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"A COMPARATIVE STUDY OF CONVENTONAL TECHNIQUES AND HAND HELD PROBE BASED ON ON POLARIZED FLOURESCENCE SPECTROSCOPY IN DETECTION OF CERVICAL PRECANCEROUS AND CANCEROUS LESIONS"

Gynaecology

KEYWORDS: Polarised fluorescence, hand held probe, cancer cervix, colposcopy, screening.

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ABSTRACT

OBJECTIVES: To diagnose early cervical neoplasia by cytology, colposcopy, histopathology and polarized fluorescence spectroscopy with aid of handheld probe and to compare the efficacy of these modalities in detection of early cervical neoplasm. METHODS: The study was conducted in 81 patients undergoing hysterectomy in UISEMH, LLR Hospital between December 2013 to October 2015. All patients were subjected to cytology and colposcopy. Each of the hysterectomized sample was evaluated by a team from IIT Kanpur with hand held probe, based on polarised fluorescence. The sample was then sent to Pathology dept of GSVM Medical College for histopathology. The findings were then collaborated, considering histopathology as the gold standard. RESULTS: The sensitivity of hand held probe in detecting precancerous lesions using co-polarized light was 92.86% while specificity was 94.87%, while for cross polarized light, sensitivity was 92.86% and specificity was 89.74%. CONCLUSION: The hand held probe based on optical spectroscopy is an excellent screening modality that helps to detect precancerous lesions of the cervix. It has the added advantage that it is minimally invasive, can be used in rural set up by paramedics after little training.

INTRODUCTION

According to WHO 2015 report cancer cervix is the 4th most common cancer among females all around the world,[1] states that every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease. Mortality due to cervical cancer is also an indicator of health inequities, as 86% of all deaths,[2] due to cervical cancer are in developing, low- and middle-income countries[3].

The accessibility of the cervix, propensity of the cells to exfoliate from precancerous lesions, the evidence from pathological studies of existence of histological changes from mild atypia through pre-malignancy to frank malignancy, the apparently prolonged natural history, the long pre-malignant phase, and the ability for detecting changes, using various screening techniques, provide perhaps the best potential for control of cervical cancer by mass population screening.[4] In case of cervical carcinoma, latent phase prior to the development of invasive cancer is on average 12 years.

HPV is necessary for the development of cervical cancer. Therefore, preventing HPV infection can prevent cervical cancer. This can be achieved by complete abstinence from sexual activity or by a vaccine.[5]

The screening tools for cancer cervix presently available are Pap Smear, liquid based cytology, HPV DNA test, Visual inspection after application of Lugol's iodine (VILI) or acetic acid (VIA), speculoscopy, colposcopy, endocervical curettage, punch biopsy and cone biopsy.

The US Preventive Services Task Force (USPSTF)[6] and American Cancer Society (ACS) together released new guidelines for cervical screening in March 2012.These apply to women who have a cervix, regardless of sexual history. They recommend screening women ages 21 to 65 years with cytology every 3 years or, for women ages 30 to 65 years who want to lengthen the screening interval, screening with a combination of cytology and HPV testing every 5 years.

Cytology (Pap smear) has limitations as high rate of false negative results due to variation in lesion distribution in the cervix, smear preparation and staining, microscopic reading, and observer bias in the interpretation and reporting. Several meta-analyses have reported quite low pap smear sensitivities - with a median of 50 per cent13-15. The sensitivity falls further for post menopausal women due to physiological changes of the cervix. In general, the low sensitivity of a single pap test makes it necessary to screen women relatively frequently - every 3 to 5 years, a proposition not suitable for developing countries where even once a life time screen is not feasible.[7]

Visual modalities of screening include Visual inspection after application of Lugol's iodine (VILI) or acetic acid (VIA). Concerns have been expressed about reproducibility and quality control of VIA

in field conditions.[8] The major disadvantage of VILI is the low specificity of the test. One of the difficulties with VILI is that staining effects persist for 30-45 min and diagnostic procedure such as colposcopy after VILI must be delayed for this period of time, if it is to be administered on the same day.

The very high sensitivity (88 to 100%) of HPV testing for high grade cancer precursors and the declining level of HPV detection in normal women over the age of 30 establishes testing for HPV as a more effective primary screen for women over this age than currently practiced cervical cytology. The extremely high NPV (99-100%) of the combination of normal cytology screen and a negative HPV test should allow safely lengthening intervals up to 8-10 years. The specificity of HPV testing for HSIL and above ranges from 57 to 89 per cent. Though the specificity of the test increases in older women, the overall specificity is low. This indicates potential for overt treatment.[7]

Colposcopy is a good diagnostic and therapeutic tool in cancer cervix but its sensitivity and specificity are affected by the observer's expertise. It is also an expensive modality for screening per se.

Most of the present screening modalities require the patient to come to the hospital. Since 80% of our population resides in rural areas, these screening modalities are almost inaccessible to them.[9] The need of the hour is to carry our screening tools to the population at risk. Optical imaging technique can image tissue with sub-cellular resolution to probe changes in epithelial cell morphology and epithelial architecture without the need for biopsy, sectioning and staining.[10]

How does optical imaging work?

- Optical method can be used to probe change associated with known hallmark of cancerous change in tissue such as epithelial cell morphology, metabolic activity and differentiation, stromal angiogenesis and epithelial stromal communication.

- In normal cervical tissue, collagen cross links give rise to bright fluorescence in the stroma when light is incident on them. Stromal fluorescence is greatly reduced in cervical precancers and cancers.

- Normal cervical epithelial cells show auto-fluorescence attributed to mitochondrial NADH (reduced NAD) and FAD. At the periphery of the cells, auto-fluorescence is due to cytokeratins.

- The unique quality of cancerous cells is continuous multiplication which is dependent on each cell running its Kreb's cycle. At the end of each Kreb's cycle, the cell gain NADH and FADH2 while it loses NAD^+ and FAD^+. *Therefore in a cancerous cell, the NADH fluorescence increases but the FAD^+ fluorescence decreases.*

- In low grade precancers, cytoplasmic fluorescence is visible in the bottom 1/3 of the epithelium and in high grade precancers, cytoplasmic fluorescence is visible throughout the lower 2/3rds of the epithelium.

- This is consistent with recent studies which show that HPV immortalized keratinocytes show increased NADH and but reduced FAD fluorescence relative to normal keratinocytes.

The hand held probe is able to detect these changes in fluorescence and hence is an effective marker of tumorigenesis.

Figure 1 represents the schematic working of the hand held probe.

AIMS

The aim of the study is to compare the efficacy of hand held probe based on polarised fluorescence, with that of cytology and colposcopy using histopathology as gold standard in detection of early cervical neoplasia.

MATERIALS AND METHODS

A total of 81 patients undergoing hysterectomy in UISEMH, LLR Hospital between December 2013 to October 2015 were studied with symptoms suspicious of cervical lesions like persistent abnormal vaginal discharge, low backache, contact bleeding, postmenopausal bleeding, menstrual disorder, and pain in abdomen. The entire procedure was explained to patients and their informed consent was taken. In every patient pelvic examination, pre-operative investigations as well as vaginal cytology & colposcopy were done pre-operatively.

Post operatively the hysterectomized uterus with cervix was examined with the hand held probe, in our department by us along with technical team from Department of Physics, Indian Institute of Technology (IIT) Kanpur. The probe is unique in its design in that it consists of a laser source, a polarizer, an analyser and a detector in one unit itself. The hand held probe consists of a laser diode (405nm, Pegasus, Shanghai, optical system co.ltd) and Xe-lamp (Newport oriel instrument USA) as light source and a miniature spectrometer (H 2000+, Ocean Optics, Inc., Dunedin, Florida), to detect fluorescence signal from the sample under both co-polarised and cross polarised light.

The tissue sample was mounted on a frame box and light from the hand held probe was incident onto it. The incident light varied 450 nm to 650 nm thus covering a significant part of the visual spectra. The reflectance pattern (based on the presence or absence of FAD^+) was detected by the probe and results were plotted.

After examining, formalin fixed samples were sent for evaluation in pathology department of GSVM Medical College.

The results of the reflectance pattern were compared with then compared with that of histopathology to calculate the sensitivity and specificity of the hand held probe probe using statistical analysis by MedCalc software (ROC analysis). Results of optical imaging were also compared with that of cytology and colposcopy. Chi square test was applied to calculate p value.

RESULTS

Table 1 shows the co-relation of cytological findings using Pap smear with histo-pathology. The sensitivity of pap smear is 77.42% while specificity is 58.33%. The positive predictive value (PPV) and negative predictive value(NPV) are 76.19% and 60% respectively.

Table 2 shows the most common colposcopic findings in the patients of our study group. Acetowhite epithelium is the most common colposcopic finding.

Table 3 shows the co-relation of colposcopic findings (using reid's index) with histo-pathology. The sensitivity of colposcopy is 97.61% and specificity is 76.92%. PPV is 82% but the NPV is 96.77%.

Fig 2 shows the Receiver Operative Characterstics curve (ROC curve) of band area around peak with co-polarization(VV) for detection of precancerous tissues using hand held probe. The sensitivity is 92.6% while specificity is 94.87%.

Fig 3 shows the Receiver Operative Characterstics curve (ROC curve) of band area around peak with cross-polarization(VH) for detection of precancerous tissues using hand held probe. The sensitivity is 92.86% while specificity is 89.74%.

DISCUSSION

Central to the prevention and control of cancer is the concept that carcinogenesis is not an event but a process, a series of discrete cellular changes that result in progressively more autonomous cellular processes. Aim of our study was to emphasize on early

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detection of cervical dysplastic lesions by optical imaging technique.

Table 1 shows that on correlating our cytology findings with histology, sensitivity was calculated to be 77.42% and specificity was 58.33%. The positive predictive value (PPV) of 76.19% was higher than negative predictive value (NPV) of 60%. The results are similar to other studies. According to Bahar Kohli et al[11], pap smear had a sensitivity of 80% and specificity of 64.29%.

Table 2 shows the results of colposcopy. In our study acetowhite lesions were the most common colposcopic finding, present in 76.54% cases. Also as the grade of dysplasia increases percentage of cases with Schiller's positive, mosaicism and punctuations increases. Papa Dasari et al[12] found that the most common feature was acetowhiteness (41.3%) followed by a combination of acetowhiteness and vascular abnormality (24.7%).

Table 3 shows the correlation of colposcopic finding to histology. We used Coppleson and Reid combined colposcopic index for scoring of lesions. The sensitivity was 97.61% while Specificity was 76.92%. Cantor SB et[13] evaluated accuracy of colpocopy in the diagnostic setting compared with the screening setting. They concluded that using a disease threshold of HSIL, colpocopy had a sensitivity of 98.3% and specificity of 45.1% in the diagnostic group when the test threshold was LSIL, and a sensitivity of 71.4% and specificity of 81.3% when test threshold was HSIL.

Figure 2 shows polarized fluorescence spectroscopy using band area around peak intensity value with co-polarised (VV) light has sensitivity 92.86% and specificity 94.87% with area under curve of ROC analysis of 0.971 at band area 2828.6 with p value 0.0001.

Figure 3 shows polarized fluorescence spectroscopy using band area around peak intensity value with cross-polarised (VH) light has sensitivity 92.86% and specificity 89.74% with area under curve of ROC analysis of 0.954 at band area 2083 with p value 0.0001.

This means that that the hand held probe is an excellent discriminator of normal tissue from all types of precancerous and cancerous lesions.

CONCLUSION

Interest in fluorescence diagnostics has increased considerably in the last few year as a result of several factor: the availability, decreasing cost and clinical suitability of the required technology, development of new exogenous fluorophores, more detailed understanding of auto-fluorescence, the need for better method of early tumor detection and commercial opportunities in a conscious health care environment. Optical imaging using both co-polarised (VV) and cross polarized (VH) alternatively we could get better discrimination between normal versus precancerous lesion. The high sensitivity and specificity of the hand held probe makes it an ideal modality to screen pre-invasive and invasive cervical cancer lesions as compared to the tedious presently existing tests like pap smear, VIA, VILI, colposcopy and histopathology.

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DECLARATION

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Conflict of interest: None declared
Ethical approval: Not required

TABLE 1: CO-RELATION OF CYTOLOGICAL FINDINGS WITH HISTOPATHOLOGICAL FINDINGS

HPE	NORMAL	INFLAMMATORY	CIN 1	CIN 2	CIN 3	CARCINOMA	TOTAL
CYTOLOGICAL							
NORMAL	-	8	4	-	-	-	12
INFLAMMATORY	-	21	2	1	-	-	24
MILD DYSPLASIA	-	8	20	2	-	-	30
MODERATE DYSPLASIA	-	2	5	4	-	-	11
SEVERE DYSPLASIA	-	-	-	1	2	-	3
CARCINOMA	-	-	-	-	-	1	1
TOTAL	-	39	31	8	2	1	

Sensitivity= 77.42%
Specificity = 58.33%
Positive Predictive Value = 76.19%
Negative Predictive Value = 60%

TABLE 2: COLPOSCOPIC FINDINGS

COLPOSCOPIC	NORMAL	INFLAM	CIN 1	CIN 2	CIN 3	CARCINOMA	TOTAL
Acetowhite	-	23	28	8	2	1	62
Mosaic	-	5	16	2	1	1	25
Punctuation	-	7	9	4	1	-	21
Atypical vessel	-	-	-	5	2	1	8
Schillers positive	-	5	20	1	1	1	28
TOTAL	**-**	**39**	**31**	**8**	**2**	**1**	

TABLE 3: CORRELATION OF COLPOSCOPIC FINDINGS IN RELATION TO HISTOPATHOLOGICAL DIAGNOSIS (USING COLPOSCOPIC INDEX) (Reid et al 1983)

COLPOSCOPIC INDEX	INFLAMMATORY	CIN 1	CIN 2	CIN 3	CARCINOMA	TOTAL
0	-	-	-	-	-	-
1	22	9	-	-	-	31
2	8	13	2	-	-	23
3	5	4	1	-	-	10
4	4	5	3	1	-	13
5	-	-	2	-	-	2
6	-	-	-	1	-	1
7	-	-	-	-	1	1
8	-	-	-	-	-	-
TOTAL	**39**	**31**	**8**	**2**	**1**	**81**

Sensitivity= 97.61%
Specificity = 76.92%
Positive Predictive Value = 82%
Negative Predictive Value=96.77%

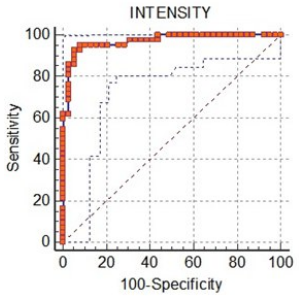

FIGURE 2: Receiver Operative Characterstics curve (ROC curve) of band area around peak with co-polarization(VV) for detection of precancerous tissues.

Area under ROC curve (AUC)	0.971
Standard error	0.0161
95% confidence interval	0.907 to 0.996
Z statistic	29.200
Significance level P (area= 0.5)	<0.0001

Youden index J	0.8773
Associated criterion	228.6
Sensitivity	92.86
Specificity	94.87

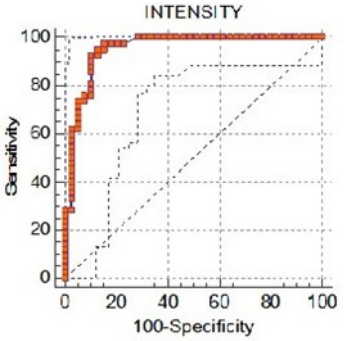

FIGURE 3: Receiver Operative Characterstics curve (ROC curve) of band area around peak with cross-polarization(VH) for detection of precancerous tissues.

Area under ROC curve (AUC)	0.954
Standard error	0.0233
95% confidence interval	0.882 to 0.988
Z statistic	19.439
Significance level P (area= 0.5)	<0.0001

Youden index J	0.8260
Associated criterion	=2083
Sensitivity	92.86
Specificity	89.74

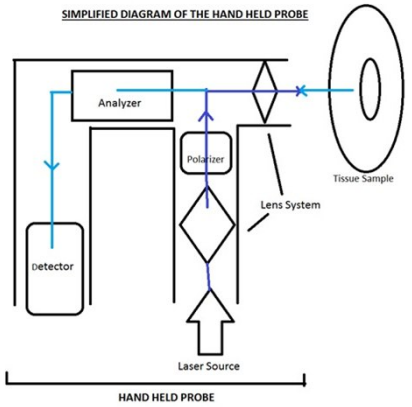

Fig 1: Simplified diagram of the working of a hand held probe

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