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Research Article

Study of Prognostic Value of Some Biomarkers in Colorectal Egyptian Patients

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ABSTRACT

This study included 89 colorectal patients and 50 healthy subjects served as controls. The serum levels of CXCL5, Cy-C, NGAL, MMP-9 and PDCD4 were measured in serum using enzyme-linked immunosorbent assay technique. Our results showed that serum CXCL5, Cy-C, NGAL and MMP-9 were significantly elevated in colorectal patients in comparison to healthy controls and this increase decreased after treatment, the elevation correlated positively with stage and progression of disease. Serum PDCD4 was significantly elevated in early stage group [group I] in comparison to controls while in late stage group [group II] its level was significantly low in comparison to healthy controls and group I. It correlated negatively with stage and progression of disease. In conclusion; Serum CXCL5, Cy-C, NGAL and MMP-9 were elevated in patients and it correlated positively with progression of disease so they could serve as a prognostic markers for colorectal cancer patients while PDCD4 decreased with progression of tumor and correlated negatively with stage; so these markers might have a role in the progression of colorectal cancer and the development of metastasis, they can be used to choose patients with a higher risk and indicates what therapies are useful for them.

Key words: Colorectal cancer, CXCL5, Cy-C, NGAL, MMP-9, PDCD4

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers in the world. It is a leading cause of deaths among cancers. The mortality rate due to colorectal cancer has not although cancer research showed much progress[1]. Several molecules have been shown to contribute to tumor invasion and spreading. Chemokines are group of small molecular weight proteins that bind to the G protein to receptors of chemokines. Chemokines play an important role in cell migration, development, immune surveillance, inflammation as well as in many pathological conditions[2]. Chemokines play a major part in regulating immune response by their involvement in the regulation of leukocyte trafficking and positioning. The binding of the chemokines to the receptors leads to a conformational change, which activates signaling pathways and promotes migration. Chemokines play an important part in cancers dissemination. They are involved in growth of cancer, formation of angiogenesis, their transition, metastasis and invasion. The formation of chemokines and their receptors is changed in many malignancies and subsequently leads to altered chemokine receptor signaling. This alteration occurs due to desregulation of the tumor suppressor genes or over activation of the oncogenes that play a role in the regulation of the chemokines[3,4]. CXCL5 is angiogenic chemokine that promote tumor growth and metastases formation. Although, metastasis is a complex process involving various factors and small molecule regulators, studies have suggested that chemokines and their receptors play a key role in metastasis[5]. Glycoproteins play an important part in the body's defense against many diseases. Lipocalins is a protein family, NGAL is a protype of lipocalins has been known as a biomarker in several benign and malignant diseases. It can be used as biomarker for diagnosis, follow-up and prognosis in cancer diseases. NGAL is expressed in several malignant diseases as the breast, thyroid, ovary[6], colon[7-9], lung[10], bile ducts, liver[8], stomach[9] and pancreas[8,11]. NGAL has been identified as a gene that was specifically and significantly overexpressed in breast cancers overexpressing the receptor tyrosine kinase HER-2 (or neu), hence the name Neu Related Lipocalin for NGAL[12]. Apoptosis is controlled by two main pathways; occurrence of apoptosis is happened through many upstream and downstream signals. Changes in the apoptotic pathways are complicated, with genetic alterations of the upstream regulatory proteins that can be targets for many drugs. Programmed cell death 4 (PDCD4) is a tumor suppressor gene which has been involved in the apoptotic mechanism and progression of tumors by interacting with translation initiation factors eIF4A and eIF4G. PDCD4 protein expression is down-regulated in human malignancies. Some mechanisms are involved in PDCD4 dysregulation; the oncogenic microRNA miR- 21 has been shown to specifically target the PDCD4 3′ untranslated region, which negatively regulates PDCD4

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Table 1: Clinicopathological characteristics of the studied colorectal cancer patients and controls

Variables	Patients	controls
Numbers	89	50
Gender	60	30
Male Female	29	20
Age (years) < 60 > 60	40	23
	49	27
T. stage T1,T2,T3,T4	60	-
	29	-
LN metastasis – ve + ve	51	-
	38	-
Distant metastasis – ve + ve	70	-
	19	-

expression. Inactive IBDs, miRNA expression profiling consistently features disease-specific signatures, and miR-21 has been pinpointed as one of the most dysregulated genes. PDCD4 is involved in IBD-associated colon carcinogenesis. Nuclear down-regulation of PDCD4 expression is controlled by IBD and IBD- associated intraepithelial neoplasia; in histology doubtful cases (differential diagnosis between regenerative and dysplastic lesions), nuclear protein down-regulation can be considered as supporting information in favor of a neoplastic intraepithelial lesion[13-16]. Matrix Metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes that cleave extracellular matrix proteins. Extracellular matrix proteins degradation has an important role in physiologic in process of embryonic development reproduction and wound healing. Also it has a role in pathogenesis of arthritis, tumor progression and metastasis. MMPs affects several molecular processes involved in cancer outcome through cleavage of proapoptotic factors, cell surface molecules, cell adhesion molecules and growth factors. MMPs can mobilize proangiogenic inhibitors, such as endostatin and angiostatin[17]. Cancer cells can produce matrix metalloproteinase -9 (MMP-9) which is a proteolytic enzyme that has the ability to degrade basement membrane and type IV collagen in cells that is important for tumor progression and metastases formation. It regulates angiogensis in cancer, positively and negatively through activation of proangiogenic factors and production of angiogenic inhibitors[18]. The aim of this work was to measure serum CXCL5, Cy-c, NGAL, PDCD4 and MMP9 in group of patients suffering from colorectal cancer (CRC) to assess their influence on disease outcome.

PATIENTS AND METHODS

This study was conducted on 89 patients with colorectal cancer their age ranged between 45-70 years beside 50 healthy persons served as control group. All patients were classified according to UICC stage classifications using resected specimens[11]. The patients were classified according to TNM stage classifications (stages I, II, III and IV). Stages I and II concerned as early stage (group I) and stages III and IV concerned as late stage (group II). Blood samples were obtained by venipuncture before and after treatment. Each sample was centrifuged at 3000g for 5 min then frozen at 80 °C until analysis. The study was conducted after the approval of research ethical committee and informed written consents were obtained from all participants before serum collection.

-Serum CXCL5 level was assayed by enzyme-linked immunosorbent assay (ELISA), Quantikine Human ENA-78; R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions.

-Serum NGAL was assayed by enzyme-linked immunosorbent assay (ELISA) catalog number SK00233-01, Aviscera Bioscience in accordance with the manufacturer's instructions.

-Serum PDCD4 was assayed by enzyme-linked immunosorbent assay (ELISA) catalog number MBS941857, MyBiosource in accordance with the manufacturer's instructions.

-Serum MMP9 was measured in sera using enzyme-linked immunosorbent assay kit (ELISA) R&D systems, MN, USA.

-Statistical analysis was performed using the SPSS software package for Windows. ANOVA was used to determine the difference between the means of the groups. Further analysis was carried out using a non- parametric test for two independent samples (Mann- Whitney U test),

Table 2: Median levels of investigated biomarkers among studied groups.

	CXCL5 (U/L)	PCD4 (U/L)	Cy-C (U/L)	NGAL (U/L)	MMP9(U/L)
Control *versus* CRC patients	0.5[a]	3.3[a]	2.9[a]	77[a]	333[a]
Control (n= 50)	1	4.36	3.46	229	836.8
CRC patients (n=92)					
Control *versus* Clinical stages	0.5[a]	3.3[a]	2.9[a]	77[a]	333[a]
before treatment	1.55	9.78	4.23	273	838.6
Control (n= 50)	3.75	3.95	9.64	773	985.4
Early stage (n= 25)					
Late Stage (n= 21)					
Control *versus* Clinical stages	0.5[a]	3.3[a]	2.9[b]	77[a]	333[a]
after treatment	0.62	4.4	3	110	550
Control (n= 50)	0.65	2.6	3.3	174	833
Early stage (n= 25)					
Late Stage (n= 21)					

Statistical analysis using ANOVA test showed [a] significant at $P<0.0001$, [b] Non-significant

Table 3: Correlation between investigated biomarkers among CRC cancer patients.

	CXCL5 (U/L)	PCD4 (U/L)	Cy-C (U/L)	NGAL (U/L)	MMP9(U/L)
CXCL5 (U/L)	---	0.185	0.738	0.638	0.401
CC	---	0.078	<0.0001	<0.0001	<0.0001
P-value					
PCD4 (U/L)	-0.738	---	0.164	0.123	0.163
CC	<0.0001	---	0.119	0.244	0.122
P-value					
Cy-C (U/L)	0.738	0.164	---	0.704	0.468
CC	<0.0001	0.119	---	<0.0001	<0.0001
P-value					
NGAL (U/L)	0.638	0.123	0.704	---	0.335
CC	<0.0001	0.244	<0.0001	---	0.001
P-value					

Significant P value <0.05

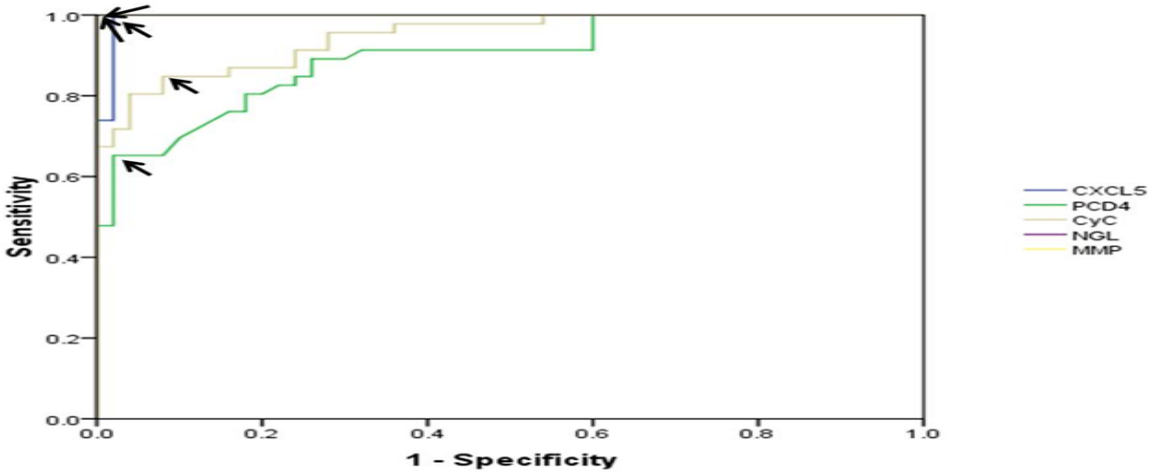

Figure 1: ROC curve that discriminates between control and CRC patients (before treatment) among all investigated biomarkers. Arrows donates the best cutoff points for investigated markers.

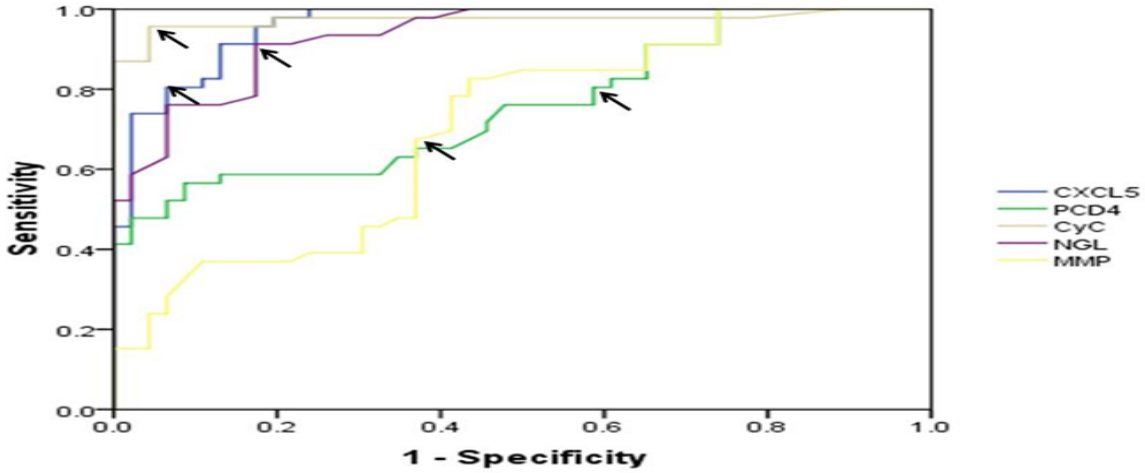

Figure 2: ROC curve that discriminates between treated and untreated CRC patients among all investigated biomarkers. Arrows donates the best cutoff points for investigated markers.

whereas t-test was used for continuous variables. Cut-off values were estimated from Receiver operating characteristic (ROC) curve. P-value < 0.05 is considered significant.

RESULTS

The clinicopathological characteristics of studied groups are shown in table (1). The median levels of studied biomarkers among studied groups are shown in table (2); the serum level of CXCL5, Cy-C, NGAL, MMP-9 and PDCD4 in healthy controls, early stage (Group I) and late stage colorectal cancer patients (Group II) before and after treatment. CXCL5 Cy-C, NGAL and MMP-9 serum levels were significantly elevated (P<0.05) in early stage group and highly significantly increased in late stage group

Table 4: Characteristics of the ROC curves among candidate biomarkers.

	AUC	95% CI (SE)	Sensitivity %	Specificity%	Cutoff - value	*P*-value
Control *versus* CRC patients						
CXCL5 (U/L)	0.995	0.952 – 0.997 (0.007)	100	98	0.78	0.0001
PCD4 (U/L)	0.896	0.817 – 0.949 (0.033)	65.22	98	4.3	0.0001
Cy-C (U/L)	0.947	0.881 – 0.982 (0.024)	84.78	92	3.93	0.0001
NGAL (U/L)	1	0.962 – 1 (0)	100	100	110	<0.0001
MMP9(U/L)	1	0.962 – 1 (0)	100	100	112	<0.0001
Treated *versus* un treated						
CXCL5 (U/L)	0.959	0.896 – 0.989 (0.022)	80.43	97.83	0.924	0.0001
PCD4 (U/L)	0.758	0.657 – 0.841 (0.050)	91.3	56.5	5.04	0.0001
Cy-C (U/L)	0.974	0.917 – 0.995 (0.0172)	95.65	95.65	3.39	0.0001
NGL (U/L)	0.933	0.861 – 0.974 (0.0274)	82.61	91.3	204	0.0001
MMP9(U/L)	0.701	0.596 – 0.792 (0.544)	65.52	82.61	783.9	0.0002

compared to control group. PDCD4 serum levels were significantly increased in early stage group before therapy, after therapy compared to the control group and increased in early stages before therapy compared to early stages after therapy and control group. In the late stage PDCD4 was significantly decreased before treatment and after treatment compared to healthy controls (P value<0.05). The correlation between CXCL5, Cy-C, NGAL, MMP-9 and PDCD4 levels and different stages of colorectal cancer are shown in table (3). CXCL5 Cy-C, NGAL, MMP-9 show positive correlation with cancer colon stages while PDCD4 shows negative correlation with cancer colon stages. The characteristics of the ROC curves among candidate biomarkers is shown in table (4); the best cut-off values were 0.78 U/L , 3.93 U/L, 110 U/L, 112 U/L and 4.3 U/L for CXCL5, Cy-C, NGAL, MMP-9 and PDCD4 respectively using ROC curve that discriminates between control and CRC patients (before treatment) among all investigated biomarkers (figure 1), while the best cut off values estimated using ROC curve that discriminates between treated and untreated CRC patients among all investigated biomarkers were 0.924 U/L, 5, 3.39 U/L, 202 U/L, 783.9 U/L and 5.04 U/L for CXCL5, Cy-C, NGAL, MMP-9 and PDCD4 respectively (figure 2).

DISCUSSION

Colorectal cancer is one of most common causes of cancer death. The process of carcinogenesis in this disease is complicated; formed of numerous genetic alterations. Staging of colorectal cancer by TNM system is the most common prognostic factor that predict the outcome of this tumor[19,20]. In this study serum CXCL5, Cy-C, NGAL, MMP-9 and PDCD4 were measured in patients with colorectal cancer to assess their value in predicting the prognosis of disease. Serum levels of CXCL5, Cy-C, NGAL, MMP-9 in our study were significantly increased according to stage and correlated with progression of disease. These results were in accordance with Kawamura et al[21] and Matsushita et al[22] who stated that preoperative serum CXCL5 is a novel predictive marker for determination of outcome of colorectal cancer patients. Several reports revealed that chemokines have a major role in carcinogenesis progression. They play a pivotal role in tumor growth, angiogenesis, tumor invasion and metastasis. The expression of chemokines and their receptors is changed in cancers and subsequently leads to aberrant chemokine receptor activation. The expression of certain chemokine receptors on the surface of the cancer cells promotes metastasis and organ specific metastasis. It had been demonstrated that CXCL5 is expressed in the liver while its receptor (CXCR2) is expressed in primary tumor, thus these molecules may be specifically associated with liver metastasis in case of colorectal cancer. Also, they found strong correlation between serum CXCL5 and liver metastasis[23,24]. Our results for Cy-c were in agreement with Barczyk et al[25] who found that the serum cytochrome c is a sensitive indicator of apoptosis as the high serum level of cytochrome indicates bad prognosis and more aggressiveness of tumor during the course of disease. Ahlemeyer et al[26] and Renz et al[27] stated that Cy-c is released from cells during treatment with chemotherapy. They stated that Cy-c is regulated by members of Bcl-2 family which have antiapoptotic action preventing its release[28,29]. In this study serum levels of PDCD4 were significantly increased in early stages of CRC but decreased significantly in late stages of disease, it correlated negatively with the disease progression. The results were in agreement with Leupold et al[30]; Yang et al[31] who found that PDCD4 regulates tumor invasion and metastasis as it overexpressed in early stages of disease and downregulated as metastasis occurs. PDCD4 overexpression in cancer cell leads to inhibition of cell proliferation and phenotypic changes, while downregulation of PDCD4 leads to development of metastases and more aggressiveness of tumor due to decreased expression of epithelial proteins and increased expression of mesenchymal proteins[32,34]. In our work serum MMP-9 and NGAL were increased according to stage, this result was in agreement with Barresia et al[35]. who suggested that the increase of NGAL can predict bad prognosis in patients with colorectal cancer. They clarified that NGAL regulates the function of matrix

metalloproteinase-9 leading to aggressiveness of tumor. NGAL is attached to 92-kDa gelatinase/MMP9. NGAL is expressed in many types of cells in response to various injuries. Serum NGAL levels correlate clearly with the severity of tissue damage. For this reason, NGAL may become one of the most promising next-generation biomarkers[36]. NGAL's ability to combine in a dimeric complex with MMP-9, results in a protective action of MMP-9 from its auto-degradation resulting in a higher gelatinolytic action of MMP-9 on extracellular matrix. So, it has been shown that NGAL may lead to cancer progression in different cancer types. Recently, Martí J et al showed the prognostic utility of NGAL mainly in metastatic CRC[37,38]. Higher levels in colon cancer cases then controls were observed by Fung KY et al but the authors concluded that it has on function as a marker for diagnosis of cancer colon as the sensitivity of NGAL was found to be 24% at 90% specificity[39]. Accordingly, although NGAL is still expressed by the majority of human neoplastic colorectal lesions, the author denotes it is not a useful biomarker for determining disease progression from adenomatous to malignant colorectal neoplasia[40]. Previous studies showed that expression of MMP-9 increased positively in correlation to TNM stages[41]. Barresia et al[35] found that increased MMP-9 in case of colorectal cancer leads to occurrence of metastases through different pathological processes and correlates with its formation in nearby lymph nodes. In conclusion; Serum CXCL5, Cy-c, NGAL and MMP-9 were elevated in CRC patient group and it correlated positively with progression of disease so they could serve as a prognostic markers for CRC patient while PDCD4 decreased with progression of tumor and correlated negatively with stage; so these markers might have a role in the progression of colorectal cancer and the development of metastasis, they can be used to choose patients with a higher risk and indicates what therapies are useful for them.

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