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ORIGINAL ARTICLE

Human papillomavirus (HPV) in Egyptian females: study by cytology, histopathology, colposcopy and molecular diagnosis of high risk types

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Abstract

Objectives: In Northern Africa, the region Egypt belongs to, about 10.7% of women are estimated to harbour cervical human papillomavirus (HPV) infection and 78.4% of invasive cancers are attributed to HPVs 16 or 18. We aimed at comparing HPV detection by ISH-PCR tissue with other conventional available cheaper techniques, finding which of them can be relied upon in a developing country like Egypt for HPV detection. *Methods:* Sixty patients were included. For them colposcopy, PAP smear, histopathology and detection of HPV using ISH PCR tissue and PCR swab were achieved. *Results:* PCR-ISH tissue was positive in 53.33%, 46.6% were negative. Pap smear was negative in 26 cases (43.33%) and 43 cases (56.67%) were positive. LSIL with perinuclear halo represented nearly half of the positive cases (16/34; 47.05%), 10 cases were diagnosed as HSIL, 4 cases as ASCUS and 4 as AGC. Histopathology was negative in 12 (20%) cases and 48 (80%) cases were positive. CIN I and CIN I+ koliocytosis represented half of the cases (30/60) and more than half of positive cases (30/48; 62.5%). Comparing the results of pap smear, histopathology, colposcopy and PCR swab with ISH PCR tissue, highly significant results were seen with sensitivity of 87.5%, 100%, 62.5% and 56.2% respectively but the specificity were 78.6%, 42.9%, 28.6% and 100% respectively. *Conclusion:* Conventional cytology and histopathology were sensitive tests for detection of HPV. This may help for early detection of cancer cervix in a developing country like Egypt. PCR swab showed the highest specificity and the lowest sensitivity.

Keywords: HPV, Egypt, ISH-PCR, pap smear, histopathology

INTRODUCTION

Cervical cancer is recognized as the third most common type of cancer in women worldwide and the second most prevalent cancer type in women in developing countries.[1] It is distinctive among human cancers by being the first found to be directly attributable to the effects of an infectious agent [HPV]. This extraordinary discovery, for which Harald zur Hausen was deservedly awarded the Nobel Prize in 2008, brings with it the potential of global cervical cancer prevention.[2] Data is not yet available on the HPV burden in the general population of Egypt. However, in Northern Africa, the region Egypt belongs to, about 10.7% of women in the general population are estimated to harbour cervical HPV infection at a given time and 78.4% of invasive cervical cancers are attributed to HPVs 16 or 18.[3]

Papillomaviruses have been detected in a wide variety of animals as well as in humans. More than 200 types of human papillomavirus (HPV) have been recognized on the basis of DNA sequence. Specific typing of HPV is depending on the type of epithelium infected and tissue tropism.[4] HPV types are often referred to as "cutaneous" or "mucosal" types.[5]

Currently, HPVs are divided into major categories depending on their level of association with cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma. The "high-risk (carcinogenic)" types are primarily 16 and 18, but also 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. HPV types 26, 53, and 66 have been categorized as 'probably high-risk', and

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HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108 are incorporated in the 'low-risk category'.[6]

Variant cervical lesions induced by HPV infection are involved in continuous pathological process, including the subclinical, latent, and persistent infection of high risk (HR)-HPV, chronic cervicitis with abnormal results of cytological examination, cervical intraepithelial neoplasia (CIN), and cervical cancer.[7] Infection with HPV is suspected by the appearance of clinical lesions and through the results of cytology, histology, and colposcopy, all of which are subjective and often inaccurate. In addition, serology is unreliable and unable to distinguish past from current infection.[8] Molecular diagnostic tests for HPV can augment screening for cervical cancer when used in conjunction with the Pap smear.[9]

Colposcopy is a diagnostic method to detect CIN and cervical cancer, following an abnormal cytology so its major role is in guiding the diagnostic biopsy. HPV testing changes the colposcopic practice as HPV screening will increasingly change the patient population referred to colposcopy.[10]

In situ hybridization of HPV DNA after PCR amplification to detect the HPV DNA (ISH-PCR) allows determining the cellular targets of the virus. *In situ*-based co-expression analyses of HPV with putative target proteins provide great insight into the molecular evolution of these viral-associated cancers. HPV DNA is present in high copy number in the precancerous lesions and is, thus, readily detected by *in situ* hybridization. However, viral integration which is typical during oncogenesis, is associated with reduced copy number of the virus, necessitating *in situ* polymerase chain reaction amplification for accurate *in situ* detection of HPV.[11]

In this study we aimed to evaluate the different methods of diagnosis of cervical HPV infection in Egyptian females by cytology, histopathology, colposcopy and HPV genotyping, to find which of these techniques can be relied upon in a developing country like Egypt with low resource settings for HPV detection.

MATERIALS AND METHODS

Sixty patients were approved to join this study. All procedures performed were in accordance with the ethical standards of the kasr alainy research committee and with the 1975 Helsinki declaration, as revised in 2008.

PAP smear

Each patient was subjected to full history taking, full clinical examination, abdominal examination and colposcopic examination. PAP smear was taken using Ayre's spatula to scrape the cervix (360 degrees). The material collected was spread on a glass slide, then immersed in 95% alcohol as a fixative for at least 20 min before staining by Papanicolaou stain. We considered a Pap smear result to be suggestive of the presence of HPV infection when we found ASUS, LSIL, HSIL or koliocytic atypia.

HPV genotyping

The DNA was extracted from the swab by DNA extraction kit (Qiagen, Germany). HPV DNA was amplified under standard conditions with the L1 consensus HPV primers MY09/MY11 (Digene, Germany). The amplification mixture contained 6.5 mM $MgCl_2$, 50 mM KCl, 2.5 U of Ampli *Taq* DNA polymerase (Roche Molecular Diagnostics, Mississauga, Canada), 200 µM (each) dATP, dCTP, dGTP, and dTTP, and 50 pmol of each primer pool. Amplifications were performed in a Thermocycler (Qiaplex, Germany) for 40 cycles with the following cycling parameters: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Positive cases were subjected for genotyping using specific primers for high risk type [16/18/31/33/35/39/45/51/52/58/59/68].

Colposcopy

Acetic acid (3%) was applied to the cervix using cotton swabs to enhance the squamocolumnar junction and transformation zone (TZ). We considered the acetowhite areas of TZ suggestive of the presence of HPV infection by the presence of three of the modified criteria of REID'S classification: (1) rapid appearance, (2) well-defined border, (3) dense white in color, (4) no uptake of iodine.

Biopsy and histopathology

Schiller's Iodine test: Lugol's iodine was applied to the cervix. It stains mature squamous epithelial cells mahogany color in estrogenized women due to high cellular glycogen content. The area with no iodine uptake especially if preceded by acetowhite area is considered abnormal and multiple biopsies were taken.

Biopsied tissue were fixed for 24 hours in 10% neutral buffered formalin then dehydrated, cleared, and embedded in paraffin wax according to routine processing procedures. Tissue sections of 5 µl thickness were cut from the paraffin

blocks and stained by Hematoxlyin and eosin stain for routine histopathological examination. We considered the histopathological findings suggestive of the presence of HPV infection when we found koliocytosis, CINI, CINII, CINIII, and SCC.

PCR - In Situ Hybridization in tissue (PCR-ISH)

First step: High risk primers were used for PCR on tissue then the amplification mixture contained 6.5 mM $MgCl_2$, 50 mM KCl, 2.5 U of Ampli *Taq* DNA polymerase (Roche Molecular Diagnostics, Mississauga, Canada), 200 µM (each) dATP, dCTP, dGTP, and dTTP, and 50 pmol of each primer pool. Amplifications were performed in a Thermocycler (Qiaplex, Germany) for 40 cycles with the following cycling parameters: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Positive cases were subjected for genotyping using specific primers for high risk type [16/18/31/33/35/39/45/51/52/58/59/68].

Second step: In Situ Hybridization. The biotinylated DNA probes for HPV types [16/18/31/33/35/39/45/51/52/58/59/68] were brought to room temperature. One drop (approximately 40 µl) of either probes was added to the appropriate tissue section and a glass coverslip was placed over the section and the probe. Slides were placed flat on the prewarmed, heating block and were incubated at 100 ± 2°C for 5 minutes. The slides were taken off the heating block and placed flat in humidified incubation chamber. The slides were incubated at 37°C for 24 hours. 800 ml of buffer was prewarmed to 37 ± 2°C. The slides were removed from the incubation chamber. The slides were individually rinsed with gentle agitation in the first container of buffer in order to remove the cover slips and rinse off excess probe. The slides were then placed in the staining rack and washed in three changes of buffer for 3 min each at 37 ± 2°C. Then the slides were removed one at a time from the rack. Excess liquid was carefully wiped away from around the outside.

Statistical analysis

Data were analyzed using SPSS version 22.0 (Statistical Product for Services Solutions). Data were summarized as percentages. For numeric variables the measures for central tendency (mean) and the measures of dispersion (standard deviation, range) were calculated. Chi-square tests were used to determine correlation for nominal variables. Determining the probability factor (P value) assessed the significance of the results. When P value levels were found to be less than 0.05 or less than 0.01, the results were considered to be statistically significant or highly significant respectively.

Standard diagnostic indices including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic efficacy were calculated as described by Galen (1980).[12]

RESULTS

This study included sixty patients with ages ranging from 20 to 50 years and a mean of 32.77 ± 7.99 years. ISH PCR was positive in 32 (53.33%) cases and 28 (46.6%) cases were negative (Fig. 1). Table 1 shows the clinical parameters of the patients and their relation

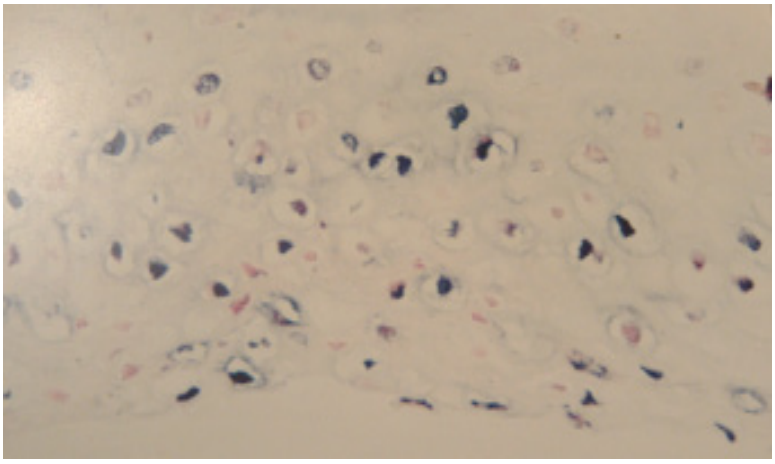

FIG. 1: A positive case of HPV by PCR *in situ* hybridization. The purplish blue nuclei deposits present in the superficial layers of the cervical epithelium denote positive infection with HPV

TABLE 1: Clinical parameters in relation to ISH PCR detection of HPV

Clinical parameter		ISH PCR		P value
		Negative (n= 28)	Positive (n= 32)	
Age	20-30 yrs. (n= 26)	12 (42.9%)	14 (43.8%)	0.352 (NS)
	30-40 yrs. (n= 24)	14 (50.0%)	10 (31.2%)	
	> 40 yrs. (n= 10)	2 (7.1%)	8 (25.0%)	
Parity	Nulli parity (n= 6)	2 (7.1%)	4 (12.5%)	0.626 (NS)
	Parity (n= 54)	26 (92.9%)	28 (87.5%)	
Risk factor	Negative (n= 36)	20 (71.4%)	16 (50.0%)	0.232 (NS)
	Positive (smokers, husband smokers, pills+smoking or pills only) (n= 24)	8 (28.6%)	16 (50.0%)	

NS: non significant

to ISH PCR detection of HPV. No statistically significant difference was found between the presence of HPV by PCR-ISH tissue and age, parity and the presence of risk factors.

Table 2 shows the correlation of HPV detection by ISH-PCR and other techniques such as cytology, histopathology, colposcopy, and PCR swap (Figs. 2, 3, 4, 5, 6, 7). Pap smear was negative in 26 (43.33 %) cases and 34 (56.67%) were positive. LSIL with perinuclear halo represented nearly half of the positive cases (16/34) 47.05%, 10 cases were diagnosed as HSIL, 5 as ASCUS and 4 as AGC. Histopathology was negative in 12 cases (20%) and 48 cases (80%) were positive. CIN I and CIN I+ koliocytosis represented half of the cases (30/60) and more than half of positive cases (30/48) 62.5%. Table 2 also showed distribution of the results of Pap smear and histopathological examination with its correlation to ISH PCR positivity.

When we compared the different types of abnormal smear with the result of ISH PCR tissue, we found 28 (87.5%) of 34 cases with abnormal cytological smear result had positive result for ISH PCR tissue. Four cases that showed negative cytological results were positive for ISH-PCR tissue. All cases with atypical glandular cells (4/4 cases; 100%) were positive for ISH PCR tissue, 14 of 16 (87%) cases with LSIL were positive for ISH-PCR and 8 (80%) of 10 cases with HSIL were positive also for ISH PCR. On comparing the different types of abnormal histopathological findings with the result of ISH PCR tissue, we found 32 (66.6%) cases from 48 with abnormal histopathological result were positive for ISH PCR tissue, CIN I with koliocytosis was (12/12) 100%, CINIII (2/2) 100% and SCC (12/12) 100%.

We found histopathology the most sensitive test for HPV diagnosis and the PCR swab the most specific test (Fig. 8).

Comparing the result of PAP smear in diagnosis of HPV with the ISH PCR tissue, the sensitivity of Pap smear was 87.5%, the specificity was 78.6%, positive predictive value (PPV) was 100% and the negative predictive value (NPV) was 66.7%. Furthermore, the results of histopathology revealed a sensitivity of 100%; specificity of 42.9%, positive predictive value of 66.7% and negative predictive value of 100%.

DISCUSSION

Cervical cancer is the third common type of cancer in women worldwide.[1] It is the first cancer found to be directly attributable to the effects of HPV.[2]

According to WHO 2013 fact sheets, Egypt has a population of 28.37 million women with ages 15 years and older who are at risk of developing cervical cancer. Cervical cancer ranks as the 13th most frequent cancer among women in Egypt and the 10th most frequent cancer among women between 15 and 44 years of age. Data is not yet available on the HPV burden in the general population of Egypt. However, in Northern Africa, the region Egypt belongs to, about 10.7% of women in the general population are diagnosed with cervical HPV at a time and 78.4% of invasive cervical cancers are attributed to HPVs 16 or 18.[3]

This is an analytical study comparing HPV detection by PCR-ISH tissue with other conventional available techniques as cytology, histopathology and colposcopy. It aimed at finding which of these techniques can be relied upon in a developing country like Egypt with low resource settings for HPV detection.

TABLE 2: Correlation of HPV detection by ISH-PCR and other techniques as cytology, histopathology, colposcopy, and PCR swab

Parameter			ISH PCR Negative (n= 28)		ISH PCR Positive (n= 32)		P value
PAP smear	**Negative** (n= 26)		22 (78.6%) TN		4(12.5%) FN		0.001**
	Positive (n= 34)	ASCUS(4)	6 (21.4%)	2	28(87.5%)	2	
		LSIL(16)		2		14	
		AGC(4)		0		4	
		HSIL(10)	FP	2	TP	8	
Histo pathology	**Negative** (n= 12)		12 (42.9%) TN		0 (0%) FN		0.003**
	Positive (n= 48)	Koi(4)		2		2	
		CIN1± koi (30) CIN1(18)	16 (57.1%)	14	32 (100%)	4	
		CIN1, koi(12)		0		12	
		CIN3(2)	FP	0	TP	2	
		SCC(12)		0		12	
Colpo scopy	**Negative** (n= 20)		8 (28.6%) TN		12 (37.5%) FN		0.605 (NS)
	Positive (n= 40)		20 (71.4%) FP		20 (62.5%) TP		
PCR SWAB	**Negative** (n= 42)		28 (100%) TN		14 (43.8%) FN		0.001**
	Positive (n= 18)		0 (0%) FP		18 (56.2%) TP		

**: highly significant, NS: non significant, koi: koilocytic change

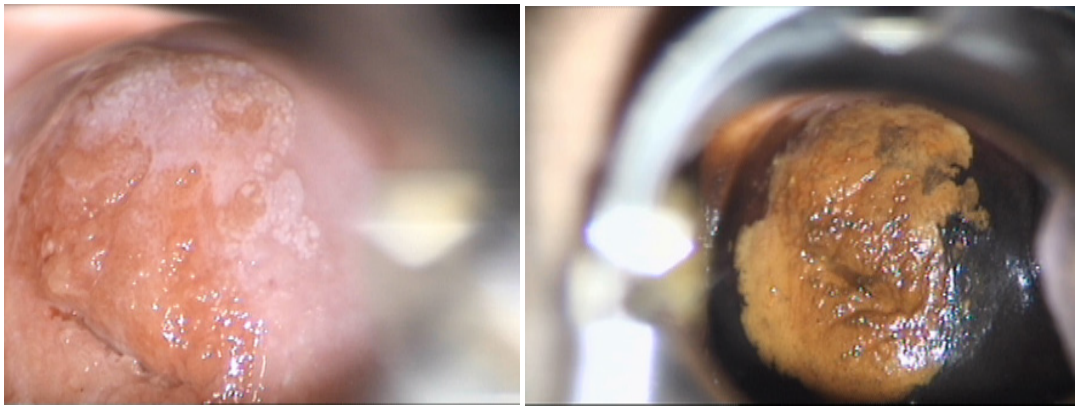

FIG. 2: (Left) Dense acetowhite area at anterior lip of the cervix. This case showed positive result with PCR-ISH, HGSIL on cytology and histopathology result show squamous cell carcinoma. (Right) The same case shows no iodine uptake after application of Lugol's iodine

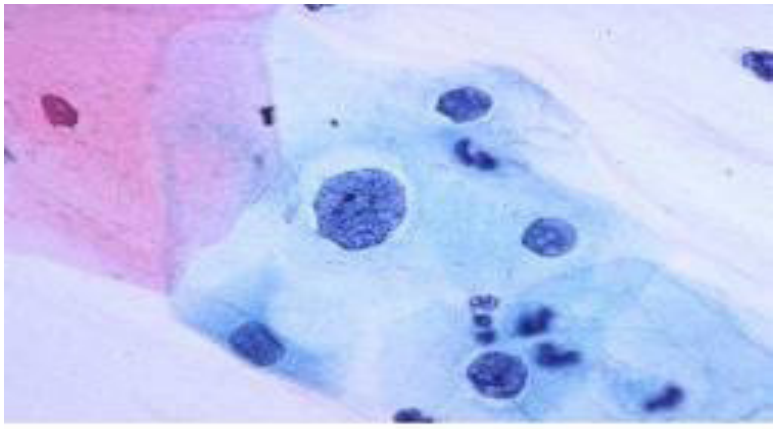

FIG. 3: Cervical cytology with cervical squamous cells showing perinuclear halo (koilocytosis), LSIL (High power view)

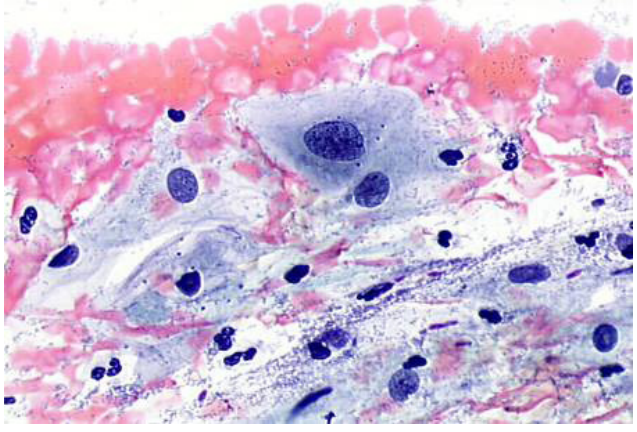

FIG. 4: Cervical cytology showing atypical squamous cell (High power View)

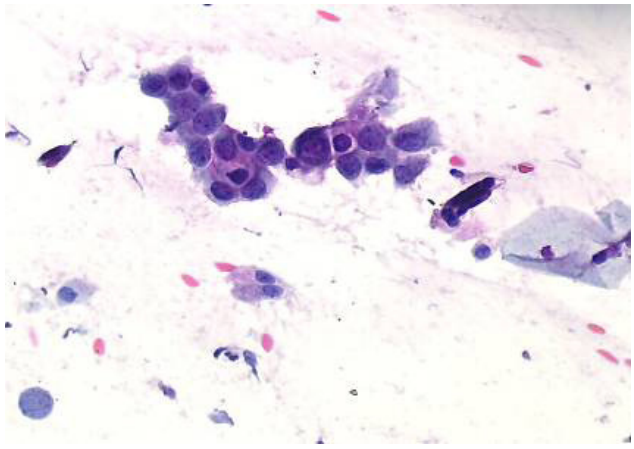

FIG. 5: Cervical cytology showing atypical glandular cell showing nuclear enlargement and hyperchromasia (High power view)

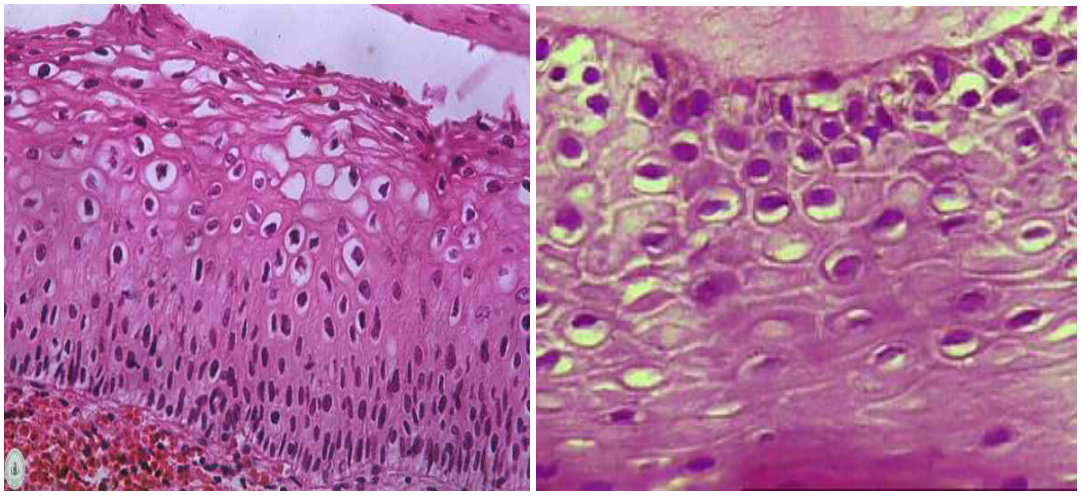

FIG. 6: (Left): Cervical tissue showing CIN 1, with koilocytosis. There is cellular and nuclear pleomorphism with nuclear enlargement and loss of polarity in the lower third of the cervical epithelium with intact basement membrane. (low power view) H&E stain. (Right): Cervical tissue showing koilocytotic changes, the cervical epithelium showing koilocyte with nuclear enlargement, irregularity of the nuclear membrane and perinuclear halo (High power view)

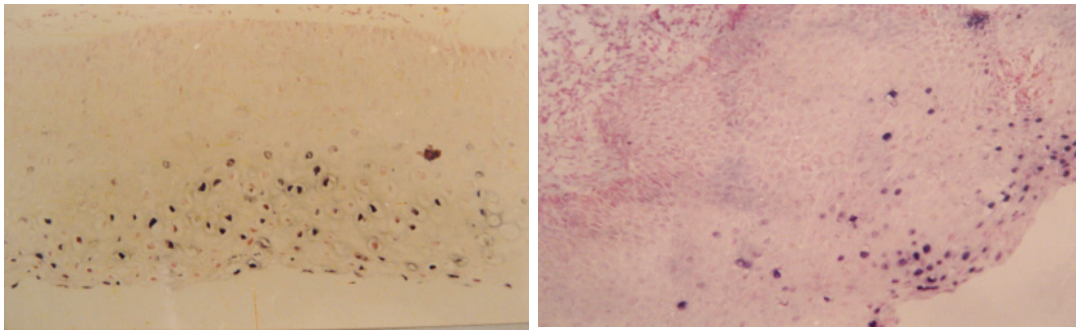

FIG. 7: (Left) A case of CIN positive for HPV by PCR *in situ* hybridization. (Right) High risk probes showing the purplish blue nuclei deposits present in the superficial layers of the cervical epithelium denoting positive infection with HPV

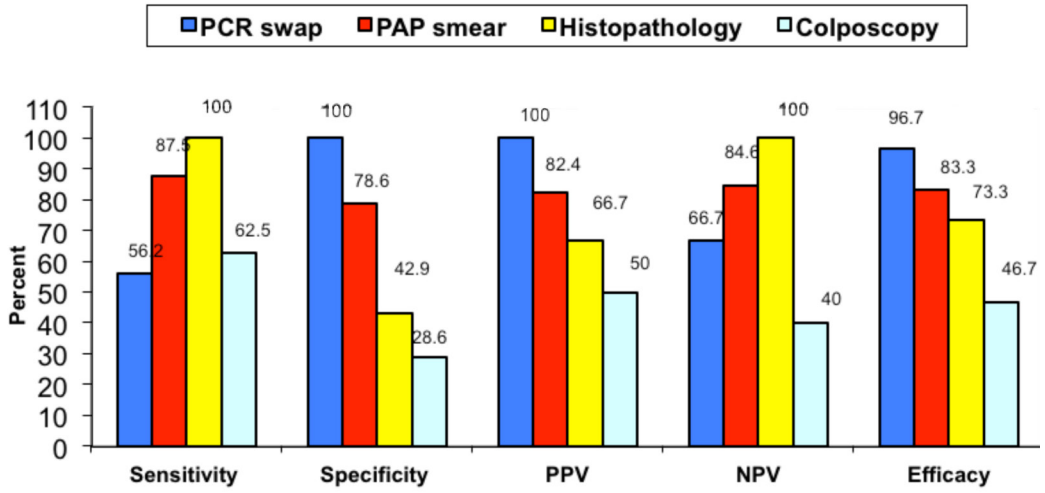

FIG. 8: Diagnostic indices (sensitivity, specificity, PPV, NPV and efficacy) of different diagnostic tools

In our study we did not find a correlation between age, parity and presence of risk factors and HPV detection. Sargent *et al* (2008)[13] found that there was a marked decline in the prevalence of HR-HPV infection with age, but the proportion due to each HPV type did not vary greatly with age. Xi *et al* (2009)[14] detected higher baseline HPV16 and HPV18 DNA load association with current smokers but not former smokers.

In our study while comparing Pap smear results with the PCR-ISH tissue, the sensitivity of Pap smear was 87.5%, the specificity was 78.6%, positive predictive value 100% and negative predictive value 66.7%. We found 28 (87.5%) of 34 cases with abnormal cytological smear result had positive result by PCR-ISH tissue. Four cases with negative cytological results were positive for ISH-PCR tissue. All cases with atypical glandular cells (4/4 cases, 100%) were positive for PCR-ISH tissue, while 14/16 (87%) cases with LSIL, and 8/10 (80%) cases with HSIL were positive also for PCR-ISH tissue. ASCUS and LSIL could also be caused by low risk HPV types. Our results showed 6 cases of ASCUS, LSIL, and HSIL (2 cases each) were HR HPV negative, a finding in agreement with Kelesidis *et al* (2011).[15] They found that 112 (86.8%) of 129 patients with abnormal cytological smears had positive ISH cervical tissues. Positive ISH signals were observed in 59 (87%) of 68 samples with LSIL, 14 (100%) of 14 with HSIL, and 3 (100%) of 3 with adenocarcinoma.

Jovanović *et al* (2012)[16] examined the correlation of HPV infection with cytology, colposcopy, and histopathological examination of cervical biopies in low- and high-grade cervical lesions. They examined 1,927 patients by colposcopy, cytology, histopathology, and HPV test verification was made in all patients. They found that among 1,927 women studied, 635 (32.95%) had abnormal cytological findings and among these only 272 (42.83%) were positive for HR-HPV. This data may represent the actual low sensitivity of cytology in detecting HPV infection as they studied a large number of cases.

The Pap smear procedure has some limitations. The false-negative rate has been reported to be as high as 20 to 30% according to Jung *et al* (2004).[4] False-negative results can occur due to a misreading of slides, especially when the cells are not spread evenly or uniformly on the slide. In addition, contaminants such as bacteria or yeast prevent the detection of abnormal cells in the specimens. When the specimens are exposed to air too long before their fixation on the slides, the cervical cells can become distorted and unreadable. Human error is probably the main cause of false interpretation from a Pap smear. The average Pap smear slide contains 50,000 to 300,000 cells to be examined. Abnormal cells can easily be missed, particularly by overworked readers, if the sample contains only a few abnormal cells within a crowded background of healthy cells.

Recognition of the problems associated with the Pap test led to attempts to develop new technology, including automated screeners, in order to reduce the need for labour-intensive manual screening. Liquid-based cervical cytology preparations and automated screening technology were developed. Studies showed that some liquid-based cytology systems had superior sensitivity for the detection of squamous intraepithelial lesions (SIL) compared with the conventional Pap test.[17]

Histopathological examination suggested HPV in 48 out of the 60 cases in this study. 32 out of these 48 were positive by ISH PCR tissue and actually these 32 cases represent all positive cases diagnosed by ISH PCR tissue in the whole study. That is why the sensitivity of histopathology for detection of HPV was 100% in our study. The low specificity (42.9%) of histopathology to detect HPV may be due to the fact that the HPV virus can be detected in tissue before it causes cytohistological changes. HPV detection by PCR-ISH tissue was seen in 50% in those diagnosed as koliocytic change only and in 22.2% in those diagnosed as CINI only. With increasing grade of severity of histopathological results the detection of HPV by ISH-PCR tissue was 100% in CINI+ koliocytic change, CINIII and in SCC cases.

The results of Menegdazzi *et al* (2009)[18] agreed with ours. They evaluated HPV type-specific distribution in 654 women from the South of Italy undergoing voluntary screening and correlated with cyto-histological abnormalities. Histological analyses were performed in 86 women who underwent colposcopy and cervical biopsy. HPV DNA was detected in 56.1% of biopsies with negative histology, 60.9% of which had HR-HPVs. The prevalence of HPV-DNA (and HR-HPV DNA) detection progressively increases with the worsening of histological pattern, with 8 (88.9%) CIN I, for HPV DNA and 7 (87.5%) for HR-HPV and (100%) CIN-II/III cases positive for HR-HPVs, including HPV-16, HPV-18, HPV-31, and HPV-51.

Mass cytology screening has shifted the presentation of cervical carcinoma from the clinical to the preclinical stage. This is an established fact. Following mass screening, there has been a reduction of 38–57% in the overall incidence of invasive carcinoma and a reduction of 67% in the incidence of clinically evident carcinoma.

Whereas in the prescreening era invasive carcinoma contributed approximately 80% of all diagnosed cases, at the present time it makes up less than 20% of the cases, the remaining being carcinomas in the intraepithelial (CIN) stage. This has resulted in an increased cure rate for the screened population and an increase in the survival times for patients with invasive carcinoma.[2]

Regarding colposcopy, we found that it had lower sensitivity (62.5%) and specificity (28.6%) than cytology and histopathological examination. It showed a positive predictive value of 50% and negative predictive value of 40%. Cox (2008)[19] in an article questioning its accuracy stated that in most studies of colposcopy the estimated sensitivity of the procedure for detection CINIII ranged from 54% to 85%. New techniques in optical spectroscopy have been developed and tested to improve the diagnostic accuracy of colposcopy. Optical spectroscopy is a real time diagnostic method that can be used in the colposcope.[20]

When we compare the PCR swap with ISH PCR tissue for detection of HPV in trying to answer the question if cervical swaps can be substitutes for biopsies, we found that cervical scrapes were less sensitive than biopsies. We found that sensitivity of swap was 56.2% but the specificity was 100%.

Conclusion and recommendations

PCR-ISH tissue is the golden standard for diagnosis of HPV. The advantage of ISH is that it is performed directly on tissue which allows localization of the target sequences and correlation with the clinical appearance. When augmented by PCR, the sensitivity can be increased to one HPV copy per cell but it has a major disadvantage of being expensive, so in countries like Egypt with low resource settings for screening of cervical cancer and HPV detection, it cannot be used on a large scale.

Our study showed that conventional cytology and histopathological examination were sensitive tests for detection of HPV. This may help for early detection of cervical cancer, as although incidence of cervical cancer in Egypt is low, the mortality rate is nearly 100% as the diagnosis is always late.

Colposcopy showed lower sensitivity and specificity to detect HPV but this can be improved by taking multiple biopsies. PCR swap showed the highest specificity and the lowest sensitivity as blood contamination and insufficient amount of cells adversely affect its result.

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Authors declare no conflict of interest.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011; 61: 69–90.
2. Rosai J. Uterus-cervix, female reproductive system. In Rosai J, editor. Rosai and Ackerman's Surgical Pathology. 10th ed. New York: Elsevier; 2011. P. 1436-59.
3. World health organization WHO, 2013. ICO (Institut Català d'Oncologia) Information centre on HPV and cancer Egypt human papillomavirus and related cancers, fact sheet [Internet]. 2013. Available from: www.hpvcentre.net.
4. Jung WW, Chun T, Sul D, *et al*. Strategies against human papillomavirus infection and cervical cancer. J Microbiol. 2004; 42: 255-66.
5. Bonnez W and Reichman RC. Infectious Disease and Their Etiologic Agents. In: GL Mandell, JE Bennett and R Dolin, editors. Principles and Practice of Infectious Diseases. Philadelphia: Churchill Livingstone. 2000. p. 1630-44.
6. Munoz N, Bosch FX, de Sanjose S, *et al*. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003; 348: 518-27.
7. Li S, Meng YH, Ting H, Shen J, Ma D. Clinical significance of human papilloma virus infection in the cervical lesions front. Front Med China. 2010; 4: 264–70.
8. Carter JJ, Koutsky LA, Hughes JP, *et al*. Comparison of human papilloma virus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis. 2000; 181: 1911-9.
9. Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol. 2005; 32 Suppl 1: S43–51.
10. Jeronimo J, Schiffman M. Colposcopy at a crossroads. Am J Obstet Gynecol. 2006; 195: 349–53.
11. Nuovo GJ. In situ detection of human papillomavirus DNA after PCR-amplification. Methods Mol Biol. 2011; 688: 35-46.
12. Galen RS: Predictive values and efficiency of laboratory testing. Pediat Clin North Am. 1980; 27: 861-9.
13. Sargent A, Bailey A, Almonte M, *et al.* Prevalence of type-specific HPV infection by age and grade of cervical cytology: data from the ARTISTIC trial. Br J Cancer. 2008; 98: 1704–9.
14. Xi LF, Koutsky LA, Castle PE, *et al*. Relationship between cigarette smoking and human papilloma virus types 16 and 18 DNA load. Cancer Epidemiol Biomarkers Prev. 2009; 18: 3490-6.
15. Kelesidis T, Aish L, Steller MA, et al. Human papillomavirus (HPV) detection using in situ hybridization in histologic samples: correlations with cytologic changes and polymerase chain reaction HPV detection. Am J Clin Pathol. 2011; 136: 119-27.
16. Jovanovic AM, Dikic SD, Jovanovic V, *et al*. Correlation of human papilloma virus infection

with cytology, colposcopy and histopathological examination of the bioptic tissue in low- and high-grade intraepithelial lesions. Eur J Gynaecol Oncol. 2012; 33: 512-6.
17. Limaye A, Connor AJ, Huang X, Luff R. Comparative analysis of conventional Papanicolaou tests and a fluid-based thin -layer method. Arch Pathol Lab Med. 2003; 127: 200-4.
18. Menegazzi P, Barzon L, Palù G, Reho E, Tagliaferro L. Human papillomavirus type distribution and correlation with cyto-histological patterns in women from the South of Italy. Infect Dis Obstet Gynecol. 2009; 2009: 198425.
19. Cox JT. More questions about the accuracy of colposcopy: what does this mean for cervical cancer prevention? Obstet Gynecol. 2008; 111: 1266-7.
20. Cardenas-Turanzas M, Freeberg JA, Benedet JL, *et al.* The clinical effectiveness of optical spectroscopy for the in vivo diagnosis of cervical intraepithelial neoplasia: where are we? Gynecol Oncol. 2007; 107*(*1 Suppl 1*):* S138–46.