RESEARCH ARTICLE

Editorial Process: Submission:06/13/2024 Acceptance:10/12/2024

Significance of *CD70, VEGF* and *CD90* Immunohistochemical Expression in Colorectal Cancer

Tarek Aboushousha[1], Merna Osama[2], Merna Mohamed[2], Yasmine Khaled[2], Hesham A Elmeligy[3], Yousra Ossama[4]*

Abstract

Background: Colorectal cancer is the 4th most reported reason for cancer death worldwide. It is a complex and multifaceted disease with diverse histopathological manifestations. *CD70* is present on activated immune cells and is upregulated in patients who have finished adjuvant therapy. VEGF controls angiogenesis and demonstrates immuno-regulatory characteristics that inhibit the anticancer activity of immune cells. CD90 is an extracellular cancer stem cell marker and regulates apoptosis, cell migration, and T cell activation. **Aim of work:** to elucidate the prognostic potential of a combined immunohistochemical assessment of *CD70*, *VEGF*, and *CD90* biomarkers in colorectal cancer tissues. **Materials and methods:** Our study is a retrospective study done on 70 paraffin blocks (45 colorectal carcinoma cases and 11 adenomas, along with 14 cases of colitis serving as controls) were subjected to conventional Hematoxylin and Eosin stain for routine histopathological assessment and immunohistochemical staining for *CD70*, *VEGF* and *CD90*. Our investigation focused on evaluating the expression of *CD70*, *VEGF*, and *CD90* in various colorectal tissues, including colitis, adenomas, and adenocarcinomas, as well as different grades of adenocarcinoma tissues, different stages of tumor invasiveness, and lymph node status. **Results:** Our research revealed distinctive expression patterns of *CD70*, *VEGF*, and *CD90* across different stages and grades of colorectal cancer. **Conclusion:** our research signifies the potential clinical utility of the *CD70*, *VEGF*, and *CD90* triad as prognostic markers in colorectal cancer. The combined analysis of these markers emerged as a potent prognostic tool, offering valuable insights into disease progression. Our research showed that epithelial CD90 is the most sensitive marker for both tumor stage and lymph node status. *CD70* and *VEGF* showed a statistically significant relation with lymph node status only and not with tumor stage.

Keywords: Cancer colon- *CD70- VEGF- CD90-* prognostic value- immunohistochemical expression

Asian Pac J Cancer Prev, **25 (10)**, 3691-3699

Introduction

Colorectal cancer (CRC) is increasing significantly all over the world. It is categorized as the second most common cancer in females and the third in males. It represents about 60% of cases that have already developed metastasis at the time of diagnosis [1].

Worldwide, CRC is the 4th most reported reason for cancer death, about 8.0 % of all cancer related deaths [2]. In the Middle East and North Africa, CRC accounts for 7.4% of the total cases of cancer [1]. Egypt likewise has a high prevalence of CRC as it was detected in 14.0% of colonoscopies. In a study done on a population in Gharbia, Egypt reported the highest rate of colorectal cancer in cases aged 40 years and younger [2]. According to the International Agency for Research on Cancer (IARC/WHO) and the World Cancer Research Fund (WCRF), smoking, drinking alcohol, being overweight, and eating processed and red meat increase the risk factors for colon cancer [3]. *CD70* is a type-II transmembrane surface antigen and a member of tumour necrosis factor super family (TNFSF). It has been identified as the best prospective cell surface protein for targeting investigations because its expression is extremely limited and only expressed on the activated immune cells [4]. *CD70* has been identified as *CD27* ligand located on chromosome 19p131 and exhibited only on antigen-presenting cells (APCs) [5]. According to Nakamura et al., 2021 study, patients with colorectal cancer who have finished adjuvant chemotherapy, *CD70* upregulation may be a unique prognostic marker and a possible therapeutic target [6]. Previous research showed that *CD70*, an immunological checkpoint molecule, is expressed in cancer-associated fibroblasts (CAFs) in CRC tissues. The *CAF CD70*-positivity serves as an independent predictive marker in CRC [7].

Vascular endothelial growth factor (VEGF), one of the pro-angiogenic factors, is a popular therapeutic target

*[1]Department of Pathology, Theodor Bilharz Research Institute, Giza, Egypt. [2]Faculty of Biotechnology, October University of Modern Science and Arts, Giza, Egypt. [3]Department of Surgery, Theodor Bilharz Research Institute, Giza, Egypt. [4]Department of Pathology, October 6 University, Giza, Egypt.*For Correspondence: yousraossama_j@hotmail.com*

because of its critical contribution to the development of abnormal tumour vasculature. VEGFs and VEGFRs control angiogenesis and vasculogenesis [8].

Additionally, VEGF demonstrates immuno-regulatory characteristics that inhibit the anticancer activity of immune cells. It plays a significant role in enhancing the rates of cell survival, migration, and proliferation, as well as the endothelial cells (ECs) differentiation, vascular permeability, and vasodilation [9]. The most appealing target in pharmacological therapy for induced angiogenesis in cancer is VEGF due to its high efficacy as a growth factor in dealing with tumour angiogenesis and its elevated level of expression in diverse tumour types. Because of this, inhibiting angiogenesis by blocking the VEGF pathway has received a lot of attention in the field of cancer treatment [9].

When it comes to tumour growth, metastasis, and patient survival, VEGF expression has been linked to an increase in the number of microvessels in colonic tumours. According to recent investigations, VEGF was discovered to be expressed in the early stages of the development of CRCs. Research has revealed that VEGF may be used to predict how a patient would respond to local radiation or conventional systemic therapy, in addition to proof suggesting that VEGF is a prognostic factor in CRC [8]. *CD90*, sometimes referred to as THY1, is a cell surface protein which weighs 25–37 KDa that is heavily N-glycosylated, glycophosphatidylinositol (GPI) anchored and free from the cytoplasmic domain. A 5591-bp gene found on human chromosome 11q23.3 codes for *CD90* (cell surface glycoprotein) [10].

CD90 was found to be an extracellular important cancer stem cell (CSC) marker for many cancers and mainly colon cancer. According to bioinformatical analysis, colonic adenocarcinoma exhibited considerably higher expression of several CSC markers, including CD90, *CD133*, *CD24*, and *CD44*, compared to nearby normal colon tissue [11]. There are many roles for *CD90*; it can act as a tumour suppressor [12], tumour promoter [13], cancer stem cell and cancer prognostic marker [14].

CD90 is mainly expressed in many certain cells such as myofibroblasts, fibroblasts, endothelial cells, mesenchymal stem cells and natural killer cells. CD90 is present under normal circumstances and plays an important role in regulating cell apoptosis, cell migration, cell adhesion, T-cell activation, fibrosis, and cell-matrix and cell-cell interaction [15].

Colonic myofibroblasts, also known as stromal cells or CD90 innate immune cells, make up to 30% of healthy lamina propria cells in the colon. They are more numerous in the stroma of CRC tumours. The number of colonic CD90 cells that produce IL-6 is increased in CRC tumours. These findings were verified by in situ examination of CRC tumours and nearby normal colonic mucosa using immunostaining and confocal microscopy, which showed that the expression of IL-6 protein is elevated inside the CRC tumours when compared to the matching normal colonic mucosa [16]. There is a relationship between these biomarkers as *CD70* overexpression on tumour cells is linked with poor prognosis [17]. *CD90* overexpression could also predict this poor prognosis and may be used as a potential prognostic biomarker for colorectal cancer patients [18]. Also, elevated level of VEGF expression has a relationship with reduced overall and disease-free survival rates combined with a higher incidence of distant metastases [19].

The aim of this study was to evaluate the immunohistochemical expression of a combination of *CD70*, *VEGF* and *CD90* biomarkers as a prognostic marker in colorectal cancer tissues. This aim was achieved by analysing the expression of the three markers in colitis, adenoma, and adenocarcinoma cases as well as different grades of adenocarcinoma tissues, stages of tumour invasive, and lymph node metastasis stages of colorectal cancer.

Materials and Methods

Case selection

Our study is a retrospective study formed on tissue sections of (70) paraffin blocks including colorectal carcinoma (45 cases) and non-cancerous tissue (11 adenomas and 14 cases of colitis taken as controls) from patients of average age 50-58 years old, taken from biopsy specimens received at the pathology department of Theodor Bilharz Research Institute.

Inclusion criteria of Cancerous tissue

Total colectomy, with full clinical data.

Exclusion criteria

Non-available blocks and absence of clinical data.

Specimens were analysed for routine and immunohistochemical study.

Histopathological study

Haematoxylin and eosin-stained sections were prepared for routine diagnosis, grading, and staging of tumours.

All sections were assessed and scored according to the WHO system. Sections were examined using light microscopes [Scope A1, Axio, Zeiss, Germany]. Photomicrographs were taken using a microscope-camera [AxioCam, MRc5, Zeiss, Germany]. All procedures were done at the pathology department of Theodor Bilharz Research Institute, Giza, Egypt.

Immunohistochemical Staining

Immunohistochemistry for *CD70*, *VEGF* and *CD90* were performed on tissue sections cut from the paraffin blocks at 4μm onto positively charged slides (Superfrost Plus, Menzel-Glaser, Germany) and stained on an automated platform (Dako Autostainer Link 48) using: 1) Anti-*CD70* monoclonal antibody (Cat. Number: YPA2585) was used at a dilution of 1:150, 2) Anti-VEGF (Chongqing Biospes, China, YMA1314) at 1:200 dilution. 3) Anti-CD90 monoclonal primary (Cat. Number: YPA2404, Chongqing Biospes, China) at 1:100 dilution. The antibodies were incubated for 2 hours at room temperature. Heat-induced antigen retrieval was used for 30 min at 97°C in the high-PH EnVision™ FLEX Target Retrieval Solution (DAKO company). Then

endogenous peroxidase blocking was done by (3% H_2O_2) for 4 minutes at 37°C. Slides were incubated with primary antibody for 40 minutes at 37°C followed by a universal secondary antibody for 20 minutes at 37°C. Slides were incubated in streptavidin-horseradish peroxidase (SA-HRP) D for 15 minutes at 37°C and then the substrate, 3,3′-diaminobenzidine tetrahydrochloride (DAB) was added for 10 minutes followed by counter-staining of tissue sections using Haematoxylin.

Assessment of biomarkers expression

Semi-quantitative analysis of *CD70* expression was evaluated by using the percentage of the epithelial cells with the immunostaining of cytoplasm.

Semi-quantitative analysis of (*VEGF*) expression was estimated by evaluating the percentage of epithelial cells showing cytoplasmic immunostaining.

Assessment of the *CD90* expression IHC score was based upon the percentage of *CD90* positive epithelial cells. Stromal expression of *CD90* was also measured separately as a percentage of positivity in stromal cells of studied specimens [20].

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 20.0 was used for data cleaning, management, and analysis. Categorical variables were presented using number and percent, whereas continuous ones were presented by mean and standard deviation (mean ± SD), with the p-value considered significant if <0.05.

Results

The experiment was conducted on 34 males and 36 females. Malignant cases were older in age, with an average of 58.23-years-old, while colitis and adenoma patients were 50-57 years old with non-significant difference (Table 1).

Malignant cases represented 64.3% of the cases in our study, while colitis and adenoma cases represented 20% and 15.7% of cases, respectively.

CD70

Adenocarcinoma cases showed the highest significant expression parameters of *CD70* compared to colitis and adenoma cases ($p < 0.001$) (Table 1, Figure 1(b) and Figure 1(d)).

In grade 2 adenocarcinoma, *CD70* showed higher expression when compared to grade 1 and grade 3, but this was not statistically significant ($p > 0.8$) (Table 1, Figure 1 (c) and Figure 1(d))

There was an increase in the percentage of *CD70* expression with increasing the stage of tumour. However, this difference was statistically non-significant ($p>0.3$) (Table 1).

As regards the lymph node metastasis, there was a significant increase in *CD70* expression with progress of N score ($p<0.05$) (Table 1).

VEGF

VEGF expression score for malignant patients was 2.36 which was more than in colitis and adenoma patients, 1.14 and 1.36 respectively, with a statistically significant

Table 1. Distribution of the Studied Cases According to the Diagnosis, Sex, Age, and Expression Scores of *CD70, VEGF & CD90.*

Diagnosis (number of cases)	M:F	Age (M±SD)	CD70 % (M±SD)	VEGF % (M±SD)	Epith. CD90 % (M±SD)	Stromal CD90 % (M±SD)
Colitis (14)	4:10	50.55 ± 9.38	16.4286 ± 14.2	57.86±18.05	13.00 ±9.77	8.00±7.14
Adenoma (11)	4:07	57 ± 7.62	17.72 ± 12.91	88.47±18.1	11.00±5.16	28.50±18.26
Adenoca. (45)	26:19:00	58.23 ± 9.8	82.22 ±16.36	92.73±9.05	19.57 ± 11.07	41.79±24.46
			p<0.001	p<0.001	P<0.05	P<0.001
Grade						
G1 (2)			75 ± 21.21	60.00±0.00	18.55± 9.21	33.42±10.08
G2 (37)			82.7 ± 15.92	88.95±18.09	19.91±11.54	40.42±25.91
G3 (6)			81.66 ± 20.41	95.00±12.25	17.50 ±8.66	50.00±11.54
			p>0.8	p<0.5	p >0.5	P >0.4
Stage						
T2 (6)			73.33 ± 13.66	91.67±11.69	30.00 ±5.77	40.00± 23.09
T3 (29)			83.79 ± 17.4	89.00±19.90	16.11 ±11.82	38.89± 23.98
T4 (8)			85 ± 13.09	91.25±11.26	23.00 ± 4.69	51.67 ± 28.40
			p>0.3	p>0.9	p <0.05	P >0.5
Lymph node						
N0 (19)			75.78 ± 18.04	83.21± 23.15	19.83±13.69	32.50±22.91
N1 (11)			84.54 ± 12.13	91.82±7.51	26.25±6.94	42.50±29.64
N2 (13)			90.76 ± 13.2	97.69±8.32	12.50 ± 4.62	55.00 ± 16.03
			P<0.05	p<0.05	p <0.05	P >0.1

M, Mean percentage; S.D, Standard deviation.

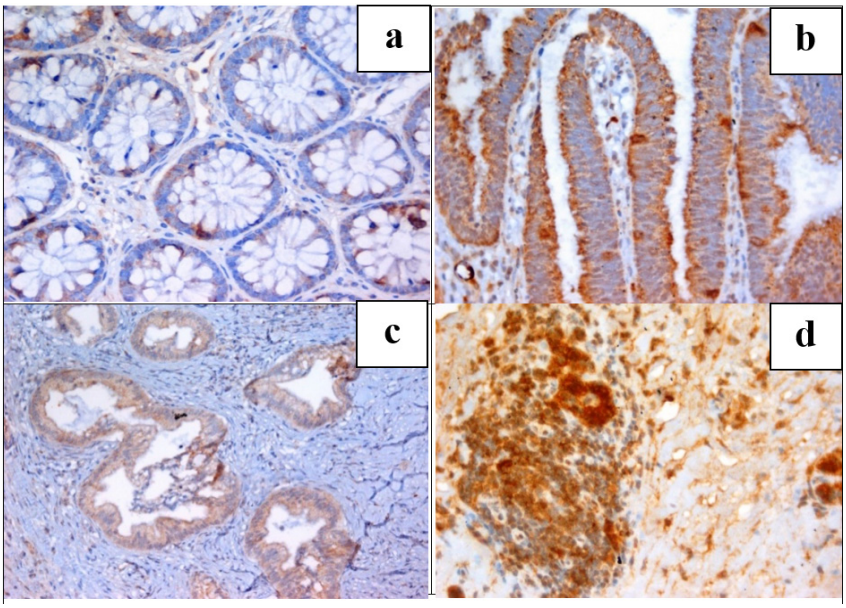

Figure 1. a: Normal colonic mucosa showing negative expression of *CD70*. b: Villous adenoma showing moderate focal expression of *CD70* within epithelial cells. c: Low-grade adenocarcinoma showing mild diffuse expression of CD70 within epithelial cells. d: Adenocarcinoma showing strong diffuse expression of *CD70* within epithelial cells and the surrounded lymphocytes (*CD70* IHC staining X400).

difference (P<0.0001) (Table 1, Figure 2(e), Figure 2(f) and Figure 2(h)).

Regarding tumour grade, the expression score of *VEGF* in grade 3 was 2.83 which was higher than grade 2 and 1 (2.30 and 2 respectively), however, the difference of VEGF expression between grades wasn't statistically significant (P>0.2) (Table 1, Figure 2(g) and Figure 2(h)).

The expression score of *VEGF* in stage 4 (2.50) was higher than stage 3 (2.31), and nearly like stage 2 (2.50), with non-significant difference (P>0.7). The mean *VEGF* score of expression in N2 was 2.69, N1 was 2.36, and N0 was 2.16, with non-significant difference (P>0.1) (Table 1).

Epithelial CD90

Adenocarcinoma cases showed the highest expression percentage of epithelial *CD90* expression compared to colitis and adenoma cases (p<0.05). Adenoma cases showed lower epithelial *CD90* expression compared to colitis cases, with statistically significant difference (Table 1).

According to the tumour grade, grade 2 adenocarcinoma, showed higher epithelial *CD90* expression, when compared to grade 1 and grade 3, but this was statistically non-significant (P>0.05) (Table 1, Figure 3(i), Figure 3(k), Figure 3(m) and Figure 3(n)).

Regarding tumour stage, T2 showed higher epithelial

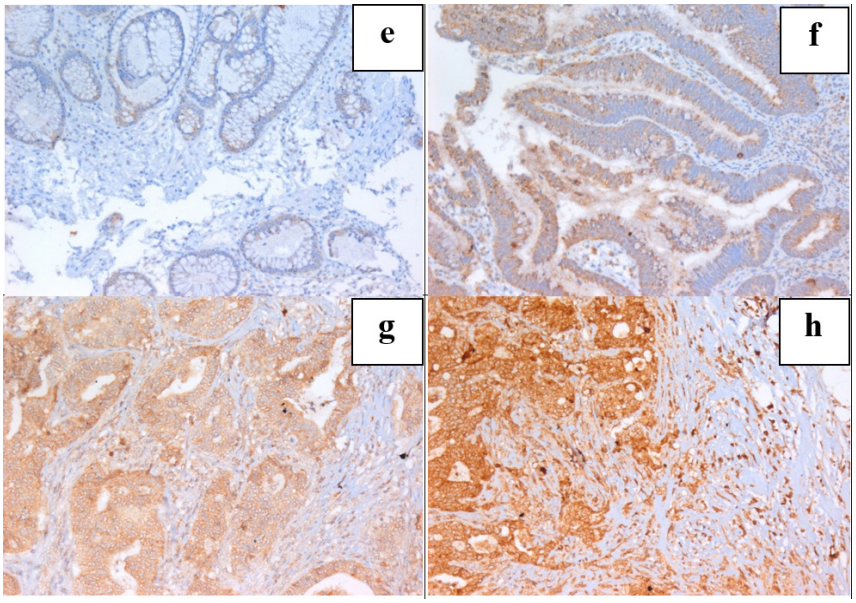

Figure 2. e: Mild colitis showing mild focal expression of *VEGF* within epithelial cells. f: Villous adenoma showing mild focal expression of *VEGF* within epithelial cells. g: Low grade adenocarcinoma showing mild diffuse expression of *VEGF* within epithelial cells h: High-grade adenocarcinoma showing strong diffuse expression of *VEGF* within epithelial cells (*VEGF* IHC staining X200).

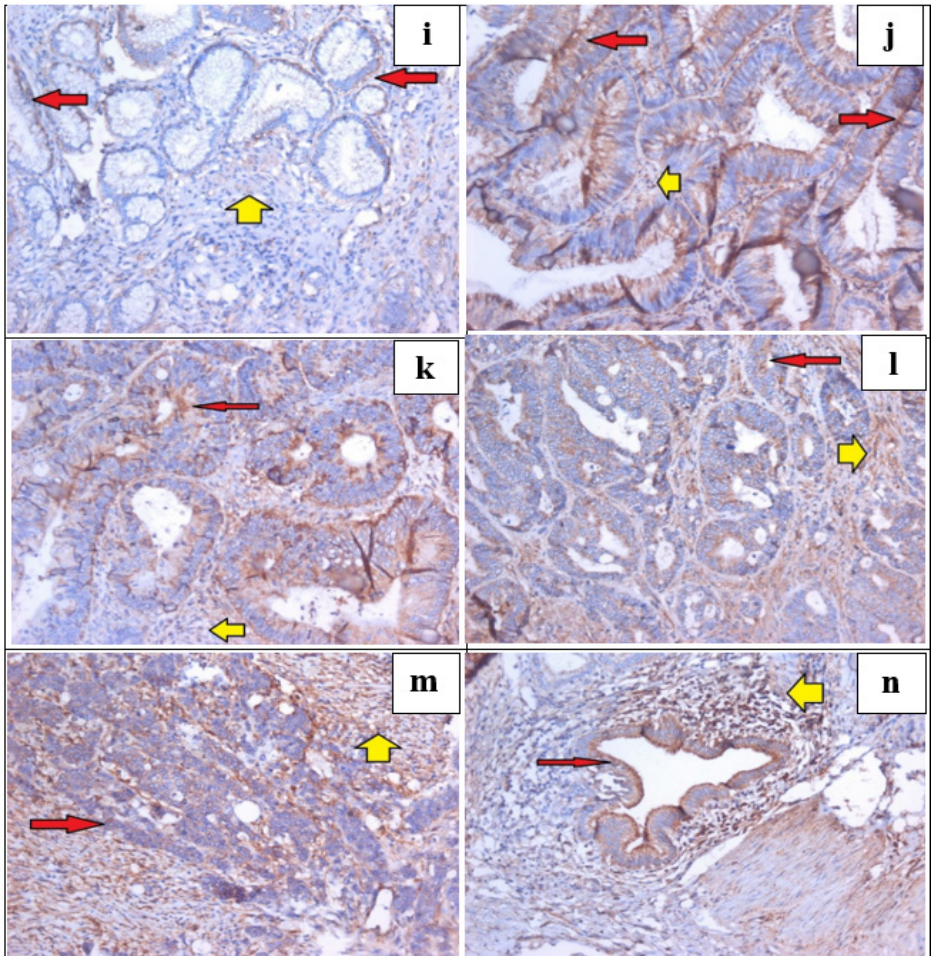

Figure 3. i: Colitis showing mild basal expression of *CD90* on epithelial cells and mild focal expression in the stroma. j: Villous adenoma showing diffuse surface expression of CD90 on epithelial cells (red arrows) and negative expression in the stroma (yellow arrow). k: Well differentiated adenocarcinoma showing surface expression of CD90 on epithelial cells (red arrow) and negative expression in the stroma (yellow arrow). l: Moderately differentiated adenocarcinoma showing focal moderate expression of *CD90* on epithelial cells (red arrow) and strong expression in the stroma (yellow arrow) m: High grade adenocarcinoma showing mild focal surface expression of *CD90* on epithelial cells (red arrow) and strong expression in the stroma (yellow arrow). n: Adenocarcinoma invading the muscle showing surface expression of *CD90* on epithelial cells (red arrow) and high expression in the stroma (yellow arrow). (*CD90* IHC staining, X200).

CD90 expression compared to (T3 and T4 stages). T4 cases showed higher epithelial *CD90* expression than T3 cases and the results were statistically significant ($p<0.05$) (Table 1).

According to Lymph node stages, N1 cases showed the highest epithelial *CD90* expression compared to N0 and N2 cases, followed by N0 cases, with statistically significant difference ($P<0.05$) (Table 1).

Stromal CD90

Adenocarcinoma cases showed the highest expression percentage of stromal *CD90* (Figure 3(i), Figure 3(m) and Figure 3(n)). Adenoma cases showed lower stromal CD90 expression than colitis cases. The results were statistically significant ($p<0.001$) (Table 1).

Regarding tumour grade, grade 3 cases showed the highest stromal CD90 expression, followed by grade 2 cases. There was an increase in the percentage of stromal CD90 expression with increasing the tumour grade but without statistically significant difference ($p>0.4$) (Table 1, Figure 3(k), Figure 3(i)).

According to tumour stage, the highest stromal CD90 expression was found in stage 4 cases. Stage 2 showed higher stromal CD90 expression than stage 3 cases with statistically non-significant difference ($P>0.5$) (Table 1).

As for lymph node stages, N2 cases showed higher stromal CD90 expression than N1 and N0 cases. N0 cases showed lower stromal CD90 expression than N1 cases. There was an increase in the percentage of stromal CD90 expression with increasing the lymph node stage of the tumor, however, this difference was statistically insignificant ($p>0.1$) (Table 1).

Discussion

Worldwide, colorectal cancer (CRC) is one of the most prevalent gastrointestinal malignancies, with high morbidity and mortality rate [21].

Our study was conducted on tissue sections of (70) paraffin blocks including colorectal carcinoma (45 cases)

and non-cancerous tissue. Immunohistochemistry for *CD70*, *VEGF* and *CD90* were performed on tissue sections cut from the paraffin blocks to clarify their association with clinicopathological features and prognosis.

In our study 36 cases were females and 34 were males. Adenocarcinoma cases were 45 cases with a mean age of 58.23 years. 14 cases were cases of colitis with a mean age of 50.55. The remaining 11 cases were adenoma cases with a mean of 57 years. This showed that the incidence of colorectal cancer increases with increasing age. This was compatible with a study done by Goodarzi et al. [22] which stated that colorectal carcinoma is considered as an age-related disorder, and it is especially clear in developed countries where colorectal cancer rates are the highest . Another study done by Balasubramanian et al. [8] revealed a link between the global incidence of adenocarcinoma and the male preponderance, with the peak incidence occurring between the ages of 51 and 60.

CD70

CD70 has been regarded as an intriguing therapeutic target for malignancies in which it is overexpressed since *CD70* is extremely limited and practically nonexistent on normal tissue. Indeed, significant *CD70* positivity have been identified in renal, melanoma, pancreatic, ovarian, and breast cancer cells [23].

The current study showed that the highest *CD70* expression was in adenocarcinoma cases, representing 82.22 % of the studied cases of adenocarcinoma, followed by adenoma cases where *CD70* expression represented 17.72 %. The mean percentage of colitis cases was 16.42.

These results were statistically significant (P<0.001) and agreed with the results of a previous study done by Perotti et al. [24]. This implies that focusing on *CD70*+ve cells may aid in the removal of malignant cells and enhance immunological performance. Still, further research is required.

The present study showed that the *CD70* expression was higher in grade 2 adenocarcinoma cases (2.83) than in grade 1 (2) and grade 3 (1.95) but without statistical significance (P>0.8).

This was compatible with what was found in a previous study done by Banias et al. [25] and was conducted on 112 cases of which 89 cases were grade 2, 8 cases were grade 3 and only 4 cases were grade 1 [25]. Also, Jacobs et al. [7] reported that *CD70* expression was highly expressed in high grade CRC than low grade and this agreed with our results .

Regarding tumour stage, the current study results showed that the mean percentage of *CD70* expression was higher in stage 3 (mean= 83.79%) and stage 4 (mean=85) than stage 2 (mean=73.33%). However, these results were statistically insignificant (p>0.3). Similar results were stated in a previous study as the distribution of CRCs' stages at diagnosis showed slight variation. There was no longer a difference between males and females after merging stages I and II and in stages III and IV [26]. This showed that *CD70* expression is unrelated to tumour stage.

According to lymph node metastasis, the mean percentage of *CD70* expression in the present study was 75.78% for cases at stages N0, 84.54 for stage N1, and 90.76 for stage N2 cases. There were statistically significant differences between *CD70* expression and lymph node metastasis (P < 0.05). Similar results were obtained by Fortea-Sanchis et al. [27] in a previous study where the stages of lymph node metastasis were measured by LODDS methods, and the results were 2 and 8 for stage N1 and stage N2 respectively and 0 for stage N0 . This emphasizes that CD 70 is a valuable prognostic marker and targeting *CD70* may reduce lymph node metastasis and enhance patient outcome.

VEGF

Nearly four decades ago, vascular endothelial growth factor (VEGF) was identified as a critical factor promoting vascular permeability and angiogenesis which is essential for tumour growth [28].

VEGF showed the highest expression in adenocarcinoma cases, followed by colitis and then adenoma. The P- value was <0.001 indicating that the results were statistically significant. This result was supported by a previous study done by Ding et al. [29] which showed that VEGF score could be exemplified in CRC.

Concerning tumour grade, the least expression score of VEGF expression was found in grade 1 in the least number of cases, grade 2 had the second least expression score of VEGF in the highest number cases, and grades 3 had the highest expression score of VEGF in the second least number of cases. P-value was <0.5 indicating statistically insignificant results. Similar results were found in a previous research done by Miao et al. [30] which documented that VEGF expression wasn't related to grades.

Regarding tumour stage, the VEGF expression score showed that stage 4 has higher expression score than stage 3; and nearly equal to stage 2. The difference among stages wasn't statistically significant (P>0.7). Similar results were found by another research done by Goi et al. [31] who stated that there were no difference among stages. In contrast to our results, other studies done by Mazeda et al. [32] showed that the expression of VEGF in stage IV tumours was significantly more intense than in stage II and III. Variation among results of different studies including the current one is probably attributed to the different study samples as regards the number of the study population, the race and socioeconomic standard of the patients.

According to the lymph node metastasis, most of cases were in stage N0 and showed the least VEGF expression. The least number of patients with the second least VEGF expression was found for N1, and the second least number of patients with the highest VEGF expression was found for N2, which means that VEGF expression in N2 is higher than N1 and followed by N0. These results were statistically significant (p-value < 0.05). Similar findings were concluded in a previous studies done by Mazeda et al. [32] who stated that VEGF expression was correlated with lymph node metastasis. VEGF is one of the main angiogenic agents in tumors, it contributes to the formation, spread, and metastasis of cancers at the early stages [33].

Vascular endothelial growth factor (VEGF) is a renowned pro-angiogenic factor upregulated in most cancer cells and has become a major target in most anti-angiogenic cancer therapies. Thus, assessing anti-VEGF therapies improves the efficacy of anti-angiogenesis cancer therapies [34].

Epithelial and Stromal CD90

Our study showed that the mean percentage of epithelial CD90 expression in adenocarcinoma cases (19.57) was higher than that in adenoma cases (11.00) and colitis cases (13.00) with statistically significant difference (P <0.05). This showed that CD 90 may have a role in cancer development [35].

An agreement was demonstrated by Kumar et al. [36] who reported in his study that a very small percentage of CD90 is present in normal tissues and organs, CD90 expression was significantly higher in carcinoma compared to normal tissues.

Regarding stromal expression of CD90, our results showed that it was 41.79 for adenocarcinoma cases, 28.50 for adenoma cases and 8.00 for colitis cases with statistically high significance (P <0.001). Similar results were stated in a study done by Huynh et al. [16] study, who found that CD90 stromal expression is highly elevated in CRC tumors. This signifies that stromal cell expressing CD90 support cancer stem cells [37].

The mean percentage of epithelial *CD90* expression in our study was 18.55 in low grade colorectal carcinoma (grade 1), 19.91 in grade 2 and 17.50 in grade 3. However, these results were statistically insignificant (P > 0.5). These results showed an agreement with Kumar et al. [36] study who reported that epithelial CD90 was observed in all grades of many tumours but the expression of epithelial CD90 was higher in high-grade tumours than low-grade tumours.

Regarding tumour grade, the mean percentage of stromal CD90 expression was higher in high grade colorectal carcinoma, grade 2 (40.42) and grade 3 (50.00), than low grade colorectal carcinoma (33.42). this demonstrates an agreement with another study done by Zhu et al. [38], and showed that the expression of stromal CD90 was increased in high-grade tumours and also stated that the overexpression of stromal CD90 in the tumour microenvironment determined the tumour aggressive, high metastatic nature and could be used as a prognostic marker as well as a therapeutic target. This study also showed that the expression of stromal CD90 was increased in high-grade tumours [38].

Regarding the tumour stages, the current study demonstrated that epithelial CD90 expression showed the highest score in stage T4 colorectal adenocarcinoma. These results were statistically significant (P <0.05) and this pointed to the association between CD90 expression and increased cancer aggressive behaviour. Our results were supported by Zhu et al. [38] who stated that CD90 is more expressed in higher tumour stages (T3 andT4) than in lower stages (T1 and T2). Also, similar results were found by Kumar et al. [39] who documented that epithelial CD90 expression was correlated with the advanced tumour stage and associated with poor survival [36]. CD90 could be a valuable prognostic marker for colorectal cancer.

In our study, stage 4 (T4) showed the highest CD90 stromal expression and stage 2 (T2) showed the least CD90 stromal expression. Nearly similar results were stated in a study done by Huynh et al. [16] which showed that stromal cells expressing the CD90 marker is highly expressed in stages T4 and T3 colorectal adenocarcinoma than in lower stage (T2) . This clarifies that stromal cells expressing CD90 support cancer stem cells and promote tumour growth [37].

In the present study, the mean percentage of epithelial CD90 expression was higher in lymph node stage N3 than N2. Statistically significant difference (P <0.05) was found between epithelial CD90 expression and lymph node status. According to this results, initial tumour CD90+ cells may have a high propensity for malignancy since they can disseminate to lymph nodes and may have a bad prognosis. This result showed an agreement with what was demonstrated by Yamaoka et al. [40] study which stated that epithelial CD90 expression is significantly associated with lymph node metastasis, meaning that CD90 expression in low lymph node stages is less than that of high lymph node stages .

In the current study, the mean CD90 stromal expression was 32.50 for cases in stage N0, 42.50 for stage N1 and 55.00 for stage N2 without statistically significant difference (P >0.1). Our results demonstrated that stromal *CD90* expression increases with increasing lymph node stage and emphasis that CD90 expression in the tumour microenvironment is associated with cancer proliferation and dissemination which agreed with what was shown in a study done by Numakura et al. [41] in which Stromal CD90 expression was associated with larger tumour size and lymph node metastasis. CD90 may be an effective therapeutic marker however further studies are needed [42].

Our study differs from others as it is the first to assess the value of combining the three markers: *CD70*, *VEGF* and *CD90* as prognostic markers for colorectal cancer, as up to our knowledge no previous studies showed this combination with the same sample size and this makes our study a promising one.

Limitation of this study include the limitation of sample size resulting from the inclusion criteria applied to the included paraffin blocks.

Our research signifies the potential clinical utility of the *CD70*, *VEGF*, and *CD90* triad as prognostic markers in colorectal cancer. The unprecedented nature of this study, coupled with compelling evidence supporting its prognostic value, highlights its significance in advancing our understanding of colorectal cancer pathogenesis to open doors for further research in targeted therapeutic interventions. Studying the three markers, we found that epithelial *CD90* had statistically significant relation with both tumor stage and lymph node status (p-value <0.05). *CD70* and *VEGF* showed statistically significant relation with lymph node status only and not with tumor stage. *CD70*, *VEGF*, epithelial and stromal *CD90* expressions were upregulated in adenocarcinoma cases than in adenoma and colitis cases and showed statistically significant relation (p-value < 0.05). However, further

larger scale studies are necessary to explore more pertinent clinical evidence in this field.

Author Contribution Statement

Conceived and designed the experiments: TA, MO, MM and YK. Did colectomy operations: HE. Performed the experiments: TA, YO, MO, MM and YK. Analyzed the data: TA, YO, MO, MM and YK. Wrote the paper: TA, YO, MO, MM, YK and HE. All authors read and approved the final manuscript.

Acknowledgements

We thank Theodore Bilharz Research Institute, Giza, Egypt.

Approval

The study was approved by Theodor Bilharz Research Institute – Research ethics committee.

Ethical Declaration

The study was approved by Theodor Bilharz Research Institute – Research ethics committee registered at the office for Human Research Protections, US Department of Health and Human Service and operates under Federal Wide Assurance No. FW A00010609.

Data availability

All data generated or analyzed during this study are included in this manuscript.

Study Registration

The study was not registered in any registering dataset.

Conflict of interest

The authors declare that they have no conflict of interests.

References

1. Kassem Nm, Emera G, Kassem H A, Medhat N, Nagdy B, Tareq M, et al. Clinicopathological features of egyptian colorectal cancer patients regarding somatic genetic mutations especially in kras gene and microsatellite instability status: A pilot study. Egypt J Med Hum Genet. 2019;20(1):20. Https://doi.Org/10.1186/s43042-019-0028-z.
2. Hassan am, khalaf am, elias aak. Colorectal cancer in egypt: Clinical, lifestyle, and socio-demographic risk factors. Al-Azhar Intern Med J. 2021;2(9):6–15. Https://doi.Org/10.21608/aimj.2021.79043.1490.
3. Lukic M, Licaj I, Laaksonen MA, Weiderpass E, Borch KB, Rylander C. The burden of colon cancer attributable to modifiable factors-the norwegian women and cancer study. Int J Cancer. 2023;152(2):195-202. https://doi.org/10.1002/ijc.34237.
4. Nilsson MB, Yang Y, Patel S, Heeke S, Le X, Aruguman T, et al. Cd70 is a novel therapeutic target for egfr mutant nsclc with acquired, emt-associated egfr tki resistance. Cancer Res. 2022;82(12_Supplement):1827-.
5. Inoue S, Ito H, Tsunoda T, Murakami H, Ebi M, Ogasawara N, et al. Cd70 expression in tumor-associated fibroblasts predicts worse survival in colorectal cancer patients. Virchows Arch. 2019;475(4):425-34. https://doi.org/10.1007/s00428-019-02565-1.
6. Nakamura K, Sho M, Akahori T, Nishiwada S, Kunishige T, Nakagawa K, et al. Clinical relevance of cd70 expression in resected pancreatic cancer: Prognostic value and therapeutic potential. Pancreatology. 2021;21(3):573-80. https://doi.org/10.1016/j.pan.2021.01.013.
7. Jacobs J, Deschoolmeester V, Zwaenepoel K, Flieswasser T, Deben C, Van den Bossche J, et al. Unveiling a cd70-positive subset of cancer-associated fibroblasts marked by pro-migratory activity and thriving regulatory t cell accumulation. Oncoimmunology. 2018;7(7):e1440167. https://doi.org/10.1080/2162402X.2018.1440167.
8. Balasubramanian S, Priyathersini N, Johnson T. Expression of vascular endothelial growth factor (vegf) in colorectal adenoma and carcinoma in a tertiary care center. Cureus. 2022;14(11):e31393. https://doi.org/10.7759/cureus.31393.
9. Ghalehbandi S, Yuzugulen J, Pranjol MZI, Pourgholami MH. The role of vegf in cancer-induced angiogenesis and research progress of drugs targeting vegf. Eur J Pharmacol. 2023;949:175586. https://doi.org/10.1016/j.ejphar.2023.175586.
10. Zhu L, Zhang W, Wang J, Liu R. Evidence of cd90+cxcr4+ cells as circulating tumor stem cells in hepatocellular carcinoma. Tumour Biol. 2015;36(7):5353-60. https://doi.org/10.1007/s13277-015-3196-6.
11. Dzobo k, senthebane d, ganz c, thomford n. The significance of cancer stem cell markers' gene expression and relevance for survival outcomes. 2020. Https://doi.Org/10.20944/preprints202005.0047.V1.
12. Krolewska-Daszczynska P, Wendlocha D, Smycz-Kubanska M, Stepien S, Mielczarek-Palacz A. Cancer stem cells markers in ovarian cancer: Clinical and therapeutic significance (review). Oncol Lett. 2022;24(6):465. https://doi.org/10.3892/ol.2022.13585.
13. Sauzay C, Voutetakis K, Chatziioannou A, Chevet E, Avril T. Cd90/thy-1, a cancer-associated cell surface signaling molecule. Front Cell Dev Biol. 2019;7:66. https://doi.org/10.3389/fcell.2019.00066.
14. Bahnassy AA, Fawzy M, El-Wakil M, Zekri AR, Abdel-Sayed A, Sheta M. Aberrant expression of cancer stem cell markers (cd44, cd90, and cd133) contributes to disease progression and reduced survival in hepatoblastoma patients: 4-year survival data. Transl Res. 2015;165(3):396-406. https://doi.org/10.1016/j.trsl.2014.07.009.
15. Chen WC, Chang YS, Hsu HP, Yen MC, Huang HL, Cho CY, et al. Therapeutics targeting cd90-integrin-ampk-cd133 signal axis in liver cancer. Oncotarget. 2015;6(40):42923-37. https://doi.org/10.18632/oncotarget.5976.
16. Huynh PT, Beswick EJ, Coronado YA, Johnson P, O'Connell MR, Watts T, et al. Cd 90+ stromal cells are the major source of il-6, which supports cancer stem-like cells and inflammation in colorectal cancer. Int J Cancer. 2016;138(8):1971-81. https://doi.org/10.1002/ijc.29939.
17. Flieswasser T, Camara-Clayette V, Danu A, Bosq J, Ribrag V, Zabrocki P, et al. Screening a broad range of solid and haematological tumour types for cd70 expression using a uniform ihc methodology as potential patient stratification method. Cancers. 2019;11(10):1611. https://doi.org/10.3390/cancers11101611.
18. Koh HM, Lee HJ, Kim DC. Prognostic and clinicopathological value of cd90 expression in cancer patients: A systematic review and meta-analysis. Transl Cancer Res. 2021;10(7):3356-63. https://doi.org/10.21037/tcr-21-266.
19. Hutajulu SH, Paramita DK, Santoso J, Sani MIA, Amalia A, Wulandari G, et al. Correlation between vascular endothelial growth factor-a expression and tumor location and invasion

in patients with colorectal cancer. J Gastrointest Oncol. 2018;9(6):1099. https://doi.org/10.21037/jgo.2018.07.01.
20. Marques-Piubelli ML, Sagert J, Pham MT, Will M, Henderson D, Tipton K, et al. Cd70 is a potential target biomarker in peripheral t-cell lymphomas. Histopathology. 2022;81(2):272-5. https://doi.org/10.1111/his.14670.
21. Komura M, Wang C, Ito S, Kato S, Ueki A, Ebi M, et al. Simultaneous expression of cd70 and postn in cancer-associated fibroblasts predicts worse survival of colorectal cancer patients. Int J Mol Sci. 2024;25(5):2537. https://doi.org/10.3390/ijms25052537.
22. Goodarzi E, Beiranvand R, Naemi H, Momenabadi V, Khazaei Z. Worldwide incidence and mortality of colorectal cancer and human development index (hdi): An ecological study. World J Cancer Res. 2019;6:8. https://doi.org/10.32113/wcrj_201911_1433.
23. Nilsson MB, Yang Y, Heeke S, Patel SA, Poteete A, Udagawa H, et al. Cd70 is a therapeutic target upregulated in emt-associated egfr tyrosine kinase inhibitor resistance. Cancer Cell. 2023;41(2):340-55. e6. https://doi.org/10.1016/j.ccell.2023.01.007.
24. Perotti V, Fabiano S, Contiero P, Michiara M, Musolino A, Boschetti L, et al. Influence of sex and age on site of onset, morphology, and site of metastasis in colorectal cancer: A population-based study on data from four italian cancer registries. Cancers (Basel). 2023;15(3):803. https://doi.org/10.3390/cancers15030803.
25. Banias L, Jung I, Bara T, Fulop Z, Simu P, Simu I, et al. Immunohistochemical-based molecular subtyping of colorectal carcinoma using maspin and markers of epithelial-mesenchymal transition. Oncol Lett. 2020;19(2):1487-95. https://doi.org/10.3892/ol.2019.11228.
26. White A, Ironmonger L, Steele RJC, Ormiston-Smith N, Crawford C, Seims A. A review of sex-related differences in colorectal cancer incidence, screening uptake, routes to diagnosis, cancer stage and survival in the uk. BMC Cancer. 2018;18(1):906. https://doi.org/10.1186/s12885-018-4786-7.
27. Fortea-Sanchis C, Martínez-Ramos D, Escrig-Sos J. The lymph node status as a prognostic factor in colon cancer: Comparative population study of classifications using the logarithm of the ratio between metastatic and nonmetastatic nodes (lodds) versus the pn-tnm classification and ganglion ratio systems. BMC cancer. 2018;18:1-11. https://doi.org/10.1186/s12885-018-5048-4.
28. Patel SA, Nilsson MB, Le X, Cascone T, Jain RK, Heymach JV. Molecular mechanisms and future implications of vegf/vegfr in cancer therapy. Clin Cancer Res. 2023;29(1):30-9. https://doi.org/10.1158/1078-0432.CCR-22-1366.
29. Ding C, Li L, Yang T, Fan X, Wu G. Combined application of anti-vegf and anti-egfr attenuates the growth and angiogenesis of colorectal cancer mainly through suppressing akt and erk signaling in mice model. BMC cancer. 2016;16:1-13. https://doi.org/10.1186/s12885-016-2834-8.
30. Miao H, Ruan S, Shen M. Vegf-c in rectal cancer tissues promotes tumor invasion and metastasis. J BUON. 2018;23(1):42-7.
31. Goi T, Nakazawa T, Hirono Y, Yamaguchi A. The prognosis was poorer in colorectal cancers that expressed both vegf and prok1 (no correlation coefficient between vegf and prok1). Oncotarget. 2015;6(30):28790. https://doi.org/10.18632/oncotarget.4744.
32. Mazeda I, Martins SF, Garcia EA, Rodrigues M, Longatto A. Vegf expression in colorectal cancer metastatic lymph nodes: Clinicopathological correlation and prognostic significance. Gastrointestinal Disorders. 2020;2(3):25.
33. Yang Y, Cao Y. The impact of VEGF on cancer metastasis and systemic disease. InSeminars in cancer biology. 2022; 86(3), 251-261. https://doi.org/10.1016/j.semcancer.2022.03.011.
34. Elebiyo TC, Rotimi D, Evbuomwan IO, Maimako RF, Iyobhebhe M, Ojo OA, et al. Reassessing vascular endothelial growth factor (vegf) in anti-angiogenic cancer therapy. Cancer Treat Res Commun. 2022;32:100620. https://doi.org/10.1016/j.ctarc.2022.100620.
35. Yang J, Zhan XZ, Malola J, Li ZY, Pawar JS, Zhang HT, et al. The multiple roles of thy-1 in cell differentiation and regeneration. Differentiation. 2020;113:38-48. https://doi.org/10.1016/j.diff.2020.03.003.
36. Kumar A, Bhanja A, Bhattacharyya J, Jaganathan BG. Multiple roles of cd90 in cancer. Tumour Biol. 2016;37(9):11611-22. https://doi.org/10.1007/s13277-016-5112-0.
37. Xue B-z, Xiang W, Zhang Q, Wang H-f, Zhou Y-j, Tian H, et al. Cd90 low glioma-associated mesenchymal stromal/stem cells promote temozolomide resistance by activating foxs1-mediated epithelial-mesenchymal transition in glioma cells. Stem Cell Res Ther. 2021;12:1-11.
38. Zhu J, Thakolwiboon S, Liu X, Zhang M, Lubman DM. Overexpression of cd90 (thy-1) in pancreatic adenocarcinoma present in the tumor microenvironment. PLoS One. 2014;9(12):e115507. https://doi.org/10.1371/journal.pone.0115507.
39. Guo Z, Li LQ, Jiang JH, Ou C, Zeng LX, Xiang BD. Cancer stem cell markers correlate with early recurrence and survival in hepatocellular carcinoma. World J Gastroenterol. 2014;20(8):2098-106. https://doi.org/10.3748/wjg.v20.i8.2098.
40. Yamaoka R, Ishii T, Kawai T, Yasuchika K, Miyauchi Y, Kojima H, et al. Cd90 expression in human intrahepatic cholangiocarcinoma is associated with lymph node metastasis and poor prognosis. J Surg Oncol. 2018;118(4):664-74. https://doi.org/10.1002/jso.25192.
41. Numakura S, Uozaki H, Kikuchi Y, Watabe S, Togashi A, Watanabe M. Mesenchymal stem cell marker expression in gastric cancer stroma. Anticancer Res. 2019;39(1):387-93. https://doi.org/10.21873/anticanres.13124.
42. Zhang K, Che S, Su Z, Zheng S, Zhang H, Yang S, et al. Cd90 promotes cell migration, viability and sphere-forming ability of hepatocellular carcinoma cells. Int J Mol Med. 2018;41(2):946-54. https://doi.org/10.3892/ijmm.2017.3314.