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Correlation of Pap Smear, Colposcopic Directed Biopsy and HPV Detection in Symptomatic Women

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Abstract:

Objective:To detect the human papillomavirus by two methods in a sample of Iraqi women complaining of multiple gynecological symptoms and to verify the most accurate way for detection of such virus. Design :A prospective study including a total number of symptomatic cases obtained from patients referred to the Iraqi National Cancer Research Center, the period from Sep.2011 to Dec. 2012 for clinical or colposcopic suspicion of HPV infection. Setting:Al-Yarmouk Teaching Hospital& Iraqi National Cancer Research Center. Population:One hundred women who suffered from different gynecological problems were subjected to Pap smear. Method:The data collected from the patients referred to the Iraqi National Cancer Research Center, Pap smear and HPV testing by PCR were done for all patients, but the colposcopy directed biopsy was done only for 45 cases which gave abnormal Pap smear results and screening of virus by immune histochemical assay. Results:The overall prevalence of PCR sensitivity for detection of HPV, in this study there was only one case of HPV positive result detected by PCR in contrast to 10 cases of positivity detected by immune histochemical, and the most positive cases were within the low–grade intraepithelial lesion. Conclusion:Our study showed the detection of HR HPV infection detected by cervical cytology is susceptible to errors. Both methods of detection of HPV (PCR and immune histochemical assay) are complex laboratory measures requiring numerous processes by humans and machines.Our study showed that histoimmune detection of HPV was higher in number compared to the PCR detection of HPV.Our results of Pap smear and colposcopy-directed biopsy were strongly comparable.We know that the PCR has higher sensitivity in detection of HPV infection,so this result need to be assessed thoroughly about the accuracy of such method in our country

Keywords:

Pap smear, human papilloma virus, colposcopy

Introduction

Cervical cancer is the second most common cancer affecting women worldwide, accounting for 473,000 new cases per year (Parkin, D. M. et al., 1993). In developing countries, it is a leading cause of death among middle—aged women, where an estimated three-quarters of the global burden occurs (Sankaranarayanan, R. et al., 1998). Although the incidence rates of this cancer in Iraq are relatively low, as in most other Islamic countries, the majority of the cases usually present in advanced stages with

poor prospects of cure. According to the latest Iraqi Cancer Registry (El- Hassani, M. et al., 1996),cervical cancer ranks 9th among the 10 most common female cancers(forming 3.6% of total female malignancies). As more than two—third of the patients had late diagnosis(i.e. stages IIb, III or IV),a feasible control strategy would be to encourage Iraqi women to seek early detection of cervical intra epithelial neoplasia (CIN)(Miller, A. B. et al., 1992).Cancer of the cervix may be prevented or detected early by regular screening with the Pap test (sometimes combined with a test for human papilloma virus(HPV).If it is detected early,cervical cancer is one of the most successfully treatable cancer .In the United State ,the cervical cancer death rate declined by almost 70% between 1955 and 1992 ,in large part due to the effectiveness of Pap test screening .The death rate from cervical cancer continued to decline until 2003 .Since then it has remained stable in white women ,but has gone down in African—American women (American Cancer Society, 2010; Bosch, X. et al., 2008). Pap smear is also used to follow–up women with minor cytological abnormalities or women after treatment of cervical precancers (Cotton, S. et al., 2010).

Table 1*: Screening intervals for national cervical screening programme(56).*

Age group(years)	Frequency of screening
25	First invitation
25-49	Three yearly
50-64	Five yearly
65+	Only screen those who have not been screened since age of 50 or those Who have had recent abnormal tests

Risk Factors for Cervical Cancer:-The most important risk factor for cervical cancer, is a group of more than 100 related viruses, some of which cause a type of growth called a papilloma, or more commonly known as warts. HPV can infect cells on the surface of the skin ,genitals, anus, mouth and throat, but not the blood or most internal organs such as the heart or lung .(17,18) Different type of HPV cause warts in different parts of the body. Some types cause common warts on the hand and feet.Other types of HPV may cause warts around the genital organs known as genital warts or condylomaacuminatum type 6 and 11 which called low risk type of HPV because they are seldom linked to cervical cancer. Certain types of HPV are called high risk types because they are strongly linked to cancers, including cancer of the cervix,vulva,and vagina in women ,the high risk types includeHPV 16,18,31,33, and 45,as well as some others.About two thirds of all cervical cancer are caused by HPV16 and 18.(17) Although HPV can be spread during sex –including vaginal intercourse anal intercourse,and oral sex –sex doesn‘t have to occur for the infection to spread.All that is needed to pass HPV from one person to another is skin to skin contact with an area of the body infected with HPV .Infection with HPV seems to be able to be spread from one part of the body to another –forexample,infection may start in the cervix and then spread to the vagina. The Pap test look for changes in cervical cells caused by HPV infection.Other tests look for the infection themselves by finding genes (DNA)from HPV in the cells. For some women,the HPVtest is used along with the Pap test as a part of screening.(19). The HPV test may also be used to help decide what to do when a women has amildly abnormal Pap test result.If the test fined a high – risk type of HPV,it may mean she will need a full evaluation with colposcopy procedure.Although there is currently no cure for HPV infection,there are ways to treat the warts and abnormal cell growth that HPV causes.

Other risk factors included:-

Smoking:-Women who smoke are about twice as likely as nonsmokers to get cervical cancer.Tobacco by-product have been found in the cervical mucus of women who smoke.(20).

Immunosuppression :-Human immunodeficiency virus (HIV),the virus that causes AIDS ,damages the body‘s immune system and places women at higher risk for HPV infection. This may explain the

increased risk of cervical cancer in women with AIDS.

Chlamydia infection :-Chlamydia is a relatively common kind of bacteria that can infect the reproductive system .It is spread by sexual contact .some studies have seen a higher risk of cervical cancer in women whose blood test results show signs of past or current chlamydia infection compared with women with normal test results.(19)

Diet:-Women with diets low in fruits and vegetable may be at increased risk for cervical cancer .Also overweight was are more likely to develop adenocarcinoma of the cervix (20).

Oral contraceptives:-There is evidence that taking oral contraceptive for long time increase the risk of cervical cancer goes up the longer a woman takes OCs.but the risk goes down again after the OCs are stopped. In one study ,the risk of cervical cancer was doubled in women who to control pills longer than 5 years, but the risk returned to normal 10 years after they were stopped (20).

Multiple full-term pregnancies:-Women who have had 3 or more full-term pregnancies have an increased risk of developing cervical cancer, one theory is that these women had to have had un protected intercourse to get pregnant, so they may have had more exposure to HPV .Also, studies have pointed to hormonal changes during pregnancy as possibly making women more susceptible to HPV infection or cancer growth, another thought that immune system of pregnant women might be weaker,allowing for HPV infection and cancer growth.(19). Women who were younger than 17 years when they had their first full-term pregnancy are almost 2 times more to get cervical cancer later in life than women who waited to get pregnant until they were 25 years or older.

Poverty:Also a risk factor for cervical cancer, many women with low incomes do not have ready access to ahealth care services,including Pap tests. This mean they may not get screened or treated for cervical pre-cancerous lesions.

Diethylstilbestrol(DES): This drug was given to some women to prevent miscarriage between 1940 and 1971 is found that may be acause for cervical cancer.Family history of cervical cancer may play arole as a risk factor for the disease(20)

The Papanicolaou (Pap)test:- The Pap test is the main screening test for cervical cancer and pre-cancerous changes.Although the Pap test has been more successful than any other screening test in preventing a cancer,it is not perfect.One of the limitationsof the Pap test is that it needs to be examined by human,so an accurate analysis of the hundreds of thousands of cells in each sample is not always possible. Conventional cytology is one way to smear the sample directly on to a glass microscope slide,which is then sent to the laboratory .For about 50 years, all cervical cytology samples were handle this way.This method works quite well and is relatively inexpensive , but it does have some drawbacks.One problem with this method is that the cells smeared onto the slide are sometimes piled up on each other,making it hard to see the cells at the bottom of the pile.(23). The most widely used system for describing Pap test results is the Bethesda System (TBS).There are 3 main categories ,some of which have sub-categories :

- Negative for intraepithelial lesion or malignancy,
- Epithelial cell abnormalities ,and
- Other malignant neoplasms

Negative for intraepithelial lesion or malignancy :-
This first category means that no signs of cancer , precancerous changes ,or other significant abnormalities were found .

Epithelial cell abnormalities :-
This second category,means that the cells lining the cervix or vagina show changes that might be cancer or pre-cancerous condition .This category is divided into several group for squamous cells and glandular cells.

The epithelial cell abnormalities for squamous cells are:-

- Atypical squamous cells(ASCs).
- Squamousintraepitheliallesions includes low grade Squamous intraepithelial lesions(SIL) (Koliocytosis&CIN1) and high grade Squamous intraepithelial lesions(CIN2 &CIN3).
- Squamous cell

Glandular cell neoplasminclude Atypical glandular cell and Adenocarcinoma.

Pap testing is not recommended for women 20 years old or younger,but if they do have a Pap test that show low grade SIL,they should have repeat Pap test in ayear.If they have a high –grade SIL,colposcopy is recommended.(14,15).

Colposcopy: Colposcopy(AncientGreek:Kolpos‘‘hollow,womb,vagina‘‘+skopos‘‘look at_‘)is medical diagnostic procedure to examine an illuminated, magnified view of the cervix and the tissues of the vagina and vulva.(Bosch, F. X. et al., 2008). Many premalignant lesion and malignant lesions in these areas have discernible characteristic which can be detect through the examination. The procedure was developed in 1925 by the german physician Hans Hinselman, with help from Dr. Helmut Wirths(Castellsague, X. et al., 2002). Is a binocular operating microscope with magnification of between 5 and20 times .It has been used to examine the cervix detail to identify CIN and preinvasive cancer.Usually acolposcopic-directed biopsy will be taken from the most abnormal area of the epithelium to confirm the diagnosis(American Cancer Society, 2012). Acetic acid also known as Glacial acetic acid,acetasol,ethylic acid and acetate.It is acolourless pungent liquid,miscible with water,widely used in the manufactured of acetic anhydride ,also in the colposcpic screening of the cervical cancer (Grubb, C. et al., 1997).

Sciller test:- Sciller test identifies normal squamous epithelium,normal,mature squamous epithelium contains abundant glycogen that stain dark brown with iodine ,the test involves the application of lugols iodine solution to the ecto cervix .The normal squamous epithelium will stain dark brown, whereas columnar epithelium, abnormal squamous and immature normal squamous epithelium are not. However conventional cytology diagnosis is often hampered by high false-negative rates and inter observer variability.(28)High risk HPV(HR-HPV)DNA testing is considered the most sensitive indicator of high –grade cervical lesions ,and the combination of HPV testing and the Pap test has a sensitivity and negative predictive value of almost 100%(29,30) HR_HPV infection, although found in almost all cervical cancer(30),is recognized as a necessary but insufficient cause of cervical carcinogenesis,in fact ,that HR-HPV infections may be self-limiting and can spontaneously regress.(31) Several types of HPV that cause subclinical infection and dysplasia can develop in to cervical cancer .However, cervical cancer is extremely rare among young adults because the immune response is effective in most cases. Early changes are found on Pap smear results and can be treated before cancer develops. Regular Pap smears combined with appropriate follow up treatment can practically eliminate the risk of developing cancer.

Polymerase Chain Reaction(PCR):-PCR is a selective target amplification assay capable of exponential and reproducible increase in the HPV sequences present in biological specimens. (24). The sensitivity and specificity of PCR-based methods can vary, depending on the DNA extraction procedures, site and type of clinical sample, sample transport and storage,primer sets,the size of the PCR product, reaction conditions and performance of the DNA polymerase used in the reaction, the spectrum of HPV DNA amplified and ability to detect multiples types. Most laboratories use PCR assays, which utilize consensus primers, directed to a conserved L1 gene, and hence able to detect all mucosal HPV types(25).PCR can theoretically produce one billion copies from a single double- stranded DNA molecule after 30 cycles of amplification. Therefore, care must be taken to avoid false–positive results derived from cross- contaminated specimens or reagents. Several procedures are available to avoid this problem while using PCR protocol for HPV DNA detection (32). The sensitivity and specificity of PCR-based methods can vary, depending mainly on the primers set ;the size of the PCR product;reaction conditions and performance of the DNA polymerase used in the reaction ;the spectrum of HPV types amplified, and ability to detect multiple types; and availability of a type specific assay. With the latter,very high sensitivities and specificities can be achieved,though detection of a wide spectrum of HPV types has been the preferred tool to generate the attributed disease risk by HPV (33) .

Aim of the study

- To verify the correlation between Pap smear, colposcopic directed biopsy,and HPV detection in symptomatic women.
- To compare the efficiency of adding histoimmune assay for further detection of HPV

Materials and method

We analyzed a consecutive series of 100 cervicovaginal samples obtained from patients referred to the Iraqi National Cancer Research Center, in the period from Sep.2011 to Dec. 2012 for clinical or colposcopic suspicion of HPV infection or previous abnormal Pap test results.Written informed consent was obtained from all patients which included the following:- Name, age, religion,address,occupation,age at menarche,age at menopause,marital status,age at marriage,age at first pregnancy,total pregnancies,number of abortions,methods of contraception,menstrual period regularity ,causes behind the visit,Gynecological history(did she have a pap smear or colposcopic examination before) and the date of pap test.

- The median patient age was 37 years ranging from 18-60
- The cytologic specimens were interpreted by using the 2001 Bethesda reporting
- The results were classified as follows:- Negative for intraepithelial lesion or malignancy, atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion(LSIL), high-grade squamous intraepithelial lesion(HSIL),and invasive squamous cell

All women were subjected to a detailed history,clinical examination, cervical cytology (Pap smear), colposcopic examination for those with abnormal Pap smear results, and HPV detection by PCR (Polymerase Chain Reaction)method. Each woman was placed in a dorsal position with her legs flexed at the hip and knee and abducted.The Cusco‘s bivalve speculum which was not lubricated then passed and fixed to visualize the cervix under a bright light source.The hook end of the Ayres‘spatula was then inserted at the external os and swept through 360 degrees) rotatory movement either in a clockwise direction to scrape the entire squamocolumnar junction of the transformation slides and promptly immerse into 95% alcohol for fixation. The smears were stained by the Papanicolaou method and read by a pathologist.The cytology report of the smears therefore read any of the following:(Normal,Low grade squamous intraepithelial lesion (LGSIL),High grade squamous intraepithelial lesion(HGSIL),Atypical squamous cell(ASC),Atypical glandular cell of uncertain significance (AGCUS). The brush was inserted 1-1.5 cm into the cervical os until the largest outer bristles of the brush touched the ectocervix ,it was rotated 3 full turns in a counterclockwise direction,brush was removed from the canal ,and inserted to the bottom of transport tube,sampler shaft was then snapped off at ascore line,leaving the brush inside the tube.tube was recapped securely by snapping it in a place,cervical specimens were sent to the testing laboratory(specimens could be held at room temperature for 2 weeks). A cervical swab collected with cyto brush was immersed in a sterile flask containing 1 ml DNAzol(Invitrogen) and sent to the PCR laboratory Some of the specimens were sent to Central Heath Laboratory, and most of them were examined by a private laboratory.The next step in our study is that any abnormal Pap smear result underwent colposcopic examination.A colposcopy is used to identify visible lesions suggestive of abnormal tissue, Its functions are as an alighted binocular microscope to magnify the view of the cervixvagina and vulvar surface, low power may be used to obtain a general impression of the surface architecture. The higher power is often necessary to identify certain vascular patterns, that may indicate the presence of more advanced precancerous or cancerous lesions.Colposcopy is performed with the patient lying back(the position known as dorsal lithotomy position).A speculum is placed in the vagina after the vulva is examined for any suspicious lesions.Three percent acetic acid is applied to the cervix using a cotton swab .The area of aceto whiteness correlates with higher nuclear density.The transformation zone is the acritical area on the cervix where many precancerous and cancerous lesions most often arise.The ability to see the transformation zone and the entire extent of any lesion visualized determined whether an adequate colposcopic examination is attainable. Areas of the cervix that turn white after the application of acetic acid or have abnormal vascular patterns are often considered for biopsy.If no lesions are visible an Iodine solution(Lugol‘s or Schiller‘s) is applied to the cervix to help highlight areas of abnormality.After complete examination, we determine the area with the highest degree of visible abnormality and obtain biopsies from these areas(Schiller‘s negative) using along biopsy instrument such as a punch forceps, or a cotton swab applied to the cervix for hemostasis. The patient warred that we will expect to have a thin

coffee-ground-like discharge for up to several days after the operation of taking biopsy.Between the

100 patients collected there was one case of females under the age of 20,therefore the screening recommendation for adolescent require that the onset of sexual activity must be specifically identified and noted on the chartThere are a variety of special circumstances that would warrant the early onset of Pap testing .Adolescents who are known or suspected of being sexually abused and those with diseases or medical treatment that compromise the immune system warrant early Pap testing. The screening recommendation for adolescent requires that the onset of sexual activity must be specifically identified and on the chart as follow:- Patient age 18 years, Age of marriage15 years, Housewife,divorced ,parity zero,no history of contraception, irregular menstrual cycle ,last menstrual period was on 31/3/2012 ,Pap testing was on ,8/4/2012,the causes behind visit were post–coital bleeding and abnormal vaginal discharge,Pap smear results were chronic cervicitis and koliocytic changes which give suspicion of HPV infection.

Results

Table (2): ***The correlation between age group and HPV result + The correlation between years of marriage and HPV results***

Age group(years)	Total number		HPV(positive)		HPV(negative)	
Age group	NO.	%	NO.	%	NO.	%
16-19	1	1.0	0	0	1	1.0
20-29	20	20.0	0	0	20	20.0
30-39	***30***	***30.0***	***1***	***1.0***	***29***	***29.0***
40-49	23	23.0	0	0	23	23.0
50-60	26	26.0	0	0	26	26.0
Total No.	100	100%	100	100%	100	100%

Table 3 ***: HPV by PCR, HPV by immune histochemistry***

Age of patients at marriage	Total NO.		HPV +ve		HPV --ve		HPV +ve	HPV--ve
	N0.	%	N0.	%	N0.	%	N0.	%
≥20	55	55.0	0	0	55	55.0	6	13.33
20-29	41	41.0	1	1.0	40	40.0	3	6.66
≥30	4	4.0	0	0	4	4.0	1	2.22

The maximum age group was 30—39 years, within this age group the HPV +ve case was found.Total no. of caese 100 case all subjected to PCR assay ,only 45 case were subjected to immune histochemical assay

Table 4 ***: Results of Pap smear***

Results of Pap smear	No	%
Cervicitis	30	30.0
Squamous metaplasia	25	25.0
Koliocytic changes	25	25.0
Dysplastic changes	20	20.0
Total No.	100	100%

Dysplastic changes type	ASC-US	1	5.0
	C1N1	16	80.0
	C1N2	2	10.0
	C1N3	-	-
	CaCx	1	5.0
Total No.		20	100%

Table shows the Pap smear findings and their percentages of 100 cases.

Table 5: ***Correlation between the clinical signs and symptoms + HPV results***

Symptoms	Total number		HPV by PCR		HPV by immune histochemical	
			Positive		positive	
	No.	%	No.	%	No.	%
Post coital bleeding	43	43.0	1	2.32	8	17.77
Abnormal vaginal discharge	54	54.0	1	1.58	2	4.44
Inter menstrual bleeding	3	3.0	0	0	0	0
Total No. 100	100%	100	100%		45	100%

Table shows that from the 100 cases subjected to the PCR test there was only one case give HPV positive presented with post coital bleeding and abnormal vaginal discharge, while 10 cases were HPV positive by immune histochemical test of total number were 45 cases.

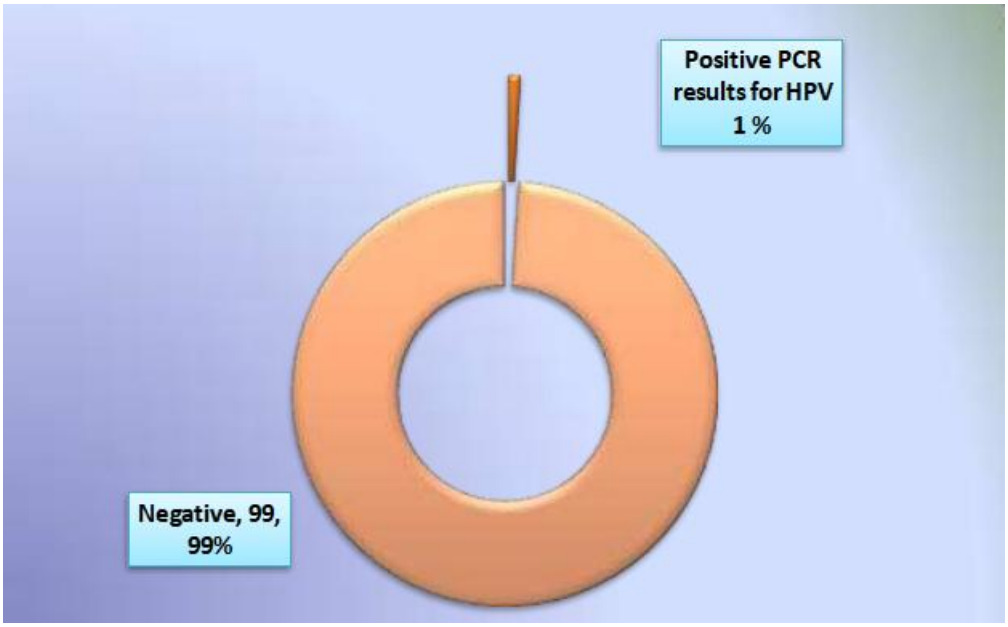

Figure 1: ***PCR results of HPV***

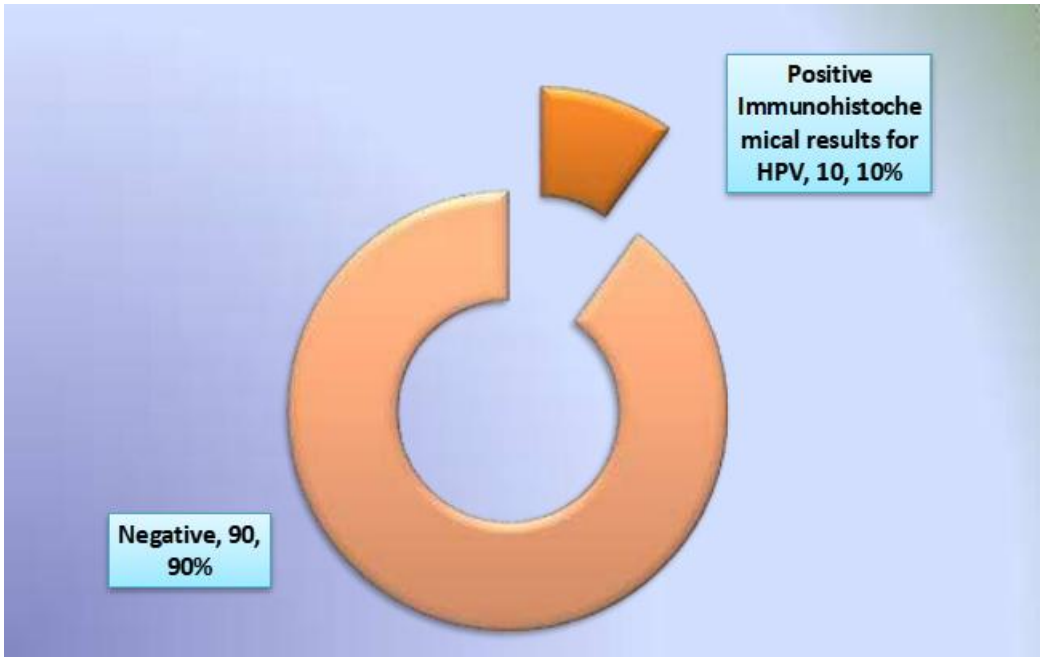

Figure 2 : *Immune histochemical results of HPV*

Table 6: *Correlation between Pap smear and colposcopic dysplastic findings*

Pap smear findings		Colposcopy directed biopsy findings					
		ASC-US	C1N1	C1N2	C1N3	CaCx	None
	ASC-US	-	-	-	-	-	1
	C1N1	-	7	-	-	-	9
	C1N2	-	1	1	-	-	-
	C1N3	-	-	-	-	-	-
	CaCx	-	-		-	1	-

Table 7: *Correlation of Parity and combined Oral Contraceptive pills with +Ve HPV findings.*

Parity	Total number		HPV +v by PCR		HPV by immunhistochemical	
	N0.	%	N0.	%	N0.	%
P 0	7	7.0	--	--	--	--
P 1	20	20.0	--	--	--	--
P 2	21	21.0	--	--	3	6.66
P 3	27	27.0	--	--	1	2.22
P 4	12	12.0	1	8.33	5	11.11
P5 and more	13	13.0	--	--	1	2.22
Combined oral contraceptive pills user(C.O.C.P)	80 cases	80.0	1	1.25	8	17.77
Total No.	100	100%	100	100%	45	100%

This table shows the relation between parity,combined oral contraceptive(C .O .C.P) user and the HPV positivity .Table showed that there was no significant correlation between the increasing the parity and the rate of HPV infection ,but significant relation with the C.O.C.P user.

Table 8: ***Correlation between the Pap smear findings and colposcopic directed tissue biopsy results***

Findings in	Pap smear findings		Immunhistochemical Findings	
	NO.*	%	NO.**	%
ASC-US	1	1.0	0	0
LSIL (CIN1,KOLIOCYTOSIS)	41#	41.0	18	40.0
HSIL (CIN2,CIN3)	2	2.0	1	2.22
SQUAMOUS CELL CARCINOMA	1	1.0	1	2,22
NEGATIVE	55	55.0	25	55.55

*Total No. of cases subjected to Pap smear test which were 100 cases,** total No. of cases subjected to colposcopy directed biopsy which were 45 cases. # Total No. of LSIL which included 16 cases of CIN1 and 25 cases of kiolocytic changes by Pap smear.

Table 9: ***Correlation between Pap smear ,colposcopy directed biopsy and immune histochemical results of HPV.***

		Immunohistochemical results for HPV			
		Positive		Negative	
		No*	%	No	%
Pap smear findings					
Koliocytic changes*	Yes	6	60.0	19	21.1
	No	4	40.0	71	78.9
Dysplastic changes*	Yes	5	50.0	15	16.7
	No	5	50.0	75	83.3
Colposcopy findings					
Koliocytic changes*	Yes	5	50.0	5	5.6
	No	5	50.0	85	94.4
Dysplastic changes*	Yes	4	40.0	6	6.7
	No	6	60.0	84	93.3
*Significant at 0.05 level using Pearson Chi-square test (P<0.01)					

*Total No. of cases which were 45 cases out of 100 cases presented with abnormal Pap smear and then subjected to colposcopy directed biopsy. From 25 cases of kiolocytic changes by Pap smear only 10 case were found by colposcopy directed biopsy, and only 10 cases out of 20 were presented with dysplastic changes by colposcopy directed biopsy. As showed there was significant relation between the results of HPV positivity by immune histochemical assay
.

Table (10):*Correlation between Pap smear findings, colposcopy directed biopsy and HPV*

	Pap smear number&%		Colposcopic directed biopsy	number&%		HPV +ve by PCR		HPV+ve by immune histochemical	
	N0.	%*		No.	%**	No.	%	No.	%
ASC-US	1	1.0		0	0	0	0	0	0

LSIL (CIN1,kolio-cytosis)	41	41.0		18	40.0	1	2.22	8	17.77
HSIL (CIN2,3)	2	2.0		1	2.22	0	0	1	2.22
Squamous cell carcinoma	1	1.0		1	2.22	0	0	1	2.22
Negative	55	55.0		25	55.5	--	---	--	--
Total No.	100	100%		45	100%	100	100%	45	100%

This table shows that one case with ASC-US by Pap smear with negative case by tissue biopsy and– ve HPV results ,one case of positive HPV detection by PCR compare to (8)cases by tissue biopsy within(LSIL) group, Negative HPV detected by PCR and one positive case by tissue biopsy in(HSIL)showed negative HPV by PCR compare to one positive case by immune histochemical assay.

Discussion

The prevalence and incidence of cervical cancer has decreased worldwide. This decrease is due to the introduction and improved penetration of cervical cancer screening programs, the quality and reliability of which are further increased by HPV testing .(53) Because HPV infections are progressively becoming a major subject in gynecological malignancy and it is the most dangerous risk factor for most common genital tract tumors we tried to point the light on this problem in our country, especially with increasing pre-invasive or invasive cervical carcinoma. In this study, we focused on the feasibility of PCR HPV detection in consecutive series of cervico vaginal samples processed by conventional Pap smear technology ,in parallel with the colposcopic directed biopsy , and to determine the benefit of detection HPV by histo immune assay and compare it to PCR HPV detection. Precancerous and cancerous lesions of the uterine cervix have been the subjects of many investigators. The most interesting component of the Bethesda System, which followed in this study ,is the division of smears showing cytological evidence of CIN or HPV into two categories: high grade and low- grade SIL.AL-ALwan(34,35,36).

PCR HPV versus cytological examination (Pap smear):-In this study the total CIN group which were 41 cases with an incidence of 41%, only one case of HPV by PCR was positive which mean that among all cases of CIN the incidence of HPV by PCR was 1% .In LGSIL which constitutes only 1% of total CIN showed a positive PCR HPV with incidence of 1% ,while there was no case of positive PCR HPV in HSIL and invasive cancer. When a correlation of cervical cytology was made with HPV positivity it was observed that 1% of cases who had LSIL in cervical cytology were positive for high risk HPV by PCR ,our findings are not in close conformity with the data given by the University of Pittsburg (Infectious Diseases and Microbiology Graduate School of public health).According to them ,HR HPV is present in more than 95% cases of invasive carcinoma ,79—90% cases of HSIL ,50—75% case LSIL ,and in 2—5% of normal smears(54). In our study the Pap smear cytology of 100 cases 55% had inflammatory conditons,1% ASC—US ,41% with LSIL ,HSIL 2% and squamous cell carcinoma 1%, there was one case withHPV +ve finding represented with the LSIL group.

PCR HPV versus Histoimmuneassay for HPV:- The relation between HPV (PCR) and chemical histoimmune HPV detection. Our study ,showed that there was 1% positive HPV by PCR in LSIL, while with histoimmune assay the incidence of HPV positivity was 17.7% in(LSIL)within 100 cases.Also the incidence of HPV positivity by histoimmune assay was 2.22% in patients with HGSIL compared to no positive case with PCR HPV method of detection in HGSIL .According to this data we can say that using the PCR only is specific for HPV diagnosis in our study.The histoimmuneassy for HPV was done for all the patients presented with abnormal Pap smears. The incidence of abnormal Pap smears was 45% of the total number 100 cases .These abnormal Pap smears were proved by colposcopic directed biopsy .In our study we reported (1%) squamous cell carcinoma of the cervix out of total group studies(TGS) .The

diagnosis was suspected clinically and smears were taken together with colposcopy directed biopsies to confirm the diagnosis. there was no case of adenocarcinoma reported .Our findings were similar to the frequency of AL-ALwan reported in 1987 which was(1.4%) of (TGS) of squamous cell carcinoma(38). This could be attributed to that in Islamic countries the circumcision art the strict observance of religion and the presence of principles and laws that prevent illegal relationships and extramarital relations may explain the lower incidence of cervical cancer in most Islamic countries compared to Western countries(37,38,.39,40,41, 42) Among these 45% of cases which undergone colposcopy directed biopsy, all had been subjected to histoimmune study . This discrepancy in the detection of HPV by this two method in our data will make us think several times before sending our patients for HPV detection by PCR.

Pap Smear Versus Colposcopic Directed Biopsy:-In Table (8)we compare Pap smear results of 100 cases and colposcopy directed biopsy. The number of patients subjected to colposcopic directed biopsy was 45 cases with an incidence of 45%. We found that by Pap smear there was only one case of ASC-US which turned to be negative by colposcopic directed biopsy. No.of cases with LSIL were 41 cases by Pap smear with an incidence of 41%,which turned to have the same findings colposcopic directed biopsy at 40%. Among patients with HSIL by Pap smear(total no.2 cases)with an incidence of 2%,turned out to have the same finding with colposcopy directed biopsy with an incidence of 2.22%.The incidence of total CIN in the present work forms 20% of total group studies according to the cytological diagnosis.CIN-1were(80%),CIN-2 were (10%) and CIN-3were(0%) from tptalCIN.Our findings are nearly similar to those detected by a thesis informed by Dr.SamiraSuhiel supervised by Prof.Dr.Nada A.AL-ALwan 2002.(37).

Concerning (COCP) the present analysis of our research showed a strong correlation between COCP&HPV infections. The only case which was HPV positive by PCR was on COCP for about 2 years while patients with HPV by histoimmune were 10 on COCP. In the present study the frequency of squamous cell carcinoma compared to other studies is less than the findings by Dr. Issraa Ali supervised by Prof.Nada A- ALwan2005.but lower than that reported by (AL- ALwan,1987),AL-Anbari-2002and Jayant et .al 1995 who reported)(1.4% of TGS) ,25% of TGS respectively.(45,46,47) Therefore ,it is important for early detection and screening for precancerous cervical lesions.AL-ALwan observed in her preliminary report on comparing the performance of colposcopy ,cervical cytology and human papillomavirus detection as screening tools for cervical cancer ,that although the colposcopic failure rate was higher than that of cytology ,no lesion was missed when both methods were used in concert . Also she observed that the efficacy of cytological screening could be improved by papillomavirus detection by PCR .(48) Although each of these methods is associated with a certain margin of error, the colposcopic failure rate remains higher than that of a good cytology test, simply because of 10%--15%(44.4% in this study) compared to 13% of N.A.S.AL-ALwan(49) of atypical lesions are situated deep into the cervical canal out of reach of the colposcope (49,50) Thus,it has been reported that the accepted rate of colposcopic accuracy in the detection of cervical cancer precursors does not exceed 80% (49,50). Errors when using the conventional Pap cytology are inevitable ,despite the excellent performance of the smear in reducing morbidity and mortality from cervical cancer over the past 40 years (49,51,52).The sources of error begin with cell sampling and relate to many aspects of sources of error begin with cell sampling and relate to many aspects of specimen preservation, slide preparation and staining (49,53).In our study all of the patients with squamous cell carcinoma (SCC) on Pap smear ,proved to have (SCC) by histopathology and similar findings were found in patients with CIN3,CIN2,CIN1 on Pap smear ,proved by histopathology ,these findings were under Dr.Samira study 2002.

Conclusions

Our study showed that HPV infections detected by cervical cytology are susceptible to
Both methods of detection of HPV(PCR and histoimmune assay)are complex laboratory measures requiring numerous processes by humans and machines.
Our study showed that histoimmune detection of HPV was higher in number compared to the PCR detection of HPV.

Our results of Pap smear and colposcopy directed biopsy were strongly
We know that the PCR has higher sensitivity in the detection of HPV infection ,so this result needs to be assessed thoroughly about the accuracy of such a method in our country.

Recommendation

More concentration on the method for assessment of the HPV by PCR since it is the most sensitive method of detection of such virus.

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