of serum albumin, Hb, lymphocytes and platelet count compared non-metastatic CRC patients and healthy subjects. There were statistically significant differences among the three studied groups regarding hemoglobin, platelets, total bilirubin, AST, ALT, CEA, CA 19-9, ESR, CRP and GDF -15 ($P< 0.001$, 0.005, < 0.001, 0.011, 0.02, < 0.001, < 0.001, < 0.001, < 0.001, < 0.001) respectively. There were no statistically significant differences among the three studied groups regarding other laboratory values.

Higher activity of ALT and AST not only refer to a problem in liver but also may refer to other diseases for example sympathies where ALT and AST are present in skeletal muscle in addition to liver **(16)**. Additionally, the elevation activity of ALT and AST may be a sign of liver metastasis where previous studies like **Wu et al., (17)**, **Ojo et al., (18)** and **Fathy et al., (19)** showed a significant increase ($P < 0.05$) in ALT and AST activities in metastatic CRC as about 50% of CRC patients will develop a metastatic disease and the most commonly affected organ by metastases in CRC is liver. The reason of low albumin level is malnutrition as it is a common problem in CRC patients due to low food intake, the higher rates of metabolism stimulated by cancer, or failure synthesis of albumin in liver **(20, 21)**.

In the current study, platelet count was significantly decreased in metastatic CRC patients compared to other groups ($P= 0.005$), a finding that was shared by other authors who showed that that platelet count decreased in metastatic CRC patients compared to normal subjects and patients with benign colorectal growth ($P< 0.05$) **Ahmed and Gupta, (22); Qian et al., (23)**. On the other hand, **Zhu et al. (24)** displayed significantly higher platelets counts among their patients with metastatic CRC, and this could be attributed to angiogenesis, metastasis and tumorigenesis. The contradictory result gained from our studymaybe due tomore advanced tumor grades and stages of our patientswho received chemotherapy and was treated with some drugs that led to drop of platelets count (thrombocytopenia).

Our results agreed with **Vocka et al., (25)** and revealed that serum GDF-15 levels in metastatic CRC patients were higher than those in non-metastatic CRC patients and healthy controls with higher statistical significance among the three groups. Among participants of our study, the serum GDF-15 in metastatic CRC group was higher than serum GDF-15 of non-metastatic CRC group than healthy control group with median 8.9 ng / ml, 7.2 ng / ml, and 1.7 ng / ml, respectively. There is a significant positive relationship between serum GDF-15 level and each of clinical stage, presence of metastasis and progression of CRC **(19)**.

Unlike to several studies, our results showed over expression of CEA and CA19-9 in non-metastatic CRC patients' group more than metastatic CRC patients' group and healthy individual. **Vocka et al., (25)** showed serum elevation of CEA and CA19-9 in metastatic CRC patients than control group. **Luo et al., (6)** also reported that the rate of positivity for CEA were significantly higher in patients with distant metastasis compared with those without distant metastasis. Although the CEA and CA19-9 are not suitable biomarkers for detection of CRC at early stages, they are still the markers of choice (especially CEA) for monitoring cancer progression **Gonzalez-Pons and Cruz-Correa (26)** until the perfect biomarker is discovered.

The elevation of CEA and CA 19-9 levels after CRC surgery is a marker for metastasis. CEA and CA 19-9 must be evaluated every 3 months (first 3 years) and every 6 months (after 3 years) and this is a gold standard method for proceeding after CRC. Also, the levels of CEA and CA 19-9 increase due to chemotherapy, so they must be evaluated every 6 weeks in patients on chemotherapy **(27).**

There were statistically significant differences between CRC and metastatic CRC groups regarding imaging of the liver, splenomegaly, IPFF, lymph node metastasis and hepatic metastasis ($P< 0.001$, < 0.001, < 0.001, 0.032 and< 0.001) respectively. There were no statistically significant differences between CRC and metastatic CRC groups regarding other imaging data in ultrasound and CT. Colonoscopy findings of the current study showed that 14 patients had their primary colonic tumor resected (46.7%). There is an obvious need for prognostic and predictive markers to determine the risk of recurrence early after liver resection and to commence more comprehensive follow-up and possibly more aggressive adjuvant treatment for patients at high risk. It would be valuable if measurement of serum or plasma concentrations of tumor markers could be used to predict the outcome of metastatic CRC after curative resection **(28)**.

Twenty-five patients presented with malignant features in colonoscopy (83.3%) and 23 patients had polyps (76.7%). Site of colorectal mass was rectum in eight patients (26.7%), left colon in 16

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patients (53.3%) and right colon in six patients (20%). There were no statistically significant differences between CRC and metastatic CRC groups regarding all colonoscopy findings.

Due to the high association between GDF-15 and the TNM staging and histological grading as well as the presence of metastasis, all CRC patients included in the current work were classified according to histopathological examination into four patients with stage I (13.33%), 11 patients with stage II (36.66%) and 15 patients with stage IV (50%). According the degree of differentiation, there were eight patients with poorly differentiated carcinoma (26.7%),and 22 patients had moderate to well differentiated carcinoma (73.3%). Twenty-eight patients had adenocarcinoma type (93.3 %) and only two had mucinous type (6.7%).

There was statistically significant difference between CRC and metastatic CRC groups regarding staging (P< 0.001). There were no statistically significant differences between the two groups regarding other histopathological examination.

Direct comparison with data from other studies is very difficult due to small number of metastatic CRC patients in earlier studies and only limited information about results of such patients separately. **Vocka et al., (25)** publishedlevels of GDF-15 in metastatic CRC patients (146.5 pg/ml), but only in 8 cases and it was measured in plasma. All studies with patients in stage IV did not present any data about the site of distant metastasis and none compared GDF-15 with standard tumor markers (CEA and CA19-9). on comparing GDF-15 with other parameters in non-metastatic colorectal carcinoma group there were direct significant correlation between GDF-15 and each of CEA, CA 19-9 and ESR (P= 0.007, 0.022 and 0.033 respectively). In metastatic colorectal carcinoma group, on comparing GDF-15 with other parameters, there were direct significant correlation between GDF-15 and each of total bilirubin and CEA (P= 0.011 and 0.048 respectively). This result was consistent with that of **Brown et al., (29)** who also showed a strong positive correlation between the serum MIC-1 (GDF-15) level and CEA (P 0.01; r = 0.765 using log serum MIC-1 and CEA).Similarly, **Vocka et al., (25); Gao et al., (30)** showed that GDF15 is an effective biomarker in patients with metastatic CRC and provides the same sensitivity as CEA giving an extra indication regarding liver metastasis.

In addition, the detection of GDF15 has some practical advantages. There were no significant differences in GDF15 levels between various components of blood, and samples need no special treatment. In serial studies, the level of GDF15 was relatively stable, comparable and reliable. The means of GDF15 detection in serum was feasible, convenient and low cost. Even so, there were several deficiencies in standard and quality assurance among different studies. Well-designed prospective studies and larger scale measurements of GDF15 are required to evaluate the value of GDF15 in CRC **(27)**.

CONCLUSION:

We conclude that high Growth differentiation factor 15 (GDF-15) can act as a valuable independent biomarker for screening CRC in comparison with CEA and other tumor biomarkers. Furthermore, an elevated GDF-15 in a cutoff value can identify CRC metastasis especially to the liver metastasis.

No Conflict of interest.

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