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RESEARCH ARTICLE

ABNORMAL PAP TESTS IN SYSTEMIC LUPUS ERYTHEMATOSIS: A CYTOPATHOLOGICAL AND HUMAN PAPILLOMAVIRUS TESTING STUDY.

Ehab F. Girbash^{2,3}, Doaa S. Atta¹, Dalia S. Fahmy¹, Shaimaa M. Abdelwahab¹, and Ibrahim Therwat¹.

1. Rheumatology And Rehabilitation.
2. Obstetrics And Gynecology, Faculty of Medicine. Zagazig University,
3. Najd Consulting Hospital, KSA.

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*Corresponding Author

Ehab F. Girbash.

Abstract

Objectives: the frequency of cervical squamous intra-epithelial lesions (SIL) in women with systemic lupus erythematosus (SLE), and the association between clinical parameters, use of immunosuppressant agents, human papillomavirus (HPV) infection, colposcopy, and development of SIL in SLE.

Methods: A retrospective case control study conducted in 150 women constituted the study group with confirmed diagnosis of SLE. The enrolled patients divided into study group (no.28) with abnormal pap smears and reference group (no.122) with normal pap smears. All patients subjected to clinical evaluation, colposcopy, directed biopsies, and HPV-ISH testing. All participants reassessed every 6 months for 2 years.

Results: mean patient age with abnormal pap smears was 44.8(P = 0.74) years with mean SLE disease duration was 9.3±7.1 years. There was past history of cervical lesions where it was positive in 89.3% (P = 0.001). SLE patients with abnormal pap smears showed significant difference regarding nephritis (P = 0.001) and high titers of Anti-double stranded DNA (P = 0.001) and Antiphospholipid antibodies (P = 0.012). SLEDAI, SDI, and immunosuppressant agent usage found significantly different among patients with abnormal pap smears. HPV Infection found significantly important (P = 0.001) when the infection was high-risk HPV, multiple HPV, or persistent HPV infection among patients with abnormal pap smears. On comparison with cytology, colposcopy, HPV-ISH results, the best sensitivity was with the use of HPV-ISH (90%) then with colposcopy (75%). In addition, the best specificity was with the use of colposcopy (99%). The highest positive predictive value was with colposcopy (94%) and the highest negative predictive value was with HPV-ISH assay (98%).

Conclusion: Patients with SLE had higher risk for HPV infection and cervical dysplasia than the general population. Thus, gynecological visits at shorter intervals (3-6 months) recommended for those patients. The use of colposcopy is useful tool in areas where the screening of HPV is not available.

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Introduction:-

Cervical cancer is the second most commonly occurring cancer in women and accounts for up to 300,000 annual deaths. The list of mentioned causes for cervical cancer in various sources includes early sexual activity, multiple sexual partners, human papilloma virus (HPV) infection, genital warts, sexually transmitted diseases, genital tract abnormalities, age, smoking, passive smoke, poor nutrition, immunodeficiency and malnutrition⁽¹⁾.

Systemic lupus erythematosus (SLE) is a multi-organ system, autoimmune disease with numerous immunological and clinical manifestations. It is believed to develop as a result of dysregulation of the immune system, ultimately leading to the clinical features of inflammation such as HPV. In addition to the clinical manifestations of SLE itself, female patients also have to contend with a heightened risk of developing abnormal cervical smears and squamous intra-epithelial lesions (SILs) of the cervix as well as other cancers⁽²⁾.

Research has shown that SLE syndrome represents a permissive effect of immunosuppression on increased host susceptibility to high cancer-risk HPV infections, the causative agents of SIL and cervical cancer⁽³⁾.

One study showed an increased incidence of cervical cancer in SLE and other studies have noted a high risk of abnormal Pap tests in women with SLE⁽⁴⁾.

Aim:-

To assess the frequency of squamous intra-epithelial lesions in women with systemic lupus erythematosus (SLE), and the association between clinical parameters, use of immuno-suppressants, HPV infection, and development of SIL in SLE.

Material and methods:-

A retrospective case control study conducted over a period from March 2013 to March 2015. 150 women constituted the study group with confirmed diagnosis of SLE attending outpatient clinics of Rheumatology and Rehabilitation department for management and follow up and referred to Obstetrics and Gynecology department outpatient clinics for early detection of SIL at Zagazig University Hospital, Egypt and Najd Consulting Hospital, KSA. The enrolled patients divided into study group (no.28) and reference group (no.122) according to result of pap smears. Informed consent obtained from all participants. The study received ethics approval from the department of Rheumatology, Zagazig University, Egypt.

All patients fulfilled 1997 American Collage of Rheumatology revised criteria for the classification of SLE⁽⁵⁾. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)⁽⁶⁾ and the Systemic Lupus International Collaborating Clinics/ ACR Damage Index⁽⁷⁾ were used to indicate disease activity and damage respectively.

Patients were eligible for the study if they were married (sexually active) and had not undergone a Pap test in the past one year or had never had a Pap test.

Patients younger than age 18 years, those who were pregnant, Patients who received HPV vaccine, virgin and those with abnormal vaginal bleeding were excluded.

Each participant was subjected according to WHO guidelines in 2013: 1) complete history taking for collection of the demographic information, and past sexual, gynecologic, and obstetric history. 2) medication history for the past 2 years at baseline. 3) general examination and local musculoskeletal examination. 4) referred gynecologic outpatient clinics for local examination to detect any cervical lesion (as ectopy, condyloma, or polyp) and make cervical pap smear for cytological evaluation and follow up. 5) colposcopy with saline technique and acetic acid 5% application, directed cervical punch biopsy from transformation zone (TZ), and HPV testing by In Situ hybridization assay^(8,9). All participants reassessed every 6 months for 2 years.

Each Pap test was done using a Ayre's spatula (Cytopath, UK) applied on ectocervix including TZ and rotated 360° and a Cytobrush (Medscand, Sweden) applied inside endocervical canal and rotated 180° and collected ectocervical and endocervical smears rapidly spreaded over a glass slide and immediately fixed in 95% ethyl alcohol for 1-3 hours. Then stained by the modified ultrafast pap staining technique with superior results ⁽¹⁰⁾.

The resulting slides were classified according to The 2001 Bethesda System terminology of cytological classification into benign changes (infective or reactive), and epithelial abnormalities. The epithelial include: 1) Atypical Squamous Cells of Undetermined Significance [ASCUS], 2) Low Squamous Intraepithelial Lesions [LIS]; including HPV infection [Koilocytosis], and Cervical Intraepithelial Neoplasia grade1 [CIN1]; and 3) High Squamous Intraepithelial Lesions [HIS] including Cervical Intra-epithelial Neoplasia grade 2&3, and carcinoma in situ [CIN2, CIN3 and CIS] ⁽¹¹⁾.

Colposcopy was done for all patients for diagnosis (saline technique for evaluation vascular pattern of TZ and detection of acetowhite lesions after acetic acid 5% application) and taking directed biopsy from detected lesions including TZ or random multiple biopsies including TZ in negative colposcopy using Kevorkian bunch biopsy forceps for histopathology and HPV testing by ISH.

HPV testing by ISH was done using BioGenex HVP-DNA fluorescented screening probe containing cocktail of HPV types 6,11, 16, 18, 31 and 33 with HPV detection using supersensitive ISH kit (BioGenex Lab. Inc., San Ramon, CA, USA). The technique was done according to manufacture guidelines. The results interpreted under standard light microscope by detecting cellular purplish precipitate in infected cells.

According to multiple studies, the status of HPV infection may be incident (which is negative at start of study "baseline" and become positive after that), persistent (detecting the same HPV type at 2 or more examinations 6 month apart), multiple, or high risk infection ^(12, 13).

Results:-

In this study, 150 women constituted the study group with confirmed diagnosis of SLE attending outpatient clinics of Rheumatology and Rehabilitation department for management and follow up. Then referred to Obstetrics and Gynecology department outpatient clinics for early detection of SIL. The enrolled patients divided into study group (no.28) and reference group (no.122) according to result of pap smears. The demographic and clinical characteristics of the study groups showed in Table 1. Mean patient age with abnormal pap smears was 44.8 (P = 0.74) years with menopause percentage 42.9% and mean SLE disease duration was 9.3±7.1 years. There was significant difference regarding past history of cervical lesions where it was positive in 89.3% (P = 0.001). In addition, SLE patients with abnormal pap smears showed significant difference regarding chronic kidney disease (nephritis) (P = 0.001) and high titers of Anti-double stranded DNA (P = 0.001) and Antiphospholipid antibodies (P = 0.012).

SLEDAI and SDI were found significantly different among patients with abnormal pap smears with values of 2.4±0.7 (P = 0.018) and 11(39.3%) (P = 0.014) respectively (Table 2). In addition, the use of Cyclophosphamide (P = 0.001) and Cyclosporin A (P = 0.022) were found significantly high among patients with abnormal pap smears.

HPV Infection pattern found significantly important (P = 0.001) when the infection was with the high-risk group, multiple HPV infection, or persistent HPV infection among patients with abnormal pap smears (Table 3).

In this study, histopathological examination of the biopsies obtained from 150 participating patients showed 20 patients with true positive result for cervical intraepithelial neoplasia (CIN) and 130 patients with true negative result for CIN. On comparison with cytology, colpo-scopy, HPV-ISH results, the best sensitivity was with the use of HPV-ISH assay (90%) then with colposcopy (75%). In addition, the best specificity was with the use of colposcopy (99%). So, the best test to diagnose CIN was colposcopy (with highest positive predictive value 94%) and the best test to exclude CIN was HPV-ISH assay (with highest negative predictive value 98%) (Table 4& 5). HPV-ISH assay found positive (intracellular purple precipitate) in 23.3% of the study group (fig 1).

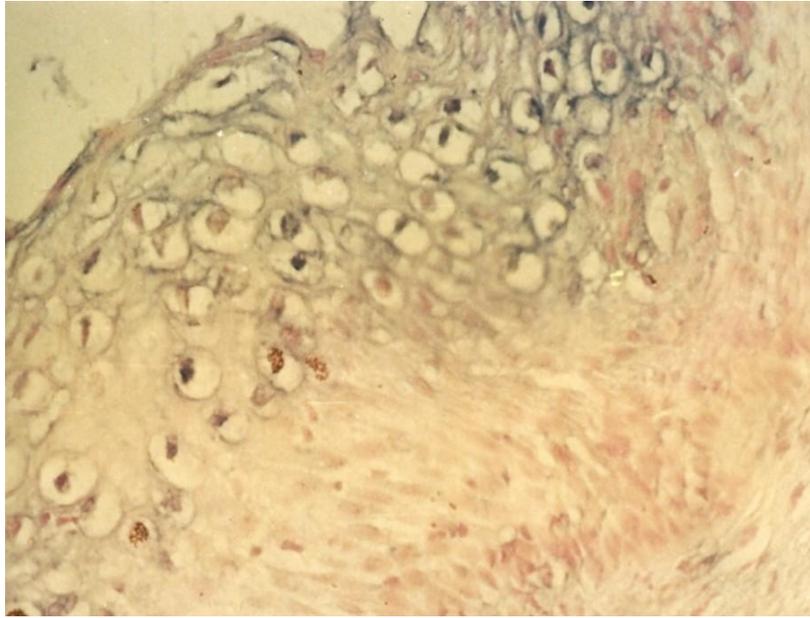


Fig 1: Patient with LSIL and positive HPV assay.

Figure 2 showed colposcopy of patient with high SIL with dense acetowhite area and well demarcated margin.



Fig 2: Patient with Colposcopy showed HSIL.

Table 1: Demographic and clinical characteristics of study groups.

Characteristic	Normal Pap smear (n=122)	Abnormal Pap smear (no=28)	P value
Age Mean \pm SD	42 \pm 6	44 \pm 8	0.740
Menopause no(%)	48 (39.3%)	12(42.9%)	0.89
Age <20 y at first coitus no(%)	22 (18%)	10(35.7%)	0.07
History of Oral contraceptive use	30(24.6%)	7(25%)	0.96
Multiparity	85(69.7%)	22(78.6%)	0.49
History of Sexually transmitted disease	3(2.4%)	1(3.6%)	0.74
SLE DURATION Mean \pm SD	10.6 \pm 6.8	9.3 \pm 7.1	0.37
History of cervical lesions	38(31.1%)	25(89.3%)	0.001
Multiple marriages	21(17.2%)	6(21.4%)	0.191
Post coital bleeding	20(16.4%)	8(28.6%)	0.184
CLINICAL FEASTURE			
Nephritis	40(32%)	16(57.1%)	0.001
Neuropsychiatric	7(5.7%)	2(7.1%)	0.681
Hematologic	100(81.9%)	24(85.7%)	0.793
Discoid rash	9(7.3%)	3(10.7%)	0.684
Malar rash	59(48.4%)	15(53.6%)	0.811
Arthritis	91(74.6%)	20(71.4%)	0.745
Photosensitivity	27(22%)	8(28.6%)	0.436
Oral ulcers	30(24.6%)	9(32.1%)	0.412
Serositis	22(17.2%)	6(21.4%)	0.673
Antinuclear Antibodies	122(100%)	28(100%)	1.000
Anti-double stranded DNA	29(23.8%)	15(53.6%)	0.001
Antiphospholipid antibodies	32(26.2%)	10(35.7%)	0.012

Table 2: Association of Clinical and Medication Features in the past 2 years and Pap smear results in SLE Patients.

Clinical and Medication Features	Normal Pap smear n=122	Abnormal Pap smear no=28	P value
SLEDAI	1.0 \pm 0.1	2.4 \pm 0.7	0.018
SDI \geq 1	17 (13.9%)	11 (39.3)	0.014
Prednisolone	117 (95.9%)	28 (100%)	0.580
Cyclophosphamide	10 (9.8%)	17 (60.7%)	0.001
Azathioprine	82 (70.5%)	14 (50%)	0.134
Cyclosporin A	108 (88.5%)	19 (67.9%)	0.022
Hydroxychloroquin	10 (9.8%)	3 (10.7%)	0.711
SLEDAI :Systemic lupus erythematosus disease activity index			
SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index			

Table 3: Association between HPV Infection patterns and Pap smear results in SLE Patients.

HPV Status Results	Normal Pap smear n=122	Abnormal Pap smear no=28	P value
Incident HPV infection (no=35)	28(22.9%)	7(25%)	0.812
Multiple HPV infection(no=30)	17(13.9%)	13(46.4%)	0.001
High risk HPV infection(no=22)	5(4.1%)	17(60.7%)	0.001
Persistent HPV infection(no=28)	10(8.2%)	18(64.3%)	0.001

Table 4: Association between histopathology, cytology, colposcopy, and HPV-ISH.

Histopathologic result	Normal (-VE) (n=130) (86.7%)	CIN1/HPV (n=15) (10%)	CIN2/CIN3 (n=5) (3.3%)
Pap smear	Negative (-VE) (n=122) (81.3%)	ASCUS/HPV/LSIL (n=24) (16%)	HSIL (n=4) (2.7%)
	True -VE (n=12) False -VE (n=8)	True +VE (n=12) False +VE (n=16)	
Colposcopy	Negative (-VE) (n=134)(89.4%)	ASCUS/HPV/LSIL (n=11)(7.3%)	HSIL (n=5)(3.3%)
	True -VE (n=129) False -VE (n=5)	True +VE (n=15) False +VE (n=1)	
HPV-ISH result	Negative (n=115) (76.7%)	Positive (n=30) (20%)	Positive (n=5) (3.3%)
	True -VE (n=113) False -VE (n=2)	True +VE (n=18) False +VE (n=17)	

Table 5: Performance values of Pap smears, HPV-ISH and Colposcopy in comparison with histopathologic results.

Performance value	Pap smear	Colposcopy	HPV-ISH
Sensitivity	60%	75%	90%
Specificity	87%	99%	87%
Positive predictive value	43%	94%	51%
Negative predictive value	93%	96%	98%

Discussion:-

Epidemiological studies have accumulated in recent years to support essential role of chronic HPV infection in the development of cervical neoplasia and progress of the benign cervical lesions into cancer cervix⁽¹⁴⁾. SLE usually affects women during reproductive age, which is the common age for cervical neoplasia.

Several studies showed increased prevalence SIL in patients with SLE. In addition, there was increased incidence of cervical premalignant lesions in patients taking Cyclo-phosphamide and other immuno-suppressive drugs⁽¹⁵⁾.

In this study, the clinical data (in table 1) showed previous cervical lesions were prevalent and statistically important among SLE patients with abnormal pap smears. This come in agreement with Tam et al that noted previous diagnosis of SLE was an independent predictive factor for abnormal pap smears, after logistic regression analysis. Moreover, found also higher prevalence of multiple HPV infections and recurrent persistent cervical lesions among SLE patients⁽¹⁾.

In this study, the lupus nephritis clinical presentation showed statistical important association with abnormal pap smears. This could explained by these patients always need immunosuppressive medications to their disease activity. This come in agreement with Cibere et al that found increased risk of multiple malignancies including cervical cancer especially with the use of immunosuppressive medications. Moreover, Bertansky et al found an increase in abnormal cervical cytology in the lupus group vs. controls ($P < 0.0008$). Furthermore, the abnormal cytopathology was accounted for by an excess of HGSIL and cancer ($OR = 3.46$; $P < 0.0003$)^(16, 17).

In this study, high titers of Anti-double stranded DNA ($P = 0.001$) and Antiphospholipid antibodies ($P = 0.012$) were found among patients with abnormal pap smears. This appear in agreement with study of Dey et al that found increased incidence of malignancies among patients with high titers of these antibodies and concluded that increased malignancy was related excess activity of SLE and increased tissue damage and was not related to immunosuppressive use. But Dey et al found these results statistically insignificant in spite of it come in agreement with multiple controlled studies⁽¹⁸⁾.

In this study, SLEDAI and SDI (table 2) found significantly different among patients with abnormal pap smears. In addition, the use of cyclophosphamide and cyclosporine A found significantly high abnormal pap smears. A univariate analysis showed that the development of SIL was associated with previous treatment of cervical lesions as

was the use of azathioprine and cyclophosphamide ever in the past 10 years. Other demographic, life style and clinical parameters (including disease activity and damage indices) and the use of other immune suppressants were not associated with development of SIL⁽¹⁹⁾. This was not in agreement with the results of this study. Another report found that in women currently receiving azathioprine, 50% had cervical atypia compared with 11% of controls⁽²⁰⁾.

In contrast, Nath et al. noticed that patients with normal smears were more likely to be receiving hydroxychloroquine⁽²¹⁾. This could potentially reflect the disease severity, since azathioprine and cyclophosphamide usually given to patients with major organ involvement, whereas hydroxyl-chloroquine usually given to patients with mild disease.

A future prospective study with a larger sample size and longer follow up duration would be required to assess the risk of SIL and the use of immunosuppressant agents.

In this study, important statistical association was found between high risk HPV (60.7%), persistent HPV (64.3%), and multiple HPV (46.4%) infections (table 3), and the high frequency of abnormal pap smears. This come in agreement with Lee et al that found high prevalence of HPV infection (24.6%) among Korean patients with SLE in comparison with 7.9% ($p < 0.001$) in healthy women⁽²²⁾. In addition, Tam et al found in SLE patient high prevalence of HPV infection and abnormal pap smears (25%) which statistically increased to 48.5% after 3 years follow up with persistent and multiple HPV infections when compared non-SLE patients with cervical dysplasia⁽²³⁾.

HPV detected by in situ hybridization technique using newly super sensitive HPV probes and detection kit which characterized by less complex and less cost when compared with detection of HPV by PCR. In addition, only ordinary light microscope needed for detection of the result with excellent sensitivity, specificity, and negative predictive value (tables 4&5). In this study, HPV-ISH assay showed high sensitivity (90%), specificity (87%), and negative predictive value (98%) with reasonable positive predictive value (51%). These results come in agreement with study of Kelesidis et al. that compared between HPV-PCR and HPV-ISH testing and found similar sensitivity, specificity, and negative predictive value with values of 89.5%, 40%, and 93.2% respectively. However found lower positive predictive value of 29% which can be explained by different HPV infection status and demographic data in studied area⁽²⁴⁾.

In addition, several studies demonstrated great advantage of HPV-ISH assay over HPV-PCR in easy detection and differentiation of HPV infection into integrated infection and episomal infection. HPV-DNA integration into cervical cell DNA, lead to persistence and rapid progression of cervical dysplasia into cervical carcinoma. However, when HPV-DNA remain as round bodies in cervical cell cytoplasm (episomal infection), there was rapid regression of cervical dysplasia in majority of patients. So, HPV-ISH assay may had better clinical and prognostic value over HPV-PCR assay^(25, 26,27).

Moreover, in this study Colposcopy showed high sensitivity (75%), specificity (99%), and excellent positive (94%) and negative (96%) predictive values. This come accordance with results of Wentzensen et al showing high sensitivity (74-87%), specificity (56-68%), and good positive and negative predictive value especially when multiple colposcopic directed biopies obtained and recommend use of colposcopy as useful tool in diagnosis and management of SILs⁽²⁸⁾.

Conclusion:-

Patients with SLE had higher risk for HPV infection and cervical dysplasia than the general population. Thus, gynecological visits at shorter intervals (3-6 months) recommended for those patients. The use of colposcopy is useful tool in areas where the screening of HPV infection is not available.

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