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The comparison of PAP smear test results of women according to anti-mullerian hormone levels

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Abstract

This study investigates OR investigated the relationship between PAP smear test results and anti-mullerian hormone (AMH) levels. The PAP smear test is used to screen for cervical cancer. This test identifies malignant or pre-malignant cells of the cervix and permits timely diagnosis and treatment. This retrospective cross-sectional study included 104 women of reproductive age who applied for a PAP smear test. This study was conducted between October 2019 and December 2020. Patients were clustered as subjects: 1) with an AMH <1ng/ml, 2) with a 1<AMH<3, and 3) with an AMH>3ng/ml. The mean age of the study group was 28.45 ± 3.31 years. There was a statistically significant association between AMH levels and PAP smear test results ($p<0.05$). AMH>3ng/ml group had a significant relationship with the AGUS PAP smear results. There was a statistically significant association between AMH between 1 and 3 and normal PAP smear test results. In conclusion, the results indicated that the serum AMH level and PAP smear test results had a significant relationship.

Keywords: Cervical cancer, anti-mullerian hormone, pap smear test, precancerous lesions

1. Introduction

The number of new cases of cervical cancer as a global public health problem is ranked 9th among all cancers, of which the incidence is estimated to be 604,127 and the mortality is estimated to be 341,831 in 2020 all over the world (1). There has been a decline in cervical cancer incidence in several high-resource countries with organized cervical screening programs (2). Approximately half a million new cervical cancer cases are detected yearly because of well-integrated human papillomavirus (HPV) vaccine programs (3, 4). PAP smear test is used for fast diagnosis of the disease and reduces its effects to a great extent. PAP smear test is a screening test to diagnose cervical cancer in apparently healthy women and is considered a health-promoting behavior (5). This test is performed every three years on women who have been or are sexually active. The implementation of the screening program in several countries for a term of five years has shown that the PAP smear test can reduce the death rate of cervical cancer by 60% (6).

Müllerian inhibiting substance (MIS), or anti-mullerian hormone (AMH) is a member of the superfamily of transforming growth factor beta (TGF-β) discovered by Alfred Jost (7, 8). AMH is produced exclusively in the ovarian small antral and preantral follicles' granulosa cells (9-11). One of the hormones which has attracted attention recently as a marker for predicting ovarian response before using assisted reproductive methods is AMH (12, 13). This hormone is produced by the ovarian granulose cells, and after puberty, its rate gradually decreases at a slow rate and disappears during menopause (14). Among the functions of this hormone are inhibition of primary follicle application, Follicle stimulating hormone (FSH)-dependent growth inhibition, and selection of perinatal follicles and small antral follicles (12).

Since AMH serum levels are associated with the number of primary antral follicles, this hormone is used to evaluate fertility potential and ovarian response in IVF, which stands for in vitro fertilization (11). We believe that there is a relationship between AMH levels and PAP smear test results. Physicians should recommend patients perform the PAP smear test by observing AMH values.

The present study assessed the relationship between AMH levels and PAP smear test results. The present study is significant in identifying the factors affecting the incidence of cervical cancer to provide preventive treatments. This study assesses the relationship between AMH levels and PAP smear test results.

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2. Materials and Methods

This retrospective cross-sectional study was approved by the local ethics committee of the university. (Date: 13/01/2021 Decision no: 2021/0027). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was performed on one hundred-four women between October 2019 and December 2020.

After the Institutional Review Board gave the approval, all surgical pathology files and PAP smear files were searched for "routine" PAP smears. We classified abnormal routine PAP smears into the following categories based on the latest Bethesda system: Atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells of undetermined significance (AGUS), low-grade squamous intraepithelial lesion (LGSIL), high grade squamous intraepithelial lesion (HGSIL), and cervicitis. We correlated each abnormal PAP smear with a tissue biopsy from the cervix, if available, for each abnormal PAP smear. We collected the grade and presence of dysplasia and the utility of immune stains on tissue biopsy, particularly HPV-16 (Clone Cam Vir-1, ready to use, BioGenex) and P16 (Clone E6H4, ready to use, Roche) in ruling out or confirming HPV infection, if available. AMH values were recorded from hospital data. Details about the serum AMH levels were collected from medical records. AMH enzyme immunoassay (Instrumentation Laboratory and Beckman-Coulter, Vienna, Austria) was used to specify serum AMH (ng/ ml). The scale for measuring hormones was nanograms per milliliter (ng/ml). Patients were clustered as subjects: 1) with an AMH<1, 2) with a 1<AMH<3, and 3) with an AMH>3 ng/ml.

2.1. Statistical analysis

The Kolmogorov-Smirnov test was performed to check the normality. The mean and standard deviations (SD) were measured to check each continuous variable, including age, body mass index (BMI), and AMH. The Mann-Whitney U test was performed to study the difference between the two groups. Pearson's chi-squared test was used to decide whether there was a statistically significant difference between the Pap smear test results and AMH levels. The Paired Samples Z-Test was used to determine the significance of AMH levels in different Pap test results. SPSS v22 was used for statistical analyses. A value of $p<0.05$ was accepted as statistically significant.

To calculate the sample size with the G-Power 3.1 program, two groups' total mean was measured based on the Mann-Whitney test with a power of 95%, an effect size of 50%, and 0.05 type 1 error for at least 92 patients (15).

3. Results

This study included one hundred and four age-matched (28.45±3.31) and BMI-matched (25.19±2.25) women. Table 1 shows descriptive statistics of study parameters. Table 2 shows the frequency of PAP smear test results of AMH values in each PAP smear test result. As stated in Table 2, the highest frequency of PAP smear results was cervicitis (37.5%), normal (34.6%), ASCUS (14.4%), AGUS (8.7%), LGSIL (3.8%), and HGSIL (1%). As stated in Table 3, a chi-square test found a statistically significant association between AMH levels and Pap smear test results ($p<0.05$). The Pairwise Z-Tests found that the percentage of women who had the normal test result was significantly higher for those who had AMH between 1 and 3 (55.3% from n = 21) than for those who had AMH greater than three and lower than one. The percentage of women with the AGUS test result was significantly higher for those with AMH greater than three (20.6% from n=7). Women with AGUS PAP smear test results had serum AMH levels (2.8±1.14). AMH levels were not significantly different in ASCUS, LGSIL, HGSIL, and cervicitis PAP smear test results. Fig. 1 shows AMH levels in different PAP smear test results. More than 50% of normal PAP smear test results appeared between one and three in AMH.

Table 1. Descriptive statistics of study parameters in women (n=104)

Study parameters	median (range) / mean ± SD
Age	28 (22-36) / 28.45±3.31
BMI	25 (20-30) / 25.19±2.25
AMH	1.85 (0.2-4.6) / 2.02±1.25

SD, standard deviation

Table 2. The frequency of PAP smear test results

Study parameters		N (%)	AMH (mean ± SD)
PAP smear test results	Normal	36 (34.6)	2.01±1.07
	AGUS	9 (8.7)	2.8±1.14
	ASCUS	15 (14.4)	2.16±1.3
	LGSIL	4 (3.8)	0.9±0.62
	HGSIL	1 (1)	0.8±0.0
	Servisit	39 (37.5)	1.94±1.38

Table 3. The relationship between PAP smear test results and AMH levels

Study parameters		AMH<1 n (%)	1<AMH<3 n (%)	AMH>3 n (%)	*p*
PAP smear test results	Normal	7 (21.9)	**21 (55.3)**†	8 (23.5)	0.010*
	AGUS	1 (3.1)	1 (2.6)	**7 (20.6)**†	
	ASCUS	5 (15.6)	4 (10.5)	6 (17.6)	
	LGSIL	2 (6.3)	2 (5.3)	0 (0.0)	
	HGSIL	1 (3.1)	0 (0.0)	0 (0.0)	
	Servisit	16 (50.0)	10 (26.3)	13 (38.2)	

*A Chi-square test. † Pairwise Z-Tests

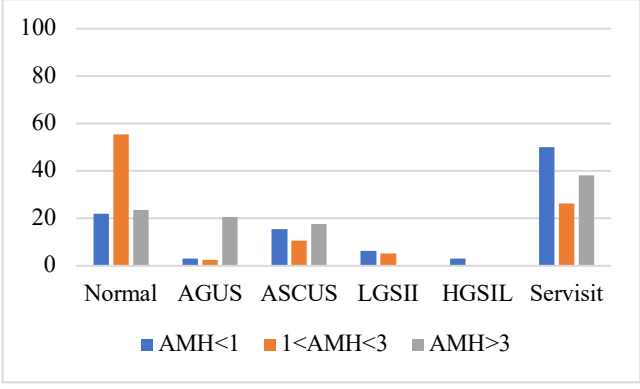

Fig. 1. AMH levels in different PAP smear test results

4. Discussion

Our study investigated the relationship between PAP smear results and AMH levels in sexually active women. Of the 104 sexually active women, 36(34.61%) had normal cytology results, and 68(65.39%) women had abnormal cytology. The frequency of abnormal cytology was 8.7% (n=9) for AGUS, 14.4% (n=15) for ASCUS, 3.8% (n=4) for LGSIL, 1% (n=1) for HGSIL, and 37.5% (n=39) for cervicitis. There was a significant association between AMH and PAP smear results. AMH levels as a marker for predicting ovarian response were between one and three in women who had normal cytology. The AGUS result was significantly higher for women who had AMH greater than three.

It is essential to know the risk factors of cervical cancer to reduce the mortality and morbidity related to the cervical cancer. The researchers have evaluated the factors affecting the PAP smear test results. Cervical cancer can be easily diagnosed and wholly treated in the early stages, but if its diagnosis is delayed, it will be challenging to treat and sometimes unsuccessful. Early diagnosis of this cancer is possible through a PAP smear test (16). The warning items that women should observe to perform this test are essential. Based on the findings, women can be suggested to take the test by monitoring their levels of AMH. However, to prove this finding, more research with a higher number of samples of different ages is needed. One can predict the qualitative and quantitative aspects of controlled ovarian stimulation and menopausal age, with the polycystic ovary syndrome (PCOS) diagnosis of serum AMH levels as a biomarker to evaluate ovarian reserve in women. Factors such as a history of ovarian surgery, PCOS, obesity, chemotherapy, and vitamin D deficiency also impact serum AMH levels (16). Several studies have been conducted on the extent and impact of these factors (16). This study aims to evaluate the possible relationship between PAP smear results and serum AMH levels. The following sections highlight a few related works investigating the relationship between serum AMH levels and the clinical characteristics of women.

Cervical cancer is one of women's leading causes of death (17). A PAP smear test is used for the early detection and diagnosis of this cancer. Researchers studied the factors influencing women's awareness of the PAP smear test for years (18). Age, multiparity, economic conditions, education, and job status were reported as factors affecting the PAP smear test (19-21). Factors affecting PAP smear test results are considered in limited studies. In our study, measures of the level of AMH in the blood and its effect on PAP smear test results were presented. AMH between one and three had a statistically significant relationship with normal PAP smear results. Therefore, knowing the AMH levels can make recommending a PAP test more serious.

Sachan et al. (22) studied the demographic profile of patients that affects pap smear results. Their study demonstrated that LGSIL and HGSIL were primarily seen in women 41–50 years of age, and multiparity (>3) is a considerable risk factor for cervical carcinoma. Sharif (23) reported low smoking rates, conservative sexual behavior, and religious norms affect the PAP smear results.

Gosh et al. (24) reported that the PAP smear results were associated with a more youthful age group, a younger age at sexual debut, and a lower socioeconomic status. The reason for the positive relationship between infections and young age is that younger women have more sexual activity, making them susceptible to sexually transmitted infections. Many studies reported socioeconomic status influencing PAP smear results because of poor hygiene and few health check-ups (19-21). Studies found that socioeconomic status and age were critical factors in cervical cancer screening that could change PAP smear test results.

We accept the limits of our examination. All samples have been recovered from a single organization, and the number of tests conducted for abnormal PAP smears was low. Another limitation is that the AMH test is more expensive than the PAP smear test and is more difficult to obtain. Physicians could use these results as good advisors and suggest patients conduct PAP smear tests by seeing AMH values in routine controls.

In conclusion, the results showed that the serum AMH level and the PAP smear test results had a statistically significant relationship. More research is required to evaluate the impact of serum AMH levels on the PAP smear test results. The research results can be used for a better understanding of cervical cancer risk factors in sexually active women. The current study will provide as a strong foundation for further investigation into the potential effects of blood AMH levels on the outcomes of PAP smear tests in the future.

Ethical Statement

The study was carried out with the permission of Medeniyet University non-interventional clinical researchs ethics committee (Date:13/01/2021 Decision no: 2021/0027).

Conflict of interest

The authors have no conflicts of interest to declare.

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Authors' contributions

Concept: İ.Ö., A.A.M., E.D., Design: İ.Ö., A.A.M., E.D., Data Collection or Processing: İ.Ö., A.A.M., E.D., Analysis or Interpretation: İ.Ö., A.A.M., E.D., Literature Search: İ.Ö., A.A.M., E.D., Writing: İ.Ö., A.A.M., E.D.

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