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Thesis Title	The Use of p16INK4a and In Situ Hybridization and Their Impact on the Healthy and Equivocal Pap Smears of the Cervix			
Year	2015			
Abstract	<p>Throughout the past thirty years, the perception of cervical carcinoma has shifted from that of a mysteriously fatal disease, to one of the sexually transmitted, human papillomavirus (HPV) related infection. HPV DNA has been found in almost all cervical invasive and preinvasive cervical neoplasms. Conventional Pap smear, which was established in the early sixties reduced effectively the morbidity and mortality related to cervical cancer. Given the lower sensitivity of the Pap cytology test, new diagnostic parameters have been established. The histological features of preinvasive cervical neoplasia (CIN1, CIN2, and CIN3) are well understood. However, misinterpretation of the morphological criteria could lead to significant variability. The aim of this study is to identify women with equivocal and normal Pap smear who are at risk for developing cervical cancer through the use of p16INK4a immunostaining and human papillomavirus (HPV) DNA testing using <i>in situ</i> hybridization (ISH).</p> <p>Materials and Methods</p> <p>In order to identify human papillomavirus infection in minor cytological and histological abnormalities, biopsies collected from 60 women 25-66 years of age with normal cervixes and Pap smears (group I), abnormal cervixes and Pap smears (group II) and with</p>			

cervical carcinoma (group III) referred to the Colposcopy Clinic in Baghdad Teaching Hospital – Medical City Complex, during the period from June 2013 through July 2014. Cytology samples collected with ThinPrep for IV liquid base cytology (LBC), punch biopsy for histopathology formalin fixed and paraffin embedded. In situ hybridization (ISH) and p16INK4a immunohistochemistry in addition to routine hematoxylin and eosin (H&E) stain were used to evaluate the histological specimens.

Results

The age, age of marriage and parity of the three groups were studied in relation to each of ISH and p16INK4a reactions.

Age distribution was highly significant among all age groups; in both ISH and p16 test results GI & GII ($p=0.00$), GI & GIII ($p=0.00$), GII & GIII ($p=0.00$) in both ISH and p16 test results. The age of marriage was not significant among the groups in both ISH and p16; GI & GII ($p=0.93$), GI & GIII ($p=0.12$), GII & GIII ($p=0.30$). The effect of parity was insignificant among all groups; GI & GII ($p=0.66$), GI & GIII ($p=0.82$), GII & GIII ($p=0.79$) in both ISH and p16 test results.

p16INK4a immunoreaction: Among group I there was a negative reaction in all the specimens collected. In specimens from group II, there was a very mild or focal p16INK4a immunoreaction in one specimen (5%), a moderate immunoreactivity in 9 specimens (45%), and a high positive reactivity in 10 specimens (50%) ($p=0.000$). Group III showed very high immunoreaction in all the specimens collected. In situ hybridization (ISH) in group I was mild positive in 4 specimens (20%) which showed diffuse signal pattern. A mild positive reaction with diffuse nuclear staining was noticed in 4 (20%) of the cervical specimens. Moderate positive reaction with diffuse staining of

the nucleus was observed in 8 (40%) of the specimens. The remaining 8 (40%) of the specimens showed both diffuse and punctate staining of the V nuclei. Among group III specimens, the nuclei in the epithelial cells showed both punctate and diffuse signal patterns. Chi square was done to compare results concerning ISH & various groups was highly significant ($p= 0.00$)

Conclusion
HPV infection is prevalent and can be missed by the conventional Pap smear. The severity of the disease was directly proportional with the age of women in this study. p16 overexpression was correlated with the positivity of ISH. This may support the use of either, or both, tests to improve the accuracy of cervical intraepithelial neoplasia (CIN) diagnosis, and help in the triage of women with equivocal lesions.