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Comparative Rating of Tumor Marker Profiles in Colon Cancer in both sex: A Clinical Study in Mosul city

SHAIMAA OBAID MOSTAFA [a,1,*], HAITHAM L. AL-HAYALI[2], MOWAFAK K. HASAN[3]

[1] Department of Biology, College of Science, Mosul University, Mosul, Iraq, Email: shysbio112@uomosul.edu.iq

[2] Department of Biology, College of Science, Mosul University, Mosul, Iraq, Email: haysbio68@uomosul.edu.iq

[3] Department of Biology, College of Science, Mosul University, Mosul, Iraq, Email: mufsbio62@uomosul.edu.iq

*[a] Corresponding Author: SHAIMAA OBAID MOSTAFA, shysbio112@uomosul.edu.iq

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ABSTRACT

Background: third most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality globally in both sex. it often begins without symptoms, in the early stages are similar to those of intestinal disorders and other diseases. Also, there is a difference in the incidence of tumors in males compared to females. Detecting it early can make a significant difference in how well patients survival. Objectives: This study seeks to identify tumor parameters that help detect the different stages of malignant tumors, and distinguish between male and female tumors. Methods: Blood samples were gathered from 86 individuals undergoing colonoscopies in hospitals across Mosul. And 37 individual's healthy persons too. and After colectomy, the malignant tumor samples were divided into three groups depending on the tumor stages. and tested using the ELISA method. with comparison control groups. Results: The findings showed Clear differences between the sexes. Males had significantly lower blood counts. and weakened immunity. the ratios such as L/M and P/L showed meaningful differences between men and women, which provides differences in the immune system response of patients according to gender. In women had higher levels of tumor markers especially in early-stage cancer. Markers like Septin-9, HIF-1α, and cf-DNA showed significant differences by tumor stage in women, while they were more accurate in men in identifying colon cancer progression. Conclusion: The diagnosis and development of colon cancer can be understood with the help of VCAM-1, Septin-9, HIF-1α, and cf-DNA. Also, cf-DNA and HIF-1α are capable of distinguishing factors such as sex and tumor stages.

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Introduction

The word "malignancy" is used to describe uncontrolled cell growth that has the potential to invade nearby tissues or spread to distant organs (Krakstad, 2021; Al-Taie and Al-Alaaf, 2025a). it arises as a result of a series of complex genetic changes that enable cells to evade programmed cell death (apoptosis), continue to multiply, and invade neighboring tissues and organs.Which is called metastasis, which spreads the disease to neighboring tissues and organs These are key factors in the development of cancer (Boaretto et al., 2023). That the term "malignancy" is used synonymously with cancer (Yadav & Srivastava, 2014).

Colon cancer (CCA) ranks as the third most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality globally in both males and females (Martínez-Gutierrez et al., 2022). CCA ranks as the third most frequently diagnosed cancer and the fourth leading cause of cancer-related mortality globally in both males and females (Martínez-Gutierrez et al., 2022). Early detection of the disease is difficult (Lee et al., 2024). Colon cancer often develops gradually, starting with benign polyps and progressing to metastatic stages that affect organs of the gastrointestinal tract (Reimers et al., 2013; Zarour et al., 2017).

Variations in white blood cell (WBC) counts may be attributed to the immune system's response and defense mechanisms. This activation may stimulate the production of monocytes and lymphocytes, leading to an increase in total white blood cells (Al-Hayali, 2020 ; Al-mushhadani et al., 2023)

A study (Youssef et al., 2024) indicated that some biochemical markers in colon cancer patients were significantly higher than in the control group. Biomarkers are essential for detecting malignant diseases. It can be used to track the growth and spread of tumors. Patients' reactions to treatment are occasionally reflected in it (Al-Taie and Al-Alaaf, 2025b).

VCAM-1 is a glycoprotein found on the surface. In 1989, it was found. It dissolves in cancer sufferers' serum. It spreads from the tumor surroundings, where endothelial cells express it in reaction to inflammatory circumstances. It has a crucial role in angiogenesis and the development of colon cancer (VanHeys et al., 2022).

VCAM-1 expression may be a biomarker because of linked to cancer stage. lymph node involvement, and tumor development (Mirzaee, 2017).

Hartwell discovered septins in 1971. Septin-9 is as a P-loop GTPase. It is essential for cytoskeletal structure, and cell cycle regulation. It is encoded by the Septin-9 gene on

chromosome 17p25. Septin-9 controls unchecked and fast cell division, In malignant tissues. Septin-9 is methylated in the blood of patients with colorectal cancer, that has become a biomarker for cancer monitoring and detection (Yildiz et al., 2019 ; Fischer et al., 2022).

HIF-1α reacts to low oxygen levels, which are frequently present in solid tumors like colon cancer. It is a component of the heterodimeric HIF-1 complex, which also contains the HIF-1β and oxygen-sensitive HIF-1α subunits. HIF-1α is essential for controlling gene expression linked to cancer cell proliferation, and resistance mechanisms (Rawłuszko-Wieczorek et al. 2014).

In colon cancer case HIF-1α is often overexpressed, and this overexpression is connected to bad prognosis and more progresive stages of the colon cancer (Chai et al., 2022). By controlling vascular endothelial growth factor (VEGF), it support cancer cell survival, proliferation, and metastasis.Its function in linking inflammation to cancer progresive, through the connection between HIF-1α and inflammatory pathways like NF-kB. it thus may becomes a possible target for therapeutic in colon cancer in future.

A cf-DNA is a complicated combination of DNA fragments reflecting a tumor's genomic traits. Released into the circulation by cancer cells. these fragments can be used for cancer detection and surveillance. (Grabuschnig et al., 2020). The patterns of 5-hydroxymethylcytosines (5hmC) in cf-DNA have shown great specificity colon cancer. So suggesting that this method is a good for diagnosis (Li et al., 2017). Raman spectroscopy has also offered insightful analysis of the chemical makeup of cf-DNA. Including those with colon cancer, this method can differentiate between the cf-DNA of healthy people and cancer patients (Papadakis et al., 2023).

2. Materials and methods

123 samples were collected from patients visiting different endoscopy units in Mosul city was as 41 cases of malignancy tumor and 45 cases without tumor and 37 healthy individuals. The participants' ages ranged from 17 to 84 years, and the samples included individuals of both sexes. of colonoscopy units in Ibn Sina Teaching Hospital, Mosul General Hospital, and from the private clinic of Dr. Abdullah Zuhair Al-Yuzbaki in Mosul City. between March 14, 2023, and March 12, 2024.

Venous blood samples were collected from the study participants before colonoscopy at the above-mentioned hospitals. Each sample size was 5 ml, distributed into two types of tubes: 3 ml in gel tubes for tumor marker analysis and 2 ml in EDTA tubes for complete blood count (CBC) analysis using the MicroCC-20Plus device on the same day of the collection. Complete blood counts were performed within one hour of sample collection to ensure accurate results and were not affected by subsequent time changes. After serum separation, samples were classified into 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 Before colostomy (BCO. II, III, and IV)), Then the serum was used to measure tumor biomarkers.

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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.

Tumor markers

This aspect of the study involved estimating six tumor markers in the serum using ELISA technology, Labtech Microplate Reader LT-4000, East Sussex, UK. The markers included Vascular Cell Adhesion Molecule 1 (VCAM-1), Septin-9, Hypoxia-Inducible Factor 1 Alpha (HIF-1α), and cf-DNA, following the guidelines provided by Shanghai Ideal Medical Technology Co., Ltd., China.

Statistical Analysis

All data are presented as means ± SD, 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 version 26 (Jalolov, 2024)

4. Result

The results showed significant differences between the biopsy groups and the control groups of males in each of white blood cell (WBC), lymphocyte counts (LYM), red blood cell (RBC), hemoglobin (Hb), Hematocrit (HCT). However, in Granulocytes (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, as shown in Table 1.

Table 1: Complete blood count of male biopsy groups

Groups / Variables	Control he. Mean± SD	Control + Mean± SD	BCO. II Mean± SD	BCO. III Mean± SD	BCO. IV Mean± SD
WBC	7.29 a ± 0.65	7.30 a ± 0.62	7.50 a ± 1.14	6.02 b ± 0.57	6.82 ab ± 0.15
LYM	2.80 a ± 0.42	2.23 b ± 0.30	1.53 c ± 0.17	1.50 c ± 0.618	1.67 c ± 0.54
MID	0.561 ab ± 0.07	0.51 abc ± 0.13	0.625 a ± 0.07	0.403 c ± 0.11	0.467 bc ± 0.13

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GRA	**3.92 b ± 0.39**	**4.57 ab ± 0.72**	**5.35 a ± 0.91**	**4.11 b ± 0.30**	**4.68 ab ± 0.55**
RBC	**5.23 a ± 0.11**	**5.32 a ± 0.16**	**4.02 c ± 0.72**	**4.33 c ± 0.18**	**4.52 b ± 0.17**
Hb	**14.57 a ± 0.44**	**14.03 a ± 1.91**	**9.62 b ± 1.45**	**9.20 b ± 2.08**	**10.00 b ± 0.20**
HCT	**44.62 a ± 2.32**	**43.24 a ± 3.79**	**30.55 b ± 4.79**	**30.76 b ± 4.6**	**32.80 b ± 1.10**
PLT	**220 a ± 11**	**222 a ± 30.4**	**209 a ± 39**	**198 a ± 3.51**	**195 a ± 5.0**

RBC ($\times 10^6/\mu l$) - Hb (g/dl)- (%) – WBC, Lymph, MID, GRA and PLT ($\times 10^3/\mu l$). SD (Standard deviation) ; control + (positive control); control he (Healthy 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 ($P \leq 0.05$)

Furthermore, when comparing the biopsy and adenoma groups with the control groups, the results showed significant differences in WBC and LYM counts for Biopsy II; monocytes (MID) for Biopsy IV; RBC counts, Hb levels, HCT, PLT, when comparing the biopsy and adenoma groups with the control groups.

No significant differences were observed in the following parameters: (GRA). As well significant differences were observed between the control groups, as shown in Table 2.

Table 2: Complete blood count of female biopsy groups

Groups / Variable	Control he. Mean± SD	Control + Mean± SD	BCO. II Mean± SD	BCO. III Mean± SD	BCO. IV Mean± SD
WBC	**6.93 b ± 1.09**	**7.04 b ± 2.24**	**10.12 a ± 1.88**	**6.48 b ± 0.54**	**8.50 ab ± 0.54**
LYM	**2.37 b ± 0.53**	**2.19 b ± 0.86**	**3.54 a ± 1.46**	**2.45 b ± 0.943**	**2.75 ab ± 0.451**
MID	**0.476 b ± 0.1**	**0.479 b ± 0.27**	**0.68 ab ± 0.31**	**0.6 ab ± 0.06**	**0.813 a ± 0.11**
GRA	**4.09 ab ± 0.87**	**4.37 ab ± 1.74**	**5.90 a ± 3.65**	**3.42 b ± 0.330**	**4.95 ab ± 0.26**
RBC	**4.66 a ± 0.20**	**4.67 a ± 0.28**	**4.32 ab ± 1.78**	**3.39 c ± 0.11**	**3.84 bc ± 0.20**
Hb	**13.04 a ± 0.61**	**13.07 a ± 1.30**	**11.6 b ± 1.40**	**8.42 c ± 1.26**	**10.53 b ± 0.33**
HCT	**39.66 a ± 1.58**	**38.86 ab ± 2.35**	**36.30 b ± 6.2**	**26.5 d ± 2.78**	**32.4 c ± 0.712**

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PLT	**239 b ± 25**	**250 b ± 44**	**393 a ± 8**	**274 b ± 33.6**	**184 c ± 21.7**

RBC ($\times 10^6/\mu l$) - Hb (g/dl)- (%) – WBC, Lymph, MID, GRA and PLT ($\times 10^3/\mu l$). SD (Standard deviation) ; control + (positive control); control he (Healthy 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 ($P \leq 0.05$)

Table 3: The percentage of lymphocytes to monocytes and platelets to lymphocytes in both sexes for all groups

Percentage / Groups	Sex	% L/M	% P/L
Control he.	**males**	**5.6**	**78.6**
	females	**5**	**101**
Control +	**males**	**4.4**	**99.6**
	females	**4.6**	**114**
BCO. II	**males**	**2.5**	**136.6**
	females	**5.2**	**111**
BCO. III	**males**	**3.7**	**132**
	females	**4.1**	**110**
BCO. IV	**males**	**3.6**	**117**
	females	**3.4**	**67**

L/M (lymphocyte to monocyte ratio) ; P/L (platelets to lymphocyte ratio). ; control + (positive control) ; control he (Healthy control), BCO. II(colon cancer stage II), BCO. III (colon cancer stage III), BCO. IV(colon cancer stage IV)

Regarding to tumor markers, the results demonstrate that males and females had significant increase in all parameters compared to the control groups, as shown in Table 4. The readings for VCAM-1 and cf-DNA, along with HIF-1α, were significantly elevated in Biopsy III and IV exhibited a notable increase in Septin-9. A significant difference was observed in HIF-1α and cf-DNA across the three biopsy groups. The VCAM-1 indicate significant differences only in the biopsy IV group.

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Table 4: Male tumor markers

Groups / Variables	Control he. Mean± SD	Control + Mean± SD	BCO. II Mean± SD	BCO. III Mean± SD	BCO. IV Mean± SD
VCAM_1	62.5 d ± 2	64.3 d ± 1.8	72.2 b ± 2	75.3 a ± 1.9	68 c ± 2
Septin_9	1.04 d ± 0.02	1.07 d ± 0.02	1.48 c ± 0.31	1.88 b ± 0.6	2.28 a ± 0.4
HIF_1_alpha	4.3 d ± 0.5	4.5 d ± 1.3	9.4 c ± 1.3	13.5 a ± 1.2	11.7 b ± 0.4
cf_DNA	53 d ± 8	67 c ± 12	96 b ± 4	105 a ± 8	88 b ± 2

VCAM-1(ng/ml) ; Septin-9(ng/ml) ; HIF-1α (pg/ml) ; cfDNA (nmol/L) ; SD (Standard deviation) ; ; control + (positive control) ; control he (Healthy control), BCO. II(colon cancer stage II), BCO. III (colon cancer stage III), BCO. IV(colon cancer stage IV) . Using the Dun can' test, the different letter indicates the significant difference at the probability level ($P \leq 0.05$)

It is worth to mention that patients tumor parameters in stage IV were highest, significant differences were found in VCAM-1, HIF-1α, and cf-DNA among the three groups. Also direct increase was observed with the progression of the disease stage, except for VCAM-1, as detailed in Table 5.

Table 5: Female tumor markers

Groups / Variables	Control he. Mean± SD	Control + Mean± SD	BCO. II Mean± SD	BCO. III Mean± SD	BCO. IV Mean± SD
VCAM_1	60.1 c ± 2.0	61.8 c ± 2.0	74.5 b ± 1.5	76.2 b ± 2.0	81.4 a ± 4.3
Septin_9	1.05 d ± 0.7	1.07 d ± 0.3	1.5 c ± 0.23	1.99 b ± 0.41	2.48 a ± 0.5
HIF_1_alpha	6.0 d ± 0.9	6.2 d ± 0.8	9.7 c ± 0.7	11.8 ± 1.6	13.8 a ± 0.9
cf_DNA	47 e ± 3	59 d ± 10	85 c ± 9	97 b ± 5	117 a ± 11

VCAM-1(ng/ml) ; Septin-9(ng/ml) ; HIF-1α (pg/ml) ; cfDNA (nmol/L) ; SD (Standard deviation) ; ; control + (positive control) ; control he (Healthy control), BCO. II(colon cancer stage II), BCO. III (colon cancer stage III), BCO. IV(colon cancer stage IV) . Using the Dun can' test, the different letter indicates the significant difference at the probability level ($P \leq 0.05$)

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Table 6: Differences in tumor parameters between the sexes

Groups / Variables	Males			Females		
	BCO. II Mean± SD	BCO. III Mean± SD	BCO. IV Mean± SD	BCO. II Mean± SD	BCO. III Mean± SD	BCO. IV Mean± SD
VCAM_1	72.2 c ± 2	75.3 b ± 1.9	68.0 d ± 2.0	74.5 c ± 1.5	76.2b ± 2.0	81.4 a ± 4.3
Septin_9	1.48 c ± 0.31	1.88c ± 0.6	2.28b ± 0.4	1.5 c ± 0.23	1.99 c ± 0.41	2.48 a ± 0.5
HIF_1_alpha	9.4c ± 1.3	13.5a ± 1.2	11.7b ± 0.4	9.7c ± 0.7	11.8b ± 1.6	13.8a ± 0.9
cf_DNA	96b ± 4	105a ± 8	88c ± 2	85c ± 9	97b ± 5	117a ± 11

VCAM-1(ng/ml) ; Septin-9(ng/ml) ; HIF-1α (pg/ml) ; cfDNA (nmol/L) ; SD (Standard deviation) ; ; control + (positive control) ; control he (Healthy control), BCO. II(colon cancer stage II), BCO. III (colon cancer stage III), BCO. IV(colon cancer stage IV) . Using the Dun can' test, the different letter indicates the significant difference at the probability level ($P \leq 0.05$)

Discussion:

A CBC is a standard blood test that reveals a patient's general health and can find anomalies suggesting cancer development or presence. Though not a direct diagnostic for colon cancer, but generally, differences in WBCs among colon cancer patients show a complicated between the immune response to the malignancy, And tumor inflammatory environment. Also, the physiological alterations brought about by the disease itself.

Often, a CBC will detect anemia, which can suggest colon cancer from persistent blood loss because tumor growth. For patients showing symptoms like rectal bleeding or unexplained lethargy, which need additional diagnostic tests (Mohammad et al., 2023).

According to Wan et al. 2022 the high LMR is linked to improved survival rates in cancer patients. However, survival results showed no correlation with LMR following treatment. LMR is derived from lymphocyte and monocyte counts, as measure of antitumor immunity. A lack of LYM is linked to a failure the tumor immune response, which results in bad clinical results in many different kinds of cancer. Conversely, tumor-infiltrating lymphocytes (TILs) are

lymphocytes that travel within tumor settings and significantly contribute to antitumor immunity via their capacity to kill cancer cells.

In study of 1,674 colorectal cancer surgery patients, found that WBCs values rise as surgery drew near, although lymphocyte levels decrease. After surgery, those with the highest WBC and low lymphocyte values had worse cancer-related survival (CRS) results. Although the relationship between inflammation and cancer is well-studied, there is little data on prediagnostic inflammatory and their association with higher cancer risk. They also found low hemoglobin levels, high platelet counts, and rising inflammatory markers seen as early as nine months before diagnosis colon cancer, that could help diagnosis cancer (Turri et al. 2023). These findings are consistent with our present result, which indicated that female WBC levels in the IV and II biopsy groups increase by 1.5 to 3.2 times when compared to the control group.

Our findings show that the lymphocyte-to-monocyte (L/M) and platelet-to-lymphocyte (P/L) ratios decreased in the biopsy groups across all three disease stages (II, III, and IV) for both males and females. This is in line with the study by (Yamamoto et al., 2021), which identified the lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) as significant prognostic markers in colon cancer, indicating a heightened inflammatory state. A low lymphocyte ratio is linked to reduced overall survival in patients with colon cancer

Complete blood count components, such as the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), are significantly higher in patients with colorectal cancer compared to those without the disease. These ratios possible cancer screening indicators (Virdee et al., 2020).

A study of 272 stage I - III colon cancer patients who had surgical removal was done by Hayama et al. (2021). Among these individuals, that variations in hemoglobin levels may given to many factors including tumor site. Often connected to bleeding, right-sided colon cancer (RCC) might cause lower hemoglobin levels than left-sided colon cancer (LCC). Over time, LCC is more likely to induce chronic, less obvious blood loss, which might develop to anemia.

The discovery of occult blood in the stool is notably more common in colorectal cancer patients than in those with control groups. This means continuous bleeding, which may be

resulting in decreases hemoglobin concentrations. (Abd El Kader et al., 2023). Lymphocytes are involved in anti-tumor immunity, while monocytes contribute to inflammation that promotes tumors (Lisanti et al., 2023; Ye and Mi, 2024).

Biological molecules known as colon cancer biomarkers can be detected in blood, various body fluids, or tissues, that indicating the presence or advancement of colon cancer. A study (Mohammad, 2012 ; Bdaiwi, 2013) reveals that certain crucial compounds can rise by as much as 50% or more in malignant tumors, including CEA, were noted in the blood serum of patients across different stages of colon cancer when compared to the control group. (Azeez et al., 2018).

Tables 4 and 5 indicate a rise in VCAM-1 levels in both males and females in biopsy samples when compared to control groups. In patients with colon cancer, heightened expression could be associated with its involvement in tumor advancement and spread. VCAM-1 serves as a cell adhesion molecule that enhances the interaction between cancer cells and their microenvironment, thereby facilitating processes such as cell migration, invasion, and metastasis, and correlates with unfavorable outcomes and treatment resistance (VanHeyst et al., 2022).

(Zhang et al., 2020) illustrated that VCAM-1 is crucial in initiating the epithelial-mesenchymal transition (EMT) program, a vital mechanism in the progression of cancer metastasis. EMT facilitates the transition of cancer cells from epithelial characteristics to mesenchymal, therefore increase their migratory and invasive abilities. This process enables cancer cells to adhere to endothelial cells, which is essential and necessary for their migration and the progression of metastasis. The study revealed that heightened expression of VCAM-1 correlated with inadequate differentiation and greater distant metastases in colorectal cancer patients, suggesting a more aggressive tumor phenotype. Additionally, it was confirmed that patients exhibiting elevated levels of VCAM-1 experienced shorter survival durations in contrast to those with reduced levels, underscoring its importance as a prognostic indicator in colon cancer.

It is important to highlight that Septin-9 levels have also risen in both males and females; these findings align with the results made by Okletey et al. (2023) regarding the various molecular mechanisms that contribute to increased Septin-9 in colon cancer patients. The

mechanisms are mainly linked to their roles in cellular structures and signaling pathways. Moreover, the tumor-causing variant stimulates the development of invadopodia, which aid in the invasion of cancer cells by breaking down the extracellular matrix (ECM).

Additionally, the findings of Peng et al. (2017) refer to that the suppression of Septin-9 expression enhances cell migration and modifies Rho A signaling, without influencing cell proliferation. Hypermethylation could be associated with the inhibition of gene expression, subsequently playing a role in the migration of cancer cells and their resistance to chemotherapy.

Hypermethylation of the *SEPT-9* gene is a prevalent genetic alteration in colorectal cancer that contributes to disease progression (Yildiz et al., 2019). Furthermore, the hypermethylation of the SEPT9 gene represents a crucial molecular mechanism in the progression of colon cancer. This genetic modification is linked to the repression of tumor suppressor genes, which play a important role in the advancement of cancer. This marker can be identified in both tissues and the peripheral blood of patients, showing its significance as a biomarker for the detection and monitoring of colorectal cancer. Hypermethylated SEPT9 found in plasma signifies the release of tumor DNA from dead colorectal cancer cells. This discovery support the link between methylation and reduced levels of Septin-9 throughout the progression of cancer (Sun et al., 2019; Leon Arellano et al., 2020). our findings also align with the work of Qu and Sun (2023), who indicated that septin-9 levels rise as the disease progresses, particularly in stages III and IV

The study conducted by El Kader et al. (2023) found that assessing this protein in patients with colorectal cancer, both prior to and three months post-surgery, resulted in a sensitivity of 96.7% and a specificity of 95.5% for differentiating between colorectal cancer cases. This is consistent with observations made when analyzing protein levels in biopsy samples in Tables 5 and 6..

Similar to other findings, the hypoxia factor HIF-1α shows increased levels in both male and female colon cancer patients when compared to control subjects. HIF-1α functions as a key regulator of the cellular response to low oxygen levels, which is a prevalent feature of solid tumors. This factor is linked to uncontrolled cell growth, processes that prevent cell death, as well as migration and invasion, all of which play a role in tumor development and spread. HIF-

1α is activated in response to low oxygen levels and is essential in cancer progression by turning on genes that help cancer cells adjust to the hypoxic conditions present in the tumor microenvironment (Pandey et al., 2025).

As noted by Chen et al. (2023), malignant colon cancer generally presents a more intense hypoxic environment, leading to a significant increase in HIF-1α levels. This is attributed to the rapid proliferation of cancer cells outpacing the development of new blood vessels.

Mäkinen et al. (2024) demonstrated that the activation of the hypoxia-inducible factor (HIF) pathway by roxadustat results in an increase in glycolysis, which is intricately associated with the metabolic alterations frequently seen in cancer cells. In a similar vein, Perepechaeva et al. (2023) established that the overexpression of HIF-1α is markedly increased in colon cancer cells when subjected to hypoxic conditions.

According to the findings of Smolarz et al. (2024), the microenvironment of solid tumors is marked by hypoxia, a phenomenon resulting from the swift growth of cancer cells coupled with inadequate blood vessel formation within the tumor. This indicates that the blood supply might be insufficient to satisfy the tumor's requirements. It was observed that under hypoxic conditions, the HIF-1α subunit accumulates and translocates to the cell nucleus, where it forms an asymmetric dimer with HIF-1β. This process plays a vital role in allowing cancer cells to adjust to anaerobic environments, as it governs the expression of genes that are key to various adaptive mechanisms, such as angiogenesis and metabolism.

A study conducted by (Chai et al., 2022) revealed that the expression rate of HIF-1α in colon cancer tissues was 80%, compared to just 14% in normal colon tissues. The findings are illustrated in tables 4, 5, and 6.

Noë et al. (2024) proposed that cf-DNA consists of small, fragmented pieces of DNA that circulate freely in the bloodstream, as opposed to being enclosed within cells. These fragments can be considered as signals that cells release into the bloodstream. cf-DNA can be derived from both healthy and malignant cells. This substance, derived from cancer cells, has the potential to aid medical professionals in identifying cancer in its initial stages. The terminal regions of these DNA fragments generally feature CC or GC base pairs. The significance of methylation and

embryonic expression is crucial for comprehending the characteristics of these fragments. Increased methylation at CpG sites leads to the presence of larger and more abundant cf-DNA fragments, suggesting a higher quantity of genetic material in the bloodstream.

(Chidharla et al., 2023) showed that cf-DNA includes circulating tumor DNA (ct-DNA) derived from cancer cells. During the process of apoptosis, tumor cells release fragments of DNA into the circulation. The blood's level of cf-DNA is a measure of the tumor The blood's level of cf-DNA is a measure of the size of the tumor. Useually increased level of it correlate with advance stages of colon cancer, also a elevated chance of recurrence. so that cf-DNA as a significant biomarker for tracking colon cancer. Moreover, elevated blood levels may indicate the presence of cancer cells, highlighting its potential utility as a biomarker for early detection of cancer. Moreover, the increased levels observed post-surgery are associated with a greater likelihood of cancer recurrence, aligning with the findings shown in Tables 4 and 5.

A study conducted by Elbaiomy and Sabry in 2023 found that ct-DNA levels rose by 62.2% in stage II patients exhibiting high-risk factors, including T4 adenomas or lympho vascular invasion, in contrast to a 28.2% increase observed in patients without such risk factors. The findings revealed an increased probability of cancer recurrence among patients exhibiting high-risk factors, showing rates of 39% compared to 19% for those lacking such factors. This data could provide insight into the rise in stage II and III male patients, as illustrated in Table 4.

According to Hu et al. (2020), the findings indicated a direct relationship between elevated serum cf-DNA concentration and cancer stage tumor size in individuals with colon cancer. This observation could elucidate the reason behind the elevated cf-DNA concentrations in male patients with stage III colon cancer when compared to other biopsy groups, as shown in Table 4. The study indicated that this significant distinction in colon cancer might serve as a biomarker to aid physicians in differentiating colon cancer.

In conclusion, the involvement of VCAM-1, Septin-9, HIF-1α, and cf-DNA, both inflammatory cell infiltration and cancer cells metastasis, owes their marked functional versatility as a target for colon cancer disease. cf-DNA can be used as a biomarker for assessing tumor progression and metastasis. Finally, the differences between the values obtained for males and females may reflect a biological indicator of gender discrimination.

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