EARLY DETECTION OF CERVICAL CANCER BASED ON HPV INFECTION IN PAP   SMEAR USING MORPHOLOGICAL METHOD

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Abstract:

In developing countries cervical cancer is a leading cause of death in females. In more than 90% of cases, these carcinomas are etiologically related to human papilloma virus infection. In developed countries HPV can be easily detected by PCR methods but these methods are very costly and not affordable. The purpose of this study is to found effective, low-cost, less time consuming method in our set up for early detection of cervical cancer. Current study was conducted in Isra university hospital, Hyderabad, Pakistan. Total 100 samples were collected from patients routinely presenting with gynecological problems to the outpatient department. All samples were fixed in alcohol immediately to avoid air drying effect. and were brought to Isra university histopathology laboratory for cytopathological examination. Total 100 Pap smear samples were examined for Cytomorphological changes for cervical malignant changes. Age categories were divided into < 30 years – ≥60 years. Cytoplasmic changes- the Vacuolation, Koilocytosis, Keratohyaline granules and cytoplasmic folding were present in 32, 64, 50 and 52 samples respectively. Koilocytosis was most frequent finding in our samples. Nuclear changes- the Size variation, Multi nucleation, prominent nucleoli and Mild hyperchromasia were present in 64, 66, 48 and 58 samples respectively. Size variation, multi nucleation and hyperchromasia were frequently observations. Cumulative Cytomorphological changes were noted in 90% of study subjects.
Conclusion: Various Cytomorphological changes like Vacuolation, Koilocytosis, Keratohyaline granules, cytoplasmic folding and nuclear changes like nuclear size variation, multi nucleation, prominent nucleoli and mild hyperchromasia as markers indicative of HPV infection and may enhance cervical cytology importance.
Key Words: *Cervical cancer, Screening, Cytology, Papsmear, Human Papilloma Virus.*

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INTRODUCTION:

Cervical cancer is the most common cancer in females throughout the world in the past. It is responsible for the largest causes of mortality in females in low-income countries[1,2].The causative agent in 90% of cervical cancers is human papilloma virus [3].Due to persistent infection with human papilloma virus ,squamous metaplasia of columnar cervical epithelium occurs and it will ultimately progress to cervical intraepithelial neoplasia(CIN).It is a precursor from which most cervical cancer develops. Human papilloma virus infect squamous cells and express two potent oncoprotiens E6 and E7.These oncoprotein inactivate tumor suppressor genesP53 and RB and increase chances for further mutations which can ultimately progress to carcinoma[4].Carcinoma of cervix is a escapable disease.Papinacolau test is a cheap method of cervical screening for early detection of precancerous lesions in cervix[5,6].This test has been most successful screening test in the world and has played great role in decreasing mortality rate . Cervical cancer incidence is increasing in Pakistan. For early detection of cervical cancer in asymptomatic patients Pap test is extremely useful. In developing countries Pap test role needs to be established for population screening [7,8]. In developed countries the incidence of morbidity and mortality of cervical carcinoma is significantly decreased. In United States, there is positive correlation between the rate of cytological screening (Pap test) and decrease in morbidity and mortaliy of cervical malignancy. The situation for Pakistan is highly alarming. Rate of screening is only 1.9% while the disease cases burden is 13.6/million populations. Cervical cytology by Pap smear is an effective tool for the cervical cancer screening of premalignant and malignant condition.[9]. Pap test is simple, safe, noninvasive and very effective for the screening instead of much invasive procedures [10,11]. Screening and awareness programs should be composing for early detection of cervical cancer. Pap smears routinely reporting includes chronic inflammation with squamous metaplasia with mild, moderate and severe dysplasia. HPV detection in developed countries is carried out by serological and immunohistochemical methods on PCR, which is a very expensive method and not affordable for poor patients. Little work has been done to study the morphological changes that starts due to HPV infection. The aim of this study was to evaluate the Cytomorphological changes in Pap smear cause by HPV infection for early detection of cervical cancer.

MATERIALS AND METHODS:

This was observational study which was conducted in Pathology deparment, Isra University hospital. Total 100 samples were collected. The sample size for the study was calculated by the formula for sampling for proportions. A detailed history was taken on patient proforma from patients routinely presenting with gynecological problems to the outpatient department.Patients with bleeding P/V, diagnosed cases of cervical cancer and other malignancies of female genital tract like endometrial and cervical cancer are excluded from study. All smears were stained by Papanicolaou's method and were examined using light microscope and various morphological changes were noted on a printed proforma. All the patients' data from clinic-pathological proforma were collected and fed in computer. The data was analyzed on SPSS version 21.0 for windows release . Continuous and categorical variables were analyzed by using appropriate test(s) of significance according to the type of variables. A P- value of <0.05 was considered as significant.

RESULTS:

100 Pap smear samples were examined for Cytomorphological changes for cervical malignant changes. The age distribution showed mean ± SD of 44.48± 11.78 years. Age categories were divided into < 30 years – 6 patients, 30
– 39.9 years – 26 patients, 40 – 49.9 years – 42 patients, 50 – 59.9 years – 8 patients and ≥60 years – 18 patients(figure.1). This shows,most of our study subjects belonged to 3rd – 4th decades. Cytomorphological changes- sub divided as cytoplasmic and nuclear changes are shown in table-3. Cytoplasmic changes- the Koilocytosis vaculation, Keratohyaline granules and cytoplasmic folding were present in 32, 64, 50 and 52 samples respectivley (P= 0.0001). Koilocytosiwas most frequent finding in our samples. Nuclear changes- the Size variation, Multi nucleation, prominent nucleoli and Mild hyperchromasia were present in 64, 66, 48 and 58 samples respectivley P= 0.0001 table.1. Size variation, multi nucleation and hyperchromasia were frequently observations. Cumulative Cytomorphological changes were noted in 90% of study subject.
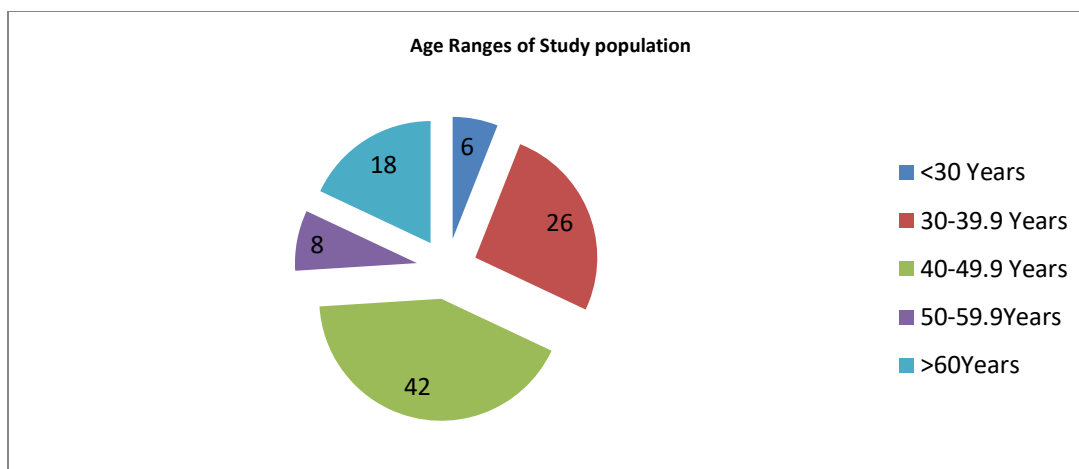
Figure.1 showing age ranges of study subjects

Table.1: Cytomorphological and nuclear changes observed on PAP smear in study subjects   (n=100)

Cytoplasmic changes Frequency and percentage		Nuclear changes Frequency and percentage	
Vacuolation	32(32%)	Size variation	64(64%)
Koilocytosis	64(64%)	Multi nucleation	66(66%)
Keratohyaline granules	50(50%)	Prominent nucleoli	48(48%)
Cytoplasmic folding	52(52%)	Mild hyperchromasia	58(58%)
P-Value	0.0001	P-Value	0.0001
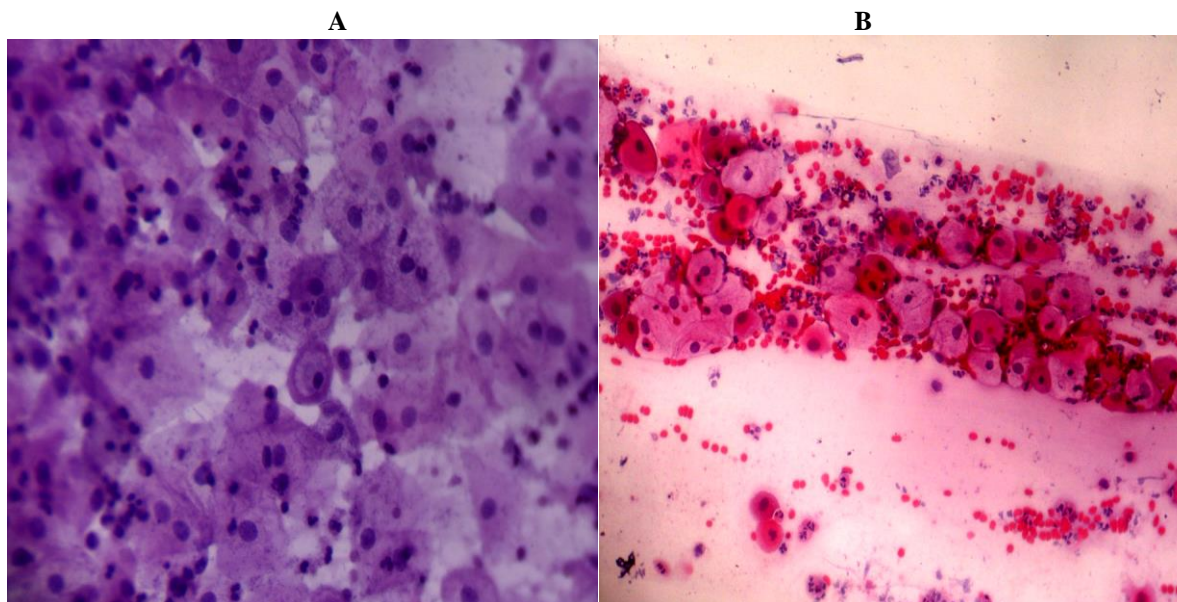
PHOTOMICROGRAPH -1.
A. Pap smear showing prominent nucleoli with bineucleation and mild hyperchromasia(Size 20x).
B. B Koilocytic effect with prominent neucloli(Size 40x)

DISCUSSION:
The present study demonstrated the Cytomorphological changes in Pap test to detect precancerous and cancerous lesions in cervix associated with HPV. Our aim is early detection and to prevent progression to cervical carcinoma . Screening of cervix should begin at age of 18 years or as soon after first sexual intercourse[12].Many causes have been recognized for the premalignant lesions of cervix but most frequently cause include persistent infection of human papilloma virus. HPV may cause benign lesion or may represent first step towards

carcinogenesis[13].In the present study we tried to evaluate the cytomorphological changes in cervical cytology associated with HPV infection as established by Bathesda. The ASCUS-LSIL Triage Study (ALTS) recommend that the Pap smear showing atypical squamous cells of undetermined significance (ASCUS) may be seen in more than 50% cases of women infected by human papilloma virus[14]. In present study 100 Pap smear samples were examined for Cytomorphological findings for cervical malignant changes. Koilocytosis was most frequent finding in our study subjects. Size variation, multi nucleation and hyperchromasia were frequent observations. The findings of present study are in juxtaposition to these studies [15, 16]. The infections manifests clinically as warts which may be hyperplastic and hyperkeratotic warts. The warts may appear as dysplastic lesions. The lesions transform into neoplastic growth [17].HPV 16 and 18 types are established cause of cervical pre malignant lesions [18]. HPV 16 is linked with cervical squamous carcinoma and HPV 18 is linked to cervical adeno-carcinoma. International Agency for Research on Cancer (IARC) has classified HPV types 16 and 18 as proved human carcinogenic agents [19]. Cytomorphological changes are useful in clinical settings performed on single Pap smear has been demonstrated by a previous study [20]. The findings are consistent to present study. As regards specificity, the Cytomorphological changes are reported to be nonspecific for the HPV infection, although they are highly suggestive and a valuable tool in screening programs [20]. Elkharashy, et al has reported age 51.50±13 years of cervical cancer patients. This is in contradistinction to our present study. In present study, the age was noted as 44.4± 11.78 years (t = 37.75, p=0.0001). He also demonstrated that abnormal cervical findings on papsmear needs HPV DNA testing by PCR [21]. Velázquez-Márquez et al from Mexico have reported age of study subject of 41.4 (64) which are consistent to the present study[22]. Afrakhteh et al from iran reported a study on abnormal cervical cytology. They reported mean age of 46 years which is higher to age noted in present study [23]. Balaha et al from eastern Saudi Arabia reported patients with abnormal cervical cytology with age range of 18 to 76 (36.5 years). The finding of mean age of this study is lesser compared to present study, but the abnormal cervical cytological findings are accordant [24]. Khattak et al from Peshawar reported that Papinacolau test is very effective means of detecting cervical intraepithelial neoplasia in curable stage[25]. In view of evidence of Cytomorphological changes of present study and review of previous studies as mentioned above, the present study suggests a role of these changes as a valuable diagnostic tool for the cervical cancer screening. If abnormal cytological changes are observed then further sophisticated investigations may be conducted for confirmation of the cervical lesions. The limitations of present study include small sample size, no molecular testing for HPV and study subjects belonged to particular ethnicity, and hence these findings cannot be generalized to other settings.

CONCLUSION:

The Cytomorphological changes; the Vacuolation, Koilocytosis, Keratohyaline granules, cytoplasmic folding, nuclear size variation, multi nucleation, prominent nucleoli and mild hyperchromasia as markers indicative of HPV infection may elevate cervical cytology importance. Cytomorphological changes especially the Koilocytosis may help to detect cervical intra epithelial lesions at earlier and treatable stage.

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