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The Significance of Carbohydrate Antigen 19-9, Carcinoembryonic Antigen, Calcium and Zinc as Biomarkers in Sera of Colorectal Cancer Patients

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Abstract

Colorectal cancer (CRC) is a disease that begins exclusively in the colon or rectum and is caused by an abnormal proliferation of glandular epithelial cells in the colon. Carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), calcium (Ca), and zinc (Zn) are recognized to play a significant role in colorectal tumor pathogenesis. The aim of this study is to detect serum concentrations of carbohydrate antigen 19-9, carcinoembryonic antigen, calcium, and zinc in CRC. The current study investigated the vital roles of carbohydrate antigen 19-9, carcinoembryonic antigen, calcium, and zinc in cancer development by comparing serum levels of colorectal cancer patients with those of the healthy control group at Erbil's Nanakaly Hospital. The concentrations of serum carbohydrate antigen 19-9, carcinoembryonic antigen, calcium, and zinc in 50 colorectal cancer patients and 50 from the healthy control group have been examined. A significant positive correlation was found between CA19-9 and CEA (p<0.0165, r = 0.3723). Also, a non-significant negative correlation between CA19-9 and calcium (P=0.77, r= - 0.0471) and zinc (P=0.6334, r= - 0.07676) was found. This study demonstrates a direct relationship between CRC and serum levels of (CA19-9, CEA, Ca, and Zn). The result showed significantly high values of serum CA19-9 (p<0.0001), CEA (p<0.0001) and significant decreases in Ca (p=0.0046) and Zn (p=0.0011) in patients with CRC when compared with healthy group. Our findings demonstrate that increased levels of CA19-9 and CEA, and decreased levels of Ca and Zn can be associated with the pathogenesis and progression of CRC.

Keywords: Carbohydrate antigen 19-9, Carcinoembryonic antigen, Calcium, Colorectal cancer, Zinc.

Introduction

Colorectal cancer (CRC) is the third most prevalent malignancy in men and women globally, the second major cause of cancer-related death, and the leading cause of death in gastrointestinal (GI) malignancies. According to the most recent study on global cancer statistics, there were 935.000 deaths and two million new cases of colorectal cancer in 2020 [1]. The CRC is becoming a major public health issue, particularly in developed countries [2]. In prospective epidemiologic investigations, indicators of systemic inflammation, obesity, and diabetes mellitus were found to be linked with colorectal cancer risk [3].

Carbohydrate antigen 19-9 (CA19-9) is the carbohydrate determinant (sialylated lacto-N-fucopentaose 119) of a circulating oligosaccharide antigen that was found using a monoclonal antibody in human colon carcinoma cells in culture. Elevated serum levels of the antigen have been detected on glycoproteins in the sera of patients with colorectal, gastric, and pancreatic malignancies [4].

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Carcinoembryonic antigen (CEA) identifies a group of complex glycoproteins that is one of about 20 similar molecules in the immunoglobulin gene superfamily (IgSF)[5]. Carcinoembryonic antigen is an embryonic tumor antigen generated in GI tissue during the human embryonic and fetal stages. The CEA is mostly released by colonic mucosal cells in adults, with a small amount reabsorbed into the blood. The CEA is functionally linked to cellular contact, cell adhesion, immunological response, and liver metastasis promotion[6,7]. The purpose of carcinoembryonic antigen monitoring following curative resection of CRC is to detect disease recurrence at an early and curable stage [8].

Calcium (Ca) is required for bone and tooth structure, the regular function of all cells, and several enzyme-controlled activities. The role of calcium as a colorectal anti-carcinogen has been extensively assessed in both *in vitro* and *in vivo* studies [9,10]. It has been proposed that calcium phosphate or ionized calcium could decrease colon carcinoma by binding free fatty acids and secondary bile acids, mainly lithocholic and deoxycholic acids, thereby reducing their effective toxic dose to the colonic epithelial cells and preventing their stimulatory effects on the intestinal mucosa proliferation[11].

Zinc (Zn) is one of the most significant metals in organisms, and Zn-binding proteins are essential for a variety of biological activities, including transcription control, cell metabolism, and cell death. Zn is essential for the functioning of transcription factors, the antioxidant defense system, and deoxyribonucleic acid repair[12]. A decrease in Zn intake can raise the risk of tumor growth by causing single- and double-strand breaks and oxidative alterations to DNA. In addition, it participates in a number of biological processes, including signal transcription, transduction, and replication [13].

Therefore, the aim of this research is to investigate the serum levels of CA19-9, CEA, Ca, and Zn and their correlation in CRC patients to find out their association with CRC pathogenesis and development.

Materials and Methods

The blood samples were collected from 100 individuals whose average age (29-65) years, who were split into two groups: Group 1 (G1): includes 50 subjects (25 men and 25 women) as a healthy control group. Group 2 (G2): includes 50 cases at stages III and IV with CRC after receiving the first and second doses of chemotherapy. There were 25 stage III and 25 stage IV patients diagnosed after taking a chemotherapy dose. All patients were taking 5-fluorouracil, oxaliplatin, and leucovorin therapy. The study was approved by the oncology department at Nanakely Hospital for the period between November 2021 and May 2022. To separate the serum from the samples, 5 ml of each sample was centrifuged at 6000 rpm for 10 minutes. Then, the serum samples were kept in a chiller at -40 ^{0}C, and storage samples were used to measure the levels of CA19-9, CEA, Ca and Zn.

Measurements of Serum CA19-9, CEA, Ca and Zn

The serum was separated by centrifugation and placed in a cooler at a low temperature. The CA19-9 and CEA serum concentrations of the cancer and healthy control groups were then assessed using Cobas e 601, and the calcium and zinc serum concentrations were determined using Statlab T. The Statlab T measurements at 578 and 546 nm for calcium and zinc respectively. The concentrations of CA19-9, and CEA in the examined samples were reported in U/mL and ng /mL respectively while the concentrations of Ca and Zn were expressed in mg/dl.

Statistical Analysis

GraphPad Prism 9.0 was used to perform the statistical analysis. The data is presented as the Mean± SEM. The T-test was applied to identify any differences between the two groups. A one-way ANOVA was performed to compare the mean values of the examined parameters between stages III and IV of CRC. The Pearson's correlation test (r) was used to determine statistical relationships between CA19-9 and related biochemical indicators in the patient group. Always using two-sided *P* values, significant differences were considered to be less than 0.05.

Results and Discussion

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The study population was 100 subjects, and all subjects were divided into three main groups (patients and control subjects) as demonstrated in Table 1 and Figs. 1,2,3 and 4. Table.1 presents the results of four parameters (CA19-9, CEA, Ca and Zn) in serum of healthy subjects and patients. There was a statistically significant increase in CA19-9 for stage III (531.6±93.54 U/mL), stage IV (909.7±122.2), when compared with control (7.743±1.255). Also, there was a significant increase in CEA for stage III (551.5±33.27), stage IV (925.7±35.73 ng/mL) when compared with control (1.120±0.1261). A significant decrease was detected in Ca for stage III (7.651 ± 0.2767 mg/dl), stage IV (7.528±0.2031 mg/dl), when compared with controls (8.607±0.2482 mg/dl). In addition, A significant decrease in Zn for stage III (85.48 ± 1.920 mg/dl), stage IV (78.85±3.258 mg/dl) in comparison with controls (92.54±2.096 mg/dl) was found.

Table 1. Serum values of CA19-9, CEA, Ca and Zn in CRC patients and healthy control group

Serum parameters	Healthy controls	Stage III	Stage IV	*P*-value
CA19-9 (U/mL)	7.753±1.255	531.6±93.54	909.7±122.2	$p<0.0001$
CEA (ng/mL)	1.120±0.1261	551.5±33.27	925.7±35.73	$p<0.0001$
Ca (mg/dl)	8.607±0.2482	7.651 ± 0.2767	7.528±0.2031	0.0046
Zn (mg/dl)	92.54±2.096	85.48 ± 1.920	78.85±3.258	*0.0011*

The CA19-9 is a carbohydrate-associated tumor antigen that was initially identified in a colon cancer cell line. It is an O-linked glycoprotein that is expressed on the cell surface as a glycolipid and is also known as sialyl Lewis-a. Carbohydrate antigen 19-9 production is strongly related to the blood group antigen called disialyl Lewis-a, which has one additional sialic acid residue than CA19-9 and is formed preferentially in normal non-malignant GI and other organ epithelial cells. However, in malignant cells, because of an aberrant sialylation pathway, sialyl Lewis-a, which is a simpler molecule, is synthesized instead [14]. These results have shown that CRC patients had a greater serum CA19-9 level than the normal groups, and there were significant differences between the CRC patients and normal subjects ($p<0.0001$). The current investigation revealed a significant rise in CA 19-9 concentrations in CRC patients, and these results agree with Lehtomäki et.al and Lakemeyer et.al [15, 16]. The elevation in the carbohydrate antigen CA19-9 could be due to the increase in its expression and its ligand protein [17].

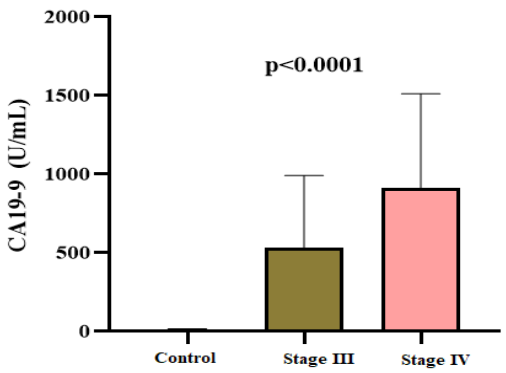

Figure 1. Concentration of CA19-9 in CRC patients and healthy subjects.

Carcinoembryonic antigen is an oncofetal antigen that is produced in large quantities by normal growing fetuses and some cancers, but only in small quantities by normal adult human cells. The primary CEA consists of a single polypeptide chain with variable carbohydrate constituents. The polypeptide chain of carcinoembryonic antigen is responsible for the majority of its antigenic expression, and the functional similarities among family members are due to the shared structure at the disulfide loop domain. The metastatic capacity of cancer cells is determined by these functions. The CEA serves in intercellular recognition and attachment as a surface antibody, binding to carcinoembryonic antigen receptors at sites of metastases[18]. Carcinoembryonic antigen was found in the circulation of colorectal cancer patients and was used as a serum marker [19,20]. The current study demonstrated a highly significant rise in CEA levels

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in colorectal carcinoma patients as compared to the control subjects as shown in Fig. 2, and these results agree with Ramphal et.al [21]. It might be due to the detection of protein in cancer [22].

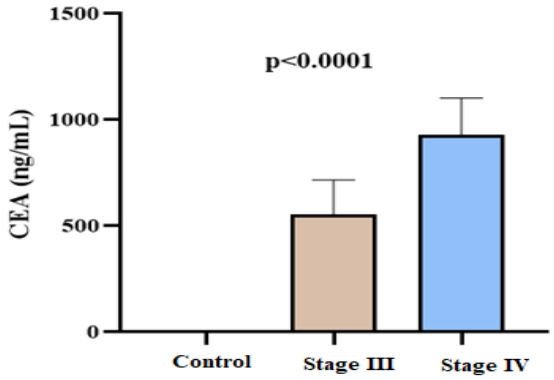

Figure 2. Concentration of CEA in CRC patients and healthy subjects.

In this study, our results showed that CRC patients had a lower serum calcium level than healthy controls as shown in Fig. 3. These results were in accordance with the data from other studies showing that mean calcium levels decreased in patients with CRC [23, 24]. Ca's beneficial effects are thought to be more pronounced in the proximal colon because Ca protects the colorectal wall from inflammation and bile acid irritation. Intracellular Ca in colorectal epithelial cells may diminish malignancy-promoting inflammatory responses, and the presence of ionized Ca in the colorectal epithelial cells may prevent the harmful and potentially irritating effects of free fatty acids and bile acid[25]. Ca reduces the risk of colorectal carcinoma by reacting with ionized fatty acids, and secondary bile acids, which have both been associated with epithelial cellular proliferation in the colon. Those with a higher fat intake, according to this theory, should gain the most from a higher calcium consumption [26,27]. Furthermore, Ca may also lower plasma inflammatory markers like interleukin-6 and protect against oxidative deoxyribonucleic acid damage in patients with colorectal adenoma[28].

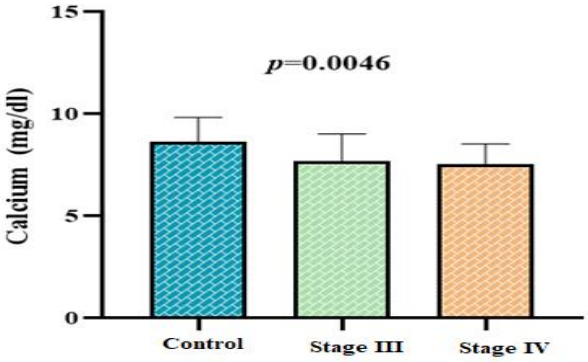

Figure 3. Concentration of calcium in CRC patients and healthy subjects.

The current study found a significantly lower Zn concentration in colorectal adenoma patients as compared to the healthy group, which is consistent with previous research [29,30] as shown in Fig. 4. Zinc may protect against CRC progression through its antioxidant effects [31]. In addition, Zn acts as an anti-carcinogen by stabilizing the structures of RNA, DNA, and the ribosome [32]. In addition, Zn is required for the action of many transcription factors and proteins that identify certain deoxyribonucleic acid sequences and modulate gene transcription. Since Zn prevents cells from damaging free radicals, a low Zn level may contribute to the degradation of colon cancer-affected tissue and cause extra damage[33]. The lowering in serum zinc levels in patients with colorectal cancer may be reduced due to the mobilization of circulating Zn to colorectal adenoma tissue and its role in the antioxidant system since the patients may be under more oxidative stress. Zinc has antioxidant properties; consequently, it could protect macromolecules against free radical induced oxidation *in vitro* and reduce the production of excessive free radicals [34, 35]. Additional explanations for decreased levels of Zn in CRC patients include impaired GI absorption, unbalanced dietary consumption, unfavorable medication effects, and greater sequestration in tumor tissue. Additional explanations for decreased levels of Zn in CRC patients include impaired GI absorption, unbalanced dietary consumption, unfavorable medication effects, and greater sequestration in tumor tissue [36].

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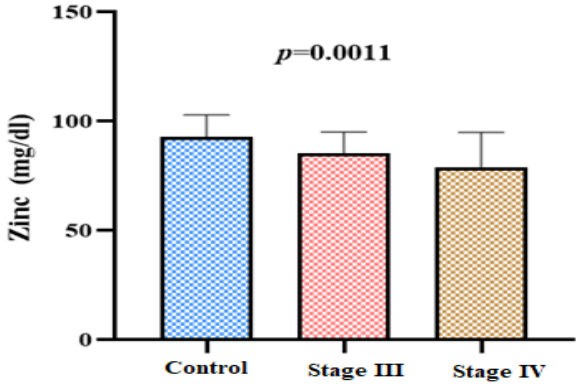

Figure 4. Concentration of zinc in colorectal adenoma patients and healthy subjects

The correlation between CA19-9 and CEA showed a positive correlation (r = 0.3723), which was statistically significant as demonstrated in Fig. 5.

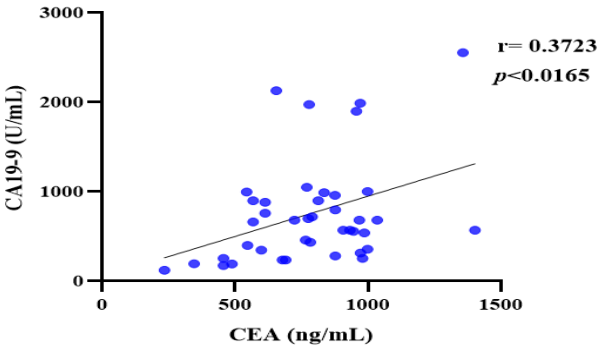

Figure 5. Shows the correlation between CA19-9 and CEA.

Figs. 6 and 7 showed a statistically non-significant negative correlation between CA19-9 and calcium (r = -0.0471) and CA19-9 and zinc (r = -0.07676).

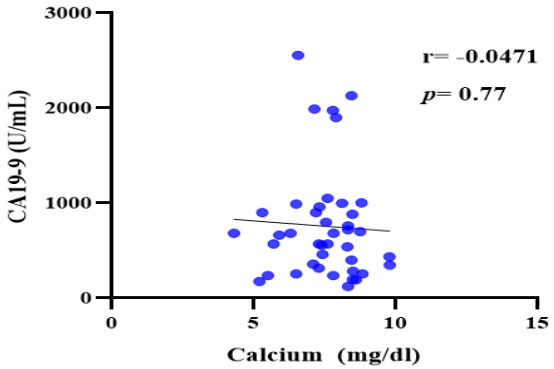

Figure 6. The correlation between CA19-9 and calcium

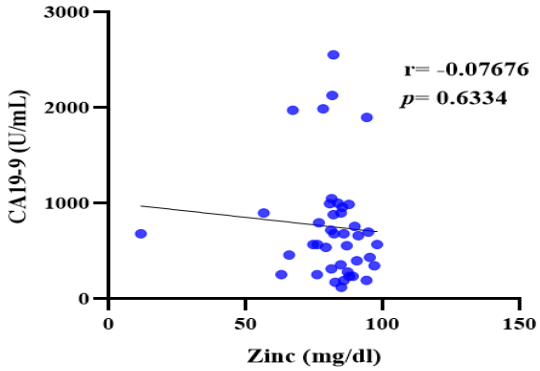

Figure 7. The correlation between CA19-9 and zinc.

Figs. 8A and 8B display the receiver operating characteristic (ROC) curve of CA19-9 and CEA performance as a potential diagnostic marker for CRC, which were statistically significant. A relatively high AUC (area under the curve) suggests that testing for CA19-9 and CEA could be helpful in detecting CRC.

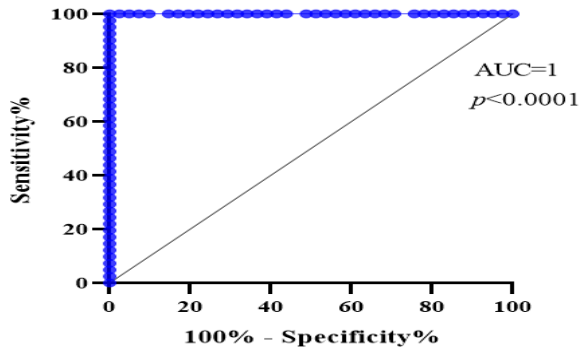

Figure 8A. The ROC curve illustrates the sensitivity and specificity of CA19-9 for the detection of CRC.

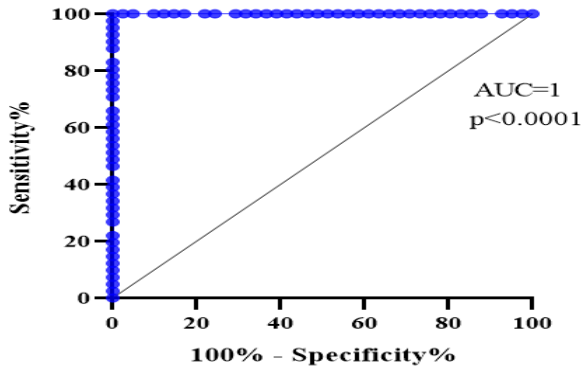

Figure 8B. The ROC curve of CEA for the detection of CRC.

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Figs. 8C and 8D display the ROC curve of calcium and zinc performance as biomarkers for CRC, which were statistically significant.

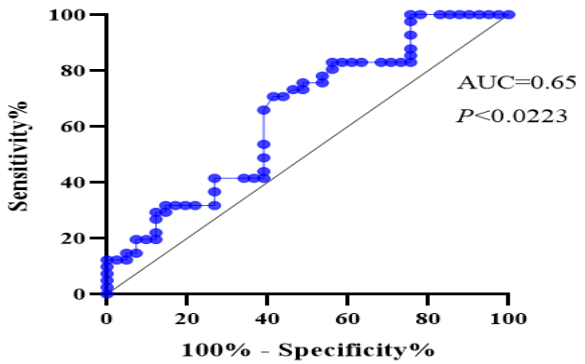

Figure 9C. The ROC curve of Ca for the detection of CRC.

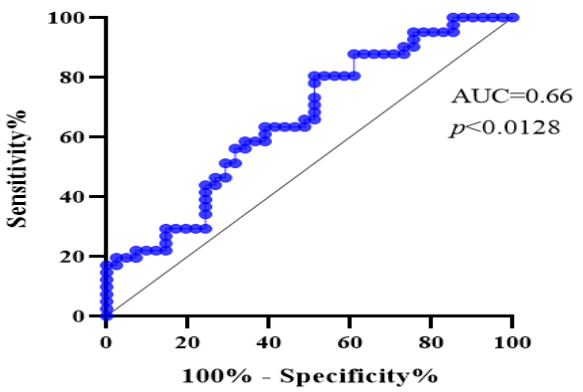

Figure 10D. The ROC curve of Zn for the detection of CRC.

Conclusion

The current original study findings indicated that CA19-9, CEA, Ca, and Zn levels in the serum of colorectal carcinoma patients differed significantly from those of randomly selected healthy subjects. According to current research, CA19-9, CEA, Ca, and Zn play a significant role in colorectal cancer pathogenesis. According to the investigation, the serum levels of CA19-9, CEA, Ca, and Zn and their correlation in CRC patients were examined to find out their association with colorectal cancer pathogenesis and development. As a result, these variables may be crucial in the development of colorectal cancer and serve as biomarkers of significance for the disease.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at Salahaddin University.
- No animal studies are present in the manuscript.
- No potentially identified images or data are present in the manuscript.

Authors' Contribution Statement

F.A.N; performed the experiment, wrote the manuscript, data analysis and performed the statistical analysis. Z.A.A. supervised the project.

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اهمية العلامات الحيوية لكل من مستضد الكربوهيدرات 19-9 ، مستضد السرطانى المضغي ، كالسيوم والزنك في أمصال مرضى سرطان القولون و المستقيم

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الخلاصة

سرطان القولون والمستقيم هو اضطراب يبدأ حصريا في القولون والمستقيم وينجم عن تكاثر الشاذ للخلايا الظهارية الغدية في القولون. من المعلوم أن مستضد الكربوهيدرات 19-9 والمستضد السرطانى المضغى والكالسيوم والزنك يلعبون دورا هاما فى امراض ورم القولون والمستقيم. الهدف من هذه الدراسة هو التحقيق في مستويات مصل لمستضد الكربوهيدرات 19-9 و المستضد السرطاني المضغي والكالسيوم والزنك فى مرضى سرطان القولون والمستقيم. في هذه الدراسة تم البحث عن الأهمية الحيوية لمستضد الكربوهيدرات 19-9 ومستضد السرطاني المضغي والكالسيوم والزنك في تطور السرطان من خلال مقارنة مستويات مصل مرضى المصابين بسرطان القولون والمستقيم مع مجموعة الاصحاء في مستشفى ناناكالي في اربيل. تم فحص مستويات مستضد الكربوهيدرات 19-9 و مستضد المصل السرطاني المضغي و الكالسيوم والزنك في 50 مريضا مصابا بسرطان القو لون والمستقيم و 50 من مجموعة الاصحاء. تم العثور على علاقة أيجابية كبيرة بين المستضد الكربوهيدرات 19-9 ومستضد السرطانى المضغي (r= ,0.0165>P). (0.37230 و ارتباط سلبي غير معنوي بين المستضد الكربوهيدرات 19-9 مع الكالسيوم (P=0.77, r= -0.0471) والزنك (P=0.6334r= -0.07676). وفقا لهذه الدراسة ، هناك علاقة مباشرة بين سرطان القولون والمستقيم ومستويات مصل الدم لكل من مستضد الكربوهيدرات 19-9 ، مستضد السرطانى المضغي ، الكالسيوم والزنك. و تظهر النتائج بشكل ملحوظ القيمة العالية لمصل المستضد الكربوهيدرات 19- 9 (p<0.0001) و مستضد السرطانى المضغي (P<0.0001) و انخفاض كبير في قيم كل من الكالسيوم (0.0011) والزنك (0.0046) في مرضى سرطان القولون والمستقيم عند مقارنتها بمجموعة الاصحاء. النتائج التي توصلنا اليها تظهر ان زيادة مستويات المستضد الكربوهيدرات 19-9 ومستضد السرطاني المضغي و انخفاض مستويات الكالسيوم والزنك يمكن ان التسبب في ظهورسرطان القولون والمستقيم وتطوره.

الكلمات المفتاحية: مستضد الكربوهيدرات 19-9 مستضد السرطانى المضغي ، كالسيوم ، سرطان القولون والمستقيم، زنك.