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Association of Pap Smear Abnormalities with Autoimmune Disorders

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Abstract: Recently, it is hypothesized that there might be an association between immunological disorders and cervical premalignant and malignant abnormalities. Related studies have been generally focused on some particular autoimmune disease, specially the Systemic Lupus Erythematosus (SLE). This study aimed at comparing the rate of Pap smear abnormalities in female patients with autoimmune diseases and normal counterparts. In a case-control setting, 118 female patients with various autoimmune diseases (the case group) and 118 healthy female counterparts (the control group) were recruited in Tabriz Imam Reza Teaching Centre in a 24 months period of time. The two groups were matched for demographics and known risk factors of cervical malignancy. Frequencies of abnormal Pap smear testing were compared between the two groups. The autoimmune disorders were SLE (74 patients), rheumatoid arthritis or RA (32 patients), systemic sclerosis or SS (7 patients) and ankylosing spondylitis or AS (5 patients) in the case group. Frequency of abnormal Pap smear testing was significantly higher in the case group comparing with that in the controls (7.6% vs. 1.7%; $p = 0.03$). Frequency of abnormal Pap smear testing was higher in the patients with SLE (8.1%) and RA (9.3%) comparing with that in the controls; However, these differences were marginally nonsignificant ($p = 0.06$ and $p = 0.07$, respectively). Frequency of cases with abnormal Pap smear testing was not statistically different between the autoimmune disorders ($p = 0.99$). Based on these findings and in conclusion, there might be an association between the autoimmune disorders and occurrence of premalignant or malignant lesions in cervix. Further studies with larger samples sizes are recommended.

Key words: Pap smear, autoimmune disorder, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, ankylosing spondylitis

INTRODUCTION

Cervical cancer is the second most common malignancy among women all over the world (Gonzalez-Losa *et al.*, 2006; Hussain *et al.*, 2009; Sultana *et al.*, 2011) and a leading cause of death in this group (Tambunan *et al.*, 2007). There are known risk factors in this regard including early and multipartner sexual contacts, smoking, use of Oral Contraceptive Pill (OCP) and immunosuppressive medications. It is clear that screening of women could decrease the incidence of cervical cancer dramatically by detection of early premalignant changes in the tissue (Rosai, 2004). One of the best screening modalities for cervical cancer is the clinical cytologic approach and the Pap smear test is an accurate noninvasive method in this regard (Beckmann, 2005; Shobeiri and Nazari, 2007; Moosavi *et al.*, 2008; Yavari *et al.*, 2009). It is previously hypothesized that there might be an association between disorders of immune system and increased risk of cervical cancer (Gibbs *et al.*, 2008). Nandigam (2006) reported a case of

cervical cancer with immunologic thrombocytopenia. There are a number of studies in the literature indicating higher frequency of abnormalities in Pap smears of women with Systemic Lupus Erythematosus (SLE) comparing with healthy counterparts (Tani *et al.*, 2011; Tam *et al.*, 2011). There is no consensus on the etiology of this association between immunological diseases and cervical cancer. Infection with Human Papillomavirus (HPV) (Lee *et al.*, 2010; Klumb *et al.*, 2010a), immunosuppressive therapy (Klumb *et al.*, 2010b) or a causal association (Mercado, 2009; Bernatsky *et al.*, 2006) have been proposed in this regard. To the best of our knowledge, there is not any study in the literature assessing association of immunological disorders (not a particular one like SLE) and cervical cancer based on the result of Pap smear. This study aimed at determining the frequency of Pap smear abnormality in a group of women with different autoimmune diseases in comparison with a well-matched group of healthy counterparts.

MATERIALS AND METHODS

Subjects: In this case-control study, 118 females patients with different autoimmune disorders and 118 matched healthy females were enrolled in Tabriz Imam Reza Educational Centre in a 24 month period of time from June 2009 to June 2011. The autoimmune disorders were definitely diagnosed by two different skilled specialists. The autoimmune diseases (in the case group) were SLE (systemic lupus erythematosus) in 74 patients, RA (rheumatoid arthritis) in 32 patients, SS (systemic sclerosis) in 7 patients and AS (ankylosing spondylitis) in 5 patients were compared with 118 healthy counterparts (the control group).

Pap smear: Pap smear testing was performed by a skilled pathologist skilled in gynecological diseases and evaluation was carried out by Liquid-Based Cytology (LBC) for cervical cancer screening. The pathologist was blind to the grouping of subjects.

Pap smear testing was performed in these steps:

- **Transferring of smear:** The content of smear was deployed in a box of preservatives. This box included an alcohol-based material for preserving cells for long times (2-18 months) in the room temperature
- **Preparation of slides:** The thin layer technology was employed for this purpose. By using a special detergent instead of applying filters, blood, mucus and any debris were cleansed. Then the preparation was centrifuged. The precipitated pellet was mixed by cell-base solution. After vibration, this liquid was used to prepare the final slide

The specimen adequacy of Pap smears were judged according to the patients' demographics, clinical information, adequate number of squamous epithelial cell and adequate number of endocervical cells in transformation zone including 5 groups of endocervical or squamous metaplastic cells (Atkinson and Silverman, 1998).

Study design and variables: The results of Pap smear testing were compared between the case and control groups. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences. The studied data were patients' demographics, obstetrical history and risk factors of cervical cancer.

Statistical analysis: Statistical evaluation was made using SPSS for Windows V 18.0 (SPSS Inc., IL, USA). Data were shown as frequency (percentage) or Mean±Standard Deviation (SD). Independent samples

T, chi-square and Fishers' Exact tests were employed for statistical analysis. The p-values less than 0.05 were regarded as significant.

RESULTS

General data and the studied variables including age, marriage status, education level, career, nulliparity, gravidity, parity, history of abortion, OCP use and smoking status were comparable between the cases and the controls, between the SLE patients and the controls and between the RA patients and the controls. These variables were also comparable between the SS/AS patients and the controls were comparable except for the history of abortion which was significantly higher in the SS/AS group comparing with the controls. The age ranged between 32-41 years in the case group (32-41 in the SLE group, 32-39 years in the RA group and 33-39 years in the SS/AS group) and 34-40 years in the controls.

The educational level ranged between 8-14 years in the case group (8-14 years in the SLE group, 9-13 years in the RA group and 8-13 years in the SS/AS group) and 8-14 years in the controls.

The gravidity ranged between 0-5 in the case group (0-5 in the SLE group, 1-4 in the RA group and 0-4 in the SS/AS group) and 0-5 in the controls.

The parity ranged between 0-3 in the case group (0-3 in the SLE group, 0-3 in the RA group and 1-3 in the SS/AS group) and 0-3 in the controls. These data are summarized and compared between the mentioned groups in Table 1.

A positive Pap smear result was present in 9 patients in the case group including 6 patients in the SLE group and 3 patients in the RA group. There was no case with the positive Pap smear result in the SS/AS group. In the control group, there were 2 patients with positive Pap smear result. The rate of positive Pap smears was significantly higher in the patients with autoimmune diseases than in the healthy counterparts (7.6% vs. 1.7%; $p = 0.03$). Although, the frequencies of patients with positive Pap smear results in the SLE (8.1%) and RA (9.3%) groups were not significantly different from that in the controls, both differences were marginal ($p = 0.06$ and 0.07 , respectively). The rate of abnormal Pap smear results were not significantly different between the groups with various autoimmune diseases ($p = 0.56$). Percentages of subjects with abnormal Pap smear result are shown in Fig. 1.

All the positive Pap smear results were of the Atypical Squamous Cells of Undetermined Significance (ASCUS) type except for 2 subjects in the case group which were of the human papillomavirus (HPV) type.

Table 1: Comparison of general data in different studied groups with the controls

Variable	Case (n = 118)	SLE (n = 74)	RA (n = 32)	SS/AS (n = 12)	Control (n = 118)	p*	p**	p***	p****
Age (year)	37.9±7.2	38.3±6.9	38.3±7.8	36.8±6.6	38.4±5.7	0.58	0.90	0.95	0.91
Married	91 (77.1)	55 (74.3)	24 (75)	12 (100)	87 (73.7)	0.55	0.49	0.59	0.09
Education (years)	10.8±3.1	11.3±2.7	11.7±1.1	10.7±1.8	10.5±3.7	0.79	0.57	0.41	0.49
House wife	107 (90.7)	67 (90.5)	29 (90.6)	11 (91.7)	102 (86.4)	0.31	0.48	0.46	0.59
Nulliparity	38 (32.2)	24 (32.4)	9 (28.1)	5 (41.7)	27 (22.9)	0.11	0.51	0.54	0.08
Gravidity	3.1±0.2	3.2±0.1	3.0±0.1	3.1±0.1	3.2±0.5	0.35	0.84	0.28	0.33
Parity	1.2±0.1	1.3±0.2	1.1±0.1	1.1±0.2	1.1±0.2	0.49	0.37	0.78	0.98
Abortion	19 (16.1)	9 (12.2)	5 (15.6)	5 (41.7)	10 (8.5)	0.07	0.09	0.12	0.04
OCP use	36 (30.5)	23 (31.1)	9 (28.1)	4 (33.3)	32 (27.1)	0.57	0.55	0.91	0.58
Smoking	7 (5.9)	4 (5.4)	2 (6.3)	1 (8.3)	3 (2.5)	0.20	0.43	0.29	0.49

Data are shown as Mean±Standard deviation or frequency (percentage). p<0.05 is considered statistically significant. The only significant difference was between the SS/AS and the control groups with regard to the history of abortion. *Case vs. control, **SLE (systemic lupus erythematosus) vs. control, ***RA (Rheumatoid arthritis) vs. control, ****SS/AS (systemic sclerosis/ankylosing spondylitis) vs. control. OCP: Oral contraceptive pill

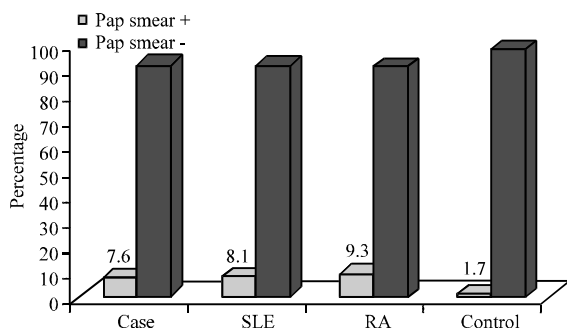


Fig. 1: Percentages of patients with positive Pap smear results in the studied groups, RA: Rheumatoid arthritis. SLE: Systemic lupus erythematosus. The rate of subjects with positive Pap smear was significantly higher in the case group vs. the controls (p = 0.03). The comparisons between the SLE groups vs. the controls (p = 0.06) and between the RA group vs. the controls (p = 0.07) were insignificant but borderline in this regard. The difference was not significantly significant between the groups with various autoimmune diseases (p = 0.56)

DISCUSSION

In the present study, the rate of positive Pap smears was significantly higher in the patients with autoimmune diseases. Although, the frequencies of patients with positive Pap smear results in the SLE and RA groups were not significantly different from that in the controls, both differences were marginal (Fig. 1). It should be noticed that the known risk factors of cervical cancer such as smoking, nulliparity and OCP use were comparable between the patients and controls and so the confounding factors were eliminated in this study. To the best of our knowledge, the available reports are only confined to those assessing possible association between the cervical cancer and the SLE by now. Blumenfeld *et al.* (1994) studied 39 female patients with SLE and reported

that the rate of cases with positive Pap smear is significantly higher in this group comparing with age-matched healthy females (35.9% vs. 5%). Comparing with our results, the rate of abnormal Pap smears is higher in both the SLE patients and the normal subjects in the mentioned study. This controversy might be due to presence of possible confounding factors which were not controlled in the Blumenfeld's series. Nevertheless, in both reports the rate of abnormal Pap smears was higher in the SLE patients comparing with normal subjects. Berthier *et al.* (1999) also reported similar results in a group of female patients with SLE. So, the result of the present study regarding a higher frequency of abnormal Pap smears in the SLE patients is in line with the mentioned report. The rate of positive Pap smear was 24.1% in female patients with SLE vs. 9.6% in controls in another series by Dhar *et al.* (2001). These rates are also higher than those in the present study. Another justification for this heterogeneity may be ethnical and racial differences between different populations. The corresponding rates were 16.5% vs. 5.7% in another series by Tam *et al.* (2004). This difference might be also justified by racial differences. Other possible etiologies in this regard are the differences in sample size, risk factors of cervical cancer, method of performing Pap smear and level of examiners' experience. On the other hand, it is not exactly known if an abnormal result of Pap smear testing is due to pathophysiology of the SLE disease itself or a consequence of related therapies (Sultan *et al.*, 2000; Bateman *et al.*, 2000; Ognenovski *et al.*, 2004). It is thought that the immunosuppressive treatments make patients vulnerable to infections and simultaneously hinder resolving a present infection in patients (Bernatsky *et al.*, 2004). This condition is a rich environment for different microbial (such as Chlamydia) and viral (such as HPV) infections (Gopalkrishna *et al.*, 2000; Finan *et al.*, 2002; Schiffman *et al.*, 1993; Berthier *et al.*, 1999). Nevertheless, some other studies concluded that association of autoimmune disease (SLE) with higher incidence of cervical cancer is independent of

immunosuppressive therapy (Blumenfeld *et al.*, 1994; Tam *et al.*, 2004). In the present study, it was not possible to definitely evaluate the effect of previous immunotherapy; because it was only based on the patients' report which could be influenced by the recall bias or misreport. Further more controlled longitudinal studies may elucidate the issue. In the present study, there was not a significant difference between various groups of autoimmune diseases with regard to the frequency of abnormal Pap smear results ($p = 0.56$) (Fig. 1). Abu-Shakra *et al.* (1996) proposed that occurrence of cervical cancer is less in SLE disease comparing with that in RA and SS. This is in contradiction with finding of the present study. It should be noticed that in the mentioned study the rate of cervical cancer has been compared between the groups with autoimmune diseases and this may be different from the abnormal Pap smear result. Small sample size specially in the SS/AS group is another justification of this heterogeneity. Further studies with larger sample sized are required in this regard.

CONCLUSION

Based on finding in this study, autoimmune diseases may increase risk of cervical cancer in these patients. This hazard is independent of conventional risk factors for such malignancies. So, it is highly recommended that relevant screening approaches such as Pap smear testing are better considered in all patients with autoimmune diseases regularly.

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