Changing pattern of epithelial cell abnormalities using revised Bethesda system

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ABSTRACT

Objective(s): In developing countries and worldwide cervical cancer is an important cause of female mortality. Reports describing the frequency and pattern of abnormal Pap smears in Saudi Arabia, using the revised Bethesda system (RBS) are very few. The current study was conducted to explore the changing pattern of epithelial cell abnormalities (ECA) detected in Pap smears (PS) in females of the Western region of Saudi Arabia at King Abdulaziz University Hospital, Jeddah using the RBS.

Materials and Methods: A retrospective study was designed to review all the PSs from the archives of Cytopathology Department at King Abdulaziz University Hospital, starting from January 2000 to October 2012 using RBS. Cytological aspects of PSs were reviewed with age distribution.

Results: Of the 15805 PS, 84 (0.53%) unsatisfactory smears were excluded. There were 2295 cases (14.52%) with ECA. In the abnormal squamous cell category the distribution of lesions was as follows: Atypical squamous cells of indeterminate significance (ASC-US) were 7.1%; atypical squamous cells, cannot exclude high squamous intraepithelial lesion (ASC-H) were 1.08%; low grade squamous intraepithelial lesion (LSIL) including human papillomavirus was 2.2%, high grade squamous intraepithelial lesion (HSIL) was 0.8% and high grade squamous intraepithelial lesion with suspicious invasion was 0.06% smears. The mean age (MA) incidence was 39,43,45,46 and 45 years respectively.

Conclusion: The percentage of abnormal PS is increasing (14.52%) over the last decade. This increase is evident by different studies conducted across Saudi Arabia. Under present circumstances the need for mass screening.

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Introduction

The worldwide incidence of cervical cancer is 15.3 / 100,000 women per year (1). Cancer of the cervix uteri is the second most common cancer among women worldwide, with an estimated 529,409 new cases and 274,883 deaths in 2008 (1). About 86% of the cases occur in developing countries, representing 13% of female cancers. The cause of this cancer is attributed to human papillomavirus in 99% of these cases (2). Worldwide, mortality rates of cervical cancer are substantially lower than incidence with a ratio of mortality to incidence to 52% (1). The highest incidence rates are reported from Eastern and Western Africa at 35.4% and 33.7% respectively. In the developing countries, cervical cancer is the most deadly of all cancers (3). There is however marked variation in the frequency of cervical cancer in the developing countries owing to the differences in screening programmes and the prevalence of risk factors (3). The lowest reported incidence rates are from Middle East and are among Muslims and Jews, compared to other religious groups (1, 3).

In Saudi Arabia the most recent estimates from cancer registry 2007 report indicate that cervical cancer ranks as the 5th most frequent cancer among women in between 30 - 44 years of age representing an incidence of 3.1% (4). Data is not yet available on HPV burden in the general population of Saudi Arabia. The overall age-standardized incidence rate is 2.2/100,000 with highest incidence of 8.1% in age group of 60-64 years. The age-standardized mortality rate is 0.8 /100,000 in women population (1). The highest incidence is reported in Jouf region with 5.6% followed by Hail region 3.5%, Najran 3.2%, Al Baha 2.2 % and the lowest in Jazan and Mekkah with 2.2% (4). There are no reported rates for other regions such as Riyadh, Eastern and Northern region, Madinah, Qassim, Asir, and Tabuk (4). Cervical intraepithelial neoplasia incidence in

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Saudi Arabia is not registered because there are no well-developed screening programs nationwide. However recent studies from different regions of the Kingdom show slight increase in the prevalence of epithelial cell abnormalities (ECA) in Pap smears (PS) (5-8). A study from Maternity and Children's Hospital, Saudi Arabia reported that cervical cancer among Saudi nations represents 33.5% of all gynecological malignancies (9).

The incidence and mortality of cervical cancer has declined dramatically ever since the introduction and wide spread utilization of PS screening test especially in countries with well-established cervical screening programs such as USA where it is considered the sixth most common cancer in women (10). Cytological evidence of all ECA can be easily, safely and economically obtained by the preparation and examination of cytology smears (11). Identification of preventable, precursor lesions by cytology could prevent further progress of the disease by simple therapeutic procedures and continued surveillance (11) and as such emphasizes the need for early detection of cervical intraepithelial neoplasia. Over the years, the original PS classification system has been revised in response to our growing knowledge of cervical cancer precursors and our understanding of the role of HPV cervical carcinogenesis (12). Standardization of PS reporting using the Bethesda system (RBS) has unified various overlapping and confusing terminologies (12) and has overcome the consequences of inter-observer subjectivity in interpretation and application of these terminologies to some extent.

The current study was conducted to explore the changing pattern of epithelial cell abnormalities (ECA) detected in PS in females of the Western region of Saudi Arabia at King Abdulaziz University Hospital, Jeddah using Bethesda system (RBS). We also compared our results to other recent studies from Arab and Asian countries.

Materials and Methods

A retrospective study targeting 15805 PSs performed during January 2000- October 2012 at King Abdulaziz University Hospital (KAUH) Jeddah was performed. The data was collected by conducting a computerized search through the cytopathology archives at KAUH Jeddah. This study included both Saudi and Non Saudi females, since KAUH is a teaching hospital of King Abdulaziz University Medical School. It is the only hospital at Jeddah providing treatment to all nationalities thereby providing the unique opportunity of studying cross cultural variations and similarities .The data was filtered indicating the following essential parameters: Date of undergoing PS, personal identity (medical record number, age, sex etc) and relevant clinical information (indicated for

routine or for gynecological symptoms).The study included all PSs performed at KAUH during the period of study. Repeated smears from the same patients with insignificant cytological changes and carrying the same diagnosis and unsatisfactory PSs were excluded from this study.

Collected data were arranged in Microsoft Excel format and were used for statistical analysis. The data were rechecked manually to avoid duplications. Statistical Package for the Social Sciences, version 15.0 (SPSS Inc., Chicago, IL, USA) program was used for analysis. Descriptive and frequency statistics were obtained for the variables studies. The total number of pap smears examined (TSE) after excluding unsatisfactory smears and the total number and percentages of abnormal pap smears (TAPS) were analyzed and correlated with the patient's age. The procedures followed in the present study were approved by and were in accordance with the ethical standards of the hospital ethical committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

Initially all PS were collected to identify the sufficient smears. A smear was considered sufficient when it was appropriately labeled having adequate sampling of the endocervical transformation zone with optimal preservation of the cells. All sufficient cervical cytology smears were reviewed and reclassified according to the 2001 RBS of PS reporting (12) (Table 1), into negative for intraepithelial lesions, positive for epithelial cell abnormalities (ECA) and sub classified into specific categories. Quality indicators such as inflammation, hemorrhage and reactive cellular changes associated with repair, squamous metaplasia, follicular cervicitis, atrophy and organisms were also identified.

In cases of ambiguous diagnoses, the cytology slides were reviewed on a multi-headed microscope by a senior cytotechnologist and two pathologists to resolve the discrepancy.

Results

A total of 15805 cervical PSs were retrieved and reported in the Department Of Cytopathology at KAUH, Jeddah in the period from January 2000 to October 2012. The study identified 15721 (99.46%) sufficient smears and 84 (0.53%) unsatisfactory smears (Table 1). The latter cases were excluded from further analysis. Of the 15721 cervical PSs, 12797 (81.15%) smears were reported as negative for ECA while 2295 (14.52%) were diagnosed as positive for ECA. The smears positive for squamous and glandular cell abnormalities were further sub classified according to the 2001 RBS (12) as shown in Table 2. Unsatisfactory smears were 0.53 % among other quality indicators and were withdrawn for further analysis as they were beyond the scope of

Table 1. Overall distribution of Pap smear categories using revised Bethesda system at KAUH,Jeddah ,Saudi Arabia

Total number of Pap smears	N=15805	%
Negative for intraepithelial lesion	12797	81.15%
Positive for epithelial cell abnormalities	2295	14.52%
Quality indicators		
Inflammation	246	1.5%
Hemorrhage	160	1.01%
Unsatisfactory	84	0.53%
Reactive cellular changes		
Associated with repair	30	0.19%
Associated with squamous metaplasia	54	0.34%
Associated with follicular cervicitis	2	0.01
Associated with organisms	42	0.26%
Atrophy	92	0.58%

Table 2. Distribution of squamous and glandular epithelial cell abnormalities using revised Bethesda system at KAUH, Jeddah, Saudi Arabia

E C A's	N	TAPS% N=2295	TPSE % N=15721	MA Incidence
Squamous cells	1796	78.25%	11.42	45.1
ASC-US	1128	49.1	7.1	39
ASC-H	170	7.4	1.08	42.7
LGSIL	352	15.3	2.2	44.8
HGSIL	126	5.5	0.8	46
HGSIL with suspicious invasion	11	0.47	0.06	45
SCC	9	0.39	0.05	48.4
Glandular cells	497	21.65	3.1	50.42
AGC NOS	381	16.6	2.4	46
AGC favoring neoplastic	15	0.65	0.09	52.57
Adenocarcinoma NOS	5	0.21	0.03	54
Adenocarcinoma endometrial	2	0.08	0.01	56
Adenocarcinoma endocervical	1	0.04	0.006	49
Malignant NOS	2	0.08	0.01	54

ECA: Epithelial cell abnormalities, ASC US: Abnormal squamous cells of undetermined significance, ASC-H: Abnormal squamous cells-high grade cannot be excluded, LGSIL: Low grade squamous intraepithelial lesion, HGSIL: High grade squamous intraepithelial lesion, MA: Mean age, AGC NOS: Atypical glandular cells not otherwise specific, AGC: Atypical glandular cells, SCC: Squamous cell carcinoma, TAPS: Percentage of total abnormal pap smears, TPSE: Percentage of total pap smears examined

Table 3. Analytical comparison of epithelial cell abnormalities in Pap smears using revised Bethesda system in recent studies from KAUH Jeddah, Saudi Arabia

Categories	Present Study	Study 2009 (13)	Study 2006 (7)
Total no. of cases N	15805	7235	5132
ECA	2295 (14.52%)	1254(17.3%)	243 (4.7)
ASC-US %	7.1%	9.3%	2.4%
ASCH	1.08%	0.8	*
LGSIL %	2.2%	2.7%	0.6%
HGSIL%	0.8%	0.9%	0.4%
SCC%	0.05%	0.06%	0.1%
AGC-NOS %	2.4%	3.2%	1.1%
AGC favor neoplastic%	0.09%	0.9%	0.06%

this study. We compared our results to the past studies from our institution in Table 3 and other recent studies from the Saudi Arabia, Arab world and other international studies in Table 4.

In comparison to the previous studies from our institution (Table 3) (7, 13) there is a significant increase in the number of smears positive for ECA from 4.7% in the study that was conducted in year 2006 to 17.3% in the study that was conducted in 2009. In the present study this number is 14.52%.

Squamous cell abnormalities have also increased from 3.5% in the year 2006 to 17.3% in 2009 to 11.42 % at the time of this study and glandular cell abnormalities from 1.10 in the year 2006 to 3.35% in 2009 to 3.1% at the time of the study.

Discussion

Cervical intraepithelial neoplasia and invasive cervical carcinoma are less common in Saudi Arabia compared to the Western countries (1, 2, 4, 8, 9) and

Table 4. Summarized distribution of epithelial cell abnormalities in Pap smears using Bethesda system in recent studies from other institutions of Saudi Arabia , Arab world and Asia

Studies	Year	Region	N =	% of ECA	% of Revised Bethesda categories					
					ASCUS	ASC-H	LGSIL	HGSIL	SCC	AGUS
other institutions of Saudi Arabia										
Elhakeem HA *et al* (5)	2005	South Western	2100	7.9	2.76	0.19	1.3	0.66	0.33	*
Abdullah LS (6)	2007	Jeddah	5746	5	1.84	0.10	1.0	0.55	0.37	0.53
Balaha MH *et al* (8)	2011	Eastern	1171	4.95	2.99	0.60	0.09	0.68	0.34	0.09
Al-Jaroudi and Hussain (14)	2010	Riyadh	241	2.9	1.20	0.42	0.84	*	*	0.42
Ahmed A (15)	2012	Eastern	7772	1.3	0.48	0.06	0.19	0.37	0.1	0.2
Present study	2012	Jeddah	15805	14.5	7.1	1.08	2.2	0.8	0.05	2.4
Arab world										
Ghazal Aswad S *et al* (16)	2006	UAE	4055	3.6	2	*	0.77	0.54	0.07	*
Kapila K *et al* (17)	2006	Kuwait	86434	4.22	2.2	*	1	0.2	0.05	0.8
Abul el-All HS *et al* (18)	2007	Egypt	5453	7.8	34.4	*	41.0	5.2	0.5	15.3
Present study	2012	Saudi Arabia	15805	14.52	7.1	1.08	2.2	0.8	0.05	2.4
Asia										
Banik U *et al* (19)	2011	Bangladesh	1699	8.18	0.18	*	6.36	1.18	*	0.12
Bal MS *et al* (20)	2012	India	300	5	0.3	*	2.7	0.7	1	*
Bukhari MH *et a l* (21)	2012	Pakistan	1000	10.2	1.01	*	4.6	2.2	1.42	0.4
Present study	2012	Saudi Arabia	15805	14.52	7.1	1.08	2.2	0.8	0.05	2.4

ECA: Epithelial cell abnormalities* - Figures not stated in the studies;
+ -- Figures were calculated excluding the insufficient cases (18) from the total number (1000) to get appropriate statistical comparison

early stages of cervical cancer are even less (4-9). The original PS reporting system contained several overlapping terminologies and in order to present a more comprehensive system it was updated in 2001 (12). The observations in the present study imply an overall increase in the number of abnormal PS with a relative and significant increase in squamous cell abnormalities especially abnormal squamous cells of undetermined significance (ASC-US). The published data of 2009 (13) showing a marked increase in ECA, squamous and glandular abnormalities raised concerns and led the cytopathologists at our institution to strictly adhere to the criteria for each diagnostic category thereby addressing issues of over interpretation with obvious improvement. This also led to study larger number of PSs to revisit the past observations. The current study, however still seems to revalidate and compliment the previous studies as regards the increase in number of ECA. Although the comparison of the previous studies are not valid due to overlap of data but it is a crude method to indicate that there is a continuous increase in the number of abnormal PSs. The increase in the total number of PSs however is explained by a number of reasons, such as the increasing awareness among the females in the Western region of Saudi Arabia regarding the importance of cervical cancer screening through campaigns from Cervical Cancer Scientific Chair in Jeddah area.

If we assess studies from other parts of Saudi Arabia (5, 7, 8, 14, 15) (Table 4) using BS in diagnosis of PSs, we notice a gradual increase in the incidence of ECA especially in the squamous cell category is being reported. Furthermore studies from the Arab world have also reported a significant increased trend in ASC-US and AGUS over the years (Table 4) (16-18). Abul el-All et al study from Egypt reported ECA of 7.8%, ASC-US of 34.4%, Low grade squamous intraepithelial lesion (LGSIL) 41%, High grade squamous intraepithelial lesion (HGSIL) 5.2%, Squamous cell carcinoma (SCC) of 0.5% and AGUS of 15.3% (18). Most of these studies indicate that there has been a definite increase in the prevalence of ECA in the current years. It is not clear whether it is due to a high level of awareness of the targeted population within these studies or it is related to the trend of acquiring a more open life style .These issues need further investigation and assessment. However the lowest reported incidence of ECA is from the eastern region, Al Khobar, wherein ASCUS detected was 0.48%, abnormal squamous cells-high grade (ACS-H) was 0.06%, LSIL was 0.19%, HSIL was 0.37%, SCC was 0.10% and AGUS was 0.25% (15).

Other recent studies from Asia report an ECA between 5-10.2% (19-21) (Table 4) and although the ASC-US category in these studies is within an acceptable range of 0.3-1 % but they show a higher incidence of LGSIL and HGSIL which could be partly explained by the variation in the population under consideration. Also it could be partly due to

subjective variation in application of ASC-US criteria early in these lesions. There are 2-3 million ASC-US, 1.25 million LGSIL and 300,000 HSIL in USA (22). Approximately three fourth of all cervical cancers in USA are squamous cell and the remaining are adenocarcinomas (3). HPV 16 and 18 account for approximately 68% of squamous cell carcinomas and 83% of adenocarcinomas. This finding warrants the need for incorporating HPV-DNA testing in the screening programme (3, 23). In most developed countries the current prevalence of cytological abnormality is between 5-10% (23, 24). HPV L1 capsid protein and hTERC gene (human telomerase RNA component) may serve as markers for the early diagnosis and prediction of cervical lesions. The increase in L1/hTERC ratio reflects the progression of cervical lesions to a certain extent (24).

Currently there is no organized cervical screening programme in Saudi Arabia (5) so information regarding the rate of PS testing in Saudi Arabia remains obscure. In the Arab world a screening rate of 14.3% (25) is reported. In a study from USA the prevalence of HPV16 was 13.3% among ASC-US, 23.6% among LSIL and 60.7% among HSIL (26). In the developing countries, we, however face an important challenge to apply HPV-based technology (3). HPV-DNA testing has higher sensitivity but lower specificity than thin layer pap screening (3). The age-specific incidence of cervical cancer peaks around the age of 40 years, which suggests that the specificity and efficacy of HPV screening is maximal when it is performed on women between 30 and 40 years of age (27). Virus-like particles (VLPs) containing HPV structural proteins are being used as vaccines and can induce genotype specific virus neutralizing antibodies for preventing HPV infections (28, 29). However, HPV infection as the current concept of the etiology of cervical cancer lacks adequate validation in terms of effective screening especially in Saudi Arabia. No population based data is available to indicate the prevalence of HPV cervical infections or their age specific incidence in Saudi Arabia. In Saudi population, co factors found contributory to cervical cancer are smoking and use of oral contraceptives (1). A recent study emphasizes the need to include patients more than 45 years of age for PAP screening (19) in developing countries suggesting that a negative history before the age of 50 years does not necessarily exclude the risk of having cervical cancer.

The limitation of the current study is that the diagnosis of ASC-US is much over the acceptable range although the ASC-US/SIL ratio of 2.3 is within the limit (30, 31). The increased trend at our institution is partly explained by the fact that the criterion for ASC-US and LSIL is quantitative and cytopathologists prefer to use the diagnosis of ASC-US rather than LSIL. The possible rationale behind this is that patients with ASC-US

diagnosis are treated for inflammation followed by a repeat PS within 6 months, while patients with LSIL diagnosis are subjected to colposcopy and biopsy, especially in the absence of routine HPV testing in ASC-US or SIL diagnosis.

Conclusion

There is evidence indicating increase in the number of abnormal PSs over the last decade. This increase is supported by different studies conducted across Saudi Arabia. It may not be a significant increase to call for nationwide mass cervical cancer screening program, which is a continuous process that requires allocation of huge resources that should be justifiable. It should, however, be obligatory for all women in the age of 35-55 to undergo PSs and those with abnormal EC findings to have a test for HPV subtype.

The data from all provinces should be evaluated thoroughly and if the results indicate considerable health problem with a definite increase in the incidence over successive years, proper documentation of such data should be enforced for more comprehensive analysis and decision on mass screening programme nationwide.

Declaration of interest

The authors state that this study or this manuscript has no conflict of interest.

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