Programmed death protein 1 (PD1) mRNA expression as a screening and diagnostic tool in colon cancer

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Abstract:

Purpose: Colon cancer is one of the most frequent malignant tumors worldwide. Studies showed that in patients with colon cancer, immune system is generally compromised. Correlation of PD1 expression and numerous types of cancer such as colorectal cancer have been well illustrated. The higher levels of PD1 expression correlates with poorer prognosis.
In our study, we **aim** to investigate the associations between PD1 gene expression and susceptibility to CRC.
Methods: This study was carried out on 50 patients with colon adenocarcinoma, 50 patients with benign colon polyp and 50 apparently healthy persons served as controls. All subjects were exposed to full history taking, general clinical examination. Complete blood count, liver and kidney function, determination of serum tumor markers (CEA and CA19-9). Estimation of PD1 Gene expression by real-time PCR was done.
Results: The mean PD1 gene expression was 5.8% in cancer patients compared to 0.9% in benign polyps group and 0.04 in normal people. The sensitivity and specificity of PD1 expression in our study was 98% and 95% respectively. Higher PD1 gene expression had statistically significant relation with tumor stage (p=0.001) and presence of metastases (p=0.003).
Conclusions: The level of PD1 can be used to differentiate between colon cancers and begin adenomas. PD1 could be used as a prognostic marker in colon cancer.

Keywords: Colorectal cancer, programmed death protein-1, real- time PCR.

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Introduction:

Colorectal cancer (CRC) is the third most common cancer worldwide after lung and breast cancers with two-thirds of all colorectal cancers occurring in the more developed regions of the world. CRC affects men and women of all racial and ethnic groups [1]. It is the second most commonly diagnosed cancer in women and the third in men [2].

Colorectal cancer (CRC) is a major cause of morbidity and mortality throughout the world with large geographical differences [3]. It is the third most commonly diagnosed cancer and the fourth most common cause of cancer related deaths in both sexes worldwide. An estimated 1.3 million people are diagnosed with CRC, and approximately700,000 people die from it annually [4].

The etiology of CRC is complex as it results from multistep carcinogenesis. These alterations can either be acquired, as happens in the sporadic forms, or inherited, as in genetic hereditary syndromes. The most famous of these syndromes are familial adenomatous polyposis and Lynch syndrome [5]. Approximately 5-6% of all CRCs are associated with germ line mutations that confer an inherited predisposition [6]. Over the past few years, there is more and more evidence that CRC is a very heterogeneous disease and that molecular and genetic features of the tumor determine the prognosis and response to treatment [7].

Colonoscopy is an invasive and uncomfortable investigation but it is still considered the most reliable screening method for diagnosis of colorectal cancer. While other markers as the fecal occult blood test, serum CA19-9 and CEA have either low sensitivity, specificity or both [8].

Tumor-infiltrating lymphocytes (TILs) are widely considered as reflection of primary host immune response against solid tumors. Evidence has shown that tumor infiltration by activated CD8+ cytotoxic T lymphocytes correlates with better survival of CRC patients [9]. Programmed death 1 protein (PD1) and its ligand (PDL1) is an immune checkpoint which is up-regulated in many tumors and their microenvironment.

It is an inhibitory pathway for suppression of T cytotoxic lymphocytes that gives cancer cells the hallmark of evasion of the immune system [10].

PD1 is a negative regulator of the immune system expressed on CD4+ T cells, CD8+ T cells, NKT cells, B cells and monocytes. This molecule is highly expressed on exhausted T cells. Studies showed that blockade of PD1 can enable T cells to proliferate and produce effector cytokines. Correlation of PD1 expression and numerous types of cancer such as colorectal cancer has been well illustrated. Furthermore, it is shown that level of PD1 expression is associated with tumor prognosis. The elevated level of PD1 expression arises the poorer prognosis in certain malignancies [11].

Patients and Methods:

Subjects:

This study was carried out at Medical Biochemistry and Molecular biology, General Surgery and Clinical Oncology and Nuclear Medicine Departments, Faculty of Medicine, Menoufia University. The study included 50 patients with colorectal adenocarcinoma named as group I, 50 patients with benign colonic polyps named as group II and 50 healthy persons named as group III.

An informed written consent was obtained from every subject participated in this study and this study was approved by the Ethical Committee of Medical Research, Faculty of Medicine, Menoufia University.

Exclusion criteria included: patients with history of inflammatory bowel disease, familial adenomatous polyposis, or hereditary non-polyposis colorectal cancer (HNPCC) and patients diagnosed with recurrent colorectal tumors or with tumors located elsewhere.

Methods:

All groups of the study were subjected to the following: Full history taking, general clinical examination, abdominal ultrasound & CT-scan for abdomen, colonoscopy, biopsy and histopathological examination (for patients only), laboratory investigations including: complete blood count that was measured with Pentra-80 automated blood counter (ABX– France –Rue du Caducee- Paris Euromedecine-BP-7290.34184 Montpellier-Cedex 4.) ,estimation of kidney functions (urea & creatinine), liver enzymes (AST, ALT), serum carcinoembryonic antigen(CEA) and serum cancer antigen (CA19-9), both detected by enzyme-linked immunosorbent assay method, using (Human CA19-9, CEA ELISA kits, ChemuxBioScience, Inc, USA) and detection of PD-1 m RNA gene expression by Real time PCR.

Clinical data including tumor stage, sites of metastasis, response evaluation by response evaluation criteria in solid tumors RECIST version 1.1 and survival status at 1 year of diagnosis.

Sample collection:

A volume of 6 ml of venous blood were withdrawn by venipuncture; and divided into 3 tubes: 1 ml for complete blood count (CBC) and 2 ml were put into EDTA containing tube for RNA extraction & detection of PD1 gene expression by real time PCR and the remaining 3 ml was put into a plain tube and allowed to clot at 37°C, centrifuged for 10 minutes at 4000 R.P.M and the serum obtained was stored at -80◦C until we use it for measuring serum urea, creatinine, AST, ALT serum carcinoembryonic antigen (CEA),determination of serum carbohydrate antigen(C19-9).

Reverse transcriptase PCR (RT-PCR):

RNA was isolated from peripheral blood leukocytes using QIAamp RNA Blood MiniKit (Qiagen, USA), then first step-PCR: Complementary DNA was synthesized using QuantiTect Reverse Transcription Kit (Qiagen,Applied Biosystems, USA),second step- PCR (real time PCR step): it was performed using QuantiTect SYBR Green PCR Kit with ready-made quantiTect Primer Assay, Qiagen. For measurement of BAFF mRNA levels, the following primers were used: forward and reverse primers of human PD1, 5’-CGTGGCCTATCCACTCCTCA-3’ and 5’-ATCCCTTGTCCCAGCCACTC-3’, respectively; forward and reverse primers B-actin 5’-TCCCTGGAGAAGAGCTACGA-3’ and 5’-TGAAGGTAGTTTCGTGGATGC-3’, respectively. PCR was conducted under the following conditions: Each reaction was performed in a final volume of 20 µL,containing 10 µLSYBR Green 2x QuantiTect PCR Master Mix 3µL cDNA, 1 µL forward primer, 1 µL reverse primer and 5 µl RNase-free H2O. The mix was incubated at 94°C for 3 min, followed by 60 cycles; denaturation at 94°C for 30 s, annealing at 55°C for 40 s, and extension at 72°C for 31 s. Data analysis was done in Applied Biosystems 7500 software version 2.0.1. The relative quantification (RQ) of gene expression performed using comparative ΔΔCtmethod(15).PD1 mRNA was normalized to the mRNA levels of house keeping gene B-actin. Melting curve was done to confirm specificity of the amplification and absence of primer dimers.

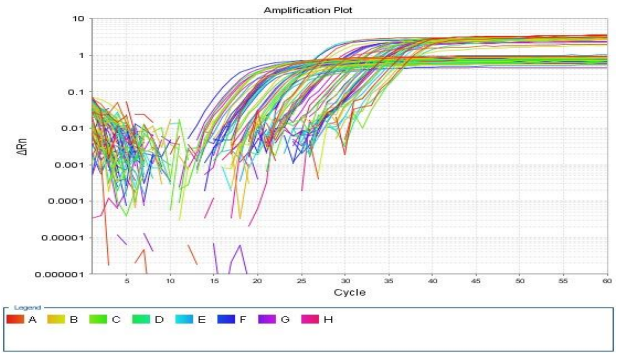

Figure 1 Amplification plot of PD1 mRNA gene expression (normalized fluorescence signal (ΔRn) plotted versus cycle number).

Statistical analysis:

The data collected was tabulated and analyzed by SPSS (statistical package for the social science) software version 20. Chi-square test is used to study the association between two qualitative variables. Student’s

t-test was used to assess the statistical significance of parametric data F-test (ANOVA)

For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) (LSD) for pairwise comparisons, Spearman's correlation was used for skewed distributed quantitative variables and Receiver operator characteristic (ROC) with respective points of maximal accuracy for sensitivity and specificity were generated to determine biomarker performance. Values less than 0.05 were considered significant.

Results:

There was no significant difference in demographic data between the three study groups including age and sex (P=0.7 and 0.55 respectively). In group 1 (cancer patients) 90% had family history. The site of the disease was equally distributed in the group (15 cases with distal rectum, right colon, left colon and 8 cases with proximal rectal cancer). The most commonly seen presenting symptom was abdominal pain (50%), followed by bleeding per rectum (24%). All of the cases had good performance (0, 1 or 2). Thirty six percent of the patients presented with metastatic disease, half of them was in both lung and liver. Complete remission was seen in 48% with standard therapies and by the end of the study 66% of the patients were alive. The most common causes of death were liver failure (5 patients), intestinal obstruction (4 patients) and respiratory failure (4 patients).

The mean hemoglobin level in cancer patients was 8.71 gm/dl compared to 11.8 gm/dl in benign conditions and normal persons (P<0.001). Likewise, mean platelet count was 184 /dl in cancer patients compared to 304 and 3018 /dl in benign cases and normal persons respectively (P<0.001).

The blood levels of AST, ALT, urea and creatinine didn't show any significant difference between the studied groups. However, CEA and CA19-9 were significantly higher in the group 1 (cancer patints). The mean CEA value was 27.1 mg/dl in cancer patients compared to 8 mg/dl in benign polyps and 1.5 mg/dl in normal people indicating a significant difference between cancer patients and the other two groups (P<0.001). Likewise, mean CA19-9 value was 29.5 mg/dl for cancer patients compared to approximately 12 mg/dl in the other two groups (P<0.001).

The mean PD1 gene expression was 5.8% in cancer patients compared to 0.9% in benign polyps group and 0.04 in normal people. This result shows significant difference in the expression of this gene between the three groups (P<0.001 between each group and the other).

The ROC curve for using these biomarkers (PD1, CEA and CA19-9) shows a 98% sensitivity and 95% specificity for PD1 with a positive predictive value of 81% and a negative predictive value of 98%. However, the sensitivity and specificity for CA19-9 were 88% and 79% respectively with a PPV of 67% and NPV of 92%. On the contrary, CEA showed a higher sensitivity and specificity than CA19-9 (94% and 93% respectively) and a PPV of 87% and a NPV of 96%.

PD1 expression showed strong correlation with age, weight, level of AST and level of tumor markers (CEA and CA19-9) in the cancer group but it didn't show any other significant correlation with other parameters. For the other two groups (benign polyps and normal persons), the level of PD1 expression didn't show any significant correlation with all parameters apart from CEA and CA19-9.

By doing subgroup analysis for the cancer patients, the level of PD1 expression was significantly higher with metastasis (P=0.003), poor survival (P=0.002), higher grade of the tumor (P=0.037) and higher tumor stage (P=0.001). The median value of expression was 7.95% in case of metastasis versus 3.98% in non metastatic patients. The median value was 7.4% in dead pateints and 3.95% in survivors. The median value for grade I, II and III were 5.6, 3.9 and 6.7% respectively while for stage II, III and IV it was 3.9, 3.9 and 7.95% respectively.

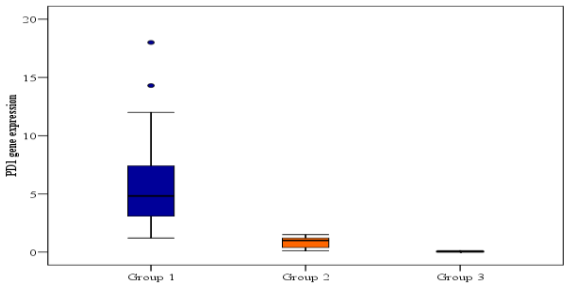

Figure (2): Comparison between the three studied groups according to PD1 gene expression

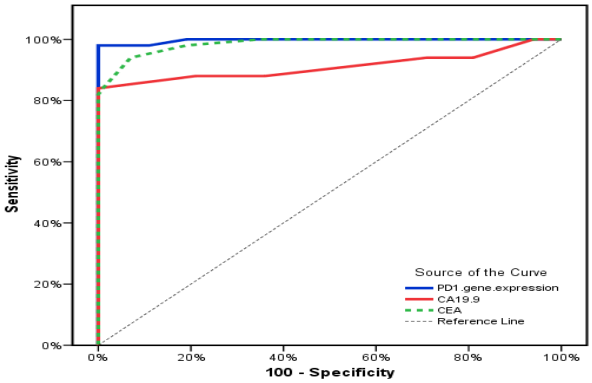

Figure (3): ROC curve for different parameters to diagnose cancer patients (n = 50) from Benign +control (n = 100)

Table (1): Comparison between the three studied groups according to demographic data

	Group 1 (n = 50)		Group 2 (n = 50)		Group 3 (n = 50)		Test of Sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	24	48.0	25	50.0	28	56.0	χ^2= 0.694	0.707
Female	26	52.0	25	50.0	22	44.0		
Age								
Min. – Max.	25.0 – 85.0		26.0 – 85.0		29.0 –76.0		F= 0.587	0.558
Mean ± SD.	55.24 ± 13.22		53.94 ± 16.49		52.34 ± 9.63			
Median (IQR)	58.50(48.0 – 65.0)		55.0(40.0 – 65.0)		50.0(45.0 – 60.0)			

χ^2: **Chi square test F**: **F for ANOVA test** p: p value for comparing between the studied groups
*: Statistically significant at p ≤ 0.05 **Group 1:Cancer,Group 2:Benign ,Group 3: control.**

Table (2): Comparison between the three studied groups according to CBC

	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 50)	F	p	Sig. bet. grps.		
						1vs. 2	1 vs. 3	2 vs. 3
Hb% (gm/dl)								
Min. – Max.	5.40 – 11.0	10.0 – 13.0	11.0 – 13.0					
Mean ± SD.	8.71 ± 1.22	11.86 ± 0.66	11.87 ± 0.69	205.540*	<0.001*	<0.001*	<0.001*	0.999
Median (IQR)	8.90(7.9 – 9.3)	12.0(11.5 – 12.0)	12.0(11.0 – 12.0)					
Platelets (10^3/mm^3)								
Min. – Max.	78.0 – 300.0	160.0 – 400.0	200.0 – 390.0					
Mean ± SD.	184.4 ± 55.11	304.0 ± 71.88	318.0 ± 39.81	82.535*	<0.001*	<0.001*	<0.001*	0.440
Median (IQR)	196.5(145.0–222.0)	300.0(250.0 – 365.0)	320.0(290.0– 350.0)					

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**
p: p value for comparing between the studied groups *: Statistically significant at p ≤ 0.05
Group 1: Cancer, Group 2: Benign, Group 3: control.

Table (3): Comparison between the three studied groups according to liver function

Liver function	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 50)	F	p
ALT (IU/L)					
Min. – Max.	18.0 – 35.0	18.0 – 35.0	20.0 – 33.0		
Mean ± SD.	24.62 ± 5.04	24.36 ± 4.56	25.62 ± 3.57	1.127	0.327
Median (IQR)	22.0(21.0 – 29.0)	22.0(22.0 – 28.0)	26.0(23.0 – 28.0)		
AST (IU/L)					
Min. – Max.	19.0 – 33.0	19.0 – 31.0	22.0 – 32.0		
Mean ± SD.	25.44 ± 3.95	26.10 ± 4.04	26.28 ± 2.50	0.768	0.466
Median (IQR)	25.0(22.0 – 30.0)	25.0(23.0 – 30.0)	27.0(24.0 – 28.0)		

F: **F for ANOVA test** p: p value for comparing between the studied groups*: Statistically significant at p ≤ 0.05 **Group 1: Cancer, Group 2: Benign, Group 3: control.**

Table (4): Comparison between the three studied groups according to renal function

Renal function	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 50)	F	p
Urea (mg/dl)					
Min. – Max.	18.0 – 36.0	18.0 – 36.0	18.0 – 36.0		
Mean ± SD.	27.10 ± 5.12	25.78 ± 5.57	26.24 ± 3.86	0.993	0.396
Median (IQR)	26.0(25.0 – 30.0)	25.0(22.0 – 28.0)	26.0(25.0 – 30.0)		
Creatinine (mg/dl)					
Min. – Max.	0.20 – 1.20	0.20 – 1.0	0.40 – 1.20		
Mean ± SD.	0.72 ± 0.22	0.68 ± 0.21	0.75 ± 0.19	1.678	0.190
Median (IQR)	0.70(0.60 – 0.90)	0.70(0.50 – 0.80)	0.75(0.60 – 0.90)		

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Tukey)**
p: p value for comparing between the studied groups *: Statistically significant at $p \leq 0.05$
Group 1: Cancer, Group 2: Benign, Group 3: control.

Table (5): Comparison between the three studied groups according to CEA (mg/dl) and CA19-9 (U/ml)

CEA (mg/dl)	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 50)	H	p	Sig. bet. grps.		
						1 vs. 2	1 vs. 3	2vs. 3
Min. – Max.	9.0 – 43.0	5.0 – 12.0	6.0 – 13.0					
Mean ± SD.	27.10 ± 11.37	8.06 ± 2.22	7.68 ± 1.53	94.816*	<0.001*	<0.001*	<0.001*	0.517
Median (IQR)	30.0(15.0 – 36.0)	8.0(6.0 – 9.0)	7.0(6.0 – 9.0)					
CA19-9 (U/ml)								
Min. – Max.	10.0 – 51.0	9.0 – 15.0	10.0 – 15.0	70.426*	<0.001*	<0.001*	<0.001*	0.191
Mean ± SD.	29.56 ± 12.27	11.92 ± 1.97	12.60 ± 1.55					
Median (IQR)	29.0(19.0 – 40.0)	12.0(10.0 – 13.0)	12.0(12.0 – 14.0)					

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$ **Group 1: Cancer, Group 2: Benign, Group 3: control.**

Table (6): Comparison between the three studied groups according to PD1 gene expression

PD1 gene expression	Group 1 (n = 50)	Group 2 (n = 50)	Group 3 (n = 50)	H	p	Sig. bet. grps.		
						1 vs. 2	1 vs. 3	2vs. 3
Min. – Max.	1.20 – 18.0	0.10 – 1.50	0.01 – 0.09					
Mean ± SD.	5.86 ± 3.53	0.90 ± 0.42	0.04 ± 0.02	131.866*	<0.001*	<0.001*	<0.001*	<0.001*
Median (IQR)	4.82(3.1 – 7.4)	1.0(0.40 – 1.2)	0.04(0.03 – 0.06)					

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test) ,**p: p value for comparing between the studied groups
*: Statistically significant at $p \leq 0.05$, **Group 1: Cancer, Group 2: Benign, Group 3: control.**

Table (7): Agreement (sensitivity, specificity) ROC curve for different parameters to diagnose cancer patients (n = 50) from Benign +control (n = 100)

	AUC	p	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
PD1 gene expression	0.988	<0.001*	0.976	1.0	>1.3	98.0	95.0	81.7	98.9
CA19-9 (U/ml)	0.911	<0.001*	0.845	.978	>14	88.0	79.0	67.7	92.9
CEA (mg/dl)	0.985	<0.001*	0.951	0.998	>10	94.0	93.0	87.0	96.9

AUC: Area Under a Curve p value: Probability value, CI: Confidence Intervals NPV: Negative predictive value PPV: Positive predictive value *: Statistically significant at $p \leq 0.05$.

Table (8): Correlation between PD1 gene expression and different parameters in each group

	PD1 gene expression					
	Group 1		Group 2		Group 3	
	r_s	p	r_s	p	r_s	p
Age	0.327	0.020*	-0.123	0.393	-0.065	0.653
Weight (kg)	0.279	0.049*	0.082	0.571	0.104	0.471
Height (cm)	0.198	0.167	-0.012	0.935	0.001	0.995
BMI (kg/m2)	0.207	0.149	0.083	0.569	0.033	0.818
L	0.113	0.433	–	–	–	–
N	0.040	0.785	–	–	–	–
L/N ratio	0.020	0.890	–	–	–	–
Hb% (gm/dl)	-0.165	0.253	-0.014	0.925	-0.037	0.796
Platelets (103/mm3)	0.243	0.089	0.258	0.071	0.162	0.260
ALT (IU/L)	0.199	0.166	0.065	0.654	-0.065	0.653
AST (IU/L)	0.324	0.022*	0.105	0.467	-0.011	0.941
Urea (mg/dl)	-0.058	0.687	-0.112	0.439	-0.252	0.077
Creatinine (mg/dl)	0.240	0.093	-0.169	0.240	-0.115	0.425
CEA (mg/dl)	0.397	0.004*	0.289	0.042*	0.539	<0.001*
CA19-9 (U/ml)	0.311	0.028*	0.523	<0.001*	0.281	0.048*

r_s: Spearman coefficient

*: Statistically significant at $p \leq 0.05$.

Table (9): Relation between PD1 gene expression and different parameters in cancer group (n= 50)

	N	PD1 gene expression			Test of Sig.	p
		Min. – Max.	Mean ± SD.	Median		
Metastasis						
Yes	**18**	2.05 – 14.30	7.74 ± 3.57	7.95	U= 142.50*	0.003*
No	**32**	1.20 – 18.0	4.81 ± 3.08	3.98		
Fate						
Live	**33**	1.20 – 10.30	4.75 ± 2.49	3.95	U= 131.5*	0.002*
Dead	**17**	2.05 – 18.0	8.02 ± 4.28	7.40		
Grade						
I	**9**	2.98 – 18.0	7.91 ± 5.44	5.60	H= 6.579*	0.037*
II	**26**	1.20 – 10.30	4.61 ± 2.42	3.94		
III	**15**	1.90 – 11.0	6.81 ± 3.10	6.70		
Stage						
Stage 2	**13**	1.80 – 7.40	4.20 ± 1.96	3.90	H= 13.501*	0.001*
Stage 3	**19**	1.20 – 12.0	4.65 ± 2.55	3.97		
Stage 4	**18**	1.80 – 18.0	8.35 ± 3.99	7.95		

H: H for **Kruskal Wallis test** **U: Mann Whitney test** p: p value for association between different categories *: Statistically significant at $p \leq 0.05$

Discussion:

Colorectal cancer (CRC) is the second most common cancer in women and the third most common cancer in men worldwide. Despite advances in treatment, CRC is still the third most frequent cause of cancer-related death [16]. A sensitive assay that can accurately diagnose the onset of cancer using non-invasively collected clinical specimens is ideal for early detection. The earlier and more accurate the diagnostic biomarker that can predict disease onset, the more valuable it becomes [17].

Programmed cell death protein 1 (PD1) is expressed by both lymphoid and non-lymphoid immune cells, and is up-regulated upon after engagement of T cell or B cell receptors on naive lymphocytes. Activation of PD 1 by its ligand PDL1 induces down-regulation of lymphocyte proliferation and cytokine production, resulting in lymphocyte deletion. Indeed, expression of PDL1 on tumour cells has been found to suppress CD8C T cell activity and to be associated with an impaired prognosis is several types of cancers [18].

The aim of this work was to evaluate the role of PD1 gene expression in patients with colorectal cancer as a diagnostic biomarker. This study involved 150 individuals, 50 patients with CRC, 50 patients with benign diseases in colon and rectum and 50 healthy persons served as controls.

In the present study, there was no significant statistical difference between the three studied groups regarding age and sex. This agreed with the study of Ghanadi et al.,Ozen et al. and Khan et al. who showed that there was no statistically significant difference between cases &control groups regarding age [19,20,21].

This is in agreement with results reported by Heitzer et al., Kim et al. and Hoda et al. who documented that there was no statistically significant difference between cases &control groups regarding sex [22, 23, 24].

The three groups were homogenous regarding epidemiological features. However, in laboratory parameters patients in cancer group had significantly lower Hb% than in the other groups this can be explained by chronic blood loss from the malignant tumor.

In this study, there was no significant statistical difference between studied groups regarding serum urea, creatinine, AST and ALT as kidney and liver diseases are parts of the exclusion criteria of our patients. The present findings revealed that there was significant statistical increase in CRC patients when compared with both benign and control groups regarding serum Tumor markers CEA and CA19-9 indicating their diagnostic value. However, there was no statistically significant difference in the level of CA 19-9 and CEA between benign and control groups.

These findings were matched with the results reported by Wang et al., Mourtzikou et al. and Zaher et al. who observed a significant increase in CA 19-9 levels in cancer patients when compared with the control. Moreover, Qi et al. stated that CA 19-9 serum levels have been proposed to differentiate benign from malignant colorectal diseases [25, 26, 27, and 28].

In contrast to our results, Al-Shuneigat et al. reported no significant difference in the serum levels of CA 19-9 in patients when compared to controls [29].

Polat et al. reported that serum CA 19-9 was not significantly different in the control and patient groups while serum CEA was significantly higher in the patient group than in the control group [30].

In the present study the ROC curve was applied to assess the diagnostic utility of CA19-9 control versus patients. It revealed that the best cutoff point for CA 19-9 is >14. It had a diagnostic sensitivity of 88%, specificity of 79%, with a positive predictive value of 67.7% and negative predictive value of 92.9%. These results confirm the values of CA19-9 in colon cancer with an acceptable sensitivity and specificity.

Qi et al. reported that the optimal cut-off values for CA 19-9 set at the point of maximum sensitivity plus specificity by ROC curve was 37 U/ml with positive predictive value (68.4%) and negative predictive value (61.3%) [28].

Regarding CEA the ROC curve revealed that the best cut off point for CEA >10. It had a diagnostic sensitivity of 94.0%, specificity of 93%, with a positive predictive value of 87% and negative predictive value of 96.9%.

Previous investigators have reported that CEA promotes cancer cell metastases and invasion by targeting the adherence junction complexes between cells and enhancing the aggregation of cells. CEA has also been demonstrated to be involved in suppressing the immunity by inducing the release of suppressor factor from normal lymphocytes. These underlying biological mechanisms may explain why patients had a high CEA levels [31].

Regarding PD1 gene expression between the studied groups the cancer group showed significantly higher gene expression compared to patients with adenomatous polyps and healthy controls. This was in agreement with Liu et al. who reported that PD-1 expression on NK cells is increased in digestive cancer patients, and is further increased when stimulated. PD1/PDL1 ligation inhibits NK-cell anti-tumor effects, thereby protecting tumor cells from being killed [32].

Programmed cell death receptor 1 (PD1) is considered as important immunosuppressive molecule and play an important role in tumor immune escape and cancer progression. PD1 played a role in immune regulation according to the functional and pathway enrichment analysis. PD1 primarily participated in the regulation of immune cell activation and proliferation, immune cell receptor complex, cell adhesion molecules, and T cell receptor signaling pathway. It is well known that regulation of immune cell activation and proliferation and the T cell receptor signaling pathway can significantly alter the immune status of the local microenvironment of the tumor. Previous studies also suggest that high levels of PD1 in TILs indicate an exhausted T cell status [33].

In order to assess the validity of PD1 expression in the diagnosis of colon cancer, Receiver operator characteristic (ROC) curve was performed and respective points of maximal accuracy for sensitivity and specificity were generated and it was found that PD1 expression was sensitive and specific tests in the diagnosis of colon cancer.

Immune checkpoint blockade targeting the programmed death1 (PD1) pathway has shown efficacy in several types of cancers including mismatch-repair-deficient colorectal carcinoma [34].

Furthermore, it is shown that level of PD1 expression is associated to tumor prognosis. The elevated level of PD1 can be used as predictive and prognostic marker as well [35]. Many anti PD1 and anti PDL1 antibodies are now available for use in the clinics [36]. Pembrolizumab is an anti PD1 antibody currently approved for use in metastatic colon cancer patients who express high microsatellite instability [37]. However, the predictive value of PD1 expression for response to anti PD1 antibodies was better seen in non-small cell lung cancer than in colon cancer [38].

In our study we noticed that there was significant positive correlation between PD1 expression level and both CEA and C19-9. We can attribute that all of them cause suppressing of the immune system by releasing the suppressor molecules from lymphocytes. So as the levels of PD1, CEA and CA19-9 get higher, cancer cells can evade the immune system more easily and this explains why poorer prognosis is noticed with the higher levels.

In the current study, expression of PD1 had been related with different disease parameters in colon cancer patients as PD1 had been over expressed in patients with stage IV disease, grade III pathology and in patients with shorter time to progression, indicating that: PD1 gene expression is directly associated with tumor aggressiveness and patients' outcomes.

This was matched with Tanaka et al. who documented that Programmed death-ligand 1 (PDL1), is an immune inhibitory ligand that is expressed on various tumor cells like colorectal cancer. Binding of PDL1 on tumor cells to PD-1 receptors on T cells blocks anti-tumor T cell activity and thus allows tumor cells to evade the host immune surveillance with advanced stages. Therefore, PDL1 and PD1 are major targets of the currently popular immune checkpoint immunotherapies [36]. During T cell activation, PD1 is expressed on the surface of T cells and causes T cell exhaustion .It is also expressed on the cell surface of B lymphocytes and natural killer cells (NKs); however, it predominantly affects CD8+ T-cells as the first line of defense against tumor cells [37].

A contradicting result to what we have found, Ajoedi et al. who documented that PD-1 mRNA expression decreased in peripheral blood of CRC patients compared to healthy individuals. PD-1 expression tends to be low in CRC with advanced stages [39].

It could be concluded that the more the increase of PD1 gene expression the more the aggressiveness and progress of colon cancer. The level of PD1 can be used to discriminate between colon cancers and begin adenoma. PD1 might have a beneficial role in colon cancer.

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