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RESEARCH ARTICLE OPEN ACCESS

Comparative appreciation of some tumour markers in colon cancer patients: A clinical study in Nineveh Governorate

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Abstract
Objective: This study aims to evaluate the diagnostic value of selected tumour markers, Septin-9, HIF-1α, and cf-DNA, in different stages of colon cancer, with an emphasis on potential differences between male and female patients.
Methods: A total of 125 individuals participated in this study. Among them, 41 patients had confirmed colon cancer and were classified into stages II, III, and IV based on pathological findings. Additionally, 45 individuals with benign colon conditions (control+) and 39 healthy individuals served as comparison groups. Blood samples were analysed using ELISA to quantify tumour marker levels. Haematological parameters, including a complete blood count and immune-related ratios such as the lymphocyte-to-monocyte (L/M) and platelet-to-lymphocyte (P/L) ratios, were also assessed.
Results: Significant sex-based differences were observed in both haematological and tumour marker profiles. Male patients exhibited lower CBC values and altered immune ratios, suggesting potential immune suppression. In females, higher levels of tumour markers were evident even at early stages of cancer. Septin-9, HIF-1α, and cf-DNA exhibited stage-specific expression patterns, with more pronounced progression trends observed in male patients.
Conclusion: Septin-9, HIF-1α, and cf-DNA may serve as valuable biomarkers for assessing colon cancer progression and understanding sex-based biological variations. These findings indicate a potential role for these markers in differentiating between cancer stages and between sexes. However, this is preliminary and hypothesis-generating, given the small sample size. Their integration into early diagnostic and monitoring protocols could enhance personalised treatment strategies, though further validation in larger cohorts is recommended.

Keywords: Colon Cancer, CBC, Septin-9, HIF-1α, cf-DNA, Colectomy

Plain English Summary

This study looked at whether certain blood-based tumour markers can help in diagnosing and monitoring colon cancer. The researchers focused on three markers: Septin-9, HIF-1α, and cell-free DNA (cf-DNA). They also examined routine blood tests like complete blood counts (CBC) and immune-related ratios to see how they varied between cancer patients and healthy or benign cases.

A total of 125 people took part. Of these, 41 had colon cancer at stages II–IV, 45 had benign colon conditions, and 39 were healthy controls. Blood samples were taken and analysed for the tumour markers and blood cell counts.

The study found clear differences between men and women. Men with colon cancer tended to have lower blood counts and changes suggesting weaker immune responses. Women, however, often showed higher levels of tumour markers even at earlier stages. All three tumour markers rose progressively with advancing cancer stage, but the patterns varied by sex. For example, Septin-9

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increased in both sexes, HIF-1α rose more in males at stage III but more in females at stage IV, while cf-DNA levels were notably higher in stage III males and stage IV females.
Routine blood test results also showed useful patterns. Ratios like lymphocyte-to-monocyte (L/M) and platelet-to-lymphocyte (P/L) declined as the disease progressed, which may reflect the immune system's struggle against cancer. Anaemia and other blood count abnormalities were more frequent in advanced stages, especially among female patients.
Overall, the findings suggest that combining simple blood tests with tumour marker measurements could improve the early detection and monitoring of colon cancer. The work also highlights important biological differences between men and women that could affect diagnosis and treatment. However, the researchers note that the sample size was relatively small and limited to hospitals in Nineveh, Iraq. Larger studies are needed before these markers can be reliably used in routine clinical care.

Introduction

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 (1). Early detection of the disease remains challenging (2). CCA often develops gradually, starting with benign polyps and progressing to metastatic stages that affect organs of the gastrointestinal tract (3, 4). The term "malignancy" is commonly used as a synonym for cancer (5) and refers to uncontrolled cell growth with the ability to invade adjacent tissues or metastasise to distant organs (6, 7). This malignancy arises due to a series of genetic and epigenetic alterations that allow cells to evade apoptosis, sustain proliferation, and invade surrounding structures—key hallmarks of cancer progression (8).

Changes in white blood cell (WBC) counts are often reflective of the host's immune response. In cancer patients, immune activation may stimulate monocyte and lymphocyte production, contributing to an overall increase in WBC count (9, 10).

Some biochemical markers are significantly elevated in colon cancer patients compared to healthy controls, playing essential roles in early detection, monitoring tumour progression, and evaluating response to treatment (11, 12). Among these, Septin-9—a P-loop GTPase first discovered by Hartwell in 1971—is involved in cytoskeletal organisation and cell cycle regulation. Encoded on chromosome 17p25, Septin-9 is frequently methylated in the blood of colorectal cancer patients and serves as a non-invasive biomarker for diagnosis and disease monitoring (13, 14).

Similarly, HIF-1α is a hypoxia-inducible factor frequently overexpressed in solid tumours like colon cancer. It plays a critical role in regulating genes related to angiogenesis, proliferation, and therapy resistance (15). Overexpression of HIF-1α is associated with poor prognosis and advanced tumour stages (16).

Another promising diagnostic avenue is the use of circulating cell-free DNA (cf-DNA), which consists of tumour-derived DNA fragments released into the bloodstream (17). These fragments can provide valuable insights into the tumour's genomic landscape. Patterns such as 5-hydroxymethylcytosines (5hmC) in cf-DNA have demonstrated high specificity for colon cancer diagnosis (18). Additionally, advanced tools like Raman spectroscopy have shown the potential to distinguish between cf-DNA from cancer patients and healthy individuals (19).

Despite these advances, sex-based differences in tumour marker expression and immune responses remain poorly understood. The present study was designed to evaluate the diagnostic potential of Septin-9, HIF-1α, and cf-DNA in colon cancer patients, with particular attention to their association with tumour stage and patient sex.

Materials and methods

Study Population and Sample Classification

A total of 125 individuals were included in this study, divided into three groups (Table 1):

Malignant tumour group (n = 41): Patients with histologically confirmed colon cancer, further classified into Stage II, Stage III, and Stage IV according to the TNM classification system (AJCC, 8th edition).

Positive control group (n = 45): Patients who underwent colonoscopy and were found to have benign colonic conditions without evidence of tumours or polyps.

Healthy control group (n = 39): Apparently healthy individuals with no clinical symptoms or history of gastrointestinal disease.

Table 1: Study Population and Sample Sizes

sample	Numbers
malignancy tumour	**41**
cases without tumour (control +)	**45**
healthy individuals	**39**

Sample Collection and Processing
Venous blood samples (5 mL) were collected from all participants before colonoscopy. Each sample was divided into two tubes:
3 ml in gel tubes for serum separation and tumour marker analysis.
2 ml in EDTA tubes for complete blood count (CBC) analysis using the MicroCC-20Plus haematology analyser (Suzhou Hightech Medical Instrument Co., Ltd., China).
CBC analyses were performed within one hour of collection to avoid temporal bias. After serum separation, samples from colon cancer patients were stored at –20°C until histopathological confirmation of tumour stage. Serum samples were then categorised into Stage II, III, and IV according to the pathology reports.

Tumour markers
This aspect of the study involved estimating three tumour markers in the serum using ELISA technology, Labtech Microplate Reader LT-4000, East Sussex, UK. The markers included Septin-9, Hypoxia-Inducible Factor 1 Alpha (HIF-1α), and cf-DNA, which were measured using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Ideal Medical Technology Co., Ltd., China). Measurements were performed on a Labtech Microplate Reader LT-4000 (East Sussex, UK).
To ensure reliability, all assays were performed in duplicate. The manufacturer's instructions were strictly followed, including calibration and internal quality controls. The detection limits for the assays were as follows: Septin-9 (0.1 ng/mL), HIF-1α (5 pg/mL), and cf-DNA (2 ng/mL). Catalog numbers were SPT9-ELISA-01, HIF1A-ELISA-02, and CFDNA-ELISA-03, respectively.

Statistical Analysis
All data were expressed as means ± SD. Differences between groups were first assessed using one-way ANOVA. Post-hoc comparisons were performed using Duncan's multiple range test to identify pairwise differences. A p-value ≤ 0.05 was considered statistically significant. Statistical analyses were conducted using SPSS software (version 26). No formal adjustment for multiple comparisons was applied (20).

Result
The highest incidence of malignant tumours was recorded among male patients with stage II, followed by female patients with stage III. The lowest incidence rate was among female patients with stages II and IV. Figure (1)

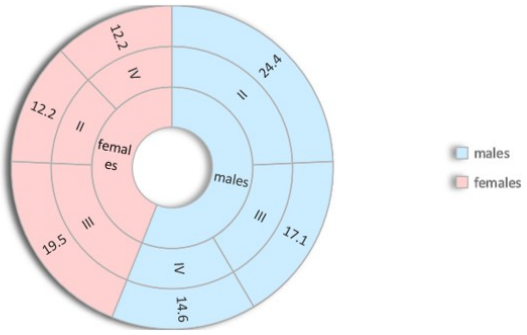

Figure 1: Rate of Infection of Males and Females according to the Stage of the Disease

The results showed significant differences between the cancer stage groups and the control groups of males in each of white blood cell (WBC), lymphocyte counts (LYM), red blood cell (RBC), and haemoglobin (Hb), the significant difference was limited to stage II only. There was no significant difference in platelet count except for the adenoma group. The lack of a substantial difference between the two control groups is worth noting, as shown in Table 2.

Table 2: Haematological Profiles of Males with Colon Cancer Patients by Tumour Stage

Groups Variables	Control he. Mean± SD	Control + Mean± SD	STAGE II Mean± SD	STAGE III Mean± SD	STAGE 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
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
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, and PLT ($\times 10^3/\mu l$). SD (Standard deviation); control + (positive control); control he (Healthy control), STAGE II (colon cancer stage II), STAGE III (colon cancer stage III), STAGE IV (colon cancer stage IV). Using Dunnett's test, the different letter indicates a significant difference at the probability level (P ≤ 0.05)

Furthermore, when comparing the groups with the control groups, the results showed p-values for WBC and LYM counts in stage II, monocytes (MID) in stage IV, and RBC counts, Hb levels, and PLT when comparing the stage with the control groups.

Significant differences were also observed between the two control groups, as shown in Table 3.

Table 3: Haematological Profiles of Female Colon Cancer Patients by Tumour Stage

Groups Variable	Control he. Mean± SD	Control + Mean± SD	STAGE II Mean± SD	STAGE III Mean± SD	STAGE 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
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
PLT	239 b ± 25	250 b ± 44	393 a ± 8	274 b ± 33.6	184 c ± 21.7

RBC (×106/µl) - Hb (g/dl)- (%) – WBC, Lymph, MID, and PLT (×103/µl). SD (Standard deviation); control + (positive control); control he (Healthy control), STAGE II (colon cancer stage II), STAGE III (colon cancer stage III), STAGE IV (colon cancer stage IV). Using Dunnett's test, the different letter indicates a significant difference at the probability level (P ≤ 0.05)

The results in Figure 2 show significant differences in the lymphocyte-to-monocyte (L/M) ratio across the different study groups. Males showed a higher L/M ratio (5.6) compared to females (5.0) in the control group. The control group had lower L/M values for both sexes. Across all three stages, L/M decreased in females as the disease progressed. The ratio was higher in females than in males in biopsy groups II and III, and vice versa in biopsy group IV.

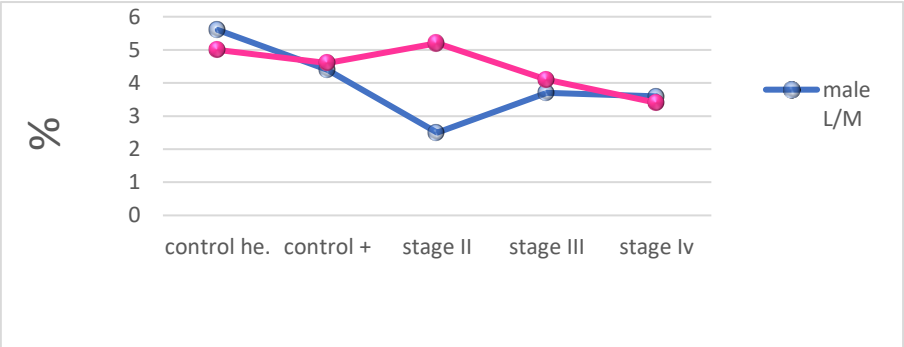

Figure 2: Percentage of lymphocytes to monocytes in males and females according to the stages of the disease

The results in Figure 3 show significant differences in the platelet-to-lymphocyte (P/L) ratio across the different study groups. Females showed a significantly higher P/L ratio than males (101 vs. 78.6) in the control group. At the same time, females had significantly lower P/L ratios across all three stages compared to males.

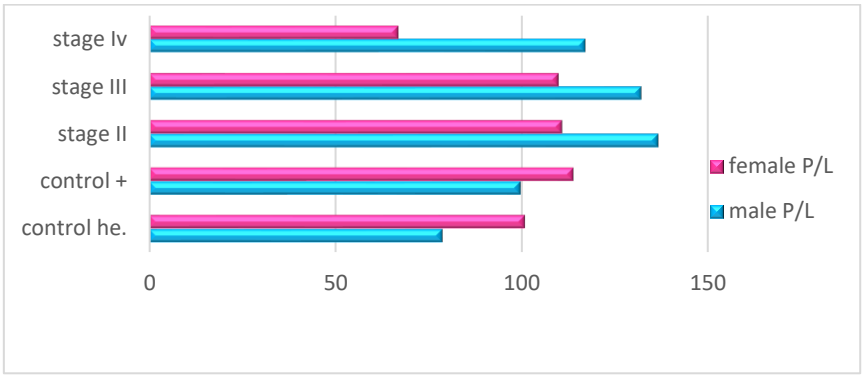

Figure 3: Percentage of platelets to lymphocytes in males and females according to the stages of the disease

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

Table 4: Biomarker Profiles of Male Patients with Colon Cancer

Groups Variables	Control he. Mean± SD	Control + Mean± SD	STAGE II Mean± SD	STAGE III Mean± SD	STAGE IV Mean± SD
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

Septin-9(ng/ml); HIF-1α (pg/ml); cfDNA (nmol/L); SD (Standard deviation); control + (positive control); control he (Healthy control), STAGE II (colon cancer stage II), STAGE III (colon cancer stage III), STAGE IV (colon cancer stage IV). Using Dunnett's test, the different letter indicates a significant difference at the probability level (P ≤ 0.05)

It is worth mentioning that tumour marker levels were highest in Stage IV patients, and effect sizes were found in HIF-1αand cf-DNA among the three groups. Also, a direct increase was observed with the progression of the disease stage, as detailed in Table 5.

Table 5: Biomarker Profiles of Female Patients with Colon Cancer

Groups Variables	Control he. Mean± SD	Control + Mean± SD	STAGE II Mean± SD	STAGE III Mean± SD	STAGE IV Mean± SD
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

Septin-9(ng/ml); HIF-1α (pg/ml); cfDNA (nmol/L); SD (Standard deviation); control + (positive control); control he (Healthy control), STAGE II (colon cancer stage II), STAGE III (colon cancer stage III), STAGE IV (colon cancer stage IV). Using Dunnett's test, the different letter indicates a significant difference at the probability level (P ≤ 0.05)

Figure 4 shows a comparison between male and female colon cancer patients with Septin-9. Septin-9 showed a relative increase for females at all stages of the disease. There was no significant difference when comparing males and females at all stages.

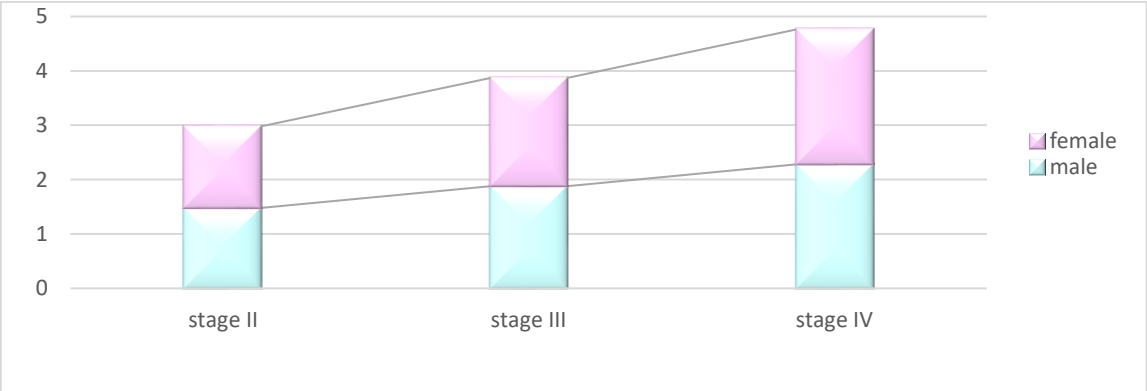

Figure 4: Sex-Based Differences in Septin-9 Expression Across Colon Cancer Stages II–IV

Figure 5 shows a comparison between male and female colon cancer patients with HIF-1α. When comparing Stage II results in both males and females, there was no significant difference, with a relative increase for females. When comparing the two Stage III groups, an important difference was found in HIF-1α, with a relative increase for males. A significant difference was also found between the two Stage IV groups, with a relative increase for females.

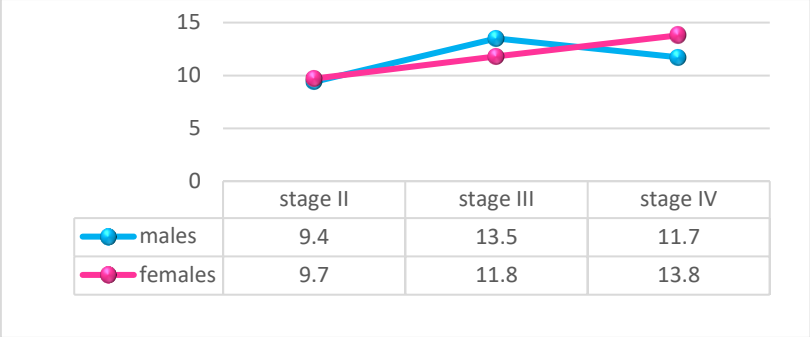

Figure 5: HIF-1α Levels in Male and Female Patients at Different Stages of Colon Cancer

Figure 6 illustrates the comparison between male and female colon cancer patients (cf-DNA). When comparing Stage II results in both males and females, a significant difference was found in both cf-DNA. When comparing the two Stage III groups, a relative increase was found for males in both cf-DNA and cf-DNA. A significant difference was also found between the two Stage IV groups in cf-DNA.

Figure 6: Comparison of cf-DNA Levels Between Male and Female Patients Across Three Disease Stages

Discussion:

A CBC is a routine haematological test that provides insight into a patient's general health and may reveal abnormalities suggestive of cancer development. Although it is not a direct diagnostic tool for colon cancer, variations in WBC counts among colon cancer patients reflect a complex interaction between the immune response, the tumour-associated inflammatory environment, and the physiological alterations caused by malignancy. Our results support this notion, as clear shifts in WBC levels were observed across tumour stages and between sexes, highlighting the influence of both disease progression and biological differences on haematological parameters.

A CBC frequently detects anaemia, which in the context of colon cancer may result from chronic blood loss due to tumour growth. For patients presenting with rectal bleeding or unexplained lethargy, additional diagnostic investigations are warranted (21). In our cohort, anaemia was more frequently observed in advanced stages, particularly among female patients, suggesting that haematological changes may provide indirect evidence of disease burden.

The lymphocyte-to-monocyte ratio (LMR) has been widely recognised as a prognostic indicator in cancer. According to (22), a high LMR is associated with improved survival, although this relationship may diminish following treatment. Since LMR reflects the balance between lymphocyte-driven antitumor immunity and monocyte-associated tumour-promoting inflammation, a reduction in lymphocyte levels may indicate impaired immune surveillance and poorer clinical outcomes. In contrast, tumour-infiltrating lymphocytes (TILs) play a pivotal role in antitumor defence by directly targeting malignant cells. In line with this, our study demonstrated a decline in LMR among colon cancer patients, reinforcing its relevance as a potential marker of immune dysregulation in disease progression.

In a large study of 1,674 colorectal cancer patients undergoing surgery, WBC counts were observed to rise before surgery, while lymphocyte levels declined. Patients with the highest WBC and lowest lymphocyte counts experienced worse cancer-related survival (CRS). Although the relationship between inflammation and cancer has been extensively studied, there is limited evidence on pre-diagnostic inflammatory alterations and their association with cancer risk. Notably, low haemoglobin, elevated platelet counts, and increased inflammatory markers were detectable up to nine months before diagnosis, suggesting

their potential role in early detection (23). Our findings are consistent with these observations, as female patients in Stages II and IV showed WBC elevations of 1.5–3.2 times higher than controls, underscoring both the prognostic and sex-related aspects of haematological changes in colon cancer.

Our findings showed that the lymphocyte-to-monocyte (L/M) and platelet-to-lymphocyte (P/L) ratios decreased consistently across Stages II, III, and IV in both male and female patients, as demonstrated in Figures 2 & 3 of our results. This observation is consistent with the study by (24), which identified both LMR and PLR as significant prognostic markers in colon cancer, reflecting an enhanced inflammatory state. A reduced lymphocyte ratio has been associated with poorer overall survival in colon cancer patients, supporting the potential clinical value of these indices as simple, cost-effective markers of prognosis.

Complete blood count–derived indices, including the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), are significantly elevated in colorectal cancer patients compared with healthy controls. These markers may therefore serve as potential indicators for cancer screening (25). Our data, as presented in Figures 2 and 3, also revealed elevated NLR and PLR values in malignant groups, reinforcing their utility as supportive diagnostic tools alongside established biomarkers.

A study of 272 Stage I–III colon cancer patients who underwent surgical resection reported that variations in haemoglobin levels were associated with tumour location (26). Right-sided colon cancer (RCC) was more frequently linked to reduced haemoglobin values compared with left-sided colon cancer (LCC), largely due to chronic or occult bleeding that may progress to anaemia. Our findings align with this observation, as decreased haemoglobin levels were more evident in advanced-stage patients, consistent with tumour-related blood loss (see Tables 2 & 3).

The detection of occult blood in stool samples is notably more common among colorectal cancer patients than controls, reflecting persistent gastrointestinal bleeding that contributes to reduced haemoglobin levels (27). Lymphocytes play a central role in antitumor immunity, whereas monocytes promote tumour-associated inflammation (28, 29). Consistent with these mechanisms, our data demonstrated lower lymphocyte counts and relatively elevated monocyte levels in tumour groups compared with healthy controls, reinforcing the link between systemic inflammation and disease progression.

Biological molecules serving as colon cancer biomarkers can be detected in blood, body fluids, or tissues, providing valuable information about the presence and progression of the disease. A study (30, 31) reported that certain compounds may increase by 50% or more in patients with malignant tumours across different stages of colon cancer when compared with controls (32).

Septin-9 is of particular interest, as our results showed elevated levels in both male and female patients, with more pronounced increases in advanced stages (Tables 5&6). These findings are consistent with (33), which described molecular mechanisms underlying Septin-9 overexpression in colon cancer, primarily through its involvement in cytoskeletal organisation and signalling pathways. Moreover, oncogenic variants of Septin-9 stimulate the development of invadopodia, thereby facilitating tumour invasion via degradation of the extracellular matrix (ECM) Additionally, the findings of (34) indicated that suppression of Septin-9 expression enhances cell migration and alters RhoA signalling, without affecting cell proliferation. These results suggest that SEPT9 hypermethylation, through its association with gene silencing, may contribute to increased tumour cell motility and resistance to chemotherapy, thereby playing a critical role in colorectal cancer progression.

Hypermethylation of the SEPT-9 gene is a prevalent alteration in colorectal cancer contributing to progression (13). Furthermore, SEPT9 hypermethylation represents a crucial molecular mechanism in colon cancer, linked to repression of tumour suppressor genes. This biomarker can be detected in both tissue and plasma samples, underscoring its value for early detection and disease monitoring. Hypermethylated SEPT9 found in plasma signifies tumour DNA release from dead cells, supporting the link between methylation and reduced Septin-9 expression during cancer progression (35, 36). Our findings also align with (37), who reported that Septin-9 levels increase with disease advancement, particularly in Stages III and IV.

Hypermethylation of the SEPT-9 gene is a common genetic alteration in colorectal cancer that contributes to tumour progression [13]. This epigenetic change is closely associated with the repression of tumour suppressor genes, underscoring its role as a key molecular mechanism in carcinogenesis. SEPT9 hypermethylation can be detected in both tissue and plasma, making it a promising non-invasive biomarker for early detection and disease monitoring. The presence of hypermethylated SEPT9 in plasma reflects the release of tumour-derived DNA, thereby linking methylation changes with reduced Septin-9 expression

during disease progression (35, 36). Our findings are in agreement with (37), who demonstrated that Septin-9 levels rise with disease advancement, particularly in Stages III and IV, further supporting its utility in monitoring tumour dynamics. Similarly, (38) reported that evaluating this protein in colorectal cancer patients before and three months after surgery yielded a sensitivity of 96.7% and a specificity of 95.5%, confirming its diagnostic value. Our results demonstrated higher Septin-9 expression in both males and females, with stage-wise increases more pronounced in advanced disease (Figure 4) Similar to other reports, HIF-1α levels were elevated in both male and female colon cancer patients compared with controls. HIF-1α functions as a central regulator of the hypoxic response in solid tumours and is associated with uncontrolled proliferation, enhanced migration and invasion, and resistance to apoptosis (39). As noted by (40), malignant colon cancers typically present with a more intense hypoxic environment, leading to increased HIF-1α expression as rapid tumour proliferation outpaces angiogenesis. (41) further demonstrated that activation of the hypoxia-inducible factor (HIF) pathway by roxadustat enhances glycolysis, a hallmark of cancer cell metabolism. Similarly, (42) reported that HIF-1α overexpression rises in colon cancer cells under hypoxic conditions. According to [43], the hypoxic tumour microenvironment results from rapid growth and poor vascularisation; under such conditions, HIF-1α accumulates, translocates to the nucleus, and dimerises with HIF-1β to regulate genes involved in angiogenesis and metabolic adaptation. As shown in Figure 5, HIF-1α levels rose progressively across stages, with males exhibiting higher values in Stage III and females showing higher levels in Stage IV, consistent with previous reports (39, 40, 41, 42, 43). A study by (16) found HIF-1α expression in 80% of colon cancer tissues compared to only 14% in normal colon tissues. Moreover, our findings regarding HIF-1α are consistent with Zhong et al., who reported its overexpression correlated with VEGF levels and advanced stages in colorectal cancer (44). In agreement with these studies, our results also showed stage-wise increases in HIF-1α levels, with more pronounced elevations in advanced stages (Tables 5 & 6) and Figure 5), underscoring its value as a marker of tumour progression.

Cell-free DNA (cf-DNA) has emerged as a promising biomarker in colorectal cancer. (45) proposed that cf-DNA consists of small DNA fragments freely circulating in the bloodstream, derived from both healthy and malignant cells, and may serve as an early detection marker. (46) further demonstrated that cf-DNA includes circulating tumour DNA (ct-DNA) released during apoptosis, with levels correlating with tumour size, disease stage, and recurrence risk. In a study of Stage II patients, (47) reported that ct-DNA levels rose by 62.2% among those with high-risk features, compared to 28.2% in those without, indicating a greater likelihood of recurrence. This is consistent with our current results shown in Tables 5 and 6 and Figure 6. According to [48], elevated cf-DNA concentrations correlated directly with tumour stage and tumour size, which may explain the higher cf-DNA levels observed in Stage III male patients in our study compared with other groups. In agreement with these findings, Tóth et al. [49] demonstrated that quantitative assessment of SEPT9 and SHOX2 methylation in plasma cf-DNA strongly correlated with tumour burden and stage. Together, these results and our data emphasise the value of cf-DNA as a minimally invasive biomarker with strong potential for staging, prognosis, and patient follow-up in colorectal cancer. Figure 6 illustrates that cf-DNA levels increased significantly with advancing stages, with notably higher concentrations in Stage III males and Stage IV females, supporting its value as a biomarker of tumour progression (45, 46, 47, 48, 49).

Study Limitations

This study has some limitations. The sample size was relatively small and drawn from a limited number of hospitals in Nineveh, which may reduce the generalizability of the results. In addition, the study covered only one year and did not include long-term follow-up to evaluate recurrence or survival. Finally, the analysis focused only on three biomarkers (Septin-9, HIF-1α, and cf-DNA), while other markers that might provide additional insights were not assessed. Moreover, no formal adjustment for multiple comparisons was applied in the statistical analysis, which may increase the likelihood of type I error and should be considered when interpreting the findings.

Conclusion

This study highlights important sex- and stage-related variations in both haematological parameters and tumour biomarkers among colon cancer patients in Nineveh Governorate. Differences in immune cell ratios, such as lymphocyte-to-monocyte and platelet-to-lymphocyte, together with altered levels of Septin-9, HIF-1α, and cf-DNA, underline the complex interaction between the immune response and tumour biology. These findings suggest that simple blood indices, when combined with molecular markers, may

contribute to better diagnostic evaluation and disease monitoring.
A key contribution of this work is its focus on an Iraqi population, where such data are scarce. To our knowledge, this is the first study from Iraq to examine sex-specific differences in these markers across different stages of colon cancer. By integrating routine haematological tests with molecular assays, the study offers a novel perspective on how locally available resources can be used to improve early detection and patient management in settings with limited diagnostic facilities.

Declarations

Ethics approval and consent to participate

The Nineveh Governorate Health Department granted official approvals in compliance with administrative protocols for the collection of blood samples, biopsy, and patient data. Participants' ages ranged from 17 to 84 years, and both sexes were represented. Recruitment took place between March 14, 2023, and March 12, 2024, from the colonoscopy units of Ibn Sina Teaching Hospital, Mosul General Hospital, and the private clinic of Dr. Abdullah Zuhair Al-Yuzbaki in Mosul, Iraq. Written informed consent was obtained from all participants. The study protocol was approved by the Institutional Ethics Committee of the Nineveh Health Directorate (Approval No. 12989/14-3-2023).

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Availability of Data

Data for this work is available from the authors and may be provided upon reasonable request.

Conflict of Interest Disclosure

Every author affirms that they have no conflicts of interest.

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Authors' contributions

MSO: Conceptualisation, Methodology, Data curation, Formal analysis, Writing – original draft.
Al-HHL: Investigation, Resources, Validation, Writing – review & editing.
HMK: Supervision, Project administration, Funding acquisition, Writing – review & editing.

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