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Comparative Estimate of Some Tumor Markers in Females with Colon Cancer: A Clinical Study in Mosul City

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ABSTRACT

Objective: *This study seeks to identify tumor parameters that help detect the different stages of malignant tumors.* ***Method:*** *Blood samples were gathered from 43 females undergoing colonoscopies in hospitals across Mosul, and 18 individuals who were healthy. After colectomy for cancer, 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 ratios such as L/M and P/L showed meaningful differences between stages, which provides differences in the immune system response of patients. In early-stage cancer, markers like Septin-9, HIF-1a, and cf-DNA showed significant differences by tumor stage in women in identifying colon cancer progression.* ***Novelty:*** *The diagnosis and development of colon cancer can be understood with the help of VCAM-1, Septin-9, HIF-1a, and cf-DNA.*

INTRODUCTION

Malignancy is used to characterize uncontrolled cell growth that has the potential to invade nearby tissues [1][2]. it grows as a result of progression 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 [3]. That the term "malignancy" is used synonymously with cancer [4].

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 [5]. Early detection of the disease is difficult [6]. CCA often develops gradually, starting with benign polyps and progressing to metastatic stages [7] [8].

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 [9] [10].

A study [11] indicated that some biochemical markers in CCA 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 [12].

VCAM-1 is a glycoprotein found on the surface. In 1989, it was found. It dissolves in cancer sufferers' serum. It has a crucial role in angiogenesis and the development of CCA [13]. Expression may be a biomarker because of linked to cancer stage. lymph node involvement, and tumor development [14].

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 [15] [16].

HIF-1α reacts to low oxygen levels, which are frequently present in solid tumors like CCA. 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 [17].

In CCA case HIF-1α is often overexpressed, and this overexpression is connected to bad prognosis and more progresive stages of the CCA [18]. 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 CCA 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. [19]. 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 [20]. Raman spectroscopy has also offered insightful analysis of the chemical makeup of cf-DNA. Including those with CCA, this method can differentiate between the cf-DNA of healthy people and cancer patients [21].

RESEARCH METHOD

61 females samples were collected from patients visiting different endoscopy units in Mosul city and healthy individuals, was as 18 cases of malignancy tumor and 25 cases without tumor and 18 healthy individuals. The participants' ages ranged from 19 to 84 years, and the samples included individuals of females 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. from March 14, 2023, to 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 CCA 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.

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 [22].

RESULTS AND DISCUSSION

Results

The incidence of colon cancer in females was approximately (44.4%) at stage III, which is the highest, and the rate was equal for stages II and IV, as shown in Figure 1. The most common site is the ascending colon, with a rate of 44.5%, which is the most common in terms of the number of infections, followed by the sigmoid colon, then the descending colon, with 33.3% and 11.1%, respectively. Multiple colon infections also constituted (11.1%), as shown in Figure 2.

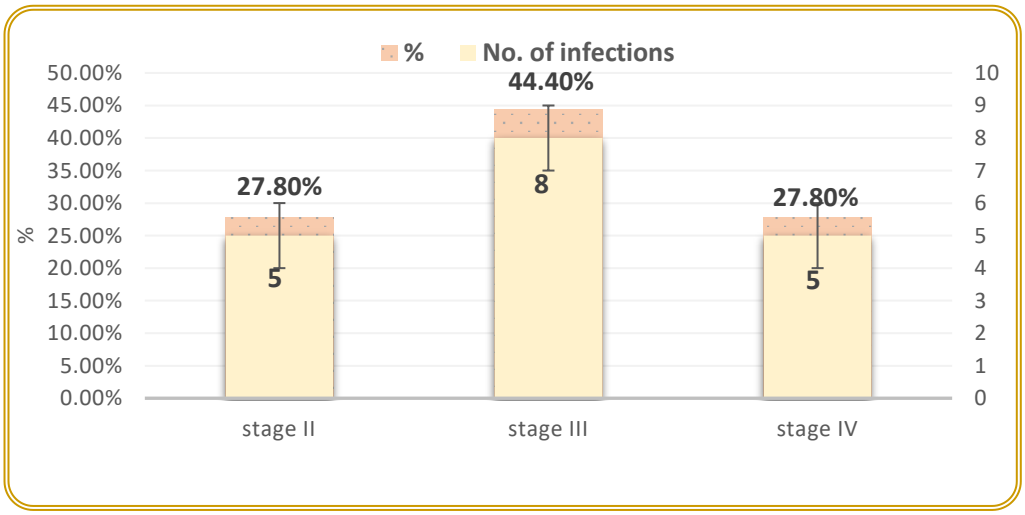

Figure 1. The number and stages of colon cancer in female.

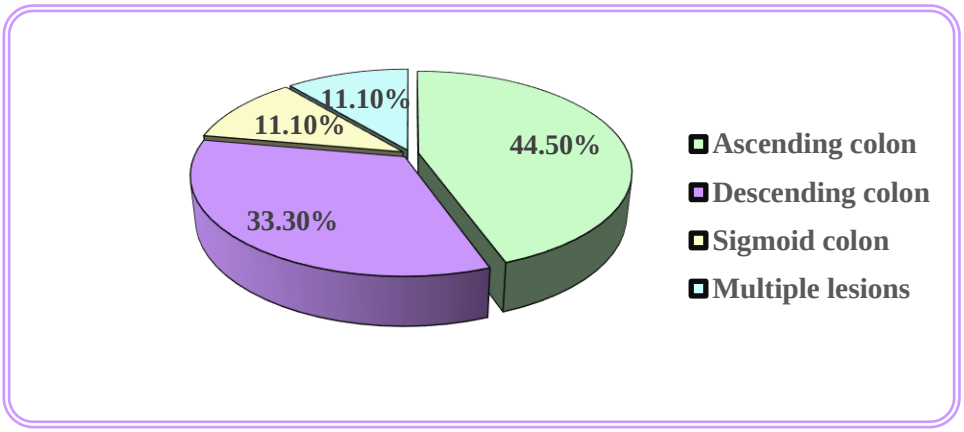

Figure 2. Percentage of malignant tumor distribution in colon parts.

The results showed significant differences between biopsy 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 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 1.

Table 1. 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
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 ≤ 0.05)

Table 2. The percentage of lymphocytes to monocytes and platelets to lymphocytes in females.

Percentage / Groups	% L/M	% P/L
Control he.	5	101
Control +	4.6	114
BCO. II	5.2	111
BCO. III	4.1	110
BCO. IV	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).

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, as detailed in Table 3.

Table 3. 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 b ± 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 ≤ 0.05)

Discussion

The prevalence of advanced malignancies, including colon cancer, was determined among a German population-based sample of 15,985 individuals aged 55–79 who

underwent colonoscopy screening, of whom 8,163 were women. Men had a two-fold increased risk of colon cancer (1.8%) compared to women (1%). The incidence of proximal and distal colon cancers was 33.3% and 32.1%, respectively, in women. 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 [23].

A CBC is a standard blood test that reveals a patient's general health. Though not a direct diagnostic for CCA, but generally, differences in WBCs among CCA patients show a complicated between the immune response to the malignancy, And tumor inflammatory environment.

Often, a CBC will detect anemia, which can suggest CCA from persistent blood loss because tumor growth. For patients showing symptoms like rectal bleeding or unexplained lethargy, which need additional diagnostic tests [24].

According to [25] the high LMR is linked to improved survival rates in cancer patients. 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 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, 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 CCA, that could help diagnosis cancer [26]. 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 in table (2) 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 females. This is in line with the study by [27], which identified the lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) as significant prognostic markers in CCA, indicating a heightened inflammatory state. A low lymphocyte ratio is linked to reduced overall survival in patients with CCA

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 [28].

A study of 272 stage I - III CCA patients who had surgical removal was done by [29]. 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 [30]. Lymphocytes are involved in anti-tumor immunity, while monocytes contribute to inflammation that promotes tumors [31] [32].

Biological molecules known as CCA biomarkers can be detected in blood, various body fluids, or tissues, that indicating the presence or advancement of CCA. A study [33] [34] 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 CCA when compared to the control group. [35].

Tables 3 indicate a rise in VCAM-1 levels in females in biopsy samples when compared to control groups. In patients with CCA, 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 [13].

[36] 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. 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.

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 [37] regarding the various molecular mechanisms that contribute to increased Septin-9 in CCA 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 [38] 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.

Hypermethylation of the *SEPT-9* gene is a prevalent genetic alteration in colorectal cancer that contributes to disease progression [15]. Furthermore, 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 [39] [40]. our findings also align with the work of [41], who indicated that septin-9 levels rise as the disease progresses, particularly in stages III and IV

The study conducted by [30] 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 Table 3.

Similar to other findings, the hypoxia factor HIF-1α shows increased levels in female CCA 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. By turning on genes that help cancer cells adjust to the hypoxic conditions present in the tumor microenvironment [42].

As noted by [43], CCA 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.

According to the findings of [44], 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.

A study conducted by [18]. Revealed that the expression rate of HIF-1α in CCA tissues was 80%, compared to just 14% in normal colon tissues. The findings are illustrated in table 3.

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 [45].

[46] 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 size of the tumor. Usually increased level of it correlate with advance stages of CCA, also a elevated chance of recurrence. so that cf-DNA as a significant biomarker for tracking CCA. 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 Table 3.

A study conducted by [47] 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.

According to [48], the findings indicated a direct relationship between elevated serum cf-DNA concentration and cancer stage tumor size in individuals with CCA. The study indicated that this significant distinction in CCA might serve as a biomarker to aid physicians in differentiating CCA.

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. Septin-9, HIF-1α, and cf-DNA can be used as a biomarker for assessing tumor progression stages and metastasis.

CONCLUSION

Fundamental Finding : The diagnosis and development of colon cancer can be understood with the help of VCAM-1, Septin-9, HIF-1α, and cf-DNA. The ratios such as L/M and P/L showed meaningful differences between stages, which provides differences in the immune system response of patients. In early-stage cancer, markers like Septin-9, HIF-1α, and cf-DNA showed significant differences by tumor stage in women in identifying colon cancer progression. Patients tumor parameters in stage IV were highest, with direct increase observed with disease progression. **Implication :** Septin-9, HIF-1α, and cf-DNA can be used as a biomarker for assessing tumor progression stages and metastasis. This substance, derived from cancer cells, has the potential to aid medical professionals in identifying cancer in its initial stages. Elevated blood levels may indicate the presence of cancer cells, highlighting potential utility for early detection. Increased levels post-surgery are associated with a greater likelihood of recurrence, reflecting aggressive tumor biology. **Limitation :** Although the relationship between inflammation and cancer is well-studied, there is little data on prediagnostic inflammatory and their association with higher cancer risk. The results are limited to female patients from Mosul hospitals and may not be generalizable. The small sample size may restrict statistical power. The cross-sectional design cannot establish causality, and some parameters such as GRA showed no significant differences. **Future Research :** Future studies should explore combining VCAM-1, Septin-9, HIF-1α, and cf-DNA with other biomarkers to enhance diagnostic accuracy. Longitudinal, multi-center studies including both genders are needed to improve generalizability and evaluate recurrence prediction. Research should investigate molecular mechanisms linking biomarkers with tumor microenvironment and develop non-invasive screening tools integrating biomarkers with imaging and clinical data.

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