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ORIGINAL RESEARCH

Can co-polarized fluorescence spectroscopy replace pap smear and colposcopy for in-vivo detection of precancerous cervical lesion in near future ?

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ABSTRACT

Background: Cervical cancer is a preventable disease due to its long invasive state and availabilities of screening programs, the aim was to diagnose the cervical precancerous lesion by cytology, colposcopy and co-polarized fluorescence spectroscopy with aid of in vivo hand held probe and to compare the efficacy of these modalities in detection of cervical precancerous lesion. **Material and Method:** It was a prospective non- randomised study conducted in 34 patients who came in the department of Obstetrics and gynaecology, LLR Hospital Kanpur between December 2016 to August 2018. All patients were subjected to conventional PAP smear followed by evaluation by using hand held probe based on co-polarised fluorescence. After that these Patients were subjected to colposcopy guided biopsy and the sample was sent to pathology department of G.S.V.M 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 96.67% and specificity was 93.35%. Sensitivity of PAP smear was 84.2% and specificity was 73.3% and colposcopy sensitivity is 73.7% and specificity was 86.7%. **Conclusion:** The hand held probe based on co-polarized fluorescence spectroscopy is an excellent screening modality that helps in detection of precancerous lesions of the cervix. It has added advantage that it is minimally invasive, can be used in rural set up by paramedics after little training and can be used for mass population screening.

Key words: Polarised fluorescence, hand held probe, Cancer cervix Screening.

INTRODUCTION

Cervical cancer is the most commonly occurring genital cancer in Indian females and it is the **second most common cancer** in women living in less developed regions after breast cancer.[1]According to WHO 2018 cervical cancer report, 570,000 new cases in 2018 representing 6.6% of all female cancers and approximately 90% of deaths from cervical cancer occurred in low and middle income countries. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early and effective screening programmes and treatment of pre invasive cervical lesions. [2]

According to ICO Information Centre on HPV and cancer (Summary Report 2014] every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 die from the disease. [3]

Central to the prevention and control of cancer is the concept that carcinogenesis is not an event but a process involving a series of discrete cellular changes that result in progressively unregulated autonomous cellular growth and failure of apoptosis or programmed cell death . Long premalignant phase, known natural history of disease and HPV as a causative agent in 99% cases and ability for detecting precancerous changes using various screening techniques, provide perhaps the best opportunity to

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prevent this deadly disease by mass population screening.[4]

The Screening tools for cervical cancer presently available are PAP smear(conventional &Liquid based cytology), HPVDNA testing, Visual inspection of cervix after Lugol's iodine (VILI) or acetic acid (VIA), Speculoscopy and cervicography but all possess some or other limitations.

Pap smear has a high rate of false negative results due to variation in lesion distribution in the cervix, smear preparation and staining, microscopic reading, and intra as well as inter observer bias in the interpretation and reporting although Bethesda system has revolutionised the nomenclature of preinvasive cervical lesion.[5] A meta-analysis shows conventional Pap smear had sensitivity of 51%, specificity 66.6%, whereas with the addition of liquid based cytology sensitivity has improved to 55.3% and specificity 77.7%respectivly.[6] The sensitivity falls further for postmenopausal 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 years, a proposition not suitable for developing countries.

There are other the screening modalities which includes visual inspection after application of acetic acid (VIA) or lugol's iodine (VILI) which are specially designed for rural settings where " see and treat" approach is adopted .Concerns have been expressed about reproducibility and quality control of VIA in field conditions.[7]In one meta-analysis sensitivity of VIA was 82.14% and specificity was 50.0%.[8]Though these techniques are being used as the current diagnostic modality to identify cervical abnormalities, and more dependent on the expertise of the person who is examining, resulting in subjective error.

Therefore an effective Screening method to diagnose cervical cancer is still being in search. which should be highly sensitive, specific, cost effective and within the reach of the targeted population. Fluorescence spectroscopy is an emerging optical technology that offers a great promise as it detects the biochemical and morphological changes with in dysplastic cervical tissue. Till date, in vitro studies by using Hand Held Probe, an instrument (fabricated by Department of Physics, IIT Kanpur) based on polarized fluorescence spectroscopy have been done to demonstrate the efficacy of fluorescence spectroscopy in detection of pre-invasive cervical lesions . From in vitro studies it was analyzed that 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%.[9]

Thus, from in vitro study it was found that hand held probe based on fluorescence spectroscopy it is as good as other screening methods therefore we are carrying it forward to in vivo detection of cervical precancerous lesion by hand held probe and Compare its efficacy with that of cytology, colposcopy, histopathology.

MATERIALS AND METHODS

It was a prospective non randomised study with 34 patients enrolled who came with complain post-coital bleeding, postmenopausal bleeding, leukoplakia, persistent vaginal discharge, irregular bleeding, conductedin outpatient department Obstetrics and gynaecology , LLR Hospital Kanpur between December 2016 to August 2018 (pregnant women's were excluded from this study). Ethical clearance was taken by ethical committee G.S.V.M Medical College Kanpur.

After taking informed and written consent, all patients were subjected to conventional PAP smear then these patients' cervix (different sites) were evaluated by atechnical team from Department of Physics, IIT Kanpur along with us by using hand held probe based on co-polarised fluorescence. 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.

After that These Patients were subjected to colposcopy and biopsy of suspicious areas and the sample was sent to pathology department of G.S.V.M Medical College for histopathological examination. The findings were then collaborated and analysed, considering histopathology as the gold standard.

How It Works

- Optical spectroscopy detects changes such as epithelial cell morphology, metabolic activity and differentiation, stromal angiogenesis and epithelial stromal communication which are hallmark of cancerous changes.
- In cervical tissue nicotinamide adenine dinucleotide hydrogen(NADH), flavin adenine dinucleotide (FAD), Collagen and Porphyrin are autofluorophores and collagen cross links give rise to high fluorescence in the stroma which is greatly reduced in cervical precancerous and cancers. At selected wavelengths (405nm) FAD is the dominating fluorophore and as the disease progresses, FAD gets converted to reduced form which does not have fluorescence. This is consistent with recent studies which show that *HPV immortalized keratinocytes show increased NADH and but reduced FAD fluorescence* relative to normal keratinocytes.

In this study diode laser light of 405 nm wavelength and xenon light (470 – 700) nm wavelength was incident on cervical tissue and co-polarized spectra is recorded by a miniature spectrometer (HR4000, Ocean Optics).Cancer detection based on co-polarized

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auto-fluorescence of FAD present in human cervical tissues, reveals a peak at 405 nm under UV light radiation. The collected signals were transferred to computer and the data was processed by Windows-based data acquisition programme. Difference in cellular chemistry associated with tissue pathology would be reflected on the fluorescence spectral profile, providing quantitative diagnostic information. To calculate the sensitivity and specificity of the hand held probe using MedCalc software (ROC analysis) statistical analysis, Results of Optical imaging were also compared with that of cytology and colposcopy.

RESULTS

In this study, the mean age for inflammatory lesions was found 36.5 ±5.7 years, whereas for CIN 1, CIN2, CIN 3, Carcinoma was 40.62±8.3**,** 46.28±5.6**,** 54.3± 9.3**,**57±11.3 yrsrespectively.

82.15% of patients who had premalignant and malignant lesions were associated with 1st coital activity at the age less than 25 years.79.4% had with parity more than two and 85.71% belonged to low socio-economic class.

Table 1 shows that when the cytological findings were correlated with histopathology, sensitivity was calculated 84.2% and specificity was 73.39% with positive predictive value 80.0% and negative predictive value 79.4%.

Table 2 shows the presence of various colposcopic findngs in the cases. Aceto white epithelium is the most common colposcopic finding in our study, present in 76.48% cases.

Table 3 shows the correlation of colposcopic finding to histopathology. We used Reid colposcopic index for scoring of lesions. The sensitivity was 73.7% while Specificity was 86.7%.

Figure 1Scatter plot shows that band area around peak intensity by co-polarization (VV) of individual patients. patients those are histologically normal presented by blue dots(series1) patients those are are histologicaly abnormal presented by red dots(series2) it shows that most of the patients with abnormal histopathology are below 3000 intensity .

Fig 2 shows ROC curve to determine the diagnostic ability of screening method between normal and CIN1/CIN2/CIN3/Carcinoma in situ sensitivity is 96.67% and specificity is 93.35%.The 95% confident interval of area under the curve is above 90%(0.974 to be premise) implies that we are >95% confident that the method will yield similar results in population also the ideal cutoff value for intensity is <2800.

Table 4 showing comparison between various diagnostic modalities in identification of precancerous lesions showing that for detection of cervical precancerous lesion sensitivity of co-polarized fluorescence spectroscopy is 96.67% and specificity is 93.55% while for PAP smear sensitivity is 84.2% and sensitivity is 73.3% and for colposcopy sensitivity is 73.7% and specificity is 86.7%.

TABLE-I. CO-RELATION OF CYTOLOGICAL FINDINGS WITH HISTOPATHOLOGICAL FINDINGS

HPE / CYTOLOGICAL	NORMAL	INFLAMMATORY	CIN 1	CIN 2	CIN 3	CARCINOMA	TOTAL
NORMAL	-	03	02	-	-	-	05
INFLAMMATORY	-	08	01	-	-	-	09
MILD DYSPLASIA	-	02	03	05	-	-	10
MODERATEDYSPLASIA	-	-	02	02	02	-	06
SEVERE DYSPLASIA	-	-	-	-	02	-	02
CARCINOMA	-	-	-	-	-	02	02
TOTAL	**-**	**13**	**08**	**07**	**04**	**02**	**34**

95% confidence interval
True Positive (TP) = 16 False Positive (FP) = 4
True Negative (TN) = 11 False Negative (FN) = 3
Sensitivity= 84.2 % (60.4-96.2) Specificity = 73.3% (44.9-92.2)
Positive Predictive Value = 80.0% (62.8-91.5)
Negative Predictive Value = 79.4% (62.1-91.3)

TABLE-II. SHOWS THE PRESENCE OF VARIOUS COLPOSCOPIC FINDNGS

COLPOSCOPIC FINDING	NORMAL	INFLAMMATORY	CIN 1	CIN 2	CIN 3	CARCINOMA	TOTAL
ACETOWHITE	-	6	10	6	2	2	26
MOSAIC	-	-	4	2	2	2	10
PUNCTUATION	-	1	3	3	1	-	8
ATYPICAL VESSEL	-	-	-	1	1	2	4
SCHILLERS POSITIVE	-	1	7	1	1	2	12

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TABLE-III. 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	08	01	-	-	-	09
2	01	02	02	-	-	05
3	04	03	01	-	-	10
4	-	01	02	-	-	03
5	-	01	02	-	-	04
6	-	-	-	03	-	03
7	-	-	-	01	-	01
8	-	-	-	-	02	02
TOTAL	**13**	**08**	**07**	**04**	**02**	**34**

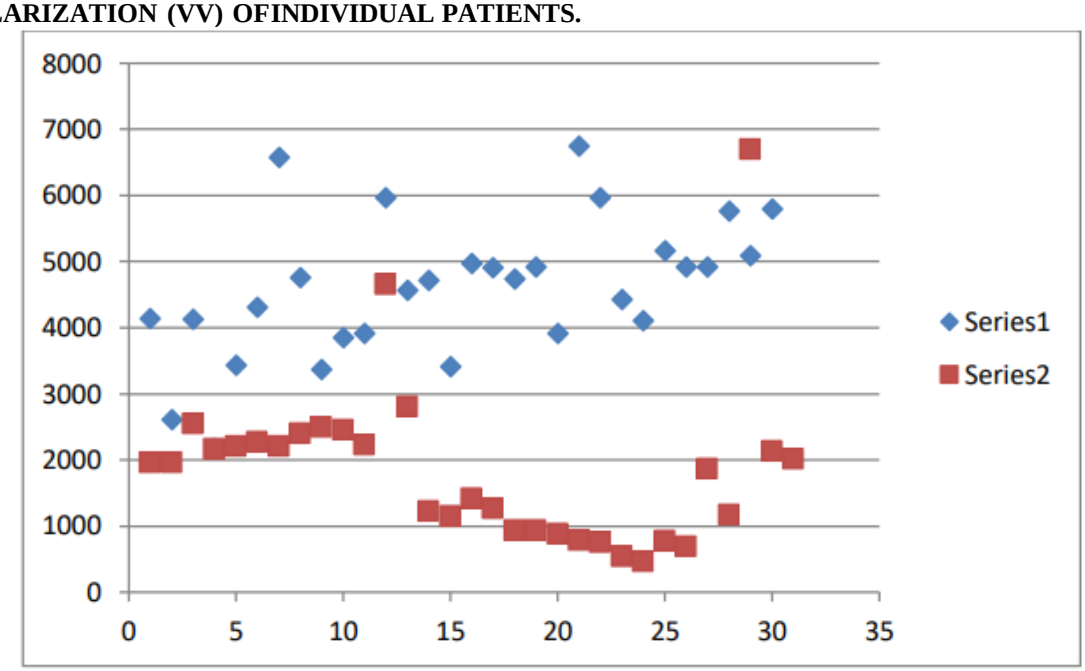
AT 95% CONFIDENCE INTERVAL

True Positive (TP) = 14 False Positive (FP) =2
True Negative (TN) = 13 False Negative (FN) = 5
Sensitivity =73.7% (48.8-90.8) Specificity = 86.7% (59.5-98.3)
Positive Predictive Value = 87.5% (65.2-96.3)
Negative Predictive Value=72.2% (54.4-84.9)

TABLE-IV. SHOWING COMPARISON BETWEEN VARIOUS DIAGNOSTIC MODALITIES IN IDENTIFICATION OF PRECANCEROUS LESIONS

	PAP SMEAR	COLPOSCPY	COLPORISED HAND HELD PROBE
SENSIVITY	84.2 %	73.7%	96.67%
SPECIFICITY	73.3%	86.7%	93.55%

Fig-I. SCATTER PLOT SHOWING BAND AREA AROUND PEAK INTENSITY BY CO-POLARIZATION (VV) OFINDIVIDUAL PATIENTS.



- patients those are histologically normal presented by blue dots (series1)
- patients those are histologicaly abnormal presented by red dots (series2)

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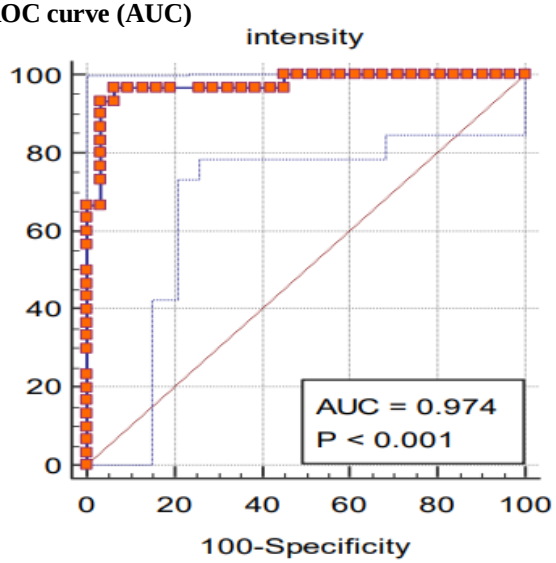
Fig-II. A RECEIVER OPERATIVE CHARACTERSTICS CURVE (ROC CURVE) OF BAND AREA AROUND PEAK WITH CO-POLARIZATION (VV) FOR DETECTION OF PRECANCEROUS TISSUES. Area under the ROC curve (AUC)



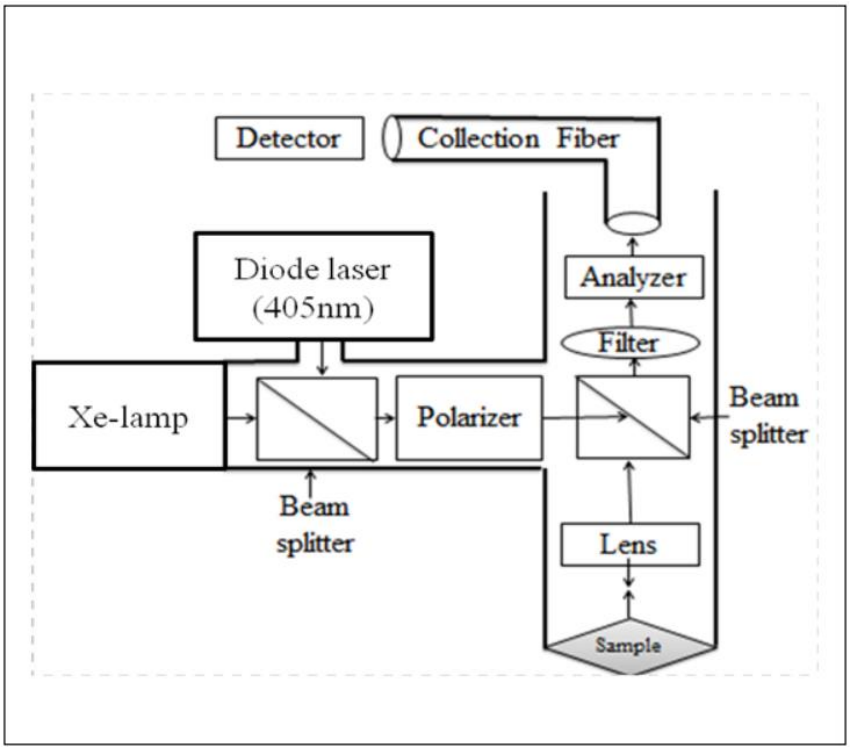
Area under the ROC curve (AUC)	0.974
Standard Error [a]	0.0185
95% Confidence interval [b]	0.897 to 0.998
z statistic	25.634
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988[b] Binomial exact

Youden index

Youden index J	0.9022
Associated criterion	≤2798.8
Sensitivity	96.67
Specificity	93.55

EXPERIMENTAL SETUP OF HAND HELD PROBE



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DISCUSSION

Our study is the first of its kind Previously in vitro studies were done which showed that 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%.

In this study it was found that incidence of cervical precancerous lesion was associated with increasing age, The mean age for inflammatory lesions was found 36.5 ±5.7 years, whereas for CIN 1, CIN2, CIN 3 ,Carcinoma was 40.62±8.3 **,** 46.28±5.6**,** 54.3 ±9.3 **,**57±11.3 yrs.

Ageat 1st coitus significantly affects the incidence of cervical lesions. 82.15% cases associated with 1st coital activity <25 year. Early sexual activity predisposes to precancerous lesions.The severity of lesion increased with increased parity, 79.4% premalignant and malignant cases were associated with parity >2 most of the cases of CIN and carcinoma were found in patients of lower socio-economic status , 85.71% cases with premalignant and malignant cytology belonged to low socio-economic class

Kamna Gupta was conducted a study to detect prevalence of cervical dysplasia in women of western UP showed a relatively high prevalence of epithelial abnormalities in cervical smears with increasing age, parity, early age at first coitus (<20 year), and lower socioeconomic status.[10]

Table 1shows that on correlating our cytology findings with histopathology, sensitivity was calculated 84.2% and specificity was 73.3%. The positive predictive value 80.0% was higher than negative predictive value 79.4%.The results were similar to other studies. According to Bahar Kohli et al PAP smear had a sensitivity of 80% and specificity of 64.29%.[11]

Table 2 shows the presence of various colposcopic findngs in the cases. Aceto white epithelium is the most common colposcopic finding, present in 76.48% cases.

Also as the grade of dysplasia increases percentage of cases with Schiller's positive, mosaicism and punctuations increases. There is more than one colposcopic finding in the same case. Papa Dasari et al found that the most common features was aceto whiteness (41.3%) followed by a combination of aceto white and vascular abnormalities(24.7%).[12]

Table 3 shows the correlation of colposcopic finding to histology. We used Reid colposcopic index for scoring of lesions. The sensitivity was 73.7% while Specificity was 86.%. Cantor SB et that for LSIL sensitivity of colposcopy was 71.4% and specificity was 81.3% and for HSIL sensitivity 98.3% and specificity 45.1%.[13]

Figure 1 Scatter plot showing band area around peak intensity by co-polarization (VV) of individual patients. patients those are histologically normal presented by blue dots(series1) patients those are are histologicaly abnormal presented by red dots(series2) it shows that most of the patients with abnormal histology are below 3000 intensity .

Figure 2 shows the ROC curve to determine the diagnostic ability of screening method between normal and CIN1/CIN2/CIN3/Carcinoma in situ. area under the curve was found 0.974 ($p<0.001$) that is very high and is also statistically significant this implies that hand held probe in in vitro trials has proved itself as an excellent discriminator between normal and abnormal cases . 95% confident interval of area under the curve is above 90%(0.974 to be premise) implies that we are >95% confident that the method will yield similar results in population also. The ideal cutoff value for intensity is <2800 because sensitivity is 96.67% and specificity is 93.35% and we want our test to be highly sensitive so that false negative cases are controlled and disease positive cases are detected by the test.

This means that the hand held probe is an excellent discriminator of normal tissue from all types of precancerous and cancerous lesions. In vivo hand held probe is as good as In vitro hand probe in Detection of cervical precancerous lesion. In Comparison with Pap Smear and Colposcopy, in vivo hand held probe based on copolarized fluorescence spectroscopy is better screening modality for Detection of cervical precancerous lesion .It is minimally invasive screening method and can be done even by paramedical staff with minimal facility and can be done at grass root level on a large population. Thus it provides better screening method for improving women' health in India by early detection of cervical neoplasia.

CONCLUSION

Interest in fluorescence diagnostics has increased considerably in the last few year as a result of several factors -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.

In vivo hand held probe based on optical imaging using co-polarised (VV) light, provides a better discrimination between normal versus abnormal lesions. In comparison to PAP smear and colposcopy, In vivo hand held probe is better screening modality in detection of cervical precancerous lesion.

At last I conclude emphasizing that diagnostic technique based on optical imaging has the potential to link the biochemical and morphologic properties of tissues to individual patient care. In particular, these techniques are fast, non-invasive and quantitative. from this study we found that like in vitro method, in vivo optical hand held probe is also an excellent method for detection of cervical precancerous lesion and can emerge as a better screening technique in near

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future but more studies are required with large sample size before extra poliating this fact.

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