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Value of Serum Growth Differentiation Factor 15 in diagnosis of Colorectal Cancer

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ABSTRACT

Background: Colorectal Cancer (CRC) is considered the third most deadly and fourth most commonly diagnosed cancer in the world.

Objective: The aim of the present study was to compare serum levels of growth differentiation factor-15 (GDF-15) in patients with CRC and in those healthy control subjects.

Patients and methods: The study included 60 subjects that were divided in two groups: Group I included 30 patients diagnosed with colorectal cancer and group II that included 30 healthy volunteers as control group. They didn`t have any acute or chronic diseases. All subjects of this study were subjected to full history taking, clinical examination and laboratory investigations. The study was conducted at Internal Medicine Department (gastroenterology and endoscopy unit), Faculty of Medicine, Zagazig University.

Results: 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 suspicious occupational exposure (21.7%) and 27 were smokers (45%). Mean BMI of all participants was 31 ± 6 kg/m^2 with no statistically significant differences between the studied groups. Regarding Hb level and GDF-15, there were statistically significant differences between CRC group and control group where Hb was higher in the control group, while GDF-15 was higher in CRC group.

Conclusion: Growth differentiation factor 15 (GDF-15) could be used as a valuable independent biomarker for screening CRC.

Keywords: Colorectal Cancer, GDF-15, CRP, CT scan.

INTRODUCTION

Colorectal cancer is the third most common malignancy in men (10%) and the second most common cancer in women (9.2%) worldwide **(1).** Early detection and diagnosis improve outcomes and give chance for effective and successful management. Early detection of hepatic metastasis is an indication for surgical intervention. Therefore, novel biomarkers for prompt detection of cancer and screening of metastatic disease are strongly needed **(2).** It has been reported that the level of GDF-15 is markedly elevated in malignant tissues and cancer lesions as compared to non-malignant normal tissues, and with basal GDF-15 serum level. Many studies show the important role of GDF-15 in CRC **(3).**

GDF-15 dysregulation is involved in the progression of colon cancer, and the serum level of GDF-15 is gradually elevated with progression of adenomatous polyps to colorectal carcinoma. A significant correlation was observed between serum GDF-15 level and each of tumor stage and progression of CRC to metastatic disease **(4).**

Therefore, this study aimed to compare serum levels of GDF-15 in patients with CRC and those healthy control subjects.

PATIENTS AND METHODS

The study was conducted 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, Zagazig University.

The study included 60 subjects that were divided in two groups: Group I included 30 patients diagnosed with colorectal cancer and group II, which included 30 healthy volunteers as control group. They didn`t have any acute or chronic diseases.

Inclusion criteria: Adult patients in age above 18 years old, both male and female who were diagnosed with colorectal carcinoma.

Exclusion criteria: Patient with other primary malignant tumors, patient with inflammatory bowel disease and patient refusal.

Operative Assessment: All subjects of this study were subjected to full history taking and complete clinical examination.

Routine laboratory investigations including:

1) Complete blood count (CBC): by automated cell counter "Sysmex XS" (Sysmex Corporation, Japan).
2) Liver function tests: serum bilirubin (total and direct), serum albumin, serum alanine transferase and aspartate transferase measured by kinetic method.
3) Kidney function test: serum creatinine and serum urea.
4) Coagulation Profile.

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5) Erythrocyte sedimentation rate (ESR).
6) Qualitative C- Reactice Protein (CRP).

Special laboratory investigation including:

Measurement of serum GDF-15 level by ELISA:

GDF15 was measured using the GDF15 direct enzyme linked immunosorbent assay Kit (Shino-Test Corporation, Kanagawa, Japan) through following the manufacturer's instructions [(5)].

Radiological investigation were performed using CT scan or MRI for primary colorectal cancer.

Ethical approval:

The study was approved by the Ethical Committee of Zagazig Faculty of Medicine. An informed consent was obtained from every patient in this research. Every patient received an explanation for the purpose of the study. All given data were used for the current medical research only. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

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) and categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). Continuous data were checked for normality by using the Shapiro- Wilk test. Independent samples Student's t-test was used to compare between two groups of normally distributed data. Chi square test was used for qualitative variables not normally distributed. One Way ANOVA test was used to compare between more than two groups of normally distributed data while Kruskal- Wallis H test was used for quantitative variable, which are not normally distributed (skewed distribution). Levene's test was used to determine the homogeneity of variance. A post - Hoc test was done using either LSD or Tamhane's T2 methods according to homogeneity of variance. All tests were two-sided, p-value $\leq$ 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS) and p-value $\geq$ 0.05 was considered statistically non-significant (NS).

RESULTS

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 suspicious occupational exposure (21.7%) and 27 were smokers (45%). Mean BMI of all participants was 31 ± 6 kg/m^2 with no statistically significant differences among the studied groups regarding all recorded socio demographic data (Table 1).

Regarding clinical presentation, there was a statistically significant difference between CRC group and control (Table 2).

Regarding laboratory investigation, there was a statistically significant difference in the values of Hb, CEA, CA 19-9, ESR and CRP between CRC group and control (Table 3).

Regarding CRC group, table **(**4) showed that there were 10 patients had focal lesions in the liver (33.3%) and three patients had cirrhotic liver (10%) but others had normal liver by imaging (56.7%). There were 12 patients had enlarged spleen (40%) and 11 patients had inra-peritoneal free fluid (36.7%).

Serum values of GDF-15 was highly significantly higher in CRC group compared to control group (Table 5).

Table (1): Comparison of socio-demographic data between the studied groups

		Total		Groups				Chi-square Test	Sig
				Control		CRC			
		N	%	N	%	N	%		
Personal History									
Age		61±9		60±9		62±9		-0.9	0.371
Sex	Female	25	41.7%	13	43.3%	12	40.0%	0.1	0.793
	Male	35	58.3%	17	56.7%	18	60.0%		
Residence	Urban	26	43.3%	16	53.3%	10	33.3%	2.4	0.118
	Rural	34	56.7%	14	46.7%	20	66.7%		
Occupation exposure	No	47	78.3%	21	70.0%	26	86.7%	2.5	0.117
	Yes	13	21.7%	9	30.0%	4	13.3%		
BMI		31±6		31±7		31±6		-0.2	0.841
Smoking	No	33	55.0%	16	53.3%	17	56.7%	0.1	0.795
	Yes	27	45.0%	14	46.7%	13	43.3%		
Medical History									
DM	No	36	60.0%	18	60.0%	18	60.0%	0.0	>0.999
	Yes	24	40.0%	12	40.0%	12	40.0%		
HTN	No	37	61.7%	21	70.0%	16	53.3%	1.8	0.184
	Yes	23	38.3%	9	30.0%	14	46.7%		
Hepatic	No	53	88.3%	28	93.3%	25	83.3%	1.5	0.228
	Yes	7	11.7%	2	6.7%	5	16.7%		
Renal	No	52	86.7%	28	93.3%	24	80.0%	2.3	0.129
	Yes	8	13.3%	2	6.7%	6	20.0%		
COPD	No	47	78.3%	26	86.7%	21	70.0%	2.5	0.117
	Yes	13	21.7%	4	13.3%	9	30.0%		
Cardiac	No	46	76.7%	26	86.7%	20	66.7%	3.4	0.067
	Yes	14	23.3%	4	13.3%	10	33.3%		
Inflammatory bowel disease (IBD)	No	44	73.3%	29	96.7%	15	50.0%	16.7	<0.001
	Yes	16	26.7%	1	3.3%	15	50.0%		

Table (2): Clinical Presentation between between the studied groups

		Total		groups				Chi-square Test	Sig
				Control		CRC			
		N	%	N	%	N	%		
Presentation									
Asymptomatic	No	29	48.3%	0	0.0%	29	96.7%	56.1	<0.001
	Yes	31	51.7%	30	100.0%	1	3.3%		
Abdominal pain	No	58	96.7%	30	100.0%	28	93.3%	2.1	0.15
	Yes	2	3.3%	0	0.0%	2	6.7%		
Bleeding per rectum	No	44	73.3%	30	100.0%	14	46.7%	21.8	<0.001
	Yes	16	26.7%	0	0.0%	16	53.3%		
Constipation	No	56	93.3%	30	100.0%	26	86.7%	4.3	0.038
	Yes	4	6.7%	0	0.0%	4	13.3%		
Diarrhea	No	58	96.7%	30	100.0%	28	93.3%	2.1	0.15
	Yes	2	3.3%	0	0.0%	2	6.7%		
Weight loss	No	57	95.0%	30	100.0%	27	90.0%	3.2	0.076
	Yes	3	5.0%	0	0.0%	3	10.0%		

Table (3): Comparison of Laboratory values between the studied groups

	Total		Groups				Chi-square Test	Sig
			Control		CRC			
	N	%	N	%	N	%		
Laboratory investigation								
Hb (g/dL)	10.6±2.0		11.9±1.4		9.3±1.5		-5.4	**<0.001**
PLT (mcL)	219±8		252±7		186±7		-2.7	0.007
WBCS (mcL)	6.55 ± 1.44		4.86 ± 0.52		4.85 ± 0.37		-0.2	0.83
Total protein (g/dL)	8.31 ± 1.89		7.54 ± 2.43		7.50 ± 1.60		-0.3	0.784
Albumin (g/L)	3.3±0.5		3.4±0.4		3.3±0.6		-0.1	0.894
Total bilirubin (µmol/L)	6.33 ± 1.82		4.42 ± 0.72		3.22 ± 0.44		-1.1	0.26
AST (U/L)	5.88 ± 1.42		5.37 ± 1.77		4.37 ± 1.32		-1.0	0.318
ALT (U/L)	5.22 ± 1.72		4.36 ± 1.40		4.19 ± 1.37		-0.8	0.398
Creatnine (mg/dL)	5.03±1.36		4.09±1.07		3.01±0.12		-1.3	0.205
Urea (mg/dl)	6.98 ± 1.21		5.05 ± 1.25		4.82 ± 0.85		-2.0	0.047
Carcinoembryonic Antigen (CEA) (ug/L)	6.13±1.23		4.09±1.07		3.23±0.12		-6.1	**<0.001**
CA 19-9 (U/mL)	7.38 ± 1.13		5.69 ± 1.67		4.87 ± 1.44		-6.3	**<0.001**
ESR (mm/hr)	11.32±2.78		8.15±2.23		7.45±1.89		-6.7	**<0.001**
CRP (mg/L)	10.42±2.99		9.58±1.13		8.36±1.67		-5.4	**<0.001**

Table (4): Ultrasonographic and CT data between studied groups

Radiology		**Total**		**group**				**Chi-square Test**	**Sig**
				Control		**CRC**			
		N	**%**	**N**	**%**	**N**	**%**		
Abdominal ultra-sound and CT									
Liver	Normal	45	75.0%	28	93.3%	17	56.7%	12.9	0.002
	cirrhotic	5	8.3%	2	6.7%	3	10.0%		
	Focal Lesion	10	16.7%	0	0.0%	10	33.3%		
Spleen	Normal	47	78.3%	29	96.7%	18	60.0%	11.9	0.001
	Enlarged	13	21.7%	1	3.3%	12	40.0%		
Intra-peritoneal free fluid	No	48	80.0%	29	96.7%	19	63.3%	10.4	0.001
	Yes	12	20.0%	1	3.3%	11	36.7%		

Table (5): Serum values of GDF-15 ng/ml between studied groups

	Total		**Group**				**Chi-square Test**	**Sig**
			Control		CRC			
	N	%	N	%	N	%		
GDF-15 (ng / ml)	2.9 (0.2-14.2)		1.7 (0.2-2.1)		7.7 (3.6-14.2)		-6.7	**< 0.001**

DISCUSSION

For patients with CRC, survival time is significantly dependent on cancer stage upon diagnosis. Therefore, improving the CRC prognosis depends upon early and accurate diagnosis [(6)]. Currently, CRC colonoscopy combined with pathological biopsy is the most accurate method of diagnosis. However, these tests are invasive and expensive, carry potential life threatening complications, and patient compliance to them is poor [(7)]. Therefore, alternative cost-effective, non-invasive, easily measurable, and accurate screening procedures are urgently required for CRC screening. Thus, the clinical applications of biomarkers in CRC are not only needed for the early detection of the disease but also are essential for prognostic stratification, surveillance, and therapy selection. The increasing emergence of adjuvant and neoadjuvant therapy approaches results in an urgent need for predictive biomarkers that guide the decision-making process [(8)]. 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 CEA, CA 19 9, CA125 and CA242 [(9)].

CEA and CA19-9 are the most common biomarkers for CRC detection despite they had low sensitivity and specificity. Interestingly, GDF-15 levels are substantially increased in various pathological conditions, including inflammation and injury. Notably, experimental and epidemiological evidence has demonstrated that GDF-15 levels are up-regulated in many types of digestive system tumors, such as CRC [(10)]. GDF-15 has received much attention as a diagnostic and prognostic biomarker in CRC. So, we aimed in this study to evaluate the serum levels of GDF-15 in the patients with colorectal cancer as compared to the healthy controls.

Our study is a case-control study conducted at Faculty of Medicine, Zagazig University, and included 60 participants classified into two equal groups: Group I included CRC patients, and Group II included healthy subjects as a control. According to socio-demographic data in our study, there were no statistically significant differences between the two studied groups. This result is consistent with a previous study by **Mehta *et al.*** [(11)] who had similar classification of CRC patients and control subjects and showed that the associations did not significantly change according to subgroups as regards age, BMI, smoking status, family history of CRC, history of polyps, and history of screening.

Regarding the medical history, there was statistically significant difference between the two studied groups concerning history with inflammatory bowel disease (IBD) ($P < 0.001$), highlighting the increased risk of CRC in IBD but there was no significant association in terms of IBD between CRC patients' group and those with metastatic characters. Otherwise, there were no statistically significant differences in DM, HTN, hepatic diseases, renal diseases, COPD, and cardiac history between the two studied groups. Several studies reported that patients with IBD are at significantly increased risk of CRC, principally resulting from the pro-neoplastic effects of chronic intestinal inflammation. Epidemiologic studies continue to highlight the increased risk of CRC in IBD [(12)].

Laboratory investigations including liver function tests (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), albumin and bilirubin), kidney function tests (urea and creatinine), complete blood counts, some tumor biomarkers (CEA, CA 19-9 and GDF-15) and inflammatory marker (ESR and CRP) were done for all serum samples of the included subjects. There were statistically significant differences between the two groups regarding hemoglobin, CEA, CA 19-9, ESR and CRP. **Hu *et al.*** [(13)] found a low level of albumin < 3.5 g/dl (hypoalbuminemia) in metastatic CRC patients compared to healthy subjects. Previous study showed elevation of C-reactive protein (CRP) concentration leading to hypoalbuminemia and this happens in many cancers including CRC [(14, 15)]. Anemia is a common prognostic factor in patients with CRC especially iron deficiency anemia whereas one of the most common symptoms of CRC is hemorrhage as blood appears in stool of patient, so the Hb level and RBC counts become decreased and leading to anemia [(16, 17 and 18)]. Besides, **Borgaonkar *et al.*** [(19)], **Schneider *et al.*** [(20)] and **Wilson *et al.*** [(21)] showed that Hb level and RBCs count are decreased in CRC patients group compared to healthy group.

In our study, there were statistically significant differences between CRC and control group regarding imaging of the liver, splenomegaly, intra-peritoneal free fluid (IPFF). Serum values of GDF-15 ng/ml showed a significant increase in CRC group compared to control group. These results agree with studies that identified serum GDF-15 as a new marker for colon cancer when compared CRC versus controls [(3, 11)].

GDF-15 dysregulation is involved in the progression of colon cancer, and research showed that the GDF-15 levels in serum gradually increase in the process of conversion from adenomatous polyps to colorectal carcinoma [(22)].

CONCLUSION

Growth differentiation factor 15 (GDF-15) could be used as a valuable independent biomarker for screening colorectal cancer (CRC).

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