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Serum Osteopontin and its Relation to Colorectal Carcinoma in Egyptian Patient

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ABSTRACT

Background: Colorectal cancer (CRC) incidence has increased worldwide recently, and the death rate from it has increased to reach 10% of cancer-related deaths. Many risk factors are expected to play a role in this high incidence such as the higher age, change in dietary habits, cigarette smoking, low physical exercise, and the increased prevalence of obesity of the populations worldwide. **Objectives:** To evaluate the role of serum level of osteopontin in prediction of colorectal carcinoma in correlation with tissue histopathology, which is the gold standard test.

Patients and Methods: This study was conducted on 80 subjects referred for colonoscopy at the Endoscopic Unit of Ain Shams University Hospitals. Patients were divided into 2 groups: Group A included 40 patients diagnosed as colorectal carcinoma as a patient group and group B that included 40 individuals with age- and sex-matched who had normal colonoscopy and was used as control group.

Results: The level of osteopontin was statistically significant higher in CRC patients than in subjects with normal colonoscopy. Also, the ROC curve for osteopontin in prediction of CRC showed the best cut of value of > 12 ng/ml with area under the curve (AUC) = 0.889, sensitivity = 85% and specificity = 77.5%.

Conclusion: Serum Osteopontin (OPN) level was detected in our study to be high in patients with CRC in comparison to patients with normal colonoscopy. Therefore, it can be used as a biomarker in the diagnosis of CRC. Also, serum osteopontin level is significantly higher in cancer patients with metastasis according to TNM staging, thus it can be used in prognosis.

Keywords: Colorectal carcinoma, Serum osteopontin, Colonoscopy, Histopathology.

INTRODUCTION

Colorectal cancer (CRC) is one of the commonest GIT malignancies and one of the causes of morbidity and mortality among different types of neoplasms, despite improvement in diagnosis and treatment [(1)]. The survival rate of patients with CRC remains poor due to distant metastasis in most cases upon discovery. Thus, early diagnostic and treatment modalities during the early stages will help to improve the prognosis, survival, and disease-free time for patients with CRC [(2)].

Common symptoms such as hematochezia and abdominal discomfort, and tests such as digital rectal examination and abdominal palpation will help to diagnose patients with CRC in the early stages [(3)]. The most common histological type accounting for almost 95% of CRCs is adenocarcinoma, whether papillary adenocarcinoma, tubular adenocarcinoma, mucinous adenocarcinoma, and signet-ring cell carcinoma. The exact etiology contributing to the CRC is still unclear. Many genes, microstructures and the microenvironment participate in tumor formation, proliferation, invasion and spread. Other factors, such as sex, age, environment, also have a role [(3)].

Treatment options for CRC include surgical removal in addition to chemotherapeutic medications such as fluorouracil, oxaliplatin, and cetuximab. These medications can be used to either shrink the tumor diameter pre-operation or serve as a post-surgical adjuvant for the treatment of patients with CRC [(3)]. Colorectal cancer can spread to the liver, bone, lung, and brain. Local relapse and distant metastasis are two main intractable problems in managing CRC in spite of the presence of many new chemotherapeutic agents. Many tumor markers-such as CEA and CA19.9- are used in clinical practice to detect and follow-up CRC patients. However, the exact value of these markers is still unclear [(4)].

Osteopontin (OPN), a phosphorylated glycoprotein, plays a role in many processes including cell adhesion, cell migratory activity, bone metabolism, and stone formation [(5)]. Additionally, OPN is found in many tumor cells, as in the stomach, large intestine, mammary gland, and lungs [(6)]. OPN is involved in neovascularization of tumor cells through an interaction with VEGF, as it triggers VEFG expression in tumor cells that stimulates neovascularization [(7)].

The aim of this study was to evaluate the role of serum level of osteopontin in prediction of presence of colorectal carcinoma in correlation with tissue histopathology, which is considered the gold standard test.

PATIENTS AND METHODS

This was a randomized-controlled prospective clinical trial conducted at the Endoscopic Unit of Ain Shams University Hospitals. It was conducted on 80 Egyptian subjects that were divided into 2 groups as follows: Group A included 40 patients diagnosed with colorectal carcinoma-by colonoscopy and confirmed by histopathology, and group B, which included 40

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individuals with age- and sex-matched who had normal colonoscopy and served as control group. Exclusion criteria included pregnancy and malignancies other than CRC.

All cases underwent a thorough medical history, with a focus on rectum bleeding, constipation, weight loss, any abdominal pain, and a family history of similar conditions. Additionally, a full clinical examination was done followed by laboratory investigations that included CBC, INR, AST, ALT, Na, K, creatinine, and urea. In addition, blood samples for serum Osteopontin were obtained. Kits were supplied (Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Co., Ltd). Radiological investigations were also done that included abdominal & pelvic ultrasonography, colonoscopy, and CT scan of the abdomen with oral and IV contrast if needed for staging. A histopathological examination was done to ascertain the type of tumor and its stage.

Ethical consent:

An approval of the study was obtained from Ain Shams University Academic and Ethical Committee (Ethics committee's reference number: 000017585). Every patient signed an informed written consent for acceptance of the study. 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

Data were collected and analyzed using SPSS software version 18 windows 7. For quantitative variables, mean, standard deviation (SD), and range were used. Numbers and percentage were used for qualitative variables. Chi-square test was used to compare qualitative variables between groups. Power of significance was evaluated as follows: Probability level (P-value) > 0.05: Insignificant, P-value ≤ 0. 05: Significant, P-value < 0. 01: Highly significant.

RESULTS

Table (1): Comparison between the two groups for demographic data

		Control group	Patients group	P-value	Sig.
		n = 40	n = 40		
Age	Mean ± SD Range	47.25 ± 18.95 18 – 79	52.25 ± 17.79 32 – 91	0.227	NS
Gender	Female Male	26 (65.0%) 14 (35.0%)	17 (42.5%) 23 (57.5%)	0.44	NS

Table (1) showed that all groups were matched as regards sex & age as there was no statistical significant difference between groups as regards sex and age.

Table (2): Comparison between the two groups regarding different clinical and history data

		Control group	Patients group	Test value*	P-value	Sig.
		n = 40	n = 40			
Bleeding per rectum	No Yes	15(37.5%) 25(62.5%)	16 (40.0%) 24 (60.0%)	0.053	0.818	NS
Recurrent anemia	No Yes	15 (37.5%) 25 (62.5%)	21 (52.5%) 19 (47.5%)	1.818	0.178	NS
Constipation	No Yes	17 (42.5%) 23 (57.5%)	23 (57.5%) 17 (42.5%)	1.800	0.180	NS
Abdominal pain	No Yes	18 (45.0%) 22 (55.0%)	24 (60.0%) 16 (40.0%)	1.805	0.179	NS
Weight loss	No Yes	33 (82.5%) 7 (17.5%)	14 (35.0%) 26 (65.0%)	18.620	0.000	HS

Table (2) showed a highly significant difference between both groups in weight loss (p-value < 0.01). But there was no significant difference between both groups in bleeding per rectum, recurrent anemia, constipation and abdominal pain (p-value > 0.05).

Table (3): Laboratory data of both groups

		Control group	Patients group	Test value•	P-value	Sig.
		n = 40	n = 40			
HGB (g/L)	Mean ± SD	10.72 ± 1.61	10.02 ± 1.97	1.736	0.087	NS
TLC (cells/mm^3)	Mean ± SD	7.09 ± 1.34	7.90 ± 1.46	-1.382	0.171	NS
Platelet (mcL)	Mean ± SD	303.33 ± 7.65	269.43 ± 8.92	1.912	0.060	NS
ALT (U/L)	Mean ± SD	20.98 ± 4.21	20.58 ± 4.35	0.148	0.883	NS
AST (U/L)	Mean ± SD	24.83 ± 1.46	24.75 ± 4.34	0.028	0.978	NS
Creatinine (mg/dl)	Mean ± SD	1.07 ± 0.02	1.07 ± 0.32	-0.068	0.946	NS
Osteopontin	Mean ± SD	15.05 ± 3.65	47.20 ± 1.91	7.406	0.000	HS

Table (3) showed that osteopontin was highly significantly increased in group 1 than in group 2 (P-value < 0.01). There was non-significant difference between both groups in CBC, ALT, AST and creatinine (p-value > 0.05).

Table (4): Comparison between The studied groups as regards histopathologic finding

Histopathology	Control group		Patients group		Test value	P-value	Sig.
	No.	%	No.	%			
Adenocarcinoma	0	0%	32	78.7%	44.000	0.000	HS
Active chronic	32	62%	1	3.8%			
Intermucosal carcinoma	0	0%	2	5%			
Villous adenoma with malignant changes	0	0%	5	12.5%			

Table (4) showed highly significant difference between both groups in histopathologic finding (P-value < 0.01), where there was increase of the number of adenocarcinoma in patient group and increase of active chronic lesion in control group.

Table (5): Correlation between osteopontin levels and laboratory parameters of colorectal cancer patient

	Osteopontin	
	r	P-value
Age (year)	0.123	0.448
HGB (g/L)	0.170	0.294
TLC	-0.239	0.138
Platelet (mcL)	-0.202	0.212
INR	0.095	0.562
ALT (U/L)	0.401*	0.010
AST (U/L)	0.144	0.376
Creatinine (mg/dl)	0.230	0.153

Correlation studies showed negative correlations between osteopontin levels and age, CBC, INR, AST, creatinine (p > 0.05). There was a significant positive correlation between osteopontin level and ALT (p < 0.05).

Table (6): Correlation between osteopontin levels and demographic, clinical, radiological and staging parameters of CRC patients

		Osteopontin (Mean ± SD)	P-value	Sig.
Gender	Female Male	44.29 ± 27.23 49.35 ± 22.57	0.525	NS
Bleeding per rectum	No Yes	52.62 ± 25.86 43.58 ± 23.31	0.257	NS
Recurrent anemia	No Yes	49.76 ± 24.83 44.37 ± 24.38	0.493	NS
Constipation	No Yes	42.96 ± 20.95 52.94 ± 28.15	0.206	NS
Abdominal pain	No Yes	47.79 ± 24.75 46.31 ± 24.76	0.854	NS
Weight loss	No Yes	41.07 ± 26.43 50.5 ± 23.17	0.250	NS
CT showing mass	No Yes	40.55 ± 23.56 49.72 ± 24.71	0.295	NS
Histopathology	Adenocarcinoma Intermucosal carcinoma Villous adenoma	45.94 ± 25.29 54.00 ± 0.0 52.8 ± 25.24	0.786	NS
Metastasis	No Yes	43.14 ± 23.09 75.6 ± 12.07	0.004	HS
T stage	1 2 3 4	36.57 ± 16.96 72.25 ± 25.39 70 ± 16.39 72 ± 15.59	0.000	HS
N stage	0 1 2	40.13 ± 19.85 75.5 ± 23.56 40 ± 9.85	0.000	HS
M stage	0 1	41.26 ± 19.7 88.8 ± 7.53	0.000	HS

Table (6) correlated osteopontin level with gender, bleeding per rectum, constipation, weight loss, abdominal pain, recurrent anemia, and CT scan showing a mass. There was a positive correlation with no statistical difference where there was a mass in the pelviabdomenal CT scan and in histopathological examination. There was a positive correlation with a highly statistical difference in staging according to TNM that indicated an increased level of osteopontin in metastasis and staging at all grades.

Table (7): The ROC curve between patients and controls as regard osteopontin

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>12	0.889	85.00	77.50	79.1	83.8

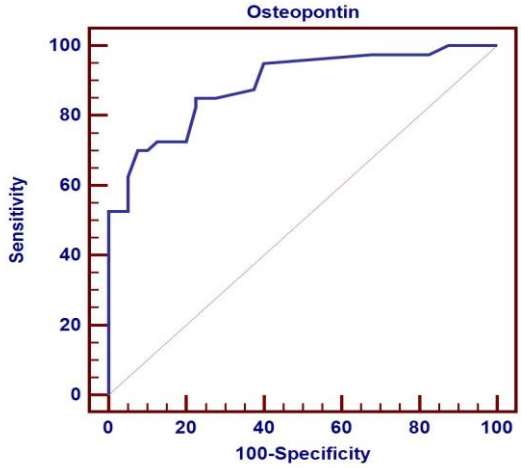

Figure (1): The ROC curve between patients and controls as regard osteopontin

Table (7) and Figure (1) showed the cut point between patients and control was =12 with sensitivity of 85%, specificity of 77.5%, positive predictive value (PPV) of 79.1% and negative predictive value (NPV) of 83.8%.

DISCUSSION

Colorectal cancer (CRC) is the third most common cancer and the fourth most common cause of cancer-related death. Its incidence is increasing year by year. Thus, early detection of CRC is essential to improve overall survival rates [8]. The most common tests for investigation of the whole colon are colonoscopy and pelviabdomenal CT with oral and IV contrast [9]. CT relies on rapid, high-resolution CT scanning of the prepared and gas-distended colon. Polyps and cancers are seen as wall thickening or by their disruption of the normal smooth wall of the colon [10]. Osteopontin (OPN) is found in different tissues and has a role in various biological processes such as inflammation, angiogenesis, and tissue remodeling. OPN expression has been seen to play a role in tumor formation and spread [11]. OPN expression has been detected in a variety of cancers, including lung cancer, breast carcinoma, esophageal cancer, endometrial cancer, gastric cancer, and malignant pleural mesothelioma [12].

Thus, our study aimed to evaluate the role of serum level of osteopontin in prediction of colorectal carcinoma in correlation with tissue histopathology which is the gold standard test for CRC detection and confirmation.

This study revealed that overall mean age of all patients was 52.25 ± 17.79 years. This study included 80 patients of whom 37 (46.25%) patients were males and 43 (53.75%) were females. In terms of sex and age, our study found no statistical significant difference between the two groups. This disagrees with the study done by **Alan *et al.*** [13] that showed minimal sex differences with higher mortality of colorectal cancer in men. They concluded that more targeted interventions are required for prevention and earlier diagnosis of CRC.

In our study, there was a highly significant difference between both groups in weight. But, there was no significant difference between both groups in bleeding per rectum, recurrent anemia, constipation and abdominal pain.

Regarding colonoscopy, it is considered the most sensitive modality for early diagnosis and treatment of some cases of CRC. Thus, screening for CRC by colonoscopy is recommended in mass population for its early detection [14]. CT scans of the abdomen and pelvis has a role in the diagnosis and management of CRC. Our study showed that CT with oral and IV contrast was one of the best radiological modalities for the diagnosis of CRC as in 72% of the patients' group a mass was detected by the CT scan. This agrees with the study of **Colvin and Lukram** [15] who concluded that the value of CT in demonstrating or excluding colorectal cancer is essential in routine practice.

Pathologically CRC is classified into adenocarcinoma, the commonest type, squamous cell, neuroendocrine, adenosquamous and undifferentiated carcinomas. Adenocarcinoma is divided into well, moderate, and poorly differentiated adenocarcinoma according to percentage of gland formation. In well differentiated adenocarcinoma, > 95% of the tumor is gland forming, while in moderately differentiated adenocarcinoma there is 50-95% gland formation. Poorly differentiated adenocarcinoma is mostly solid with < 50% gland formation [16].

Our study showed a highly significant difference between both groups in histopathological findings (P-value < 0.01) that showed an increase in the number of adenocarcinoma in patients' group and an increase in active chronic lesions in the control group. This is similar to the study of **Matthew *et al.*** [16] who found that more than 90% of colorectal carcinomas are adenocarcinoma, which originate from the epithelium of the colonic mucosa.

Our study showed a highly significant difference between both groups in osteopontin as there was an increase in OPN level in group 1 than in group 2. This agrees with meta-analysis study by **Zhao *et al.*** [17] that included 15 studies with 1698 CRC patients. They concluded that there is a significant association between increased OPN levels and the high tumor grade. Also they found that OPN level increases with the increase in the depth of the invasion. In addition, going with our findings, **Eschrich *et al.*** [18] study found that OPN expression was high in CRC. Their study was done on 13 normal colonic tissues, 9 adenomas, 120 1^{ry} colonic neoplasms, and 10 associated with hepatic secondaries. OPN expression was strongly elevated in 1^{ry} colonic neoplasms and hepatic secondaries, but not in pre-cancerous lesions and UICC stage I tumors.

Our study showed a positive correlation between OPN level and cases of bleeding per rectum, cases of constipation, cases of weight loss, cases of abdominal pain, and cases of recurrent anemia.

In correlating OPN level with staging according to TNM we found a positive correlation with a highly statistical difference. That indicates an increased level of osteopontin in metastasis and staging at all grades. This agrees with the study of **Jing *et al.*** [19] that was done on tissue specimens. They confirmed that OPN level is increased in CRC. Also, they found that its level increased significantly as the tumor stage increased and with the lymph node metastasis. Our study disagrees with the study of **Jing *et al.*** [19] who found that overexpression of OPN was not significantly correlated with different histological types, however our study showed a positive correlation between osteopontin level and histopathologic types.

CONCLUSION

Serum osteopontin (OPN) level in our study was found to be high in patients with CRC in comparison to patients with normal colonoscopy. Therefore, it can be used as a biomarker in the diagnosis of CRC. Also, serum osteopontin level is significantly higher in cancer

patients with metastasis according to TNM staging, and thus it can be used in prognosis.

Abbreviations:
ALT: Alanine Aminotransferase, ANOVA: Analysis of variance, AST: Aspartate Aminotransferase, AUC: Area under curve, CA19.9: Cancer antigen 19.9, CBC: Complete Blood Count, CEA: Carcinoembryonic antigen, CRC: Colorectal cancer, ELISA: Enzyme Linked Immunosorbent Assay, GIT: Gastrointestinal Tract, INR: International Normalized Ratio, K: Potassium, Na: Sodium, NPV: Negative Predictive value, OPN: Serum Osteopontin, PPV: Positive Predictive value, ROC-curve: Receiver Operating Characteristic curve, UICC: Union for International Cancer Control and VEGF: Vascular endothelial growth factor,

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REFERENCES

1. **Ferlay J, Soerjomataram I, Dikshit R *et al.* (2015):** Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Patterns in GLOBOCAN 2012. International Journal of Cancer, 136: 359-86.
2. **Laubert T, Freitag-Wolf S, Linnebacher M *et al.* (2015):** Stage-Specific Frequency and Prognostic Significance of Aneuploidy in Patients with Sporadic Colorectal Cancer— A Meta-Analysis and Current Overview. International Journal of Colorectal Disease, 30: 1015-1028.
3. **King L, Love C, Sieber O *et al.* (2016):** Differential RNA-Seq Analysis Comparing APC-Defective and APC-Restored SW480 Colorectal Cancer Cells. Genomics Data, 7: 293-296.
4. **Bolocan A, Ion D, Ciocan D *et al.* (2012):** Prognostic and Predictive Factors in Colorectal Cancer. Chirurgia (Bucur), 107: 555-563.
5. **Ichikawa H, Imano M, Takeyama Y *et al.* (2009):** Involvement of osteopontin as a core protein in cholesterol gallstone formation. J Hepatobiliary Pancreat Surg., 16: 197–203.
6. **Imano M, Satou T, Itoh T *et al.* (2009):** Immunohistochemical expression of osteopontin in gastric cancer. J Gastrointest Surg., 13: 1577–1582.
7. **Chakraborty G, Jain S, Kundu G (2008):** Osteopontin promotes vascular endothelial growth factor-dependent breast tumor growth and angiogenesis via autocrine and paracrine mechanisms. Cancer Res., 68: 152–161.
8. **Brenner H, Stock C, Hoffmeister M (2014):** Effect of screening sigmoidoscopy and screening colonoscopy on colorectal cancer incidence and mortality: systematic review and meta-analysis of randomised controlled trials and observational studies. BMJ., 348: g2467.
9. **Wolff W (1989):** Colonoscopy: history and development. Am J Gastroenterol., 84 (9): 1017–25.
10. **Pickhardt P, Kim D (2013):** CT colonography: pitfalls in interpretation. Radiol Clin North Am., 51 (1): 69–88.
11. **Denhardt D, Giachelli C, Rittling S (2001):** Role of osteopontin in cellular signaling and toxicant injury. Annu Rev Pharmacol Toxicol., 41: 723–749.
12. **Cho H, Kang E, Kim Y *et al.* (2009):** Diagnostic and prognostic impact of osteopontin expression in endometrial cancer. Cancer Invest., 27: 313–323.
13. **Alan W, Lucy I, Robert J *et al.* (2018):** A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the UK. BMC Cancer, 18 (1): 1-1.
14. **John K, Constantine V, George M (2015):** Indian Association of Surgical Oncology. Colonoscopy in Colorectal Cancer Screening: Current Aspects. Indian Journal of Surgical Oncology, 6 (3): 237-50.
15. **Colvin H, Lukram A, Sohail I *et al.* (2013):** The performance of routine computed tomography for the detection of colorectal cancer. The Annals of The Royal College of Surgeons of England, 95 (7): 473-6.
16. **Matthew F, Sreelakshmi R, Sergei F *et al.* (2012):** Colorectal carcinoma: Pathologic aspects. J Gastrointest Oncol., 3 (3): 153-73.
17. **Zhao M, Liang F, Zhang B *et al.* (2015):** The impact of osteopontin on prognosis and clinicopathology of colorectal cancer patients: A systematic meta-analysis. Sci Rep., 5: 1-8.
18. **Eschrich S, Yang I, Bloom G *et al.* (2005):** Molecular staging for survival prediction of colorectal cancer patients. J Clin Oncol., 23: 3526–35.
19. **Jing L, Guang-Zhi Y, Zi-Man Z *et al.* (2012):** Osteopontin is overexpressed in colorectal carcinoma and is correlated with P53 by immunohistochemistry. Experimental and Therapeutic Medicine, 3 (4): 621-4.