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ORIGINAL ARTICLE

The Association of Relative Risk of High Risk HPV with Cervical Abnormalities in Bahrain

Khalid Ahmad Al-Sindi[1], Mulazim Hussain Bukhari[2], Ebtisam N. Buali[3], Suhail I. Baithun[4], Duaa A. Abduljabbar[5], Mohamed A. Alhamar[6], Mandeep Bedi[7]

[1]Professor of Pathology, King Hamad University Hospital, Building 2345, Road 2835, Block 228, Busaiteen, P. O. Box 24343, Kingdom of Bahrain, [2]Professor of Pathology, Punjab Medical College, Faisal Abad, Pakistan, [3]Cytotechnologist, King Hamad University Hospital, Building 2345, Road 2835, Block 228, Busaiteen, P. O. Box 24343, Kingdom of Bahrain, [4]Histopathologist, King Hamad University Hospital, Building 2345, Road 2835, Block 228, Busaiteen, P. O. Box 24343, Kingdom of Bahrain, [5]Senior House Officer – Internal Medicine, King Hamad University Hospital, Building 2345, Road 2835, Block 228, Busaiteen, P. O. Box 24343, Kingdom of Bahrain, [6]Senior House Officer – Histopathology, King Hamad University Hospital, Building 2345, Road 2835, Block 228, Busaitee n, P. O. Box 24343, Kingdom of Bahrain, [7]Mandeep Bedi Pathologist and Department in charge Kolkata Police Hospitals, Kolkata, West Bengal, India

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Khalid Ahmad Al-Sindi

ABSTRACT

Background: The study was conducted to see the association of cervical abnormalities in relation to high risk Human Papilloma Virus (HR-HPV) infection at King Hamad University Hospital, Bahrain.

Materials and Methods: It was a retrospective cohort study completed in 3 years at King Hamad University Hospital, Department Pathology Lab and other private Hospitals of Bahrain. Patients of Obstetrics and Gynecology out patient's clinic and other requesting private hospitals were included in this study.A non-probability purposive sampling technique was used for this retrospective review of 160pathology reports and HPV cervista reports. Data was collected from I-Seha and patients Al-care, and was transferred and assessed SPSS-version 22.

Results: There were 160 cases in total, who were examined for HPV–HR DNA using Cervista molecular testing. There were 73 cases were Positive for HPV and 87 cases negative for HPV. The minimum age of patiesnt's was 20 years while the max was 70 years. The mean age was 42.5 years. HR-HPV was detected in (100%) all cervical HSIL cases and in 71% of LSIL cases. Cervical intraepithelial lesion CIN2/3+ was significantly associated with HR-HPV positive cases. Compared to HPV positive cases, here was no cervical intraepithelial lesion (CIN) of any grade found in HR_HPV negative cases. There were only four cases with LSIL found to be R-HPV positive, which may be associated with Low-risk HPV infection.

Conclusion: There was strongest association of cervical neoplastic lesions with high risk HPV to control.

INTRODUCTION

Cervical cancer is still the commonest malignancy among female, with 528,000 new cases and 266,000 deaths each year worldwide.[1]

For the effective screening method of cervical lesions, the Papanicolaou cervical cytology is used to detect premalignant and malignant conditions. It is included in the screening program for evaluation of all patients with abnormal cervical smears in developed countries. This test is adopted because it is a simple and cost-effective technique for the detection of early changes in the cervical epithelium. Unfortunately, many developing countries lack the ability to carry out widespread Pap screening.[3-6]

According to Centers for Disease Control and Prevention, Cervical cancer mortality in the United States has decreased over the last five decades by 70%, largely as a result of the introduction of the Papanicolaou (Pap) test It is now understood that persistence infection with high risk HPV and immunosuppression is

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Corresponding Author:
Mulazim Hussain Bukhari, MBBS, DCP, MPhil, FCPS, PhD, Prof of Pathology HOD, Punjab Medical College Faisal Abad, Pakistan,
Phone: +92-3008414743. E-mail: mulazim.hussain@gmail.com

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necessary for the development of cervical cancer and its precancerous lesion. Epidemiologic case series have shown that nearly 100% of cervical cancer cases, test positive for HPV. According to Bethesda system 2012, HPV-related squamous intraepithelial lesions(SIL) which is diagnosed by PAP test cytology are divided into the low squamous intraepithelial lesion (LSIL) encompasses the histological diagnosis of cervical intraepithelial neoplasms 1 (CIN1), and High grade squamous intraepithelial lesion (HSIL) encompasses cervical intraepithelial neoplasia (CIN2 and CIN3).[7]

Many techniques have been introduced to detect DNA and RNA in high risk HPV infected cells. The most popular techniques, are DNA amplification using Hybrid capture 2, Real time Polymerase chain Reaction using cobas HPV test and GeneXpert,a messenger RNA detection technique using Aptima HPV assay,and Signal amliphication Invader technology using Cervista test. The study was conducted to detect high risk HPV using Invader molecular technology and to see any association of different known risk factors for neoplastic (premalignant and malignant) cervical Lesions.

MATERIALS AND METHODS

Study Design

It was retrospective cohort study.

Study Universe and Study Population

Sample size was calculated according to the prevalence of HPV and 160 cases were selected out of 7500 Papanicolaou (Pap) smears of cervical cytology, who attended the Gynecology Clinics of King Hamad University Hospital Bahrain,and other requesting private hospitals from October 2012 to October 2014.

Data Collection

Cases of cervical abnormalities were selected according to the Bethesda System.[2] These cases of cervical lesions were followed for biopsy (punch biopsy, conization and hysterectomies).

The study was conducted according to the standardized protocol of the International Agency for Research on Cancer (IARC) HPV Prevalence Surveys.[8] Ethical approval of ethical principles for medical research for human subjects for the study was received from the Review board of ethical committee of the University, Bahrain.

Inclusion Criteria

1. All cases tested for HR-HPV using cervista HR-HPV molecular technique from the period October 2012 until October 2014 that have vaginal and cervical (THINPREP) Pap smear or Histologic result.
2. Cases suspicious for Cervical Pathology on Pap smear categorized by Bethesda System.
3. Woman with age 21 to 65 year with ASCUC cytology who are tested for HPV to determine the need for referral to colposcopy.
4. Woman age age 30 and older to adjunctively screen for the presence or absence of HR-HPV.
5. Women younger the 21 age who attend the clinic for cinical indications
6. Women older that 65 age with clinical indications.

Exclusion Criteria

1. Virgin females
2. Infective (non specific infections) smears
3. Women under age 30 with normal cervical cytology and no clinical indication.
4. Women older that age 65 with normal cervical cytology and no clinical indication.

High-risk HPV Testing Method

HR-HPV was performed using the below method

Product Name: Cervista HPV HR & Genfied DNA Extraction test.

Manufacturer : Third wave Technologies, Hologic Inc, 502 Rosa Rd, Madison, WI 53719

FDA Approval Date: March 31, 2009.

ThinPrep® Pap Test PreservCyt® Solution PAP test was used.

CervistaTM HPV HR is a qualitative method. It uses the Invader® chemistry, a signal amplification method for detection of specific nucleic acid sequences.

It can detect up to 14 high-risk HPV types includes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.A positive result indicates that at least one of the 14 high-risk types is present in the DNA sample (cervista manuel).

Diagnostic criteria

Papanicolaou tests for all patients were evaluated using manual cytotechnologist review. Papanicolaou tests suspicious for reactive, reparative, or dysplastic changes were reviewed by competent anatomic pathologists. Cytologic findings were classified according to the 2001 Bethesda System terminology for reporting results of cervical cytology. Abnormal cytological lesions were followed by histopathology cervical biopsy, and cervical conization by loop electrosurgical excision procedure (LEEP).

All HR-HPV DNA testing was performed on patients following ACS, SCCP, ASCP, 2012 Guidelines where in

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patients with atypical squamous cells of undetermined significance (ASCUS) cervical cytology results are tested for HPV to determine the need for referral to colposcopy. HPV is also tested in women 30 years and older as a co-testing with cervical cytology to adjunctively screen to assess the presence or absence of high-risk HPV.

Results were categorized as either Cervista HPV HR negative or Cervista HPV HR positive based on Carboxyfluorescein dye (FAM) fluorescent signal that lies either above an empirically derived cut-off value or below it. For each reaction, a positive result is represented by a FAMfluorescent signal that lies above an empirically derived cut-off value. (cervista).[9,10]

Statistical Analysis

Data was entered and analyzed through SPSS version 16 by researcher herself. Quantitative variable like age was presented in form of mean ± SD with respect to type of cancer and year. The qualitative variables like gender, type of cancer etc., were presented in form of frequency tables and appropriate charts.

Simple percentage of the positive lesions was calculated and compared with histopathology reports. The accuracy rate, sensitivity, specificity, positive predictive value, negative predictive value, false positive rate, and false negative rate were calculated by definition. The chi-square test was performed for comparative analysis and the results were considered statistically significant at p-values <0.05. Fisher exact test was used to determine the statistical significance of the difference.

Table 1: Cytology results vs HPV test results

Pap test diagnosis	Prevalence (%)	No. of HR-HPV positive cases	No. of HR-HPV negative cases
No cytology result	1 (0.6)	1	0
UNSAT	4 (2.5)	1	3
Negative	74 (46)	17	57
ASCUS	50 (31)	28	22
LSIL	14 (9)	10	4
HSIL	13 (8)	13	0
ASC-H	2 (1)	2	0
AGC	2 (1)	1	1
Total no. of cytology cases	160 (100%)	73 (46%)	87 (54%)

UNSAT: Unsatisfactory smears, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low grade squamous cell intraepithelial lesions, HSIL: High grade squamous cell intraepithelial lesions, AGCUS: Atypical glandular cells of undetermined significance

RESULTS

There were 160 cases in total, who were examined for HPV–HR DNA using Cervista molecular testing.The minimum age of patients was 20 years while the max was 70 years. The mean age was 42.5 years. There were 73 cases Positive for HPV and 87 cases negative for HPV. There were 23 (31%) Bahraini women out of 73(46%) women with positive high risk HPV. The second most common females were of Philippines women, then Indian and British women comprising of 15 (21%), 07 (10%) and 05 (7%) respectively.

Out of 73 (46%) HPV positive cases, 17(23%) cases were normal showing no cytology problems. 28 (38%) cases were ASCUS. 10 (17%)cases were LSIL, 13 (18%) cases were HSIL, 2 (3%)cases were ASC-H and one cases (1%) was AGC. (Table 1)

On histology follow up, out of 73 cases with positive HPV result, 30 cases have histopathology follow up (41%). From the 30 cases, 5 cases were normal (17%), 8 cases showed koilocytic changes (27%), 6 cases showed CIN1) 20%, 2 cases showed (CINII) (7%), 8 cases showed (CINIII) 27% and I cases showed unsatisfactory result (3.3%). HR-HPV was detected in all cervical HSIL cases and in 71% of LSIL cases. Cervical intraepithelial lesion CIN2/3+ was significantly associated with HR_HPV positive cases (Tables 1 and 2 and Figures 1-3).

Compared to HPV positive cases, 87 cases (54%) were HPV negative. Out of 87 cases 57 cases were normal (66%),

Table 2: HPV-related cervical lesion HPV positivity and histologic correlation

Pap test diagnosis	Prevalence N0=160 (100%) cases (%)	HR-HPV positive rate (%)	Histology follow up (%)							
			No follow up	Inadequate	Normal	CIN or invasive carcinomas				
						Koilocyte only	CIN I	CIN II	CIN III	CA
UNSAT	4 (2.5)	1	4 (100)	-	-	-	-	-	-	-
Negative	74 (46)	17 (23)	71 (69)	-	-	2 (3)	1 (1)	-	-	-
ASCUS	50 (31)	28 (56)	42 (84)	1 (2)	4 (8)	1 (2)	1 (2)	-	1 (2)	-
LSIL	14 (9)	10 (71)	7 (50)	-	-	4 (29)	2 (14)	-	1 (7)	-
HSIL	13 (8)	13 (100)	4 (31)	-	-	-	1 (8)	2 (15)	6 (46)	
ASC-H	2 (1)	2 (100)	0 (0)	-	1 (50)	-	1 (50)	-	-	-
AGC	2 (1)	1 (50)	2 (100)	-	-	-	-	-	-	-
No cytology	1 (0.6)	1 (100)	0 (0)	-	-	1 (100)	-	-	-	-

UNSAT: Unsatisfactory smears, ASCUS: Atypical squamous cells of undetermined significance, LSIL: Low grade squamous cell intraepithelial lesions, HSIL: High grade squamous cell intraepithelial lesions, AGCUS: Atypical glandular cells of undetermined significance

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Table 3: Relative risk with HPV in 160 patients

Risk	Disease present (symptomatic patients) (%)	Disease absent (asymptomatic patients) (%)	Total (%)
Positve HPV-DNA	36 (49)	37 (51)	73 (100)
Negative HPV-DNA	16 (18)	71 (82)	87 (100)

RR (is the ratio of the probability of an event occurring in HPV positive cases in an exposed group to the probability of the event occurring in a comparison, non-exposed group in HPV negative cases)=2.3

showing no cytological or histopathological problems. The ASCUS was seen in 22 (25%) cases. Only 4 cases (5%) were with low-grade abnormalities (LSIL).

Confection with other STD was seen in 2 cases with history of herpes infection in HPV positive cases.

The relative risk was 2.3 (An RR of > 1 means the event was more likely to occur in the HPV Positive group than in the HPV negative group) (Table 3).

DISCUSSION

Cervical cancer is the 4th commonest female malignancy in the world. A wide range of regional diversity in the incidence of cervical cancer in different parts of the world is evident from several reports.[11]

In Bahrain, there have been no studies showing the extent of the incidence of this virus in the community, perhaps because cervical cancer is not frequently seen in the country. Unlike the rest of the world, cervical cancer is considered locally the eleventh most prevalent cancer among women.[12]

In addition, a high prevalence of HPV was in more other women working in Bahrain (70%) as compared to the natives (30%). It was less seen in Bahraini population because HPV is considered to be a sexually transmitted disease and Arab society is known to be a conservative one when it comes to adult sexuality, which is supposed to exist only after marriage.Among other non-Bahraini workingwomen Philippians were bearing more PHV infections and cervical abnormalities (21%) as compared to another and then comes Indians and British women which comprise 10% and 7% respectively.

We used Cervista techniquesfor the detection of this high risk HPV. Our study showed a relatively high prevalence of cervical abnormalities with HPV infection among women residing in Bahrain. For traditional reasons, only married, divorced, and widowed females were registered in this cohort study. All of them visited Gynecology Clinics of King Hamad University Hospital Bahrain, and other requesting private hospitals for routine and follow up gynecological examination.

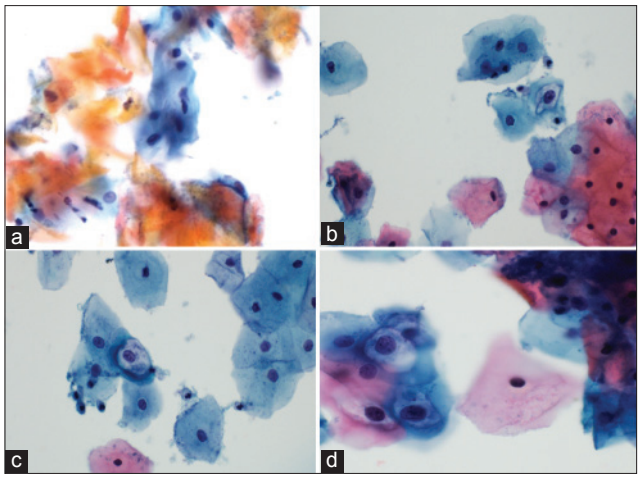

Figure 1: ASCUS: (a-d) Nuclear changes are more marked than reactive, less than LSIL (Pap stain 10x, 20x and 40x)

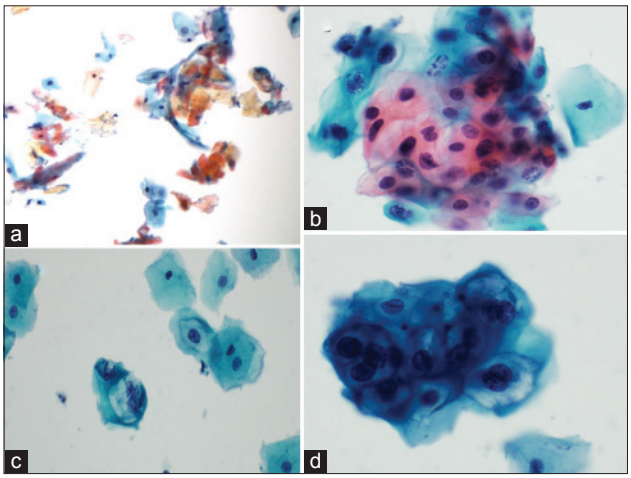

Figure 2: Microphotograph of LSIL: (a); The cells appearing predominantly single, in flat sheets or cobblestone arrangements (Pap stain 10x), (b-d); Nuclei are hyperchromatic enlarged 3-4 times the size of a normal intermediate cell nucleus with irregular nuclear membrane with perinuclear halo in some cells (Pap stain 20x and 40x)

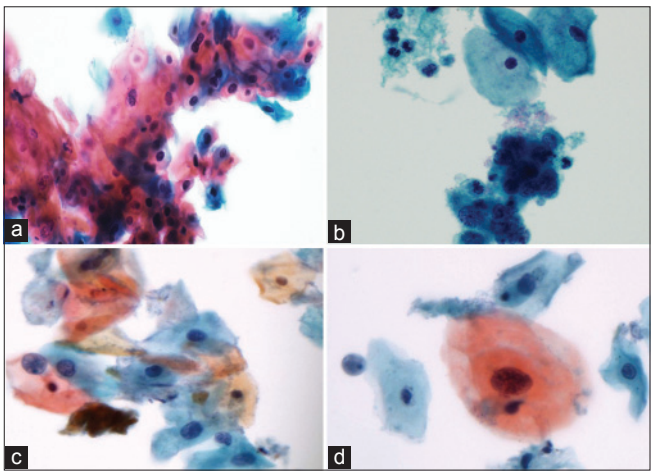

Figure 3: Miceophotograph of Papanicolaou stain, A-D HSIL cells with nuclear enlargement, irregular nuclear outlines and coarse chromatin. HSIL (5x, 10x, 20x, 40x)

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In this general screening, we found koilocytic lesions, ASCUS, AGS, LSIL and HSIL in our HPV positive and negative cases.

There were 160and minimum age of patients was 20 years while the max was 70 years. The mean age was 42.5 years. Abnormal cytology smears were followed by Histopathology.

In different studies the age trends of cervical lesions seemed to high at a relatively younger age in North America (<30 years), compared with 25 to 40 years in Europe and Middle East, Africa, Asia, and Central and South America. Age patterns of low-grade lesions generally declined after a peak in the younger age groups (20-30 years).[11] Our findings shows that HPV prevalence is age dependent with the highest rate among women age 30-39 (42%)and the lowest rate among women age 50 and above (14%). Our findings are inconsistent with Dunne et al, that shows decreasing in HPV prevalence among women age 50 and above to 39% (Dunne). However, the highest rate of HPV prevalence in Dunne study occurs among women age 20-24 which comprise 54% of total cases, while with our study, the highest rate occurs among women age 30-39. This can be due the conservative society of Muslims and Arabs when it comes to adult sexuality, which normally start after marriage and with the average age of 27.

In our study, HSIL is encountered in 8% of all cytology cases. HPV testing is not necessary for women with HSIL. However, HPV test was performed according to ASCP guidelines which approved routine Pap and HPV contesting for age group 30 and above. All women with HSIL have been tested positive for HR-HPV.Only 61% cases of HSL were followed up by biopsy and histology. Out of these cases 22% showed CIN2 and 67% showed CIN3. As persistence, high risk HPV is the major cause of cervical cancer and its precancerous lesions it makes sense to introduce HR-HPV testing in the management guidelines for cervical abnormalities to identify women most at risk.

The relative risk was 2.3; 95% confidence interval (95%CI) means the event of cervical abnormalities were more seen in the HPV Positive patients as compared to the HPV negative patients. This showed a stronger association of these lesions with HPV infection. Out of HPV negative cases, most of the cases were normal, showing no cytological or histopathological problems.

Most LSIL 80% are associated with HR-risk HPV, while 20% of LSIL cases are associated with LR-HPV infection. Almost all HSIL cases and cervical cancer are associated with HR-HPV.

Only mild degree cervical abnormalities were seen in this group (LSIL) which could be related to Low risk HPV infection. No high grade cervical lesions were seen in our study in HPV negative patients. The findings of association of high risk HPV with precursor cervical lesions for cervical cancer are consistent with Boldrini etal (2014) and others. The prevalence is low as compared to Al-Awadhi, a Kuwaiti study.[13]

Confection with other STD was seen in 2 cases with history of herpes infection.

Out of HPV Positive cases, most of the patients showed disease (ASCUS, LSIL to HSIL and AGC). Total abnormal cases with HPV were 74% while 2 cases (3%) were inadequate with unsatisfactory findings and even with our neighbor country Saudi Arabia.[14-16]

CONCLUSION

No High grad abnormalities were associated with Negative HVP result. The precancerous cervical abnormalities were associated with high risk HPV.

Authorship

Khalid Al Sindi was the principal researcher and collected the data, deigned the research Protocol and helped in designing the research protocol, MHB, helped is writing and finalizing the manuscript. Ebtisam N Buali, Suhail I Baithun, Duaa A Abduljabbar, Mohamed d, all did the lab work and helped preparation of slides and HPV detection techniques Mandeep Bedi gave the final touch of the manuscript

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