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Serological Evaluation of Vitamin B12 and Homocysteine Levels in Colorectal Cancer Patients and their Relationships to Global DNA Methylation and P53 Expression

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Abstract

Colorectal cancer (CRC) is the most deadly disease, and has a high rate of mortality. Interestingly, biological biomarkers is binifical in the early diagnosis and treatment of CRC. This study was designed to measure the changes in the Vitamin B12 (Vit. B12) and Homocysteine (Hcy) at serological levels according to the clinical-outcome of CRC patients as well as to examine its correlation with global 5-methylcytosine (5mC) and P53 expression that associated with progression of CRC disease. The current study was included 30 healthy volunteers who served as controls and 60 patients with CRC. The blood samples were collected and serum levels of Vit. B12 and Hcy were measured using ELISA assay. DNA and RNA were also extracted, and the levels of global DNA methylation and P53 expression were evaluated. The results revealed a significant increases in Hcy levels in CRC patients compared to healthy controls was observed (373.67±17.67 and 83.16±6.11 pg/ml), respectively. In contrast , no significant change was observed in the levels of Vit. B12 in CRC patients compared to healthy controls (565.66±32.21 and 1178.76±28.93 pg/ml), respectively. They were accompanied by significantly reduced levels of 5mC in CRC patients in compared to controsl (0.31±0.02 and 0.46±0.03, respectively), with a positive correlation between 5mC and the different stages of CRC. In addition, the results showed a positive correlation between 5mC and Vit. B12 but not Hcy. The results of P53 expression showed no significant differences concerninig to healthy control. Furthermore, there was no correlation between Hcy, global DNA methylation, and P53 gene expression. In conclusion, the present study indicates that the changes in Vit.B12, Hcy, and 5mC levels at the different pathogenesis outcomes of CRC disease play an important role as a risk factor for CRC progression.

Keywords: DNA methylation, Colorectal cancer, homocysteine, P53 gene expression, Vit. B12

التقييم المصلي لمستويات فيتامين B12 والهموسيستين لمرضى سرطان القولون والمستقيم وعلاقاتهم بميثيلة الحمض النووي وتعبير P53

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الخلاصة

يعد سرطان القولون والمستقيم (CRC) من أكثر الأمراض فتكًا، وله معدل وفيات مرتفع. ومن المثير للاهتمام أن تقدير المؤشرات الحيوية البيولوجية مفيد في التشخيص المبكر وعلاج سرطان قولون و المستقيم. تم تصميم هذه الدراسة لقياس التغيرات في المستويات المصلية لفيتامين B12 والهوموسيستين (Hcy) وفقًا للنتائج السريرية لمرضى سرطان قولون و المستقيم وكذلك لفحص ارتباطها مع مثيلة الحمض النوويي5mC و تعبير P53 وارتباطهما بتطور مرض CRC. شملت الدراسة الحالية 30 شخصا متطوعا سليمًا كمجموعة سيطرة لأغراض المقارنة و60 مريضًا مصابين بـ CRC. تم تقييم مستويات B12 و Hcy في مصل مجاميع الدراسة كمياً باستخدام تقنية الامتزاز المناعي (ELISA). كما تم تقييم مستويات مثيلة الحمض النووي وتعبير P53. وقد لوحظت زيادة كبيرة في مستويات Hcy لدى مرضى CRC مقارنة بمجموعة السيطرة (373.67 ± 17.67 و 83.16 ± 6.11 بيكوغرام / مل) على التوالي. كما أظهرت النتائج عدم وجود فرق معنوي في مستوى فيتامين B12 في مرضى سرطان القولون والمستقيم مقارنة بمجموعة السيطرة (565.66 ± 32.21 و 1178.76 ± 28.93 بيكوغرام / مل) على التوالي. لوحظ انخفاض ملحوظ في مستويات 5mC في مرضى CRC مقارنة بمجموعة السيطرة (0.31 ± 0.02 و 0.46 ± 0.03) على التوالي، مع وجود علاقة إيجابية بين مستوى 5mC والمراحل المختلفة للمرض. كما أظهرت النتائج وجود علاقة إيجابية بين 5mC وفيتامين B12 ولكن ليس Hcy. أظهرت نتائج P53 عدم وجود فروق ذات دلالة إحصائية فيما يتعلق بمجموعة السيطرة. أيضًا، لم يكن هناك ارتباط بين Hcy وميثيلة الحمض النووي والتعبير الجيني P53. تشير دراستنا إلى أن التغييرات في مستويات Vit.B12 وHcy و5mC في المراحل المرضية المختلفة لمرض CRC تلعب دورًا مهمًا كعامل خطر لتطور CRC.

Introduction

Colorectal Cancer (CRC) is the second-most deadly and third-most prevalent cancer that affects an estimated 1.9 million people annually. Colorectal cancer is more prevalent in highly developed nations and is rising in middle- and low-income countries as a result of westernization. According to a study, 0.9 million CRC-related fatalities were reported in 2020 [1]. It is believed that CRC pathogenesis is caused by various pathways such as suppressor pathway, or chromosomal instability, which is linked to the accumulation of mutations that can turn on the oncogenes (KRAS) and turn off the tumor suppressor genes such as TP53 and SMAD4, ultimately leading to neoplastic transformation [1]. Additionally, aberrant DNA methylation is considered another pathway of CRC disease development [2, 3] However, the subtype of colorectal cancer affects prognosis and therapy response because it is a heterogeneous disease [1]. Homocysteine (Hcy) has a sulfur group created by many different types of cells all across the human body through the demethylation of methionine. Abnormal Hcy levels have been significantly linked to the etiology of CRC. Higher levels of Hcy have been identified as a risk factor for CRC incidence[4].

Regarding Vit.B12, which has been closely associated with the metabolism of methionine, it is a crucial element in biological methylation events like DNA methylation. The connection between Vit. B12 and DNA methylation are still unclear. Researchers focused on the link between Vit. B12 and the risk of CRC [5]. According to a large numbers of epigenome studies several genes' regulation can be affected by supplements like Vit. B12. However, many studies confirm the unique relationship between Vit. B12 and aberrant epigenetic status are linked to colorectal tumorigenesis [6]. Recently, epigenetics gained important scientific attention since it has added a new dimension to genomic and proteomic research[7]. Oncogene, tumor suppressor genes, and DNA repair mechanism-related gene alterations may

contribute to the development of CRC [8]. Consequently, the short arm of chromosome 17 encodes the tumor suppressor gene (TS P53), widely known as the genome's guardian (17p13.1). The P53 is a critical mediator of apoptotic death that is triggered in response to different stress signals, such as gene damage or activation of oncogene in CRC. However, P53 signaling is deactivated frequently by changing regulators of P53 or by P53 mutations, which can cause a heterogeneous response. The method selected depends on several factors, such as the availability of interaction, the modification of enzymes, and the P53 protein level. These elements control the expression of P53 target genes [9].

This study was designed to measure the changes in the Vit. B12 and Hcy at serological levels and their relation to the clinical-outcome of CRC patients, as well as to examine their correlation with the changes of global DNA 5-methylcytosine (5mC) and P53 expression that is associated with the progression of CRC disease.

2. Materials and Methods

2.1. Subjects

A total of 60 Iraqi CRC patients (32 male, 28 female) were attended at Oncology Teaching Hospital, Medical City Hospital, and Baghdad, Iraq, during the period between February 2022 and September 2022. In addition, 30 healthy individuals (12 male, 18 female) as a healthy control group. This study was approved by the ethical committee of the Department of Biology, College of Science, University of Bagdad, Iraq, with authorization reference number CSEC/1021/0098 on October 29, 2021. Blood samples were collected from all participants in this study in order to estimate the serum levels of Hcy and Vit. B12, P53 gene expression and global 5mC in studied groups. Colorectal cancer patients' stages and grading were classified and identified based on TNM and the World Health Organization (WHO). The status of CRC patients was also estimated based on the ECOG classification [10].

2.2. Blood samples

2.2.1. Blood sample and assay of immunological markers

A five ml vein blood samples was drawn from each participant (3 ml from the 5ml for serum collection and 2 ml placed in EDTA-containing tube for RNA extraction) using disposable syringes. Gel clot activator vacuum tubes were used for clotting. Then samples were centrifuged at 3000 rpm for 10 minutes to aspirate the serum. The serum was then dispensed into Eppendroff tubes using a micropipette, and the tubes were then refrigerated at -20C^{o} for later immunological investigations. One hundred microliters of diluted sample was added to separate wells in a microwell plate for the immunological serum markers studies. The vitamin B12 levels in the serum were measured using (Monobind Vitamin B12 AccuBind ELISA Kits, USA) and homocysteine levels were measured using commercial ELISA assay (Human Fine Test Biotech, ELISA kit, China), following the manufacturer’s instructions.

2.2.2. Extraction of DNA

Genomic DNA was extracted from blood samples following the manufacturer's instructions by (Quick-DNA™ Miniprep Kit; ZYMO RESEARCH; USA). In each case, the DNA size was determined by agarose-gel electrophoresis, and the purity was assessed based on the absorbance measurements at 260 and 280 nm.

2.2.3. Expression of P53 gene assay

For the assay of P53 expression, 20 ul of samples containing RT buffer were prepared as a final volume. The (TRI Reagent®; ZYMO RESEARCH; USA) was supplied as a 2X master

mix with an integrated antibody-mediated hot start, dNTPs, SYBR Green I fluorescent dye, MgCl2,, and stabilizers. The P53 primer sequence for cDNA amplification is:

Table1: Oligonucleotide primers of P53 used in the qPCR.

Gene	5` Sequence 3`	Tm	GC%	Reference
P53	AGA GTC TAT AGG CCC ACC CC	60	55	[11]
	GCT CGA CGC TAG GAT CTG AC	61	50	
GAPDH	AATGGGCAGCCGTTAGGAAA	57	50	[12]
	GCGCCCAATACGACCAAATC	56	50	

Master Mix was utilized together with the KAPA SYBR FAST qPCR Kits, nuclease-free water, cDNA samples, and an endogenous control called GAPDH that is used as a reference gene (Applied Biosystems Foster City, CA, USA).

2.2.4. Assay of DNA methylation

Following our prior procedure, we used the DNA Quantification kit (The MethylFlashTM methylated DNA quantification Kit (Epigentek, USA)) to examine the global DNA methylation levels. Using a microplate spectrophotometer, the 5-methylcytosine (5mC) antigen-antibody complex was analyzed at 450 nm and was used to quantify the total 5mC content in DNA extracted blood.

2.2.5. Statistical analysis

The SPSS version 24.0 program was used for all statistical analysis. The data were expressed as Mean ± SE. The three parameters or more were compared using the ANOVA. T Student tests were also used to compare the two numerical or categorical parameters. Differences with P values <0.05 were considered to be statistically significant.

3. Results

3.1. The distribution of vitamin B12 and homocysteine levels between patients and controls

The results of the serum levels of Vit-B12 and Hcy showed that Vit-B12 levels decreased in CRC patients but there were no significant differences observed at ($P \geq 0.05$). However, a significant increase was found between CRC patients and healthy controls in the levels of Hcy ($P \leq 0.001$) as shown in table2.

Table 2: The distribution of the levels of Vit-B12 and Homocysteine between patients and controls

Parameters	CRC Mean± S.E.	Healthy Control Mean± S.E.	P-value
Vit-B12 (pg/ml)	565.66±32.21	1178.76±28.93	0.238
Hcy (pg/ml)	373.67±17.67	83.16±6.11	0.001

3.2. Homocysteine and vitamin B12 levels in CRC patients in relation to clinic-pathological variables

The general characteristics of participants were shown in table 3, there was a statistically significant relation in the levels of Vit. B12 and Hcy among patients' features according to TNM stage in CRC groups, and the participants in the low-risk group have higher mean levels of Vit. B12 and Hcy. In addition, the relation between the levels of Vit. B12 and Hcy showed no significant differences among patient's features according to grading and tissue invasion in CRC groups, except for the Hcy among patient's and grading as shown in table 3.

Table 3: Homocysteine and vitamin B12 levels in CRC patients in relation to clinic-pathological variables

Variables	Vitamin B12 (pg/ml) Mean± S.E.	Homocysteine (pmol/ml) Mean± S.E.
TNM Stage		
Low level (I+II)	625.15± 54.68	401.96±35.84
High level (III+ IV)	538.09±39.44	360.49±19.81
P-value	0.001	0.001
Grading		
Grade 1-2	412.75± 55.74	457.20 ± 25.13
Grade 3-4	583.01± 63.21	373.04 ± 31.85
P-value	0.051	0.044
Tissue invasion		
Low invasion T1+T2	601.92± 74.58	353.11± 30.628
High invasion T3+T4	485.9 ± 46.82	399.4428± 23.19
P-value	0.175	0.23

The results are presented as mean ± standard error
Low L. (I+II): low-level stage of the disease, stage I and stage II
High L.(III+ IV): advanced levels of the disease, stage III and IV
T: refers to the local extent of the untreated primary tumor at the time of diagnosis and initial workup

3.3. Measurements of homocysteine and vitamin B12 levels according to age and gender

The results illustrated in table 4 showed higher mean levels of Vit. B12 in the age group <40 in the low-level stages (I+II), while higher mean levels in high-level stages (III +IV) was shown in the age group (61-80) with significant differences ($p \leq 0.001$). The results of homocysteine showed a higher mean level in the age group (61-80) in the low level stage (I+II), while higher mean levels of homocysteine in the high-level stage (III +IV) was shown in age group (41-60) with significant differences at ($p \leq 0.001$). Regarding to gender of the patients, Vit. B12 showed higher mean levels in male groups in both (low and high level stages), whereas females results showed higher mean levels of homocysteine in groups in both (low and high-level stages), and there was statistically significant. Differences ($P \leq 0.001$) in the levels of Vit-B12 and homocysteine among patients, as shown in table 4.

Table 4: Correlations of vitamin-B12 and homocysteine with demographic and TNM stage of CRC patients

Variable		Vitamin B12 (pg/ml) Mean± S.E.	Homocysteine (pmol/ml) Mean± S.E.
Age groups			
Low L. (I+II)	**<40**	1148.871 ± 0.00 [a]	213.53±0.00 [a]
	40-60	645.81±50.29 [a]	369.98±55.72 [b]
	61-80	533.86±89.63 [a]	465.48±38.26 [b]
P-value		**0.001**	**0.001**
High L.(III+ IV)	**<40**	475.09±107.16 [c]	337.64±31.51 [b]
	40-60	541.35±63.46 [b]	381.71±34.81 [b]
	61-80	560.77±58.70 [b]	213.53±0.00 [a]
P-value		**0.001**	**0.001**
Gender			
Low L. (I+II)	**Male**	715.52±65.10 [b]	356.74±51.24 [a]
	Female	470.23±68.51 [c]	479.47±25.02 [a]
P-value		**0.001**	**0.001**
High L.(III+ IV)	**Male**	667.72±40.77 [a]	354.86±29.28 [b]
	Female	401.98±54.54 [c]	366.41±27.26 [b]
P-value		**0.001**	**0.001**

The results are presented as mean ± standard error.
***Different letters mean there are significant differences between genders**
Low L. (I+II): low-level stage of the disease, stage I and stage II
High L.(III+ IV): advanced levels of the disease, stage III and IV

3.4. Statistical analysis of DNA methylation and P53 expression

The results of global DNA methylation (5mC) showed a highly significant reduction (P-value = 0.01) in the levels of 5mC in CRC patients compared with the control group (0.31±0.02 and 0.46±0.03, respectively), as shown in figure1-A. Conversely, the p53 gene expression was elevated in CRC patients than in the control group with no significant difference when compared with the control group (Figure1-B)

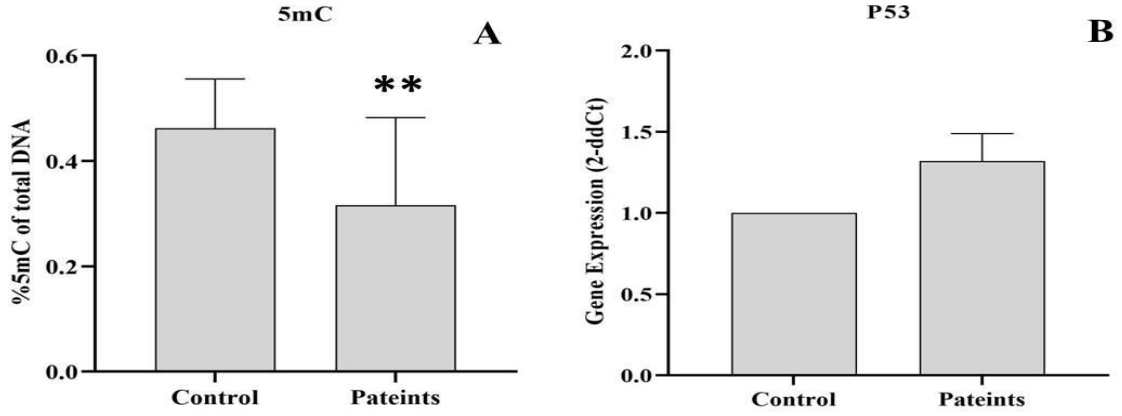

Figure 1: Statistical analysis of DNA methylation and P53expression values in CRC patients and healthy control. A: levels of 5mC global DNA in the colorectal cancer and control groups
B: P53 expression in the colorectal cancer and control groups
**Refers to significance at P ≤0.01

3.5. Evaluation of DNA methylation and P53 expression in CRC patients in relation to clinic-pathological variables

The results of 5mC and P53.gene expression indicated that there was no significant link between tumor aggressiveness and 5mC DNA methylation and P53gene expression in the CRC groups. Even though there were no detectable clinic-pathologic differences between the patients' groups with different levels of the disease (low-level stages (I+II), low level Grades 1-2, high-level stages (III+IV), and high-level grades 3-4) and the P53 expression. The only significant link was shown between DNA methylation and tumor stage aggressiveness of CRC ($P \leq 0.01$), as shown in table 5.

Table 5: DNA methylation and P53 expression in CRC patients in relation to clinic-pathological variables.

Variables	DNA methylation Mean± S.E.	P53 expression Mean± S.E.
TNM Stage		
Low level (I+II)	0.29±0.04	1.52±0.34
High level (III+ IV)	0.32±0.03	1.28±0.18
P-value	**0.072**	**0.778**
Grading		
Grade 1-2	0.319±0.02	1.37±0.17
Grade 3-4	0.312±0.05	1.34±0.41
P-value	**0.908**	**0.953**
Tissue invasion		
Low invasion T1+T2	0.319± 0.04	1.5± 0.31
High invasion T3+T4	0.314± 0.03	1.38± 0.18
P-value	**0.942**	**0.74**

The results are presented as mean ± standard error
Low L. (I+II): low-level stage of the disease, stage I and stage II
High L.(III+ IV): advanced levels of the disease, stage III and IV
T: refers to the local extent of the untreated primary tumor at the time of diagnosis and initial workup

3.6. Evaluation of the relation between DNA methylation, P53 expression, age groups and gender in CRC patients according to clinic-pathological variables

DNA methylation and P53 expression showed no significant differences among all ages of the studied groups ($P \geq 0.05$). Regarding gender, the results of DNA methylation showed significant differences between gender of both clinic-pathologic levels (low-level and high-level stages) ($P \leq 0.001$), while gene expression of P53 shows no significant differences in the gender of studied groups ($P \geq 0.05$) as shown in table 6.

Table 6: DNA methylation and P53 expression in CRC patients in relation to clinic-pathological of tumor according to the age groups and gender

Variable		DNA methylation Mean± S.E.	P53 expression Mean± S.E.
Age groups			
Low L. (I+II)	**<40**	0.22±0.05 [a]	2.32± 0.76 [a]
	40-60	0.32±0.06 [a]	2.00±0.84 [a]
	61-80	0.25±0.06 [a]	1.20±0.16 [a]
High L.(III+ IV)	**<40**	0.27±0.04 [a]	1.12±0.22 [a]
	40-60	0.27±0.03 [a]	1.60 ± 0.18[b]
	61-80	0.39±0.08 [a]	0.97±0.16 [b]
P-value		**0.686**	**0.552**
Gender			
Low L. (I+II)	**Male**	0.38±0.06 [a]	1.83±0.21 [a]
	Female	0.24±0.05 [a]	1.52±034 [a]
High L.(III+ IV)	**Male**	0.87±0.00 [b]	2.28±0.34 [a]
	Female	0.29±0.02 [a]	1.98±0.18 [a]
P-value		**0.003**	**0.793**

The results are presented as mean ± standard error
*Different letters mean there are significant differences between age groups and genders
Low L. (I+II): low-level stage of the disease, stage I and stage II
High L.(III+ IV): advanced levels of the disease, stage III and IV

However, Pearson correlation was used to evaluate the strength relationship between the studied parameters and the percentages, which are presented as a median of individual dots. Vitamin B12 and Hcy were negatively and significantly associated with healthy control ($P \leq 0.001$) as shown in figure2-A. Vitamin B12 and DNA methylation in the tumor area were positively and significantly associated with healthy control ($P \leq 0.01$) as shown in figure 2-B. Vitamin B12 and P53 expression in the tumor area were not correlated and significantly no associated with control ($P \geq 0.05$) (Figure2-C). Figure2-D showed that homocysteine and DNA methylation in the tumor area were negatively and significantly no associated with the control ($P \geq 0.05$). Homocysteine and P53 expression in the tumor area were not correlated and significantly not associated with control ($P \geq 0.05$) (Figure 2-E). DNA methylation and P53 expression in the tumor area were negatively and significantly not associated with the control ($P \geq 0.05$) as shown in figure2-F.

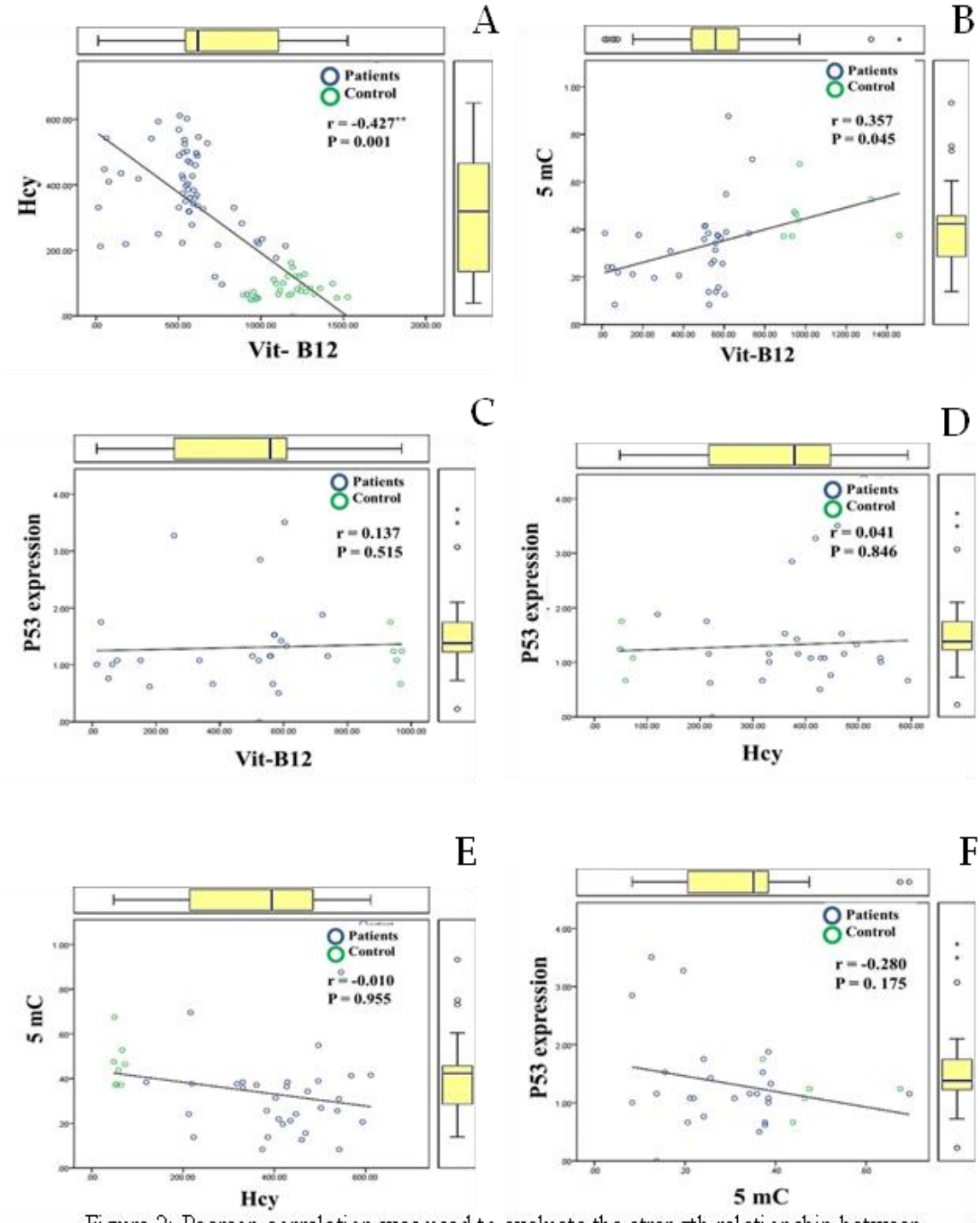

Figure 2: Pearson correlation was used to evaluate the strength relationship between parameters studied and the percentages are presented as median of individual dots. The results of correlation are significant at the level of 0.05.

Discussion

Homocysteine metabolism can be controlled by the concentration of vitamin B and their related (Vitamins B2, B6, B9, and B12), which serve as substrates or cofactors for enzymes metabolic process. Folate has the ability to donate one carbon group, which can be used in nucleotide synthesis and DNA methylation, also folate decreases status linked with the development of cancer innumerous organs such as colon and rectum [13]. Previous results showed that patients with advanced and metastatic CRC have lower levels of circulating Vit.B12 and showed better overall survival and slower rates of progression of the disease

than those with normal / higher levels [14]. However, numerous studies have attempted to point out the precise link between circulating vitamin B12 and CRC risk [15]. The current study showed increased level of Hcy and this matched with previously reported results by Al-Rubaye [16]. In contrast, a previous study showed that patients with inflammatory bowel disease frequently have hyperhomocysteinemia (>12–15 mol/L), which is caused by either decreased absorption of folate and its related (Vitamins B9, B2, B6 and B12), which are all necessary for homocysteine metabolism and one, carbon metabolism pathways or increased requirements for these vitamins [17].

Many studies indicated that increased homocysteine levels undermine the health of the body, compromising epigenetic modifications necessary for DNA synthesis and healthy aging. They are an independent predictor of all-cause death for every (5mol/Letter) increase in homocysteine, the risk of death increased by (32%) and the risk of heart diseases by (52%) [18]. Numerous researchers examined the connection between dietary folate supplement, fiber intake and cancer risk. For example, higher Hcy and lower folate levels were linked to a higher risk of CRC [19]. The dual role of one carbon metabolism in carcinogenesis, which has been hypothesized, is reflected by a substantial tendency toward the negative relationship between vitamin B12 levels and the incidence of early-stage CRC, but not the higher stage of CRC. Folate is believed to speed up the growth of tumors that are already present but have not yet turned malignant [20, 21].

A previous study reported that Vit. B12 and methionine intakes are associated with a lower risk of colorectal cancer in women. However, folate, vitamin B2 and vitamin B6 were shown to be inversely correlated with colorectal cancer in both sexes [22]. The results of the previous study concluded that high levels of cysteine minimize infection with CRC, and lower 5mC is related to advanced malignancy grade in colon cancer [17]. However, Hcy has the potential to work as tumor biomarker for a number of malignancies including CRC [23]. Although the determination of circulating levels of proteins, inflammatory cytokines have to be taken into consideration as markers for staging of the cancers [24].

The current study demonstrated a low level of 5mC. It was suggested that early in the process of oncogenesis, DNA hypomethylation might take place followed by hypermethylation [25]. In addition, the current analysis indicates a small elevation of mean P53 expression but non-significant differences. However, in contrast to our results, a previous study found a strong correlation between the overexpression of the P53 gene in tumor tissue and the development of colorectal cancer (CRC). Even though there is evidence linking colorectal cancer to the P53 gene, additional molecular research, such as epigenetics and microRNA analysis, is necessary to completely understand the association between colorectal cancer and molecular biomarkers [26].

A study by Ng *et al.* 2022 revealed significant increases in 5mC levels in patients with advanced-grade tumors and this came in disagreement with the current study. Also, our results showed the levels of global 5mC decreased significantly and this decrease was associated negatively with T-stage and these findings are in line with the majority of studies on global DNA methylation levels in CRC illness [27]. A previous study found that eighty percent of CRCs that have strong P53 expression were characterized by missense mutations and suggest that gain function, compared to 53% of CRCs in the non-expression of P53 group which suggests loss of function [28].
High amounts of circulating Vit. B12 were linked to lower LINE1 methylation in tumor CRC; therefore, our findings are indicated that DNA methylation in CRC sufferers may be

influenced by circulating Vit. B12 levels. The connection between Vit. B12 levels and methylation process in the tumor area, suggests employed as a prognostic factor in CRC [6]. Other studies suggested that there is no evidence linking the Vit. B12 to DNA methylation, prior findings based on the epigenetic landscape imply that. Vitamin B12.may operate as an epigenetic helper that affects specific cell cycle pathways and the proliferative characteristics of CRC [5].

Previous research revealed that plasma Hcy levels in CRC patients in remission were much lower at the study's conclusion than they were at baseline. These changes were negatively linked with the changes in the global cell-free DNA methylation level, indicating that a high Hcy level can coexist with widespread cell-free DNA hypomethylation [29, 30] Epigenetic changes such as global DNA hypomethylation and gene-specific hypermethylation have been of great interest to cancer researchers since they are key features of various malignancies, including colorectal and prostate cancer [31]. Expression levels of some genes were association with some of the clinical features like BRCA1 in breast cancer patients [32]. One of the most well-known pathways in aging research is the P53 pathway, which controls the cell cycle and interacts with proteins involved in controlling DNA methylation. P53 is considered the most promising biological marker for monitoring aging at the moment, further research is needed to completely understand the specific genes and mechanisms of aging-related DNA methylation and gene expression [33].

Conclusion

This study indicated that levels of Vit. B12 and Hcy in CRC-suffering patients might be used as prognostic risk factors that could be utilized in the diagnosis of CRC disease, and this study also indicated statistically significant differences between CRC-diagnosed patients and healthy controls in levels of 5mC. However, further research might perform extensive study explore the correlation between DNA methylation and demethylation patterns in genes related to CRC and focus on new therapies that may aid in detecting new sides of the disease pathogenesis and determine novel targets for therapy.

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