Özgün Çalışma / Original Article

INFLAMMATORY HEMATOLOGICAL MARKERS IN PREDICTION OF CERVICAL INTRAEPITHELIAL NEOPLASIA RECURRENCE IN NEGATIVE SURGICAL MARGINS

CERRAHİ SINIRI NEGATİF OLGULARDA SERVİKAL İNTRAEPİTELYAL NEOPLAZİ NÜKSÜNÜ ÖNGÖRMEDE İNFLAMATUAR HEMATOLOJİK BELİRTEÇLERİN YERİ

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ABSTRACT

AIM: This study aimed to evaluate the predictive value of inflammatory hematological markers in cervical intraepithelial neoplasia recurrence after conization surgery.

MATERIAL AND METHOD: In this retrospective study, patients who underwent conization due to cervical intraepithelial neoplasia were analyzed. Inclusion criteria were cervical intraepithelial neoplasia in the conization specimen with negative surgical margins and at least 12 months follow-up period at our clinical database. Demographic, clinical, pathological data, and preoperative complete blood count findings of the patients were extracted from medical files.

RESULTS: There were a total of 115 patients divided into two groups: the recurrence group (n = 30) and the control group (n =85). There were no statistically significant differences between the two groups in terms of cervical intraepithelial neoplasia pathology grades. There was statistically increased number of postconization endocervical positive results in the recurrence group. In the recurrence group, the mean of recurrence free survival was 10.5 months. There were no statistically significant differences in the preoperative neutrophil-lymphocyte ratio and in other hematologic parameters between the groups. In logistic regression analysis, only postconization endocervical curettage positivity was predictive in estimating recurrence (p=0.002, %95 CI 0.084-0.365).

CONCLUSION: Preoperative neutrophil-lymphocyte ratio and other inflammatory markers were not predictive of recurrence after conization in women with negative surgical margins. Positive postconization endocervical curettage was the only prognostic factor associated with recurrence after excisional therapy.

Keywords: cervical intraepithelial neoplasia; conization; endocervical curettage; neutrophil-lymphocyte ratio; recurrence

ÖZET

AMAÇ: Bu çalışmada konizasyon cerrahisi sonrası servikal intraepitelyal neoplazi nüksünü öngörmede preoperatif inflamatuar hematolojik belirteçlerin prediktif değerinin araştırılması amaçlanmıştır.

GEREÇ VE YÖNTEM: Bu retrospektif çalışmada, servikal intraepitelyal neoplazi patolojisine bağlı konizasyon uygulanan hastalar analiz edilmiştir. Çalışmaya konizasyon materyalinde servikal intraepitelyal neoplazi saptanıp cerrahi sınırları negatif olan ve klinik veri tabanımızda en az 12 aylık takip süresi olan hastalar dahil edilmiştir. Hastaların demografik, klinik, patolojik verileri ve preoperatif tam kan sayımı bulguları tıbbi dosyalardan çıkarılmıştır.

BULGULAR: Toplamda mevcut olan 115 hasta iki gruba ayrılmıştır: rekürrens grubu (n = 30) ve kontrol grubu (n = 85). İki grup arasında servikal intraepitelyal neoplazi patoloji dereceleri açısından istatistiksel olarak anlamlı fark saptanmamıştır. Rekürrens grubunda kontrol grubuna göre postkonizasyon endoservikal küretaj pozitif sonuç sayısı artmış olarak bulunmuştur. Nüks grubunda rekürrenssiz sağkalım ortalaması 10.5 aydı. İki grup arasında preoperatif nötrofil-lenfosit oranı ve diğer hematolojik parametreler açısından istatistiksel olarak anlamlı fark saptanmamıştır. Lojistik regresyon analizinde rekürrens tahmininde sadece postkonizasyon endoservikal küretaj pozitifliği belirleyici olarak bulunmuştur (p = 0.002, % 95 CI 0.084-0.365).

SONUÇ: Preoperatif nötrofil-lenfosit oranı ve diğer inflamatuar belirteçler cerrahi sınırları temiz konizasyon olgularında nüksü öngörmede etkin bulunmamıştır. Pozitif postkonizasyon endoservikal küretaj, eksizyonel tedaviden sonra nüks ile ilişkili tek prognostik faktör olarak bulunmuştur.

Anahtar Kelimeler: servikal intraepitelyal neoplazi; konizasyon; endoservikal küretaj; nötrofil-lenfosit oranı; rekürrens

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INTRODUCTION

Cervical intraepithelial neoplasia (CIN) is a preinvasive squamous lesion of the uterine cervix diagnosed by histopathological examination following cervical biopsy. The main target of CIN treatment is to avoid progression to invasive cervical cancer. Excisional surgical treatment modalities by loop electrosurgical excision procedure (LEEP), laser conization or cold-knife conization (CKC) are the most common preferred treatment procedures (1). There are a limited number of factors that can be used to estimate the CIN recurrence rate. Positive surgical margins, glandular involvement, persistent humanpapilloma virus (HPV) infection, and age were found as prognostic factors for CIN recurrence after treatment (2-7).

HPV infection was found in 99.7% of cervical cancer patients, however, most women infected with HPV do not develop high-grade CIN or cervical cancer (8). Persistent HPV infections lead to invasive diseases. On the other hand, there are serious concerns about chronic infections and the systemic inflammatory response of body in development and progression of the tumorogenesis (9). Leucocytes and their subgroups, cytokines, and chemokines are involved in the cancer related systemic inflammatory response (9). In recent years, an elevated peripheral neutrophil-lymphocyte ratio (NLR) along with monocyte to lymphocyte ratio (MLR), platelet to lymphocyte ratio (PLR), and mean platelet volume (MPV) have been shown as poor prognostic factors in various cancers including cervical cancer (10-13). Thus, the aim of this study was to determine the prognostic value of peripheral blood inflammatory hematological markers on the recurrence of CIN after conization.

MATERIALS AND METHODS

The present study is a retrospective cohort study of patients who underwent CKC due to CIN pathology at Ankara Dr. Zekai Tahir Burak Women's Health Education and Research Hospital from 2013 to 2017. The study protocol was approved by the Institutional Review Board of the hospital (7/07.27.2018). Demographic, clinical, pathological data, and preoperative complete blood count (CBC) findings of the patients were extracted from their records. Inclusion criteria were CIN in the conization specimen with negative surgical margins and at least 12 months follow-up period at our clinical database. Patients with positive margins in the conization specimen, missing medical records, absence of preoperative complete blood count (CBC) analysis within the 15 days prior to surgery and patients with a follow-up at less than 12 months were excluded. Other exclusion criteria were malign lesions in conization specimen, patients who underwent hysterectomy for all reasons, previous or current history of systemic infection or hematologic disease, and use of glucocorticoid or immunosuppressive drugs. The positive surgical margin was defined as if the distance between the CIN lesion and the margin of the resection was less than 1 mm. Recurrence-free survival (RFS) was defined as the duration in months between the date of conization and the date of first recurrence. Routine preoperative CBC tests performed within 15 days prior to surgery were recorded. The NLR was calculated as dividing the absolute neutrophil count by the absolute lymphocyte count and similar calculations were done for MLR and PLR. After standard conization procedure, endocervical curettage (ECC) was performed to assess residual disease in the remaining cervix.

The analysis of data was performed by the SPSS software version 23.0 (SPSS, Inc., Chicago, IL). Data were checked for normality by histogram and in terms of Skiwness and Curtosis. Independent sample T tests and Chi-square tests were used for comparing the demographic and clinical features of the patients. Receiver operating characteristic (ROC) curve was used to determine the diagnostic value of NLR, PLR, MLR, MPV, and Hb on predicting recurrence. Kaplan-Meier method was used to evaluate the impact of demographic and clinical features on RFS. Univariate and multivariate regression analyses were used to determine the factors affecting recurrence. Cox proportional hazard model was used to evaluate the factors affecting RFS. A p value < 0.05 was used for statistical significance and all p values were two sided.

RESULTS

We identified 136 patients with at least 12 months follow-up period in our database during the study period. Of them, 19 patients were excluded due to surgical margin positivity and two patients for immunosuppressive drug use. Therefore, there were 115 patients meeting the inclusion and exclusion criteria. Two groups were performed according to whether there was a recurrence during the follow-up controls: the recurrence group (n=30) and the control group (n=85). The baseline characteristics of the groups are shown in the **Table 1**. There were no statistically significant differences between the two groups in terms of age, BMI, gravidity, parity, smoking status, presence of chronic disease, and menopausal status.

The pathological findings of the conization specimens of the two groups are shown in **Table 2.** There were no statistically significant differences in terms of CIN pathology grades between the two groups. However, there was statistically increased number of ECC positive results in the recurrence group when compared to the control group. The duration of follow-up was not different between the two groups. In the recurrence group, the mean of RFS was 10.5 months and the histologic grade of recurrence was CIN I with 74%, CIN II with 10%, and CIN III with 16%.

When the two groups were compared according to the preoperative hematologic inflammatory markers, there were no statistically significant differences in terms of NLR, MLR, PLR, and in other hematologic parameters **(Table 3)**. In ROC analysis, Hb, NLR, MLR, PLR and MPV were not statistically significant in predicting recurrence.

Table 1: The baseline characteristics of the two groups. The values are given mean ± SD, median (min. – max.), and number (%), as appropriate.

	Recurrence group (n= 30)	Control group (n = 85)	p
Age	42.06 ± 7.61	42.61 ± 7.52	0.737
BMI	25.76 ± 5.6	26.21 ± 4	0.760
Gravidity	2 (0 - 7)	3 (1 - 8)	0.357
Parity	2 (0 - 7)	2 (1 - 5)	0.957
Smoking status (n, %)			
• Non-Smoking	13 (43.3)	50 (58.8)	0.362
• Smoking	17 (56.7)	35 (41.2)	
Chronic disease (n, %)			
• No	26 (86.6)	78 (91.8)	0.610
• Yes	4 (13.4)	7 (8.2)	
Menopausal status (n, %)			
• Premenopausal	25 (83.3)	73 (85.9)	0.689
• Postmenopausal	5 (16.7)	12 (14.1)	

BMI Body mass index.

Table 3: Preoperative hematologic findings of the two groups. The values are given mean ± SD and median (min. – max.), as appropriate.

	Recurrence group (n= 30)	Control group (n = 85)	p	Reference values
Hb (g/dL)	13.21 ± 1.41	13.29 ± 1.5	0.786	12.5 – 17.2
MCV (fL) (mean ± SD)	85.93 ± 7.04	85.11 ± 8.4	0.635	80 – 101
WBC (/uL)	7969 ± 1752.26	8171.41 ± 1857.69	0.604	3600 – 10500
Neutrophil count (/uL)	4946.66 ± 1459.5	5015.88 ± 1599.9	0.835	1500 - 7700
Lymphocyte count (/uL)	2313.33 ± 575.19	2444.58 ± 676.62	0.345	1100- 4000
Monocyte count (/uL)	500 (200 - 1000)	500 (100-900)	0.888	100 - 900
Eosinophil % (%)	1.55 (0.1 - 4.60)	1.30 (0- 5.8)	0.477	0.5 – 5.5
Platelet count (10³/uL)	274.6 ± 71.51	278.32 ±64.77	0.792	160 - 400
NLR	2.11 (1.25 - 6.18)	2.03 (1.04 - 9.5)	0.819	
MLR	0.244 ± 0.104	0.227 ± 0.097	0.416	
PLR	120 ± 37	120 ± 43	0.782	
MPV (fL)	8.9 (7.6 - 10.3)	9.1 (6.5 – 13.8)	0.413	6 -10

Hb Hemoglobin; MCV Mean Corpuscular Volume; WBC White Blood Cell; NLR Neutrophil / Lymphocyte Ratio; MLR Monocyte / Lymphocyte Ratio; PLR Platelet / Lymphocyte Ratio; MPV Mean Platelet Volume.

When univariate analysis was performed with demographic, pathologic and hematologic variables for the prediction of recurrence, only ECC positivity was predictive in estimating recurrence (p=0.002, %95 CI 0.084-0.365). When we grouped the patients as ECC negative and positive cases; being ECC negative was

Table 2: Pathologic characteristics of the two study groups. The values are given median (min. – max.) and number (%), as appropriate.

	Recurrence group (n= 30)	Control group (n = 85)	p
Pathology (n, %)			
CIN I	3 (10)	16 (18.8)	0.349
CIN II	6 (20)	15 (17.6)	
CIN III	21 (70)	54 (63.5)	
ECC (n, %)			
ECC negative		80 (94.1)	
ECC positive	23 (76.6)		**0.007**
1. CIN I	4 (13.4)	4 (4.7)	
2. CIN II	-	1 (1.2)	
3. CIN III	3 (10)	-	
Follow-up (months)			
Median (min-max)	36.5 (12-60)	35 (15-60)	0.628
Recurrence time (months)			
Median (min-max)	10.5 (5-54)	-	-
Recurrence Pathology (n,%)			
CIN I	22 (73.3)	-	-
CIN II	3 (10)	-	-
CIN III	5 (16.7)	-	-

CIN cervical intraepithelial neoplasia; ECC endocervical curettage

effective and statistically significant on RFS (p=0.003) **(Figure 1)**. The median RFS of ECC negative patients were 34 months (min-max: 5-60) and 21.5 months (min-max: 7-51) for ECC positive patients. The odds of recurrence of ECC positive patients were approximately 5 times higher than ECC negative ones (OR=4.87, CI 1.412-16.79; p=0.007). The result of cox proportional hazard model showed that ECC positivity increases the hazard of recurrence (HR=0.295, 95% CI 0.125- 0.694; p=0.005).
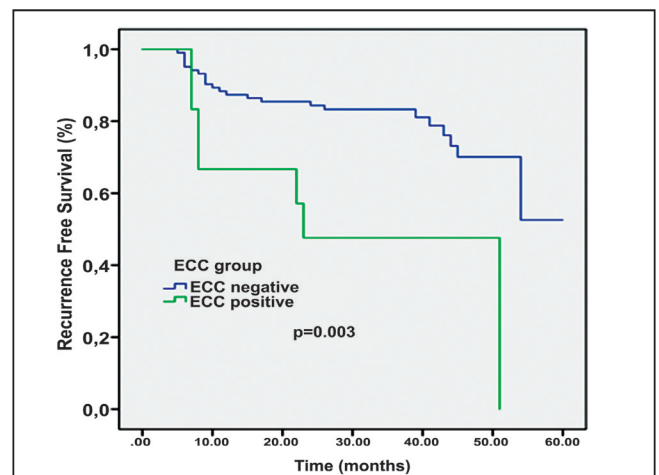

Figure 1: Kaplan-Meier plot of recurrence free survival according to the endocervical curettage.

DISCUSSION

Neutrophil to lymphocyte ratio is a simple novel biomarker for assessing the inflammatory response between neutrophil-dependent tumor promoting inflammation

and lymphocyte-associated anti-tumor immune response (14). Therefore, a higher level of pretreatment NLR was found to be associated with poor prognosis (15). In our study, we did not find any associations between preoperative NLR and CIN recurrence in patients with negative surgical margins during the initial conization. Our results are in contrast with the two other studies in the literature. In the first study, Chun et al. found a cut-off value of 2.1 for NLR in ROC analysis and according to the this cut-off value RFS was statistically higher in the low NLR group when compared to the high NLR group (p=0.0125).(16) They found NLR (HR=7.66, 95% CI 2.34-25.10, p=0.001), absolute eosinophil count (HR= 6.91, 95% CI 1.82-26.24, p=0.005), Hb (HR=0.21, 95% CI 0.07-0.65, p=0.007), and mean corpuscular volume (HR= 0.27, 95% CI 0.08-0.92, p=0.037) as prognostic factors in multivariate Cox regression analysis. (16) In the second study, Farzaneh et al. identified a cut-off value 1.9 for NLR and the recurrence rate was 4.8% in NLR low group in contrast to 28.6% in the NLR high group.(17) Besides, the high NLR (HR=4.55, 95% CI 1.97-10.51, p<0.001), white blood cell (WBC) count levels (HR=1.27, 95% CI 1.04-1.55, p=0.017), and absolute lymphocyte count (HR=0.258, 95% CI 0.121-0.549, p<0.001) were associated with significantly decreased RFS in multivariate Cox regression analysis. Our study consisted of a relatively small number of patients than the two studies mentioned above and this might have lead to the conflicting results between our study and others.

The major difference between our study and these two studies is we did not include patients with positive surgical margins in conization specimens. Our hospital protocol is to perform reconization in patients with positive margins. Marginal involvement in conization specimen is a well-documented prognostic factor of recurrence (3-5, 7). Furthermore, in a meta-analysis, 18% of women with margin involvement developed high-grade CIN after treatment, while the ratio was 3% in women with negative margins (18). Thus, we did not include patients with positive margins in our study. In Chun's study, the rate of positive margins was 14.8% and there was no comment about the treatment approaches in these cases. It was not featured as a prognostic factor for RFS in multivariate Cox regression analysis, though not specified as whether it was explicitly analyzed (16). On the other hand, in Farzaneh's study, the rate of margin involvement was 9.1% (17). They clearly stated that their general approach was reconization and only patients with negative margins were entered in the follow-ups. Although they did not find any protective effect of reconization in decreasing recurrence rate, less cervical tissue excision (measured as cm2) and margin involvement were associated with higher recurrence in multivariate logistic regression and decreased RFS in Cox regression models (OR=6.70, 95% CI 1.49-30.04, p=0.013 and HR=4.52; 95% CI 1.89-10.84, p=0.001; respectively).

We found only ECC involvement performed at the conization as a prognostic factor in recurrence. We routinely perform ECC after conization; however,

this approach has not been generally accepted. The significance of residual disease in the cervix above the conization site has been studied in the literature with conflicting results. Suzuki et al. concluded that ECC at the time of conization did not offer any advantages due to its low sensitivity (42.9%) and positive predictive value (PPV) (54.5%, κ = 0.284; p = 0.053) in predicting residual disease (19). However, Schneider et al. found that postconization ECC showed high specificity (0.94) and positive predictive value (0.88) in the subgroup of women 50 years or older (20). Another study showed that positive ECC was associated with a worse outcome if cone margins were also positive, but not in cases with negative cone margins (21). Moreover, Lu et al. found that post-cone ECC was found to be the most statistically significant factor for predicting persistent disease (OR 7.940, 95% CI 3.428 –18.390, p < 0.001) in CIN III patients (3). Our results also showed positive postconization ECC as a prognostic factor in recurrence even if in negative cone margins. In our cohort, the recurrence rate was found to be 26.6%. High rate of postconization ECC positivity, and selection bias due to the retrospective design of the study might be the reasons for this high rate.

There were several shortcomings of our study. It was a retrospective study with limited number of patients performed at a single institution. HPV subtypes were not defined in our records. Prospective studies should be performed to confirm our results.

CONCLUSION

Preoperative NLR and other inflammatory markers were not predictive of recurrence after conization in women with negative surgical margins. Positive postconization ECC was the only prognostic factor associated with recurrence after excisional therapy.

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