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ORIGINAL ARTICLE

Association of Endocrine, Metabolic, and Trace-Element Profiles with Stage I–III Colon Cancer in Iraqi Male Patients: A Cross-Sectional Case-Control Study

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ABSTRACT

Colorectal cancer is often associated with endocrine and metabolic disturbances. These alterations reflect interactions between the tumor and the host. The aim of this study was to evaluate hormonal, metabolic, and trace-element profiles in male patients with colon cancer (stages I–III). The associations between these biomarkers and disease stage were also examined. In this cross-sectional, case-control study, 300 histopathologically confirmed male patients with colon cancer (stages I–III) and 100 age-matched healthy controls (50–70 years) were enrolled. Serum levels of thyroid hormones (T3, T4, TSH), parathyroid hormone (PTH), parathyroid hormone–related peptide (PTHrP), calcium, vitamin D, insulin-like growth factor-1 (IGF-1), β-catenin, ferritin, and trace elements (Zn, Cu, Se, Mg, Mn) were measured using enzyme-linked immunosorbent assay and atomic absorption spectrophotometry. Statistical analyses including one-way ANOVA with post hoc testing, false discovery rate correction, correlation analysis, principal component analysis, and multivariate regression were used. Several endocrine and metabolic markers differed significantly between cancer patients and controls. Compared with controls, cancer patients showed thyroid dysfunction, reduced PTH with elevated calcium and PTHrP, pronounced vitamin D deficiency, and higher IGF-1 and β-catenin levels. Moreover, trace-element analysis revealed reduced zinc, selenium, magnesium, manganese, and ferritin levels alongside elevated copper concentrations. Multivariate analyses revealed stage-related biomarker patterns and complex relationship among markers. Colon cancer in men was associated with coordinated endocrine, metabolic, and trace-element alterations detectable from early stages, supporting the value of integrated biomarker profiling.

Keywords: β-Catenin, IGF-1, Colon Cancer, Diagnostic Biomarkers, Male Patients

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Introduction

Colorectal cancer (CRC) is highly prevalent and remains a leading cause of cancer-related mortality worldwide, however it is potentially preventable through effective screening and early detection (1). It arises from the colon lining and, in most cases, initially presents as benign adenomatous polyps that may transform into malignant lesions over time (2,3). CRC develops through genetic and epigenetic alterations and is influenced by environmental factors. While most CRC cases arise without a family history, inherited conditions such as familial adenomatous polyposis and Lynch syndrome increase susceptibility to CRC (4). In addition, lifestyle factors including high consumption of red and processed meat, low fiber intake, obesity, physical inactivity, smoking, and excessive alcohol use contribute to disease risk (5,6).

Chronic inflammation plays an important role in colorectal carcinogenesis. Similar to molecular alterations that have been reported to several malignancies, the adenoma–carcinoma sequence reflects the gradual accumulation of molecular alterations, including mutations in *APC* and *KRAS*, dysregulation of *p53*, and abnormalities in the Wnt signaling pathway (7,8). In addition, molecular features such as the CpG island methylator phenotype and microsatellite instability have been studied and have been shown that may influence disease behavior and response to therapy (9).

Early detection is critical for improving patient outcomes. In this regards, colonoscopy remains the gold standard for detecting precancerous lesions (10). Non-invasive stool-based tests serve as a valuable alternative, particularly for initial screening phases (11). Treatment strategies are determined primarily by the disease stage. Early-stage colon cancer is managed through surgery. In contrast, advanced stages are treated with adjuvant chemotherapy, targeted therapy, or immunotherapy (12). Clear clinical benefits have been reported with immunotherapy in selected patient groups. This effect is particularly evident in tumors characterized by microsatellite instability–high (MSI-H) status or mismatch repair deficiency (dMMR) (13). Recent advances in molecular profiling have supported the use of precision medicine strategies. With these approaches, treatment can be tailored to the specific molecular characteristics of each tumor (14). In addition to tumor genomics, biochemical alterations may also reflect interactions between the tumor and the host. Therefore, tumor behavior is influenced not only by genetic changes but also by systemic metabolic and biochemical disturbances. Trace elements such as zinc, selenium, copper, magnesium, and iron are known to play important roles in colorectal cancer biology. These elements participate in several essential cellular processes, such as the regulation of oxidative stress, control of cell proliferation, maintenance of DNA repair mechanisms, and modulation of immune responses. Several clinical studies have reported reduced circulating levels of zinc and selenium in patients with colorectal cancer, whereas copper levels are often elevated. These findings suggest that disturbances in trace-element balance may contribute to tumor development and disease progression (15).

Although endocrine markers, vitamins, and trace elements have been studied individually in colorectal cancer, integrated analyses remain limited. This is particularly true in locally characterized populations where combined endocrine–metabolic–trace element profiles are examined in relation to clinicopathologic stages I–III within a standardized dataset. In addition, the simultaneous evaluation of thyroid and parathyroid hormones, vitamin D level, IGF-1, β-catenin, and trace-element profiles has not been systematically investigated in Iraqi male patients with colon cancer. In the present study, these biomarkers were integrated and analyzed within a single standardized cohort of Iraqi male patients with colon cancer.

Methods

Study Design and Participants

Over a one-year period, we conducted a cross-sectional case–control study to survey endocrine, metabolic, and trace-element profiles in male patients with CRC. A total of 400 men aged 50–70 years were included in the study. The cohort consisted of 300 patients with colon cancer and 100 healthy controls. Patients were recruited from several oncology centers across Iraq. All cancer cases were histopathologically confirmed using biopsy or surgical specimens to ensure diagnostic accuracy. Tumor staging was performed according to the 2012 American Joint Committee on Cancer (AJCC) TNM classification system. Patients were recruited from multiple oncology centers across Iraq. All cancer cases were histopathologically confirmed using biopsy or surgical specimens to ensure

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diagnostic accuracy. Tumors were staged according to the 2012 American Joint Committee on Cancer (AJCC) TNM classification system. Based on pathological staging, patients were categorized into stage I (n = 100), stage II (n = 100), and stage III (n = 100). Stage I included T1–T2 tumors without lymph node involvement (N0) or distant metastasis (M0). Stage II comprised T3–T4 tumors without nodal involvement (N0) or metastasis (M0). Stage III included tumors of any T category with regional lymph node involvement (N1–N2) but no distant metastasis (M0). Control subjects were healthy adult males recruited through community outreach programs

Inclusion and Exclusion Criteria

Eligible participants were male individuals aged between 50 and 70 years. Cancer patients were required to have a histopathologically confirmed diagnosis of stages I–III colon cancer and to be treatment-naïve at the time of blood sampling (no prior chemotherapy, radiotherapy, immunotherapy, or targeted therapy). Control participants were required to have no prior history of malignancy. They were required to have no history of malignancy and no evidence of colorectal polyps, adenomas, or other neoplastic lesions, as confirmed by screening colonoscopy.

Participants were excluded if they had chronic systemic diseases, including diabetes mellitus, hypertension, chronic kidney disease (stage 3–5), chronic liver disease, cardiovascular disease, metabolic disorders, autoimmune disease, or urinary tract disorders. Additional exclusion criteria included acute or chronic inflammatory or infectious conditions; prior colorectal polyps, adenomas, or inflammatory bowel disease; use of medications known to affect endocrine, thyroid, calcium, vitamin D, or trace-element homeostasis (e.g., steroids, hormone therapy, or chelating agents); and incomplete clinical or laboratory data. Individuals receiving medications known to affect thyroid function, calcium metabolism, vitamin D status, or trace-element homeostasis were also excluded. These criteria were applied to minimize potential confounding effects on endocrine and metabolic parameters.

Ethical Considerations

The study protocol was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the Iraqi Ministry of Health (ethical approval code: UT879; approval date: 3 November 2023; file number: A-10-8821-1). Written informed consent was obtained from all participants prior to enrollment. Confidentiality of personal data and anonymity of samples were ensured throughout the study.

Sample Collection and Processing

After an overnight fasting period of at least 12 hours, 10 mL of venous blood was collected aseptically from each participant into plain vacutainer tubes without anticoagulant. Samples were allowed to clot at room temperature for approximately 30 minutes and were subsequently centrifuged at 3000 × g for 10 minutes. Serum was carefully separated and aliquoted.

For biochemical and trace-element analyses, 200 µL of serum was transferred into trace-element–free polypropylene microcentrifuge tubes and stored at −80 °C until analysis. To minimize trace-element contamination, all glassware and plastic consumables were pre-treated with 10% nitric acid, thoroughly rinsed with deionized water, and air-dried prior to use.

Biomarker Selection

Biomarkers were selected based on their established or proposed roles in colorectal carcinogenesis, endocrine dysregulation, calcium homeostasis, oxidative stress, and tumor-associated metabolic alterations. The evaluated parameters included components of the thyroid axis (triiodothyronine, thyroxine, and thyroid-stimulating hormone), markers of calcium metabolism (parathyroid hormone, parathyroid hormone–related peptide, and serum calcium), growth and signaling markers (vitamin D, insulin-like growth factor 1, and β-catenin), trace elements (zinc, copper, selenium, magnesium, and manganese), and ferritin as an indicator of iron metabolism.

Biochemical and Hormonal Assays

Serum concentrations of triiodothyronine, thyroxine, thyroid-stimulating hormone, parathyroid hormone, vitamin D, testosterone, and cortisol were measured using commercially available enzyme-linked immunosorbent assay kits obtained from DRG Instruments GmbH (Germany). Serum levels of insulin-like growth factor 1 (IGF-1), β-catenin, and parathyroid hormone–related peptide (PTHrP) were

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measured using ELISA kits (Elabscience Biotechnology; USA). Serum testosterone and cortisol concentrations were assessed using ELISA kits (DRG Instruments GmbH, Germany), according to the manufacturers' instructions. Serum testosterone and cortisol levels were measured using ELISA kits (DRG Instruments GmbH, Germany) according to the manufacturer's instructions. Testosterone was evaluated as an indicator of gonadal endocrine function. Cortisol was measured as a marker of hypothalamic–pituitary–adrenal (HPA) axis activity and systemic stress. All samples were analyzed in duplicate. Absorbance was recorded at 450 nm using a microplate reader (BioTek Instruments, USA). Standard curves were prepared using the supplied standards, and concentrations were calculated from these curves. Quality control samples at low, medium, and high concentrations were included in each assay run. The intra-assay and inter-assay coefficients of variation were kept below 10% to ensure reliable measurements.

Determination of Trace Elements by Atomic Absorption Spectrophotometry

Serum levels of zinc, copper, selenium, magnesium, and manganese were measured using atomic absorption spectrophotometry (PerkinElmer Analyst 400; USA). Serum iron was measured using a colorimetric enzymatic assay based on the ferrozine method, according to the manufacturer's instructions (Roche Diagnostics). Before analysis, serum samples were diluted 1:5 with 0.1% nitric acid. The mean recovery ranged from 95% to 105%, indicating good accuracy. The relative standard deviation was below 5%, showing good precision. The detection limits ranged from 0.001 to 0.01 µg/mL, depending on the element

Statistical Analysis

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., USA). Data distribution was assessed using the Shapiro–Wilk test. Homogeneity of variances was evaluated with Levene's test. Continuous variables were expressed as mean ± standard deviation. Group differences were analyzed using one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's post hoc test was used for pairwise comparisons. Effect sizes were estimated using partial eta squared. To reduce the risk of type I error across the biomarker panel, multiple comparisons were controlled using the Benjamini–Hochberg false discovery rate correction when appropriate. Associations between biomarkers were assessed using Pearson's correlation coefficient. These relationships were visualized using correlation heatmaps. Principal component analysis was performed to explore multivariate relationships among biomarkers. The variance explained by each component was reported. Multivariate linear regression analysis was used to examine independent associations between selected biomarkers and cancer stage after adjustment for age. A p-value < 0.05 was considered statistically significant.

Results

Thyroid Function Parameters

Serum concentrations of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) are summarized in Table 1. Statistically significant differences were observed among the four study groups for all thyroid parameters (one-way ANOVA, $p < 0.05$). Mean serum T3 levels differed significantly across groups. The lowest values observed in the control group and stage I patients, with progressively elevated concentrations recorded in stages II and III. Significant intergroup variation was also observed on serum T4 levels; mean concentrations were lower in stage I patients than in controls, while intermediate values were observed in stages II and III. Serum TSH concentrations were significantly elevated in all cancer stages compared with controls, with peak mean levels observed in stage III patients. Significant pairwise differences between cancer stages and controls for TSH were confirmed by post hoc analysis. Furthermore, stage-dependent differences in T3 and T4 were identified (Table 1.) A moderate to large effect of disease stage on TSH levels was identified by effect size analysis.

Parathyroid Hormone, PTHrP, and Calcium Levels

Serum concentrations of PTH, PTHrP, and calcium are presented in Table 2. Significant differences among groups were observed for all three parameters ($p < 0.05$). PTH levels were significantly lower in all colon cancer groups compared with controls, with the lowest mean concentrations recorded

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in stage I patients. In contrast, serum calcium levels were significantly higher in cancer patients than in controls, with the highest mean values observed in stage III patients. Serum PTHrP concentrations were markedly elevated in all cancer stages relative to controls. While no statistically significant differences between stages I and II, stage III exhibited slightly lower concentrations; however, theses remained significantly elevated levels compared with controls. Significant differences between each cancer group and controls were confirmed for PTH, calcium, and PTHrP using a Post hoc comparisons analysis. Furthermore, a strong association between disease status and alterations in calcium homeostasis markers was indicated by effect size estimates.

Table 1. Thyroid function parameters in colon cancer patients and healthy controls

Group	n	T3 (ng/mL)	T4 (nmol/L)	TSH (µIU/mL)
Stage I	100	2.37 ± 0.11	82.11 ± 7.73	8.57 ± 1.13
Stage II	100	3.61 ± 0.10	102.72 ± 11.11	8.30 ± 1.24
Stage III	100	5.66 ± 0.17	111.86 ± 13.29	5.92 ± 0.62
Controls	100	1.25 ± 0.27	117.69 ± 14.75	2.74 ± 0.09

Table 2. Parathyroid hormone, calcium, and PTHrP levels in study groups

Group	n	PTH (pg/mL)	Calcium (mg/dL)	PTHrP (ng/mL)
Stage I	100	14.73 ± 2.46	11.67 ± 1.34	11.02 ± 0.99
Stage II	100	16.81 ± 3.55	11.20 ± 2.07	10.75 ± 0.74
Stage III	100	23.95 ± 4.44	10.29 ± 1.90	8.60 ± 0.69
Controls	100	56.83 ± 3.98	9.08 ± 2.48	4.20 ± 0.70

Values are presented as mean ± standard deviation.Group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Statistically significant differences were observed among groups for all parameters ($p < 0.05$).

Vitamin D, IGF-1, and β-Catenin

Serum levels of vitamin D, IGF-1, and β-catenin are shown in Table 3. We observed significant differences among study groups for all three biomarkers ($p < 0.05$). Vitamin D concentrations were significantly lower in all colon cancer stages compared with controls.

However, no significant differences were observed among stages I, II, and III, suggesting a consistent reduction across disease stages. Serum IGF-1 levels were significantly higher in stages I and II compared with controls. However, intermediate values were observed in stage III, though these remained significantly higher than control levels. Conversely, β-Catenin concentrations peaked in stage I and demonstrated a gradual decrease across stages II and III, while remaining significantly elevated than control levels. Post hoc testing confirmed significant differences between cancer groups and controls for all three markers. Effect size analysis suggested a moderate effect of disease stage on IGF-1 and β-catenin levels.

Trace Elements and Ferritin

Serum concentrations of zinc, copper, selenium, magnesium, manganese, and ferritin are presented in Table 4. Significant intergroup differences were observed for all measured elements ($p < 0.05$).

Zinc levels were significantly lower in all cancer stages compared with controls, with the lowest concentrations in stage III patients. Copper concentrations were significantly higher in all cancer groups compared to controls and demonstrated a progressive increase with advancing stage. Selenium levels were significantly lower in cancer patients compared with controls, with a stage-dependent decline. Ferritin concentrations were also significantly decreased in all cancer groups relative to controls. Magnesium levels were significantly lower in cancer patients than in controls, although differences among

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cancer stages were less noticeable. Manganese concentrations were also significantly reduced in all cancer stages compared with controls. Post hoc analyses confirmed significant differences between cancer groups and controls for all trace elements and ferritin. Effect size estimates showed large effects for copper, zinc, selenium, and ferritin.

Serum Iron, Testosterone, and Cortisol

Serum iron, testosterone, and cortisol levels were evaluated to enhance complement endocrine and metabolic profiling and to support subsequent multivariate analyses. Descriptive statistics are summarized in Table 5. Serum iron, testosterone, and cortisol levels differed between colon cancer patients and healthy controls, and showed associations with key disease-related biomarkers. Serum testosterone concentrations were reduced in cancer patients relative to controls, while serum cortisol levels were higher in cancer patients. These differences were generally consistent across cancer stages, although stage-dependent gradients were less pronounced than those observed for other endocrine and metabolic biomarkers.

Table 3. Vitamin D, IGF-1, and β-catenin concentrations

Group	n	Vitamin D (ng/mL)	IGF-1 (ng/mL)	β-catenin (ng/mL)
Stage I	100	17.12 ± 2.89	34.10 ± 6.91	0.57 ± 0.13
Stage II	100	16.85 ± 3.84	27.53 ± 5.29	0.48 ± 0.10
Stage III	100	16.64 ± 2.79	23.47 ± 4.32	0.43 ± 0.09
Controls	100	32.91 ± 2.64	21.65 ± 4.98	0.35 ± 0.08

Values are mean ± SD. All biomarkers differed significantly between cancer patients and controls ($p < 0.05$). No significant differences in vitamin D levels were observed among cancer stages.

Table 4. Serum trace elements and ferritin concentrations

Group	n	Zn (ng/mL)	Cu (ng/mL)	Se (ng/mL)	Mg (ng/mL)	Mn (ng/mL)	Ferritin (ng/mL)
Stage I	100	0.62 ± 0.09	1522 ± 32	66.21 ± 5.45	16545 ± 105	2.45 ± 0.96	6.11 ± 1.65
Stage II	100	0.44 ± 0.08	1601 ± 40	41.21 ± 4.98	13501 ± 85	1.91 ± 0.54	5.24 ± 1.52
Stage III	100	0.23 ± 0.06	1695 ± 45	31.21 ± 4.69	12011 ± 55	1.45 ± 0.34	3.11 ± 0.92
Controls	100	1.01 ± 0.17	1200 ± 189	120.43 ± 23.67	22553 ± 2005	9.15 ± 3.22	137 ± 19.54

Values are mean ± SD. Significant differences were observed between cancer patients and controls for all trace elements and ferritin ($p < 0.05$).

Table 5. Pearson correlation coefficients among selected biomarkers

Parameter	Fe	Zn	Cu	Mg	Se	Ca	Ferritin	Vitamin D	Testosterone	Cortisol
Iron (Fe)	1	−0.03	0.34	0.21	−0.72	0.13	−0.55	0.64	0.46	−0.17
Zinc (Zn)		1	−0.03	−0.62	−0.32	−0.04	−0.42	−0.28	0.06	0.00
Copper (Cu)			1	−0.57	−0.51	0.72	0.03	−0.48	−0.08	−0.19
Magnesium (Mg)				1	0.87	0.70	−0.34	−0.09	−0.25	0.00
Selenium (Se)					1	0.26	−0.41	0.23	−0.14	−0.04
Calcium (Ca)						1	−0.37	−0.08	−0.63	−0.18

Values represent Pearson's correlation coefficients (r). Statistical significance was assessed after false discovery rate (FDR) correction. Only correlations with adjusted $p < 0.05$ were considered significant and visualized in the heatmap (Figure 1).

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Correlation Analysis

Pearson correlation analysis was performed to examine relationships among endocrine, metabolic, and trace-element parameters. Results are summarized in Table 5 and visualized using a correlation heatmap (Figure 1).

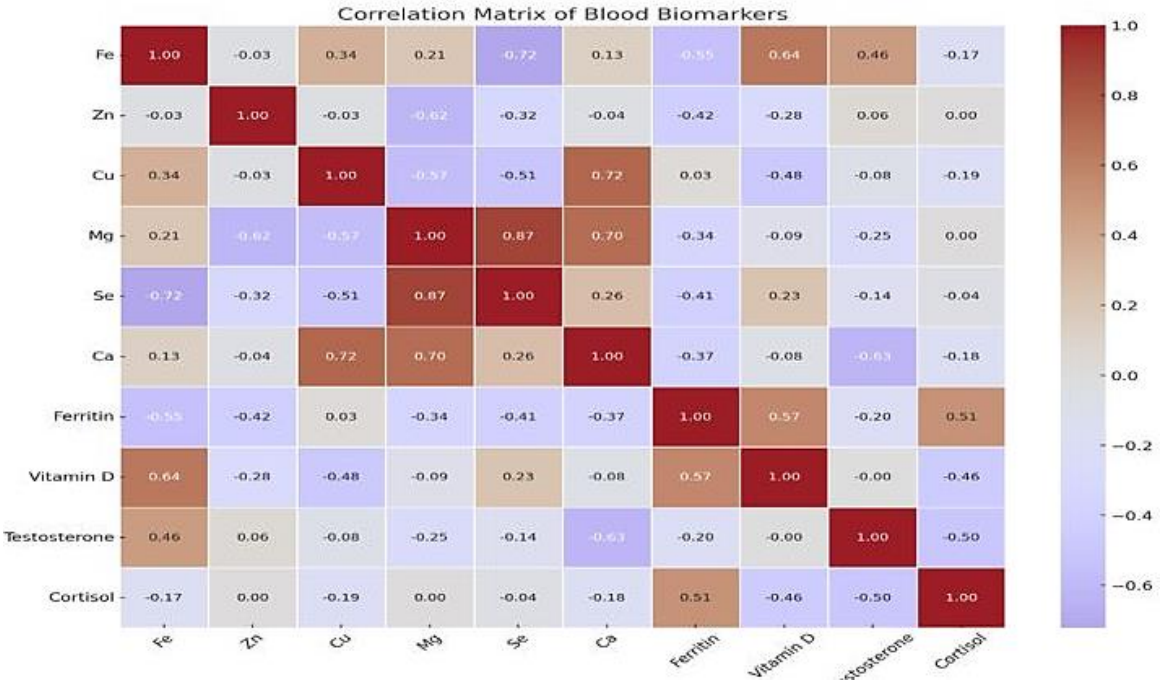

Figure 1. Correlation matrix of endocrine, metabolic, and trace-element biomarkers in colon cancer patients. The heatmap shows pairwise Pearson correlation coefficients (*r*) among selected serum biomarkers measured in male patients with colon cancer. The analyzed markers included iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg), selenium (Se), calcium (Ca), ferritin, vitamin D, testosterone, and cortisol. Correlation coefficients range from −1 to +1 and are shown using a color scale. Red represents positive correlations, whereas blue represents negative correlations. The number in each cell indicates the corresponding Pearson's *r*. Only correlations that remained statistically significant after false discovery rate (FDR) correction (adjusted $p < 0.05$) were included in the analysis. The matrix shows complex relationships among mineral metabolism, endocrine pathways, and stress-related hormones. These patterns supported the subsequent multivariate analyses.

Several statistically significant correlations were identified after false discovery rate correction. Positive correlations were observed between magnesium and selenium and between copper and calcium Negative correlations were identified between zinc and magnesium, iron and selenium, and calcium and testosterone. Ferritin showed a positive correlation with vitamin D and cortisol. Correlation patterns differed across biomarker categories, reflecting complex interrelationships among endocrine, metabolic, and trace-element parameters.

Principal Component Analysis

We used Principal component analysis (PCA) to examine multivariate patterns among the measured biomarkers. The first two principal components explained a substantial proportion of the total variance in the dataset (Figure 2). Biomarkers with similar correlation patterns clustered together, whereas those with opposing trends were located farther apart in the PCA space. The PCA plot showed partial separation between cancer patients and controls. Although the distributions of cancer stages overlapped, they displayed distinguishable patterns along the principal components.

Multivariate Regression Analysis

We also performed multivariate linear regression analyses to evaluate independent associations between selected biomarkers and cancer stage after adjustment

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for age. Results reveaked that cancer stage remained significantly associated with serum levels of calcium, copper, zinc, IGF-1, and β-catenin after adjustment. However, it has been observed that the age was not a significant predictor in most models.

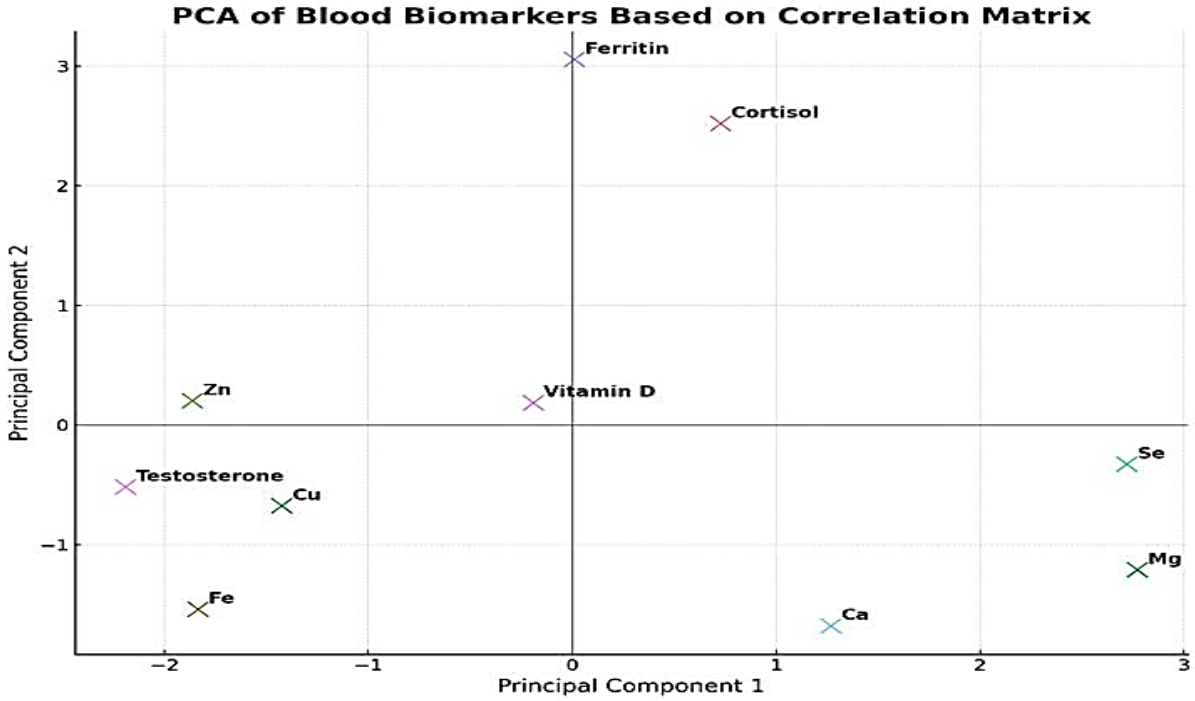

Figure 2. Principal component analysis of endocrine, metabolic, and trace-element biomarkers in colon cancer patients. Principal component analysis (PCA) was performed using the correlation matrix of serum biomarkers measured in male patients with colon cancer. The analyzed biomarkers included iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg), selenium (Se), calcium (Ca), ferritin, vitamin D, testosterone, and cortisol. The scatter plot shows the loadings of each biomarker on the first two principal components (PC1 and PC2). Together, these components explain a large proportion of the total variance in the dataset. Biomarkers located close to each other show similar correlation patterns. In contrast, biomarkers positioned far apart or in opposite quadrants indicate different relationships. The PCA shows clustering of mineral-related biomarkers and separation of endocrine and stress-related markers. This pattern reflects the multivariate structure underlying the observed biochemical alterations.

Discussion

This study provides an integrated evaluation of endocrine, metabolic, and trace-element alterations in male patients with stage I–III colon cancer. Moreover, the results of the study highlight stage-associated biochemical patterns and inter-marker relationships. By combining hormonal axes, growth signaling markers, vitamin D status, and trace elements within a single cohort, these findings offer a systems-level perspective on tumor–host metabolic interactions that goes beyond single-biomarker analyses.

Serum T3, T4, and TSH levels differed significantly among the study groups. Cancer patients, particularly those in advanced stages, showed higher TSH concentrations. Altered thyroid hormone metabolism has been linked to cancer progression and poorer prognosis. This may reflect adaptive endocrine responses to systemic illness or impaired peripheral hormone conversion (16-18). In non-thyroidal illness syndromes (NTIS), which often occur in severe or chronic diseases, normal feedback regulation of the hypothalamic–pituitary–thyroid axis is disrupted. This leads to abnormal TSH, T3, and T4 patterns that do not follow classical negative feedback mechanisms, such as changes in deiodinase activity or hormone transport (17-18). Although our cross-sectional study cannot establish causality, these findings suggest that thyroid

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axis disturbances accompany colorectal cancer. They likely reflect systemic illness or tumor-related stress rather than primary thyroid gland dysfunction.

Marked reductions in circulating PTH levels alongside elevated calcium and PTHrP concentrations were identified in colon cancer patients relative to controls. These findings are consistent with the well-established role of PTHrP in malignancy-associated hypercalcemia, whereby tumor-derived PTHrP mimics PTH activity at target tissues while suppressing endogenous PTH secretion. Although primary hyperparathyroidism has been occasionally associated with colorectal malignancy, most evidence supports a paraneoplastic mechanism mediated by PTHrP rather than intrinsic parathyroid dysfunction (19). These data support this paradigm, indicating that alterations in calcium metabolism are evident even in non-metastatic colon cancer.

Vitamin D levels were significantly lower in patients across all cancer stages compared with healthy controls. However, no significant differences were observed between the different stages of the disease. This observation is consistent with epidemiological and experimental studies indicating that vitamin D deficiency is associated with an increased risk of colorectal cancer and impaired cellular differentiation (20,21). The absence of stage-dependent variation suggests that vitamin D deficiency may represent a systemic host-related factor rather than a biomarker of tumor progression. While our findings show an association between low vitamin D levels and colon cancer, we acknowledge that without longitudinal or prospective follow-up data, it is not possible to determine whether vitamin D deficiency reflects a systemic host factor or a marker of tumor progression; this limitation aligns with prior observational research showing inverse vitamin D–CRC associations but inconsistent evidence on progression and survival outcomes in longitudinal cohorts and trials (22).

In contrast, IGF-1 and β-catenin levels showed stage-associated alterations. Elevated IGF-1 concentrations in cancer patients are consistent with its known role in promoting cell proliferation and inhibiting apoptosis through PI3K–AKT and MAPK signaling pathways (23,24). Similarly, increased circulating β-catenin levels may reflect activation of the Wnt/β-catenin pathway, a central driver of colorectal carcinogenesis and tumor progression (24,25), , however, the mechanisms underlying its presence in serum are not fully established. β-catenin is predominantly an intracellular/nuclear effector of canonical Wnt signaling; however, tumor cells can release β-catenin into the extracellular space associated with extracellular vesicles, including exosomes, which can carry intracellular proteins into circulation and protect them from degradation, whereas free β-catenin or degradation fragments may also circulate as proteolytic byproducts (26). Although primarily a signaling molecule, detection of β-catenin in serum may therefore reflect heightened tumor turnover or vesicle-mediated export from malignant tissue, consistent with findings in translational studies. Although immunohistochemistry (IHC) of tumor tissue remains the standard for evaluating β-catenin localization and intensity, we measured circulating β-catenin in serum to assess systemic tumor-associated protein alterations non-invasively, consistent with prior studies that have detected higher serum β-catenin levels in colorectal cancer patients and explored its potential as a biomarker of tumor biology (25).

Colon cancer patients showed significant reductions in zinc, selenium, magnesium, manganese, and ferritin levels, along with increased copper concentrations. These results are generally in line with previous studies, which have reported similar trace-element imbalances in colorectal cancer especially lower zinc and selenium and higher copper or Cu/Zn ratios (27-29). Zinc and selenium play key roles in antioxidant defense, DNA repair, and immune function. Deficiencies in these elements may worsen oxidative stress and increase genomic instability in cancer patients (30).

The observed increase in copper is notable because copper plays important roles in angiogenesis, redox signaling, and tumor metabolism (29). Recent studies suggest that copper homeostasis is closely linked to cancer cell survival and may represent a potential therapeutic target (29). Although inflammation often leads to elevated ferritin levels, the reduced ferritin observed in cancer patients may reflect increased iron use by proliferating tumor cells or disrupted iron storage (29). In the present study, correlation and principal component analyses revealed complex multivariate relationships among endocrine, metabolic, and trace-element markers. Positive correlations between magnesium and selenium, and between copper and calcium, suggest coordinated regulation of mineral

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metabolism in the tumor microenvironment. In addition, negative correlations involving zinc, iron, and selenium further support the idea of competitive or compensatory trace-element dynamics in cancer (28). Principal component analysis showed partial separation between cancer patients and controls. This supports the idea that integrated biomarker profiles capture disease-associated metabolic states more effectively than individual markers. These multivariate patterns highlight the potential of combined biomarker panels to improve our understanding of tumor–host interactions, although validation in independent cohorts is essentially needed.

These findings show that important endocrine, metabolic, and trace-element changes occur even in early-stage colon cancer. However, several limitations should be considered. First, the cross-sectional design does not allow conclusions about causality or prognosis. Second, only male participants were included, which limits the generalizability of the results to women. In addition, the control group was defined based on clinical history rather than longitudinal molecular monitoring. Another limitation is that key molecular tumor features such as microsatellite instability and oncogenic mutations (e.g., *KRAS* and *BRAF*) were not evaluated. These factors may influence systemic biomarker profiles.

Future studies should include longitudinal follow-up, molecular stratification, and functional analyses. These approaches may help clarify the diagnostic, prognostic, and therapeutic value of these biomarkers.

Ultimately, the finding of the present study emphasized again that colon cancer in men is marked by coordinated disturbances across endocrine axes, pathways of growth signaling, calcium homeostasis, and trace-element concentrations. These alterations are detectable even in early stages. They include thyroid dysfunction, PTHrP-related calcium dysregulation, reduced vitamin D, elevated IGF-1 and β-catenin, and trace-element imbalances, collectively reflecting systemic metabolic remodeling during tumorigenesis. Integrated biomarker profiling revealed stage-associated patterns and complex inter-marker relationships that were not evident from single-marker analyses. Despite the cross-sectional design and lack of molecular stratification, these findings support future longitudinal and mechanistic studies that combine endocrine–metabolic panels with molecular tumor features to enhance biological understanding and guide biomarker development.

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