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Blood cell indices in High-Grade Squamous Intraepithelial Lesions

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Abstract

Low patient motivation and relatively long procedures can sometimes limit the gains of the PAP test. At this point, the clinician may need additional stimulating tests to persuade patients to perform screening tests. The aim of this study was to determine the predictive value of hematological parameters for the high-grade squamous intraepithelial lesion (HG-SIL). Ninety-one patients possessing HG-SIL with complete blood counts and 111 healthy patients with normal cervical cytology were reviewed retrospectively. The age, WBC, and RBC count were significantly different in patients with HG-SIL than in controls. A cutoff value was found 42.5 for age (42% sensitivity and 58 % specificity, AUC: 0.604), 8.65 for WBC (44% sensitivity and 68% specificity, AUC: 0.570), and 4.47 for RBC (65% sensitivity and 58% specificity, AUC: 0.628) in ROC curve analysis. The results of the study show that the age of the patients, RBC, and WBC counts are important in precancerous intraepithelial lesions. Clinicians should warn female patients in the risk group to have regular gynecological examinations and screenings.

Keywords: PAP smear, Age, cervical cancer, RBC, WBC

Introduction

Cervical cancer is one of the most common women's cancers and largely preventable when early diagnosed [1]. Cervical cancer is reported to be the third most common gynecological cancer. According to the last GLOBOCAN data, the incidence of cervical cancer in Southern Africa is 43.1/100.000, in eastern Africa, it is 40.1/100.000, in western Africa 29.6/100.000 in Middle Africa 36.8/100.000, in Southeastern Asia 17.2/100.000, and in Central and Eastern Europe, it is 16/100.000 in descending order. A total of 2356 cases of invasive cervical cancer were annually diagnosed and about 1280 deaths occurred in Turkey [2].

Pap smear and HPV screening tests, which are used in the early diagnosis of cervical cancer, have been performed by clinicians for many years. Thanks to these screening tests, various studies have reported a decrease in the incidence and mortality rate of cervical cancer [3,4].

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Although national population-based cervical cancer screenings are meticulously applied, low patient motivation and relatively long procedures limit the gains of screening tests [5-7]. The relationship between chronic inflammation and cancer has been known for a long time. It has been now thought that the risk of cancer is increased in individuals affected by various chronic inflammatory diseases including those with infectious and non-communicable etiologies. In some cases, chronic inflammation can increase the stem cell pool that may be particularly susceptible to transformation. In addition, activated immune cells produce reactive oxygen species (ROS) that are directly genotoxic. ROS are produced in large amounts by activated inflammatory cells such as neutrophils and macrophages, during inflammatory reactions aimed at destroying germs and removing dead and unwanted materials [8]. At this point, oxidative stress can also be implicated in cancer development. Free iron is able to catalyze ROS formation. Under normal circumstances, the reactivity of iron is minimized by their binding to storage and transport proteins (e.g., transferrin, ferritin, and lactoferrin), which prevents these metals from participating in reactions that generate ROS [9].

Chronic epithelial injury often leads to metaplasia with the replacement of one mature cell type with a second one. Inflammation has been reported to be a facilitator in cervical

oncogenesis, and severe inflammation has been shown to be associated with cervical cytological anomalies [10]. Hemogram test parameters, which are easily used by clinicians for both screening and diagnostic purposes, can be a guide in predicting neoplastic lesions, considering the above. In the literature, it has been shown that hematological parameters can be used for predictive and prognostic purposes in cervical cancer patients [11,12]. However, according to the literature, there is not research presenting the comparison of advanced precancerous lesions (HG-SIL) and normal pap smear results. Our study aims to investigate the predictive value of hemogram parameters by comparing the values of patients with advanced precancerous lesions and normal healthy individuals.

Materials and Methods

This is a retrospective study including pap smear results at a tertiary, university- Research and Application Center between 2012-2020. Appropriate permission for the study was obtained from the University Medical Faculty Ethics Committee (approval no: 2020/175). Medical Faculty database was searched for eight years, and all PAP smear results were identified.

Selection and Description of Participants

The inclusion criteria were 1) High-grade SIL confirmation in case of abnormal PAP smear result 2) Normal PAP result without inflammation in the control case 3) Whole blood count result on the day of smear sampling 4) No previous diagnosis of malignant tumor history from other organs or tissues. We excluded the patients without inadequate samples or clinical information from the initial study group. Hematoxylin and eosin-stained and abnormal PAP slides were reviewed by the pathologist (SKC) to confirm the diagnosis and record histological features. Immunohistochemical findings (HPV, P16, and Ki67) were also used to support the diagnosis [13]. A total of 202 patients had sufficient information and pathology material and constituted the study (Figure 1).

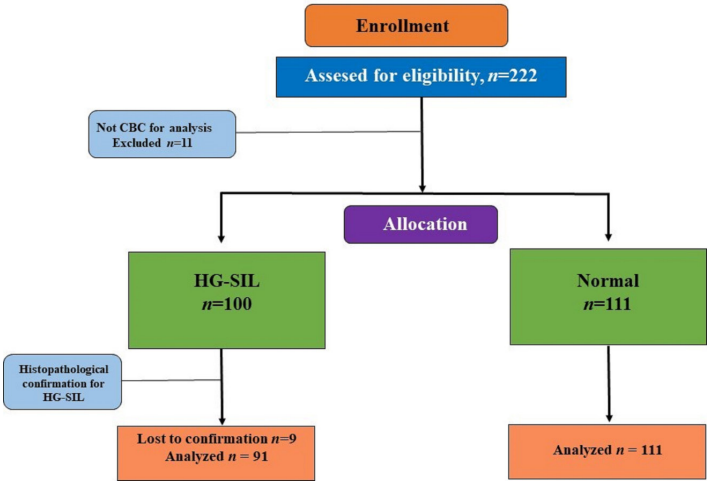

Figure 1. Patient selection with sufficient information and pathology material

Statistical analyzes

Statistical analyzes were done using SPSS for Windows software package. Descriptive statistics were shown as the mean + SD notation. The independent samples t-test was used to compare the groups. Receiver operating characteristics (ROC) analysis was performed to calculate the cutoff values for variables between patient and control groups. Logistic regression analysis was also used to investigate the multivariate relationship of clinical laboratory measurements predicting HG-SIL. Differences were considered significant at $P<0.05$.

Results

A total of 202 patients including 91 HG-SIL and 111 normal (mean age 41.2±9.2 years) were included in our study. There was a significant difference in terms of age (43.4 in HG-SIL vs 39.4 in control; p=0.002) between the two groups. Among the hematological parameters; white blood cell count was higher in patients with HG-SIL (p=0.033). Red blood cell count was lower compared to the control group (p=0.001). According to the analysis results; There was no significant difference in Neutrophil, Lymphocyte numbers and Neutrophil to lymphocyte ratio between the HG-SIL and Control group patients (respectively, p=0.084, p=0.182, p=0.093). At the same time, according to the hemogram analysis of the two groups; There was no significant difference in mean platelet volume, Platelet to lymphocyte ratio, Eosinophil to lymphocyte ratio (respectively, p=0.164, p =0.254, p=0.620). The patients' age and all mean hemogram parameters were listed in Table 1.

ROC curve analysis demonstrated that patient age, WBC and RBC were predictive of HG-SIL with a cutoff value of 42.5 for age (42% sensitivity and 58 % specificity, AUC: 0.604), 8.65 for WBC (44% sensitivity and 68% specificity, AUC: 0.570), and 4.47 for RBC (65% sensitivity and 58% specificity, AUC: 0.628), (Figure 2 and 3).

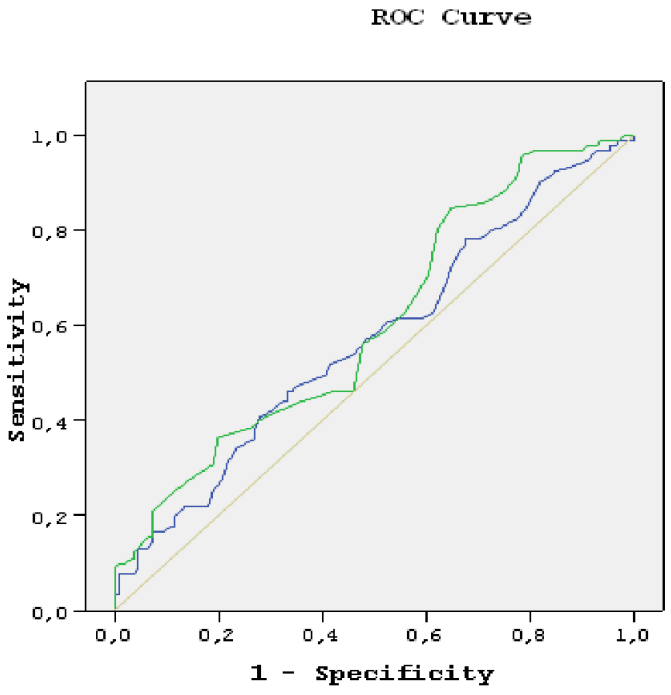

Figure 2. The receiver operating characteristic (ROC) curves of age (green) and WBC (blue) for HG-SIL

We also performed a logistic regression analysis. The Nagelkerke R square indicated that 16% of the variation in having HG-SIL is explained by the risk factors included in the model (Table 2). The overall percentage of the classification ability of the model is 65.8%.

Table 1. Comparison of age and blood parameters in patients with HSIL and controls

	HG-SIL n=91	Control n=111	p value
Age (years)	43.44	39.44	**0.002**
White blood cell (103/µL)	8.60	7.83	**0.033**
Red blood cell (106/µL)	4.33	4.51	**0.001**
Hemoglobin (g/dL)	12.20	12.45	0.241
Hematocrit (%)	36.6	37.5	0.130
MCV	84.75	83.22	0.149
MCHC	33.22	33.19	0.875
Red cell distribution width	14.65	14.64	0.992
Platelet count (µL)	269.64	291.41	0.057
Mean platelet volüme	8.68	8.47	0.164
Platelet distribution width	16.7	16.6	0.661
Neutrophil	5.52	4.94	0.084
Lymphocyte	2.30	2.16	0.182
Monocyte	0.55	0.52	0.378
Eosinophil	0.16	0.15	0.733
Basophil	0.06	0.04	0.449
Neutrophil to lymphocyte ratio	3.34	2.49	0.093
Platelet to lymphocyte ratio	133.97	144.32	0.254
Eosinophil to lymphocyte ratio	0.068	0.071	0.620

Table 2. Binary logistic regression model for HG-SIL

							95.0% C.I.for EXP(B)	
	B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**	**Lower**	**Upper**
Age	-.052	.017	9.214	1	.002	.949	.917	.982
WBC	-.147	.063	5.474	1	.019	.863	.763	.976
RBC	1.235	.392	9.907	1	.002	3.439	1.594	7.421
Constant	-1.898	1.882	1.017	1	.313	.150		

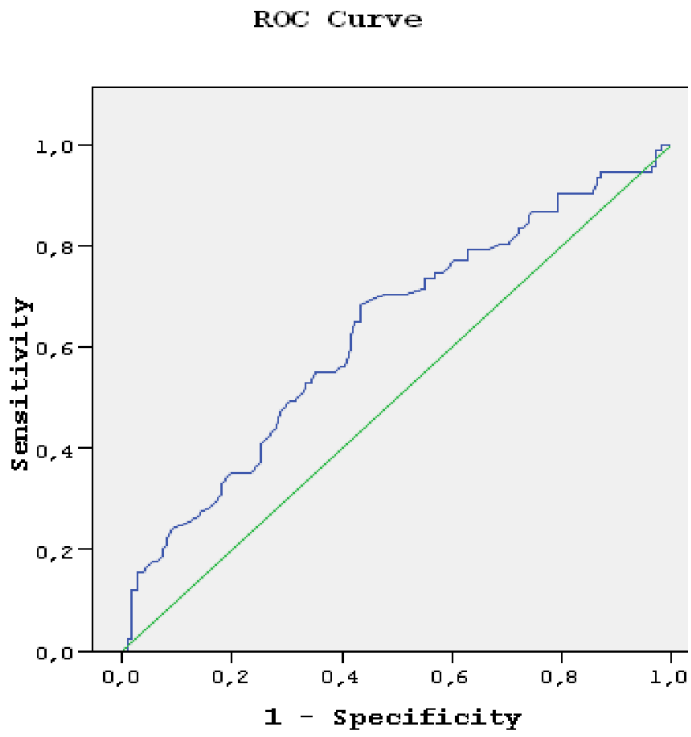

Figure 3. The receiver operating characteristic (ROC) curve of red blood cell count for HG-SIL

Discussion

In our study, the NLR, PLR and RDW values were not found to be significantly different in the HG-SIL and control groups. Some authors stated that NLR, PLR and RDW do not have sufficient prognostic value for early-stage lesions. They add that these values make significant differences in advanced metastases and recurrent disease [14-16]. Considering that the HG-SIL patient group in our study did not have advanced-stage cancer, we can say that the results of our study are consistent with the literature.

With the help of HPV and Pap smear tests, very good progress has been made in the early diagnosis of cervical cancer. However, researchs still show that some patients are not aware of the advantages of early diagnosis [17]. Clinicians can draw patients' attention with simple and stimulating laboratory results that help in early prediction of cancer development. Although there was no significant difference in inflammation markers between HG-SIL and the control group in our study results, the significant decrease in RBC in the group with HG-SIL compared to the control group is remarkable. The relationship between low red blood cell count and cancer has been shown in animal studies. It has been suggested that the decrease in RBC count is due to increased RBC lysis due to altered cellular fluidity properties of RBC in tumor-bearing animals. In the same study, it was observed that leukocyte counts

increased in rats carrying tumors [18]. In addition to low red blood cell count, the fact that the leukocyte count was significantly higher in the HG-SIL group compared to the control group in our study results suggests that there are some micromolecular changes in HG-SIL patients. It is also reported that the presence of systemic inflammation, which cannot be explained by any other reason, causes a significant irregularity in the general serum measurements of iron used in red blood cell production [19]. Cell studies say that tumor cells hold more iron than normal cells [20,21]. The use of iron by ROS, which has an important role in neoplastic pathogenesis, has been the subject of many cancer studies [22].

In our study, the WBC count in the HG-SIL group was found to be significantly higher than that of the control group patients. The relationship between WBC and cancer development has been studied in many studies. According to the results of a case control study conducted with a large patient population in Korea, it has been determined that patients with breast cancer have significantly higher WBC numbers regardless of their menopausal status [23]. Similar to our study results, there are studies in which increased white blood cell count is associated with cancer risk [24-26]. In another remarkable study, it is said that the high WBC count during the diagnosis of recurrence in patients with cervical cancer is an independent prognostic factor [27]. Some processes occurring at the molecular level explain this relationship. Vascular endothelial growth factor, which plays a role in cancer development, is found in the blood plasma, especially in the elements formed, especially leukocytes [28]. These data are said to provide evidence for the relationship between circulating WBC count and subsequent cancer, a widely available marker of inflammation [29].

In our study, when the patients were evaluated according to age, it was found that the age of the patients in the HG-SIL group was significantly higher than the control group. Recent evidence suggests that aging leads to both degenerative and hyperplastic pathologies, most likely by promoting chronic inflammation [30].

As mentioned above, correlational studies of hemogram parameters with cervical neoplasia provide support to the literature with predictive results in prognosis, pre-and post-treatment follow-up, and survival in advanced-stage cancers. On the other hand, in precancerous lesions, the number of studies that may warn the patient and the physician are limited. The most important part of combating cervical cancer is early diagnosis. Although NLR, PLR, RDW levels are not guiding in early stage cervical cancer, the age of the patients, RBC and WBC counts are important in precancerous intraepithelial lesions. The gold standard of early diagnosis of cervical cancer is still; Pap smear and HPV screening tests. Although many studies report that hematological parameters are guiding physicians, detailed gynecological examinations and regular screenings are evidence-based information in early diagnosis of cervical cancer. Further studies are needed for determining laboratory parameters for early stage cancer development in cervical cancer.

Limitations

Low-grade squamous intraepithelial lesions may regress spontaneously. Therefore, such lesions can be followed-up without a biopsy. Due to this algorithmic approach, our patients with LG-SIL were lower compared to those with HGSIL in our database, and the fact that such cases were not included in the study constituted the limitations of our study. In addition, we could not evaluate the parameters that are effective in neoplastic processes such as iron, ferritin, transferrin, which are not routinely checked in every patient who underwent smear examination.

Conclusion

The results of the study show that the age of the patients, RBC and WBC counts are important in precancerous intraepithelial lesions. NLR, PLR, RDW levels are not guiding in early stage cervical cancer. It is essential that women are encouraged to have regular gynecological examination and screening tests. We recommend careful monitoring of older women with low RBC and high WBC. However, further studies including a larger number of cases are needed to support the study results.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical approval
Appropriate permission for the study was obtained from the University Medical Faculty Ethics Committee (approval no: 2020/175).

References

1. Pimple SA, Mishra GA. Global strategies for cervical cancer prevention and screening. Minerva Ginecol. 2019;71:313-20.
2. GLOBOCAN. https://gco.iarc.fr/today/data/factsheets/cancers/23-Cervix-uteri-fact-sheet.pdf. 2018 (Access date: 11/10/2020)
3. Obeidat BR, Amarin ZO, Alzaghal L. Awareness, practice and attitude to cervical Papanicolaou smear among female health care workers in Jordan. Eur J Cancer Care (Engl). 2012;21:372-6.
4. Huh WK. The changing face of cervical cancer screening in the United States. J Natl Compr Canc Netw. 2010;8:1329-30.
5. TR. Ministry of Health. General directorate of public health. Cancer Department. Available from: https://hsgm.saglik.gov.tr/tr/kanser-tarama-standartlari/listesi/serviks-kanseri-tarama-program%C4%B1-ulusal-standartlar%C4%B1.html
6. Yeo C, Fang H, Thilagamangai, et al. Factors affecting Pap smear uptake in a maternity hospital: A descriptive cross-sectional study. J Adv Nurs. 2018;74:2533-43.
7. Schneider V. Criticism of the Pap Smear as a diagnostic tool in cervical cancer screening. Acta Cytol. 2017;61:338-44.
8. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21:363-83.
9. Koskenkorva-Frank TS, Weiss G, Koppenol WH et al. The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. Free Radic Biol Med. 2013;65:1174-94.
10. Long T, Long L, Chen Y, et al. Severe cervical inflammation and high-grade squamous intraepithelial lesions: a cross-sectional study. Arch Gynecol Obstet. 2021;303:547-56.
11. Leal-Garza CH, Cerda-Flores RM, Leal-Elizondo E, et al. Micronuclei in cervical smears and peripheral blood lymphocytes from women with and without cervical uterine cancer. Mutat Res. 2002;515:57-62.
12. Wang D, Wu M, Feng FZ, et al. Pretreatment neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios do not predict survival in patients with cervical cancer treated with neoadjuvant chemotherapy and radical hysterectomy. Chin Med J (Engl). 2013;126:1464-68.
13. Çalişkan E, Coşkun SK, Öztürk CE et al. Analysis of HPV genotypes and liquid-based cervical cytology: Results from a tertiary academic center in northwestern Turkey. Jpn J Infect Dis.2021;74:69-72.

14. Basim P, Yuksel M. Peripheral inflammatory biomarkers as predictors of recurrence in surgically-treated anogenital condylomata acuminata patients. Int J STD AIDS. 2020;31:1380-8

15. Tas M, Yavuz A, Ak M et al. Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio in Discriminating Precancerous Pathologies from Cervical Cancer. J Oncol. 2019;2476082.

16. Wang L, Jia J, Lin L, et al. Predictive value of hematological markers of systemic inflammation for managing cervical cancer. Oncotarget. 2017;8:44824-32.

17. Süleyman G, Gürgen S, Aypak C. HPV Frequency and Protection Behavior of Women with HPV Screening Between 30-65 Years in a Primary Health Care Center. Konuralp Med J. 2020;12:1-4.

18. Quemener V, Bansard JY, Delamaire M, et al. Red blood cell polyamines, anaemia and tumour growth in the rat. Eur J Cancer. 1996;32:316-21.

19. McSorley ST, Jones DC McMillan D. TalwarQuantitative data on the magnitude of the systemic inflammatory response and its relationship with serum measures of iron status. Transl Res. 2016;176:119-26.

20. Pinnix ZK, Miller LD, Wang W, et al. Ferroportin and iron regulation in breast cancer progression and prognosis. Sci Transl Med. 2010;2: 43-56.

21. Coffman LG, Parsonage D, D'Agostino R, et al. Regulatory effects of ferritin on angiogenesis. Proc Natl Acad Sci USA. 2009;106:570–5.

22. Bystrom LM, Guzman ML, Rivella S. Iron and reactive oxygen species: friends or foes of cancer cells? Antioxid Redox Signal. 2014;20:1917-24.

23. Chen L, Zhang F, Sheng XG, et al. Peripheral platelet/lymphocyte ratio predicts lymph node metastasis and acts as a superior prognostic factor for cervical cancer when combined with neutrophil: Lymphocyte. Med (Baltimore). 2016;95:e4381.

24. Kose M, Celik F, Kose SK, et al. Could the platelet-to-lymphocyte ratio be a novel marker for predicting invasiveness of cervical pathologies? Asian Pac J Cancer Prev. 2015;16:923-6.

25. Chun S, Shin K, Kim KH, et al. The Neutrophil-Lymphocyte Ratio Predicts Recurrence of Cervical Intraepithelial Neoplasia. J Cancer. 2017;8:2205-11.

26. Lee YY, Choi CH, Kim HJ, et al. Pretreatment neutrophil:lymphocyte ratio as a prognostic factor in cervical carcinoma. Anticancer Res. 2012;32:1555-61.

27. Prabawa IPY, Bhargah A, Liwang F, et al. Pretreatment Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) as a Predictive Value of Hematological Markers in Cervical Cancer. Asian Pac J Cancer Prev. 2019;20:863-8.

28. Holub K, Biete A. Impact of systemic inflammation biomarkers on the survival outcomes of cervical cancer patients. Clin Transl Oncol. 2019;21:836-44.

29. Shen WJ, Fu S, Li N, et al. Decreased Mean Platelet Volume is Associated with Cervical Cancer Development. Asian Pac J Cancer Prev. 2017;18:1769-72.

30. Bırgı SD, Gultekin M, Yuce D, et al. Definitive Treatment for Locally Advanced Cervical Cancer: A Retrospective Analysis from A Singe Institution. UHOD. 2018;28:19-29.