



# Demographic Characteristics and the Folate, Vitamin B12 and Vitamin D Serum Levels in Cervical Intraepithelial Lesions

## Servikal İntraepithelial Lezyonlarda Demografik Özellikler, Folat, Vitamin B12 ve Vitamin D Serum Seviyeleri

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### ABSTRACT

**Objective:** Human papillomavirus (HPV) infection is necessary but not sufficient for the development of cervical cancer, and some other factors are known to play a role in this progression. Here, we aimed to investigate the serum levels of folate, vitamin B12, and vitamin D, and the demographic characteristics in cervical cancer precursor lesions.

**Material and Methods:** Patients who had undergone colposcopy and tested for the serum levels of folate, vitamin B12, and vitamin D were included in this study. Based on histological outcomes, patients were grouped as those with cervical intraepithelial neoplasia grade 1 or lesser abnormalities ( $\leq$  CIN 1) and those with cervical intraepithelial neoplasia grade 2 or higher abnormalities ( $\geq$  CIN 2). Among these two groups, patients were compared in terms of demographic characteristics such as age; parity; abortus number; menopausal status; smoker status; use of intrauterine devices; postcoital bleeding complaint and the serum levels of folate, vitamin B12, and vitamin D.

**Results:** 83 patients were enrolled in the present study. The median age of the study population was 41 years and 22 patients (26.5%) were currently smokers. None of the patients were using any vitamin or folate supplements. According to the highest grade of histology outcomes; 67 (80.7%) patients had  $\leq$  CIN 1 and 16 (19.3%) patients had  $\geq$  CIN 2 lesions. The serum levels of folate, vitamin B12, and vitamin D did not differ between the groups. Current smoking was the only parameter related with the occurrence of  $\geq$  CIN 2 lesions (36.4% vs. 13.1%;  $p=0.018$ ). In addition, the occurrence of  $\geq$  CIN 2 lesions increased with the duration of smoking ( $p=0.031$ ).

**Conclusion:** Folate, and vitamins B12 and D are not risk factors for  $\geq$  CIN 2 lesions when present at average or subnormal levels. Current smoking is a cofactor for the occurrence of  $\geq$  CIN 2 lesions. Thus, the risk increases with the duration of smoking. Smoking cessation should be suggested in women with cervical premalignant lesions.

**Key Words:** Cervical cancer, Folate, Vitamin B12, Vitamin D, Smoking

### ÖZ

**Amaç:** Serviks kanseri gelişiminde Human papilloma virüs (HPV) en önemli faktör olmakla beraber bu süreçte kofaktörler de rol oynamaktadır. Çalışmamızda, servikal intraepithelial lezyonlarda demografik özellikleri, folat, B12 vitamini, D vitamini serum seviyelerini araştırdık.

**Gereç ve Yöntemler:** Çalışmaya kolposkopi yapılan ve folat, B12 vitamini ve D vitamini serum düzeyleri ölçülmüş hastalar dahil edildi. Histoloji sonuçlarına göre hastalar, servikal intraepitelyal neoplazi derece 1 veya normal ( $\leq$  CIN 1) ve servikal intraepitelyal neoplazi derece 2 veya daha yüksek anormallikler ( $\geq$  CIN 2) olan iki gruba ayrıldı. Bu iki grup arasında, hastaların yaşı, paritesi, abortus sayısı, menopozal durumu, sigara kullanımı, rahim içi araç kullanımı, postkoital kanama şikayeti gibi demografik özelliklerinin yanı sıra, folat, B12 vitamini ve D vitamini serum seviyeleri karşılaştırıldı.

**Bulgular:** 83 hasta çalışma kapsamında değerlendirildi. Hastaların ortalama yaşı 41 idi ve 22'si (%26,5) hâlen sigara içiyordu. Hastaların hiçbiri herhangi bir vitamin veya folik asit takviyesi almıyordu. Histoloji sonuçlarının en yüksek derecesine göre; 67 (%80,7) hastada  $\leq$  CIN 1 ve 16 (%19,3) hastada

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≥ CIN 2 lezyonu tespit edildi. Serum folat, B12 vitamini ve D vitamini düzeyleri gruplar arasında farklılık göstermedi. Mevcut sigara kullanımı ≥ CIN 2 lezyonlarının ortaya çıkmasına bağlı olan tek parametre idi (%36,4'e karşılık %13,1; p=0,018). Ayrıca, ≥ CIN 2 lezyonlarının ortaya çıkmasının sigara içme süresi ile arttığı belirlendi (p=0,031).

**Sonuç:** Folat, B12 ve D vitaminleri, vücutta ortalama veya subnormal seviyelerde bulunduğu ≥ CIN 2 lezyonları için risk faktörü değildir. Sigara kullanımı, serviks kanseri gelişiminde önemli bir kofaktördür. Risk, sigara kullanım süresi ile artmaktadır. Hastalara sigara kullanımının sonlandırılması önerilmektedir.

**Anahtar Sözcükler:** Serviks kanseri, Folat, Vitamin B12, Vitamin D, Sigara

## INTRODUCTION

A total of 571,000 new cases and 311,000 deaths worldwide annually are attributed to cervical cancer, a disease that continues to grow in importance as a health problem (1). Cervical cancer develops following the onset of a human papillomavirus (HPV) infection. In this process, the HPV infection persists and eventually progresses to cervical intraepithelial neoplasia and then cancer (2). Importantly, however, HPV infection alone is necessary but not sufficient for the progression from HPV infection to cervical cancer, as some cofactors also play key roles (3). The mechanisms of these cofactors in cervical cancer pathogenesis remain unclear but are thought to involve the influence of cellular immunity effects on HPV clearance and disease progression (4).

Serum levels of folate, vitamin B12, and vitamin D were previously reported to be associated with cervical cancer pathogenesis (5-11). However, conflicting results are present for serum levels of folate and vitamin B12, while studies for vitamin D are limited in number (12-14). In addition, the effects of those biochemical markers may differ between countries depending on genetics and variations in the serum levels of vitamins amongst the populations (15). Therefore, in the present study, we aimed to analyse the impacts of serum levels of folate, vitamin B12, vitamin D, and demographic characteristics in the cervical cancer precursor lesions.

## MATERIAL and METHODS

After approval of the present study by Akdeniz University Ethics Committee on 28/8/2019-786, female patients who had undergone colposcopy at our hospital's gynaecological oncology outpatient clinic between the years 2016-2019 were evaluated. The study has been conducted in compliance with the principles of the Helsinki Declaration. Women who had undergone colposcopy and tested for the serum levels of folate, vitamin B12, and vitamin D were included in the study. All women underwent liquid-based cytology prior to colposcopy. Demographic characteristics such as age; parity; abortus number; menopausal status; smoker status; current contraception method; and postcoital bleeding complaint were also recorded.

All cytologies, colposcopic biopsies, histological evaluations, and biochemistry tests were performed at our hospital. Serum levels of folate, vitamin D, and B12 were measured either on the day of colposcopy or within a few days before the colposcopy. Biopsy specimens were collected from all abnormal areas and endocervical sampling was performed according to the decision of the colposcopist in cases of absent or insufficient transformation zone observation during colposcopy. Following colposcopic biopsy and endocervical sampling, patients with the appropriate indication underwent the loop electrosurgical excision procedure (LEEP). Cytological and histological evaluations were performed by gynaecopathologists. Cytologies were reported as normal, atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells suspicious for high-grade squamous intraepithelial lesions (ASC-H), low-grade squamous intraepithelial lesions (LSIL), or high-grade squamous intraepithelial lesions (HSIL).

Results from colposcopic biopsies, endocervical samplings, and LEEP histologies were reported as either normal [no cervical intraepithelial neoplasia (CIN) or cancer], CIN grade 1, CIN grade 2, CIN grade 3, or cancer. For the colposcopic biopsy, endocervical sampling, and LEEP examinations, the highest degree of cervical premalignant lesion was considered for histological outcome. Based on the histological outcomes, patients were divided into two groups, those with ≤ CIN 1 and those with ≥ CIN 2 lesions. ≤ CIN 1 lesions included CIN 1 and normal histology results, whereas ≥ CIN 2 lesions included CIN 2, CIN 3, and cervical cancer. In these two groups, patients were compared in terms of demographic characteristics such as age; parity; abortus number; menopausal status; smoking status; use of intrauterine devices; postcoital bleeding complaint and serum levels of folate, vitamin B12, and vitamin D.

## Statistical method

The Statistical Package for the Social Sciences version 23.0 for Windows software program (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Frequencies of categorical variables were found using descriptive statistics. Continuous variables were evaluated with an analytical method (Kolmogorov–Smirnov test) to assess whether they

were normally distributed or not. As all were abnormally distributed, parameters were expressed as medians and percentiles. The response variable was histologically diagnosed  $\geq$  CIN 2 lesions. The Mann-Whitney U test for abnormally distributed parameters and chi-squared test for categorical variables, respectively, were used to evaluate the relationship of said categorical and continuous variables with the response variable. Finally, p-values of less than 0.05 were defined as statistically significant.

## RESULTS

A total of 83 patients were enrolled in the present study. None of the included women were using any vitamin or folate supplements. Continuous variables (i.e., age; gravidity; parity; abortus; and serum levels of folate, vitamin B12, and vitamin D) were found to distributed abnormally

and therefore were expressed as medians and percentiles. The median age of the study population was 41 years and 26.5% (n=22) were currently smokers. Demographic characteristics, and the folate and vitamins B12 and D serum levels of the study population are given in Table I.

When considering the highest degree of histology outcome following colposcopic biopsy, endocervical sampling, and LEEP, 67 (80.7%) patients had  $\leq$  CIN 1 and 16 (19.3%) patients had  $\geq$  CIN 2 lesions. The relationship of demographic characteristics and folate, vitamin B12, and vitamin D serum levels with the occurrence of  $\geq$  CIN 2 lesions are presented in Table II. Among the studied parameters, smoking was the only parameter associated with the occurrence of  $\geq$  CIN 2 lesions. In total, 36.4% of smokers compared with 13.1% of nonsmokers presented  $\geq$  CIN 2 lesions (p=0.018).

**Table I:** Demographic characteristics, folate, vitamins B12 and D serum levels of the study population.

Characteristic	Parameters	Values (%)
Age (years)	Median	41
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	32-48
Gravidity	Median	2
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	1-4
Parity	Median	2
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	0-2
Abortus	Median	0
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	0-1
Use of intrauterine device	Present	28 (33.7)
	Absent	55 (66.3)
Smoking habitus	Non-smoker	61 (73.5)
	Smoker	22 (26.5)
	<1 year	1 (1.2)
	1-5 years	2 (2.4)
	6-10 years	7 (8.4)
	11-20 years	7 (8.4)
	21-30 years	3 (3.6)
>30 years	2 (2.4)	
Menopausal status	Premenopause	56 (67.5)
	Postmenopause	27 (32.5)
Postcoital bleeding	Negative	78 (94.0)
	Positive	5 (6.0)
Folate	Median	8.4700
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	6.2950-11.1400
Vitamin B12	Median	297.00
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	235.000-384.000
Vitamin D	Median	16.10
	25 <sup>th</sup> -75 <sup>th</sup> percentiles	9.2000-22.6900

**Kolmogorov-Smirnov p values:** Age; 0.006, **gravidity:** <0.001, **parity:** <0.001, **abortus:** <0.001, **folate:** <0.001, **vitamin B12:** <0.001, **vitamin D:** 0.005.

The occurrence of  $\geq$  CIN 2 lesions were also associated with the duration of smoking (Table III). Further,  $\geq$  CIN 2 lesions were seen more frequently in women who had been smoking for a longer time ( $p=0.031$ ).

## DISCUSSION

In the present study, smoking was the only parameter identified as related with the occurrence of  $\geq$  CIN 2 lesions. In addition, we determined that the occurrence of  $\geq$  CIN 2 lesions increased with the duration of smoking. We did not find any relationship between folate, vitamin B12, and vitamin D levels and the occurrence of  $\geq$  CIN 2 lesions in

our population, although none of the women in our study were taking such supplements.

Higher levels of vitamin B12 and folate have been linked with decreased DNA damage by inhibiting HPV integration into the cell genome (16-17). However, some studies investigating the levels of folate and vitamin B12 report inconsistent results (7,9,12,13). Sedjo et al. found no relationship between serum folate and vitamin B12 in the progression of HPV and CIN (12). Elsewhere, it was found that vitamin B12 and folate tended to be lower but not significantly different in CIN 3 and cancer patients as

**Table II:** The relationship of demographic characteristics and folate, vitamin B12, and vitamin D serum levels with the occurrence of  $\geq$  CIN 2 lesions.

Characteristics	Parameters	Histology		Univariate analysis p value
		$\leq$ CIN 1	$\geq$ CIN 2	
Age (years)	Median	41	40	0.223 <sup>a</sup>
	IQR	15.00	11.75	
Gravidity	Median	2	2	0.170 <sup>a</sup>
	IQR	2	2	
Parity	Median	2	2	0.188 <sup>a</sup>
	IQR	1	1	
Abortus	Median	0	0	0.602 <sup>a</sup>
	IQR	1	1	
Smoking habitus	Non-smoker	53 (86.9)	8 (13.1)	0.018 <sup>b</sup>
	Smoker	14 (63.6)	8 (36.4)	
Use of intrauterine device	Present	22 (78.6)	6 (21.4)	0.723 <sup>b</sup>
	Absent	45 (81.8)	10 (19.2)	
Menopausal status	Premenopause	43 (76.8)	13 (23.2)	0.190 <sup>b</sup>
	Postmenopause	24 (88.9)	3 (11.1)	
Postcoital bleeding	Negative	62 (79.5)	16 (20.5)	0.578 <sup>c</sup>
	Positive	5 (100.0)	0 (0)	
Folate	Median	8.6600	7.4600	0.603 <sup>c</sup>
	IQR	4.85	6.01	
Vitamin B12	Median	297.000	299.000	0.768 <sup>c</sup>
	IQR	126.00	287.00	
Vitamin D	Median	16.100	16.390	0.972 <sup>c</sup>
	IQR	13.70	15.52	

**IQR:** interquartile range; **CIN:** cervical intraepithelial neoplasia; **a:** Mann-Whitney U test, **b:** Pearson's Chi-square test. **c:** Fisher's exact test.

**Table III:** Relationship of duration of smoking with the occurrence of  $\geq$  CIN 2 lesions.

Histology	Never	<1 year	1-5 years	6-10 years	11-20 years	21-30 years	>30 years
$\leq$ CIN 1	53 (79.1)	1 (1.5)	2 (3.0)	5 (7.5)	3 (4.5)	1 (1.5)	2 (3.0)
$\geq$ CIN 2	8 (50.0)	0	0	2 (12.5)	4 (25.0)	2 (12.5)	0

**CIN:** cervical intraepithelial neoplasia.

compared with the control group with lesser lesions (13). In contrast, it was shown that the serum folate level tended to be lower in  $\geq$  CIN 2 but not different in CIN 1 patients (7). However, Piyathilake et al. documented that folate has a protective effect against the progression of cervical cancer precursor lesions only when its concentration is very high and there is sufficient vitamin B12 present as well (9). When plasma folate was at supraphysiological concentrations ( $>19.8$  ng/mL) with sufficient plasma vitamin B12 ( $\geq 200.6$  pg/mL), a decrease in the occurrence of  $\geq$  CIN 2 was observed when compared with women with plasma folate levels of up to 19.8 ng/mL and plasma vitamin B12 levels of less than 200.6 pg/mL (odds ratio: 0.3;  $p=0.04$ ) (9). The same authors also reported this protective effect in terms of HPV positivity only at highest tertile levels of both folate and vitamin B12 in their population (14). In the present study, we documented no difference in the levels of vitamin B12 and folate in patients with  $\leq$  CIN 1 and  $\geq$  CIN 2. However, the median folate level of our study population was less than half of the folate level that achieved a protective effect as described by Piyathilake et al. (9). Our study confirms the noneffectiveness of folate and vitamin B12 at average or subnormal levels on preventing the progression of cervical premalignant lesions.

There are few studies that have analysed the effects of vitamin D in cervical precancerous lesions (10,11,14). Vitamin D is thought to be effective in the immune system in promoting the clearance of HPV (10). Studies have suggested the effectiveness of Vitamin D in preinvasive lesions depending on the grade of CIN. In a recent study conducted involving patients with CIN 1 lesions, it was shown that six months of vitamin D supplementation promoted the regression of lesions when compared with no vitamin D supplementation (84.6% vs. 53.8%;  $p = 0.001$ ) (11). Conversely, Hosono et al. suggested that there was no difference in daily vitamin D intake in patients with CIN 3 lesions versus patients without preinvasive lesions (14). Similar to our study, serum levels of vitamin D were investigated by Ozgu et al., who documented the low levels of vitamin D in HPV-positive patients as compared with in HPV-negative patients (11). In the present study, we recorded low levels of vitamin D in both groups but could not find a difference in the levels of vitamin D between patients with  $\geq$  CIN 2 and  $\leq$  CIN 1 lesions, respectively. Based on the previous research and our findings, we assume that vitamin D may have a protective effect on the clearance of CIN 1 and HPV infection but not for high-grade lesions.

There is strong evidence that smoking is associated with cancer (18). Smoking increases the risk of carrying HPV in parallel with the increase in the amount of cigarettes smoked per day (19). Thus, women who smoke 15 or more cigarettes per day have a twofold higher risk of developing

HPV infection than nonsmokers (19). However, Syrjanen et al. showed that smoking is not a risk factor for  $\geq$  CIN 2 lesions except in those patients who are at high risk for HPV positivity (20). Similarly, Harris et al. concluded that smoking increases the progression of HPV to CIN lesions; however, no effect on the development of HPV lesions was observed (21). In our study, we found that smoking in parallel with disease duration increases the risk of  $\geq$  CIN 2 lesions. In our analysis, 36.4% of smokers as compared with 13.1% of nonsmokers presented  $\geq$  CIN 2 lesions ( $p = 0.018$ ), while especially those patients who smoked more than five years were at risk for developing  $\geq$  CIN 2 lesions.

As they were not tested, we were unable to analyse the effects of the well-known important cervical cancer risk factors like HPV positivity and HPV subtypes, as HPV 16 and 18 are more likely to be related with cervical cancer (22). Further, we could not investigate the role of sexual behaviour like age at first sexual intercourse or the number of sexual partners due to sociocultural reasons. However, our study remains valuable due to its analysis of the effects of smoking and serum levels of folate, vitamin B12, and vitamin D in the same study population because all of these factors have effects on cellular immunity and DNA damage, which have a potential role in cervical cancer pathogenesis. Moreover, our study group was not using any supplementation including vitamins or folate; therefore, our study population provides data on the levels and outcomes of micronutrients at average or subnormal levels in relation to the progression of cervical premalignant lesions.

## CONCLUSION

Current smoking is a risk factor for the progression of cervical cancer, and the risk increases with the duration of smoking. Folate and vitamins B12 and D are not risk factors for  $\geq$  CIN 2 lesions when present at average or subnormal levels in the body. Finally, smoking cessation should be suggested in patients with cervical premalignant lesions.

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## Conflict of interest statement

The authors declare no conflicts of interest.

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## Ethics Committee Approval

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## Author Contributions

Surgical and medical practices- T.Ş., H.A.T.; Concept- T.Ş., H.A.T.; Data collection and analysis- T.Ş., H.A.T.; Literature Search- T.Ş., H.A.T.; Writing- T.Ş., H.A.T.

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