

The cytological pattern of cervical smears of a series of Saudi women in one gynecologic center

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Abstract. – OBJECTIVE: Cervical intraepithelial neoplasia (CIN) is a type of abnormal cervical epithelial development that can lead to cervical malignancy. Thus, the purpose of this study was to examine the cytological pattern of cervical smears from a group of Saudi women who visited a single gynecologic center.

PATIENTS AND METHODS: From May 2020 to May 2021, 300 women were referred to the cytopathology laboratory and their cytological materials were collected. Because of gynecologic concerns, the women in the study were referred for Pap smears. As part of the obligatory investigations in conjunction with the clinical assessment, a Pap smear is requested.

RESULTS: CIN of various grades was found in 11% of the women. High-grade Squamous Intraepithelial Lesions (HSIL) were the most detected modifications, followed by Atypical Squamous Cells of Undetermined Significance (ASUS) and Low-grade Squamous Intraepithelial Lesions (LSIL), accounting for 36.4%, 33.3%, and 30.3%, respectively. In 23.7% of the women, inflammatory cell infiltrations were found. Infection with *Candida Albicans* was identified in 2.3% of the women. In 15.8% of the patients, cytological evidence of bacterial infection was found.

CONCLUSIONS: The prevalence of CIN has been growing in Saudi Arabia in recent years, implying a probable involvement in the spread

of HPV. Most CIN cases associated with middle age point to HPV infection at a younger age. There is no link between estrogen and/or progesterone levels and CIN.

Key Words:

Cervical cancer, Pap smear, CIN, Saudi Arabia.

Introduction

Cervical cancer is the fourth most common female cancer in the world. Aside from the Human Papillomavirus (HPV), which is responsible for up to 70% of cases, various genetic and epigenetic variables have been linked to the development of cervical cancer^{1,2}. Cervical cancer literature and risk factors are still limited in many Middle Eastern nations, including Gulf Cooperation Council countries³. In these countries, much more must be done in terms of cervical cancer prevention and early detection. It was discovered that only 7.6% of Saudi women aged 25 to 49 received Pap smear tests⁴.

According to Saudi Arabian reports, most cervical cancer patients arrive with advanced stages of the disease due to the lack of cervical screening programs and HPV vaccination⁵. Cer-

vical cancer is presumably preventable if appropriate screening and precautionary practices are put in place. The main disadvantages for Saudi women in this context are a lack of awareness and knowledge⁶.

Despite its limitations, the Pap test is largely acknowledged as a viable screening tool for the early diagnosis of cervical cellular abnormal alterations. Women with abnormal pap smears should have additional testing (e.g., colposcopy) and an appropriate follow-up regimen. The Pap cytology method is the most safe, cost-effective, and dependable method for detecting, valuing, and managing cervical epithelial neoplastic changes⁷. As a result, the purpose of this paper was to assess the cytological pattern of cervical smears from a group of Saudi women who visited a single gynecologic center.

Patients and Methods

From May 2020 to May 2021, 300 women were referred to the cytopathology laboratory at the maternity hospital in Al-Madinah, Saudi Arabia, for cytological materials. Because of gynecologic concerns, the women in the study were referred for Pap smears. As part of the obligatory investigations in conjunction with the clinical assessment, a Pap smear is requested. Each patient was requested to sign a documented consent before sample collection.

Scraping the ectocervix's transformation zone yielded cytological materials. The collected materials were evenly smeared on a clean glass slide and immediately fixed in 95% ethyl alcohol while still wet. The smears were stained using the Papanicolaou method (Pap. Method) after fixation; the dried smears were hydrated in descending ethyl alcohol concentrations (90%, then 70%, then 50%, and finally to distilled water (DW) for two minutes in each session). The smears were then stained in Harris' hematoxylin (nuclear stain) for five minutes, rinsed in DW, differentiated in 0.5% aqueous hydrochloric acid for ten seconds (to eliminate residual stain atoms), and immediately rinsed in DW to stop decolorization. The smears were then blued in alkaline water for five seconds before being dehydrated in escalating ethyl alcohol concentrations of 50%, 70%, 90%, and 95% for two minutes each. The smears were then stained with Pap. OrangeG6 (cytoplasmic stain) was applied for two minutes, followed by a rinse in 95% ethyl alcohol and three minutes of dyeing in

EA50 (cytoplasmic stain). The smears were then treated with 95% and 100% ethyl alcohol, cleaned in Xylene, and mounted in Distrene Polystyrene Xylene (DPX).

Each sample was examined for staining quality prior to diagnosis, and only those with acceptable quality were diagnosed. Two cytopathologists independently interpreted each swab. The diagnosis is based on the American technique for detecting cervical cytological atypia, which has been described elsewhere⁸.

Ethical Consent

Before the interview, each participant provided informed consent. The proposal for the current study was authorized by the Ethical Committee at the College of Medicine, University Hail, Saudi Arabia. We confirm that all techniques were carried out in conformity with the relevant rules and regulations (HREC 00130/CM-UOH.04/20). We confirm that we followed the Helsinki Declaration criteria.

Statistical Analysis

Data were first organized in an Excel spreadsheet before being moved to SPSS software for analysis to obtain frequencies, percentages, means, cross-tabulations, and the Chi-square test. A *p*-value of 0.05 is considered statistically significant when the 95% confidence interval is used.

Results

Cervical smears from 300 women aged 20 to 70 years were analyzed cytologically. As indicated in Figure 1, the majority of women were aged 31-40 years, followed by 41-50, 51-60, 20-30, and > 60 years, representing 83/300 (27.7%), 80/300 (26.7%), 73/300 (24.3%), 32/300 (10.7%), and 32/300 (10.7%), respectively.

Cervical intraepithelial neoplasia (CIN), epithelial proliferative atypical alterations, was found in 33/300 (11%) women in this study. High-grade Squamous Intraepithelial Lesion (HSIL), Atypical Squamous Cells of Undetermined Significance (ASUS), and Low-grade Squamous Intraepithelial Lesion (LSIL) were the most frequently seen alterations, accounting for 12/33 (36.4%), 11/33 (33.3%), and 10/33 (30.3%), respectively. The age groups 31-40 years, 41-50 years, and 20-30 years saw the most HSIL cases, accounting for 5/12 (41.7%), 3/12 (25%), and

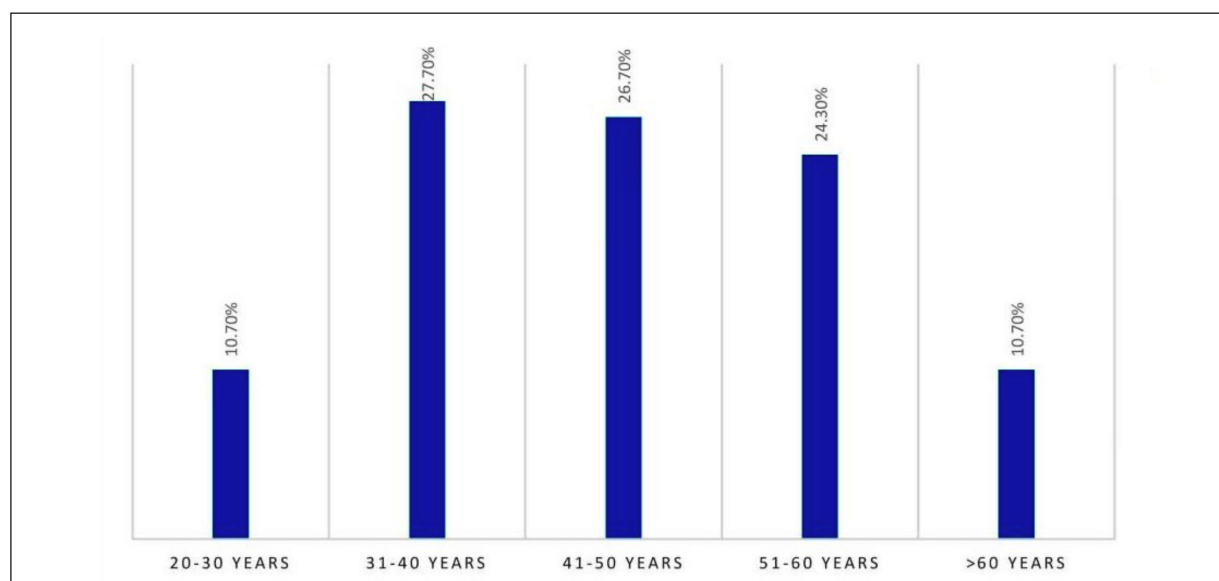


Figure 1. Description of the study population by age.

2/12 (16.7%), respectively. 4/11 (36.4%) of those aged 51-60 had ASUS. LSIL was most common in people aged 41 to 50, as shown in Table I and Figure 2.

Inflammatory cell infiltrations were found in 71/300 (23.7%) patients. The age groups 41-50 years, 31-40 years, and 51-60 years had the highest prevalence of inflammatory conditions, accounting for 26/71 (36.6%), 20/71 (28.2%), and 11/71 (15.5%), respectively.

Candida Albicans infection was discovered in seven women. Four of the seven patients (57%) were between the ages of 31 and 40, while the remaining three (7%) were between the ages of 41 and 50.

Cytological evidence of bacterial infection was found in 19/300 (15.8%) of the patients. Around 10/19 (52.6%) were between the ages of 31 and 50, while 4/19 (21%) were between the ages of 51 and 60 (Table II and Figure 3). The distribution of study patients was summarized

Table I. Distribution of the study subjects by age and cytological changes.

Variable	20-30 years	31-40	41-50	51-60	> 60	Total
<i>Epithelial cells Status</i>						
LSIL	1	2	5	2	0	10
ASUS	2	1	2	4	2	11
HSIL	2	5	3	1	1	12
Normal	27	75	70	66	29	267
Total	32	83	80	73	32	300
<i>Inflammatory cells infiltrate</i>						
Yes	8	20	26	11	6	71
No	24	63	54	62	26	229
Total	32	83	80	73	32	300
<i>Candida Albicans</i>						
Yes	0	4	3	0	0	7
No	32	79	77	73	32	293
Total	32	83	80	73	32	300
<i>Bacterial infection</i>						
Yes	2	5	5	4	3	19
No	30	78	75	69	29	281

HSIL: High-grade Squamous Intraepithelial Lesions; ASUS: Atypical Squamous Cells of Undetermined Significance; LSIL: Low-grade Squamous Intraepithelial Lesions.

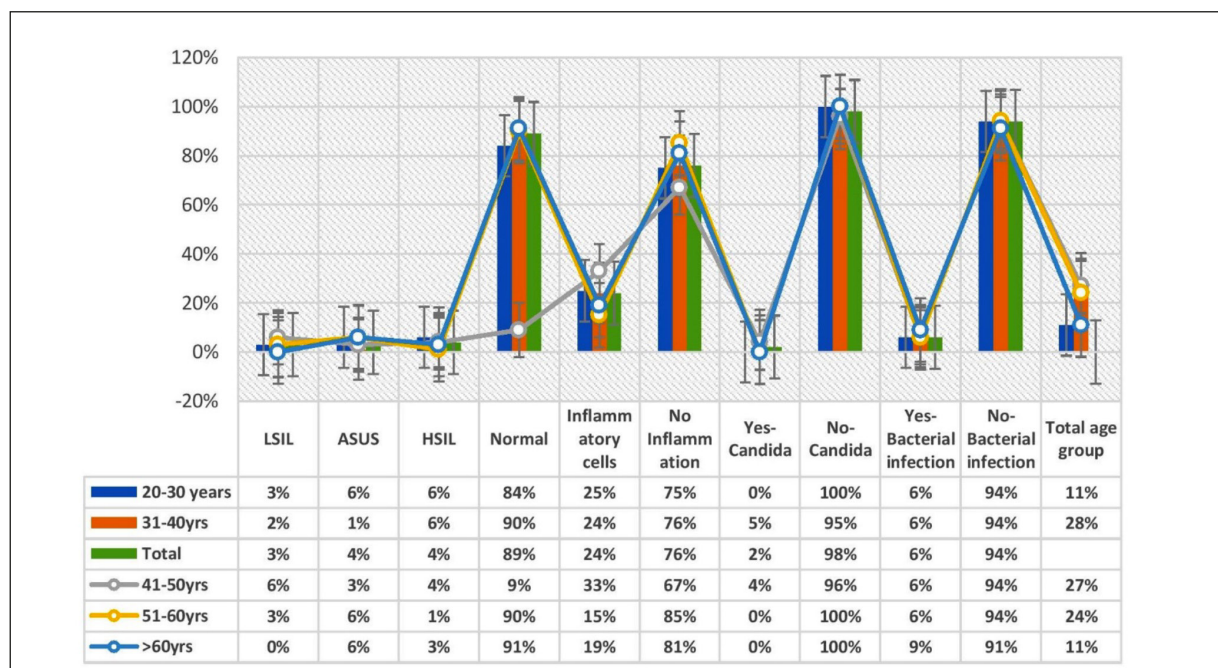


Figure 2. Description of the study subjects by age and cytological changes. HSIL: High-grade Squamous Intraepithelial Lesions; ASUS: Atypical Squamous Cells of Undetermined Significance; LSIL: Low-grade Squamous Intraepithelial Lesions.

by cytological alterations, infections, and hormonal conditions. Inflammatory cell infiltration was observed in 3/10 (30%) of LSIL cases and 3/11 (27.3%) of ASUS cases. Inflammatory cell

infiltrations were observed in 1/10 (ten percent) of LSIL. 1/11 (9%) of the ASUS were estrogen receptor-positive. Only 1/10 (10%) of the LSIL were progesterone receptor positive.

Table II. Distribution of the study subjects by cytological changes and infections and hormonal status.

Variable	LISL	ASUS	HSIL	Normal	Total
Inflammation					
Yes	3	3	0	65	71
No	7	8	12	202	229
Total	10	11	12	267	300
Bacterial					
Yes	0	0	0	19	19
No	10	11	12	248	281
Total	10	11	12	267	300
Candida Albicans					
Yes	1	0	0	6	7
No	9	11	12	261	293
Total	10	11	12	267	300
Hormone status					
Estrogen					
Positive	0	1	0	16	17
Negative	10	10	12	251	283
Total	10	11	12	267	300
Progesterone					
Positive	1	0	0	9	10
Negative	9	11	12	258	290
Total	10	11	12	267	300

HSIL: High-grade Squamous Intraepithelial Lesions; ASUS: Atypical Squamous Cells of Undetermined Significance; LSIL: Low-grade Squamous Intraepithelial Lesions.

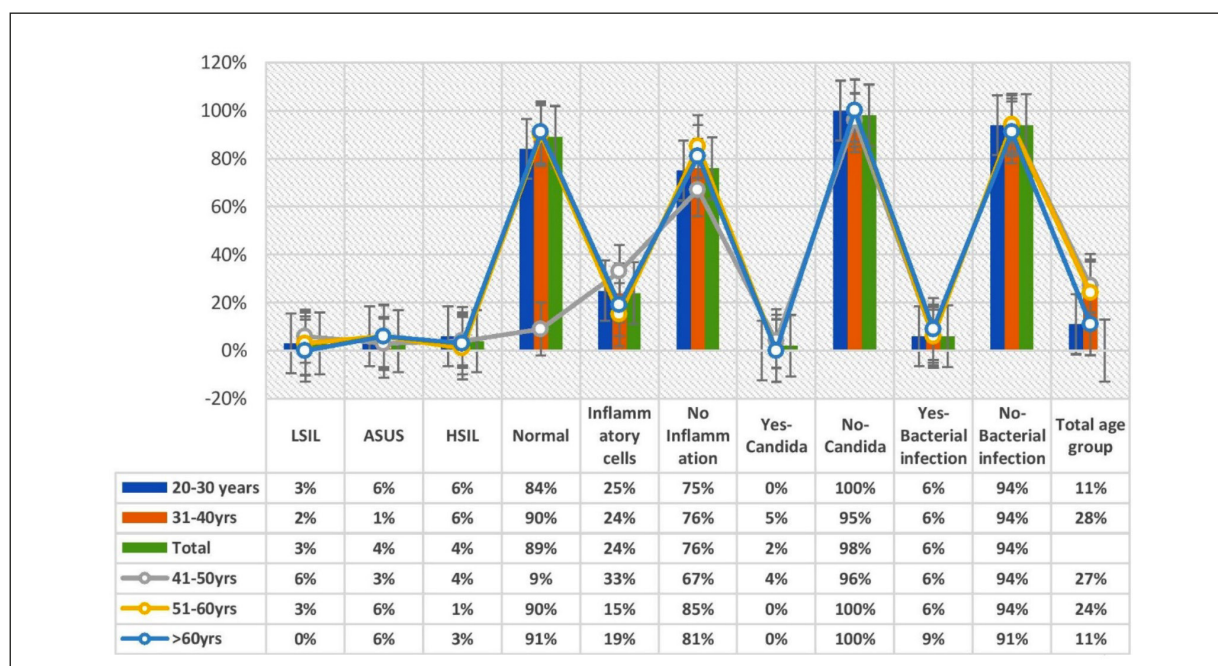


Figure 3. Description of the study subjects by cytological changes and infections and hormonal status. HSIL: High-grade Squamous Intraepithelial Lesions; ASUS: Atypical Squamous Cells of Undetermined Significance; LSIL: Low-grade Squamous Intraepithelial Lesions.

Discussion

In this group of women, cytological proliferative alterations are seen 11% of them. However, a variety of circumstances, such as the prevalence of HPV, multi-sexual activity and barriers, and the prevalence of comorbid conditions that go along with it, affect the prevalence rates of cervical cytological atypia. In one study, 31 women were examined, and 25.8% had cervical cytological atypia, including 12.4% LSIL, 6.2% ASUS, and 3.1% HSIL⁹. According to a meta-analysis, the overall prevalence rates of HPV subtypes 16 and 18 were 4.4% and 2.8% in women with normal cervical smears, 12% and 4.4% in women with ASUS cervical cytological atypia, 14.5% and 10% in women with LSIL cervical cytological atypia, and 31.9% in women with HSIL atypia, respectively¹⁰. Another study from Saudi Arabia found a substantially lower prevalence of cervical cytological atypia (2.4%) than we did in this study. The study found LSIL atypia in 36.8% of the cases, ASUS atypia in 15.9%, and HSIL in 5.3% of the cases¹¹.

According to the results of our investigation, HSIL had a larger percentage than ASUS, LSIL, and then HSIL –36.4%, 33.3%, and 30.3%, respectively. Regardless of the presence or absence

of HPV, a study¹² that examined data from Saudi Arabia on cervical epithelial abnormalities by cytology noted an escalating trend of aberrant cervical epithelial growth in recent years, notably glandular epithelial abnormalities. Cervical epithelial abnormalities, on the other hand, may be influenced by a number of variables that work together to cause cervical precancerous and cancerous lesions. A Saudi Arabian study found that family history [OR 95% CI = 4.26 (1.433-12.4)], vaginal bleeding [OR 95% CI = 3.959 (1.272-12.318)], hypertension [OR 95% CI = 4.554 (1.606-12.912)], and atypical cervical cytology [OR 95% CI =13.985 (5.108-38.284)] all increased the risk of cervical cancer¹³.

According to the current study’s findings, HSIL is becoming more common in the age groups 31-40 years (41.7%), 41-50 years (25%), and 20-30 years (16.7%). On the other hand, ASUS was most common in those aged 51-60 years (36.4%), whereas LSIL was most common in people aged 41-50 years (50%). Abnormal cervical pap smears are highly correlated with middle age and are frequently related to a woman’s degree of sexual activity¹⁴; these abnormalities are significantly associated with concealed high-risk human papillomavirus (HR-HPV) infection. According to one study¹⁵, HSIL in the age group 21-29 years was

10.54%, whereas it was 19.85% in the age group 40-49 years. Furthermore, human papillomavirus type 16 (HPV16) and 18 were linked to more than 15% of HSIL, with the age range 40-49 years accounting for 48.5%. Based on a recent Saudi study¹⁶, the prevalence of HR-HPV is relatively low in Saudi Arabia, and HR-HPV infection is more common among elderly Saudi women.

HPV DNA testing is now used as the primary cervical cancer screening method in the majority of high-income countries, and it has been shown to improve screening service quality. However, in low-income nations, this is not currently the case. When HPV DNA testing is integrated into cervical cancer screening utilizing triage with visual inspection with acetic acid (VIA), HIV status should be taken into account when considering future follow-up¹⁷.

In this study, 23.7% of the women had inflammatory cell infiltrations. Inflammatory cells in a cervical smear may suggest abnormal cervical epithelial cells, such as cervical intraepithelial neoplasia (CIN) or cervical cancer. Some stimuli, such as CD68 and macrophage-specific markers, activate these inflammatory cells¹⁸.

Infection with *Candida Albicans* was identified in 2.3% of the women. *Candida Albicans* morphologic evidence can be seen in cervical smears; however, certain infections can only be found in fungal culture¹⁹. It has been demonstrated that fungal and bacterial infections are widespread in the cervix of CIN patients, independent of CIN grade²⁰.

In 15.8% of patients, cytological evidence of bacterial infection was found. Cervical bacterial infection is frequently linked to CIN, especially CIN III, and precancerous lesions. Cervicovaginal bacterial and fungal species are associated with the host epithelial microenvironment and may be involved in cervical neoplasia²¹. Furthermore, several bacteria have been associated with the primary histological forms of epithelial ovarian cancer, including serous ovarian cancer and ovarian clear cell carcinoma^{22,23}. The current study found no link between CIN and estrogen and/or progesterone status.

However, the current study's findings may highlight the importance of implementing cervical screening programs, which may be required to begin HPV vaccination. Saudi women are less likely to accept Pap tests. This could be linked to social stigma, a lack of awareness, and ongoing cervical prevention and early detection efforts²⁴.

Conclusions

The prevalence of CIN has been growing in Saudi Arabia in recent years, implying a probable involvement in the spread of HPV. Most CIN cases associated with middle age point to HPV infection at a younger age. There is no link between estrogen and/or progesterone levels and CIN.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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Ethics Approval

The proposal for the current study was authorized by the Ethical Committee at the College of Medicine, University Hail, Saudi Arabia. We confirm that all techniques were carried out in conformity with the relevant rules and regulations (HREC 00130/CM-UOH.04/20). We confirm that we followed the Helsinki Declaration criteria.

Informed Consent

Before the interview, each participant provided informed consent.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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