MMSL
Military Medical Science Letters
Since 1925

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2024, 93(3), 274-287
ISSN 2571-113X (Online)
DOI: 10.31482/mmsl.2023.034

ORIGINAL ARTICLE

THE ROLE OF SERUM MIDKINE IN THE DIAGNOSIS AND PROGNOSIS OF THE COLORECTAL CARCINOMA

Ali H. Abd-Allah [1] ✉, Haider A. Jabbar [1,2], Mazen J. Ibrahim [2]

[1] Department of Clinical Biochemistry, College of Medicine, University; of Al- Qadisiyah, Iraq
[2] Department of Oncology, College of Medicine, University of Baghdad, Iraq

Received 31st May 2023.
Accepted 31st July 2023.
Published 2nd September 2024.

Summary

Background: The second-leading source of neoplasm-related death and a primary factor in gastrointestinal cancer, colorectal cancer (CCR) affects both genders globally. Poor eating behaviours, tobacco, an intestinal inflammatory disorder, swellings, inherited characteristics, and the elderly all increase the threat of acquiring this malignancy. The illness is more hostile in patients detected at earlier ages, although 90% of patients with colorectal tumours are older than 50, with a median oldness of 64 years. American Cancer Association estimates that it caused more than 49,700 fatalities in 2015.
Objectives: Study the correlation of midkine with carcinoembryonic antigen (CEA), liver function tests, and white blood cell count in patients with colorectal carcinoma.
Methods: The serum midkine and CEA of all subjects were measured by the ELISA technique, Liver enzymes were measured by colourimetric methods and neutrophils, and lymphocytes were measured by an Electrical Impedance Cell Counting method (automated machine).
Conclusion: The study results of the correlation between serum midkine and other parameters in colorectal carcinoma patients show a significant positive correlation of midkine with CEA, liver enzyme, neutrophils, and lymphocytes.

Key words: Colorectal carcinoma; Liver enzymes; Lymphocytes; Neutrophils; Midkine

Introduction

One of the most prevalent carcinomas globally is colorectal carcinoma (CRC) (1). Adenomas are the main source of CRCs. The development of an adenoma into cancer is thought to occur over at least ten years. Because of this, screening is crucial to their avoidance. CRC is rarely diagnosed in people under the age of 40. In 90% of the cases, the illness strikes people over 50 years. Colonoscopy is frequently advised as a monitoring method beginning at age 50 years (2, 3).

Currently accounting for 13% of all malignant cancers, it is the most prevalent gastrointestinal tract tumour. It is also the second most prevalent source of neoplasm-related mortality worldwide, affecting both males and females equally in developed and developing countries, and it is predicted to surpass heart disease death rates in the years to come (4, 5).

✉ University of Al- Qadisiyah, Iraq, College of Medicine, Department of Clinical Biochemistry
az91664@gmail.com
☎ +964 750 030 9601

The fact that the 5-year overall existence from this malignancy stays under 50%, despite recent advances in novel therapies, emphasises the need to advance early recognition, predictive, and prophetic biomarkers that can be utilised in repetitive clinical practice to reduce the ailment and death associated with this sickness (6).

CRC accounts for 10% of universal tumour occurrence and 9.4% of cancer mortality, just below lung malignancy, which will account for 18% of cancer-related mortalities in 2020. The number of novel CRC cases worldwide is anticipated to touch 3.2 million in 2040 based on forecasts of ageing, increasing inhabitants, and human advancement. The increased exposure to environmental risk factors brought on by the westernisation of lifestyle and diet is the main cause of the rise in the prevalence of CRC (7, 8).

Colorectal cancer is another most prevalent female tumour and the third most prevalent cancer in males. In 2020, an additional 1.9 million cases were reported (9). The colorectal tumour is the second most pervasive cancer-related cause of mortality, accounting for an estimated 935,00 cancer fatalities each year (10). It is one of the malignancies whose prevalence is rising and accounts for 11% of all cancer cases worldwide (11).

CRC is the third main tumour-related cause of mortality globally, with a projected 419,536 deaths for women and 515,637 deaths for men in 2020. CRC impacts more than 5.25 million people worldwide (5-year survival), just somewhat fewer than breast tumours, which account for 7.79 million tumour cases. 0.94 million people died from CRC in 2020 (12). In the United States, mutually the incidence and fatality ratio has been gradually falling (13). About 151,030 novel cases of large bowel malignancy are reported each year, 106,180 of which are colon malignancy cases, and the rest are rectal malignancy cases (14).

It ranks third among male malignancies and second among female malignancies in frequency. By 2035, there will be 2.5 million new cases of colorectal cancer worldwide, with women experiencing a 25% lower incidence and mortality rate than men (15). According to the Iraqi National Cancer Registry (INCR), the general (men and women) CRC index percentage (CIP) grew from 2.28 to 6.18 per 100 000 people in 2000 and 2019, correspondingly, with a yearly percentage change (APC) of 5.11% (16).

Midkine (MK) is a growth agent and a potential tumour parameter for various tumour types. MK exhibits strong physiological expression during embryogenesis. Since healthy people continue to produce MK at a low level, a background amount in peripheral blood should be considered (17). The serum content is a rough indicator of the level of midkine expression in a tumour and is simple to measure because MK is highly soluble in blood. Since large-scale demographic studies haven't been conducted for S-MK, a consistent reference range has not been established (18). S-MK regional difference is also conceivable (19). Numerous cancers, including colorectal carcinoma, oesophagal squamous cell carcinoma, pancreas cancer, and stomach cancer, have high MK expression (20). MK is an angiogenic, pro-mitotic, an anti-apoptotic factor, exhibiting chemotactic action towards neutrophils and monocytes. It suppresses T-regulatory cells, functions as an antimicrobial, and induces the expression of metalloproteinases, extracellular matrix elements, and proinflammatory cytokines. Both at the protein and mRNA levels, MK is overexpressed in neoplastic colorectal tissue associated with nearby normal colon-rectal tissue (21). In adults, expression of the midkine protein is just presented in the renal in extremely small amounts. In contraindication, several solid tumours (e.g. oral, oesophagal, gastric tumour, pancreatic tumour, colorectal tumour, prostate tumour, lung tumour, cervical tumour, brain tumour, neuroblastoma, and Wilm's tumour) express elevated midkine levels, regardless of tissue kind. In CRC, MK expression is discovered even at precancerous stages, i.e., in adenomas and adenocarcinomas (22). MK participate in neoangiogenesis and tumour cell differentiation, however, suppressing apoptosis. Moreover, MK suppresses the interactivity with T cells and participates in the expression of proinflammatory cytokines like IL-8 and TGF-beta. MK modifies the extracellular matrix, which induces tumour cell migration (23). MK plays crucial roles in tumour initiation and progression by regulating cell initiation, cell movement, cell persistence, angiogenesis, and the conversion of fibroblasts (24). Growth factors induce tissue reproduction and promote malignant transformation. Overexpression of growth factors has been found in numerous human tumours, and this phenomenon is often believed to be a cause of carcinogenesis (25).

Carcinoembryonic antigen (CEA) has been identified as a tumour parameter and a glycoprotein that may be found in the blood and tumour cells of adenocarcinomas since 1965. CEA is an intracellular adhesion protein generated in fetal gut tissue and epithelial tumour cells that aid in angiogenesis. It has a half-life of one to three days; Colorectal

tumours, breast tumours, gastric tumours, lung tumours, ovarian tumours, and pancreatic tumours have increased CEA heights in the blood. However, many non-malignant diseases, such as tobacco use, drinking, chronic inflammatory bowel disease, pancreatitis, and hepatic disease, can elevate CEA (26). CEA is a group of toughly bound glycoproteins expressed in intestinal tissue from the human germinal stage to the fetus. CEA is mostly generated by adult colon mucosal cells, with a tiny quantity paid by different cells. There is a tiny quantity of extent in the blood. It has a two-day half-life in the blood. CEA is a diagnostic marker in clinical practice as an embryonic tumour antigen since it is highly expressed in colorectal tumours (27, 28).

The NLR, which measures the proportional difference among baseline neutrophil and lymphocyte numbers, has been identified as a possible marker of bad prognosis in various malignancies, including colon and rectal tumours. These biomarkers of systematic inflammatory reaction have been vastly investigated as helpful indicators for the prediction of patients with cancer (29). Many malignancies are brought by prolonged contagion; this participates to about 15% of all malignancies globally. Continuous reactions to long conditions and environmental toxins lead to a chronic inflammatory reaction. Thus, inflammatory responses play a crucial role in carcinogenesis. Numbers of inflammatory cells, like neutrophils, lymphocytes, platelets, and monocytes, in addition to innate immune system coding molecules, are implicated in tumour progression (30). Peripheral blood elements might celebrate patients' inflammatory and immune responses to virulent cancers and are climacteric for determining cancer patients' therapy response and clinical results. Inflammation-related markers that estimate the systemic inflammatory reaction have generated predictive value autonomous of the TNM staging system. Between these markers is the peripheral blood NLR (31). The function of inflammation in the tumour is now fully understood and depicted at various stages of neoplasm growth (initiation, stimulation, attack, and metastasis). Functional provocative cells are foundations of reactive oxygen species and reactive nitrogen species that can enhance DNA destruction and genome variability, thus stimulating tumour initiation (32). In recent years, inflammatory blood parameters have appeared as diagnostic and predictive indicators, mainly the neutrophil-to-lymphocyte ratio (NLR) and the ratio among the absolute neutrophil and lymphocyte amounts (33).

Materials and Methods

Subjects: The study was conducted on individuals who were clinically and laboratory diagnosed with colorectal cancer and who attended the Oncology Teaching Hospital/ Medical City and Gastroenterology and Hepatology Teaching Hospital /Medical City in Baghdad – Iraq, Samples and all information were taken from the patients, as well as healthy people were selected for the study. Laboratory tests were carried out in the laboratories of the Clinical Biochemistry Branch / College of the Medicine / University of Al-Qadisiyah. Also, some laboratory tests were conducted in the Clinical Chemistry Unit / Laboratory Division of the Oncology Teaching Hospital. One hundred and twenty-nine individuals participated in the study between September 2022 and May 2023 (for sample collection), divided into two groups: patients subject: sixty people are patients with colorectal carcinoma selected from the Oncology Teaching Hospital and Gastroenterology and Hepatology Teaching Hospital after confirming their clinical and laboratory diagnoses. Control subject: Sixty-nine healthy people who do not have any disease. They were established after asking people and conducting all required laboratory analyses.

Blood Sample Collection: Each participant was taken six millilitres of blood drawn from a vein and placed in two test tubes: 2ml with an EDTA tube for neutrophils and lymphocytes and NLR and 4ml with a gel tube for biochemical analysis. Whole blood is processed and subjected to the necessary studies directly. At the same time, blood samples in gel tubes were centrifuged for ten minutes at a force of 3000 x g to obtain a sample (serum), which was then kept in three separate Eppendorf tubes at -44 °C in the deep freeze until the analysis time.

Inclusion Criteria: Patients with colorectal cancer included those newly diagnosed and those with metastatic cancer.

Exclusion Criteria: Patients with inflammatory bowel illness, pancreatic Cancer, breast cancer, or ovarian cancer were excluded from the study.

Statistical analysis: Data were together, compiled, analysed, and then presented utilising Microsoft Office Excel 2016 and the statistical package for social sciences (SPSS) version 25.

Measurable (definite) variations were expressed by utilising statistics and ratios. In contrast, numerical variations were tested for normality employing the Kolmogorov-Smirnov check before being presented as mean (a guide of central tendency) and standard deviation (a principle of dispersion) for normally spread numeric variation and median (a direction of central propensity) in addition to inter-quartile range (a guide of distribution) for abnormally distributed numeric variations.

The following was subjected to statistical Analysis:

1. When fewer than 20% of the cells had an anticipated count of fewer than 5, Chi-square was utilised to determine the relationship among any two categorical variables. The Yates adjustment test was used instead of the chi-square test where it was inapplicable (i.e. when more than 20 per cent of the cells had a predictable count of fewer than 5).

2. Examples from different sources The Mann-Whitney U test was employed in place of the t-test when the numeric variables were not normally distributed to estimate the difference in mean among any two groups.

3. To recognise the cutoff value that foretells a positive result, the receiver operator characteristic (ROC) curve analysis was utilised, along with its associated area under the curve (AUC), precision level, sensitivity degree, specificity degree, and degree of significance (P).

P-values of 0.05 or lower were used to denote significance. P-values of 0.01 or less were regarded as having strong relevance.

Results

Demographic parameters and CRC: As indicated in Table 1, There was no significant alteration in mean age between the patient and control groups, 52.97 ± 11.92 years versus 51.81 ± 12.61 years, respectively ($p = 0.561$). In addition, there was no significant alteration in the proportions of men and women between both subjects ($p = 0.660$).

Table 1. Demographic correlation of individuals with colorectal Cancer and control subject.

Characteristic	Colorectal Cancer *n* = 60	Control group *n* = 69	*p*
Age (years)			
Mean ± SD	52.97 ± 11.92	51.81 ± 12.61	0.561 I
Range	20 - 77	21 - 65	NS
Gender			
Male, *n* (%)	36 (60.0 %)	44 (63.8 %)	0.660 C
Female, *n* (%)	24 (40.0 %)	25 (36.2 %)	NS

n: number of cases; **SD**: standard deviation; **I**: independent samples t-test; **C**: chi-square test; **NS**: not significant; ***: significant at $p \leq 0.001$

Biochemical parameters and CRC: As indicated in Table 2, the Mean serum AST was significantly higher in the patient's subject compared to a healthy subject, 36.40 ± 18.58 IU/L versus 22.02 ± 12.21 IU/L, respectively ($p < 0.001$). In addition, mean serum ALT was significantly higher in the patient's subject compared to a healthy subject, 34.27 ± 9.89 IU/L versus 21.73 ± 7.40 IU/L, respectively ($p < 0.001$). Furthermore, mean serum ALP was significantly higher in the patient's subject compared to a control subject, 114.32 ± 40.10 IU/L versus 95.08 ± 27.26IU/L, respectively ($p = 0.002$).

Mean serum TSP was significantly lower in the patient's subject compared to a healthy subject, 60.23 ± 18.33 g/dl versus 67.12 ± 8.31 g/dl, respectively ($p = 0.006$). However, mean serum TSB was significantly higher in the patient's subject than in the healthy subject, 0.65 ± 0.60 mg/dl versus 0.43 ± 0.38 mg/dl, respectively ($p = 0.017$).

Table 2. Comparison of liver parameters between individuals with colorectal tumour and control subject.

Characteristic	Colorectal Cancer *n* = 60	Control group *n* = 69	*p*
AST (IU/L)			
Mean ± SD	34.27 ± 9.89	21.73 ± 7.40	<0.001 I ***
Range	18.6 - 58.3	10.3 - 43	
ALT (IU/L)			
Mean ± SD	36.40 ± 18.58	22.02 ± 12.21	<0.001 I ***
Range	16.7 - 83.7	4.7 - 61.4	
ALP (IU/L)			
Mean ± SD	114.32 ± 40.10	95.08 ± 27.26	0.002 I **
Range	41.6 - 210.3	42.2 - 169.3	
TSP (g/dl)			
Mean ± SD	60.23 ± 18.33	67.12 ± 8.31	0.006 I **
Range	23.9 - 93	41.7 - 87.8	
TSB (mg/dl)			
Mean ± SD	0.65 ± 0.60	0.43 ± 0.38	0.017 I *
Range	0.3 - 2.5	0.1 - 2.6	

n: number of cases; **SD**: standard deviation; **AST**: aspartate transaminase; **ALT**: alanine transaminase; **ALP**: alkaline phosphatase; **TSP**: total serum protein; **TSB**: total serum bilirubin; **I**: independent samples t-test;
*: significant at p ≤ 0.05; **: significant at p ≤ 0.01; ***: significant at p ≤ 0.001

Haematological parameters and CRC: As indicated in Table 3, the Neutrophil count was significantly higher in the patient's subject compared to a healthy subject, 7.23 ± 3.98 $X10^9$/L versus 4.41 ± 1.71 $X10^9$/L, respectively (p <0.001). In addition, lymphocyte count was significantly lower in the patient's subject compared to a healthy subject, 1.76 ± 1.37 $X10^9$/L versus 2.74 ± 1.00 $X10^9$/L, respectively (p <0.001). Moreover, the neutrophil to lymphocyte ratio (NLR) was significantly higher in the patient's subject in comparison with a control subject, 13.91 ± 6.59 versus 1.85 ± 1.02, respectively (p <0.001).

Table 3. Comparison of haematological parameters between individuals with colorectal Cancer and control subject.

Characteristic	Colorectal Cancer *n* = 60	Control group *n* = 69	*p*
Neutrophils $X10^9$/L			
Mean ± SD	7.23 ± 3.98	4.41 ± 1.71	<0.001 I ***
Range	2 - 15.5	2 - 7.2	
Lymphocyte $X10^9$/L			
Mean ± SD	1.76 ± 1.37	2.74 ± 1.00	<0.001 I ***
Range	0.1 - 4.5	1.5 - 4.5	
NLR			
Mean ± SD	13.91 ± 6.59	1.85 ± 1.02	<0.001 I ***
Range	0.49 - 59	0.44 - 4.5	

NLR: neutrophil to lymphocyte ratio; **SD**: standard deviation; ***n***: number of cases; **I**: independent samples t-test;
***: significant at p ≤ 0.001

Tumour marker CEA and CRC: As indicated in Table 4, Mean serum CEA was significantly higher in the patient's subject in comparison with the control subject, 133.28 ± 94.33 µg/dl versus 1.77 ± 1.07 µg/dl, respectively (p <0.001).

Table 4. Comparison of CEA between individuals with colorectal tumours and healthy group.

Characteristic	Colorectal Cancer *n* = 60	Control group *n* = 69	*p*
CEA (µg/L)			
Mean ± SD	133.28 ± 94.33	1.77 ± 1.07	<0.001 I ***
Range	14.7 - 300.2	0.18 - 4.2	

CEA: carcinoembryonic antigen; **SD**: standard deviation; ***n***: number of cases; **I**: independent t-test;
***: significant at $p \leq 0.001$

Serum midkine and CRC: As indicated in Table 5, the Mean serum midkine was significantly higher in the patient's subject in comparison with a control subject, 393.75 ± 41.18 pg/dl versus 244.65 ± 62.07 pg/dl, respectively (p <0.001).

Table 5. Correlation of serum midkine between patients with colorectal tumour and the control subject.

Characteristic	Colorectal Cancer *n* = 60	Control group *n* = 69	*p*
Midkine (pg/ml)			
Mean ± SD	393.75 ± 41.18	244.65 ± 62.07	<0.001 I ***
Range	301 - 454	150 - 352	

SD: standard deviation; ***n***: number of cases; **I**: Independent sample t-test;
***: significant at p ≤ 0.001

A receiver operational characteristic (ROC) curve investigation was carried out to find the best cutoff value of serum midkine that can foresee a positive diagnosis of colorectal carcinoma in terms of sensitivity and specificity. The outcomes are presented in Figure 1 and Table 6. The cutoff value was > 342 pg/ml with a sensitivity of 91.7%, specificity of 95.7 % and accuracy of 98.0 %. The area under the curve was 0.980 (>0.7). Thus, serum midkine is an excellent diagnostic aid for colorectal cancer.

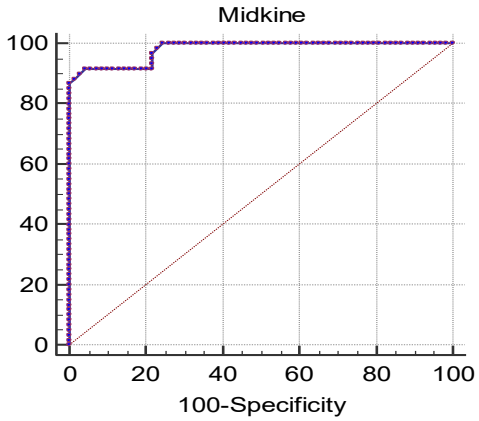

Figure 1. Receiver operational characteristic (ROC) curve investigation to find the best cutoff value of serum midkine that can foresee a positive diagnosis of colorectal carcinoma in terms of sensitivity and specificity.

Table 6. Characteristics of ROC analysis concerning serum midkine.

Characteristic	Result
Cutoff value	> 342 pg/ml
AUC	0.980
95 % CI	0.939 to 0.997
P	<0.001***
Sensitivity %	91.7
Specificity %	95.7
Accuracy %	98.0

Stage and grade prevalence of CRC: The frequency distribution of people with colorectal cancer, as claimed by a grade of disease, is shown in Figure 2A. Patients were categorised into eight conditions of well-differentiated grade I Cancer (13.3%), 43 conditions of moderately differentiated grade II tumours (71.7%) and nine conditions of poorly differentiated grade III cancer (15.0 %).

The frequency distribution of colorectal cancer individuals according to the disease stage is shown in Figure 2B. Patients were categorised into 13 conditions of stage II disease (21.7%), 17 states of stage III disease (28.3%) and 30 conditions of stage IV disease (50.0%).

The frequency distribution of individuals with colorectal cancer as claimed to metastasise is shown in Figure 2C. Fifty % of cases (30 in number) had metastasis. The site of metastasis is shown in Table 7.

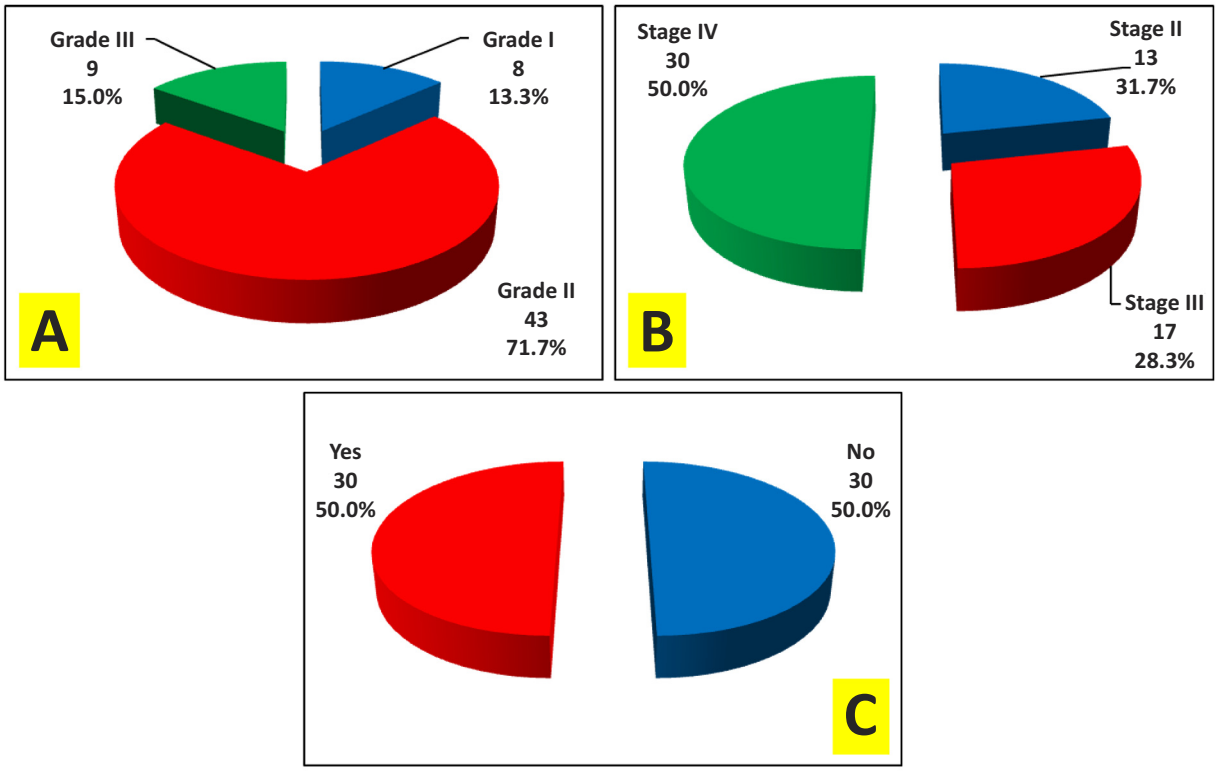

Figure 2. The distribution frequency of colorectal cancer (A) Frequency distribution of individuals with colorectal cancer according to a grade of disease. (B) frequency distribution of individuals with colorectal cancer according to the stage of the disease. (C) Frequency distribution of individuals with colorectal cancer according to metastasis.

The most common site was the liver accounting for 46.7% of cases, followed by the lung (16.7%), then by bone (13.3%), then by peritoneum, uterus and ovary (6.7% for each) and finally by spleen (3.3%).

Table 7. Frequency of sites of metastasis.

Site	Number of cases	%
Liver	14	46.7
Lung	5	16.7
Bone	4	13.3
Peritoneum	2	6.7
Uterine	2	6.7
Ovary	2	6.7
Spleen	1	3.3

Correlation study: As indicated in Table 8, the grade of disease was negatively linked to lymphocyte numbers and positively related to NLR and AST. The stage of disease was negatively associated with lymphocyte number and completely related to neutrophil numbers, NLR, AST, ALP and CEA. Metastasis was negatively related to lymphocyte numbers and positively correlated to neutrophil count, NLR, AST, ALT, ALP and CEA.

Table 8. Correlations of Grade, stage and metastasis to other Characteristics of patients.

Characteristic	Grade		Stage		Metastasis	
	r	*p*	*r*	*p*	*r*	*p*
Gender	0.234	0.071	0.199	0.127	0.000	1.000
Age	0.201	0.124	0.241	0.063	0.192	0.142
Neutrophils	0.235	0.071	0.739	<0.001***	0.446	<0.001***
Lymphocyte	-0.463	<0.001***	-0.732	<0.001***	-0.445	<0.001***
NLR	0.388	0.002 **	0.765	<0.001***	0.467	<0.001***
AST	0.320	0.013*	0.329	0.010**	0.410	0.001 ***
ALT	0.207	0.112	0.222	0.088	0.359	0.005**
ALP	0.150	0.251	0.301	0.019*	0.432	0.001***
TSP	-0.050	0.703	-0.125	0.342	-0.204	0.118
TSB	0.211	0.106	0.026	0.845	0.220	0.091
CEA	0.320	0.013	0.755	<0.001***	0.433	0.001***
Midkine	-0.043	0.742	0.187	0.152	0.243	0.062

NLR: neutrophil to lymphocyte ratio; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase; **TSP**: total serum protein; **TSB**: total serum bilirubin; **CEA**: carcinoembryonic antigen; *: significant at p ≤ 0.05; **: significant at p ≤ 0.01; ***: significant at p ≤ 0.001

Discussion

In this study, there was no significance in the age and gender between individuals with colorectal tumours and the healthy subject, respectively ($p = 0.561$), ($p = 0.660$). The mean age of individuals with colorectal tumours in the present study is comparable to that obtained by previous studies (34, 35). The colorectal tumour is the third ultimate prevalent neoplasm globally and the second most prevalent cause of tumour-related death (36). Over the past few decades, the United States and other high-income nations have seen an alarming rise in states of early-onset colon-rectal tumours, defined as a diagnosis in individuals younger than 50 (37). Early-onset colon-rectal tumours now account for about 10% of all new diagnoses of this neoplasm, and an accompanying rise in colon-rectal tumour–related death during the past decade has also been detected among younger individuals (38, 39).

This study showed significantly higher liver function test levels in colorectal tumour individuals with hepatic metastases compared to the control subject. Previous studies show elevated liver function tests in colorectal tumour individuals with hepatic metastases (40). The present study shows significant elevation in serum ALT, AST, and ALP in colorectal tumour individuals with hepatic metastases compared to the control subject, which was established by previous studies (41, 42). In addition, previous studies show increased ALP levels in colorectal cancer individuals with bone metastases (43). In addition, this study shows low serum TSP levels in colorectal cancer individuals compared to the control group, and this result was established by previous studies that indicated low levels of TSP in individuals with colorectal carcinoma compared to control subjects (44).

About 15% of the CRC states will have advanced metastases at identification, and 50% of the individuals with locally progressive illness will induce metachronous metastases, driving them to death in fewer than two years of follow-up, despite good operating and adjuvant therapy (44). Different pathologic, clinical, and biological factors determine the outcome of CRC. Between them, the cancerous stage (TNM organisation) is the most specific factor in determining the prognosis of CRC individuals. Initial stages (Stage 1) are related to a good prognosis, with a 5-year persistence rate near 90% (45). Many factors determine that patients with identical cancerous stages present different results. Between them, the presurgical feeding condition has been concurrent with the long-term oncologic prognosis of recurrence of death (46).

Metastasis refers to invading tumour cells from the origin site to additional body parts.in the present study, The most common location was the liver, accounting for 46.7% of cases, followed by the lung (16.7%), then by bone (13.3%), then by peritoneum, uterus and ovary (6.7% for each) and finally by spleen (3.3%). The proportions of disease metastasis in the present study are comparable to that seen in other studies (47).

In this study, there was elevated neutrophils count, and low lymphocytes count in individuals with colorectal carcinoma when compared with a control subject, additionally there was significantly higher NLR in colorectal tumour individuals when compared to the healthy subject, and previous studies confirmed these results (48-50).

Many studies established that inflammatory reaction plays a critical function in the growth of the tumour microenvironment, a few variations of provocative cells might be an index for progress, and changes of cellular immune elements in peripheral venous blood could reveal tumour irritation condition for indicating persistent prediction (51, 52). Systemic inflammatory reaction plays a critical function as a leading reason for neoplastic practicability, and it was vigorously involved in the formation and proliferation of different tumours (53). We know peripheral blood components, including leukocytes, neutrophils, lymphocytes, and thrombocytes, can indicate systemic inflammation. NLR and the PLR have been established as the prognosis index for various malignancies like biliary tract and gastric cancer (54). Systemic inflammation has been associated with the bad progress of colorectal tumours (55).

In this study, serum CEA was considerably increased in colorectal tumour individuals compared to the control subject. CEA is a readily presented cancer parameter that aids in managing colon-rectal tumours. Higher preoperative CEA levels independently predict whole and disease-free persistence rates (56-58). CEA has been utilised postsurgical to index tumour surveillance. Additionally, individuals with early-stage colon cancer that is node-negative but who also have increased presurgical CEA levels would have comparable progress to that of patients with node-positive illness, probably as a result of cancer upstaging, and might thus be nominees for adjuvant treatment (59). CEA is a glycoprotein engaged in adherence normally generated by the gut throughout foetal growth (60). It is a cell surface glycoprotein which aids as functional colon tumour ligands which are climacteric to the metastatic distribution of colonic tumours (61, 62). Presently, serum CEA levels have been commended by the "National Institute of Clinical Excellence European Group on Tumour Markers" and the "American Society of Clinical Oncology" for surveillance following therapeutic amputation of colorectal tumours (63).

In this study, serum midkine was highly elevated in colorectal cancer individuals compared to the control subject. This study has shown the cutoff value was > 342 pg/ml with a sensitivity of 91.7%, specificity of 95.7% and accuracy of 98.0%. The area under the curve was 0.980 (>0.7). Thus, serum midkine is an excellent diagnostic aid for colorectal cancer. Several other studies have revealed serum midkine was aggressively increased in colorectal carcinoma patients (64-66).

Midkine, a cytokine participant of the heparin-bound development elements family, was a highly expressed element throughout the initial discrimination stage in embryogenesis and was weak or unnoticeable in healthy mature tissues. MK has numerous biotic functions, such as propagation, immigration, anti-apoptosis, angiogenesis, attack, and metastasis (67). MK is one of the main irregular secreted proteins elevated in the initial stages of various tumours (68). Serum MK level was discovered to be high in precancerous lesions and the initial stage of carcinoma growth. The role of midkine in CRC established that serum MK level in CRC individuals abnormally increases where increased expression of MK can stimulate CRC cell propagation, suppress CRC cell apoptosis and induce angiogenesis and cell attack (69). MK overexpression has been described in different tumour kinds for malignancies in all main organs and tissue, extending from the main prevalent tumours to some of the rarest ones. Overexpression of the midkine gene and the midkine protein within the tumour is a typical property of neoplasm (70). High expression of MK in tumour tissues was spotted in several tumours and is related to a bad prognosis. High MK blood levels are also identified in various tumours and are a predictive factor in multiple tumours (71).

Moreover, MK had numerous mechanisms in the CRC growth manner. These comprise stimulation of tumour cell production, cell existence, anti-apoptosis, tumorigenesis, and epithelial-mesenchymal transition. Serum MK concentration is predictable to increase concerning the presence of MK-expressing tumours (65).

Numerous studies presented that these actions of MK resulted from stimulation of the mitogen-activated protein kinase and phosphatidyl inositol 3-kinase /Akt pathways by the MK receptor anaplastic lymphoma kinase, stimulation of the extracellular signal-regulated kinase 1/2, and PI3K pathways through protein tyrosine phosphatase ζ (another MK receptor), stimulation of the Janus tyrosine kinase /signal transducer, and stimulator of transcription (STAT) pathway, in addition to stimulation of Notch signalling (72).

In the present study, the grade of disease was positively correlated to lymphocyte count, NLR and AST. In contrast, the stage of illness was positively related to CEA, NLR, AST, ALP, neutrophils and lymphocytes. The positive correlation of the location of disease with liver enzymes can be attributed to liver metastasis causing damage to liver cells and liberation of their enzymes into circulation.

The positive correlation of grade and disease stage with increasing NLR follows the findings of several previous authors (31, 73). High concentrations of blood neutrophils are seen in individuals with advanced tumours and are related to worse survival (74, 75). Also, there is a great indication of a negative predictive value of the neutrophil to lymphocyte ratio in colorectal tumours. Many studies have revealed that higher NLR was related to worse survival (76-79), and the latest meta-analysis found that higher NLR was related to both poorer disease-free survival and general survival (76). In addition to aforementioned reasonable explanation about MK association with cancer, cancer cells undergo hypoxia due to overgrowth and hypoxia by itself could be responsible for modulation of cell-released trophic factors (80, 81).

Conclusion

The study found a statistically considerable variation in the average neutrophil computation, lymphocyte computation, and NLR between patients diagnosed with colorectal carcinoma and healthy control individuals. In addition, The study observed notable alterations in the serum level of liver function tests among patients with colorectal carcinoma. The mean AST, ALT, ALP, and TSB levels were significantly elevated, while the serum level of TSP exhibited a significant decrease in the patients' subjects compared to the control subject. Moreover, The present study reveals that patients diagnosed with colorectal carcinoma show a significantly elevated serum CEA and midkine level compared to the control group. This observation suggests that serum CEA and midkine measurement could be reliable predictors of colorectal carcinoma.

Acknowledgement

The authors are grateful to the Clinical Biochemistry Department / College of the Medicine / University; of Al-Qadisiyah and all the staff of the Oncology Teaching Hospital/ Medical City and Gastroenterology and Hepatology Teaching Hospital /Medical City in Baghdad – Iraq, for providing the research facilities.

Funding

Self-funded.

Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the College of Medicine/University of Al-Qadissiya with approval number (UoQ/CoM 30/4408 on 28.11.2022).

Reference

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. Thong MSY, Doege D, Weißer L, et al. Persisting Deficits in Health-Related Quality of Life of Colorectal Cancer Survivors 14–24 Years Post-Diagnosis: A Population-Based Study. Curr Oncol. 2023;30(3):3373–3390.
3. Gallardo FP, Onishi ET, Lira FI, et al. Translation, validation and cultural adaptation of" the Eustachian tube dysfunction Questionnaire-7"(ETDQ-7) to Brazilian Portuguese (BR). Braz J Otorhinolaryngol. 2019;85:456–464.
4. Dobre M, Dinu DE, Panaitescu E, et al. KRAS gene mutations prognostic factor in colorectal cancer. Rom J Morphol Embryol. 2015;56(2 Suppl):671–678.
5. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2021. Ca Cancer J Clin. 2021;71(1):7–33.
6. Dalerba P. Estimating the Economic Value of CDX2 as a Predictive Biomarker to Guide Treatment Decisions in Stage II Colon Cancer. Value Heal. 2022;25(3):382–384.
7. Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. Nat Rev Gastroenterol Hepatol. 2019;16(12):713–732.
8. Murphy N, Moreno V, Hughes DJ, et al. Lifestyle and dietary environmental factors in colorectal cancer susceptibility. Mol Aspects Med. 2019;69:2–9.
9. Li M, Pei J, Xu M, et al. Changing incidence and projections of thyroid cancer in mainland China, 1983–2032: evidence from cancer incidence in five continents. Cancer Causes Control. 2021;32(10):1095–1105.
10. Siegel RL, Torre LA, Soerjomataram I, et al. Global patterns and trends in colorectal cancer incidence in young adults. Gut. 2019;68(12):2179–2185.
11. Wong MCS, Huang J, Lok V, et al. Differences in incidence and mortality trends of colorectal cancer worldwide based on sex, age, and anatomic location. Clin Gastroenterol Hepatol. 2021;19(5):955–966.
12. Berzins SP, Wallace ME, Kannourakis G, et al. A role for MAIT cells in colorectal cancer. Front Immunol. 2020;11:949.
13. Henley SJ, Ward EM, Scott S, et al. Annual report to the nation on the status of cancer, part I: National cancer statistics. Cancer. 2020;126(10):2225–2249.
14. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7–33.
15. Bray F, Ferlay J, Soerjomataram I, et al. Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin. 2020;70(4):313.
16. Ibrahem S, Ahmed H, Zangana S. Trends in colorectal Cancer in Iraq over two decades: incidence, mortality, topography and morphology. Ann Saudi Med. 2022;42(4):252–261.
17. Isaksson GL, Nielsen MB, Hinrichs GR, et al. Proteinuria is accompanied by intratubular complement activation and apical membrane deposition of C3dg and C5b-9 in kidney transplant recipients. Am J Physiol Physiol. 2022;322(2):150–163.
18. Jones DR. Measuring midkine: the utility of midkine as a biomarker in cancer and other diseases. Br J Pharmacol. 2014;171(12):2925–2939.
19. Diakowska D, Krzystek-Korpacka M. Local and systemic interleukin-32 in esophageal, gastric, and colorectal cancers: Clinical and diagnostic significance. Diagnostics. 2020;10(10):785.
20. Shiratori F, Ito M, Yajima S, et al. The effectiveness of serum midkine in detecting esophageal squamous cell carcinoma. Esophagus. 2019;16(3):246–251.

21. Krzystek-Korpacka M, Diakowska D, Neubauer K, et al. Circulating midkine in malignant and non-malignant colorectal diseases. Cytokine. 2013;64(1):158–164.
22. Cai Y, Lv Y, Mo Z, et al. Multiple pathophysiological roles of midkine in human disease. Cytokine. 2020;135:155242.
23. Kemper M, Hentschel W, Graß J, et al. Serum Midkine is a clinical significant biomarker for colorectal cancer and associated with poor survival. Cancer Med. 2020;9(6):2010–2018.
24. Bilir A, Aynacioglu AS, Tuna MY. The possible interactions and therapeutic roles of lithium chloride and midkine on cancer treatment. Crit Rev Oncog. 2019;24(1).
25. Alvi S, Hande A, Chaudhary M, et al. The assessment of expression of midkine in epithelial dysplasia and oral squamous cell carcinoma. J Datta Meghe Inst Med Sci Univ. 2019;14(4):378.
26. Elessawi DF, Alkady MM, Ibrahim IM. Diagnostic and prognostic value of serum IL-23 in colorectal cancer. Arab J Gastroenterol. 2019;20(2):65–68.
27. Fang Y-J, Lee LJ-H, Luo K-H, et al. The Association of Carcinoembryonic Antigen (CEA) and Air Pollutants—A Population-Based Study. Atmosphere (Basel). 2022;13(3):466.
28. Huang C-S, Chen C-Y, Huang L-K, et al. Prognostic value of postoperative serum carcinoembryonic antigen levels in colorectal cancer patients who smoke. PLoS One. 2020;15(6):e0233687.
29. Hayama T, Hashiguchi Y, Okada Y, et al. Significance of the 7th postoperative day neutrophil-to-lymphocyte ratio in colorectal cancer. Int J Colorectal Dis. 2020;35:119–124.
30. Liposits G, Skuladottir H, Ryg J, et al. The prognostic value of pre-treatment circulating biomarkers of systemic inflammation (CRP, dNLR, YKL-40, and IL-6) in vulnerable older patients with metastatic colorectal cancer receiving palliative chemotherapy—The Randomised NORDIC9-Study. J Clin Med. 2022;11(19):5603.
31. Xia L, Li W, Zhai J, et al. Significance of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio and prognostic nutritional index for predicting clinical outcomes in T1–2 rectal cancer. BMC Cancer. 2020;20(1):1–11.
32. Corbeau I, Jacot W, Guiu S. Neutrophil to lymphocyte ratio as prognostic and predictive factor in breast cancer patients: a systematic review. Cancers (Basel). 2020;12(4):958.
33. Xue LB, Liu YH, Zhang B, et al. Prognostic role of high neutrophil-to-lymphocyte ratio in breast cancer patients receiving neoadjuvant chemotherapy: meta-analysis. Medicine (Baltimore). 2019;98(1).
34. MH H, Juma ASM, Ubeid MH, et al. Age and Gender in Relation to Colorectal Cancer in Najef Province: A Histopathological Study'. Acta Sci Pharm Sci. 2021;5(3).
35. Mohammed Ibraheem N, Qahtan Alrawi N, Banoosh AKA. Assessment the Characteristic Features of Colorectal Cancer among Patients Attending Tikrit Teaching Hospital from 2009-2013. Kirkuk J Med Sci. 2021;6(1):125–135.
36. Giaquinto AN, Miller KD, Tossas KY, et al. Cancer statistics for African American/black people 2022. CA Cancer J Clin. 2022;72(3):202–229.
37. Vuik FER, Nieuwenburg SA V, Bardou M, et al. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. Gut. 2019;68(10):1820–1826.
38. Sinicrope FA. Increasing incidence of early-onset colorectal cancer. N Engl J Med. 2022;386(16):1547–1558.
39. Collaborative R, Zaborowski AM, Abdile A, et al. Characteristics of early-onset vs late-onset colorectal cancer: a review. JAMA Surg. 2021;156(9):865–874.
40. Wu X-Z, Ma F, Wang X-L. Serological diagnostic factors for liver metastasis in patients with colorectal cancer. World J Gastroenterol WJG. 2010;16(32):4084.
41. Lubotzky A, Zemmour H, Neiman D, et al. Liquid biopsy reveals collateral tissue damage in cancer. JCI insight. 2022;7(2).
42. Gong Z, Zhang X, Geng Q, et al. AKP and GGT level can provide an early prediction of first-line treatment efficacy in colorectal cancer patients with hepatic metastases. Biomark Med. 2021;15(10):697–713.
43. Park HS, Chun YJ, Kim HS, et al. Clinical features and KRAS mutation in colorectal cancer with bone metastasis. Sci Rep. 2020;10(1):21180.
44. Castro M-J, Jiménez J-M, López M, et al. Impact of Preoperative Total Proteins and Glycated Hemoglobin on Recurrences after Early Colorectal Cancer. Nutrients. 2021;13(2):711.
45. Dekker E, Tanis PJ, Vleugels JL, et al. Pure-AMC. Lancet. 2019;394:1467–1480.
46. Muscaritoli M, Arends J, Aapro M. From guidelines to clinical practice: a roadmap for oncologists for nutrition therapy for cancer patients. Ther Adv Med Oncol. 2019;11:1758835919880084.
47. Benson AB, Venook AP, Al-Hawary MM, et al. Colon cancer, version 2.2021, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw. 2021;19(3):329–359.

48. Yamamoto T, Kawada K, Obama K. Inflammation-related biomarkers for the prediction of prognosis in colorectal cancer patients. Int J Mol Sci. 2021;22(15):8002.
49. An S, Shim H, Kim K, et al. Pretreatment inflammatory markers predicting treatment outcomes in colorectal cancer. Ann Coloproctol. 2022;38(2):97.
50. Lin N, Li J, Yao X, et al. Prognostic value of neutrophil-to-lymphocyte ratio in colorectal cancer liver metastasis: A meta-analysis of results from multivariate analysis. Int J Surg. 2022;106959.
51. Xia C, Dong X, Li H, et al. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J (Engl). 2022;135(05):584–590.
52. Chi Z, Li Z, Cheng L, et al. Comparison of long-term outcomes after laparoscopic-assisted and open colectomy for splenic flexure cancer. J buon. 2018;23(2):322–328.
53. Lucas C, Barnich N, Nguyen HTT. Microbiota, inflammation and colorectal cancer. Int J Mol Sci. 2017;18(6):1310.
54. Zhou D, Wu Y, Zhu Y, et al. The prognostic value of neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio in metastatic gastric cancer treated with systemic chemotherapy. J cancer. 2020;11(14):4205.
55. Ming-Sheng F, Mei-Ling D, Xun-Quan C, et al. Preoperative neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and CEA as the potential prognostic biomarkers for colorectal cancer. Can J Gastroenterol Hepatol. 2022;2022.
56. Takagawa R, Fujii S, Ohta M, et al. Preoperative serum carcinoembryonic antigen level as a predictive factor of recurrence after curative resection of colorectal cancer. Ann Surg Oncol. 2008;15:3433–3439.
57. Wang W-S, Lin J-K, Chiou T-J, et al. Preoperative carcinoembryonic antigen level as an independent prognostic factor in colorectal Cancer: Taiwan experience. Jpn J Clin Oncol. 2000;30(1):12–16.
58. Wiratkapun S, Kraemer M, Seow-Choen F, et al. High preoperative serum carcinoembryonic antigen predicts metastatic recurrence in potentially curative colonic cancer: results of a five-year study. Dis colon rectum. 2001;44(2):231–235.
59. Thirunavukarasu P, Talati C, Munjal S, et al. Effect of incorporation of pretreatment serum carcinoembryonic antigen levels into AJCC staging for colon cancer on 5-year survival. JAMA Surg. 2015;150(8):747–755.
60. Flamini E, Mercatali L, Nanni O, et al. Free DNA and carcinoembryonic antigen serum levels: an important combination for diagnosis of colorectal cancer. Clin Cancer Res. 2006;12(23):6985–6988.
61. Tan E, Gouvas N, Nicholls RJ, et al. Diagnostic precision of carcinoembryonic antigen in the detection of recurrence of colorectal cancer. Surg Oncol. 2009;18(1):15–24.
62. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med. 1965;122(3):467–481.
63. Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem. 2001;47(4):624–630.
64. Krzystek-Korpacka M, Gorska S, DiakOwska D, et al. Midkine is up-regulated in both cancerous and inflamed bowel, reflecting lymph node metastasis in colorectal cancer and clinical activity of ulcerative colitis. Cytokine. 2017;89:68–75.
65. Ji L, Gu L, Zhang X, et al.. Evaluation of serum midkine and carcinoembryonic antigen as diagnostic biomarkers for rectal cancer and synchronous metastasis. Transl Cancer Res. 2020;9(4):2814.
66. Dik B, Coskun D, Er A. Protective Effect of Nerium oleander Distillate and Tarantula cubensis Alcoholic Extract on Cancer Biomarkers in Colon and Liver Tissues of Rats with Experimental Colon Cancer. Anti-Cancer Agents Med Chem (Formerly Curr Med Chem Agents). 2022;22(10):1962–1969.
67. Jing X, Cui X, Liang H et al. Diagnostic accuracy of ELISA for detecting serum Midkine in cancer patients. PLoS One. 2017;12(7):e0180511.
68. Zhang B, Li B, Kong L, et al. Diagnostic accuracy of midkine on hepatocellular carcinoma: A meta-analysis. PLoS One. 2019;14(10):e0223514.
69. Komata Y, Tsubota S, Sakamoto K, et al. Screening of novel Midkine binding protein by BioID2-based proximity labeling. Nagoya J Med Sci. 2021;83(3):495.
70. Ibrahim NA, Hamam AM. Role of Midkine in Predicting Malignancy in Patient with Solitary Thyroid Nodule. J Cancer Tumor Intl. 2019;9(6):1–10.
71. Kadomatsu K, Kishida S, Tsubota S. The heparin-binding growth factor midkine: the biological activities and candidate receptors. J Biochem. 2013;153(6):511–521.
72. Xu C, Zhu S, Wu M, et al. Functional receptors and intracellular signal pathways of midkine (MK) and pleiotrophin (PTN). Biol Pharm Bull. 2014;37(4):511–520.

73. Turri G, Barresi V, Valdegamberi A, et al. Clinical significance of preoperative inflammatory markers in prediction of prognosis in node-negative colon cancer: correlation between neutrophil-to-lymphocyte ratio and poorly differentiated clusters. Biomedicines. 2021;9(1):94.
74. Swierczak A, Mouchemore KA, Hamilton JA, et al. Neutrophils: important contributors to tumor progression and metastasis. Cancer Metastasis Rev. 2015;34:735–751.
75. Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. Cancer Res. 2019;79(18):4557–4566.
76. Li H, Zhao Y, Zheng F. Prognostic significance of elevated preoperative neutrophil-to-lymphocyte ratio for patients with colorectal cancer undergoing curative surgery: A meta-analysis. Medicine (Baltimore). 2019;98(3).
77. Cruz-Ramos M, del Puerto-Nevado L, Zheng B, et al. Prognostic significance of neutrophil-to lymphocyte ratio and platelet-to lymphocyte ratio in older patients with metastatic colorectal cancer. J Geriatr Oncol. 2019;10(5):742–748.
78. Sun Y, Zhang Y, Huang Z, et al. Combination of preoperative plasma fibrinogen and neutrophil-to-lymphocyte ratio (the F-NLR score) as a prognostic marker of locally advanced rectal cancer following preoperative Chemoradiotherapy. World J Surg. 2020;44:1975–1984.
79. Yoshida D, Minami K, Sugiyama M, et al. Prognostic impact of the neutrophil-to-lymphocyte ratio in stage I-II rectal cancer patients. J Surg Res. 2020;245:281–287.
80. Merkhan MM, Shephard MT, Forsyth NR. Physoxia alters human mesenchymal stem cell secretome. Journal of Tissue Engineering. 2021 Oct;12:20417314211056132.
81. Shephard MT, Merkhan MM, Forsyth NR. Human Mesenchymal Stem Cell Secretome Driven T Cell Immunomodulation Is IL-10 Dependent. International Journal of Molecular Sciences. 2022 Nov 6;23(21):13596.