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Evaluation of Vitamin D and Some Liver Enzymes in Iraqi Colorectal Cancer Patients and those Without Colorectal Cancer

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***Abstract*—Colorectal cancer (CRC) is a major global health concern, described as the uncontrolled growing of cells in the colon or rectum. Vitamin D, metabolized in the liver and has garnered attention for its potential protective effects against CRC. The aim of this research is to evaluate vitamin D levels and its link with some liver enzymes in Iraqi participants with colorectal cancer. Overall, 70 colorectal cancer cases-divided into 35 newly diagnosed and 35 under medication were included, along with 30 individuals without colorectal cancer as controls. Blood sample were collected from Iraqi CRC patients and controls. Serum levels of vitamin D and liver biomarkers were analyzed using Cobas C311 and Chemiluminescence Immunoassay technology. The result showed significant variances in vitamin D levels between the studied groups (p=0.0001), with an inverse relationship between vitamin D and alkaline phosphatase (r=-0.33, p=0.0007). This study suggests that vitamin D could have a potential regulatory role in colorectal cancer patients.**

***Keywords*—biomarkers, Chemiluminescence immunoassay, colorectal cancer, liver enzyme, vitamin D.**

I. INTRODUCTION

Colorectal cancer (CRC) is a frequently occurring cancer affecting the gastrointestinal tract and has shown rising rates in both incidence and mortality in recent decades [1]. In Iraq, CRC rank as the second most common cancer after breast cancer, and third most common cancer site after breast and lung cancer among Iraqi males and females [2]. In 2019, the incidence rate for CRC was reported at 5.95 per 100,000, making it one of the top five cancers in the country, alongside, breast, brain, lung, and leukemia [3]. Similarly, Mortality rates also increased, from 1.25 to 1.77 per 100,000 during the same period, indicating a growing public health concern [4]. An earlier cross-sectional analysis indicated that only 6.1% of adults had undergone colonoscopy, indicating low screening rates in Iraqi adults [5].

Vitamin D, a fat-soluble micronutrient, is vital for regulating the calcium-phosphorous homeostasis, ensuring normal calcium levels and support the bone health [6-7]. Moreover, Vitamin D exist in two major forms, D3 (cholecalciferol), which is typically derived from animals and is also synthesized in the human skin when exposed to sunlight, and D2 (ergocalciferol), which is mainly found in plants and fungi. Both types of vitamin D are essential for human health by safeguarding it from rickets, osteomalacia, bone demineralization, hypertension, certain cancers, and autoimmune diseases [6-8]. In CRC, Vitamin D is important due to its effects on cellular processes and its association with disease risk and outcomes[9]. According to studies, vitamin D insufficiency (less than 20 ng/mL) rises the danger of CRC about 5.5 times [10]. Conversely, vitamin D showed promise role in reduction of cancer risk and management, Epidemiological studies indicated that vitamin D may have a crucial role against CRC [11]. Similarly, [12] demonstrated that elevated levels of vitamin D may correlate with improved survival rates and disease-free outcomes. Additionally, calcitriol, the active form of vitamin D, has presented ability to constrain the spread of colon cancer cells [13].

Liver enzymes are important biochemical indicators to assess liver condition, diagnose liver syndromes, and predict clinical outcomes. Some liver biomarkers such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) are frequently measured to determine liver illness, as they are secreted into the blood afterward hepatocyte damage [14]. Previous study have shown that elevated levels of ALP, ALT and AST (within reference values) are connected with a less risk of developing CRC [15], proposing an association between liver metabolic health and cancer danger. The liver is central to vitamin D metabolism. Liver dysfunction can diminish the transformation of vitamin D into 25-hydroxyvitamin D [25(OH)D], causing low serum vitamin D [16]. Moreover, previous researches have revealed that the elevation in liver enzymes (ALT, AST) particularly in chronic liver sickness and non-alcoholic fatty liver disease (NAFLD) is connected with low 25-hydroxyvitamin D [25(OH)D] levels[16-17]. Despite this, limited studies has investigated the association between vitamin D and liver enzymes levels (ALP, AST, and ALT) in colorectal cancer, mainly within middle eastern populations. The presented study was conducted to evaluation vitamin D status in Iraqi patients with colorectal

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cancer and asses its association with liver enzymes (ALP, AST and ALT).

II. Methods

A. Study Subject

The current study included 70 colorectal cancer patients and 30 individuals as controls. Blood specimens and data were gathered from AL-Amal Hospital for Radiation and Nuclear Medicine and the Digestive and Liver Disease Teaching Hospital in Medical City, Baghdad, Iraq, from January to April 2025.The studied groups were primarily categorized into 3 groups: Group I included 35 newly diagnosed patients (before treatment). Group II included 35 CRC patients undergoing treatment (chemotherapy and radiotherapy). Group III consisted of 30 individuals without CRC and with no history of cancer or any gastrointestinal problems such as inflammatory bowel disease (IBD), celiac disease, and GI infections.

- Inclusion criteria: involved CRC patients before cancer treatment and patients who had received treatment (chemotherapy and radiotherapy).
- Exclusion Criteria: Patients with diabetes, heart diseases, kidney failure, and gastrointestinal disorder.

The necessary permissions were acquired from the Middle Technical University, College of Health and Medical Technology, Baghdad. Ethics authorization numbered: 71 date:11/01/2025. Every participant provided consent.

B. Estimation of Serum Vitamin D and Liver Biomarkers Levels

Peripheral Blood specimens were collected from each individual; 10 mL of blood was drawn intravenously and placed in a vacuum gel tube, then allowed to stand for approximately 15 to 20 minutes to clot. Subsequently, serum specimens were isolated by centrifugation; (For 15 minutes at 3000 rpm). Afterward to dividing into smaller quantities placed in 4 eppendorf tubes, all sera were stored at -80°C until needed. The levels of ALP, ALT, AST and vitamin D were assessed using Cobas C311 Kit from Roche (USA) and Chemiluminescence Immunoassay Analyzer (CLIA) kit from Mindray (China).

C. Statistical Analysis

Study variables were compared among the three groups using SPSS (2019) program [17]. ALP, AST, ALT and vitamin D levels were performed using a t-test and One-way ANOVA with LSD post-hoc analysis. The variation in percentages was calculated by chi-square test, with p-value of ≤ 0.05 considered statistically significant. correlation coefficient was used to assess the association between biomarkers.

III. Results

As shown in Table 1, the gender differences were statistically non-significant among the studied groups (p = 0.502 NS). However, the mean age of participants varied between groups. Newly diagnosed CRC patients were older (62.11 ± 2.08) compared with those undergoing treatment (53.91 ± 2.32) and individuals without CRC (52.17 ± 2.83). This differences in mean age was statistically significant (p= 0.0087). A notable reduction was observed in vitamin D levels in both the newly diagnosed CRC group and patients under medication, compared to healthy controls, with P-value less than 0.001. Additionally, Vitamin D levels in newly diagnosed CRC group were lower when compared to patients under treatment (as shown in Fig.1).

As shown in Table 2 and Fig.2, Significant differences were reported in the means of ALP (101.78 ± 4.67; 37.39 ± 2.53) and AST (89.37 ± 3.24, 32.25 ± 2.14) levels in CRC patients compared to controls (68.53 ± 1.76; 25.32 ± 1.01) respectively. ALT mean (38.17 ± 3.57) showed significant differences in the newly diagnosed CRC patients in contrast to both patients under medication and the control group (27.98 ± 1.94; 25.16 ± 0.96). In Table 3 and Fig.3, an Inverse statistical correlation was demonstrated between alkaline phosphatase and vitamin D (rho = -0.33, p = 0.0007). By contrast, the result showed no association between vitamin D with both AST and ALT among the participants of the presented study (rho = - 0.10, p = 0.3064; rho = -0.16, p = 0.1068) respectively.

TABLE 1. CRC patients and the non- CRC controls characterized by gender, mean age, and serum vatimin D levels

group	N	Gender (M/F)	Mean age ± SE	Vitamin D mean ± SE (ng/mL)
Newly Diagnosed	35	19/16	62.11 ± 2.08a	19.18 ±1.46 c
Undergoing Treatment	35	19/16	53.91 ± 2.32 b	27.26 ±2.12 b
Control (without CRC)	30	16/14	52.17 ± 2.83b	34.66 ±1.47 a
Least significant difference L.S.D	--	--	--	4.906
P-value		--	0.0087 **	0.0001**
Values marked with different letters within the same column show statistical variances ** (Statistically highly significant),				

TABLE 2. Mean ± SD of ALP, AST, and ALT in different study groups

Group	Means ± SE		
	ALP (IU/L)	*AST (IU/L)*	*ALT (IU/L)*
Newly diagnosed	101.78 ±4.67 a	37.39 ± 2.53 a	38.17 ± 3.57 a
Under-medication	89.37 ±3.24 b	32.25 ± 2.14 a	27.98 ± 1.94 b
Control (without CRC)	68.53 ±1.76 c	25.32 ± 1.01 b	25.16 ± 0.96 b
Least significant difference test (L.S.D.)	10.056	5.847	7.082
P-value	≤0.001**	≤ 0.001**	≤ 0.001**
** Statistically highly significant			

IV. Discussion

Despite that vitamin D is best recognized for its primary role in calcium uptake and bone health, It also increasingly known for its anti-inflammatory properties in various diseases including cancer [18]. Vitamin D levels in the presented study showed statistically differences across the studied groups (as shown in Table 1 and Fig.1), these observations mirroring previous studies conducted in Iraq [20-21]. Low vitamin D levels were related to worse cancer-specific survival and overall survival [19]. Vitamin D

deficiency can be related to several biological and environmental factors; such as the chronic inflammation, dietary influences, and the impact of obesity-related [21-22]. Additionally, Genetic variations can influence the metabolism and receptor activity of vitamin, Contributing to the observed deficiencies in CRC patients [20]. Conversely, a study suggested that vitamin D may has a potential role in diagnosing and treating CRC due to its ability to hinder the development and spread of cancer cells in colon; additionally, it reduces the expression of oncogenes [13].

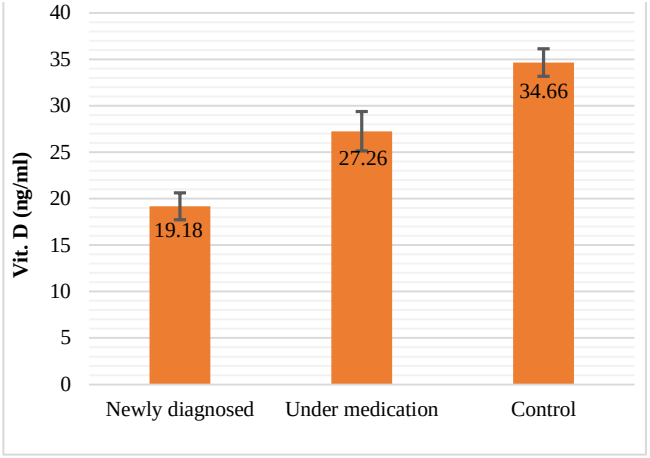


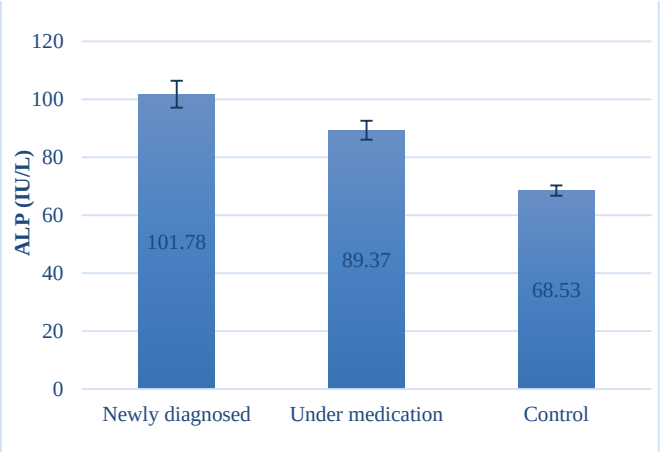
Fig. 1. Vitamin D levels relating to the studied groups.

Alkaline phosphatase (ALP) is an enzyme associated with membrane that facilitates the hydrolysis of phosphate monoesters, generating inorganic phosphate, especially at alkaline PH conditions. It is found in numerous tissues, markedly the liver, kidney, intestine, bone and placenta [21]. In the presented study, ALP means displayed significant statistically differences in both the newly diagnosed CRC patients and those under treatment patients when compared to controls with P value less than 0.001. as presented in Table II. although the means of ALP in CRC studied groups continued within the normal range, it showed elevated levels in patients in contrast to controls with a high statistical differences ($p = \leq 0.001$). Similar outcomes were reported in previous Iraqi studies [22-23]. Additionally, a study was published in 2011 established that ALP levels were considerably higher in CRC patients both pre and post chemotherapy compared to healthy individuals. Despite the elevation, ALP levels stayed within the normal reference range [22]. Elevated ALP levels are significantly associated with liver metastases in CRC patients. Moreover, patients with ALP levels above 160 U/L are 12 times extra likely to have liver metastases compared to those with lower levels. In contrast, ALP levels are often within the normal range in patients without liver metastases [23]. These findings support our findings and suggest that the slight elevation in ALP levels (as shown in Fg.2a) could indicate the absence of liver involvement. furthermore, Aspartate Aminotransferase (AST), an enzyme that is used alongside ALT to evaluate liver function, showed a statistically significant differences in the newly diagnosed CRC group when compared to the control group ($P: \leq 0.001$). Although the AST mean was within normal limits, AST levels may be higher than the normal range due to liver function deficiency or tumor load [24]. Whereas CRC patients under treatment showed non-significant differences when compared to the newly diagnosed CRC patients, as presented in Table 2 and Fig.2.b. These results are consistent with a recent Iraqi study where AST levels exhibited no significant difference between patients under treatment and control group [25]. However, the study did not involve the newly diagnosed CRC patients in the studied groups. Similarly, Alanine Aminotransferase (ALT) is an enzyme chiefly found in the liver. Compared to control group, ALT mean indicated significant differences in newly diagnosed group when compared to the under treatment CRC patients and the healthy group (as shown in Fig.2c). The notable elevations in liver enzymes (ALP, ALT, and AST) in newly diagnosed CRC patients compared to controls and the decrease in those biomarkers levels in patients under treatment as presented in this study, despite being within normal clinical ranges. May triggered by the tumor existence that can lead to worsen liver dysfunction. Additionally, their regularization post-treatment may suggest effective managing of liver function. Observing these levels is vital for forecasting treatment effectiveness[28-29].

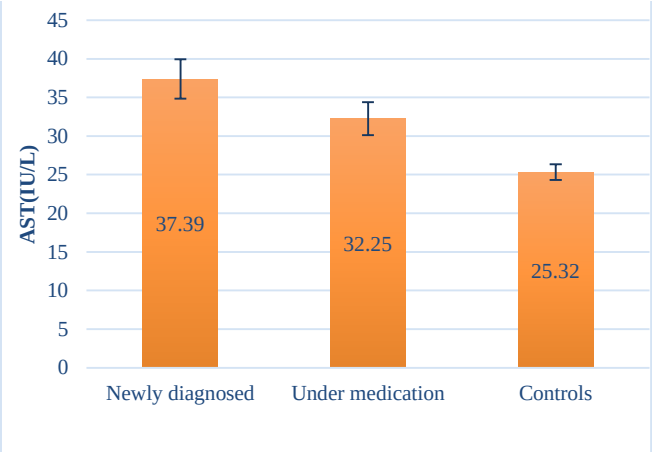
TABLE 3. SPEARMAN CORRELATION BETWEEN VITAMIN D AND LIVER BIOMARKERS IN THE STUDIED GROUPS

Parameters	Spearman correlation	P-value
Vit. D and ALP	- 0.33 **	0.0007**
Vit. D and AST	- 0.10	0.3064
Vit. D and ALT	- 0.16	0.1068
** Statistically highly significant		

Alkaline phosphatase (ALP) presented a statistically significant inverse correlation with vitamin D ($r = -0.33$) as presented in Table 3 and Fig.3. This finding is align with large clinical study that have proposed a connection between low vitamin D and high or normal ALP levels [26]. In CRC, Long-lasting inflammation may act as modulator in the relationship between vitamin D and alkaline phosphatase (ALP). Vitamin D sufficiency aids suppress inflammation, which in turn may steadying bone metabolism [27] leading to reduce ALP elevations. Conversely, based on previous study, low vitamin D levels may contribute to heightened inflammation, which can increase ALP [28]. In this study, none of the CRC patients had bone metastasis, supporting the suggestion that the slight increase (within normal threshold) may be due to low vitamin D levels. It is recognized that vitamin D insufficiency can lead to an increase in bone resorption [29]. In addition, the correlation between vitamin D and both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were weak and non-significant ($r = -0.10$, $p = 0.3064$, $r = -0.16$, $p = 0.1068$). These finding consistent with study conducted in Egypt that found a negative correlation between vitamin D and both AST and ALT in CRC patients [29]. Fewer studies were established regarding the relationship between vitamin D and liver enzyme changes. Since patients lack liver metastases, this negative correlation may reflect the fact that AST and ALT are more sensitive to direct liver injury or metastasis rather than to vitamin D status. Furthermore, the influence of vitamin D on these enzymes may be slight compared to ALP.



(a)



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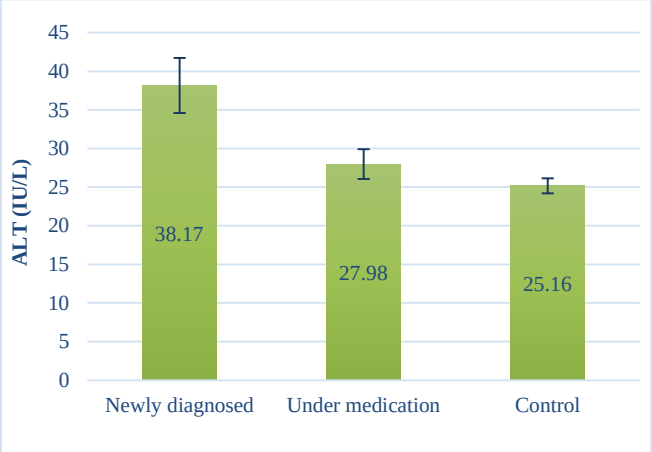

(c)

Fig. 2. Liver enzymes levels relating to the study groups (a) alkaline phosphatase (b) aspartate aminotransferase (AST) (c) alanine aminotransferase (ALT).

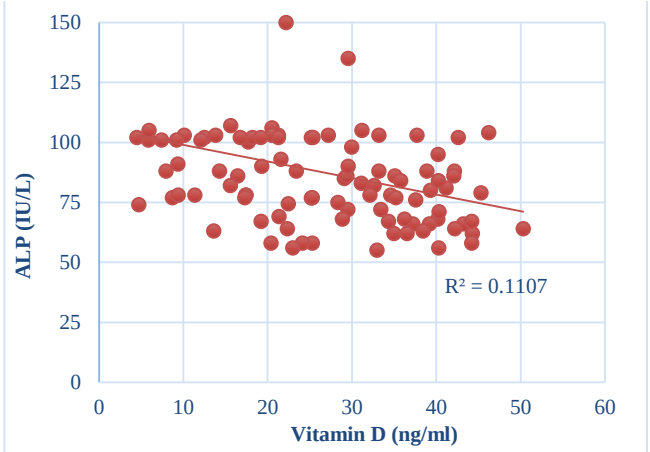

Fig. 3. Correlation between ALP and vitamin D in the studied groups.

V. Conclusion

Serum levels of vitamin D were significantly low in CRC patients in contrast to the individuals without CRC and with newly diagnosed patients exhibiting the lowest mean among the studied groups. Furthermore, liver enzymes ALP and AST were elevated in CRC patients relative to controls, although values remain within normal values. A contrary correlation was reported between vitamin D and Alkaline phosphatase proposes that low vitamin D may have an influence on ALP levels. Further longitudinal studies with data on vitamin D supplementation are required to determine the possible regulatory role of vitamin D levels on liver biomarkers in CRC patients.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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