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Evaluation of metalloprotenase-1 in early diagnosis of colon cancer

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Colorectal cancer (CRC) is treatable disease if diagnosed early. However, current screening methods are suboptimal and no serum-based test is sufficient for widespread use. Here we evaluated the efficacy of metalloprotenase-1 (MMP-1) as a non-invasive marker for early CRC diagnosis. One hundred ninety five patients had undergone colonoscopy examination were inclusive in the study (120 CRC and 75 benign conditions patients). In addition, 56 healthy individuals were included. MMP-1 was identified based on SDS-PAGE followed by Western blotting and then all samples were tested using ELISA for MMP-1 levels quantification. Area under the receiver operating characteristic curve (AUC) was applied for evaluation the diagnostic power of MMP-1. The MMP-1 level was significantly lower ($P < 0.0001$) in colon cancer patients (3.0±0.3 µg/mL) compared to patients with benign disorders (4.6±0.9 µg/mL) and healthy controls (5.7±0.8 µg/mL). Decreased MMP-1 levels were associated with CRC progression including late stages, positive lymph node invasion, distant organ metastasis and high grades. When differentiate colon cancer from all non-cancerous individuals, MMP-1 yielded AUC of 0.84 with 75.0% sensitivity and 82.5% specificity. Also MMP-1 had 70.0% sensitivity, 75.0% specificity and AUC of 0.79 when differentiate colon cancer from benign disorders. Moreover, for differentiating CRC patients with early stages and those with low grade from patients with benign disorders MMP-1 had AUC of 0.77 and 0.73 respectively. MMP-1 can be used as an effective biomarker particularly for early detection of CRC and for differentiation early stages from benign disorders.

Keywords: Colorectal cancer, Diagnosis, Biomarkers, Matrix metalloproteinase, MMP-1.

INTRODUCTION

Colorectal cancer (CRC) is the 3rd most popular cancer among males and females with an estimated 140,250 new cases and 50,630 deaths in 2018 (Cabo et al. 2020). It is a major public health issue by its high incidence and mortality rate in people over 50 years old, perhaps as a result of environmental conditions, diet, and aging (Diler, 2019). Currently, regular screening of CRC using colonoscopy (an invasive method) is the gold standard procedure for diagnosis and prevention of CRC but, colonoscopy might pose a risk of tear in the wall of colon or rectum (Sornapudi et al. 2019). Also, it may be costly, deterring many patients from using it as ordinary screening (Bhatt and Emuakhagbon, 2019).

Additionally, other screening methods including flexible sigmoidoscopy, endoscopy, magnetic resonance imaging, fecal occult blood tests and fecal immunochemical test have inherent cons, like false positives and false negatives results (Pakiet et al. 2019; Woudstra et

al. 2019). Moreover, it had been indicated that serum tumor markers levels such as carcinoembryonic antigen (CEA) and cancer antigen 19-9 (CA19-9), are closely related to the CRC prognosis (Jia et al. 2019). But unfortunately, the elevated serum of these tumor markers is not highly specific for CRC as various other gastrointestinal disorders contribute to high CEA levels and can be associated with false-positive results. Also, the other basic harms of these tumor markers are their failure to localize the lesions (Ardekani et al. 2019). Therefore, novel CRC biomarkers should be developed that will further enhance the detection and follow-up (Oh and Joo, 2020).

On the other hand, matrix metalloproteinases (MMPs) are a family of zinc and calcium-dependent endopeptidases related to many physiological and pathological processes (Zhang et al. 2019). Their roles in cancer progression had been explained by the extracellular matrix (ECM) degradation. Among them, MMP-1 also defined as collagenase-1, has been indicated to play a crucial role in degrading tumor cells, cleavage of collagenous ECM in different pathological and physiological processes, and promoter polymorphisms in several cancers (Wang et al., 2020). In CRC, MMP-1 expression suggested to be a prognostic factor (Ju et al. 2019). Therefore, this study aimed to identify MMP-1 in CRC patients and establish its ability for early CRC diagnosis in particularly to distinguish between different stages of CRC and benign disorders. Also, we aimed to investigate the association between MMP-1 and CRC severity features including late stage, high grade, positive lymph node, and positive distant metastasis.

MATERIALS AND METHODS

Samples

This study is carried out on 195 serum samples from Egyptian individuals with colon diseases (113 males and 82 females). They were collected from the Oncology Center, Mansoura University Hospitals, Mansoura, Egypt. Patients were classified into 2 groups; benign group includes 75 patients and colon cancer group includes 120 patients. Tumor features were classified according to the Union for International Cancer Control, Tumor-Node-Metastasis (TNM) Staging System (Compton and Greene, 2004). Also, 56 serum samples from age- and sex-matched healthy Egyptian individuals were collected and used in the experimental investigation as controls. Neither of patients with benign tumors nor of healthy had a history of any other advanced cancer. This study was approved by the ethical guidelines of the Helsinki Declaration.

Laboratory assays

Blood samples of this study were collected by vein puncture from all patients. Sera were obtained after centrifugation at 4000 rpm for 15 minutes processed and stored at -20°C till use. As previously described, MMP-1 was identified based on SDS-PAGE followed by Western blotting (Attallah et al. 2015). Additionally, all samples were tested using ELISA for MMP-1 according to Attallah et al. (2017).

Statistical analysis

All statistical analyses were done by statistical software package SPSS 15.0. Data were expressed as mean ± standard deviation (SD), differences in continuous variables were assessed using student *t-test* or *ANOVA* and X^2 tests for categorical variables. All tests were two-tailed and statistical significance assessed at 0.05 level. Receiver operating characteristic (ROC) curves and AUC were used to determine the best cutoff values for optimal colon cancer detection and MMP-1 diagnostic performance (Kumar and Indrayan, 2011). MMP-1 sensitivity and specificity were calculated by standard formulae (Griner et al. 1981).

RESULTS

Characteristics of included patients and controls

Characteristics of all participants enrolled in this study are shown in Table 1. According to tumor depth penetration, the 120 colon cancer patients were 56 (46.7%) with early tumor stages (T1-T2) and 64 (53.3%) with late tumor stages (T3-T4). Consistently, colon cancer patients consisted of 75 (62.2%) without lymph node invasion (N0) and 45 (37.5%) with lymph node invasion (N1-N2). In addition, they were classified into two main groups according to distant organ metastasis: 86 (71.7%) cases were without distant organ metastasis (M0) and 34 (28.3%) with distant organ metastasis (M1). A total of 70 cases (58.3%) had low tumor grade (G1-G2), and 50 (41.7%) cases had high tumor grade (G3-G4).

Identification of MMP-1

Selected serum samples from all participants were analyzed by SDS-PAGE and western blot analysis, using antibodies that specifically recognize MMP-1 (Figure 1A), demonstrated that this protein migrates as a single band of the expected molecular weight 245 KDa in serum samples from patients with benign conditions, colon cancer and healthy individuals.

Determination of MMP-1 using ELISA

The mean level of MMP-1 was significantly lower ($P < 0.0001$) in colon cancer patients (3.0 µg/mL) versus patients with benign disease (4.6 µg/mL) and healthy controls (5.7 µg/mL) (Figure 1B). As shown in Figure 2(A-D) it was found that these decreased MMP-1 levels were significantly associated with some tumor severity features including, late stages (2.5±0.6 *vs* 3.5±0.7 µg/mL, $P < 0.0001$), lymph node invasion (2.3±0.5 *vs* 3.4±0.6 µg/mL, $P < 0.0001$), distant organ metastasis (2.5±0.4 *vs* 3.2±0.4 µg/mL, $P= 0.017$) and high histological grades (2.6±0.3 *vs* 3.2±0.4 µg/mL, $P= 0.014$).

Diagnostic performances of MMP-1

Using ROC curve, the utility of the serum MMP-1 level for diagnosing colon cancer were examined. MMP-1 has sensitivity of 75.0%, specificity of 82.5%, and AUC of 0.84 when discriminating colon cancer patients from all non-cancerous individuals. Also, MMP-1 was 70.0% sensitive and 75.0% specific for discriminating cancer patients from patients with benign disorders with an AUC of 0.79 (Figure 3A, B). For discriminating patients with early stage from patients with benign disorders, MMP-1 has sensitivity of 68.0% and specificity of 73.0% with 0.77 AUC. Also, MMP-1 yielded an AUC of 0.73, sensitivity of 67.5% and specificity of 73.0% in differentiating patients with low grade from those with benign disorders (Figure 3C, D).

Table 1: Characteristics of included patients and healthy individuals

Participants	No
Healthy individuals	56
Age (years)	48.6 ± 12.4
Gender (male/female)	32/24
Benign conditions patients	75
Age (years)	43.8 ± 13.6
Gender (male/female)	50/25
Colon cancer patients	120
Age (years)	51.2 ± 11.8
Gender (male/female)	63/57
TNM classification	
Tumor stage (T1-T2/ T3-T4)	56/64
Lymph node invasion (N0/N1-N2)	75/45
Metastasis (M0/M1)	86/34
Tumor grade (G_1-G_2/ G_3-G_4)	70/50

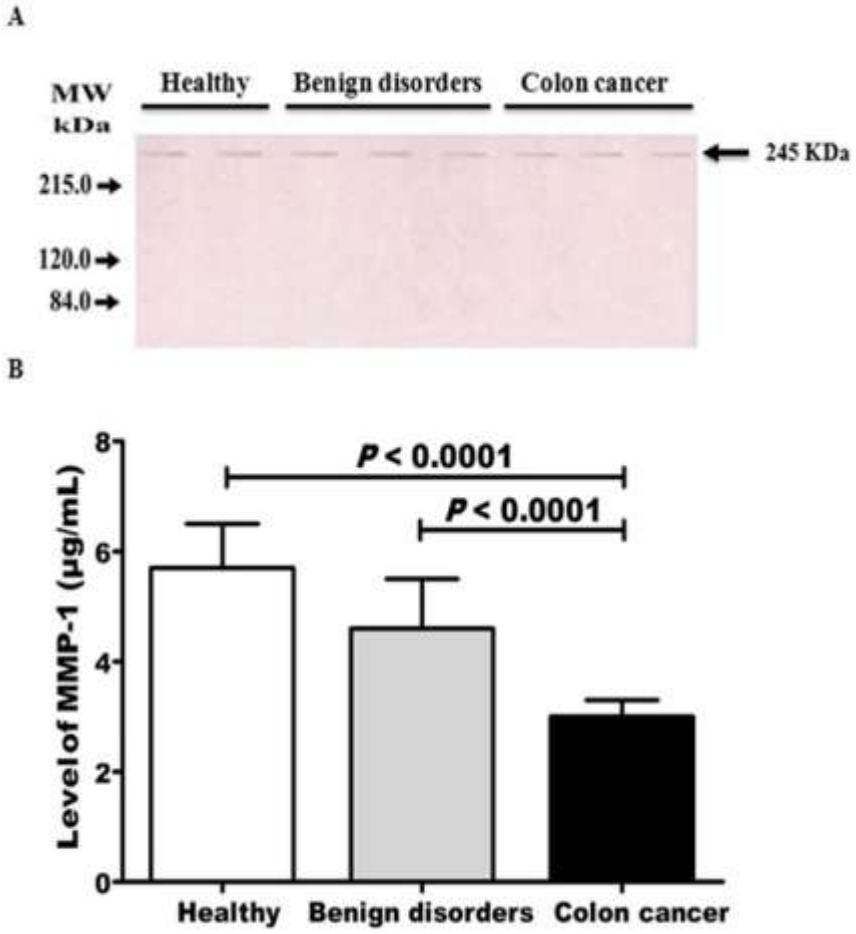

Figure 1: Matrix metalloproteinase-1 in colon cancer. (A) Expression of serum MMP-1 at 245 kDa in colon cancer, benign diseases, and healthy individuals detected by Western blotting. (B) Levels of MMP-1 in health, benign, and colon cancer groups.

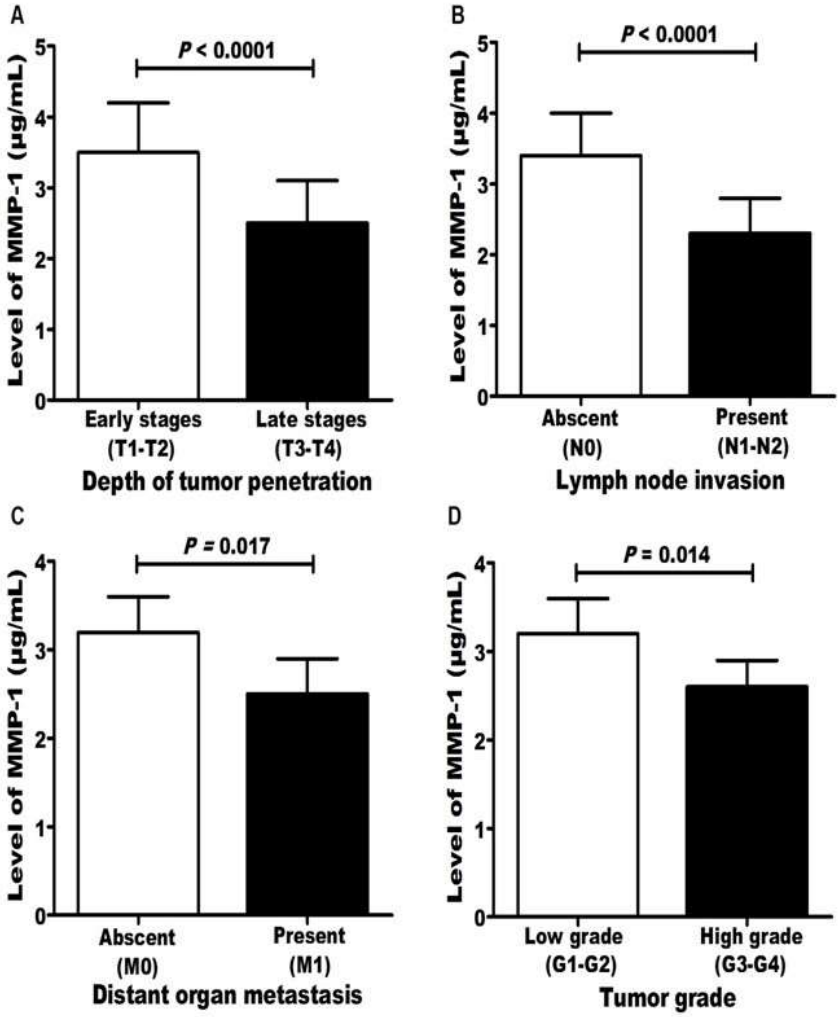

Figure 2: Distribution of circulating matrix metalloproteinase-1 values according to (A) depth of tumor penetration, (B) lymph node invasion, (C) distant organ metastasis and (D) tumor grade.

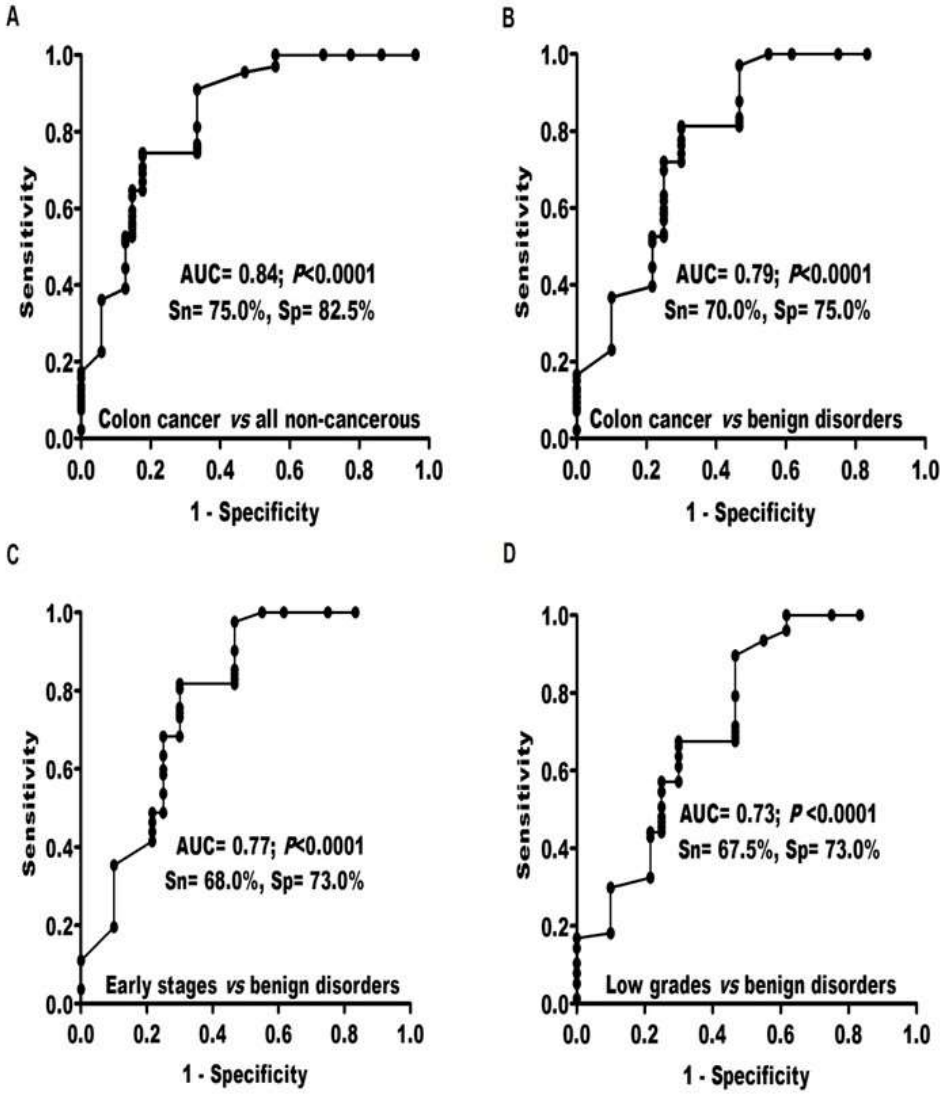

Figure 3: Area under receiver-operating characteristic curve of MMP-1 for separating (A) colon cancer patients from all non-cancer controls, (B) Patients with colon cancer from benign disorders, (C) Patients with early stages from benign disorders and (D) Patients with low grades from benign disorders. AUC: area under the receiver operating characteristic curve, Sn: sensitivity and Sp: specificity.

DISCUSSION

The incidence rate of CRC is increasing globally (Ladabaum et al., 2020). Diagnosis of CRC patients at earlier or middle stages have a better prognosis than those at late stages (Feng et al., 2019). However, there has been limited clinical success in developing effective, non-invasive diagnostic approaches for CRC early detection (Luo et al., 2020).

In this study, a single immunoreactive band for MMP-1 was shown at 245 kDa due to their binding with their respective mono-specific antibodies. These results were corresponding to as previously described results (Attallah et al., 2020). Also, other study showed that MMP-1 molecular mass were >200 kDa (Grinnell et al., 1998). Also Richardson et al showed that MMP-1 was shown at 245 kDa (Richardson et al., 1987).

Here, MMP-1 level was found to be significantly decreased (P <0.0001) in patients with colon cancer when compared to patients with benign or healthy individuals. The decreased

MMP-1 levels were associated with sever tumor features including late stages, positive lymph node invasion, distant organ metastasis, and high grades of colon cancer. In case of benign conditions, the decreased levels of MMP-1 in our patients with colon were agree with results of Stumpf et al who also found a statistically significant reduction of MMP-1 expression in benign group. Also, the downregulation of MMP-1 might be very important for the development of diverticulitis (benign tumor of colon) (Stumpf et al., 2001).

High expression of MMP-1 is confined to the primary tumors (at its organ of origin), as MMP-1 plays a more important role in the early stage of the invasive process while being less evident in metastatic lesions. In line with this view, there is a linear decrease in MMP-1 expression parallel with an increasing stage (Bendardaf et al., 2007). This is due to the high expression of their specific inhibitors tissue inhibitor metalloproteinase (TIMPs) (Benjamin and Khalil, 2012). Moreover, CRC promotes TIMP-1 synthesis in cancer cells than normal counterparts, which ultimately increases the development of cancers. Furthermore, TIMP-1 induces CRC growth, accumulation of Cancer-Associated Fibroblasts (CAFs) and inhibits apoptosis (Kuppusamy et al., 2017). This explains the decrease MMP-1 in metastatic cancer as TIMP-1 is co-expressed with MMPs and contributes to the regulation of local MMPs activity, which may lead to reduced MMPs activity and also related to a worse outcome (Lee et al., 2011).

Using the ROC curve which is the recommended method for assessing the accuracy of a diagnostic test (Swets, 1986). Our study showed that, decreased MMP-1 could be effective in CRC diagnosis and had a sensitivity of 75.0%, a specificity of 82.5%, and AUC of 0.84 when discriminating colon cancer patients from all non-cancerous individuals. This is well comparable to other colon cancer established biomarkers such as CA 19-9 (62.1% sensitivity, 89.3% specificity, 0.739 AUC), CEA (65.0% sensitivity, 90.7% specificity, 0.790 AUC) and CA 72-4 (45.2% sensitivity, 96.0% specificity, 0.746 AUC) to differentiate colon cancers from healthy control (Ning et al., 2018).

Interestingly, MMP-1 was 70.0% sensitive and 75.0% specific when discriminating colon cancer patients from patients with benign disorders with an AUC of 0.79. This is comparable to CEA (54.2% sensitivity, 68.7% specificity, 0.58 AUC), CA19-9 (64.2% sensitivity, 70.6% specificity, 0.60 AUC) and Cytokeratin-1 (70.8% sensitivity, 73.7% specificity, 0.75 AUC) to differentiate CRC from benign disorders (Attallah et al., 2018). Other study indicated that, CEA (64.5% sensitivity, 89.2% specificity 0.789 AUC) also; CA 19-9 (47.8% sensitivity , 90.1% specificity 0.690 AUC) for detection of CRC *vs* benign (Zhang et al., 2015).

In addition, MMP-1 can be a very important blood marker to detect the early stage of CRC (AUC = 0.77, 68.0% sensitivity, 73.0% specificity) and low grade (AUC=0.73, 67.5% sensitivity, 73.0% specificity) from patients with benign disorders. In detecting CRC early stage the sensitivity of CEA ranged from 43-69%, CA 19-9 from 18-65%, CA72-4 from 9-31%, P53 from 15-28% and CA125 57.1% (Hundt et al., 2007; Lech et al., 2016; Kopylov et al., 2020). Similar small sensitivity ranges were observed for vascular endothelial growth factor (VEGF) (35%-86%), Interleukin 3 (IL-3) (55%), tumor M2 pyruvate kinase (M2-PK) (45%-76%) and TIMP-1 (55%) in most studies (Hundt et al., 2007).

CONCLUSION

MMP-1 can be a useful indicator for clinical assessment of CRC. Also, serum MMP1 is superior to other markers for the diagnosis of CRC patients. This superiority is attributed to the higher sensitivity and specificity particularly in early stages. Further large scale studies are needed to evaluate the potential usefulness of combination of MMP-1 and other CRC established markers for the examination and monitoring of patients with CRC.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Attallah AM, El-Far M and Abdelrazek MA were chief investigators who conceptualized and designed the study. They equally participated in all parts of this work. Atwa RA was investigator who collected data from the literature and collected samples. Atwa RA and Abdelrazek MA carried on with different experiments and techniques. Abdelrazek MA performed all data

statistical analysis. All authors read, reviewed and approved the final manuscript.

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