Human Papillomavirus DNA in a Sample of Iraqi Women with Positive Visual Inspection by Acetic Acid

Halah Abdulwahhab Mohammed[1], Wasan Fawzi Sanad[2], Buthaina Ahmed Alwan[3]

[1]Specialist Obstetrics and Gynecology, Ministry of Health, Baghdad, Iraq, [2]Specialist Obstetrics and Gynecology, Fallujah Teaching Hospital, Ministry of Health, AlAnbar, Iraq,[3]Specialist Obstetrics and Gynecology, Abu Garib Hospital, Ministry of Health, Baghdad,Iraq.

Abstract

Background: This study has conducted in Baghdad Teaching Hospital, Department of Obstetrics and Gyanecology, Al-Habebyia H. outpatient,Ibn-Albalady H. outpatient and 6 Primary Health Care Centers in Baghdad between June 2019 to March 2020.A Prospective and multicentric study.

Aim of the Study: To asses the prevalence of HPV in a sample of Iraqi women.

Patients and methods: The study include 268 married women between the age of 30 years old and more, who were attending the outpatient department. Both screening tests; naked eye visual inspection with acetic acid &HPV test were performed.

Results: The study included 268 women.The positivity rate of HPV for all women survived was 8.9% ,19 % of women with positive VIA ,81% with negative VIA.All the positive VIA cases(19 %)and some ofnegative VIA cases(18.6%) were randomly selected(because of their symptoms) underwentHPV test. After comparing the results of HPV test and VIA results, the sensitivity of VIA 95.8 %The specificity of VIAwas 63.6%. Positive predictive values was 45.1%. Negative predictive values was 98%.Accuracy rate 71.3%.

Conclusions:

1. The prevalence of HPV infection among a sample of Iraqis women was very low.

2. Estimation of HPV infection would provide valuable data particularly to introduce the strategies for the screening of cervical cancer and HPV vaccination in Iraq.

3.VIA with HPV DNA test may be a suitable substitute to Pap. smear as a screening tests for premalignant and malignant disease of the cervix.

Keywords: *HPV, Iraqi women, DNA,acetic acid.*

Introduction

Routine cytological screening and treatment of women has resulted in a dramatic decline in cervical cancer deaths over the past four decades in wealthier countries. In developing countries cervical cancer is preventable by screeningasymptomatic women for precancerous cervical lesions and treating the lesions before they progress to invasive disease. In other words, those deaths are largely preventable. The cure

Corresponding author
Specialist Obstetrics and Gynecology, Abu Garib Hospital, Ministry of Health, Baghdad, Iraq
email: wasanfawazi7@icloud.com

rate for invasive cervical cancer is closely related to the stage of disease at diagnosis and the availability of treatment,ifleft untreated cervical cancer is almost fatal.(1)

Human papillomavirus plays a major role in the development of cervical cancers. Also increasing evidence suggests that HPV oncoproteins may be a critical component of continued cancer cell proliferation. Unlike low-risk serotypes, oncogenic HPV serotypes can integrate into the human genome. As a result, with infection, oncogenic HPV's early replication proteins E1 and E2 enable the virus to replicate within cervical cells. These proteins are expressed in high levels early inHPV infection. They can lead to cytologic changes detected as low-grade squamous intraepithelial (LSIL) cytologic findings on Pap smears(2).

Cancer subtypes

Though squamous cell carcinoma is the cervical cancer with the most incidence, the incidence of adenocarcinoma of the cervix has been increasing in recent decades(3).

Human papillomavirus

Detection of HPV is associated with a 250 fold increase risk of high grade CIN, while persistent high-risk HPV infection increase the risk of developing CIN 300 fold (4) In fact, most of the behavioral and sexual risk factors for cervicalneoplasia become statistically insignificant as independent variable s after adjusting for HPV infection(5). persisting HPV type after treatment have anincreased risk of residual CIN(2,6). pre-cancerouslesion become more severe, the koilocytes disappear, the HPV copy numbers decrease, and the capsid antigen disappears, indicating that the virusisnotcapable of reproducinginlessdifferentiatedcells.Instead, portions of the HPV DNA become integrated into the hostcell. Integration of the transcriptionally active DNA into the host cell appears tobeessential to malignant growth(4).There have been remarkable improvements in both the sensitivity and specificity of HPV in the last three decades(7).

Direct detection of HPV DNA can be done histologically by either:

a-nucleic acid amplification via polymerase chain reaction (PCR)(8)

Prevention of Ca. Cervix

Since the most common form of cervical cancer starts with pre-cancerous changes, there are two types of prevention:

1.Primary prevention by vaccination:

Although cervical cancer is a preventable disease and is completely curable if detected at an early stage, cytological screening alone will not lead to the, The tetravalent HPV vaccine is most effective if given before any sexual exposure, but sexually active women can receive and benefit from vaccination. Tetravalent vaccine is not recommended

The second one (Cervarix) is a bivalent HPV vaccine of HPV types 16/18, used at 0,1 and 6 months as a 0.5 ml intramuscular injection.The efficacy of these vaccines in preventing persistent HPV infection has been found to range between 90% and 100%, and immunity provided has been shown to last for in excess of six years. (9).

2.Secondary prevention by early diagnosis of precancerous lesions(screening):

Cervical cytology screening programs in the developed countriesof Europe and North America have been followed by substantialreduction in disease burden. However, screening programs do notexist in most developing countries.The success of prevention programs based on cytology screeningdepends upon availability of adequate technical personnel, goodquality cytology smears, adequate laboratory services with internaland external quality control, a good organisation to ensure highcoverage of the target population with screening and

diagnosis,treatment and follow-up of screen-positive women. In many developingcountries, technical, manpower and financial resources are inadequate to provide the necessary infrastructure. The difficulties in introducing cervical cytology screening in low-resource settings have prompted the evaluation of simple and inexpensive non cytological methods of detecting precursor lesions, such as visual inspection of the cervix after application of 3–5% acetic acid (VIA) or after the application of Lugol's iodine (VILI)[10]

Types of visual detection:

1- Naked eye inspection after application of 3-5% acetic acid (VIA):

The acetic acid coagulates protein of cytoplasm and nuclei and since abnormal epithelium is of a high nuclear density, this prevents light from passing through the epithelium, which thus appears white[5], the provider can recommend further treatment as needed.

2-Visual inspection after the application of Lugol's iodine (VILI):

If iodine is applied to the cervix, precancerous and cancerous lesions appear well-defined, thick, and mustard or saffron-yellow in colour, while squamous epithelium stains brown or black, and columnar epithelium retains its normal pink colour.[1]

Aim of the study

To asses the prevalence of HPV in a sample of Iraqi women.

Materials & Methods

Study Design:

This is a Prospective study which has conducted in Baghdad Teaching Hospital, Department of Obstetrics and Gynaecology, Al-Habebyia H. outpatient,Ibn-Albalady H. outpatient and 6 Primary Health Care Centers in Baghdad between June 2019 to March 2021.

268 women were enrolled in the study, some of them were complaining from different gynaecological problems and others had no complaint.

Inclusion criteria:

268 married women ages equal or more than 30 years. Written informed consent was obtained from all participants. Information on demographics and risk factors was obtained by a self-administered questionnaire.All patients examinedby VIA and HPV DNA test done for all positive VIA patients &for some patients with negative VIA & had complaint.

Exclusion criteria:

Women who were lacked a cervix (hysterectomies), were pregnant, had a history of cervical cancer, or were unable to provide consent were excluded.

Materials:

vaginal speculum (Cusco's speculum), a sterile rubber gloves, adequate light source about 100 watt(flash light), cotton swabs,labelled positive charge slides, Ayres spatula, Cuplan`s jar contain 95% ethyl alcohol. Freshly prepared 5% acetic acid solution (5ml of glacial acetic acid with 95 ml of distilled water).

The woman is asked to lie in a modified lithiotomy position onto the examination table after she has emptied her bladder.Inspection of the external genitalia was done for the presence of lesions. Thenfull pelvic examination was done in the usual way. A sterile Cusco's speculumwas carefully inserted in the vagina, and avoid use antiseptic solution forsterilization of genetilia. Inspection of the cervix was done for cervicitis,ectropian, nabothian cyst, cervical ulcer or erosion, polyp, outgrowth, andbleeding for which the women is giving treatment and appointment for reassesment. The four vaginal fornices then examined to make sure that they arefree from any growth. VIA was done for all patients and it involve gentle application of 5% acetic acid. The woman is informed

that she might feel a slight stingingsensation. After 1□2 minutes a naked eye evaluation was performed under 100-watt illumination. The transformation zone is carefully checked for any dense non movable acetowhite areas in the mucosa. If acetowhite areas are identified on the cervix after 1-2 minute, the test is positive then HPV smear was done for those positive VIApatients&those who had complaint on the same day using a conventional disposable wooden Ayre□s spatula, scrape the cervix around theentire transformation zone and smearing the cells onto a labeled glass positive charge slides, 2 slides for each patient. The smear is fixed with 95% ethyl alcohol for 20- 30 minutes. these slides were sent to laboratory to be examined.

Criteria for categorization

VIA findings were categorised as negative when any of the following findings were observed:

- No acetowhite lesions, or faint, ill-defined, bluish white or doubtful lesions.
- Acetowhitening on cervical polyps or on nabothian cysts
- Dot- or streak-like acetowhitening on the cervix.
- White line-like prominent squamocolumnarjunction (SCJ) after application of acetic acid.
- Angular or geographic acetowhite lesions far away from the SCJ or the external os, if SCJ was not visible (satellite lesions) .

VIA was categorized as positive when any of the following were observed:

- Well-defined, opaque, acetowhite lesions touching the SCJ or the external os, if SCJ was not visible.
- A large circumferential acetowhite lesion surrounding the external os.
- Pre-existing wart or leukoplakia turning intensely white after application of acetic acid.
- Ulceroproliferative growth turning densely acetowhite after application of acetic acid[10].

The woman after completition of the tests can go back to her normal activities.

When the results of HPV test appear, follow up the patients was done.

In Situ Hybridization for the Detection of Hpv-16, HPV-18

Principle of the Test: In situ hybridization is a method of localizing and detecting specific DNA or RNA sequences in morphologically preserved tissue sections. Briefly, the method involved deproteinization of fixed tissue sections mounted on slides hybridization of a biotinylated probe to the target sequence, the hybridized probe was then detected by addition of a streptavidin – alkalinephosphatase (streptavidin-AP) conjugate (DNA probe hybridization/Detection system in situ kit,Dako biotech, USA).

Upon addition of the single component BCIP/ NBT solution (substrate) which is 5-brom-4 chloro-3 indolyl phosphate/Nitro blue tetrazolium, an intense blue signal appeared at the specific site of the hybridized probe. This streptavidin-AP conjugate directly linked to the biotinylated probe provides a rapid and highly sensitive detection method.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences- version 20).

Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different percentages (qualitative data) were tested using chi-square test (c^2-test) with application of Yet's correction or Fisher Exact test whenever applicable.

Statistical significance was considered whenever the P value was equal or less than 0.05.

The sensitivity, specificity, positive predictive value, negative predictivevalue and accuracy rate were calculated.

Sensitivity= True positive / (True positive+False negative).

Specificity= True negative/ (False Positive+True negative

Results

Women under study. The total number of women included in this study was 268they had an agespectrum between 30-54 years old. The number of women in the reproductiveage was 264(98.5%)while the number of post menopause was 4 (1.5%), the mean±SD for age was 36.4±5.2. the mean±SD for age of first sexual intercourse (years) was 19.9±4.1, grand multiparous 134the mean±SD was 3.8±2.0. Themajority of patients were non-smoker 266 (99.3%), and most of them 232(86.6%) were not use oral contraceptive pills.

Table 1 shows VIA results for all 268 women under study. 51(19%) women hadpositive VIA test, the remaining 217(81%) had negative VIA test.

Table 2 show that 24 (23.8%) had positive HPV test, the remaining 77(76.2%) had negative HPV test.

Table 3 shows that 23 women with positive VIAhad HPV positive test&28 women with positive VIA hadHPV negative test, onewoman with negative VIA had HPV positive testand 49 women with negative VIAhad HPV negative test. the sensitivity of VIA was 95.8%, the specificity 63.6%,the positive predictive value 45.1%, the negative predictive value 98%. Figure 1.

Table 1: VIA results

VIA	No.	%
Positive	51	19%
Negative	217	81%
Total	268	100%

Table 2: HPV results

HPV	No.	%
Positive	24	23.8
Negative	77	76.2
Total	101	100%

Table 3: Comparism between VIA test outcome and HPV resultsHPV

		Positive	Negative	P value
VIA	Positive	23	28	PPV 45.1
	Negative	1	49	NPPV 98.0
		Sensitivity 95.8	Specificity 63.6	

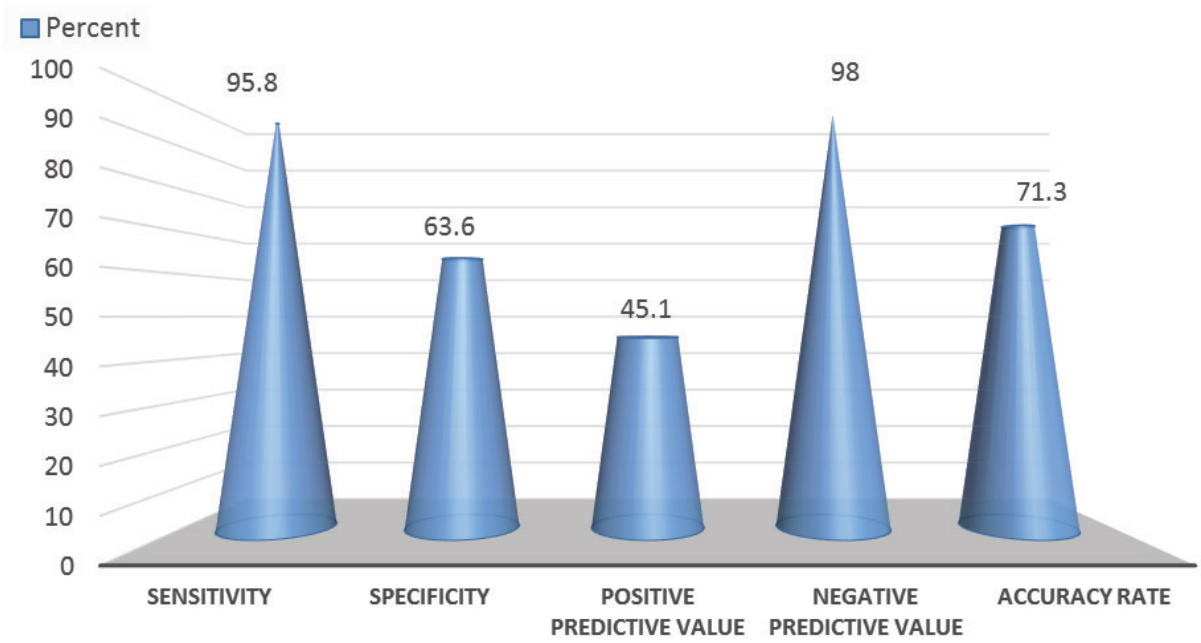

Figure 1: This figure show VIA statistical results

Discussion

Infection with oncogenic HPV has been identified as the underlying cause of cervical cancer therefore there is interest in the use of HPV DNA testing as a primary screening test for cervical cancer. The overall prevalence of HPV among cervical cancers in a large international study was more than 99%, the highest attributable fraction ever identified for a specific cause of cancer [12].

In our study the positivity rate ofHPV infection in all survied women was 8.9%, all viral types wereofthe high risk genotype (type16&18), other types of HPV not included in our study because of difficulty in availability of the kits& probs of HPV tests, so we chose the most oncogenic types.

Inameta-analysissummarizingthegloballiteraturef orwomen with negative cytology results, **de Sanjosé et al** in July 2007, the worldwide prevalence of women harboring HPV DNA was 10.4% [13]. In other studies the rates range from 7.6% to 27%, depending on the region [14,15] while in June2010arandomized trial in Turkey by**Eren et al** [16], found that the prevalence of HPV amongTurkishwomenwas16.5%, multiplegenotypes were found in 35.8% of the infected women, and 75% of the typeswerehigh risk. While in Japan study done by **Inoue** in 2010[17], the prevalence was14.5%.Another randomized trial done by **Fernandes et al** in Brazil in 2009, shows that the overall HPV prevalence in Brazil was 48%[18].

A similar study in Iran was done by **Khodakarami, Net al.** in 2012shows that the prevalence of HPV was 7.8%[19].

In our study the results showed the highest incidence of HPV infection among patient aged between 40-44 years old, this results also found in Turkish study [16].

AlthoughagecurvesforHPVinfectiondiffer notably acrossregions, HPV prevalence is strongly associated with ageworldwide.

Inallworld regions, HPV prevalence was highest in women younger than 35 years of age, decreasing in women of older age [13, 14].

Oral contraception has been proclaimed as a risk factor of cervical cancer on prolonged use by high-risk HPV positive women.In our study OCCPS was used

by 36 women(13.4%) ,only 1 of them hadpositiveHPV test (which is statisticallynot significant).

Syrjanen K et al 2006 suggest that the use of OCCPS is not an independentrisk factor for cervical cancer or its precursors but sexual behavior is different among OCCPS users andnonusers ofcontraception, these factors predispose women to HR-HPV, high grade CIN and determine the outcome of their cervical disease / HR-HPV infection[20]

0.7% of women in our study were smoker, 99.3% of women were not smoker,all of themhad disease negative, which not reach statistically significant level. **Bosch FX et al** 2007 study state that smoking is a co-factor that modify the risk among HPV DNA positive women include the use of occps for five or more years[21].

Syrjanen K et al 2007 in Finland study conclude that cigarette smoking is not an independent risk factor of HSIL, but the increased risk ascribed to smoking is mediated by acquisition of high risk \HPV,of which current smoking was an independent predictor in multivariate model[22].

], the sensitivity of VIA and Pap smear were 74.3% and 37.1% respectively, and also by **Cohn *et al***the sensitivity of VIA was 76.7% which is higher than the sensitivity of Pap.smear 44.3%. Also, by **Rana *et al***2010 [23] reported that there was no statistically significant difference between the specificity ofVIA and cervical cytology.

Conclusion

1.The prevalence of HPV infection among a sample of Iraqi women was very low.

2. Estimation of HPV infection would provide valuable data particularly to introduce the strategies for the screening of cervical cancer and HPV vaccination in Iraq.

3.VIA with HPV DNA test may be a suitable substitute to Pap. smear as a screening tests for premalignant and malignant disease of the cervix.

Declarations Conflict of Interest the authors declare that the reareno potential conflicts of interestrelatedtothestudy.

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Ethical Clearance: This research has exemption asitaroutine treatment (nonewmaterialswereused).

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