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Abstract

Colorectal cancer (CRC) is a disease that begins exclusively in the colon or rectum and is caused by the abnormal growth of glandular epithelial cells within the colon. Homocysteine, vitamin B6, and vitamin D are recognized to play a significant role in colorectal tumor pathogenesis. The aim of this study to investigate serum levels of Hcy, vitamin D and vitamin B6in colorectal cancer.

Keywords

Homocysteine, vitamin B6, Vitamin D, Colorectal cancer

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RESEARCH ARTICLE

Investigation of Homocysteine, Vitamin B_6 and Vitamin D among Patients with Colorectal Cancer

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ABSTRACT

Background and objectives: Colorectal cancer (CRC) is a disease that begins exclusively in the colon or rectum and is caused by the abnormal growth of glandular epithelial cells within the colon. Homocysteine, vitamin B_6, and vitamin D are recognized to play a significant role in colorectal tumor pathogenesis. The aim of this study to investigate serum levels of Hcy, vitamin D and vitamin B_6 in colorectal cancer.

Methods: The current study investigated the vital roles of homocysteine, vitamin B_6 and vitamin D in cancer development by comparing serum levels of colorectal cancer patients with those of the healthy control group at Nanakaly hospital. The levels of serum homocysteine (Hcy), vitamin B_6 and vitamin D in 50 colorectal cancer patients and 50 from the healthy control group have been examined.

Results: The result shows significantly high values of serum homocysteine ($p<0.0001$) and significant decreases in values of vitamin B_6 and vitamin D ($p<0.0001$and 0.0010) respectively, in patients with CRC when compared with the healthy group.

Conclusions: Our findings demonstrate that increased levels of homocysteine and decreased levels of vitamin B_6 and vitamin D could be associated with the pathogenesis and progression of colorectal cancer.

Key Words: Homocysteine, vitamin B_6, Vitamin D, Colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) is the third most common type of cancer diagnosed. It is the second most frequent cancer in men, after prostatic adenocarcinoma, and the third most prevalent cancer in women, after breast adenocarcinoma (Torre et al., 2015, Ferlay et al., 2019, Morimoto et al., 2022). According to the latest Global Cancer Statistics report, in 2020 there will be roughly 2 million new cases of CRC and 935,000 fatalities (Sung et al., 2021). CRC is becoming an increasingly significant public health issue, particularly in wealthy countries (Arem et al., 2013). In prospective epidemiologic studies, markers of obesity , inflammation, and diabetes were connected to an increased risk of CRC (Tarasiuk et al., 2018).

Homocysteine (Hcy), an amino acid containing sulfur, is generated as a metabolic intermediate by many cells in the body as a yield of demethylation during the metabolism of methionine. Plasma levels of Hcy are controlled by genetic variations of its major metabolic enzymes (Phelip et al., 2008, Mühl et al., 2022). Methylenetetrahydrofolate reductase is a critical enzyme in Hcy metabolism, regulating intracellular folate pools for the production and methylation of deoxyribonucleic acid. Vitamins B_6, Vitamin B_9, and B_{12}, are essential for the chemical processes required for the breakdown of Hcy. Elevated total homocysteine may serve as an important indicator of cancer and a sensitive indicator of recurrence. The correlation between serum levels of homocysteine and several carcinogenesis markers in breast, colon , and pancreatic cancers suggests that total serum Hcy levels, such as malignancy markers, indicate the activity of cancer cells or the rate of fast proliferation of malignancy cells (Mendonça et al., 2018). In addition, cell tissue culture research indicated that the growth of tumor cells causes hyperhomocysteinemia (Keshteli et al., 2015, Mühl et al., 2022).

Vitamin B_6 (Vit.B_6) or pyridoxine is a water-soluble vitamin that is crucial for cellular metabolism in general (Hellmann and Mooney, 2010). Pyridoxal 5-phosphate (PLP), the active form of vitamin B_6, is provided as a co-factor in over 150 enzymatic reactions that act as catalysts in important metabolic reactions, such as production, transformation, and destruction of amino acids, production of one carbon unit, trans-sulfuration, formation of tetrapyrrolic substances and polyamines, biosynthesis, and degradation of neuro-transmitters (Di Salvo et al., 2011). Vitamin B_6 can neutralize oxygen reactive species (ROS) while not being classified as a typical antioxidant (Mascolo and Vernì, 2020). Additionally, vitamin B_6 inhibits angiogenesis, lowers nitric oxide, and decreases oxidative stress, all of which are associated with suppressing tumorigenesis. Decreased vitamin B_6 levels may also be connected with chronic inflammation, a potentially colon cancer risk factor, depending on the significantly lower PLP levels found in patients with rheumatoid arthritis and inflammatory bowel disease (IBD) in comparison to the general population (Zhang et al., 2013, Gylling et al., 2017).

Vitamin D (Vit.D), a fat-soluble vitamin that can be generated in the skin by the sun's radiation or taken through food or supplementation. The liver converts both vitamin D isoforms, which are obtained through diet, supplements, or skin production, into 25(OH)D, the main form of vitamin D that circulates in the body. A 25(OH)D blood test is considered the most accurate predictor of vitamin D levels for a variety of outcomes, including the development of carcinoma (Hernández-Alonso et al., 2021). The high incidence of CRC is closely related to Vit.D deficiency, and Vit.D supplements can prevent the onset and progression of colorectal malignancy (Vaughan-Shaw et al., 2020). Additionally, vitamin D contributes to reversing colorectal cancer primarily by regulating intestinal flora, particularly Akkermansia muciniphila-mediated colon barrier integrity (Zhou et al., 2020). Therefore, this study aimed to investigate the serum Hcy, vitamin B_6, and vitamin D levels and its correlation in CRC patients, to find out its association with the CRC pathogenesis and developing.

Materials and Methods

The investigation was conducted on fifty patients between July 2021 and January 2022; their ages ranged from 29 to 65 years at stages III and IV after taking the first and second chemotherapy doses. The work was performed at the Nanakaly hospital in the governorate of Erbil. In addition, samples were taken from fifty healthy volunteers (as determined by health professionals) and their analytical data was contributed to our study as age-specific control groups (28–65). Randomizations were considered during sample selection. Throughout the study period, blood samples have been drawn from the cubital vein at varying times. The samples were centrifuged to extract the serum, which was then analyzed for Hcy, vitamin B_6 and vitamin D levels.

Measurements of serum Hcy, vitamin B_6 and vitamin D

The serum was separated by centrifugation and placed in a cooler at a low temperature. The homocysteine and vitamin B_6 serum concentrations of the cancer and control groups were then assessed using ELISA (Enzyme-Linked Immunosorbent Assay), and the vitamin D serum concentration was determined using Statlab T. The ELISA reader (ELISA reader BioTek TS 800) measurements at 450 nm for Hcy and vitamin B_6 and Statlab T measurements at 700 nm for vitamin D elicited the analytical results from the human ELISA kit. The concentration of Hcy in the examined samples was reported in µmol/L, while the concentrations of vitamin B_6 and vitamin D were expressed in ng/mL.

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 used to evaluate the comparison between the two groups. Two-sided P values were used, and significance differences were assumed to be less than $P<0.05$.

Results and Discussion

The study population was 100 subjects, and all subjects were divided into two main groups (patients and healthy subjects) as illustrated in (Table1 and Fig.1). Table 1 presents the results of three parameters (Hcy, Vit.B_6, and Vit.D) from the serum of patients and normal controls. There was a statistically significant increase in Hcy (69.83±2.276 µmol/L), and a significant decrease in Vit.B_6, and Vit.D (0.2664 ±0.03301 and 22.43±1.945 ng/mL) respectively in the mean level of cases with CRC when compared to healthy group (7.295±0.3939, 5.617±0.6904, and 34.21±2.850) respectively.

Table 1: Serum levels of Hcy, Vit.B_6, and Vit. D in patients with healthy control

Serum parameters	*Control*	*CRC patient*	*P-value*
Hcy (µmol/L)	7.295±0.3939	69.83±2.276	<0.0001
Vitamin B_6 (ng/mL)	5.617±0.6904	0.2664 ± 0.03301	<0.0001
Vitamin D (ng/mL)	34.21±2.850	22.43 ± 1.945	0.0010

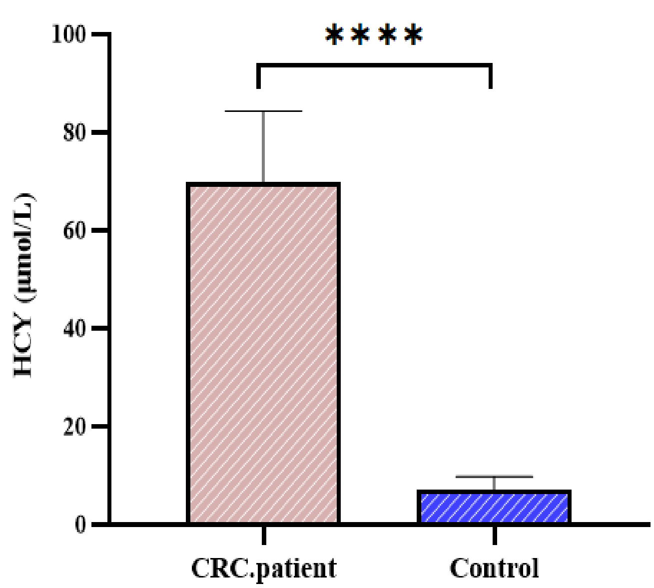

Fig. 1: Serum Hcy level in the patients with CRC and healthy control group

Homocysteine is an intermediate compound needed in the trans-sulfuration process that produces cysteine from methionine. Methionine synthase (MTR) is encoded by the MTRgene and catalyzes the remethylation of homocysteine to methionine to keep up adequate intercellular folate levels, required for deoxyribonucleic acid synthesis, methylation, and homeostasis (Lissowska et al., 2007). Our data findings are consistent with previous studies by numerous researchers from various countries, which showed that patients with colorectal cancer had significantly elevated total serum homocysteine levels (Miller et al., 2013, Liu et al., 2018, Mühl et al., 2022).

The increasing release of homocysteine into the plasma of colorectal cancer patients may be a result of reduced metabolic activity, poor nutrition, or deficient absorption, especially of folic acid, which impacts the methionine cycle. Additionally, due to the rapid growth of tumor cells, more folic acid and Vit.B_{12} may be taken, resulting in decreased concentrations of folate and vitamin B_{12}, which further impacts the metabolism of Hcy and raises levels of homocysteine in plasma (Figueiredo et al., 2009).

The same table (Table 1) and Fig. (2) show the levels of vitamin B_6 of the CRC patients and normal group. The mean ± standard error of CRC was (0.2664±0.03301 ng/ml), whereas in the control group it was (5.617±0.6904 ng/ml). These results have shown that CRC patients had a lower serum vitamin B_6 level than the normal groups, and there were significant differences between the CRC patients and normal subjects ($p<0.0001$). Our results were in accordance with the data from other studies showing that mean vitamin B_6 levels decrease in those with colorectal carcinoma in comparison to normal individuals (Jia et al., 2017). Pyridoxine could play a crucial role in CRC progression. Decreases in vitamin B_6 levels raise the risk of CRC. It could be according to one-carbon metabolism, which includes the transfer of one-carbon groups for synthesis and deoxyribonucleic acid methylation. A deficiency in vitamin B6 has been linked to a significantly altered one-carbon metabolic pathway. (Larsson et al., 2010, Zhang et al., 2013). Vitamin B_6 may also inhibit CRC by lowering angiogenesis, cell proliferation, nitric oxide synthesis , oxidative stress, and inflammation (Larsson et al., 2010).

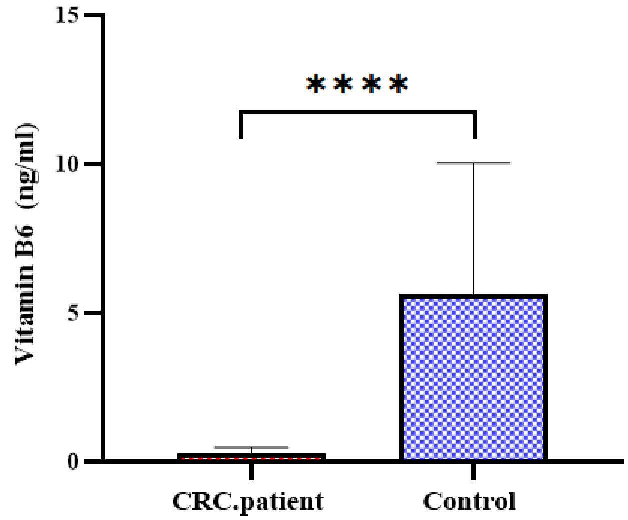

Fig.2: Serum vitamin B_6 level in the patient with CRC and healthy control group

Another parameter which considered in those CRC patients is Vitamin D. The total serum level of vitamin D is shown in (Table1 and Fig. 3) for both groups, and the concentration of vitamin D in CRC patients and control subjects was (22.43±1.945 ng/mL) and (34.21± 2.850) respectively. In this study, our results have shown that CRC patients had a lower serum vitamin D level than healthy controls. This result was agreement with other studies showing that concentration of vitamin D decreased in patients with colorectal carcinoma than in the healthy control group (Huang et al., 2020, Boughanem et al., 2021). The modulation of the immune response is one of the major significant functions of vitamin D (Chun et al., 2014, Medrano et al., 2018). This involves supporting cells of the host immune response, such as monocytes and dendritic cells, to defend against infection by bacteria such as tuberculosis (TB). In addition, Vit.D reduces overreactions of adaptive immune system cells, such as T cell activation, which can lead to auto-immune disorders like varied IBD or sclerosis (Dankers et al., 2017, Lu et al., 2019). In contrast, those with IBD and low Vitamin D have a dramatically elevated risk of CRC development (Ananthakrishnan et al., 2014). Preclinical research supports the role of Vit.D in tumor prevention by exerting anti-proliferative, pro-apoptotic, anti-invasion, anti-angiogenic, anti-metastatic, and anti-inflammatory actions on cancer cells (Feldman et al., 2014).The decrease of Vitamin D progresses in major chronic diseases, including various types of malignancies (Mühl et al., 2022). Figs. 4A, B, and C display the ROC curve for CRC detection based on Hcy, vitamin B_6, and vitamin D quantification (50 CRC cases compared with 50 control subjects). A relatively high AUC (area under the curve) suggests that testing for Hcy, vitamin B_6 and Vit.D could be helpful in CRC detection.

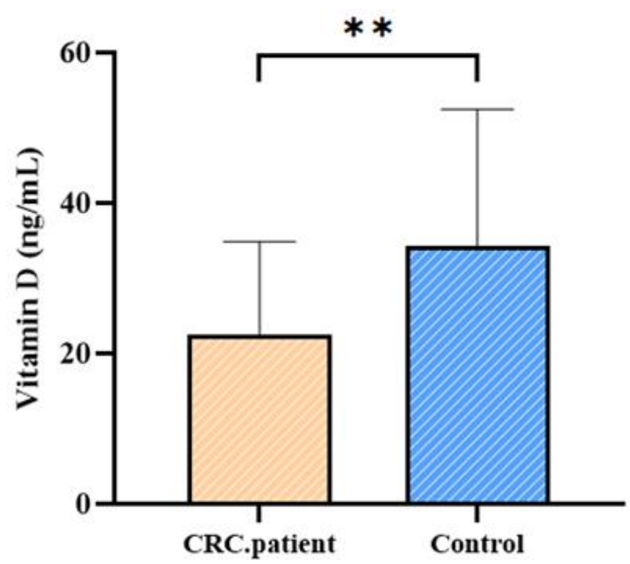

Fig. 3: Serum vitamin D level in the patient with CRC and healthy control group

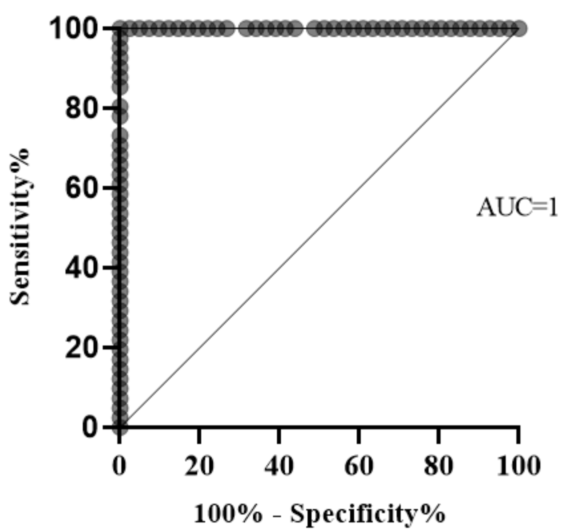

Fig. 4A: The ROC curve of Hcy

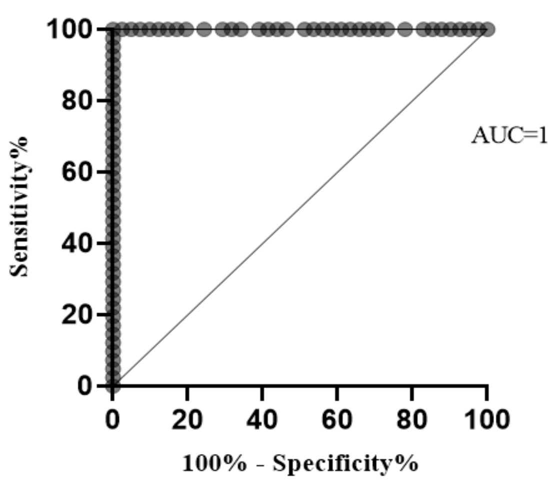

Fig. 4B: The ROC curve of vitamin B_6

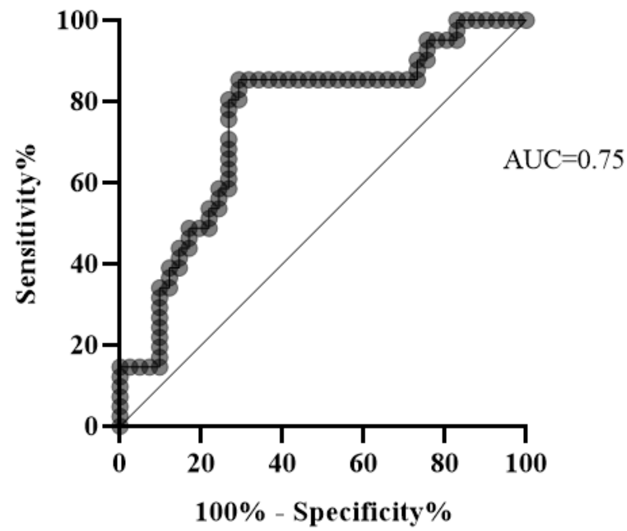

Figure 4C: The ROC curve of vitamin D

The correlation between Hcy and vitamin B_6 showed there was a negative correlation (0.04161), which was statistically non-significant (Fig.5). Also, the correlation between Hcy and vitamin D showed there was a positive correlation (r=0.0054), which was statistically non-significant (Fig.6).

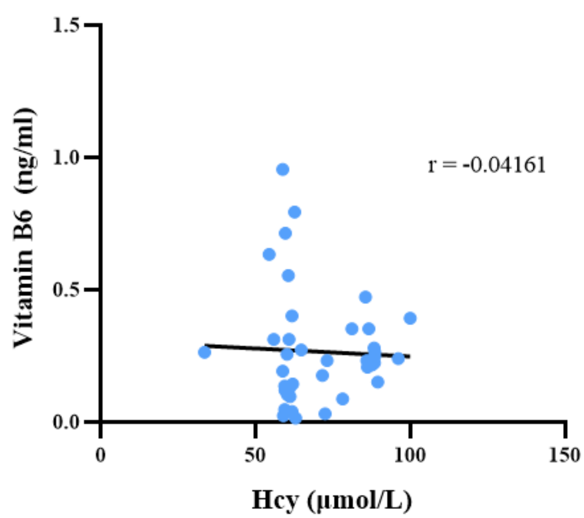

Fig. 5: correlation between Hcy and vitamin B_6

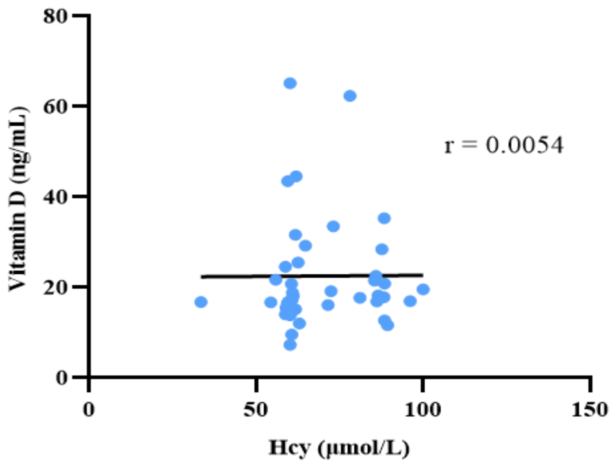

Fig. 6: Correlation between Hcy and vitamin D

Conclusions

The results of the present study indicate that homocysteine, pyridoxine and vitamin D levels in the serum of colorectal carcinoma patients differed significantly from those of randomly selected healthy controls. Therefore, these parameters play a crucial role in colorectal carcinoma progression and can be used as biomarkers for CRC.

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