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Increased Serum Calprotectin is Associated with Colorectal Carcinoma Development and Severity

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Abstract

Powerful and reliable colorectal cancer (CRC) prognostic biomarkers that can be related to the risk of the disease progression and severity is still a great challenge. This study aimed to evaluate serum calprotectin (CP) potential association with CRC development and poor outcomes. Results revealed that elevated serum CP was significantly (P<0.05) related to CRC (45.74 (27.4-58.8) ng/mL) development compared to healthy controls (6.1 (4.3-8.8) ng/mL) and patients with benign polyps (32.5 (29.2-38.8) ng/mL). Compared to CEA (AUC=0.713), it had a superior ability (AUC=0.808) to differentiate CRC cases. Serum CP elevated levels (ng/mL) was significantly associated with tumor aggressiveness features like late stages (47.5 (28.7-70.5) *vs.* 43.4 (24.8-51.9); P=0.0032), lymph node invasion (45.1 (22.6-87.1) *vs.* 46.2 (27.7-56.9); P=0.0344), distant metastasis (43.6 (19.6-109.9) *vs.* 46.2 (27.7-56.9); P=0.0423), high grades (54.6 (38.2-87.4) *vs.* 39.0 (22.0-48.9); P=0.0001), and large size (46.7 (31.3-51.6) *vs.* 26.7 (17.7-42.4); P=0.0113). CP was also significantly correlated with CEA (r =0.399; P =0.002) in CRC patients. In conclusion, serum CP protein appears to be a reliable CRC biomarker that could act in clinical practice as a non-specific marker for monitoring CRC progression and preventing poor outcomes.

Key words: Colorectal cancer; Blood marker; Serum calprotectin; Aggressiveness; Poor outcomes

1. Introduction

Worldwide, colorectal cancer (CRC) is the 3rd most frequent malignancy and the 2nd most common cause of tumor-related death [1]. Throughout the world, >1.9 million CRC patients occurred, and about 0.9 million cases died due to CRC in 2020 [1]. CRC is a heterogenous group of tumors that exhibit diverse clinicopathological outcomes and characteristics [2].

Despite advancement in CRC management via surgery, chemotherapy, radiotherapy and immunotherapy, CRC prognosis varies extremely between cases with five-year survival rates ranging from 90-10% depending on the disease stage at diagnosis [2, 3]. Compared to advanced CRC, early-stage of the disease have higher overall survival rates [4]. The main priority objectives for improving quality of life and survival rates are early diagnosis provision, regular follow-up and appropriate timely therapy [4]. Moreover, evaluating and searching for alternative optimal approaches or great sensitive biomarkers to determine CRC proliferation with potential prognostic ability seems very important [5].

Calprotectin (CP) is a neutrophil granulocytes common (40-50% of the total protein) cytosolic, calcium binding protein, and it is produced through neutrophil turnover and activation [6]. So, it is an important biomarker of neutrophil-induced inflammation [6, 7]. Faecal calprotectin (FC) is a biomarker that is being used to differentiate colon functional from organic diseases [8]. Former reports have found that CRC cases have elevated FC levels, and that FC backs to normal concentrations post resection surgery [9]. During colorectal carcinogenesis as an early step in neoplastic transformation, Luley et al. findings support the role of elevated tissue and serum CP expression [10]. Furthermore in CRC, CP expression is closely associated with inflammatory response and highlighted biological possible link between neoplastic transformation and inflammation [10].

The primary aim of this study is to evaluate serum CP potential role as a reliable biomarker for differentiating CRC from benign colorectal polyps and healthy controls. Also, we aimed to assess the association between serum CP and poor disease progression and outcomes including advanced stages, lymph node invasion, large tumor size, high histological grades, distant metastasis and elevated levels of established tumor markers carcinoembryonic antigen (CEA).

2. Material and methods

2.1. Study group

All cases included in this study (n=100) were Egyptian patients undergoing diagnostic colonoscopy at Mansoura Oncology center, Mansoura University, Egypt either for screening purposes or due to gastrointestinal symptoms. They were classified into 70 CRC cases and 30 cases with benign colon polyps. In addition, a total of 30 healthy individuals were included as controls. Diagnosis of CRC and benign disorders was mainly based on colonoscopy and in some cases on computed tomography. CRC classification and staging were performed according to the international Tumor-Node-Metastasis (TNM) system [11]. None of healthy controls or benign cases had a history of any malignancies. This work was approved by the ethics and scientific committees of Port Said University and was in accordance with the ethical guidelines of the "Helsinki Declaration".

2.2. Sample collection and laboratory tests

From all participants, after blood (10 mL) withdrawing, serum samples were obtained via centrifugation (4500 rpm, 15 minutes). Fresh sera was tested for liver enzymes activities [alanine (ALT) and aspartate aminotransferase (AST)] and serum bilirubin, albumin, urea and creatinine levels using automatic biochemistry analyzer (Hitachi, Japan) and related commercial kits. Using automated analyzer (Sysmex, Japan), another blood part (treated with EDTA-K3) was used for complete blood count. By commercial ELISA assay kits and according to the industrial prescript, serum CEA (MyBioSource, San Diego, USA) and CP (cat

number: E4010Hu, Bioassay Technology Laboratory, Korea) were measured.

2.3. Statistical analysis

Variables were expressed as mean±SD, median (interquartile range) or absolute numbers, appropriately. All analyses were performed using GraphPad version 9.0 and SPSS version 21. *Kruskal-Wallis*, student *t-test* and *ANOVA* tests were used appropriately to assess differences between groups. $P<0.05$ is significant. Serum CP diagnostic utility was evaluated using area under the receiver operating characteristic (ROC) curve. Correlation between CP and other tumor feature and CEA levels was evaluated by Pearson and Spearman correlation coefficients, appropriately.

3. Results

3.1. Patients' characteristics

CRC cases were gender- (P =0.304) and age-matched (P =0.313) with patients with benign polyps and healthy controls. Patient's characteristics including hematological and clinical data are summarized in Table 1. There was no significant ($P>0.05$) difference between CRC cases and controls in hematological, liver and kidney related variables and this may be owing to the exclusion of any chronic disorders. Elevated CEA levels (P=0.036) were significantly related to CRC cases (Table 1). All CRC patients were classified based on the TNM staging system, tumor differentiation degree and tumor size (Table 1).

Table 1. Characteristics of cases and controls

Variables	Colorectal cancer	Benign	Healthy	*P* value
Number	100	30	30	—
Gender (males/females)	70/30	20/10	19/11	0.304
Mean age ±SD, years	51.01±12.7	49.5±10.5	47.9±5.1	0.313
Hemoglobin (g/dL)	11.8±1.7	11.75±2.15	11.85±2.81	0.260
RBCs ($\times10^{12}$/L)	4.3±0.61	4.41±0.56	4.52±0.61	0.610
WBCs ($\times10^{9}$/L)	7.6±2.4	7.1±1.9	6.59±1.7	0.213
Platelet count ($\times10^{9}$/L)	273.2±52.2	265±65.5	281±59.6	0.172
ALT (U/L)	26.6±10.1	25.5±4.1	25.2±8.5	0.433
AST(U/L)	30.1±10.2	32.2±8.5	29.13±7.4	0.099
Bilirubin (mg/dL)	0.82±0.31	0.71±0.16	0.67±0.20	0.605
Albumin (g/dL)	3.74±0.81	3.91±0.45	4.1±0.35	0.125
Creatinine (mg/dL)	1.07±0.38	0.88±0.25	0.76±0.14	0.402
Urea (mg/dL)	30.5±9.3	29.8±6.0	25.6±4.9	0.622
CEA (U/L)	6.8 (2.1-15.5)	3 (2-6.5)	2 (1-3.37)	0.036
Tumor stage (Early/Late)	45/55	—	—	—
Lymph node (Negative/Positive)	62/38	—	—	—
Distant metastasis (Negative/Positive)	77/23	—	—	—
Histological grade (Low/High)	48/52	—	—	—
Tumor size (Small/Large)	40/60	—	—	—

Normally and non-normally distributed data were expressed as mean± SD and median (interquartile range), respectively. Early stage: T1-T2; Late stage: T3-T4; Low grade: (G1-G2); High grade: G3; Small tumor: ≤ 5 cm; Large tumor: >5 cm; RBC: red blood cell; WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CEA: carcinoembryonic antigen. Significant differences were determined using *ANOVA* and *Kruskal-Wallis* test, appropriately. $P<0.05$ was significant

3.2. Elevated serum calprotectin was associated with colorectal cancer

Elevated CP was related to CRC development (Figure 1), as high CP levels (45.74 (27.4-58.8) ng/mL) were significantly (P<0.05) related to CRC cases compared to healthy controls (6.1 (4.3-8.8) ng/mL) and patients with benign polyps (32.5 (29.2-38.8) ng/mL). Compared to CEA (AUC=0.713; 95%CI (0.612-0.815); Figure 2A), serum CP had superior ability (AUC=0.808; 95%CI (0.737-0.879); Figure 2B) to differentiate CRC cases from all non-cancer individuals.

3.3. Serum calprotectin was associated with tumor severity

In CRC cases, serum CP levels (ng/mL) was significantly (P<0.05) affected tumor aggressiveness including late stages (47.5 (28.7-70.5) *vs.* 43.4 (24.8-51.9); P=0.0032), lymph node invasion (45.1 (22.6-87.1) *vs.* 46.2 (27.7-56.9); P=0.0344), distant metastasis (43.6 (19.6-109.9) *vs.* 46.2 (27.7-56.9); P=0.0423), high grades (54.6 (38.2-87.4) *vs.* 39.0 (22.0-48.9); P=0.0001), and large size (46.7 (31.3-51.6) *vs.* 26.7 (17.7-42.4); P=0.0113) (Figure 3). Besides its significant correlation (P<0.05) with these tumor features (Table 2), CP was also significantly correlated with CEA (r =0.399; P =0.002; Figure 4) in CRC patients.

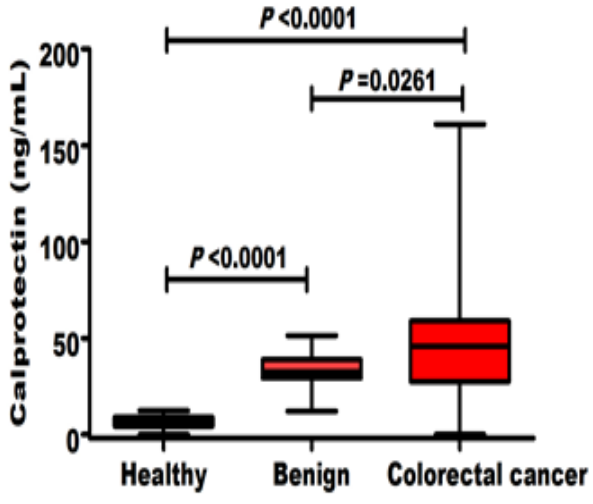

Figure 1. Serum calprotectin and colorectal cancer development. P<0.05 was significant.

4. Discussion

It was reported that most CRCs originates from proliferative, precancerous, benign growth called polyps [12]. During polyp development slow phases, varied mutations begin to accumulate and transform some polyps into CRC [13]. Early CRC stages are curable by surgery, however, after tumor invasion to distant organs or lymph nodes, CRC prognosis becomes poor [13]. As CRC prognosis is stage dependent and disease early-stages have higher overall survival compared to advanced stages [4], therefore, understanding factors that influence CRC onset and progression plays a crucial role in preventing and treating CRC [14].

Recent studies reported that increased pre-diagnostic faecal CP levels were common in CRC patients in close proximity to diagnosis [8]. Despite its high sensitivity, the low specificity prevents faecal CP from being used in CRC diagnosis or screening [15]. Moreover, a potential problem with protein detection in stool samples is patients' unwillingness about stool handling, and this could be a marked hindrance in clinical practice and test acceptability in everyday and in patient compliance [16]. Some studies underlines that serum CP strongly matched faecal CP and they were good predictors of inflammatory bowel disease (IBD) [17]. Thus, this study aimed to evaluate serum CP, as an easy sample, potential association with CRC development (compared to benign polyps and healthy controls) and also its association with the disease severity and poor outcomes.

Our findings revealed that elevated serum CP was significantly (P<0.05) related to CRC (45.74 (27.4-58.8) ng/mL) development compared to healthy controls (6.1 (4.3-8.8) ng/mL) and patients with benign polyps (32.5 (29.2-38.8) ng/mL). It had a good superior ability (AUC=0.808) to differentiate CRC cases compared to CEA (AUC=0.713). From another hand, elevated serum CP levels (ng/mL) was significantly (P<0.05) associated with tumor aggressiveness features like late stages, lymph node invasion, distant metastasis, high grades, and large size. CP was also significantly correlated with CEA (r =0.399; P =0.002) in CRC patients.

CP is a cytoplasmic protein found in the monocytes and neutrophils. Beside the main association of serum CP increased concentration and inflammatory conditions, elevated serum CP was reported to be related with some tumor cases including lymphoma [18], thyroid carcinoma [19], ovarian cancer [20], breast cancer [21]. Regarding IBD, several studies reported that elevated serum CP was correlated with active IBD [22]. For example, during the active phase of Crohn's disease and ulcerative colitis, Mori et al. found that levels of serum CP were higher in cases compared to controls [23]. Another study also found that serum CP was higher in IBD cases compared to healthy controls and serum CP was superior to C-reactive protein in revealing ulcerative colitis severity [24].

In patients with no colorectal pathology, a recent meta-analysis including nineteen studies hypothesized that CP would be lowest with a sequential rise through neoplasia stages [15]. Compared to patients with no colorectal pathology, many of these studies found higher median CP levels specifically in adenomas and CP levels were lower in adenomas compared to CRC [15]. The same meta-analysis reported that CRC cases are 5-fold more likely (P<0.001) than controls to have an increased CP (OR 5.19,

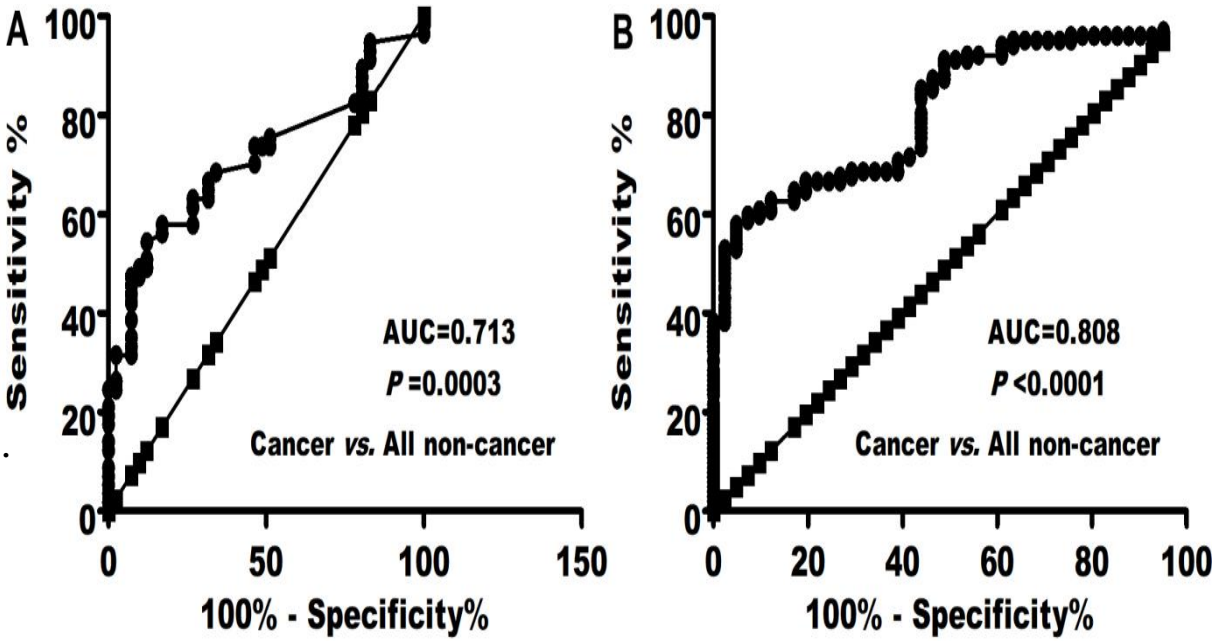

Figure 2: ROC analysis of (A) serum CEA and (B) serum calprotectin to differentiate CRC cases from all non-cancer individuals. P<0.05 was significant. All non-cancer: patients with benign polyps and healthy controls combined.

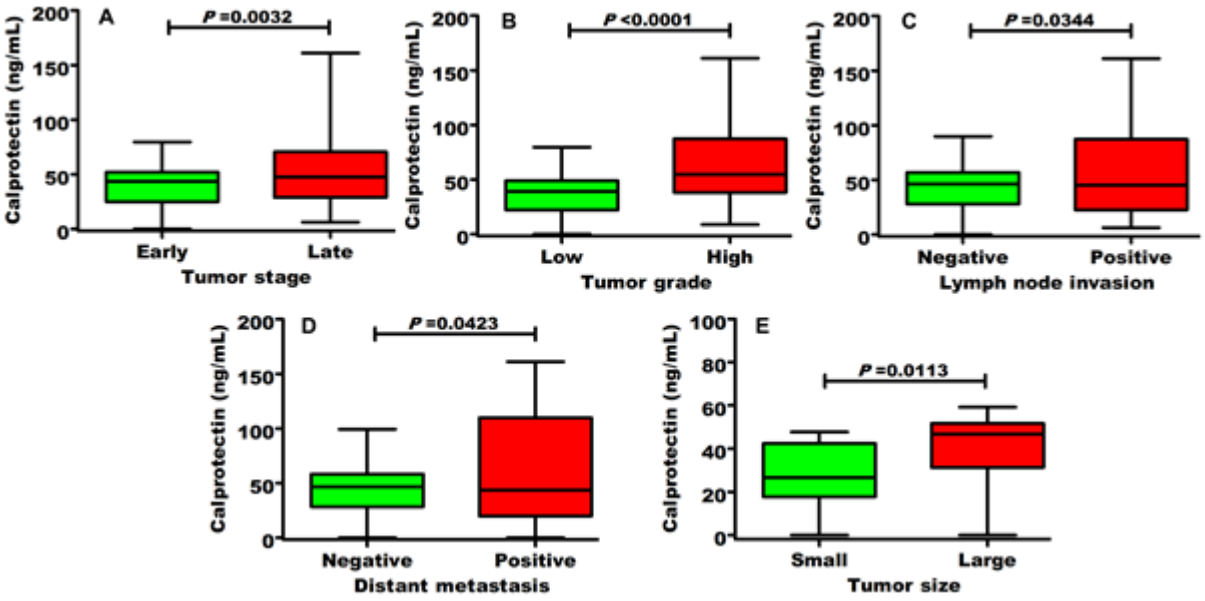

Figure 3: Serum calprotectin and CRC progression according to (A) tumor stages, (B) histological grades, (C) lymph node invasion, (D) distant metastasis and (E) tumor size. Significant difference was determined using *Kruskal-Wallis* test. $P<0.05$ was significant.

95% CI 3.12–8.62) [15]. Recently, Calero et al. found that serum CP significantly differentiate CRC patients from IBD and benign polyps [25]. They devised a predictive model, including serum CP with other blood biomarkers, with great accuracy [25].

There are fewer studies reporting CP's association to CRC histopathology or stage. For example, Lehman et al. found that CP was correlated with T-stage as patients with advanced stages (T3/4) having significantly greater CP levels than early stages (T1/2) [26]. Also, another studies found that CRC cases with Dukes A stage had lower CP levels compared to Dukes B–D stages [9, 27].

5. Conclusions

Although, the reason for CRC patients have increased serum CP levels is not known, our obtained findings demonstrated that serum CP has a potential association with CRC development and, interestingly, its elevated levels were related to CRC severity and aggressiveness behavior. Thus, it could be helpful in preventing poor disease progression.

Table 2. Correlation between calprotectin and tumor features

Factor correlated with calprotectin	Correlation coefficient	*P* value
Tumor stage	0.273	0.006
Tumor grade	0.406	0.0001
Lymph node invasion	0.210	0.043
Distant metastasis	0.201	0.042
Tumor size	0.363	0.011
CEA	0.399	0.002

Pearson correlation was used for variables with interval scale, while Spearman correlation was used for variables with ordinal scales.

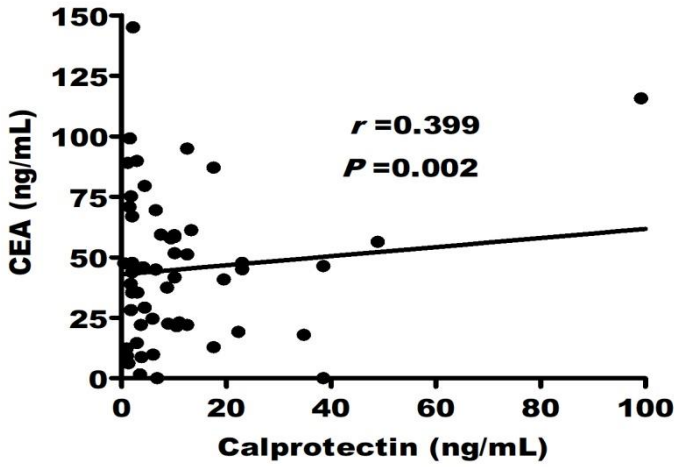

Figure 4. Correlation between serum calprotectin and CEA levels in colorectal cancer patients.

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Disclosure statement

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References

[1] Roshandel G, Ghasemi-Kebria F, Malekzadeh R. Colorectal Cancer: Epidemiology, Risk Factors, and Prevention. *Cancers (Basel)*. 2024;16(8):doi: 10.3390/cancers16081530

[2] Chen K, Collins G, Wang H, Toh JWT. Pathological Features and Prognostication in Colorectal Cancer. *Curr Oncol*. 2021;28(6):5356-5383. doi: 10.3390/curroncol28060447

[3] Roushdy DA, shaker og, Shousha WG, Fouda MS, Diab KR, Abdo SM. The dual effect of the long non coding RNA hoxa transcript at the distal tip and miR-216a on colorectal cancer. *Egyptian Journal of Chemistry*. 2024;doi: 10.21608/ejchem.2024.293873.9788

[4] Escrich V, Romero-Aranda C, López R, de Toro M, Metola Á, Ezcurra B, et al. Unprocessed snRNAs Are a Prognostic Biomarker and Correlate with a Poorer Prognosis in Colorectal Cancer. *Cancers (Basel)*. 2024;16(13):doi: 10.3390/cancers16132340

[5] Kasprzak A. Prognostic Biomarkers of Cell Proliferation in Colorectal Cancer (CRC): From Immunohistochemistry to Molecular Biology Techniques. *Cancers (Basel)*. 2023;15(18):doi: 10.3390/cancers15184570

[6] Keller D, Mester P, Räth U, Krautbauer S, Schmid S, Greifenberg V, et al. Calprotectin, a Promising Serological Biomarker for the Early Diagnosis of Superinfections with Multidrug-Resistant Bacteria in Patients with COVID-19. *Int J Mol Sci*. 2024;25(17):doi: 10.3390/ijms25179294

[7] Pruenster M, Vogl T, Roth J, Sperandio M. S100A8/A9: From basic science to clinical application. *Pharmacol Ther*. 2016;167(120-131. doi: 10.1016/j.pharmthera.2016.07.015

[8] Blad N, Palmqvist R, Karling P. Pre-diagnostic faecal calprotectin levels in patients with colorectal cancer: a retrospective study. *BMC Cancer*. 2022;22(1):315. doi: 10.1186/s12885-022-09440-4

[9] Kristinsson J, Armbruster CH, Ugstad M, Kriwanek S, Nygaard K, Tøn H, et al. Fecal excretion of calprotectin in colorectal cancer:

relationship to tumor characteristics. *Scand J Gastroenterol*. 2001;36(2):202-7. doi: 10.1080/003655201750065979

[10] Luley K, Noack F, Lehnert H, Homann N. Local calprotectin production in colorectal cancer and polyps--active neutrophil recruitment in carcinogenesis. *Int J Colorectal Dis*. 2011;26(5):603-7. doi: 10.1007/s00384-011-1165-0

[11] Greene FL. TNM staging for malignancies of the digestive tract: 2003 changes and beyond. *Semin Surg Oncol*. 2003;21(1):23-9. doi: 10.1002/ssu.10018

[12] Zheng J, Jin H, Tu Y. Differences in circulating lymphocyte subpopulations among patients with inflammatory polyps, colorectal adenomas and colorectal cancer. *Arab J Gastroenterol*. 2024;25(2):129-134. doi: 10.1016/j.ajg.2023.12.013

[13] Khan U, Chowdhury S, Billah MM, Islam KMD, Thorlacius H, Rahman M. Neutrophil Extracellular Traps in Colorectal Cancer Progression and Metastasis. *Int J Mol Sci*. 2021;22(14):doi: 10.3390/ijms22147260

[14] Kim SH, Park DH, Lim YJ. Impact of Diet on Colorectal Cancer Progression and Prevention: From Nutrients to Neoplasms. *Korean J Gastroenterol*. 2023;82(2):73-83. doi: 10.4166/kjg.2023.079

[15] Ross FA, Park JH, Mansouri D, Combet E, Horgan PG, McMillan DC, et al. The role of faecal calprotectin in diagnosis and staging of colorectal neoplasia: a systematic review and meta-analysis. *BMC Gastroenterol*. 2022;22(1):176. doi: 10.1186/s12876-022-02220-1

[16] Attallah AM, Ismail H, Ibrahim GG, Abdel-Raouf M, El-Waseef AM, Abdel-Wahab M. Use of a novel enzyme immunoassay based on detection of circulating antigen in serum for diagnosis of Helicobacter pylori infection. *Clin Diagn Lab Immunol*. 2004;11(4):775-9. doi: 10.1128/cdli.11.4.775-779.2004

[17] Kalla R, Kennedy NA, Ventham NT, Boyapati RK, Adams AT, Nimmo ER, et al. Serum Calprotectin: A Novel Diagnostic and Prognostic Marker in Inflammatory Bowel Diseases. *Am J Gastroenterol*. 2016;111(12):1796-1805. doi: 10.1038/ajg.2016.342

[18] Sincan G, Ayvaz E, Erdem F, Kiziltunç A. The importance of serum calprotectin level in patients with lymphoma. *Iraqi Journal of Hematology*. 2023;12(1):doi:

[19] Tabur S, Korkmaz H, Özkaya M, Elboğa U, Tarakçıoglu M, Aksoy N, et al. Serum calprotectin: a new potential biomarker for thyroid papillary carcinoma. *Tumour Biol*. 2015;36(10):7549-56. doi: 10.1007/s13277-015-3468-1

[20] Petsa A, Pergialiotis V, Konstantopoulos P, Katsichti A, Petrou K, Giannopoulos A, et al. Serum Calprotectin and Prealbumin Levels among Ovarian Cancer Patients Aged 30 - 45 Years. *Open Journal of Obstetrics and Gynecology*. 2017;07(303-311. doi: 10.4236/ojog.2017.73032

[21] Baydar E, Celikkol A, GÜRdal S, Seber S. Predictive Value of Serum Calprotectin Level in Response to Treatment, a New Inflammatory Marker in Patients with Breast Cancer Requesting Neoadjuvant Treatment. *Namık Kemal Tıp Dergisi*. 2023;11(12-16. doi: 10.4274/nkmj.galenos.2023.61587

[22] Saviano A, Migneco A, Brigida M, Petruzziello C, Zanza C, Savioli G, et al. Serum Calprotectin in the Evaluation of Gastrointestinal Diseases: An Ace up Your Sleeve? *Medicina (Kaunas)*. 2024;60(5):doi: 10.3390/medicina60050762

[23]Mori A, Mitsuyama K, Sakemi R, Yoshioka S, Fukunaga S, Kuwaki K, et al. Evaluation of Serum Calprotectin Levels in Patients with Inflammatory Bowel Disease. *Kurume Med J*. 2021;66(4):209-215. doi: 10.2739/kurumemedj.MS664009

[24] Okada K, Okabe M, Kimura Y, Itoh H, Ikemoto M. Serum S100A8/A9 as a Potentially Sensitive Biomarker for Inflammatory Bowel Disease. *Lab Med*. 2019;50(4):370-380. doi: 10.1093/labmed/lmz003

[25] Bayo Calero J, Castaño López MA, Casado Monge PG, Díaz Portillo J, Bejarano García A, Navarro Roldán F. Analysis of blood markers for early colorectal cancer diagnosis. *J Gastrointest Oncol*. 2022;13(5):2259-2268. doi: 10.21037/jgo-21-747

[26] Lehmann FS, Trapani F, Fueglistaler I, Terracciano LM, von Flüe M, Cathomas G, et al. Clinical and histopathological correlations of fecal calprotectin release in colorectal carcinoma. *World J Gastroenterol*. 2014;20(17):4994-9. doi: 10.3748/wjg.v20.i17.4994

[27] Karl J, Wild N, Tacke M, Andres H, Garczarek U, Rollinger W, et al. Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers. *Clin Gastroenterol Hepatol*. 2008;6(10):1122-8. doi: 10.1016/j.cgh.2008.04.021