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Study of tumor endothelial marker 1 in patients with colorectal cancer

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Abstract
Background: TEM1 represents a transmembrane glycoprotein expressed on developing as well as adult tissues going through active physiological or pathological angiogenesis. This work was aimed at assessing TEM1 levels as a diagnostic as well as prognostic serum biomarker among CRC cases.
Methods: Our case- control study involved sixty subjects. Subjects underwent a categorization into the following groups: Group I: Colorectal cancer patients (n=30): diagnosed with CRC. Group II: control group (n=30): healthy individuals free of colorectal cancer as a control group. Tumor markers carcinoembryonic antigen (CEA) as well as carbohydrate antigen 19-9 (CA19-9), TEM1, histological grading of tumor, specific investigations, abdomen ultrasonography, CT and MRI were performed to all participants.
Results: The serum TEM1 levels exhibited significantly greater values within in CRC group as opposed to the control group. Serum TEM1 levels also showed significantly greater values within CRC cases having grade III+IV as opposed to others having tumor grade I + II, tumor size T_3+T_4 than those with tumor size T_1 +T_2, lymph node (LN) infiltration N1 + N2 than those without LN infiltration N0, tumor metastases M1 than those without tumor metastases M0 and tumor metastases (TNM) stage (III) than those with TNM stage (I+II).
Conclusion: Serum TEM1 levels exhibited a significant rise in CRC cases, which suggested that serum TEM1 could bring potential benefits as a diagnostic biomarker for CRC. High serum TEM1 levels were significantly linked to poor clinicopathological features. Therefore, it is an adverse indicator for CRC prognosis, and it could be used as a prognostic marker of malignancy.

Keywords: Cancer, colorectal, diagnostic, prognostic, tumor endothelial marker 1

Introduction
Colorectal cancer (CRC) ranks as the third most frequently occuring cancer globally, accounting for 1.4 million new cases along with approximately recorded 700,000 deaths yearly [1].
The National Cancer Institute registry at Cairo University indicates that in Egypt, CRC accounts for around 6.5% of all malignant tumors. it occurs frequently in Egypt, and is being detected in around 14.0% of all colonoscopies [2].
The CRC morbidity and mortality occurrence rates are continued to rise as a result of population aging along with major negative impacts linked to lifestyle-related variables [3].
Currently, several national initiatives seeking to enhance the CRC early identification prior to progression, have achieved favorable results [4].
Although there have been ongoing advancements as regards diagnostic techniques, involving flexible sigmoidoscopy, stool-based testing, blood markers, as well as colonoscopy, the National Cancer Institute's data addresses a considerable proportion of CRC cases being detected at advanced stages [5].
The two predominant serum markers utilized in monitoring and diagnosing CRC involve carcinoembryonic antigen (CEA) as well as carbohydrate antigen 19-9 (CA 19-9). They exhibit inadequate sensitivity, particularly during the first CRC phases. Hence, identifying serum biomarkers that are both sensitive as well as specific remains crucial for the early noninvasive CRC detection [6].
TEM exhibit significant changes as regards both morphologic and genetic phenotype involving structural chromosomal alterations as well as mutations.

These alterations distinguish them from normal endothelium (NECs). They possess stem cell-like origin, making them crucial for detecting tumor neo-angiogenesis [6].

Tumor endothelial marker 1 (TEM1), Endosialin, CD 248) represents a transmembrane glycoprotein that are found on the cells' surfaces. It is often present in both developing as well as adult tissues going through active physiological or pathological angiogenesis. TEM1 expression exhibits a significant greater value within tumors' vasculature as opposed to the healthy tissues' vessels (Kontsekova *et al*., 2016) [12].

TEM1 gene switch off led to disrupted tumor vascularization, reduced growth, invasion, as well as metastasis within human CRC cells [7].

TEM1 tissue overexpression was observed within CRC cases as opposed to healthy controls along with in rectal cancer tissues while performing a comparison among stage I tumors and tumors at stages II, III, and IV based on the TNM classification [8].

This work was aimed at evaluating TEM1 serum levels as a diagnostic and prognostic serum biomarker in patients with CRC.

Patients and Methods: Our case- control study involved sixty subjects. It commenced following the Tanta University Ethical Committee's approval. All subjects were asked to fill an informed consent.

We excluded cases having radiotherapy, chemotherapy, or chemoradiotherapy preoperatively or with prior history of malignancy. Our research's participants went through a categorization into these groups: Group I: CRC cases (n=30): those diagnosed with CRC. Their mean age was 51.53 ± 4.24 years. Group II: controls' group (n=30): It involved thirty healthy individuals free of colorectal cancer as a control group. Their mean age was 50.23 ± 4.19 years.

All participants underwent a comprehensive medical history, clinical assessment, lab tests (tumor markers CEA and CA19-9, imaging techniques (abdomen ultrasonography, abdomen CT and MRI), histological grading of tumor and specific investigations (assessing TEM1 serum levels utilizing enzyme linked immunosorbent assay (ELISA) technique).

Collecting blood was carried out in a sterile environment. Each individual had 3 milliliters of venous blood drawn utilizing a disposable sterile plastic syringe and were collected in plain tube. Centrifuging was then accomplished for a period of fifteen min at 3000 R.P.M for separating serum. Storage was then done at -20 until assay of TEM1

Measurement of serum level of TEM1

Using ELISA Kit for the quantitative detection of TEM1, commercially available by SunRed Company (SUNRED., CHINA). Three wells of standard, blank, samples were prepared as manufacturer guides. 40µl from were added from each sample, followed by the addition of TEM1-antibody10µl as well as Streptavidin-HRP 50µl. Subsequently, the sealing membrane was securely closed, delicately agitated, and placed in an incubator for a duration of 60 min at 37. After removal, Incorporating Chromogen solution A 50µl &chromogen solution B 50µl was accomplished to the wells, followed by a gentle mixing as well as incubation for 10 min at 37 ºC avoiding exposure to light. Then, Reaction ceased when adding stop solution 50µl (A quick color change from blue to yellow occurred). Measuring the optical density (OD) was accomplished at a wavelength of 450 nm in fifteen min after incorporating the stop solution. The concentration of the samples was determined directly from this standard curve.

Statistical analysis: Data went through a statistical analysis utilizing SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative variables were displayed as mean as well as SD then a comparison among both groups was established utilizing unpaired Student's t-test. Qualitative variables were displayed as frequency as well as percentage (%) then went through analysis utilizing the Chi-square or Fisher's exact test when appropriate. A two-tailed P value of less than 0.05 was deemed to show a statistically significance.

Results

No statistically significant variation was documented among the studied groups regarding age as well as sex (P= 0.147, 0.432, respectively). The serum TEM1 levels in CRC group were significantly higher as opposed to to controls (P= 0.001). Table 1

Table 1: Comparison among all group regarding demographic data and serum TEM1 level

		Patient (n=30)	Control (n=30)	P. value
Age (years) (Mean± SD)		51.53±4.24	50.23±4.19	0.147
Sex N (%)	Male	16 (53.3%)	19 (63.3%)	0.432
	Female	14 (46.7%)	11 (36.7%)	
TEM 1 (ng/ml) (Mean ± SD)		8.69±1.64	2.08±0.76	0.001*

Data is exhibited as Mean ± SD or frequency (%). * significant as P value < 0.05. TEM 1: Tumor endothelial marker 1.

Serum TEM1 levels exhibited significant greater values within colorectal cases having grade III+IV as opposed to others having tumor grade 1 + II (P= 0.001). Table 2

Table 2: Association between serum TEM1 level as well as tumor grade in colorectal cancer group

	Grade (I+II) (n=17)	Grade (III+IV) (n=13)	P. value
TEM 1	7.37 ± 0.74	10.40 ± 0.39	0.001*

Data is exhibited as Mean ± SD. * significant as P value < 0.05. TEM 1: Tumor endothelial marker 1.

Serum TEM1 levels exhibited significantly greater values within CRC cases having tumor size T_3+ T_4 as opposed to others having tumor size T_1 +T_2 (P= 0.001). Table 3

Table 3: Association between serum TEM1 level as well as tumor size in colorectal cancer group

	T 1 + T 2 (n=16)	T 3 + T 4 (n=14)	P. value
TEM 1	7.32 ± 0.73	10.25 ± 0.69	0.001*

Data is exhibited as Mean ± SD. * significant as P value < 0.05. TEM 1: Tumor endothelial marker 1.

Serum TEM1 levels exhibited significantly greater values within CRC cases having lymph node (LN) infiltration N1 + N2 as opposed to others having LN infiltration N0 (P= 0.001). Table 4

Table 4: Association between serum TEM1 level as well as LN infiltration in colorectal cancer (CRC) group

	N 0 (n=17)	N 1+2 (n=13)	P. value
TEM 1	7.37 ± 0.74	10.40 ± 0.39	0.001*

Data is exhibited as Mean ± SD. * significant as P value < 0.05. TEM 1: Tumor endothelial marker 1.

Serum TEM1 levels showed significantly greater values within CRC cases having tumor metastases M1 as opposed to others with no tumor metastases M0. Table 5

Table 5: Correlation between serum TEM1 level as well as tumor metastases in colorectal cancer group

	T 1 + T 2 (n=16)	T 3 + T 4 (n=14)	P. value
TEM 1	8.46 ± 1.58	10.70 ± 0.10	0.022*

Data is exhibited as Mean ± SD. * significant as P value < 0.05. TEM 1: Tumor endothelial marker 1.

Serum TEM1 levels showed significantly greater values within CRC cases having TNM stage (III) as opposed to others having TNM stage (I+II) (P= 0.001). Table 6

Table 6: Correlation between serum TEM1 level as well as tumor metastases in colorectal cancer group

	TNM stage (I+II) (n=14)	TNM stage (III) (n=16)	P. value
TEM 1	7.21 ± 0.71	9.98 ±0.97	0.001*

Data is exhibited as Mean ± SD. * significant as P value < 0.05. TEM 1: Tumor endothelial marker TNM: tumor metastases.

Discussion

TEM1 is a glycoprotein that is found in the endothelium and/or stroma of different cancer types [9]. TEM1 is elevated in fibrotic diseases such as rheumatoid arthritis, fibrotic kidney diseases, liver fibrosis in addition to several human carcinomas' types such as lungs, pancreas, breasts, urinary bladder, brain glioma, as well as melanoma (Tomkowicz *et al*., 2014) [13].

In the current study, serum TEM1 levels in CRC exhibited significantly greater values as opposed to controls. This agreed with Pietrzyk and Wdowiak *et al*. [3] who proved that an elevated serum TEM1 levels was documented in among CRC cases as opposed to the healthy controls. Also, Zhang *et al*. [10] observed that stroma'TEM1 expression exhibited a significant rise from distant or nearby normal mucosa to the tumor within CRC cases.

Also, Macfadyen *et al*., 2017 [14] showed that the stroma's TEM1 expression was elevated within both radiotherapy-treated as well as non-radiotherapy-treated cancers as opposed to normal mucosa.

Prior research have addressed that TEM1 expression is found on breast tumors' pericytes, brain gliomas, melanoma metastases as well as metastatic carcinomas that exhibit high invasive activities (Davies *et al*., 2014) [15].

The present study showed that serum TEM1 levels exhibited significantly greater values within CRC cases having grade (III+IV) as opposed to others having tumor grade (1 + II). This was in agreement with Pietrzyk and Wdowiak *et al*. [3] who found that TEM1 concentration in serum exhibited significantly greater values within CRC cases having an advanced stage (stage III + IV) as opposed to others developing an early stage (stage I + II) group. Additionally, Zhang *et al* [10] observed that the stroma's TEM1 expression was often documented within advanced TNM stages.

The present study revealed that serum TEM1 levels exhibited significantly greater values within CRC cases developing advanced tumors' size (T3+T4) as opposed to others developing early tumors' size (T1 +T2). This finding agreed with Pietrzyk and Wdowiak *et al*. [3] addressing that serum TEM1 concentrations were T stage dependent. Additionally, they exhibited an increase after the T stages' development.

Our research addressed that, serum TEM1 concentrations exhibited significantly greater values within CRC cases with LN infiltration (N1 + N2) than those without LN infiltration (N0). Pietrzyk and Wdowiak *et al*. [3] also addressed, the serum TEM1 levels exhibited a significant correlation with the N stages' development. Zhang *et al*. [10] also found positive TEM1expression in colorectal tumor cells was more often documented in tumors showing infiltrative growth pattern as opposed to expansive growth ones.

In the current study, serum TEM1 concentrations exhibited significantly greater values within CRC cases having tumor metastases (M1) as opposed to others those without tumor metastases (M0). This was in agreement with Pietrzyk and Wdowiak *et al*. [3] who reported that serum TEM1 concentrations showed greater values within the M1 subgroup as opposed to the M0 subgroup. This could be explained by Chen *et al*. [11] who revealed that tumor-associated endothelial cells (TEC) proliferation, migration as well as differentiation possess a primary role while forming new blood vessels along with CRC cells' spread induced by tumor-derived interleukin-33 (IL-33) as well as TEM1.

Our study also addressed that, serum TEM1 concentrations exhibited significantly greater values within CRC cases developing advanced TNM stage (III) than those with TNM stage (I+II). This was in accordance with Pietrzyk and Wdowiak *et al*. [3] who demonstrated that serum TEM1 levels showed a strong correlation with the TNM staging classification, addressing that serum TEM1 level could bring potential benefits as a valuable biomarker for cancer progression.

The poor prognosis for CRC patients with elevated serum TEM1 could be explained by various mechanisms: the interaction between TEM1 and ECM proteins facilitated cell adhesion along with migration. Additionally, TEM1 is bound to fibronectin (FN), thus mediating cells' attachment as well as movement in cancerous tissues. TEM1 is simultaneously expressed with ECM components as well as enzymes included in ECM remodeling, involving collagens I, III, V, XIII, MMP-2, MMP-14, as well as lysyl oxidase (LOX). Such molecules also contribute to angiogenesis as well as epithelial-mesenchymal transition (Winkler *et al*., 2020) [16].

Our study recommended that serum TEM1 could be a valuable addition to the frequently utilized markers, involving CEA as well as CA 19-9 in diagnosis of CRC patients and may act as a therapeutic target for CRC in the future.

Conclusion

Serum TEM1 levels exhibited a significant rise in CRC cases, which suggested that serum TEM1 could bring potential benefits as a diagnostic biomarker for CRC. High serum TEM1 levels were significantly linked to poor clinicopathological features. Therefore, it is an adverse indicator for CRC prognosis, and it could be used as a prognostic marker of malignancy.

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Conflict of Interest: Nil

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