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Human Papillomavirus DNA Versus Papanicolaou Screening Tests for Cervical Cancer in a Sample of Iraqi Patients

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Abstract: Background: To determine whether testing for DNA of oncogenic human papilloma viruses (HPV) is superior to the Papanicolaou (Pap) test for cervical cancer screening. We report here the first screening round of the Iraqi Cervical Cancer Screening Trial. **Setting:** This study has conducted at Baghdad Teaching Hospital, Department of Obstetrics and Gynecology. **Study Design:** A Prospective study. **Patient and Methods:** The study population consist of 60 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. We compared HPV testing and conventional pap testing with that of colposcopy and punch biopsy. **Results:** The number of women enrolled at the study, was 60 women. The mean age at enrollment was 39. 78 year and standard deviation is 8. 912. The minimum age at marriage, the maximum age, was12 and 42 respectively. The mean was 20. 87 and std. deviation was 5. 990. Parity of the women enrolled at the study, the minimum was 0, the maximum was 11, the mean was 4. 05, and standard deviation was 2. 480. The number of women used OCSPS in our study is 39 (65%) and 21 women(35%) out of 60 did not. Sixteen women (26. 7%) were smoker and 44 (73. 3%) were not smoker. Manual workers between women in our study were 32 (53. 3%), clerks were 22 women (36. 7%) and health workers number were 6 (10. 0). Sexual transmitted diseases (as documented clinically or by culture, serological and immunological tests) was reported in 14 women (23. 3%). Condom using by male partner was reported in 26 (43. 3%) and in 34 women (56. 7%) condom was not used by the women's partner. By conventional pap test 7 cases (13. 2%) out of 53 was positive [6 cases (85. 7%) were diagnosed as CIN II, CINIII or CA (3 cases were diagnosed as HSIL by pap found to be sequmous cell cacinoma by biopsy and one case diagnosed as cacinoma by pap test and biopsy) &only one case (14. 3%) was normal (table10). Pap test was negative in 46 cases out of 53 total (86. 8 %), 21 cases (45. 7%) were diagnosed as CINII or CINIII and 25 cases (54. 3%) of the negative pap proved to be normal or CINI by biopsy. **Conclusion: -**1. HPV DNA testing has a better sensitivity than, that of cytology. Sensitivity of the test was 89%. 2. Combination of HPV DNA test with Pap test increase the sensitivity of Pap from 22% to 89%. 3. HPV types 16, 18, 11 &6 has been detected in our community by using ISH technique, and HPV types 16 &18 (high risk)were statistically significantly associated with HSIL & Cancer (P value=0. 010), while HPV 6&11(low-risk) were not associated with these lesions(P value =0. 318, 0. 669)respectively.

Keywords: HPV DNA test, Pap test, Sensitivity.

INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide, after breast cancer, with an estimated 529, 828 new cases and 275128 deaths in 2008as shown in table (1). About 86% of the cases occur in developing countries, representing 13% of female cancers (Szentirmay, Z. *et al*., 2007). Worldwide, mortality rates of cervical cancer are substantially lower than incidence with a ratio of mortality to incidence to 52% (Koliopoulos, G. *et al*., 2007). The majority of cases are squamous cell carcinoma and adenocarcinoma are less common (Lytwyn, A. *et al*., 2000).

Table 1: Key statistics in the World

Population	World	Developing regions	Developed regions
Women at risk for cervical cancer (Female population aged >=15 years)in thousand	2336986	1811, 867	525120
Annual number of new cases of Cervical cancer	529828	453321	76507
Annual number of Cervical cancer death	275128	241969	33159
Projected number of new Cervical cancer in 2025	720060	668875	81868

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Projected number of Cervical cancer in 2025	395095	380653	38291

Human Papillomavirus and Related Cancers, Summary Report Update. Novemberb15, 2010.

Iraq has a population of 8. 21 millions women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 311 women are diagnosed with cervical cancer and 212 die from the disease (Table 2). Cervical cancer ranks as the 10th most frequent cancer among women in Iraq, and the 7th most frequent cancer among women between 15 and 44 years of age as shown in figure(1&2)(Agorastos, T. *et al*., 2000).

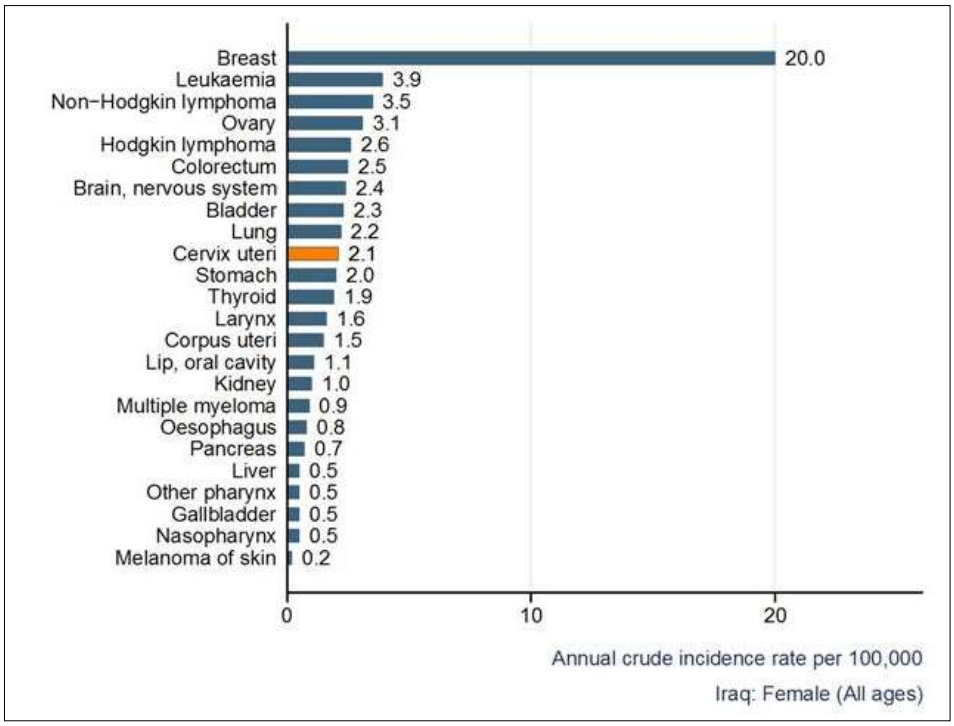

Figure 1: Incidence of cervical cancer compared to other cancers in women of all ages in Iraq. Annual crude incidence rate per 100, 000, Iraq: Female (All ages), Data source: IARC, Globocan, 2008.

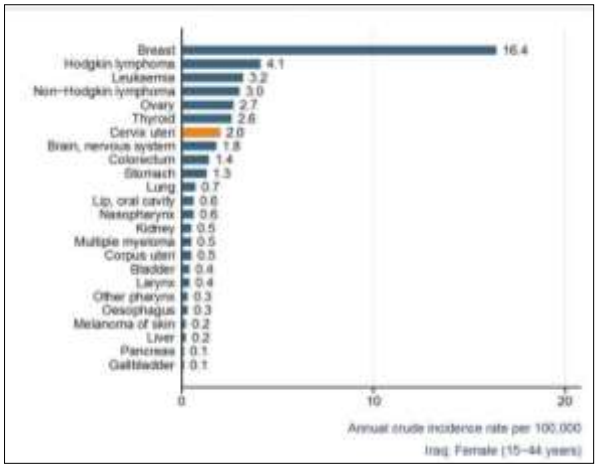

Figure 2: Age- specific cervical cancer incidence compared to age- specific incidence of other cancers among women 15-44 years of age in Iraq. Annual crude incidence rate per 100, 000. Iraq: Female(15-44 years). Data sources: Iarc, Globocan 2008.

According to WHO data the projected number of new cases of cervical cancer in Iraq by age group in 2025 is 568 cases, so there will be increase by more than 72% in age group(+65years) and 85% in age group(0-64 years) than cases reported in 2008(Koliopoulos, G. *et al*., 2007).

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Mortality: Cervical cancer mortality compared to other cancers in women of all ages in Iraq is 11th cause of death and 8th most frequent among women 15- 44 years of age in Iraq (figure 3&4) (Agorastos, T. *et al*., 2000).

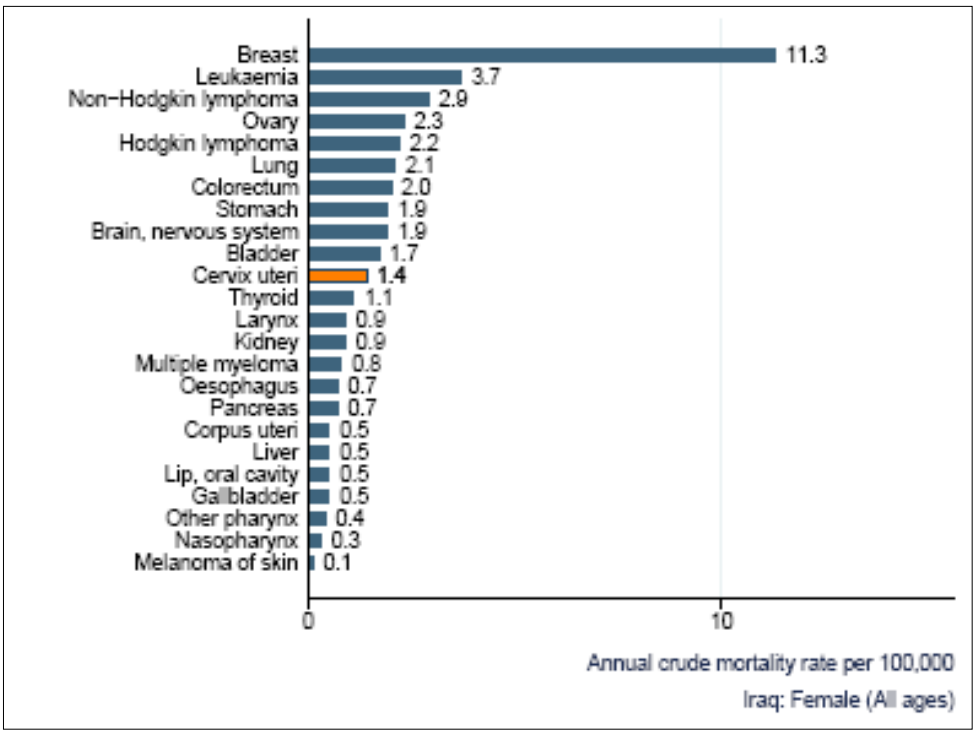

Figure 3: Cervical cancer mortality compared to other cancers in women of all ages in Iraq. sources: IRAC, Globocan, 2008.

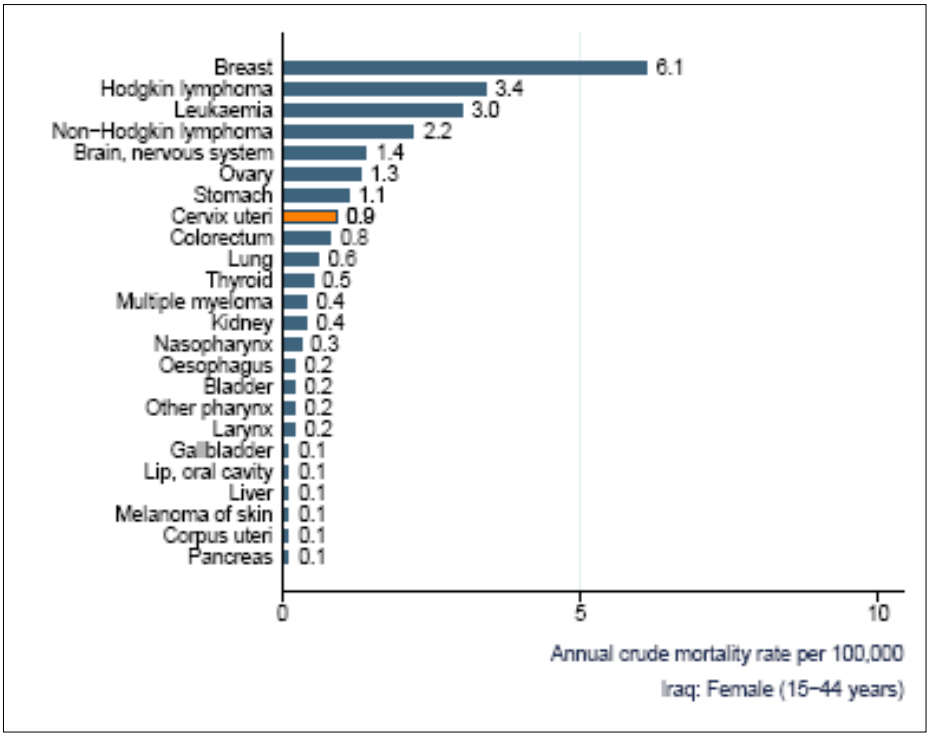

Figure4: Age-specific mortality rates of cervical cancer compared to age-specific mortality rates of other cancers among women15-44years of age in Iraq. Data sources: IRAC, Globocan, 2008

Estimated number of deaths of cervical cancer in Iraq by age group in 2008 is 212 in 2008 and projected cases in 2025 will be 393, that is mean 91% increase in rate of death in age group(0-64 years) and more than 70% increase in age group(+65 years) (Agorastos, T. *et al*., 2007).

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Histologic Types:

Squamous Cell Carcinoma: The two most common histologic subtypes of cervical cancer are squamous cell and adenocarcinoma. Of these, squamous cell tumors predominate, comprise 85%of all cervical cancers, and arise from the ectocervix. Over the past 30 years, there has been a decrease in the incidence of squamous cell cancers and an increase in the incidence of cervical adenocarcinomas. These changes may be attributed to an improved method of screening for early squamous lesions of the cervix and an increase in the prevalence of HPV (Kitchener, H.C. *et al*., 2009).

Adenocarcinoma: In contrast to squamous cell cervical carcinoma, adenocarcinomas comprise 10 to 15 % of cervical cancers and arise from the endocervical mucus-producing glandular cells. Because of this origin within the endocervix, adenocarcinomas are often occult and may be advanced before becoming clinically evident(Kitchener, H.C. *et al*., 2009). It exhibit a variety of histologic patterns composed of diverse cell types Of these, *mucinous endocervical adenocarcinomas* are the most commin. *Endometrioid adenocarcinomas* are the second most frequently identified and display glands resembling those of the endometrium. *Minimal deviation adenocarcinoma* is characterized by cytologically bland glands that are abnormal in size and shape. They contain an increased number of glands positioned at a deeper level than normal endocervical glands(Kitchener, H.C. *et al*., 2009).

Mixed Cervical Carcinomas: These cervical malignancies are rare and histologically classified as adenosquamous, adenoid cystic, adenoid basal epithelioma, and glassy cell carcinoma. *Adenosquamous carcinomas* do not differ grossly from adenocarcinomas of the cervix. The squamous component is poorly differentiated and shows little keratinization. *Glassy cell carcinoma* describes a form of poorly differentiated adenocarcinoma in which cells display cytoplasm with a ground-glass appearance and a prominent nucleus with rounded nucleoli. *Adenoid cystic carcinoma* usually presents as a hard friable mass. Histologically this tumor resembles adenocarcinoma with adenocystic differentiation. Lastly, of this rare group of mixed tumors, *adenoid basal epitheliomas* typically behave in a benign fashion. Histologically, these tumors are characterized by nests and cords of small oval cells with a peripheral palisading arrangement(Kitchener, H.C. *et al*., 2009).

Neuroendocrine Tumors of the Cervix: These malignancies include large cell and small cell tumors of the cervix. Large cell neuroendocrine tumors are highly aggressive and even early stage cancers have a relatively low disease-free survival rate despite treatment with radical hysterectomy and adjuvant chemotherapy. In contrast, small cell neuroendocrine carcinoma contains a uniform population of small cells with a high nuclear: cytoplasm ratio and resemble small cell carcinoma of the lung. Uncommonly, endocrine and paraendocrine tumors are associated with these neuroendocrine tumors(Kitchener, H.C. *et al*., 2009).

Other Malignant Tumors: Rarely, the cervix may be the site of sarcomas and malignant lymphomas. Most of these tumors present as a bleeding cervical mass. Initially, differentiation of cervical sarcomas from primary uterine sarcoma requires careful pathologic examination and localization of the tumor's primary bulk. Cervical leiomyosarcomas and cervical stromal sarcomas have a poor prognosis, similar to uterine sarcomas. Because these tumors are rare, statements regarding treatment of cervical sarcomas are limited. Most cases are managed with multimodality treatment(Kitchener, H.C. *et al*., 2009). In Iraq most frequent histological types of cervical cancer according to data from Ministry Of Health, Iraqi Cancer Board are shown in table. 2:

Table 2: Histological Classification of Cervical cancer in Iraq:

Histological types	Percentage
Squamous cell carcinoma	71. 02%
Adenocarcinoma	9. 09%
Carcinoma	1. 70%
Intraepithelial neoplasia of cervix	1. 14%

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Leiomyosarcoma		0. 57%
Adenosquamous carcinoma		0. 57%
Transitional cell carcinoma		0. 57%
Small cell carcinoma		0. 57%
Carcinoma undifferentiated		0. 57%
No histology		14. 20%

Applied Anatomy of Cervix: The Cervix: Is the lower fibro muscular portion of the uterus. It is cylindrical or conical in shape measuring 3to 4 cm in length, and 2. 5 cm in diameter. The portion of the cervix exposed to vagina is ectocervix or portiovaginalis. The cervix varies in size and shape depending on the women's age, parity and hormonal status. In parus women, it is bulky and the external os appears as wide, gaping, transverse slit where as in nulliparous women, the external os resembles a small circular opening in the center of the cervix. The external os opens into the endocervical canal, which is approximately 2 to 3 cm in length and opens proximally into endometrial cavity at the internal os. The space surrounding the cervix in the vaginal cavity is called the vaginal fornix, the part between the cervix and the lateral vaginal wall is the lateral fornix, and that between anterior and posterior walls of the vagina and the cervix are termed the anterior and posterior fornix, respectively. The stroma of the cervix is composed of dense, fibro muscular tissue through which vascular, lymphatic and nerves supplies to the cervix pass and form a complex plexus(Wheeler, C. M. *et al*., 2009). The arterial supply of the cervix is derived from internal iliac arteries through the cervical and vaginal branches of the uterine arteries. The veins of the cervix run parallel to the arteries and drain into the hypo gastric venous plexus. The lymphatic vessels from the cervix drain into the common, external and internal iliac nodes, obturator and parametrical nodes. The nerve supply to the cervix is derived from the hypo gastric plexus, the endocervix has extensive sensory nerve endings, while they are very few in the ectocervix (Wheeler, C. M. *et al*., 2009).

Squamous and Columnar Epithelia: Colposcopically, the squamous epithelium of the cervix appears as a featureless, smooth, pale pink surface. Blood vessels lie below this layer and therefore are not visible or are seen only as a fine capillary network. The mucin-secreting columnar epithelium of the endocervix appears red and velvety due to the proximity of blood vessels beneath the one-cell-layer-thick epithelium. The columnar epithelium is characterized by infoldings or clefts and is commonly referred to as "glandular". This is technically incorrect, as true glands, consisting of acini and ducts, are not present. Nonetheless, "glandular"abnormalities are reported in the Bethesda nomenclature for cervical cytology(Kitchener, H.C. *et al*., 2009).

Squamocolumnar Junction: During embryogenesis, upward migration of stratified squamous epithelium from the urogenital sinus and vaginal plate is thought to replace müllerian epithelium. This process usually ends at the external cervical os, forming the original squamocolumnar junction (SCJ). In a minority, such as those with in utero DES exposure, this migration is incomplete, leading to location of the SCJ in the upper vagina. The location of the SCJ varies with age and hormonal status. It everts outward onto the ectocervix during adolescence, pregnancy, and with use of combination hormonal contraceptives. It regresses into the endocervical canal with menopause and other low-estrogen states such as prolonged lactation and use of progestin-only contraceptives(Kitchener, H.C. *et al*., 2009).

The rise in estrogen at puberty leads to glycogenation of the nonkeratinized squamous epithelium of the LGT. Glycogen provides a carbohydrate source for lactobacilli, which dominate the normal vaginal flora in reproductive-aged women. The lactobacilli produce lactic acid, lowering the vaginal pH to less than 4. 5. The exposure of the columnar epithelium to this low pH stimulates squamous metaplasia, the conversion of one type of normal epithelium (columnar) into another (squamous). Squamous metaplasia is a normal process and occurs most

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actively immediately adjacent to the original SCJ, creating a zone of metaplastic epithelium termed the *transformation zone* (TZ), between the original SCJ and the columnar epithelium(Kitchener, H.C. *et al*., 2009).

Transformation Zone and Cervical Neoplasia: Nearly all cervical neoplasia, both squamous and columnar, develops within the transformation zone, usually adjacent to the new SCJ. Theoretically, cervical cells undergoing metaplasia are particularly vulnerable to the oncogenic effects of HPV and co-carcinogens. Metaplasia is most active during adolescence and pregnancy. This may explain why early age of sexual activity and first pregnancy are known risk factors for cervical cancer(Kitchener, H.C. *et al*., 2009).

Pathophysiology: Squamous cell carcinoma of the cervix typically arises at the squamocolumnar junction from a pre-existing dysplastic lesion, which in most cases follows infection with HPV (Kitchener, H.C. *et al*., 2009). Although most women readily clear this virus, those with persistent infection may develop preinvasive dysplastic cervical disease. In general, progression from dysplasia to invasive cancer requires several years, but wide variation exists. The molecular alterations involved with cervical carcinogenesis are complex and not fully understood. Uncovering these additional common molecular events has been difficult, and studies demonstrate vast heterogeneity. Accordingly, carcinogenesis is suspected to result from the interactive effects between environmental insults, host immunity, and somatic cell genomic variations (Kitchener, H.C. *et al*., 2009). 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 in HPV infection. They can lead to cytologic changes detected as low-grade squamous intraepithelial (LSIL) cytologic findings on Pap smears(figure5)(Kitchener, H.C. *et al*., 2009).

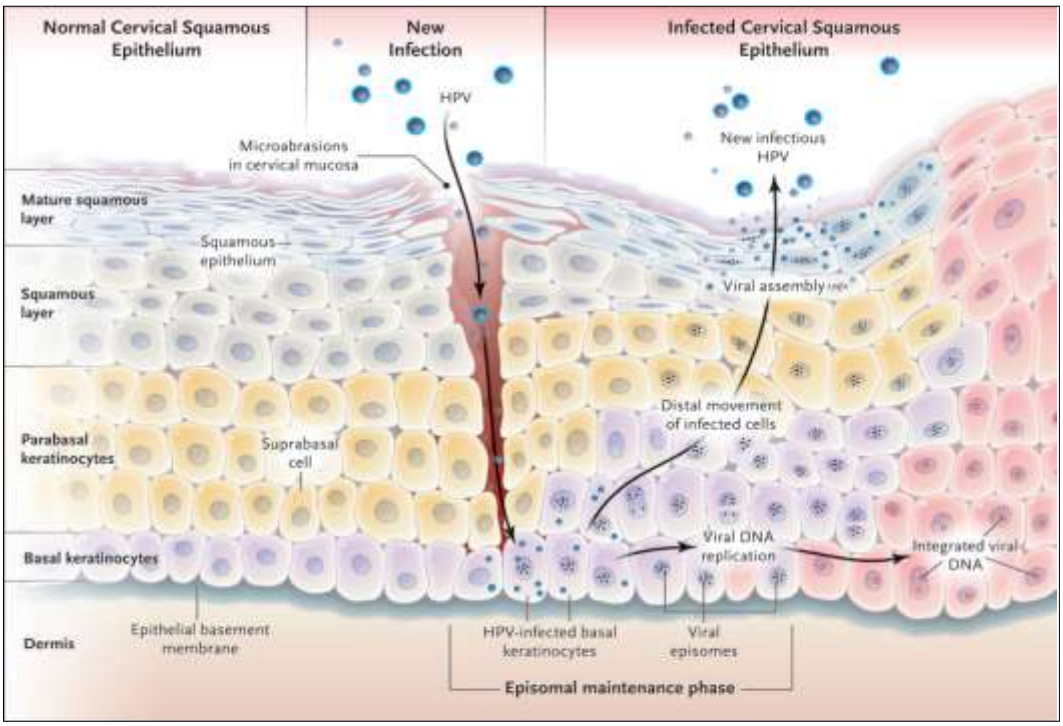

Figure 5: Human Papillomavirus Life Cycle in the Squamous Epithelium. Adapted from Frazer. 18The New England Journal of Medicine. Downloaded from nejm. org on June 14, 2011

Amplification of viral replication and subsequent transformation of normal cells into tumor cells may follow. Specifically, viral gene products E6 and E7 oncoproteins are implicated in this transformation (Figure 6). E7 protein binds to the retinoblastoma (Rb) tumor suppressor protein, whereas E6 binds to the p53 tumor suppressor protein. In both instances, binding leads to

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degradation of these suppressor proteins. The E6 effect of p53 degradation is well studied and linked with the proliferation and immortalization of cervical cells.

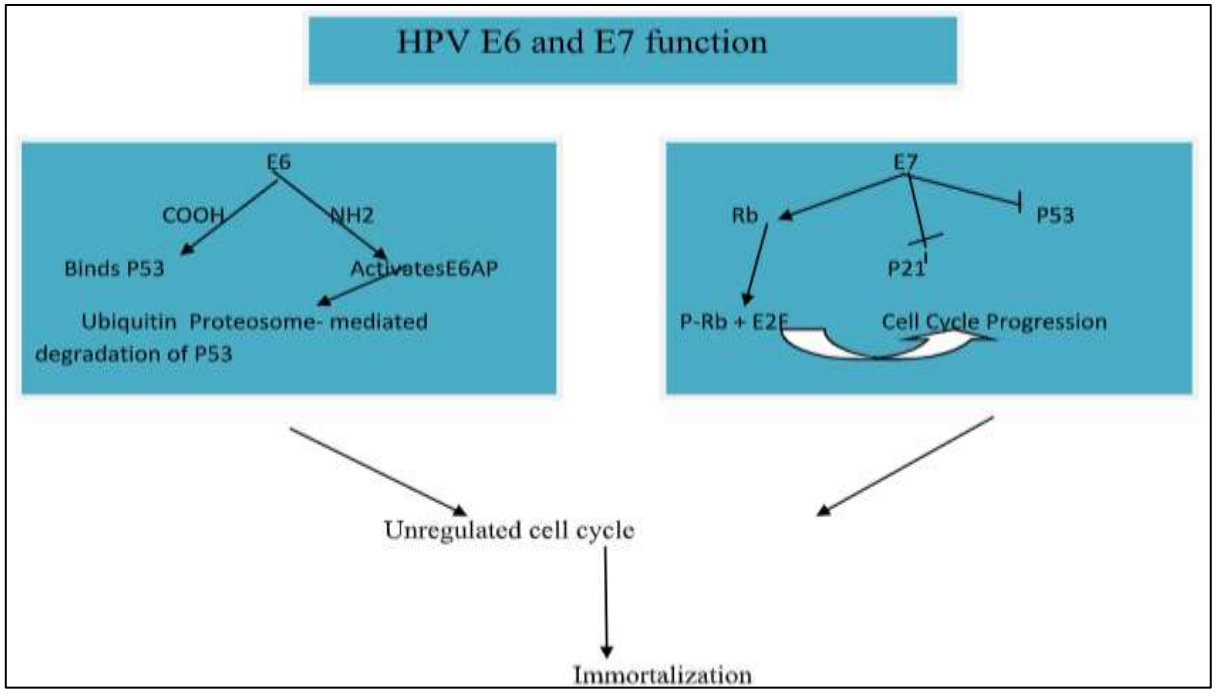

Figure 6: E6 and E7 oncoproteins and p53, p21, and retinoblastoma (Rb) tumor suppressor proteins.

On the left, viral oncoprotein E6directly binds p53 & activates E6AP to degrade p53 tumor suppressor protein. On the right, E7 oncoprotein phosphorylates Rb protein resulting in release of E2F transcription factors, which are involved in cell cycle progression. E7 has shown to downregulate p21 tumor suppressor protein production and to subvert p53 function. The cumulative effect of oncoproteins E6 and E7 eventually result in cell cycle alteration, promoting uncontrolled cell proliferation.

Risk Factors For cervical cancer: Identifiable risk factors for cervical intraepithelial neoplasia are useful in the development of cervical cancer screening and prevention programs (Table 3).

Table 3: Risk Factors for Cervical Neoplasia

Demographic risk factors
1. Ethnicity (Latin American countries, U. S. minorities)
2. Low socioeconomic status
3. Age
Behavioral risk factors
1. Infrequent or absent cancer screening Pap tests
2. Early marriage.
3. Multiple sexual partners
4. Male partner who has had multiple sexual partners
5. Tobacco smoking

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6. Dietary deficiencies
Medical risk factors
1. Cervical high-risk human papillomavirus infection
2. Parity
3. Immunosuppression

Age: In Iraq, the median age of cervical cancer diagnosis is the middle to late forties (Porras C, *et al.*, 2009), approximately a decade later than CIN. Theoretically, HPV infection in an older woman is more likely to be persistent than transient. Older age also allows accumulation of mutations that can lead to cellular malignant transformation. Additionally, decreased needs for prenatal care and contraception cause older women to access cancer prevention programs less often.

Behavior: The most consistently recognized behavioral risks for cervical neoplasia are noted in Table 3. Such behaviors increase the risk of acquiring oncogenic HPV infection. For many years, epidemiologic evidence has linked sexual behavior such as early marriage, multiple sexual partners, and male partner promiscuity with cervical neoplasia(Kitchener, H.C. *et al*., 2009).

Tobacco Smoking: Tobacco smoking increases the risk of cervical cancer among HPV-positive women. Nicotine and its major metabolite cotinine are found in the cervical mucus of women and in the semen of men who smoke. These chemicals may cause alterations that promote HPV-driven cellular transformation and neoplasia. In a case-controlled study, Becker and colleagues (1994) implicated current smoking, high-number pack-years of use, and smoking at the time of menarche as etiologic factors associated with neoplasia. According to WHO data the prevalence of smoking any tobacco by women in 2008 is 1. 9 and prevalence of cigarette smoking is 1. 3% (Agorastos, T. *et al*., 2005).

Dietary Deficiencies: Although data are inconclusive, dietary deficiencies of certain vitamins such as A, C, E, beta carotene, and folic acid may alter cellular resistance to HPV infection, promoting viral infection persistence and cervical neoplasia (Kitchener, H.C. *et al*., 2009).

Medical Risk Factors: Combination Oral Contraception (COC) and Parity: Study results linking cervical neoplasia and these risk factors are conflicting. It has been reported that steroid hormones found in COC may affect the HPV genome and increase viral expression of oncoproteins E6 and E7 (Manchanda, 2010). During pregnancy, immunosuppression and hormonal influences on cervical epithelium combined with trauma related to vaginal deliveries have been suggested as etiologic factors associated with the development of cervical neoplasia. However, analysis of young women enrolled in the Atypical Squamous Cell/Low-grade Squamous Intraepithelial Lesion Triage Study (ALTS) found that non injectable hormonal contraceptives, pregnancy, and parity had little effect on acquisition of high-risk HPV infection or development of CIN III (Ellerbrock, 2000). Moreover, epithelial cell cancers are generally not influenced by hormonal factors(Kitchener, H.C. *et al*., 2009). In Iraq, total fertility rate per women2. 8%, oral contraceptive use is 14. 6%(Agorastos, T. *et al*., 2005).

Immunosuppression: Studies consistently suggest that HIV-positive women have much higher rates of CIN compared with HIV-negative women (Abu, J. *et al*., 2005). . In women infected with HIV, up to 60% of Pap smears exhibit cytologic abnormalities and as many as 40% have colposcopic evidence of dysplasia. In addition, transplant recipients treated with immunosuppressive medications have a 5- 6% risk of developing a neoplasm after transplantation, and most of these neoplasms are associated with oncogenic DNA viruses(Kitchener, H.C. *et al*., 2009).

Inadequate Screening: Cervical cancer prevention requires cytologic identification and then eradication of cancer precursor or early invasive lesions. A report reviewing the screening histories of 481 women with invasive cervical cancer in Connecticut from 1985 through 1990 found that 52% of women had histories of suboptimal screening: 28. 5%had never been screened, and 23. 5% of those screened had their

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last Pap test 5 or more years before their cancer diagnosis(Kitchener, H.C. *et al*., 2009).

Human Papillomavirus: Human papillomavirus is a non enveloped DNA virus with a protein capsid. It infects epithelial cells exclusively and approximately 30 to 40 HPV types have an affinity for infecting the lower anogenital tract.

Viral Life Cycle: The circular, double-stranded HPV genome consists of only nine identified open reading frames (ORF). The "early"(E) genes govern functions early in the viral life cycle such as DNA maintenance, replication, and transcription. The "late"(L) genes encode capsid proteins needed late in the viral life cycle to complete assembly into new, infectious viral particles. Completion of the viral life cycle takes place only within an intact squamous epithelium. Early genes are expressed in the lower layers and late genes are expressed in the more superficial layers, in synchrony with epithelial differentiation. Viral replication is completed within the most superficial epithelial layers. HPV is a non lytic virus, and therefore infectiousness depends upon desquamation of infected cells (Figure 7).

Figure 7: Morphology of HPV virus

Viral Types:
HPVs have been classified into three oncogenic risk groups:
1. Low oncogenic risk group: including type 6, 11, 42, 43 and 44 and are associated with benign lesions, i. e. condyloma acuminate and CIN I.
2. Intermediate oncogenic risk group: includes types 33, 35, 39, 45, 51 and 56 are found in high grade lesions, but rarely in invasive cancer.
3. High oncogenic risk group: includes types 16, 18, and 31 and detected in CIN II and III with invasive cancer cervix, vulva, anus and penis.

Transmission: Transmission of genital HPV usually requires sexual contact with the genital skin, mucous membranes, or body fluids of a partner with either warts or subclinical infection (Manchanda, R. *et al*., 2010). Little is known about the infectivity of subclinical HPV, but it is assumed to be high, especially in the presence of high viral counts. Through microabrasion of the genital epithelium during sexual contact, HPV likely gains access to the basal cell layer. Once infected, the basal cells become a viral reservoir. Genital HPV infection is multifocal, involving more than one lower reproductive tract site in most cases. Therefore, neoplasia at one genital site increases risk of neoplasia elsewhere within the lower genital tract. High-risk HPV cervical infection is not seen in women who have not experienced penetrative sexual contact, although they may occasionally test positive for non-oncogenic or low-risk types at the vulva or vagina, perhaps due to vaginal tampon use or digital penetration. Oral-genital and hand-genital transmissions are possible, but appear to be far less common than with genital-genital, particularly penile-vaginal penetrative contact. Nonsexual transmission of genital HPV types is theoretically possible, but is probably rare in sexually active adults. Women who have sex with women frequently report past sexual experiences with men. This subgroup of women have rates of high risk HPV positivity, abnormal cervical cytology, and high-grade cervical neoplasia similar to those of heterosexual women, but undergo cervical

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cancer screening less often. Those who have never had sex with men appear to be at similar risk, implying that digital, oral, and object contact place them at risk of HPV infection. Therefore, women who are sexually active should undergo cervical cancer screening according to current recommendations regardless of sexual orientation.

Congenital Infection: Congenital HPV infection by vertical infection from mother to infant develops rarely. Conjunctival, laryngeal, vulvar, or perianal warts present at birth or that develop within 1 to 3 years of birth are most likely due to perinatal exposure to maternal HPV in the absence of sexual abuse. Infection is not related to the presence of maternal genital warts or route of delivery. Accordingly, cesarean delivery is recommended for HPV-related infection only in cases of large genital warts that would likely obstruct delivery or avulse with cervical dilation. Genital warts that develop in children after infancy are always cause to seriously consider sexual abuse. However, infection by nonsexual contact, autoinoculation, or fomite transfer is also possible, as evidenced by the finding of nongenital HPV types in a significant minority of cases.

Outcome of HPV Infection: Genital HPV infection results in a variety of outcomes. Infection may be *latent* or *expressed*. Expression is either *productive*, with formation of new virus, or *neoplastic*, causing preinvasive disease or malignancy. Most productive and neoplastic infections are subclinical, rather than clinically apparent, as with genital warts or obvious malignancy. Finally, HPV infection can be *transient* or *persistent.* Neoplasia is the least common outcome of genital HPV infection.

Latent Infection: *Latent infection* refers to that in which cells are infected, but HPV remains quiescent. There are no tissue effects, as the virus is not reproducing. Little is known about the incidence, natural history, or significance of latent HPV infection, as the virus is present below detectable levels.

Expressed Productive Infection: *Productive infections* have little or no malignant potential because eventual host cell death is required to complete the viral life cycle. The intact, circular HPV genome remains unintegrated into the infected cell's chromosomes and its oncogenes are expressed at only very low levels. In both the female and male genital tracts, productive HPV infections produce either visible genital warts, called *condylomata acuminata*, or much more commonly, subclinical infections known as low-grade squamous intraepithelial lesions (LSILs).

Expressed Neoplastic Infection: In cancerous lesions, the circular HPV genome integrates linearly at random locations into a host chromosome and unrestrained transcription of the E6 and E7 oncogenes followsTheir products, the E6 and E7 oncoproteins, interfere with the function and accelerate degradation of p53 and pRB, key host tumor suppressor proteins. This leaves the infected cell vulnerable to malignant transformation by loss of cell cycle control, cellular proliferation, and accumulation of DNA mutations. In preinvasive lesions, normal epithelial differentiation is modified. The degree of abnormal epithelial maturation that results is used to grade lesion histology as mild, moderate, or severe cervical intraepithelial neoplasia. The average age at diagnosis of low-grade cervical disease is younger than that of high-grade lesions and invasive cancers, and it has long been assumed that a disease continuum exists. An alternative theory proposes that low-grade lesions are generally transient and non oncogenic, whereas high-grade lesions and cancers are monoclonal, arising de novo without prerequisite low-grade disease. This may explain why some cancers are diagnosed soon after negative cytologic screening(Kitchener, H.C. *et al*., 2009).

Incidence: Genital HPV is the most common sexually transmitted infection. Worldwide, populations vary in point prevalence from 2 to 44 %. The Centers for Disease Control and Prevention (2002) estimates that the risk of a woman acquiring genital HPV by age 50 is greater than 80%. The prevalence of genital warts is approximately 1%and cytologic abnormalities 4 to 5%, with both more common in high-risk groups. Thus, subclinical, infection is far more common than genital warts. Most incident HPV infections occur in young women and adolescents under age 25.

Prevention of HPV infection: HPV infection is the most prevalent sexually transmitted infection and unlike other STDs use of condom and safe sex practice are not effective in preventing this infection. Papilloma virus live in pubic area and cells living the vagina and cervix. Condom does not cover pubic area. Even dead cells shed during intercourse can contain the virus and remain active for days. The infecting virus may remain dormant

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and it can not be known when it will become active(Wheeler, C. M. *et al*., 2009).

I) Primary prevention:

1. Counseling and vigorous efforts to discourage adolescent, especially young girls from starting smoking and early initiation of sexual activity.
2. Life style and behavior modification& Diet rich in vitamins C, A, E and folic acid.
3. 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 eradication of cancer (Bosch, F. X. *et al*., 2002). Tow prophylactic HPV vaccines have been found to be safe, immunogenic and efficacious at providing protection from typec-specific HPV infection (same source). These products are directly against virus type that cause anogenital tract disease and are derived from expression of the major caspid protein (L1) gene in tissue culture. When expressed using appropriate vectors and tissue culture systems, L1 self-assembles into a Virus Likes Particles that cannot be distinguished morphologically or antigenic ally from its wild type counterpart (13). In 2006, the Food And Drug Association approved (Gardasil, Merk) a vaccine that is highly effective, which containing HPV types 6, 11, 16 and 18 has been licensed in the United States and recommended by the Centers For Disease Control and Prevention for administration to girls and young women 9-26 years of age (Alvarez-Salas, L. M. *et al*., 2003). 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 for pregnant women. It can be provided to women who are breast feeding. It is administered at 0, 2 and 6 months as a 0. 5 ml intramuscular injection. The tetravalent vaccine has the added advantage of protecting against low-risk HPV types 6/11, which cause genital warts. 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. Mathematical modeling suggests immunity persists for 20-30 years. A recent meta-analysis found that following vaccination, the relative risk of HPV 16 and 18 infections was 0. 13 and 0. 22, respectively. Polyvalent vaccines developed in the future are likely to increase efficacy. The vaccine does not protect against infection of the women has already encountered the strain and it provides no protection against all types of HPV strains So women may still at risk even after they have been vaccinated. The vaccine has been tested only on young women and cannot be given to women older than 26 years.

There are two types of vaccine strategies:

1. Protective vaccine: Is most effective method, if available it should be given before the start of sexual activity. The drawback of this vaccines that as it is against one type of virus, it will not protect from other type, therefore a mixture of several types would have to be created(Wheeler, C. M. *et al*., 2009).

2. Therapeutic vaccine: This type will boost immune system of the infected women, it may cause cancer to regress and even disappear. Until a protective vaccine is widely available, primary prevention must focus on changing sexual practices and behaviors that will increase the risk of infection. Regular screening is essential for women how have had HPV vaccine. The vaccine is not intended to replace the screening programs, but to help prevent HPV, the virus known to cause cervical cancer. Although Gardasil protects against 2 types of HPV that cause 70% of cervical cancer cases. there are other strains that can cause cervical cancer. The vaccine will not protect against these other strains. Also some women may not fully protect by the vaccine, especially those with a weakened immune system. Gardasil requires a series of three shots given over a 6 month period. some women may not finish the series, or may get them at the wrong time, which may cause them not to be fully protected. Women may have already been infected with HPV prior to getting the vaccine.

II) Secondary Prevention:

This is very important, the secondary prevention is done by

1. Early diagnosis of precancerous lesions.

2. Treating the lesions cost effectively before it progresses to cancer.

3. Antiviral agents: Most of the cervical cancer are positive for high risk HPV. Therefore, there is a need for newer approaches to treat HPV infections. Tow noval approaches for inactivating gene expression involve ribozymes and oligonucleotides. Methods for identification of target gene involved in neoplastic transformation

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and tumor growth have been established, and these will lead to therapeutic approaches without any damage to normal cellular RNA molecules, which is often associated with conventional therapeutics. Ribozymes and oligonucleotides represent rational antiviral approaches for inhibiting the growth of cervical lesions and carcinoma by interfering with E6|E7 RNA production. These antiviral can inhibit the growth of HPV 16 and 18 immortalized cells, and tumor cells by eliminating E6|E7 transcripts. Also reduction of E6 and E7 expression is sufficient to induce cervical cervical carcinoma cells to undergo apoptosis and suggest that transfection of cervical cancer cells with HPV -16 E6 and E7 antisense RNA is a potential approach to treat HPV-16 positive cervical cancers (Eileen, M. *et al*., 2003).

Cervical Screening Technologies:

Conventional Pap Collection: George Papanicolaou first introduced cytology to the world in 1928. In the cells exfoliated from female genital tract, characteristic cellular changes associated with cervical carcinoma are noted. The technique was refined by Papanicolaou and Traut in 1932. Ayre introduced wooden spatula in 1946 to scrape the cervix and harvest the cells from TZ to cervix and soon thereafter it was found that not only invasive but preinvasive cancer can also be detected by this method. There are presently two cervical cytology techniques in use: conventional and liquid-based. The conventional Pap test is a smear of cells made directly from collection device to glass slide at the time of sampling. Goodman and Hutchinson (1996) demonstrated that most cellular material remains on the collection device and is discarded after a single conventional smear is prepared. Although examination of the excess material ordinarily discarded did not result in additional diagnoses of HSIL or cancer, the discarding of most cervical material sampled has raised concern with this method.

Liquid-Based Pap Collection: The imperfect sensitivity and variable smear quality of conventional Pap collection have driven the development of thin-layer liquid-based cytology (LBC) during the past decade. Liquid-based cytology collects cells in a liquid transport medium that is subsequently processed to produce an even monolayer of cells on a glass slide. There are currently two LBC products marketed in the U. S. : ThinPrep 2000 (Cytyc Corp., Boxborough, MA) and SurePath (TriPath Imaging, Inc., Burlington, NC). Both products are FDA approved as alternatives to the conventional Pap test. The number of cells, between 50, 000 and 75, 000, and the area of the slide covered with cells are less than with a conventional smear. However, obscuring blood, mucus, debris, and cellular overlap are largely eliminated. Theoretically, abnormal cells that might be few in number, clustered, and obscured on a conventional smear will be randomly and evenly distributed over the area of the LBC slide and thus be more visible for detection. In addition, most or all of the collected cellular material is available for laboratory processing and is not discarded in the sampling process.

Residual LBC specimens can undergo testing for HPV, herpes simplex virus, *Neisseria gonorrhoeae*, and *Chlamydia trachomatis.* ThinPrep is FDA approved for reflex HPV testing and it is possible that SurePath will gain similar approval in the future(Kitchener, H.C. *et al*., 2009).

Comparison of Conventional and Liquid-Based Cytology: Liquid-based cytology now accounts for most of Pap tests performed in the world. Both LBC products are FDA-approved to claim a 65%increased detection rate of HSIL compared with conventional smears, as well as decreased unsatisfactory sample rates. Furthermore, there is evidence that LBC is more sensitive and accurate for the detection of both squamous lesions and adenocarcinomas of the cervix. Comparison studies show variable results with respect to atypical squamous cell detection rates. Although the preponderance of numerous studies show an increase in sensitivity by LBC technology, controversy exists about data significance because of study methodologies. Ronco and colleagues (2006) have published the first randomized controlled trial that compares conventional Pap testing to LBC in a screening population. Although LBC decreased the unsatisfactory Pap rate, its sensitivity was similar to that of conventional Pap tests, but with a lower positive predictive value.

Screening Guidelines: Current cervical cancer screening guidelines are more evidence-based and comprehensive than in the past. The three agencies offering guidelines are the American College of Obstetricians and Gynecologists, the American Cancer Society (ACS), and the British Society of Colposcopy and Cervical Pathology(BSCCP) guidelines are shown in table 4:

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Table 4: BSCCP guidelines

	ACS	ACOG	BSCCP
Initiation of screening	Approximately 3 years after onset of vaginal intercourse; no later than age 21	See ACS	25 years
Screening intervals for women at average risk	Age 30: annual if conventional smear; every 2 years if liquid based test Age >30: every 2 to 3 years after 3 consecutive negative tests	Age 30: annual Age >30: see ACS	25-49 years: at least every 3 years, 50-64 years: every 5 years, `65 years: those not screened since 50 and those with recent abnormal tests
Screening intervals for women at higher risk	HIV + or other immunocompromised state: 2 tests during first year after immune disease diagnosis, then annually (per CDC)	HIV +: see ACS Other immunocompromised states, DES: may require more frequent screening History of CIN II or III or cervical cancer: annual	No specific recommendations
Discontinuation of screening	Age 70: consider if 3 documented negative (and no abnormal) tests in prior 10 years Continue if screening history uncertain, history of cervical cancer, DES, recent HPV +, HIV + status, other immunocompromised state	Age 70 in low-risk w Continue if high risk, sexually active, history of multiple sexual partners, or history of abnormal cytology omen	Age 65 if not otherwise at high risk for cervical cancer
Screening after Hysterectomy	Not indicated if removal confirmed for benign indication Subtotal hysterectomy: continue screening per guidelines	Not indicated if removal confirmed with benign pathology and past negative cytologies See ACS	Not recommended if total hysterectomy for benign disease See ACS

HPV DNA testing: The fundamental basis of DNA probe technology is the exquisitely specific recognition of target nucleic acid sequences by complementary probe nucleic acid sequences. HPV Detection technologyThe histopathology gives limited idea of HPV status; including the epithelial hyperplasia (acanthosis) & degeneration & cytoplasmic vacuolization (koilcytosis) in terminally differentiated cells. Detection of HPV common antigen of the major capsid protein could be achived by immunocytochemical staining being confined to the nucleus of infected cells &in

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cytoplasm of koilocytic cells (Moench . *et al*., 1985).

These Tests are:

A-Hybrid Capture 2(HC 2) test: Is a single amplification test. It relies on RNA-"hybrids "that are recognized by RNA-DNA antibodies conjugates to alkaline phosphares. The antibody conjugates recognize the RNA-DNA hybrids, and hundreds of antibodies can coat a single genomic hybrid. Each alkaline phosphates enzyme reacts with chemiluminescent substrate to produce a steady stream of photons that are counted by aluminometer. The test has demonstrated highly reptoducible results across study sites. Cross reactivity can be a problem in HC2.

B- Polymerase chain reaction(PCR0 test: Is a target-amplified test. It uses technology that creates extacopies of the desired target sequence before detection of the amplified products, or amplicons, by more traditional methods. Sequencing amplicons from various regions of the HPV genome has produced the ability to differentiate between minor type variants present in different populations and to better understand the persistence of HPV in various groups of women. PCR is prone to inhibitory substances that can interfere with amplification and decrease sensitivity.

C-In Situ Hybridization: Introduction: In situ hybridization (ISH) is a method of localizing and detecting specific DNA or RNA sequences in morphologically preserved tissue sections or cell preparation by hybridizing the complementary strand of nucleotide probe to the sequence of interest (Gall, J. G . *et al*., 1969). The first ISH studies were performed in the late 1960s to detect amplified DNA targets in cell nuclei (Pardue, M. L . *et al*., 1981). The technique was then used as a suitable method for detecting individual genes on chromosome preparations as well as for localizing infectious agents in individual cell populations (Angerrer, L. M . *et al*., 1981). Later on, ISH was used to detect messenger RNA in morphologically distinct cell population (Eissa, S . *et al*., 1998).

Hybridization Basic Principle: Detection of DNA or RNA in cells by ISH relies on the base-pairing properties of nucleic acids. Two strands of DNA or RNA having the appropriate complementary sequence can form a double-stranded structure by the formation of multiple base pairs, with bases on one strand binding non-covalently to bases on the other strand (Ferris, D. G . *et al*., 2022). In situ hybridization makes use of the high specify of complementary nucleic acid binding to identify infectious agents (viral and non-viral) in tissue sections, to localize mRNAs (oncogenes and tumor suppressor genes) within individual cells and to detect specific DNA sequences in the genome of the cells.

Colposcopy: Colposcopy is an outpatient procedure that is simple, quick, and well-tolerated. It allows examination of the lower genital tract and anus with a microscope to further evaluate abnormal Pap test results and visible epithelial abnormalities. This allows identification and management of premalignant lesions. Colposcopic examination of the cervix remains the clinical standard in the evaluation of patients with abnormal cervical cytology. However, its sensitivity and reproducibility have recently come into question (Runowiez, C. D . *et al*., 2007).

Colposcope: There are many styles of colposcopes, but they all operate similarly. The colposcope consists of a stereoscopic viewing system with magnification settings ranging from three- to 40-fold attached to a freely moveable stand. A high-intensity halogen light provides illumination. Use of a green (red-free) light filter emphasizes contrast by causing the color red to appear black, aiding the examination of vascular patterns (table 5).

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Table 5: Clinical Considerations Directing Colposcopy

Clinical objectives
Provide a magnified view of the lower genital tract
Identify squamocolumnar junction of the cervix
Detect lesions suspicious for neoplasia
Direct biopsy of lesions
Monitor patients with a current or past history of lower genital tract neoplasia
Clinical indications
Grossly visible genital tract lesions
Abnormal cervical cytology
History of in utero diethylstilbestrol exposure
Contraindications
None
Relative contraindications
Anticoagulant therapy if patient requires biopsy
Upper or lower reproductive tract infection
Uncontrolled severe hypertension
Uncooperative or overly anxious patient

AIM OF THE STUDY: -

1. Detection of HPV 16, 18, 6 and 11 using in Iraqi patients.
2. To determine relation of the above HPV types with cervical malignant and premalignant lesions.
3. To determine whether testing for DNA of HPV (Human Papilloma Virus) is superior to the Papaniclaou (Pap) test for cervical cancer screening in Iraq.

Study Design: This is a Prospective study which has conducted at Baghdad Teaching Hospital, Department of Obstetrics and Gynecology.

Participant: The sample consist of 60 women ages equal or more than 30 years who attended obstetrics & gynecological outpatient clinic in Baghdad teaching hospital between July 2009 to February 2011.

Inclusion criteria: Sixty 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 had Conventional Pap, HPV DNA tests and Colposcopical directed biopsies. The results of the Pap and HPV were compared to Colposcopical directed biopsies.

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Table 6: Demographical factors of the Study Population

	Minimum	Maximum	Mean	Std. Deviation	No of patients
Age	30	65	39. 78	8. 912	60
Age at marriage	12	42	20. 87	5. 990	60
Parity	0	11	4. 05	2. 480	60

Table7: Risk factors of the Study Population

	Frequency	**Percent**	**Valid Percent**	**Cumulative Percent**
OCCPS: Yes	39	65. 0	65. 0	65. 0
No	21	35. 0	35. 0	100. 0
Smoker: Yes	16	26. 7	26. 7	26. 7
No	44	73. 3	73. 3	100. 0
STD. s: Yes	14	23. 3	23. 3	23. 3
No	46	76. 7	76. 7	100. 0
Condom:				
Yes	26	43. 3	43. 3	43. 3
No	34	56. 7	56. 7	100. 0
Job:				
Manualworkers				
Clerk	32	53. 3	53. 3	53. 3
Health workers	22	36. 7	36. 7	90. 0
	6	10. 0	10. 0	100. 0
Total:	60	100. 0	100. 0	

Exclusion criteria: Women who were lacked a cervix (hysterectomies), were pregnant, had a history of cervical cancer, had undergone Pap testing in the previous year, or were unable to provide consent were excluded.

Blinding: The Pap tests were read at the participating sites by cytopathologists without knowledge of the patient's status as a participant or her HPV test result. The colposcopists and pathologists evaluating the biopsy specimens were unaware of the screening _ test results.

MATERIALS:

Equipment: All equipments used throughout this study are listed in table (8).

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Table 8: Equipments used throughout the study

Equipment	Manufacturing company
Absorbent wips Arye's spatula Bottles containing formaline	
Centrifuge Colposcopy Cotton- tipped swab sticks	Ziess/Germany(NO 6627103998) model opmi-pico
Coverslips Examination gloves of various sizes, including latex free gloves Endocrvical speculum Endocervical curette	Germany
Eppendorff tube Gauze pads(4x4)	
Glass staining jars	England
Gloves	Hungary
Graduated cylinder	England
Humidity chamber	Locally made
Incubator Jar containing alcohol for cervical smear fixation Kidney tray Large cotton-tipped swab sticks	Memmert, Germany
Light microscope	Novex (Holland)
Microtom	Leitz (Wetzlor) Germany
Oven	B. T Memmert
Pap pen	Bio Genex (USA)
Parafilm	Germany

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Positively charged microscopic slides	Fisher Scientific (USA)
Precision pipette 50-200 µl and tips	Fin pipette (Finland)
Precision pipette 200-1000 µl and tips Punch biopsy forceps(3 sizes)	Slamed (Germany)
Slides	Germany
Slides holders Sponge holding forceps	England
Timer	England
Tissue paper Vaginal side-wall retractor Vaginal speculum	Germany

Reagents and Solutions:

1. Specific reagents of ISH technique:

a. DNA probe Hybridization/Detection system-In situ kit (Maxim biotech, USA). The kite contains the following items:

- Biotinylated House Keeping Gene Probe.
- Citric buffer, PH 6. 0(100X).
- Detergent wash buffer (20X).
- DNase and RNase free diluents.
- . Hybridization solution
- . Proteinase K.
- Protein block (20X).
- Streptavidin-alkaline phosphatases conjugate.
- Substrate.

b. Biotinylated cDNA probes for HPV-16, HPV - 18, HPV- 6 and HPV- 11 (Maxim biotech, USA).

2. General reagents and solutions: All are listed in table (9)

Table 9: General reagents and solutions used throughout the study

General reagents and solutions	Brand
Absolute ethanol	BDH (England)
Counter stain (eosin)	Hopkins and Williams
Distilled water Disinfectant solution	
HCL 1% Lugols' iodine: Crystalline Iodine 2g, Potassium Iodide 4g, Distilled Water 200ml	BDH (England
Mounting medium Normal saline Three-five percent acetic acid	BDH (England)

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Xylene	Merck (Germany)

Screening Tests:
1. Conventional Pap test. (Apprndix III)
2. ISH.
3. Colposcopy.

1. Conventional Pap tests were used, with results reported or reclassified according to the 2001 Bethesda System terminology. According tothe Bethesda system, squamous- cell abnormalities are classified as atypical (i. e., ASCUS; or atypical squamous cells, cannot exclude high- grade squamous intraepithial lesion [known as ASC-H], low-grade squamous intraepithelial lesion, high-grade squamous intraepithelial lesion, or carcinoma. Glandular-cell abnormalities are classified as atypical AGC, adenocarcinoma in situ, or adenocarcinoma.

In this study, a result of ASCUS, AGC, or worse was considered positive.

Conventional Pap tests comprises three main stages:
- Collection of cervical smears.
- Fixing and staining the smear samples.
- Cytological examination and interpretation. The samples are collected by clinical team and smears are sent for cytological examination.
- Sampling Device is Ayres' spatula.

Technique:
1. Patient was placed in lithotomy position and labia parted.
2. Non- lubricated suitable (self- retaining) speculum was introduced into the introitus to visualize the entire cervix clearly and completely.
3. the spatula was placed in position and rotated 360 degree clockwise to take sample from ectocervix and endocervix.
4. Specimen was spread evenly avoiding clumps on glass slides (on both conventional glass slides and positively charged slides. The slides were immediately fixed 95% ethanol 2 hours to over night.
5. Air drying was avoided.
6. Smears was stained by Pap-stain (Appendix III) for the purpose of detection of atypical and malignant cells.

2. In Situ Hybridization for the Detection of Hpv-16, HPV-18, HPV-6 and HPV-11:

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 – alkaline phosphatase (streptavidin-AP) conjugate (DNA probe hybridization/Detection system in situ kit 2003). 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.

In Situ Hybridization Procedure:
A. Prehybridization steps:
1. Samples were backed overnight at 70˚C (slides were kept vertically).
2. Deparaffinization and rehydration was done by serial dipping the slides in glass staining jars containing the followings:
a. Xylene for 5minutes.
b. Absolute ethanol for 2minutes.
c. 95% ethanol for 1minute.
d. 70% ethanol for 1minute.
e. Distilled water for1minute.
3. Slides were left to dry for 5minutes at 37˚C.
4. The slides were placed in citric buffer solution on a hot plat at 98˚C for 15minutes.
5. The slides were washed in distilled water three times, 2minutes each.
6. Deproteinization was performed by placing 10µl of freshly diluted (1X) proteinase K solution onto each tissue section, and the slides were incubated for 10minutes at 37˚C.
7. 5 minutes the slides were washed in a detergent wash.
8. The slides were washed in distilled water three times, 2minutes each.
9. The slides were then dehydrated by serial dipping them in glass staining jars containing the followings:
a. 70% ethanol for 1minute.
b. 95% ethanol for 1minute.
c. Absolute ethanol for 1minute.
10. Slides were left to dry for 5minutes at 37˚C.

II. Hybridization step:

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1. Hybridization was performed by placing 5µl of probe onto each smear after denaturation of probe at 95c for 8-10 minute, ice quitus, and then slides were covered by cover slips with avoidance of trapping any air bubbles.
2. Slides were placed in an oven at 95˚C for 8-10 minutes, for denaturation of tissue (DNA).
3. Slides were placed in humid chamber and incubated at 37˚C overnight to allow hybridization of the probe with the target nucleic acid.

III. Post hybridization steps

1. In the next morning, slides were soaked in prewarmed protein block buffer (1X) at 37˚C until the cover slips fall off, and then slides were allowed to remain in the buffer for 3 minutes.
2. Slides were placed in prewarmed protein block buffer (2X) for 3minutes at 37˚C. Then slides were drained and blotted.
3. One to two drops of streptavidin-AP conjugate were placed on each smear. Slides were placed in humid chamber, and then incubated for 20minutes at 37˚C.
4. Slides were rinsed in pre wormed detergent wash buffer for 5minutes; they were then drained and blotted.
5. One to two drops of the substrate were placed on each tissue smear. Slides were incubated in humid chamber at room temperature (10-40˚C) until the intense blue color development was optimal.
6. Slides were rinsed in 2-3 changes of distilled water.
7. Eosin was added for one minute as a counter stain.
8. Slides were washed with running tap water.
9. Dehydration of tissue sections was done by serial dipping of slides in glass staining jars containing the following:
a. 95% ethanol for 1 minute.
b. Absolute ethanol for 2 minutes.
c. Xylene for 1 minute.
10. Sections were mounted with permanent mounting medium, then covered with cover slips and left to dry overnight.

Evaluation of the In Situ Hybridization Signal: In situ hybridization was carried out with a non-radioactive method for molecular markers (HPV-16, HPV-18, HPV-6 and HPV-11). Proper use of this hybridization/detection system will gave an intense blue stain at the specific site of the hybridization probe in positive test tissue. Quantification of different molecular markers in situ hybridization signal was evaluated under light microscopy at high power (X400)whereas the counting of positive cells was performed at (X1000).

Diagnostic Procedure: Participants were referred for colposcopy within 2 weeks to confirm the diagnosis. Colposcopists followed a standardized protocol that included ectocervical biopsies of all abnormal – appearing cervical regions, and at least two biopsies of normal – appearing ectocervical epithelium. Seven patients failed to attend colposcopy appointments and were excluded from the study.

STATISTICAL ANALYSIS

We compare the results of HPV DNA, Pap test with results of colposcopically directed bunch biopsies, as a method of verification. Statistical Package for Social Sciences –version 17 (SPSS. 17) was used for data input and analysis. Continuous variables were expressed as mean and standard deviation (SD), and discrete variables were expressed as numbers and percentages. Chi square test for goodness of fit was used to test the distribution of discrete variables. Students T- test for two independent variables was used to test the significance of difference between continuous variables. P value less than 0. 05was considered significant.

RESULTS

The number of women enrolled at the study, was 60 women. The mean age at enrollment was 39. 78 year and standard deviation is 8. 912. The minimum age at marriage, the maximum age, was12 and 42 respectively. The mean was 20. 87 and std. deviation was 5. 990. Parity of the women enrolled at the study, the minimum was 0, the maximum was 11, the mean was 4. 05, and standard deviation was 2. 480. The number of women used OCSPS in our study is 39 (65%) and 21 women(35%) out of 60 did not. Sixteen women (26. 7%) were smoker and 44 (73. 3%) were not smoker. Manual workers between women in our study were 32 (53. 3%), clerks were 22 women (36. 7%) and health workers number were 6 (10. 0). Sexual transmitted diseases (as documented clinically or by culture, serological and immunological tests) was reported in 14 women (23. 3%). Condom using by male partner was reported in 26 (43. 3%) and in 34 women (56. 7%) condom was not used by the women's partner. By conventional pap test 7 cases (13. 2%) out of 53 was positive [6 cases (85. 7%) were diagnosed as

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CIN II, CINIII or CA (3 cases were diagnosed as HSIL by pap found to be sequmous cell cacinoma by biopsy and one case diagnosed as cacinoma by pap test and biopsy) &only one case (14. 3%) was normal (table10). Pap test was negative in 46 cases out of 53 total (86. 8 %), 21 cases (45. 7%) were diagnosed as CINII or CINIII and 25 cases (54. 3%) of the negative pap proved to be normal or CINI by biopsy (table11).

Table10: The relationship between Conventional Pap results and histopathological finding in colposcopy directed biopsies

			Colposcopical directed biopsies				**Total**
			Normal	**CIN I**	**CIN II + III**	**Ca cervix**	
PAP Normal		Count	4	7	12	0	23
		% within PAP	17. 4%	30. 4%	52. 2%	. 0%	100. 0%
		% within Colpo	44. 4%	41. 2%	52. 2%	. 0%	43. 4%
	ASC-US	Count	1	2	4	0	7
		% within PAP	14. 3%	28. 6%	57. 1%	. 0%	100. 0%
		% within Colpo	11. 1%	11. 8%	17. 4%	. 0%	13. 2%
	LSIL	Count	4	7	5	0	16
		% within PAP	25. 0%	43. 8%	31. 3%	. 0%	100. 0%
		% within Colpo	44. 4%	41. 2%	21. 7%	. 0%	30. 2%
	HSIL	Count	0	1	2	3	6
		% within PAP	. 0%	16. 7%	33. 3%	50. 0%	100. 0%
		% within Colpo	. 0%	5. 9%	8. 7%	75. 0%	11. 3%
	CA	Count	0	0	0	1	1
		% within PAP	. 0%	. 0%	. 0%	100. 0%	100. 0%
		% within Colpo	. 0%	. 0%	. 0%	25. 0%	1. 9%
Total		Count	9	17	23	4	53
		% within PAP	17. 0%	32. 1%	43. 4%	7. 5%	100. 0%
		% within Colpo	100. 0%	100. 0%	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	34. 274	12	. 001

*The P- value is significant at the 0. 05 level.

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Table11: The crosstab between Conventional Pap test and Colposcopical Directed biopsies:

			Colposcopical directed biopsies		**Total**
			Dis +ve	Dis -ve	
PAP	Dis +ve	Count	6	1	7
		% within PAPgp	85. 7%	14. 3%	100. 0%
		% within ColpoGP	22. 2%	3. 8%	13. 2%
	Dis -ve	Count	21	25	46
		% within PAPgp	45. 7%	54. 3%	100. 0%
		% within ColpoGP	77. 8%	96. 2%	86. 8%
Total		Count	27	26	53
		% within PAPgp	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3. 902	1	. 100

*The P- value is significant at the 0. 05 level.

Screening [95% CI]
Sensitivity: 0. 22 [0. 09; 0. 43]
Specificity: 0. 96 [0. 78; 1. 00]
Predictive value of the positive result: 0. 86 [0. 42; 0. 99]
Predictive value of the negative result: 0. 54 [0. 39; 0. 69]

HPV 16 DNA test was positive in 32 patients (60. 45) out of 53 total cases(because 7 cases were missed cases) and negative in 21 women (39. 6%) as shown in(table12). Of these 32 positive women, 16 women (50%) were diagnosed as CIN IIorIII by colposcopical directed biopsy and 4 cases (12. 5%) were diagnosed as invasive squmous cervical cancer, so totally 20 cases out of 32 positive cases were diagnosed as CINII, CINIII or ca. (62. 5%). Ten cases out of 32 positive were diagnosed as CINI by colposcopical directed biopsies as shown in (table13). Only 2 cases out of 32 positive were normal as proved by biopsies. Twenty one cases(39. 6%) out of 53 were HPV 16 DNA ISH negative, 7 cases (33. 3%) out of 21 cases negative were diagnosed as having CIN II or CINIII by colpo. directed biopsies, 4 of them(out of 7) were HPV 18 DNA test positive, One of them (out of 7) were HPV 6 & 11 DNA test positive, and 2 out of 7 cases HPV 16 negative were negative for all4 types of HPV DNA TESTS (6, 11, 16 & 18). Seven cases (33. 4%) out of 21 negative cases were diagnosed as CIN Iand another 7 cases (33. 3%) were normal.

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Table 12: The relationship between HPV16 infection and histopathological finding in colposcopy directed biopsies:

			Colposcopical directed biopsies				**Total**
			Normal	CIN I	CIN II + III	Ca cervix	
HPV 16	+ve	Count	2	10	16	4	32
		% within HPV 16	6. 3%	31. 3%	50. 0%	12. 5%	100. 0%
		% within Colpo2	22. 2%	58. 8%	69. 6%	100. 0%	60. 4%
	-ve	Count	7	7	7	0	21
		% within HPV 16	33. 3%	33. 3%	33. 3%	. 0%	100. 0%
		% within Colpo2	77. 8%	41. 2%	30. 4%	. 0%	39. 6%
Total		Count	9	17	23	4	53
		% within HPV 16	17. 0%	32. 1%	43. 4%	7. 5%	100. 0%
		% within Colpo2	100. 0%	100. 0%	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8. 931	3	. 030

*The P-value is significant at the 0. 05 level

Table 13: The crosstab between HPV16 infection and colposcopy directed biopsies:

			Colposcopy directed biopsies		**Total**
			Dis +ve	Dis -ve	
HPV 16	+ve	Count	20	12	32
		% within HPV 16	62. 5%	37. 5%	100. 0%
		% within ColpoGP	74. 1%	46. 2%	60. 4%
	-ve	Count	7	14	21
		% within HPV 16	33. 3%	66. 7%	100. 0%
		% within ColpoGP	25. 9%	53. 8%	39. 6%
Total		Count	27	26	53
		% within HPV 16	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

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*The P-value is significant at the 0. 05level.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4. 316	1	. 038

Screening [95% CI]
Sensitivity: 0. 74 [0. 53; 0. 88]
Specificity: 0. 54 [0. 34; 0. 73]
Predictive value of positive result: 0. 63 [0. 44; 0. 78]
Predictive value of negative result: 0. 67 [0. 43; 0. 85]

HPV DNA 18 test was positive in 32 cases(60. 4%) out of 53(Table14). 21 cases out of 32 (65. 7%) were diagnosed as CINII, CINIIIor cacinoma. (18 of 21 (56. 3%) were diagnosed as CINII or CINIII and 3 cases (9. 4%) were invasive cancer. HPV 18 DNA test was negative in 21 cases (39. 6%) of total cases (Table15), 5 cases (23. 8%) were diagnosed as having CINII or CINIII by colposcopical. directed biopsy (4 had HPV 16 DNA test positive, and one case had HPV DNA 6 & 11 positive). One case of malignancy (4. 8%) had HPV 18 DNA negative but had HPV 16 positive. Nine cases of 21 negative(42. 9%) were diagnosed as CINI and 6 cases (42. 9%) were normal.

Table14: The relationship between HPV 18 infection and histopathological finding in colposcopy directed biopsies:

			Colposcopical directed biopsies				**Total**
			Normal	CIN I	CIN II + III	Ca cervix	
HPV18	+ve	Count	3	8	18	3	32
		% within HPV18	9. 4%	25. 0%	56. 3%	9. 4%	100. 0%
		% within Colpo2	33. 3%	47. 1%	78. 3%	75. 0%	60. 4%
	-ve	Count	6	9	5	1	21
		% within HPV18	28. 6%	42. 9%	23. 8%	4. 8%	100. 0%
		% within Colpo2	66. 7%	52. 9%	21. 7%	25. 0%	39. 6%
Total		Count	9	17	23	4	53
		% within HPV18	17. 0%	32. 1%	43. 4%	7. 5%	100. 0%
		% within Colpo2	100. 0%	100. 0%	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7. 444	3	. 059

*The P-value is significant at the 0. 05 level

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Table15: The cross tab between HPV18 and colposcopical directed biopsies:

			Colposcopy directed biopsies		**Total**
			Dis +ve	Dis -ve	
HPV18	+ve	Count	21	11	32
		% within HPV18	65. 6%	34. 4%	100. 0%
		% within ColpoGP	77. 8%	42. 3%	60. 4%
	-ve	Count	6	15	21
		% within HPV18	28. 6%	71. 4%	100. 0%
		% within ColpoGP	22. 2%	57. 7%	39. 6%
Total		Count	27	26	53
		% within HPV18	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

*The P-value is significant at the 0. 05 level.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6. 632	1	. 010

Screening [95% CI]
Sensitivity: 0. 89 [0. 70; 0. 97]
Specificity: 0. 42 [0. 24; 0. 63]
Predictive value of +ve result: 0. 62 [0. 45; 0. 76]
Predictive value of -ve result: 0. 79 [0. 49; 0. 94]

HPV16 & 18 DNA test were positivein 39 cases(73. 6%) out of 53case (table16). Twenty four cases (61. 6%) out of 39 positive cases were diagnosed as having CINII, CINII &CA by biopsy[20 case (51. 3%) were diagnosed as CINII or CINIII, 4 cases (10. 3%) as cancer]. Twelve cases out of 39 case positive were diagnosed as CINI (30. 8%) and 3 cases (7. 7%) were normal (table17). HPV 16 and 18 DNA test were negative in 14 cases out of 53 total cases (26. 4%), 3 cases (21. 4%) were diagnosed as CINII, or CINIII by colposcopical Directed biopsy & none of them was malignant. Five cases (35. 7%) out of 14 cases negative were diagnosed as CINI, 6 cases (42. 9%) were normal.

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Table16: The relationship between HPV16 and or 18 infection and histopathological finding in colposcopy directed biopsies:

			Colposcopical directed biopsies				**Total**
			Normal	CIN I	CIN II + III	Ca cervix	
HPV16_18	+ve	Count	3	12	20	4	39
		% within HPV16_18	7. 7%	30. 8%	51. 3%	10. 3%	100. 0%
		% within Colpo2	33. 3%	70. 6%	87. 0%	100. 0%	73. 6%
	-ve	Count	6	5	3	0	14
		% within HPV16_18	42. 9%	35. 7%	21. 4%	. 0%	100. 0%
		% within Colpo2	66. 7%	29. 4%	13. 0%	. 0%	26. 4%
Total		Count	9	17	23	4	53
		% within HPV16_18	17. 0%	32. 1%	43. 4%	7. 5%	100. 0%
		% within Colpo2	100. 0%	100. 0%	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11. 132	3	. 011

*The P-value is significant at the 0. 05 level

Table17: The crosstab betweenHPV16&or18 and colposcopy directed biopsies:

			Colposcopy directed biopsies		**Total**
			Dis +ve	Dis -ve	
HPV16_18	+ve	Count	24	15	39
		% within HPV16_18	61. 5%	38. 5%	100. 0%
		% within ColpoGP	88. 9%	57. 7%	73. 6%
	-ve	Count	3	11	14
		% within HPV16_18	21. 4%	78. 6%	100. 0%
		% within ColpoGP	11. 1%	42. 3%	26. 4%
Total		Count	27	26	53
		% within HPV16_18	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

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*TheP-value is significant at the0. 05level.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6. 632	1	. 010

Screening [95% CI]
Sensitivity: 0. 89 [0. 70; 0. 97]
Specificity: 0. 42 [0. 24; 0. 63]
Predictive value of the positive result: 0. 62 [0. 45; 0. 76]
Predictive value of the negative result: 0. 79 [0. 49; 0. 94]

HPV 6 DNA test was positive in 22 cases (41. 5 %) out of 53 total cases (table18), 13 of them (59. 1 %) were diagnosed as having CINII, CINIII or cancer by biopsy(12 cases (54. 5%) were diagnosed as CINII or CINIII & one case (4. 5 %) as cancer. (table19), 9 cases out of 22 positive (40. 9%) were diagnosed as CINI or normal [8 cases as CINI (36. 4%) and only one case (4. 5%) was normal]. HPV 6 DNA test was negative in 31 cases (58. 5%) out of 53 total, 14 case (45. 2%) were diagnosed as CINII, CINIII or cancer [11 cases(35. 5%) CINII or CINIII and 3 cases (9. 7%0) as cancer]. Seventeen cases out of 31 case negative (54. 8 %) were diagnosed as CINI or normal[9 cases (29 %) as CINI, 8 cases (25. 8 %) were normal.

Table18: The relationship between HPV6 infection and histopathological finding in colposcopy directed biopsies:

			Colposcopical directed biopsies				**Total**
			Normal	CIN I	CIN II + III	Ca cervix	
HPV6	+ve	Count	1	8	12	1	22
		% within HPV6	4. 5%	36. 4%	54. 5%	4. 5%	100. 0%
		% within Colpo2	11. 1%	47. 1%	52. 2%	25. 0%	41. 5%
	-ve	Count	8	9	11	3	31
		% within HPV6	25. 8%	29. 0%	35. 5%	9. 7%	100. 0%
		% within Colpo2	88. 9%	52. 9%	47. 8%	75. 0%	58. 5%
Total		Count	9	17	23	4	53
		% within HPV6	17. 0%	32. 1%	43. 4%	7. 5%	100. 0%
		% within Colpo2	100. 0%	100. 0%	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5. 167	3	. 160

*The P-value is significant at the 0. 05 level

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Table19: The crosstab between HPV6 and colposcopy directed biopsies:

			Colposcopy directed biopsies		**Total**
			Dis +ve	Dis -ve	
HPV6	+ve	Count	13	9	22
		% within HPV6	59. 1%	40. 9%	100. 0%
		% within ColpoGP	48. 1%	34. 6%	41. 5%
	-ve	Count	14	17	31
		% within HPV6	45. 2%	54. 8%	100. 0%
		% within ColpoGP	51. 9%	65. 4%	58. 5%
Total		Count	27	26	53
		% within HPV6	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

*P-value is significant at the 0. 05level.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	. 999	1	. 318

Screening [95% CI]
Sensitivity: 0. 48 [0. 29; 0. 68]
Specificity: 0. 65 [0. 44; 0. 82]
Predictive value of +ve result: 0. 59 [0. 37; 0. 79]
Predictive value of -ve result: 0. 55 [0. 36; 0. 72]

HPV 11 DNA test was positive in 24 cases (45. 3 %) out of 53(table20), 13 cases (54. 2 %) were CINII, CINIII or cancer by biopsy [11 case (45. 8 %) as CINII or CINIII and 2 cases (8. 3%) as cancer.], 9 cases (40. 9%) were diagnosed as CINI or normal by biopsy [8 cases (33. 3%) as CINI and 3 cases (12. 5 %) as normal](table21). HPV 11 DNA test was negative in 29 cases (54. 7 %) out of 53 total cases, 14 cases of them (48. 3%) were diagnosed as CINII, CINIII or cancer [12 case (41. 4%) as CINII, CINIII and 2 cases (6. 9%) as cancer], 15 cases of the 29 cases negative were diagnosed as CINI or normal by colposcopical directed biopsy (51. 7%)[9 cases (30. 9%) were CINI, 6 cases (20. 7 %) as normal].

Table 20: The relationship between HPV11 infection and histopathological finding in colposcopy directed biopsies:

			Colposcopical directed biopsies				**Total**
			Normal	CIN I	CIN II + III	Ca cervix	
HPV11	+ve	Count	3	8	11	2	24
		% within HPV11	12. 5%	33. 3%	45. 8%	8. 3%	100. 0%

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		% within Colpo2	33. 3%	47. 1%	47. 8%	50. 0%	45. 3%
	-ve	Count	6	9	12	2	29
		% within HPV11	20. 7%	31. 0%	41. 4%	6. 9%	100. 0%
		% within Colpo2	66. 7%	52. 9%	52. 2%	50. 0%	54. 7%
Total		Count	9	17	23	4	53
		% within HPV11	17. 0%	32. 1%	43. 4%	7. 5%	100. 0%
		% within Colpo2	100. 0%	100. 0%	100. 0%	100. 0%	100. 0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	. 636	3	. 888

*The P-value is significant at the 0. 05 level.

Table 21: The crosstab between HPV11 infection and histopatholgical finding in Colposcopy directed biopsies:

			Colposcopical directed biopsies		**Total**
			Dis +ve	Dis -ve	
HPV11	+ve	Count	13	11	24
		% within HPV11	54. 2%	45. 8%	100. 0%
		% within ColpoGP	48. 1%	42. 3%	45. 3%
	-ve	Count	14	15	29
		% within HPV11	48. 3%	51. 7%	100. 0%
		% within ColpoGP	51. 9%	57. 7%	54. 7%
Total		Count	27	26	53
		% within HPV11	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

*The P-value is significant at the 0. 05level.

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	. 182	1	. 669

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Screening [95% CI]
Sensitivity: 0. 48 [0. 29; 0. 68]
Specificity: 0. 58 [0. 37; 0. 76]
Predictive value of +ve result: 0. 54 [0. 33; 0. 74]
Predictive value of -ve result: 0. 52 [0. 33; 0. 70]

Pap test and HPV 16, 18 DNA test were positive in 40 cases (75. 5%) out of 53 total cases, 24 cases out of 40 case positive (60. 0%) were diagnosed as CINII, CINIII or cancer and in 16 cases (40. 0%) diagnosed as normal by colposcopical Directed biopsy(table22). Pap test and HPV test were negative in 13 cases (24. 5 %) out of 53 total, 3 cases (23. 1%) were diagnosed as CINII, CINIII or cancer, 10 cases (76. 9%) were diagnosed as normal by colposcopical directed biopsy.

Table 22: The relationship between Conventional Pap test, HPV16&or 18infection and histopathological finding in colposcopy directed biopsies:

			Colposcopical directed biopsies		Total
			Dis +ve	Dis -ve	
PAP_16_18	Dis +ve	Count	24	16	40
		% within PAP_16_18	60. 0%	40. 0%	100. 0%
		% within ColpoGP	88. 9%	61. 5%	75. 5%
	Dis -ve	Count	3	10	13
		% within PAP_16_18	23. 1%	76. 9%	100. 0%
		% within ColpoGP	11. 1%	38. 5%	24. 5%
Total		Count	27	26	53
		% within PAP_16_18	50. 9%	49. 1%	100. 0%
		% within ColpoGP	100. 0%	100. 0%	100. 0%

*The P-value is significant at the 0. 05 level

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5. 352	1	. 021

Screening [95% CI]
Sensitivity: 0. 89 [0. 70; 0. 97]
Specificity: 0. 38 [0. 21; 0. 59]
Predictive value of +ve result: 0. 60 [0. 43; 0. 75]
Predictive value of -ve result: 0. 77 [0. 46; 0. 94]

Occps was used by 33 women (62. 3%) out of 53 women enrolled in our study, 18 women (54. 5%) were diagnosed as HSIL or ca by colposcopical. directed biopsy and 15 cases (45. 5%) were normal. Twenty women out of 53 total (37. 7%) not used occps, 9 of them (45. 0%) were HSIL and 11 cases (55. 0%) were normal. P- value was 0. 500 which is statistically not significant. Fifteen women (28. 3%) out of 53 total cases are smoker, 10 of them (66. 7%) were diagnosed as HSIL or ca and 5 cases (33. 3%) had normal biopsy, 38 cases (71. 7%) out of 53 total number of women are not

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smoker, 17 women of them (44. 7%) were diagnosed as HSIL or ca and 21 cases (55. 3%) were proved to have normal biopsy. P-value was 0. 150 which is statistically not significant. Manual workers included house wives number are 28 women in our study (52. 8%), 12 of them (42. 9%) were diagnosed as HSIL or ca and 16 women (57. 1%) were normal. Clerk are 20 women out of 53 total number (37. 7%), 11 of them (55. 0%) were HSIL or ca and 9 women(45. 0%) are normal. Health workers are 5 women(9. 4%), 4 women of them proved to have HSIL(80. 05) and 1 women (20. 0%) is normal, so P-value of the job was 0. 279 which is statistically not significant. Therteen women out of 53 total cases (24. 5%) are proved to have STD, 9 cases of them (69. 2%) have HSIL or ca by biopsy and 4 cases (30. 8%) have normal biopsy. 40 women included in our study not have STD (75. 5%). 18 cases of them (45. 0%) have HSIL by colpo. directed biopsy and 22cases (55. 0%) have normal biopsy. P-value is 0. 129 which is statistically not significant. Twenty four women (45. 3%) in our study their partners used condom, 13 women of them(54. 2%) have HSIL by biopsy and the rest 11 women (45. 8%) have normal biopsy. 29 women (54. 7%) out of 53 total their partners not used condom, 14 women (48. 3%) have HSIL biopsy and 15 women (51. 7%) have normal biopsy. P-value is 0. 669 which is statistically not significant.

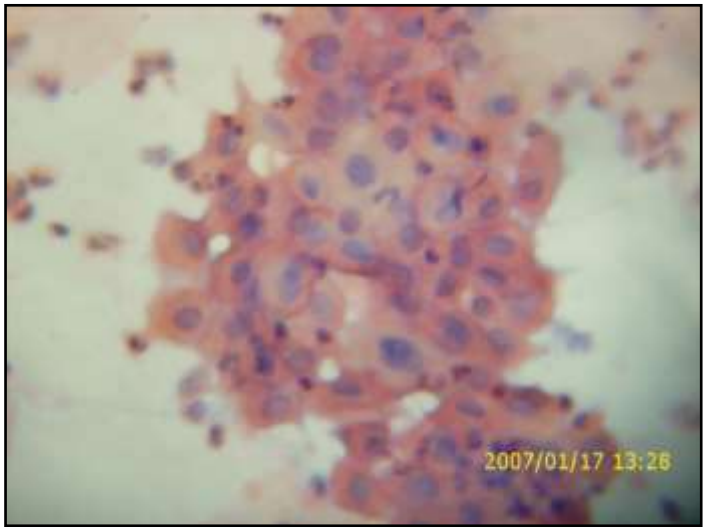

Figure 8 A: Pap stain of cervical cancer: A: Sheets of sequmous metaplastic cells showing moderate dysplasia (CIN II) X1000.

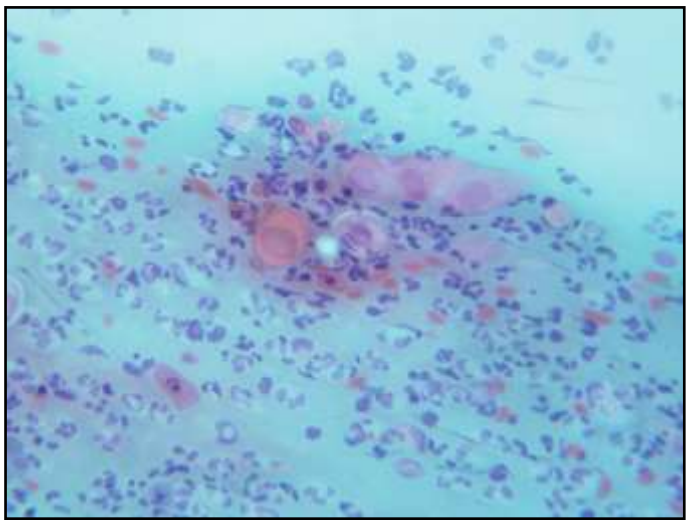

Figure 8: B: Sever dysplasia, cytoplasmic easinophilia, epitotic bodies and inflammatory changes X1000

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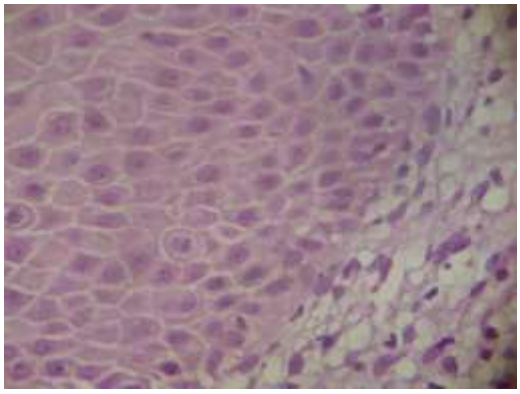

Figur 8 C: Punch biopsy: Sever dysplastic cells with one showing mitotic figure.

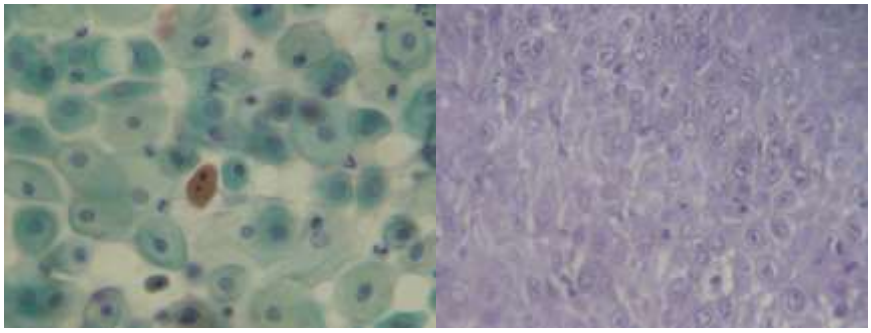

Figure 9: A: Pap smear: H&E stain showing sever dysplastic cells with nucleus in mitosis and cytoplasmic inclusion, case was diagnosed as Sequmous cell carcinoma. B: Punch biopsy of the same patient: shoming polymorphic nucleus with mitotic figure &abnormal mitosis. a case of moderate differentiated Sequmous cell carcinoma.

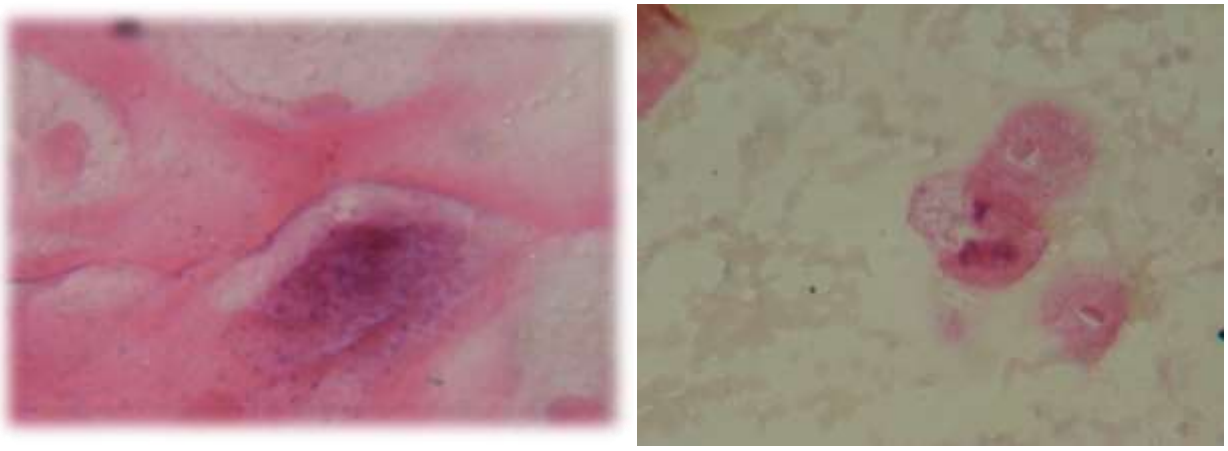

Figure 10 A: HPV 11

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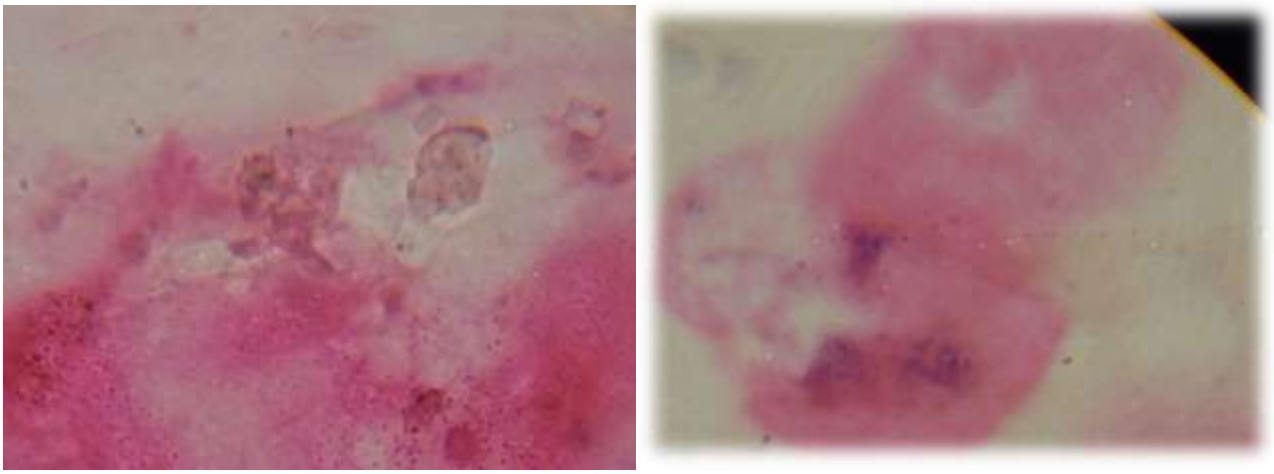

Figure 10 B: HPV 6

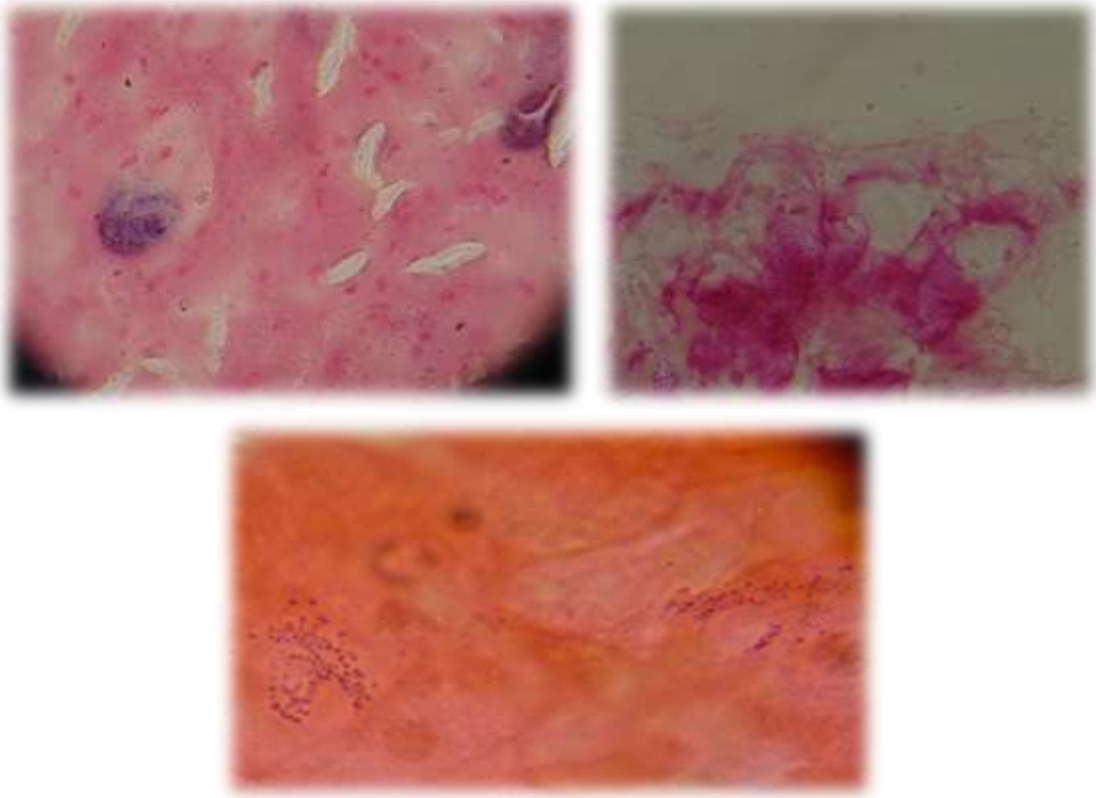

Figure 11: HPV-16 Positive signals

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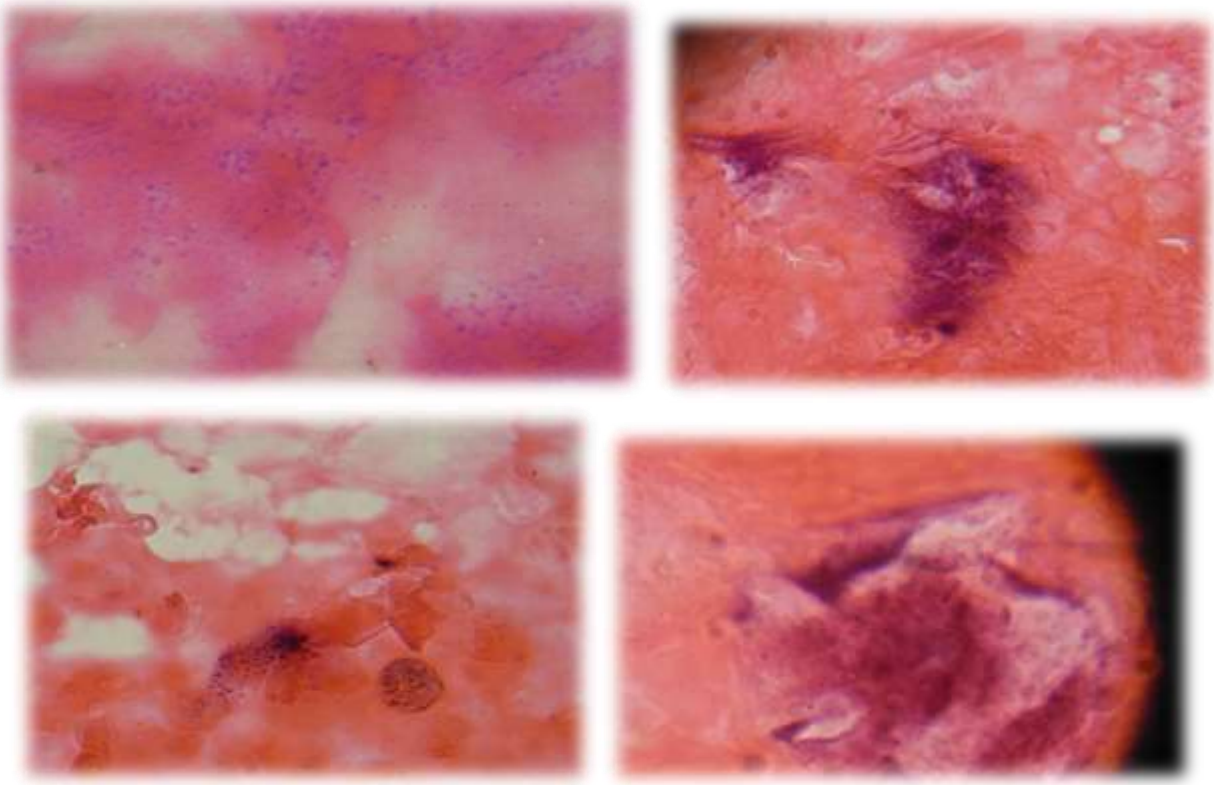

Figure 12: HPV- 18positive signals.

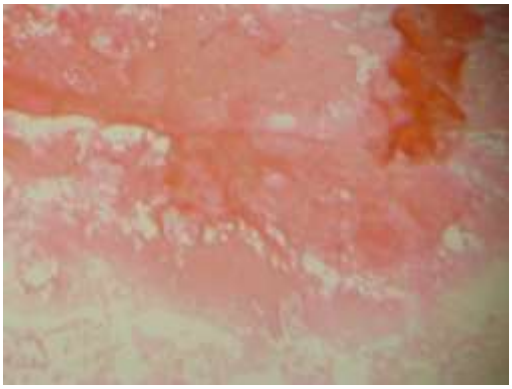

Figure 13: HPV Negative signals.

DISCUSSION

In 1943, Papanicolaou and Traut published their famous monograph on vaginal cytology as a screening method for cervical cancer. Since then, the Pap smear has become the most commonly used method to screen for cervical neoplasia, and it is the best screening tool ever introduced for any cancer. The remarkable success achieved in preventing cervical cancer is largely attributable to cytologic screening tests and presence of precancerous lesions. With the introduction of organized cervical cytological –screening programs, the incidence of cervical cancer has been dramatically reduced. However, high- quality cytology- based screening programs require highly trained personnel and some specialized equipment. Cytological screening tests for cervical neoplasia also have limitations, especially their limited sensitivity, which results in the need for additional cervical cytological tests at regular intervals. Cervical cytological testing has significantly reduced the rates of cervical cancer in many resource- rich countries as compared with resource-poor regions of the world, where cervical cancer affects disproportionately more women who have limited access to organized cytological-screening programs. Because infection with oncogenic human papillomavirus(HPV) has been identified as the underlying cause of cervical cancer, there is interest in the use of HPV DNA testing as a primary screening test for cervical cancer. The overall prevalence of HPV among

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cervical cancers in a large international study was more than 99%, the highest attributable fraction ever identified for a specific cause of cancer. With currently available technology, HPV DNA testing is highly reproducible, is easily monitored, and provides an objective outcome. HPV DNA testing is substantially more sensitive than cytological testing in detecting high-grade cervical intraepithelial neoplasia (Gong, M.Y. *et al*., 2008). The ultimate goal in cervical screening is to reduce the incidence of and mortality from invasive cervical cancer worldwide with a cost –effective and readily available test. The optimal approach will depend on the prevalence of disease, access to screening, and available resources. In this study we choose to use one of the most sensitive molecular method for in situ viral detection (Gong, M.Y. *et al*., 2008). Recent study by (Ming Gong etal) to evaluate (ISH) assy for detection of HPVs comparing with PCR assay showed that ISH and PCR had good agreement in detection of HPV DNA and without significant difference (Mayrand, M.H. *et al*., 2007). We report here the results of our study which designed to compare Pap testing with HPV testing as stand- alone screening tests for cervical –cancer precursors. The sensitivity of Pap test (22%) was significantly lower than the sensitivity of the HPV DNA test (89%). HPV DNA testing had, however, a lower specificity (42%) than conventional cervical cytological testing (96%) which is agreed by Mayrand *et al* (Canadian Cervical Cancer Screening Trial, 2007) but with little difference due to their training in performing and reading Pap smears, (sensitivity of the Pap test (55. 4%) & HPV DNA test (94. 6%) [(25),] and these findings are consistent with previous split-sample and in community- based study in Mexico which revealed that HPV DNA testing for cervical cancers screening is over 50% more sensitive than cytology testing (overall the sensitivity of HPV DNA testing was 93. 3% versus only 40% for cytology test[(26)]. We suggest that the higher sensitivity and the more "up stream"focus on cervical carcinogenesis conferred by HPV DNA testing relative to Pap testing should safely permit prolongation of screening intervals. However, since this was the first screening round in Iraq, we could not address the length of protection afforded by a negative HPV DNA test, but experience from the LAMS study by Derchain SF *et al* implicate that HPV DNA testing for high risk types might be a safe enough approach to warrant extension of the screening interval of high risk HPV DNA negative test in low- resource settings. Although some women will inevitably contract high risk HPV, the process to develop HSIL will be long enough to enable their detection at the next screening round (e. g. after three years)[(27)]. As an adjunct to the Pap test in routine screening, HPV DNA testing was a more sensitive indicator for prevalent high-grade CIN than either conventional or liquid cytology. A combination of HPV DNA and Pap testing had almost 100% sensitivity and negative predictive value. The specificity of the combined tests was slightly lower than the specificity of the Pap test (Lorinez, A.T. *et al*., 2003). Lorincz AT, 2003 state that one double- negative HPV DNA and Pap test indicated a higher prognostic assurance against risk of future CIN III than three subsequent negative conventional Pap tests and may safely allow three-years or longer screening intervals for such low-risk women thus saving considerable cost for health systems. It appears that HPV DNA testing is on the way to become a common testing strategy in cervical cancer prevention programs. Szentirmay Z *et al*, 2007 in their Hungarian study state that negative cytology completed with negative HPVDNA test means the lack of cancer risk even in the case of previously removed CIN or carcinoma, and appositive HPV test detected after conization associated with negative cytology finding indicates a risk of 70% of the development of CIN within 2 years[(30)]. Using both tests (HPV DNA testing with cytological testing) raises the initial cost, which may limit the applicability of widespread screening in resource-poor countries[(31)], however we think according to our study using both tests increase the sensitivity of the screening to 89%, so diagnosis the premalignant lesion early which decrease the cost of treating invasive cervical cancers which most of our patients presented with and decrease the health care resources. Also we cannot determine the impact of using both tests on the screening cost as we have no comprehensive screening program which involve all our population. Lytwyn A *et al* (University of Toronto, 2000) in a community-based randomized trial, they compared the test performance of HPV DNA testing with that of 6-month repeat Pap test in detecting histologically confirmed CINII or III, they found that sensitivity of HPV DNA test was 87. 5%, repeat Pap smear showing HSIL sensitivity was 11. 1% (p= o. oo4), and repeat Pap smear showing ASCUS, LSIL was 55. 6% (p=o. 16), Corresponding specificities were 50. 6%, 95. 2%(p=o. oo2) and 55. 6% (p=o. 61), they conclude that HPV DNA testing was more costy but was associated with significantly less loss to follow-up. It may detect more cases of

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CINII or III in women with low- grade cytological abnormalities[32]. Across-sectional study was performed by Agorastos T *et al* in northern Greece in 2006 to compare the performance of HPV DNA test using polymerase chain reaction against routine conventional Papanicolaou smear for the detection of low- and high- grade cervical intraepithelial neoplasia in a low risk population, they showed that HPV DNA testing had a significantly better sensitivity than the Pap test in detecting CIN (75% versus 50% for high- grade lesion and 81. 2% versus50% for lesion of any grade, respectively). So the Greek experience conclude that HPV DNA testing could be useful in screening women at low risk for cervical cancer, either as an adjunct tool to augment existing cytology programs or as a unique test of its own[33]. Prof Henry C Kitchener *et al*, 2007 disagree with us, they compare HPV testing in combination with liquid-based cytology in primary cervical screening (ARTISTIC): a randomized controlled trial, and they conclude that combination of two tests resulted in a significantly lower detection rate of CIN3 as compared with liquid-based cytology alone, so co-testing did not detect a higher rate of CINII or CINIII than cytology alone (Wheeler, C.M. *et al*., 2009). In our study we discovered 4 cases of invasive cancer, 3 of them had HPV 16 & 18 positive & one case had only 16 positive, although small number of cases, these results are agreed with the results of USA, 2009 study which conclude that HPV 16 & 18 caused the majority of invasive cervical cancer in population sample of US women, they conclude that the most common HPV genotypes detected in invasive cancer were HPV type 16 (53. 2%)and HPV 18(13. 1%)[35]. Porras C *et al*2009 agree with this finding (HPV 16 is a stronger carcinogen than other HPV types) [36]. HPV16 test were negative in 7 cases of HSIL, in 4 of them HPV 18 were positive, in 2 cases HPV 6 & 11 were positive and in one case all 4 types of HPV were negative, so in the last 3 cases there may be infection with other high grade HPV type that we didn't test it or there is a possibility that a few cervical cancers may arise from a non- viral source. Cox JT from University of California, USA1995 agree with us, he maintained in his study that most cervical cancers contain high risk types, up to 15% of such cancers test negative for HPV DNA, raising the possibility that a few, usually more aggressive, cervical cancers may arise from anon- viral source (Syrjanen, K. *et al*., 2006). we classify cases as disease positive which include HSIL & CA, disease negative group which include LSIL & normal (because the risk of progression to cancer in LSIL was 1%, HSIL progress to CA in 13. 5%) (Wheeler, C. M. *et al*., 2009). In our study HPV 16 was positive in 62. 5% of disease positive group while HPV18 in 65. 6% and negative in 33. 3% of disease positive group while HPV 18 negative in 28. 6% (P = 0. 038 for HPV 16 & P=0. 008 for HPV 18 which is statistically significant). HPV 16 and or 18 were positive in 61. 5% of disease positive group and negative in only 3 cases of disease positive group(21. 4%)(P=0. 010 which is statistically significant). HPV 6 & 11 DNA test had sensitivity of 48%, specificity 65%, 58% respectively (P=0. 318, 0. 669, respectively, which is statistically insignificant)so no need to test these viruses in cervical screening programs because they were low-risk types &they are associated with benign lesions, i. e. Condyloma acuminate and CIN(Wheeler, C. M. *et al*., 2009).

HPV-16 &18 /risk factors: 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 62. 3% of the cases, 54. 5% had disease positive and 45. 5% had disease negative, 37. 7% of women not used OCCPS, 45% of them had disease positive and 55% had disease negative(P= 0. 500, which is statistically not significant). our finding is agreed with finding of Syrjanen K *et al* 2006 which suggest that the use of OCCPS is not an independent risk factor for cervical cancer or its precursors but sexual behavior is different among OCCPS users, non users and in nonusers of contraception, these factors predispose women to HR-HPV, high grade CIN and determine the outcome of their cervical disease / HR-HPV infection[38]. Kjellberg L *et al*2000 agree with us, they found that prolonged oral contraceptive use and sexual history were associated with HSIL in univariate analysis, but these associations lost significance after taking HPV into account[39]. 28. 3% of women in our study were smoker, 66. 7% had disease positive and 33. 3% had disease negative, 71. 7% of women were not smoker, 44. 7% were had disease positive and 55. 3% had disease negative, (P= 0. 150) which not reach statistically significant level. . This finding is disagreed by Kjellberg L *et al* 2000 study that state smoking appeared to be the most significant environmental risk factor for cervical neoplasia (Guarisi, *et al*., 2009 R.). Guarisi R *et al* 2009 conclude that smoking status was not associated with the risk of developing CIN(hazard ratio=0. 73;95%CI= O. 40- 1. 33) (Roteli-Martins, C. M .

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et al., 1998). Bosch, F.X. *et al.*, 2007 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(Roteli-Martins, C. M. *et al*., 1998). conclude in their Brazillian study that the severity of CIN lesions was clearly related to two fundamental risk factors, high-risk HPV types and current cigarette smoking, these two risk factors were closely interrelated in that the high-risk HPV types were significantly more frequent in current smokers than in non-smokers, suggesting the possibility of a synergistic action between these two risk factors in cervical carcinogenesis. 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(Kwasniewska, A. *et al.*, 2007). In our study manual workers include house wife represent 52. 8% of women, 42. 9% had disease positive and 57. 1% had disease negative clerk represent 37. 7% of women, 55. 0% had disease positive and 45% had disease negative, health workers represents 9. 4% of women, 50. 9% had disease positive and 49. 1% had disease negative so job of women had (P=0. 279) which is statistically not significant. We didn't found any studies analyses the role of job. STD were positive in 24. 5% of women, 69. 2% had disease positive and 30. 8% had disease negative, 75. 5% didn't had STD, 50. 9% had disease positive and 49. 1% had disease negative, (P=0. 129) which is statistically not significant. Kwasniewska A *et al* 2009 agree with us in the Poland study, they state that no correlation was found between frequency of occurrence of HPV and Chlamydia trachomatis and of HPV and HSV-2 detected in paraffin-sectioned samples for cervical carcinoma (Naučer, P. *et al.*, 2009). Bosch, F.X. *et al.*, in their Spain study state that previous exposure to sexually transmitted diseases such As Chlamydia Trachomatis and Herpes Simplex Virus type 2, smoking and high parity(five or more full term pregnancies) are co-factors that modify the risk among HPV DNA positive women, but exposure to HIV are at high risk for HPV infection, HPV DNA persistency and progression of HPV lesions to cervical cancer(Syrjanen, K. *et al.*, 2007). 45. 3% of women in our study their partners used condom, 54. 2% had disease positive, 45. 8% had disease negative and54. 7% of women their partners not used condoms, 48. 1% had disease positive and 51. 7% were disease negative (P=0. 669)which is statistically not significant. Nidhi Gupta 2009 agree with us, she said that HPV unlike other STDs, use of condom is not effective in preventing this infection because HPV lives in pubic area and cells living the vagina and cervix, condoms doesn't cover pubic area and even dead cells shed during intercourse can contain the virus and remain active for days, the infecting virus may remain dormant and it cannot be known when it will become active(Kwasniewska, A. *et al.*, 2009; Naučer, P. *et al.*, 2009). We facing some difficulties in our study in form of availability of kits and probes of HPV DNA tests which is in ported from outside of the country because it is not available in Iraq, so we take sample of sixty women because it was costy and because of the difficult follow up of the patients, we lose seven cases whose failed to attend Colposcopy dates.

CONCLUSION

1. HPV DNA testing has a better sensitivity than, that of cytology. Sensitivity of the test was 89%.
2. Combination of HPV DNA test with Pap test increase the sensitivity of Pap from 22% to 89%.
3. HPV types 16, 18, 11 &6 has been detected in our community by using ISH technique, and HPV types 16 &18 (high risk)were statistically significantly associated with HSIL & Cancer (P value=0. 010), while HPV 6&11(low-risk) were not associated with these lesions(P value =0. 318, 0. 669)respectively.

RECOMMENDATION

1. Application of HPV DNA test in cervical cancer screening Programs.
2. We recommend application of this study using large sample size to determine the epidemiology of HPV types in Iragi patients.
3. Extent the limit of HPV DNA test to include all 14 high risk types to determine if we need to introduce HPV vaccine and in the future.

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