Original Article

The Role of Biomarkers Vascular Cell Adhesion Molecule-1 (VACM-1), Septin-9, Hypoxia-Inducible Factor-1 (HIF-1) and C-Reactive Protein (CRP) in Distinguishing between Benign and Malignant Male Colon Tumors

Biomarkers, Septin-9, Hypoxia-Inducible and CRP for Differentiate of Colon Tumors

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ABSTRACT

Objective: To assess the effectiveness of VACM-1, Septin-9, HIF-1, and CRP as potential biomarkers in distinguishing malignant from benign colon tumors in male individuals.

Study Design: Meta-analysis study

Place and Duration of Study: This study was conducted at the Colonoscopy Units in Ibn Sina Teaching Hospital, Aljumhuriy Hospital, Mosul General Hospital, Al-Salam Hospital, and Research Hospitals at Mosul University, as well as from the private clinic of Dr. Abdullah Zuhair Al-Yuzbaki in Mosul City from 1st April 2023 to 31st March 2024.

Methods: A total of 45 patients who underwent colonoscopies were enrolled. Participants ranged in age from 18 to 78 years. Venous samples were collected from each patient before colonoscopy and analyzed for complete blood counts, and tumor markers using ELISA. Samples were classified into benign and malignant tumors, and healthy patients were controls. Tumor markers in the analysis included VCAM-1, Septin-9, HIF-1α, and CRP.

Result: Compared to biopsy groups (before colostomy) results, the lower values observed in the adenoma group suggest a relative stability in the tumor state. Males demonstrated a more pronounced pattern of anemia and immune dysfunction, especially during the initial phases of colon cancer. Statistically significant alterations were observed in blood parameters and tumor markers among male adenoma patients. The variations in immune ratios, including L/M and P/L, underscore the differences in overall immune and inflammatory responses among males at various stages of the disease. VCAM-1, Septin-9, HIF-1α, and CRP also recorded a significant difference in distinguishing between benign and malignant tumors and between adenoma and control groups. VCAM-1, Septin-9, and HIF-1α measures exhibited good effectiveness in identifying various three stages of colon cancer, compared to the adenoma and control groups. This may indicate the effectiveness of alterations in the tumor microenvironment.

Conclusion: Vascular cell adhesion molecule-1, septin-9, and HIF-1α demonstrate high clinical value as biomarkers in tracking the progression and metastasis of colon cancer. Their effectiveness in diagnostically distinguishing between benign and malignant tumors enhances their feasibility as potential clinical evaluation and therapeutic monitoring targets.

Key Words: Adenoma, Colon cancer, Biopsy, VCAM-1, Septin-9, HIF-1α, CRP

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INTRODUCTION

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Benign tumors (adenomas) do not invade surrounding tissues or spread to distant sites, often encapsulated and resemble the original tissue, unlike malignant tumors, which are characterized by aggressive behavior and a harmful effect on the body. Overall, benign tumors are less dangerous and can be surgically removed, however, some have the potential to transform into malignant tumors.[1]

"Malignancy or cancer" refers to the abnormal growth of cells, with the potential to spread and invade other parts of the body, a known as metastasis. Cancer cells can resist apoptosis, and invade surrounding tissues. These cells acquire these characteristics due to complex

genetic changes, contributes to the formation and progression of tumors.[2]

Colon cancer is the third most common type of cancer and the fourth leading cause of cancer-related deaths worldwide for both genders. The disease is challenging to detect in its early stages. It often progresses gradually from benign polyps or cysts to late-stage metastases.[3] Yousif et al[4] also indicated that some biochemical indicators in colon cancer patients were significantly higher than those in the control group. Biomarkers are crucial in the early detection of malignant diseases.

Colon cancer biomarkers are biological molecules found in blood, other body fluids, or tissues that signal the presence or progression of colon cancer. These biomarkers are crucial for the early detection of the disease, assessing its prognosis in patients with colon cancer.[5] Badiwi[6] also indicated that some vital compounds increase by more than 50% in malignant tumors compared to benign tumors. It was observed that tumor marker in the serum of patients of colon cancer, were elevated compared to those in the control group.[7]

VCAM-1 (CD106) is a surface protein belonging to the immunoglobulin family. Evidence suggests its association with angiogenesis and tumor metastasis, particularly in colon cancer. (sVCAM-1) in the serum of cancer patients are an indicator of the tumor environment and contribute to enhancing tumor survival.[8]

The methylated septin-9 (mSeptin-9) is superior to traditional biomarkers such as CEA in diagnosing colorectal cancer at various stages. and after surgery, with a 100% correlation with recurrence or metasta.[9]

HIF-1α is a hypoxia-sensitive transcription factor activated in solid tumor environments, such as colon cancer. It contributes to regulating gene expression associated with cancer cell survival, proliferation.[11] Under hypoxia, it to stabilize and bind to HIF-1β in the nucleus. The resulting complex activates the transcription of genes involved in angiogenesis, metabolism, and cell survival.[10]

C-reactive protein is a diagnostic biomarker and contributor to inflammation associated with colon cancer development. It regulates the signals of immune cells and may influence tumor initiation, progression, and the body's response to it.[11] Elevated levels of are associated with tissue damage resulting from an acute inflammatory response, reflecting a potential role in promoting tumor growth and metastasis.[12]

METHODS

This meta-analysis study was conducted at Colonoscopy Units in Ibn Sina Teaching Hospital, Aljumhuriy Hospital, Mosul General Hospital, Al-Salam Hospital, and Research Hospitals at Mosul University, as well as from the private clinic of Dr. Abdullah Zuhair Al-Yuzbaki in Mosul City, Iraq. A total of 64 patients and ages from 18-78 years were enrolled. 5ml of venous blood samples were collected from the study participants before colonoscopy at the hospitals. 3 ml in gel tubes for tumor marker analysis and 2 ml in EDTA tubes for (CBC) analysis using the MicroCC-20Plus device on the same day of the collection were performed within one hour of sample collection. After serum separation, samples were classified into two main categories based on histopathological biopsy reports: benign tumors and malignant tumors. For the malignant tumor groups, according to the colon cancer staging system, these groups was classified into tumor stages based on histopathological results: Stages II, III, and IV (The serum was stored in deep freeze until the histopathological report of the patients' colectomy was obtained, and the groups were divided into biopsies II, III, and IV), Then the serum was used to measure tumor biomarkers. In addition, 5 ml blood samples were collected from patients whose colonoscopies showed no evidence of tumors or polyps. This group was considered a positive control group; ultimately, and healthy people without any disease symptoms formed the healthy control group. Six tumor markers in the serum using ELISA technology, Labtech Microplate Reader LT-4000, East Sussex, UK were noted. The markers included Vascular Cell Adhesion Molecule 1 (VCAM-1), Septin-9, Hypoxia-Inducible Factor 1 Alpha (HIF-1α), and C-Reactive Protein (CRP), following the guidelines provided by Shanghai Ideal Medical Technology Co., Ltd., China. The differences between groups were analyzed by using the Duncan test, one-way ANOVA at the level of statistical significance $P \leq 0.05$ by SPSS-26.

RESULTS

There were 23 (92%) malignant tumors including 2 samples taken from surgical procedures. Benign tumors accounted for 2 (6%) of all biopsy samples (Fig. 1). Stage IV represented the lowest number, with 5 (21.8%) cases followed by Stage III 8 (34.8%) cases, while Stage II had the highest incidence rate 10 (43.4%) [Fig. 2).

The results showed significant differences between the biopsy groups and the control groups of males in each of (WBC), (LYM), (RBC), (Hb), (MCH), (MCV), (HCT), and (MPV),. In contrast, in (GRA), the significant difference was limited to biopsy II only. There was no significant difference in platelet count except for the adenoma group. The lack of substantial difference between the two control groups is worth noting (Table 1). The L/M% ratio for the fourth-stage patients increased compared to the benign tumor; in contrast, the benign tumor showed an increase in the P/L% ratio (Table 2).

The readings for VCAM-1 and HIF-1α were significantly elevated in biopsy III. Biopsy IV exhibited

a notable increase in Septin-9, while CRP showed an increase in stage III over stage II, but CRP was not statistically significant. There was no significant difference between the two control groups, and a significant difference was found between the three biopsy groups and the two control groups in all biomarkers. A significant difference was observed in VCAM-1, Septin-9, and HIF-1α across the three biopsy groups compared with the control groups. And there was no significant difference between the biopsy groups in CRP (Table 3).

Table No. 1: Complete blood count of male adenoma and biopsy groups

Variable	Healthy control	Control^{+}	Adenoma	Biopsy II	Biopsy III	Biopsy IV
WBC	7.29±0.65	7.30±0.62	6.56±0.65	7.50±1.14	6.02±0.57	6.82±0.15
LYM	2.80±0.42	2.23±0.30	2.01±1.23	1.53±0.17	1.50±0.618	1.67±0.54
MID	0.561±0.07	0.51±0.13	0.640±0.12	0.625±0.07	0.403±0.11	0.467±0.13
GRA	3.92±0.39	4.57±0.72	3.91±0.29	5.35±0.91	4.11±0.30	4.68±0.55
RBC	5.23±0.11	5.32±0.16	3.43±1.13	4.02±0.72	4.33±0.18	4.52±0.17
Hb	14.57±0.44	14.03±1.91	7.90±0.56	9.62±1.45	9.20±2.08	10.00±0.20
MCHC	33.20±0.7	32.21±2.46	31.85±1.82	31.50±0.50	29.70±2.48	30.43±0.65
MCH	27.94±0.76	26.32±3.55	23.20±1.97	24.10±0.83	21.17±3.95	22.10±1.30
MCV	84.26±1.76	81.16±6.71	72.70±9.61	76.52±2.08	70.83±7.91	72.6±2.70
RDW - CV	12.13±0.29	12.73±1.13	14.20±1.69	13.15±0.19	13.83±1.10	13.26±0.45
RDW - SD	42.25±1.23	40.05±3.4	41.80±1.56	40.07±1.92	38.23±2.87	37.13±0.35
HCT	44.62±2.32	43.24±3.79	24.90±1.97	30.55±4.79	30.76±4.6	32.80±1.10
PLT	220±11	222±30.4	278±9.89	209 b ± 39	198±3.51	195±5.0
MPV	7.92±0.25	8.42±0.66	7.10±1.13	7.55±0.58	7.83±0.85	8.70±0.40
PDW	12.70±0.56	14.14±1.58	13.20±1.69	12.90±1.89	14.63±2.05	16.70±0.60
PCT	0.17±0.007	0.186±0.03	0.196±0.007	0.161±0.04	0.149±0.049	0.10±0.009

RBC ($\times 10^6$/µl), Hb (g/dl), PCV and PDW (%), MCV (ϑl), MCH (pg), MCHC (g/L), WBC, Lymph, MID, GRA and PLT ($\times 10^3$/µl). Control+ (Positive control); Biopsy II (Colon cancer stage II), Biopsy III (Colon cancer stage III), Biopsy IV (Colon cancer stage IV). Using the Dun can' test, the different letter indicates the significant difference at the probability level (P≤0.05)

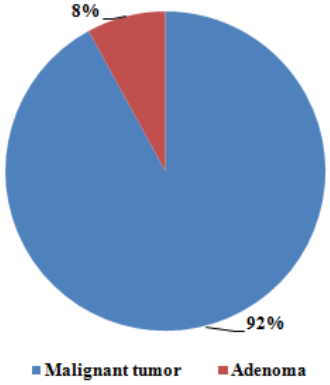

Figure No. 1: Percentage of malignant and benign tumors

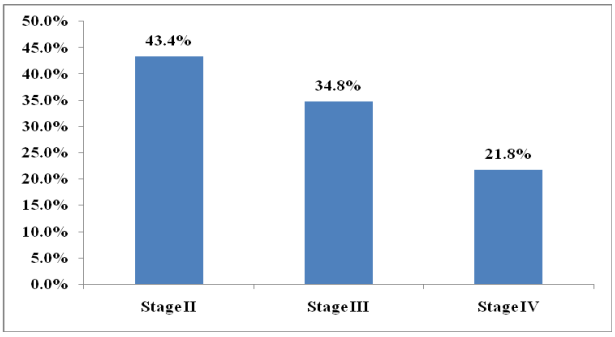

Figure No. 2: Percentage of infections

Table No. 2: The percentage of lymphocytes to monocytes and platelets to lymphocytes in the male groups

Groups	Percent lymphocyte to monocyte ratio	Percent platelets to lymphocyte ratio
Healthy Control	5.6	78.6
Positive Control	4.4	99.6
Adenoma	3.1	134.3
Biopsy II	2.5	136.6
Biopsy III	3.7	132
Biopsy IV	3.6	117

Table No. 3: Differences in tumor markers among control, benign tumor and biopsy groups in males

Variable	Healthy control	Positive control	Adenoma	Biopsy II	Biopsy III	Biopsy IV
VCAM -1	62.5±2	64.3±1.8	38.2±3.5	72.2±2	75.3±1.9	68±2
Septin -9	1.04±0.02	1.07±0.02	0.65±0.05	1.48±0.31	1.88±0.6	2.28±0.4
HIF-1 -alpha	4.3±0.5	4.5±1.3	3.6±0.6	9.4±1.3	13.5±1.2	11.7±0.4
CRP	1.74±0.03	1.81±0.14	1.38±0.11	2.10±0.05	2.11±0.07	2.05±0.04

DISCUSSION

The prevalence of advanced malignancies, including colon cancer, was determined among a German population-based sample of 15,985 participants in colonoscopy screening aged 55–79 years, of whom 7,822 were male. Men had a two-fold increased risk of colon cancer (1.8%) compared to women (1%). Men also had a higher prevalence of advanced and non-advanced adenomas (13.4%) and (24.6%), respectively. It has been suggested that estrogen may reduce the risk of proximal and distal colon cancers by increasing apoptosis in cell lines, which may explain a significant portion of the gender differences in cancer risk.[13]

In a descriptive study conducted by collecting data on 760 male patients who visited the colonoscopy unit in Somalia, it was found that 50 cases of cancer were present, or 8.5% of the cases, and adenomas constituted 40 of the 50 malignant tumors.[14]

The variation in white blood cell counts in mice may be attributed to immune system activation, which induces a positive immune response in the form of increased lymphocytes and monocytes, a hallmark of white blood cell count variations.[15,16]

Wan et al[17] indicated that a high lymphocyte-to-monocyte ratio (LMR) is associated with improved survival rates in cancer patients. LMR is an indicator of antitumor immunity and tumor burden. At the same time, tumor-infiltrating lymphocytes contribute to enhanced immune response, and their low levels are associated with impaired immunity and adverse clinical outcomes.

A recent study by Turri et al[18] showed that a high preoperative white blood cell count and low lymphocyte count were associated with worse postoperative survival outcomes. Our study findings support these observations, as white blood cell counts were 1.5-3.2-fold higher in women in the fourth and second biopsy groups compared to the control group.

Our results showed decreased L/M and P/L ratios in males with advanced disease stages, consistent with the study by Yamamoto et al[19], that demonstrated the importance of LMR and PLR as prognostic indicators in colon cancer, where decreased LMR indicates a severe inflammatory state and decreased overall and disease-free survival rates.

Abd El Kader et al[20] noted that occult blood in the stool is more common in colon cancer patients, reflecting decreased hemoglobin. Monocytes also contribute to tumor-associated inflammation, while lymphocytes play a role in anti-cancer immunity.[21]

Adhesion molecules play a pivotal role in cell growth, differentiation, and migration and contribute to the transmission of cellular signals. Their involvement in these mechanisms is important in tumor progression and vascular spread. Elevated VCAM-1 levels in colon cancer patients are associated with enhanced tumor growth and metastasis by facilitating the interaction of cancer cells with their surrounding environment. Its elevation is an indicator of poor prognosis colon cancer.[8]

Zhang et al[22] reported that VCAM-1 contributes to activating the epithelial-mesenchymal transition (EMT) program, which promotes cancer cell migration and invasion. Its high expression is associated with poor differentiation, increased metastasis, and an aggressive tumor pattern in colorectal cancer, as well as poor survival rates, highlighting its importance as a prognostic factor.

Elevated levels of Septin-9 were observed in males, consistent with the findings of, who linked this to molecular mechanisms related to the cytoskeleton and signaling pathways. The oncogenic form of the protein contributes to enhanced cancer cell invasion by degrading the extracellular matrix (ECM). Also the results are consistent with Qu & Sun.[23] It was mentioned that septin-9 increases with the progression of the disease stages, especially the III and IV Stages.

As well, Peng et al[24] found that suppressing the expression of Septin-9 nine facilitates cell migration and alters Rho A signaling while having no effect on cell proliferation. Hypermethylation may be linked to the suppression of gene expression, which in turn contributes to cancer cell migration.

Hypermethylation of the SEPT9 gene is a pivotal mechanism in the development of colon cancer, leading to the inactivation of tumor suppressor genes. It can be detected in blood, making it a promising biomarker for diagnosis and monitoring. demonstrated that measuring protein levels before and three months after surgery showed 96.7% sensitivity and 95.5% specificity in distinguishing malignant from non-malignant tumors, which is consistent with the results of our study.[25]

Like others, hypoxia factor HIF-1α is also elevated in male colon cancer patients compared to control subjects. HIF-1α is a master regulator of the cellular response to hypoxia, a common characteristic of solid tumors. This factor is associated with dysregulated cell

proliferation and anti-apoptotic processes, contributing to tumor growth, metastasis, migration, and invasion.[10] C-reactive protein (CRP) elevated CRP has been linked to increased tumor invasiveness as a result of the systemic inflammatory response.[26]

That systemic inflammation assists in assessing risk factors associated with distant metastases in colon cancer. An increase in CRP in breast cancer patients compared to benign tumors, which induce a more significant inflammatory response as a result of tissue invasion and damage, which strengthens the interpretation of the results of our study.[3]

CONCLUSION

Given the role of tumor markers VCAM-1, Septin-9, and HIF-1α it can be used as biomarkers for assessing tumor progression, metastasis and then differentiating between benign and malignant tumors.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Shaimaa Obaid Mostafa, Haitham L Al-Hayali
Drafting or Revising Critically:	Shaimaa Obaid Mostafa , Mowafak K. Hasan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

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