RESEARCH ARTICLE

Diagnostic Value of Protein Ki67 (MIB-1) in Atypical Pap Smears of Postmenopausal Women

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Abstract

Background: Atrophic epithelium of cervix sampled from postmenopausal women may mimic high-grade cervical intraepithelial neoplasia in Papanicolaou-stained (Pap) smears. Ki-67 (MIB-1) protein presents on proliferating cells, and percentage of cells with positive nuclei provides a reliable tool for rapid evaluation of the growth fraction. The aim of this study was to determine the diagnostic value of protein Ki67 staining in atypical pap smears of postmenopausal women. Methods: In a case-control setting, pap smears of 75 women with an atypical pap smear (case group) and 75 with normal pap smears (controls) were obtained before and after estrogen treatment. Afterward, samples were exposed to the monoclonal antibody Ki-67 (MIB-1) and the immunohistochemically demonstrated Ki-67+ cells were compared. Results: Mean ages of cases and controls were 60.4±4.5 and 59.9±4.3 years respectively (P=0.50). There was one (2.7%) positive Ki-67 specimen in the case group, without any positive Ki-67 specimen in the control group (P=0.50). Conclusions: Measurement of proliferative activity index in Pap smears restrained with MIB1 is a simple, reliable, and cost-effective method for excluding negatives. This would imply that it might allow a substantial reduction of diagnostic estrogen courses and subsequent Pap smears in postmenopausal women with atypical findings.

Keywords: Vaginal smears - Ki-67 antigen - postmenopause - negative predictive potential

Asian Pac J Cancer Prev, **14 (8)**, 4815-4818

Introduction

Cervical cancer is the sixth prevalent solid neoplastic tumor in America. Median age of diagnosis is 52.2 years, and age distribution is bimodal, picks at 35 to 39 and 60 to 64 years. Annually, 500000 death is caused by cervical cancer (Gibbs and Danforth, 2008; Ghojazadeh et al., 2013) and every year about half million women suffering from invasive cervical cancer are diagnosed all around the world (Moore and Tajima, 2004; Vu and Bui, 2012; Zhang et al., 2012). In Iran, diagnosis and treatment is done just based on clinical symptoms and Pap smear with sensitivity and accuracy of 45% and 65%, respectively (Baghiani, 2003).

Relationship between human papilloma virus (HPV) infection, dysplasia and cervical carcinoma has been well known (Bosch et al., 2002; Do et al., 2009; Ghojazadeh et al., 2012). Infection of cervical cells by HPV induces some changes in function and manifestation of host genes which may can be used as screening and diagnostic tools for cervical cancer (Keating et al., 2001; Munoz et al., 2003; Amirnia et al., 2012; Ghojazadeh et al., 2012). These interactions cause setting off of cellular cycle which manifests through abnormal expression of proteins dependent to this cycle, such as Ki-67 (Davey and Zarbo, 2003). Ki-67 presents normally in limited amount on basal and parabasal layers of cervical squamous epithelium (Wong, 2010). In abnormal condition like dysplasia and carcinoma, this protein is overexpressed in upper third of basal epithelium and thus number of positive cells increases (Davey and Zarbo, 2003). During recent decade, most of the large studies have evaluated the diagnostic value of Ki-67 in carcinoma of cervix (Brown and Gatter, 2002; Hanprasertpong et al., 2010; Son et al., 2012). This protein is used in routine cervical pathology for evaluation of adequate harvest of Cervical Intraepithelial Neoplasia (CIN) and distinguishing CIN from postmenopausal atrophy (Bulten et al., 2000). Totally it should be mentioned that in cervical smear prepared from women suffering from mucosal atrophy in their menopause age, the distinction between atrophic epithelial cell groups and cell groups undergoing neoplastic changes only based on cytomorphological criteria may be very difficult, leading to therapeutic errors. In fact, postmenopausal

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atypical change might be due to topical estrogen therapy. So, immunohistochemically investigation of Ki-67 manifestation in prepared Pap smear could help to distinguish atrophic changes and neoplastic capable changes in this group of women; negative staining of Ki-67 will accurately reject the probability of neoplastic changes in analyzed samples.

This study was conducted to determine the diagnostic value of protein Ki-67 staining in abnormal pap smears of postmenopausal women

Materials and Methods

Study design and population

During the present case – control study which was held in pathology center of Tabriz Imam Reza hospital for a period of 24 months from Sep 2010 to Nov 2012, 75 abnormal Pap smear samples of menopausal women (case group) and 75 normal Pap smear samples of menopausal women (control group) were analyzed in base of Ki-67 manifestation.

In this study, 75 samples were enrolled in each group. Research population includes all normal and abnormal Pap smear samples of menopausal women visiting the desired center. Also 75 normal Pap smear samples of menopausal women which were assimilated with the first group according to age were considered as control group.

Although the procedure was under the guidance of specialist, the study didn't associate with any additional cost to the patients. Also Pap smear test is a routine screening test for women in these ages and was prescribed based on the doctor's assessment. All participants have signed a written consent and the study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (TUMS), which was in compliance with Helsinki Declaration.

Age, marriage age, number of children, smoking background, family background of cervix cancer, background of genital infections, Oral Contraceptive Pill (OCP) consumption background, cases with Ki-67+ results, and using Ki-67 for predicting neoplastic lesions, all were considered as study goals, based on resulted information.

Sample collection and tests performed

Seventy Five abnormal Pap smear samples of menopausal women were collected and evaluated using cytological analysis. Collected samples were classified based on Bethesda system (ASCUS, LSIL, and HSIL).

Fifty micro liter of centrifuged Pap smear prepared sample and thin layers on gloss lams covered by poly-L-lysine were formed. Samples were fixed in methanol. Immunohistochemical analysis of samples was conducted using anti Ki-67 antibody (Dako, Aligent Technology Company, United Kingdom) and lams were incubated for 30 minutes. Second antibody was added for 25 minutes. Streptavidin-biotin complex reagent (Dako) was added for 25 minutes and formed solution was placed under diaminobenzidine – hydrogen peroxide solution. Samples were washed using phosphate saline and were stained by hematoxylin.

Immunohistochemical analysis was conducted using optical microscope. In a field with high zoom (400) all cells were counted. Number of strained cells was also counted and their percentage was calculated.

Those cells were considered as positive, whose nucleuses were strained in spotted form or homogenously. Just cytoplasm straining has not been considered as positive result.Number of positive cases was compared in samples of both groups. In case group, after taking primary Pap smear sample, estrogen therapy was prescribed for two weeks, and Pap smear sample was prepared and analyzed again after this period. The results of this secondary analysis have been considered as definite diagnosis.

Statistical analysis

Statistical analysis was performed by SPSS software package version 16.0 for windows (SPSS Inc., Chicago, USA). Quantitative data were presented as mean±Standard Deviation (SD), while qualitative data were demonstrated as frequency and percent (%). Comparison of quantitative data has been conducted using independent samples T-test or Mann-Whitney U-test and comparison of qualitative data has been conducted using Chi-square test or Fischer exact test.

Results

In secondary analysis of Pap smear samples of case group after estrogen therapy, none of samples had preneoplastic or neoplastic lesions. Related demographic characteristics are shown in Table 1. Number of patients having family history of cervix cancer, was 2 person in case group and 1 person in control group, we neither found any meaningful differences (P=0.500 and OR=0.493). Twenty one patients in case group had a background of genital infection while this number was 13 patients in other group. These number did not show significant differences (P=0.119, OR=0.539).

Thirty two patients in case group and 28 patients in control group had history of using OCP before study. These statistics showed no significant differences. Just one patient, from control group of all 150 patients, had positive result of Ki-67 test. Other demographic risk factors were also shown in Table 1. The results of Ki-67 test, which was to prevent neoplastic lesions showed 0 true positive case, 149 true negative cases, one false positive case and 0 false negative case. So, the property of Ki-67 test is 99.3% (95%CI: 96.3-100%) and PPV is 100%.

For using Ki-67 for predicting neoplastic lesions we had 149 true negative cases and one false positive case. We had no true positive and false negative cases (specificity: 99.3% (95%CI: 96.3-100%), PPV: 100%).

Discussion

In this study, we investigated diagnostic value of straining protein Ki-67 (MIB-1) in abnormal Pap smears of menopausal women. None of abnormal Pap smear samples were Ki-67 positive, in analysis. After a period of estrogen therapy and reanalyzing Pap smear, none of

Table 1. Some Demographic and Risk Factors of Studied Groups.

Variables		Study Groups		P
		Case Group N (%)	Control Group N (%)	
Age	50-54	4 (5.3)	4 (5.3)	0.50
	55-59	34 (45.3)	38 (50.7)	
	60-64	20 (26.7)	21 (28)	
	65-69	13 (17.3)	8 (10.7)	
	70≤	4 (5.3)	4 (5.3)	
Marriage Age	15-19	35 (46.7)	34 (45.3)	0.22
	20-24	25 (33.3)	26 (34.7)	
	25-29	9 (12)	8 (10.7)	
	30≤	6 (8)	7 (9.3)	
No of Children	Mean±SD	2.8±1.8	3.0±2.2	0.48
	Min-Max	0-8	0-6	-
Positive Smoking History		5 (6.7)	2 (2.7)	0.22
Family History of Cervix Cancer		2 (2.7)	1 (1.3)	0.50
Positive Genital Infection		21 (28.0)	13 (17.3)	0.11
OCP Consumption Background		32 (42.7)	28 (37.3)	0.50

*OCP: Oral Contraceptive Pill ; SD: Standard Deviation

samples showed neoplastic or preneoplastic lesions. In the group with normal Pap smear, sample was Ki-67 positive just in 1 case (1.3%). Accordingly, specificity and PPV of this test for distinguishing neoplastic and non-neoplastic Pap smears were 99.3% and 100% respectively.

Bulten et.al by similar study analyzed 83 samples of cervical biopsy and Pap smear in menopausal women in terms of Ki-67 presentation and activity. This report showed that percentage of Ki-67+ in CIN samples was significantly higher than atrophic and normal samples. Activity evaluation also managed to distinguish between neoplastic or preneoplastic samples and non-neoplastic samples completely (Bulten et al., 2000).

In another study, Pap smear samples of 30 menopausal women were analyzed based on Ki-67 incidence and its activity index. Using this parameter managed to distinguish cervical atrophic lesions from CIN in 100%. In this study, two cases were required to repeat smear. thus, analysis of Ki-67 manifestation for distinguishing neoplastic and non-neoplastic cervical lesions has known as a simple, suitable, and accurate method (Bulten et al., 2000).

In another study, abnormal Pap smears in menopausal women were analyzed in terms of Ki-67 and proliferating cell nuclear antigen incidence. Finally, it was found that Ki-67 positive density in neoplastic cases was significantly higher and there was a direct relationship between this parameter and neoplastic intensity (Goel et al., 2005).

In another study by analyzing cervical samples of menopausal women, using status determination of Ki-67 parameter has suggested as an accurate method for distinguishing atrophy and neoplastic lesions in cervical samples (Qiao et al., 2005).

Ki-67 incidence in Pap smear samples with endocervical granular atypia showed that analyzing Ki-67 status for distinguishing neoplastic and non-neoplastic lesions was helpful method (Boon et al., 2004).

Studying the distribution of positive Ki-67 samples in patients with neoplastic cervical lesions revealed that Ki-67 was significantly higher than non-neoplastic lesions. Based on this finding, using this parameter is suggested for this case (Siemens et al., 2011).

Other studies concluded that analyzing Ki-67 expression in suspicious Pap smear samples of menopause women could be helpful in distinguishing neoplastic lesion from non-neoplastic (Smedts, 2001; Pahuja et al., 2004). Some other studies have shown that positive staining of Ki-67 in cervical tissue could be helpful in distinguishing natural epithelium and CIN lesions (Mimica et al., 2010; Samir et al., 2011; Son et al., 2012).

In study of Ki-67 antigen immunostaining in squamous intraepithelial lesions of the cervix, it was reported that Ki-67 incidence in normal cervical samples is significantly lower than CIN samples (Jackson et al., 2012).

In all mentioned studies, evaluation of Ki-67 in Pap smear or cervical biopsy samples has been suggested for distinguishing neoplastic and non-neoplastic lesions. Most of studies have had diagnostic difficulty in menopausal women. In this group, it was too difficult to trust on primary Pap smear samples for absolute distinguishing and diagnosing neoplastic and non-neoplastic lesions, due to high prevalence of cervical atrophy. In the other hand, probability of neoplastic lesion formation in this age group increases significantly (Petry et al., 2011).

Two studies have shown that Ki-67 positivity decreases significantly in Pap smear samples showing atrophy and as a result, opportunity for differentiation of this non-neoplastic state and high risk cases in menopausal women is provided (Atkins, 2011; Gertych et al., 2012). So, using noninvasive methods such as analyzing Ki-67 in menopausal women is very important. It has been shown that analyzing Ki-67 incidence status could significantly decrease the need for estrogen therapy and subsequent analyzing processes in these people (Gupta and Rajwanshi, 2013).

The results of current study approve this report. One of the studies which is so similar to current investigation, has been conducted by Ejersbo et al. (1999) in that study, 386 abnormal Pap smear samples of menopausal women, either reanalyzed after an estrogen therapy period, or analyzed using colposcopy and biopsy or endocervical curettage. All non-neoplastic samples were negative in terms of Ki-67 presentation. Finally, it has been concluded that analyzing Ki-67 status in atrophic samples was so helpful for diagnosing non-neoplastic lesions and decreases false positive cases in cytological analyses up to 86% (Ejersbo et al., 1999). The results of our study are highly close to the results of the mentioned study. In our study, also, all samples with primary abnormal Pap smear without neoplastic state became negative in terms of Ki-67 incidence. Thus, it could be concluded that it is possible to assure about non-pathological condition in case of negative Ki-67 staining in menopausal women with initial suspicious Pap smear. In the other hand, one sample of normal Pap smears turned out to be false positive in terms of Ki-67 expression (false positive sample) which means positivity of Pap smear sample for Ki-67 in menopausal women does not necessarily show having got neoplastic lesion.

One of the limitations of current study was lack of neoplastic sample in subsequent follow ups. In fact, analyzing the role of Ki-67 presentation in suspicious Pap smear samples taken from menopausal women was not

possible for this study and consequently, it would not be possible to determine sensitivity and Negative Predictive Value (NPV) of this test. Such limitations also existed in other studies. In fact, conducting further studies on samples with true abnormal Pap smear would be helpful in determining diagnosis accuracy of Ki-67 staining in menopausal women (Ejersbo et al., 1999).

In conclusions, measurement of proliferative activity index in MIB1 restrained Pap smears is a simple, reliable, safe, and probably also cost-effective method to obtain a substantial reduction of diagnostic estrogen courses and subsequent Pap smears in postmenopausal women with an atypical Pap smear.

Acknowledgements

The authors would like to thank Dr. Nariman Nezami (The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins Hospital, Baltimore, MD, USA.) for his help.

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