in vivo *36*: 2414-2421 (2022)
doi:10.21873/invivo.12975

Evaluation of CD4+:CD8+ Ratio in Patients With Cervical Cancer and the Levels of Inflammatory Markers

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Abstract. *Background/Aim: Cervical cancer remains a major public health concern. The ratio of CD4+:CD8+ T-cells is used to evaluate the immune system function. This study aimed to explore the CD4+:CD8+ T-cell ratio in relation to the glycemic status, inflammatory markers, vitamin D, and vitamin B12 in patients with early diagnosed cervical cancer. Patients and Methods: This is a cross-sectional study. Blood samples were collected for flow cytometry analysis. Information regarding Papanicolaou (Pap) smears and colposcopy investigations were collected from 152 women with type 2 diabetes admitted to East Jeddah Hospital, Jeddah, Saudi Arabia, between January 2018 and January 2021. Results: Patients with early cervical carcinoma and a higher CD4+:CD8+ ratio (>1.2) had a higher C-reactive protein (CRP) level than those with a lower CD4+:CD8+ ratio (Mean±SD=13.75±13.3 vs. 10.85±8.1; p-value=0.034). Patients with early cervical carcinoma, diabetes, and higher CD4+:CD8+ ratio (>1.2) had a higher blood HbA1c percent than those with a lower CD4+:CD8+ ratio. Conclusion: A high CD4+:CD8+ T-cells ratio was associated with an increased HbA1c% and CRP levels in women with diabetes diagnosed with early cervical carcinoma, which can induce inflammation in early diagnosed patients with cervical cancer.*

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Key Words: Cervical cancer, diabetic women, C-Reactive protein, early cervical carcinoma.

Cervical cancer is a significant public health issue, as it represents the fourth leading cause of cancer incidence and mortality in women around the world. Cervical cancer affects nearly 600,000 individuals and kills about 300,000 women each year, mainly middle-aged women and those living in lower resource areas (1). The human papillomavirus (HPV) is a significant cancer risk factor. Various studies have linked recurrent HPV infections, immunodeficiency, and environmental factors to an elevated cervical cancer risk (2). Diabetes mellitus has been recognized as a cancer risk factor. In various epidemiological studies, diabetes has been shown to play a predictive role in the prognosis of a variety of cancers. Therefore, various studies have examined the prognostic role of diabetes in cervical cancer prognosis (3, 4). Antitumor immune responses are principally controlled by cell-mediated immunity. Both CD4+ and CD8+ T lymphocytes must be activated for an efficient immune response to eradicate tumor cells. It would be beneficial if there was a large number of CD8+ tumor-infiltrating lymphocytes (TILs) invading the tumor site. CD4+ TILs would be necessary as well because CD8+ T cells frequently require CD4+ T cells to function effectively. The CD4+:CD8+ T cell ratio is most likely a critical component for optimal TIL function; however, this ratio varies depending on the type of cancer (5). Early identification, differential diagnosis, and medication impact monitoring are achieved by the C-reactive protein (CRP) test. CRP's role in the early diagnosis of cervical cancer recurrence has already been established (6). Higher serum CRP levels have been associated with poor survival in patients with various

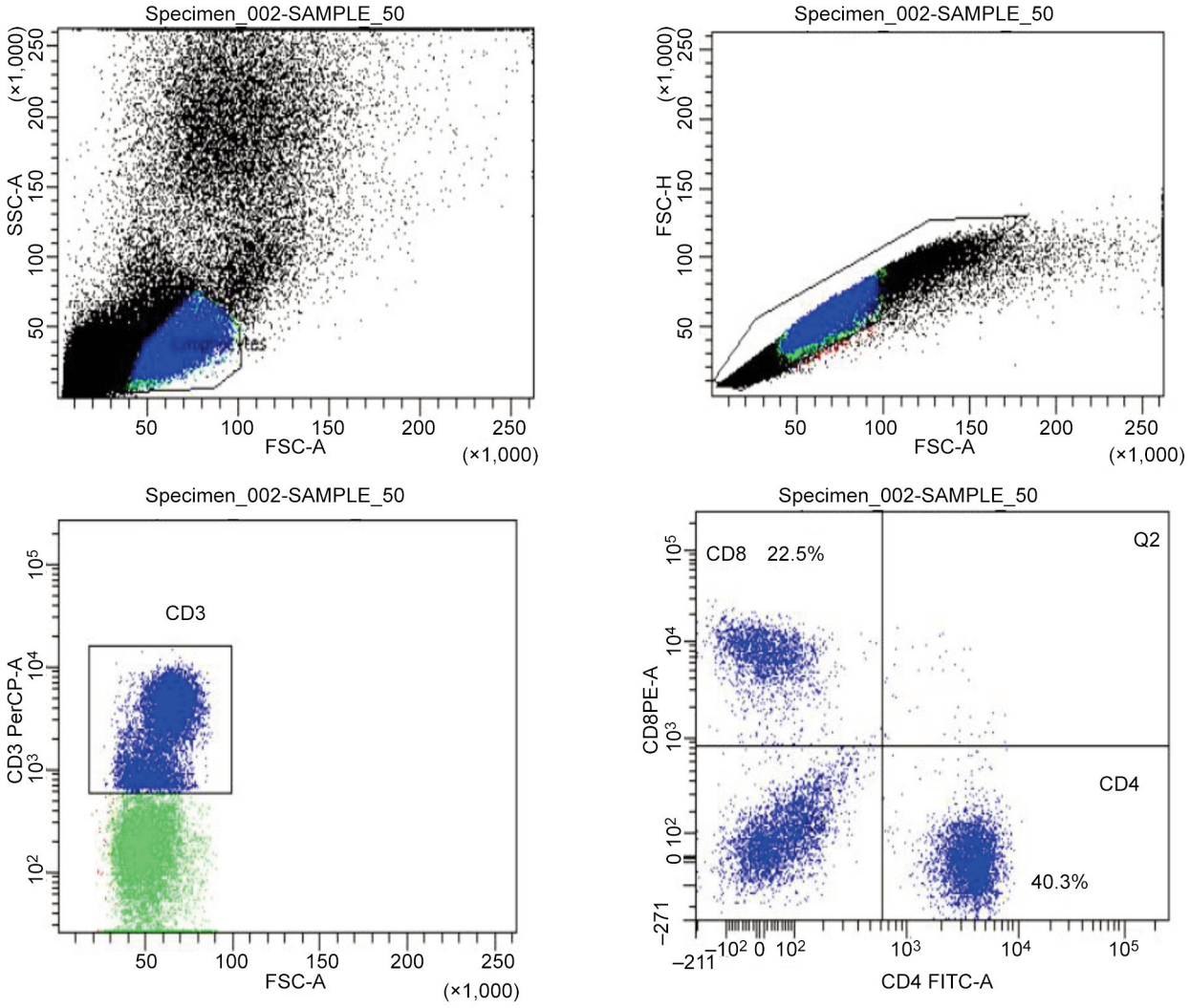

Figure 1. *Assessment of CD4+:CD8+ ratio. FACS canto was used for evaluation of CD4+:CD8+ ratio. The current patient has 3,261 CD4+ cells/mm^3 and 1,811 CD8+cells/mm^3, which results in a ratio of 1.8.*

gynecological cancers, including cervical, ovarian, and endometrial cancer (7-9). Vitamin D has shown antitumor value against a variety of cancers. It suppresses the human cervical cancer oncogene (HCCR-1) and enhances p21 expression, causing cell cycle arrest at the G1 phase (10). Treatment with vitamin D for six months reduced the severity of cervical intraepithelial neoplasia grade 1 (CIN1) and improved insulin metabolic markers (11). The anti-inflammatory properties of vitamin D and enhanced expression of insulin receptors and/or proteins in the insulin signaling cascade may play a significant role in the effect of vitamin D on insulin production and sensitivity (12). Increased folate and adequate vitamin B12 have been associated with a lower risk of pre-neoplastic lesions in the cervix. Folate and vitamin B12 may have a role in neoplasia by influencing DNA synthesis and repair. Folate plays a role in DNA methylation, which can affect gene stability and expression (13). Cancer is the most common cause of venous thromboembolism (VTE). There has been a relationship between cancer and the activation of clotting factors. The most prevalent cancer-related adverse effect is VTE, which is also the second greatest cause of death in cancer patients. Cancerous cervical tumors had the highest mean D-dimer levels, suggesting a different mechanism of VTE in gynecologic cancers. The elevated D-dimer level may display tumor progression and increased mortality (14). Serum ferritin levels may help determine the extent of cancer beyond the cervix and the prognosis of cervical cancer patients. The development of elevated ferritin levels was closely associated with a poor prognosis (15). Finding

markers for a precise diagnostic and prognostic assessment is critical to provide cervical cancer patients with exact specific treatment (16).

The present study aimed to explore the ratio of CD4+:CD8+ T-cells in early diagnosed cervical cancer patients, and evaluate the findings according to glycemic status, inflammatory markers, vitamin D, and vitamin B12.

Patients and Methods

Study design and participants. The current study is a cross-sectional study. Information regarding Papanicolaou (Pap) smears and colposcopy investigations were obtained from 152 women with type 2 diabetes admitted to East Jeddah Hospital, Jeddah, Saudi Arabia, between January 2018 and August 2021. Pap smear and colposcopy results as well as cytological and histological samples were reviewed by the pathologist of the department of pathology, East Jeddah Hospital. Abnormalities in the Pap smear and colposcopy results were evaluated in a manner consistent with the Bethesda System classification (17). Colposcopy investigations were performed by a colposcopist, and the results were evaluated according to the International Federation of Cervical Pathology and Colposcopy recommendations (18). Early diagnoses of carcinoma of the cervix were made by liquid-based cytology (19). The study was approved by the Ministry of Health in the Kingdom of Saudi Arabia (registration number HAP-02-T-067).

Data collection. Demographic and clinical data were collected from hospital records, stored in an electronic database, and independently examined by two researchers. Patient data that were linked to early cervical carcinoma were obtained, such as age and gravidity. Inclusion criteria were: early cervical carcinoma with cytology and colposcope confirmation of low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions, invasive cervical cancers, and adenocarcinoma. Exclusion criteria were: history of pelvic radiation or hysterectomy, hepatitis B or hepatitis C positive serology, immunocompromised patients either confirmed or clinically suspected immunosuppression, immunosuppressive medications and other severe systemic diseases.

Blood samples. A total of 10 ml of venous blood samples were taken from each member of the study group after 10 hours of fasting and allocated into two portions. The first part was collected in plain tubes for serum isolation and centrifuged, and then serum was stored at –20˚C until used for biochemical analysis. The second part was collected in EDTA tubes to be used for estimation of glycosylated hemoglobin (HbA1c), evaluation of CD4+ and CD8+ lymphocyte counts, and calculation of the ratio.

Assessment of CD4+ and CD8+ lymphocytes by flow cytometry. Flow cytometry is the standard method for determining CD4+ and CD8+ percentages and absolute counts (Figure 1). Lymphocytes were gated, then doublet cells were isolated, following that CD3+ T cells were gated and expressed only CD4+ and CD8+ to calculate the ratio. The CD4:CD8 ratio is estimated by dividing the number of CD4+ T cells by the number of CD8+ T cells; normal values for CD4 lymphocytes range from 500 to 1200/mm^3 and CD8 lymphocytes range from 150 to 1,000/mm^3. In immunocompetent individuals, this ratio is usually greater than one (20).

Table I. *Characteristics of cervical cancer patients. Most of the cases were age 40-60 years, with vitamin D deficiency, normal Vitamin B, and with HbA1c>6.5.*

Characteristics	Frequency Cases N (%)	Median	Chi-Square *p*-Value
Age (years)			
<40	22 (14.5%)	37 (24-38)	<0.001*
40-60	114 (75)	46 (41-59)	
>60	16 (10.5)	68 (62-89)	
Vitamin D (mg/ml)			
>50	5 (3.3)	59.6 (55.5-70.3)	<0.001*
30-49	20 (13.1)	36.3 (30.8-48.1)	
<30	127 (83.5)	15.3 (4.3-29.8)	
Vitamin B12 (pg/ml)			
>240	119 (78.3)	481 (247-1,476)	<0.001*
150-240	19 (12.5)	216 (156-234)	
<150	14 (9.2)	132 (10.10-149)	
Glycemic status (HbA1c %)			
Non	30 (19.7)	5.3 (4.8-5.9)	<0.001*
Pre	65 (42.7)	6.2 (6-6.5)	
Diabetic	57 (37.5)	7 (6.6-10.4)	

Pearson's chi squared test. *Significant *p*-value<0.05.

Biochemical analysis. Glycated hemoglobin (HbA1c) was analyzed using the automated glycosylated hemoglobin analyzer (Bio-Rad, New York, NY, USA). Serum CRP was evaluated by the immunoturbidimetric method (CRP II Latex X2, Denka, Tokyo, Japan), utilizing an autoanalyzer (Toshiba, Tokyo, Japan). Serum ferritin levels were estimated using an ELISA kit (BioVendor, Laboratorní medicína, Brno, Czech Republic, Cat. No. RCD012R). Serum D-dimer was measured using a human Abcam ELISA kit (Cat No. ab260076). Serum 25(OH) D levels were determined using an Abcam human vitamin D ELISA kit (Seattle, WA, USA, Cat No. ab213966), following the manufacturer's protocol. 25(OH) D is commonly used as an indicator of vitamin D status in the body. Chemiluminescence immunoassay was used to determine serum vitamin B12 levels (21).

Statistical analysis. Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS, Chicago, IL, USA). Quantitative data were expressed as mean±standard deviation (SD). Qualitative data were expressed as frequency and percentage. Correlation and linear regression were assessed by JASP 0.14. by scatter correlation diagrams.

Results

Characteristics of early cervical carcinoma women (152) included in the study are shown in Table I. Most women were in the middle age between 40 to 60 years (114 patients, representing 75% of the study sample). Regarding vitamin D levels, 119 patients showed low vitamin D levels (83.55% of the study sample). Regarding vitamin B12 levels, 119 patients showed high levels (representing 78.3%). Furthermore, 65 patients (42.76%) were pre-diabetic (Table I).

Table II. *Inflammatory markers. Mean values of CRP, Ferritin, D-dimer, Vitamin D, Vitamin B12, and glycemic status in patients according to their CD4+:CD8+ ratio.*

Markers		CD4+:CD8+ ratio				
		≤1.2		>1.2		
		Mean±SD	Frequency	Mean±SD	Frequency	Un-paired *t*-test
CRP (mg/dl)		10.85±8.1	98.20%	13.75±13.3	100%	0.034*
Ferritin (ng/ml)		743±65	91.6%	853±65	93%	0.153
D-dimer (ng/ml)		2±3	78%	2.575±4.5	76.5%	0.305
Vitamin D (mg/ml)						
	>50	60±3.25	3%	61.8±7.6	5%	0.29
	30-49	36.4±5.9	12%	38.5±5.2	13.55%	0.28
	<30	17.4±6	85%	16.4±6.9	81.45%	0.7
Vitamin B12 (pg/ml)	>240	545±34	74.40%	583±90	7.50%	0.353
	150-240	207.4±13	11.86%	212.1±25	10.75%	0.326
	<150	129±19	13.74%	102.3±34	81.72%	0.239
Glycemic status (HbA1c %)	Non	5.35±0.25	22.50%	5.34±0.34	15.25%	0.982
	Pre	6.15±0.25	36.50%	6.21±0.14	52.50%	0.093
	Diabetic	7±0.5	41%	7.26±0.9	32.25%	<0.001*

Frequency: percentage of cases with abnormal levels of this marker. *t*-test, calculated by comparing means. *Significant *p*-value <0.05.

Early cervical carcinoma women with an increased CD4+:CD8+ ratio (>1.2) showed significantly higher CRP levels than did women with early cervical carcinoma with a decreased CD4+:CD8+ ratio (<1.2) (Mean±SD=13.75±13.3 *vs.* 10.85±8.1; *p*-value=0.034). In addition, women with diabetes and increased CD4+:CD8+ ratio showed significantly higher blood HbA1c percent (>1.2) than did women with a decreased CD4+:CD8+ ratio (<1.2) (Mean±SD=7±0.5 *vs.* 7.26±0.9; *p*-value<0.001). Moreover, a comparison of the levels of the other parameters including ferritin, D-dimer, Vitamin D, and Vitamin B12 revealed no significant difference between the two groups of women patients with early cervical carcinoma (Table II).

In the current research, we investigated the correlation between the CD4+:CD8+ ratio and the other parameters included in the study. A positive correlation was observed between the CD4+:CD8+ ratio and HbA1C, CRP, and ferritin (Figure 2). These values indicate that a higher ratio of these cells can increase the levels of those markers in patients diagnosed with early cervical cancer. A negative correlation between the ratio of CD4+:CD8+ T-cells and D-dimer and Vitamin D was also observed (Figure 2).

Discussion

CD4+ and CD8+ T cells are the most common lymphocytes in cell-mediated immunity; they play a crucial role in the formation of efficient immune responses against cancer. The distribution of T cell subtypes in blood and tumor tissue, as well as draining lymph nodes, can be utilized to evaluate the immune system in cancer (22).

Seventy-five percent of the early cervical carcinoma women in this study were between the ages of 40 and 60 years. In accordance with these results, Quinn *et al.* (23) found that cervical carcinoma affected 54 percent of women aged 50 years, 33 percent of women aged from 50 to 69 years, and the older women, particularly those over the age of 70, have significantly lower survival rates when classified by stage and histology.

Regarding CRP levels, our results revealed a significant increase in serum CRP levels in women with diabetes diagnosed with early cervical carcinoma. The most common biomarker of the systemic inflammatory response in cancer patients is the CRP levels in the blood. CRP levels may have elevated as a result of increased production of pro-inflammatory cytokines, certainly of pro-inflammatory and immune-regulatory factors such as TNF-α and IL-6, which promote inflammation (24, 25). In a variety of cancers, including cancer of the cervix, serum CRP levels are related to tumor stages. The stronger the inflammatory response and the higher the CRP serum level, the greater the tumor stage (20).

Results of the present study showed that 83.55% of women with early cervical carcinoma had low vitamin D levels (<30) with a median of 15.3 (range=4.3-29.8 ng/ml). Ozgu *et al.* (26) revealed a statistically significant decrease in 25(OH) D levels in a comparison between HPV positive patients with cervical cancer and the control group; they highlighted how a vitamin D deficiency could lead to chronic HPV infection and, as a result, to cervical intraepithelial neoplasia. According to a study by Friedrich *et al.* (27), vitamin D receptor (VDR) expression was substantially

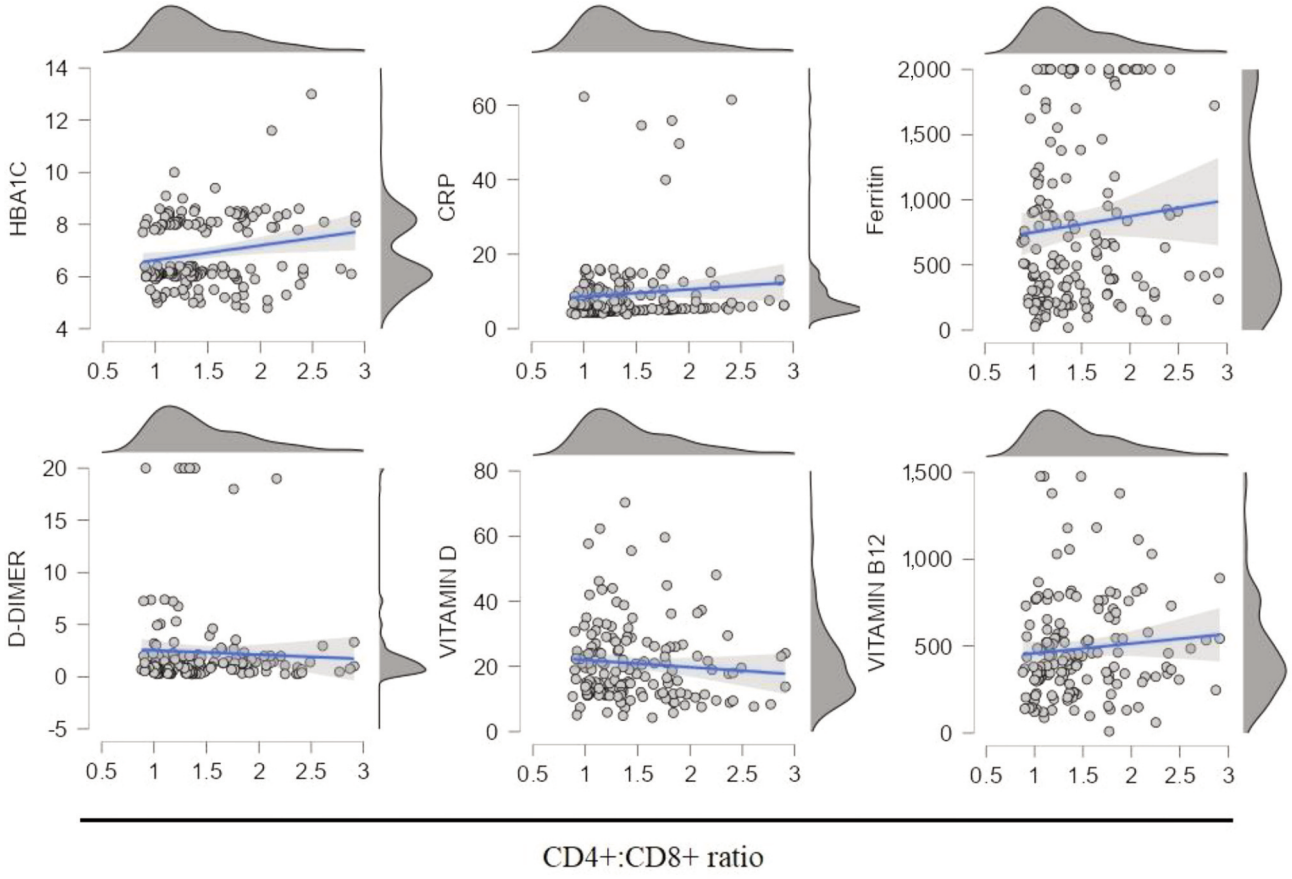

Figure 2. *Correlation of CD4+:CD8+ ratio with inflammatory markers. Scatter plots were used to study the correlation between the ratio of CD4+:CD8+ lymphocytes and inflammatory markers. HBA1C, CRP, ferritin, and Vitamin B12 levels show positive correlation with the increase of the ratio of CD4+:CD8+ lymphocytes. While D-dimer and Vitamin D show negative correlation with the ratio of CD4+:CD8+ lymphocytes. CRP, and D-dimer show linear correlation, and HBA1C shows slightly positive correlation.*

higher in cervical carcinoma tissue than in normal cervix tissue at the protein level, while no statistically significant associations between the VDR status and histopathology were discovered.

The results of the present study showed that 78.3% of studied cases had vitamin B12 >240 pg/ml with a median of 481 pg/ml (range=247-1,476 pg/ml). In contrast to our results, Kwanbunjan *et al*. (28) reported that women with normal cytological smears showed significantly increased serum vitamin B12 levels than those with both high- and low-grade cervical dysplasia. Low levels of vitamin B12 in the blood were associated with a higher incidence of low-grade cervical dysplasia (OR=4.08; 95%CI=1.41-11.79; p<0.05) and high-grade cervical dysplasia (OR=3.53; 95%CI=1.24-10.04; p<0.05).

There was a highly statistically significant difference in CD4+:CD8+ ratio in patients with diabetes compared to controls, as shown in Table II. Diabetic ketoacidosis and hyperglycemia patients had higher levels of proinflammatory cytokines and activated CD4+ and CD8+ T lymphocytes. With low effective insulin levels and high glucose and free fatty acid levels, diabetes promotes oxidative stress and an inflammatory environment (29).

The current study results showed a positive correlation between a high CD4+:CD8+ ratio and HbA1c (Figure 2). Jiamset *et al*. (30) stated that patients with type 2 diabetes have a poorer cervical cancer prognosis than those without diabetes. Vavallo *et al*. (31) also reported that hyperglycemia in patients with type 2 diabetes may have a role in tumor progression by promoting DNA damage and activating various signaling pathways linked to carcinogenesis and metastasis. Contrary to our results, Zhang *et al*. (32) revealed that, in individuals with diabetes and impaired glucose tolerance (IGT), CD4+ and the CD4+:CD8+ ratio was significantly low. Fasting blood glucose and HbA1c were inversely correlated with CD4+ T cells and with the CD4+:CD8+ ratio.

Furthermore, the current results showed a positive correlation between a high CD4+:CD8+ ratio and CRP levels (Figure 2). Schmid *et al*. (33) stated that patients with

gynecological cancers—such as cervical, ovarian, and endometrial cancer—have worse overall survival when their serum CRP levels are high. Also, Wang *et al*. (34) found that high CRP levels were associated with a poor prognosis in early curable cervical cancer.

Our results showed a negative correlation between a high CD4+:CD8+ ratio and vitamin D (Figure 2). Vitamin D and its metabolites have anti-inflammatory activities; therefore, a deficiency could play a role in HPV DNA persistence and cervical intraepithelial neoplasia. Serum vitamin D might play a protective role in cervicovaginal HPV infection (26). Vitamin D inhibits the development of tumor growth and the infiltration of cytotoxic CD8+ T lymphocytes in tumors (35).

Our results showed a positive non-linear correlation between the CD4+:CD8+ ratio and vitamin B12 (Figure 2). Vitamin B12 deficiency has been linked to increased risk of high-grade cervical lesions. There was a decrease in the absolute number of lymphocytes, notably CD8+ cells, in vitamin B12-deficient patients and an increase in the CD4+:CD8+ ratio. Vitamin B12 treatment resulted in an increase in lymphocytes, notably CD8+ cells and a substantial increase in natural killer cell activity in patients (36).

Our results showed a negative correlation between a high CD4+:CD8+ ratio and D-Dimer (Figure 2). Before treatment, the levels of plasma D-dimer could be a predictor of cervical cancer (37). In addition, there was a negative correlation between high CD4+:CD8+ ratio and ferritin (Figure 2). Serum ferritin is a predictor of cancers such as malignant lymphoma, cervical cancer, breast cancer, and other types (38).

There are limitations to our study, such as the absence of data about HPV infections, which are a common risk factor in cervical cancer. The sample size is small since cervical cancer is uncommon in Saudi Arabia. Also, there was a shortage in the recorded data regarding the status of other vitamins. It is not specified whether all patients refrained from taking these vitamins at least 3 months before the investigations or not. Therefore, we suggest further prospective studies that will consider these points.

Conclusion

Our study has found that a high CD4+:CD8+ ratio was associated with increased HbA1c% and CRP levels in women with diabetes diagnosed with early cervical carcinoma. This can lead to an increase in the inflammatory response in those patients. The outcomes show that investigation of these parameters could be helpful for the prediction of cervical carcinoma among patients with diabetes and may support the improvement of the treatment policy.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

MA, AG, MS, AS, AA, NA, AE, KA, AA, OA, AA: participated equally. AA, HA, MH, SA, EA: samples collection and inflammatory markers analysis.

Acknowledgements

The Authors would like to thank the deanship of scientific research for financially supporting this project (project no. 1-440-6143), all medical staff in King Faisal hospital, and everyone participated in this study.

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Received May 21, 2022
Revised June 13, 2022
Accepted June 14, 2022