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Is there a Role for Surrogate Markers of Pre-Malignant Lesions of Cervix in India?

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Cancer of the cervix is the most common cancer among Indian women. Unfortunately, we do not have an organised screening programme in our country such as that is used in the western countries, with an emphasis on repeated screening using Pap smear. This paper discusses the limitations of the Pap smear in the Indian context and explores the option of using surrogate markers of dysplasia for identifying high risk women.

Key words: Cervical cancer, Dysplasia, Pap smear, Surrogate markers, Human Papilloma Virus, p16, Telomerase, Ki67, Cyclin E, Visual inspection with acetic acid

Introduction

The Problem

Cancer of the cervix is the most common cancer among Indian women. The projected annual incidence of new cases is above 1,00,000/year. In the US effective screening using Pap smears has reduced the mortality due to cervical cancer by 40% (Ries 1998). However, in Cuba wherein cervical cytology screening programme has been in existence since late 60's, there has been no reduction in incidence or mortality from cervical cancer (Sankaranarayanan et al. 2001). A comparative trend in the annual incidence of cervical cancer in the major metropolis of Delhi, Mumbai, Chennai, Bangalore and Bhopal is shown in figure 1. An interesting feature of the figure is that there has been no rise in the incidence of cervical cancer in these cities, in spite of not having any organized screening programme. More importantly, in Chennai and in Bangalore, the Age Adjusted Rate (AAR) have fallen significantly (In Chennai from 43.1 to 29.4; in Bangalore from 31.7 to 21.3). The data suggests that with improvement in socio-economic status including education and a lower parity, the incidence of cervical cancer is likely to fall, but this may be only up to a point and for further reduction, intervention will be mandatory.

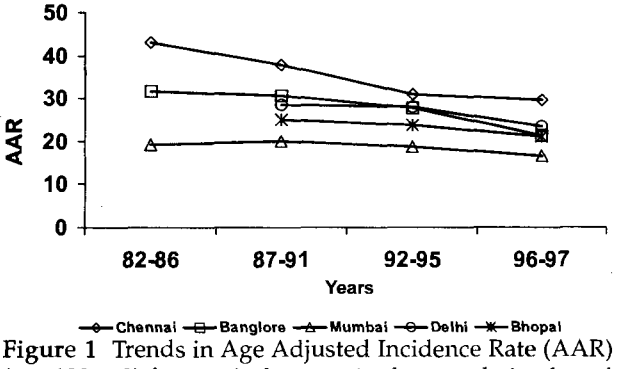

Figure 1 Trends in Age Adjusted Incidence Rate (AAR) (per 100,000) for cervical cancer in the population based Indian cancer registries.

The National Cancer Control Programme of the Government of India has focused on cervical cancer, but has little to show by way of cancer control throughout the country. Pockets of activity exist especially in Regional Cancer Centers, but they hardly cover 5% of the total population of the country.

The western model for cervical cancer control with its primary thrust on Pap smear testing of all sexually active women once every two to five years, may not be practical in our country, yet. The reasons include:

i. The large population of at risk women who need to be covered under the programme,

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with its financial implications. The Indian population stands above one billion. Even if one decides to screen women between the age of 30 - 60 years, the number of women required to be screened would be about 10,00,00,000 - 15,00,00,000. The screening may need to be repeated once every 2-5 years.

ii. The paucity of trained personnel in interpretation of Pap smears.

iii. Lack of quality control to oversee the Pap smear results.

iv. Follow-up measures not being available in or near the place of residence of the women tested. In the event of detecting an abnormal cervix, the patients need to travel considerable distance (district or Government Medical colleges or Regional cancer centers located usually in the capitals of the states). Detection of an asymptomatic pre-malignant lesion in a women worker, be it, in the rural region or in semi-urban, has its own implications in the woman not opting to come for treatment to a referral center far removed from her place of residence. She may have to spend a considerable time in a new environment to undergo the treatment, forgoing her wages for the period. All these factors along with the asymptomatic nature of her lesion, lead to poor motivation for the women to have follow up treatment. This would need to be addressed whatever the screening programme be. The management facility should be available in the Primary Health centers itself, which would make it easier for the women to undergo the prescribed line of treatment.

Therefore, an approach which can be automated, made available locally (either Primary Health Centers [PHC] or District Hospitals), cost-effective, simple and robust is needed for identifying the abnormal pre-malignant lesions. Equally important is the need to establish the treatment facility for the pre-malignant lesions detected, at the PHC level itself.

Pap Smear: Its Advantages and Limitations

The Papanicolaou technique for cervical smears has been responsible for the dramatic reduction in the incidence of invasive cervical cancers in the women in western countries that have adopted this approach of screening. The technique is simple but requires trained personnel for interpretation. The scoring of the abnormal smears was done based on the morphology and the extent to which the dysplastic cells were present relative to the thickness of the epithelium. Thus in Cervical Intraepithelial Neoplasia I (CIN I), the dysplastic cells were limited to the lower one third of the epithelium; in CIN II, this was extending up to the upper third of the epithelium. In CIN III, the dysplastic cells extended beyond the upper third of the epithelium and in Carcinoma in situ (CIS) it involves the full thickness of the epithelium. In addition, to the above categories, borderline cases existed. In view of the limitations of the above scoring system, an alternate system was developed (Bethesda) - categorizing the abnormalities as Low grade Squamous Intraepithelial Lesion (LG-SIL) and High Grade Squamous Intraepithelial Lesion (HG-SIL). Again, a borderline atypical squamous or glandular cells of undetermined significance (ASCUS or AGUS) category was defined (table 1). The major limitation of the Pap smear has been the inter-observer variability, with relatively low rates of concordance amongst cytologists, especially in the border line cases. In addition, it has a mean sensitivity of 58% and a mean specificity of 69% (Fahey et al. 1995). Nanda et al. (2000) analysed data from 12 studies and showed that the sensitivity ranges from 30-87% and specificity from 86-100%. To a certain extent this may be glossed over by the frequent screening programme (once every year to once every 5 years) followed in the western countries. However, the sheer magnitude of the population in our country, does not make this approach practical. A more realistic target would be to have at least the women aged between 35 - 65 years of age at least once during their lifetime, with a sensitive and reliable test. A once in a life time screening has been projected to yield a reduction of around 25% in the incidence of cervical cancer (Prabhakar 1992, Murthy et al. 1993).

Surrogate Markers of Pre-Malignant Lesions of Cervix

The need for a better diagnostic approach, which has less inter-observer variation and with high positive and negative predictive value, is therefore

Table 1 *Classification of Pap smear Abnormalities*

Description	CIN Grading	Bethesda System
Normal	Normal	Normal
Atypia Reactive or Neoplastic	Atypia	Atypical squamous or glandular cells of undetermined significance -ASCUS or AGUS
HPV	HPV	Low-Grade Squamous intraepithelial lesion (SIL)
Atypia with HPV	Atypia, "condylomatous atypia" and "koilocytic atypia"	Low-Grade SIL
Mild Dysplasia	CIN I (Dysplasia limited to lower 1/3 of the epithelium)	Low-Grade SIL
Moderate Dysplasia	CIN II (Dysplasia limited to lower 2/3 of the epithelium)	High-Grade SIL
Severe dysplasia	CIN III (Dysplasia in more than 2/3 of the epithelium)	High-Grade SIL
Carcinoma-in-situ	Carcinoma-in-situ (CIS) (Dysplasia involving full thickness of the epithelium)	High-Grade SIL
Invasive cancer	Invasive cancer	Invasive cancer

mandatory. A high positive predictive value refers in this case to identifying women at high risk who should be evaluated further as they run the risk of developing cancer; while a high negative predictive value, would help avoid further testing of women at a low risk for developing cancer. This would also avoid the anxiety of having to live with an "abnormal test result". To this end several groups around the world have looked for markers which could help identify and distinguish the pre-malignant lesions from non-pre-malignant lesions. A partial list of such surrogate markers is given in table 2.

Markers Related to Human Papilloma Virus (HPV) Aetiology

High Risk HPV types

Rationale

It has been clearly shown that HPV high-risk types are an important factor in cervical tumourigenesis. In most studies that have used highly sensitive method of detection of HPV high risk (HR) types (HPV 16,18,33,31,58 etc.), nearly 99% of the cancer cases have been found to be associated with the high risk HPV types (Walboomers et al. 1999). Detection of HPV high risk types in the pre-malignant lesions would identify those women who are likely to progress. Conversely, in the absence of HPV high risk subtypes, the risk to develop caner in women is very low. However, the atypical squamous cells of undetermined significance/ Low grade squamous intraepithelial lesions triage study (ALTS) group, have shown that 83% of the 532 samples of LSIL were high risk HPV positive by the Hybrid capture II test. This limits the usefulness of the high risk HPV testing in LSIL (ALTS group 2000).

Table 2 *A Partial List of Surrogate Markers for Pre-Malignant Lesions of Cervix*

Markers related to HPV aetiology	Markers of proliferation	Others
High risk HPV types	Telomerase	MN/CA9
E6/E7 protein	Cyclin E	NMP179
p16	Minichromosome maintenance proteins	
	Ki67 (MIB)	

Methodology

Polymerase chain reaction (PCR) based assays (PCR-ELISA, Multiplex PCR, HPV Chip) and hybridisation (Hybrid capture II).

Limitations

1. Cost - The cost of PCR based testing for a single sample, can vary depending on whether one would like to know the individual HPV type/s (cost above Rs. 3000) or whether it is sufficient to know high risk versus low risk HPV infection (cost around Rs.1000 to 1500). The Hybrid capture test is likely to cost around Rs.1500, when it is done commercially. Both do not include the initial cost of setting up the

equipments for the analysis. In comparison the cost of a single Pap smear testing costs around Rs.250.

2. Trained personnel needed for the Molecular investigation, especially with regard to the PCR based approach.
3. Although, High risk HPV are critical in the tumourigenesis, nearly 70% of the infected young women may clear the infection (Ho et al. 1995). Thus, while the assay will have a high negative predictive value, its positive predictive value (those who are likely to develop cancer) is low.

HPV Protein Expression Detection

Rationale

Once the high risk HPV infection occurs, HPV E6 and E7 protein expression commence. E6 and E7 are critical proteins in HPV induced malignancy, as they neutralise the function of the two major tumour suppressor genes, p53 and Rb proteins, respectively.

Methodology

Immuno-histochemistry using monoclonal antibodies raised against E6 and E7 protein. Pathologists in the District Hospital can do the immuno-histochemistry (IHC).

Limitations

1. Nonavailability of good reliable monoclonal antibodies to E6 and E7.
2. Cost could range from Rs.500 to 750, for each marker.
3. E6 and E7 antigenicity differ with different High-risk HPV subtypes.

Detection of Antibodies to HPV Proteins

Rationale and Limitations

Serological assays such as ELISA for detection of HPV antibodies have been evaluated in the past and have generally not found to be very satisfactory. The primary reason is knowing if the antibody response titers are a reflection of an active infection or one of the past. With the use of Viral Like Particles (VLP) as antigens for ELISA type assays, detection of antibody response is feasible (Seilles et al. 1999). However, the wide variation in the antibody response, makes it difficult to use this assay, as an indicator of pre-malignant lesions.

Detection of p16/MTS1 Protein

Rationale

p16 is an important tumour suppressor gene playing a crucial role in regulation of G1 phase of mitosis. p16 can inhibit the function of Cyclin D1-CDK4, preventing it from phosphorylating Retinoblastoma (Rb) which is bound to E2F (figure 2). In high risk HPV infection, E7 protein binds to Rb and inactivates it, thereby allowing the E2F available for promoting entry into S phase. p16 is over-expressed by the cell in an attempt to inhibit the G1 phase progression, but is unable to do so, as the Rb is not available for functional inhibition of entry into S-phase.

Studies have shown that p16 over-expression occurs in dysplasias and in invasive cervical cancers (Klaes et al. 2001). In normal cervical epithelium p16 is not expressed in detectable amounts. The presence of p16 in dysplastic cervical epithelium would indicate that the high risk HPV infection has occurred and it has already started to produce E7 protein, leading to inactivation of Rb protein. Thus the detection of p16 would be indicative of progression of HPV infection to a critical stage.

In the study reported by Klaes et al. (2001), all CIN II lesions (n=32), all CIN III lesions (n=60) and 58/60 invasive cancers were found to have high levels of expression for p16 in both cytoplasm and/or nucleus of the cells, diffusely. In CIN I lesions which were associated with Low risk (LR)-HPV (n=15), staining was negative or was only focal; in contrast 13/15, HR-HPV associated CIN I were found to have strong and diffuse expression for p16.

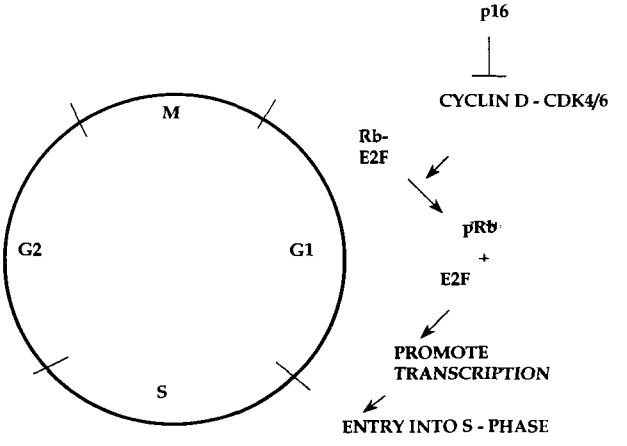

Figure 2 Role of p16 in cell cycle regulation.

In our own study, none of the 30 normal cervical epithelium showed immuno-histochemically detectable p16 nuclear expression.

CIN –I:- Only one of the 12 CIN – I (8.3%) lesion showed focal nuclear expression for p16. Cytoplasmic staining was evident in the basal cells of the normal cervical epithelium.

CIN –II:- Among the 8 cases histologically diagnosed as CIN-II, 2 cases (25%) showed immuno-histochemically detectable focal nuclear expression for p16.

CIN –III:- Of the 36 cases diagnosed as CIN-III, 31 (86%) showed diffuse pattern of nuclear expression for p16. Adjacent normal areas showed no detectable expression for p16. Dysplastic areas however showed a clear demarcation with strong diffuse p16 nuclear immuno-expression. Expression of p16 in CIN lesions of cervical tumours seemed to show a progressive increase in intensity and percentage of tumour cells with increasing dysplasia. Association of p16 nuclear immuno-expression with grade of CIN was statistically significant (table 3) ($p<0.000001$).

Invasive Cervical Cancers:- Among the 142 cases diagnosed as invasive cervical cancer, 121/142 (85.2%) showed diffuse pattern of nuclear expression for p16.

Methodology

Immuno-histochemical detection using a three layered Avidin Biotin Complex (ABC) system. The method can be used on both cervical scrape smears and on biopsy specimen. In view of the clear brown staining of the p16 positive cells, inter-observer variation is reduced.

Limitations

1. Needs trained personnel for interpreting the slides. However, as the staining following the immuno-peroxidase reaction is clear cut, inter-observer variation is bound to be less.
2. Cost is likely to be around Rs.500 to Rs.750.

Table 3 *Expression of P16 in CINs and Invasive Tumours*

	p16 +VE	p16 –VE
CIN I (n=12)	1 [8.3](only focal expression)	11 [91.6]
CIN II (n=8)	2 [25] (only focal expression)	6 [75]
CIN III/CIS (n=36)	31[86.1] (diffuse)	5 [13.8] ♣
Invasive Tumours (n=142)	121 [85.2] (diffuse)	21 [14.7] ♦

The numbers within brackets denote percentages. Statistical significance assessed by chi square test. ♣ -p<0.000001; p=0.0000

3. It will need to be validated by a study with large number of different grades of CIN's and with follow-up of these individuals.

Markers of Cell Proliferation

Telomerase

Rationale

Telomerase is a ribonucleoprotein which serves to synthesize tandem repeats of the sequence TTAGGG, which make up telomeric DNA. These sequences are present at the ends of the chromosomes and serve to prevent abnormalities of chromosomes in dividing cells (end to end fusions, rearrangements etc.). As the cell undergoes several divisions, there is progressive shortening of the telomeric end, as new repeats are not synthesized. Therefore the number of divisions a cell can undergo without having its telomeric end elongated is fixed. Thus the telomeres also act as a molecular clock heralding cellular senescence. (figure 3). In the stem cells of the haematopoietic system, germ cells and basal cells of epidermis there is telomerase activity, which is necessary for normal function of these cells.

In cancers and pre-cancerous lesions, there is inappropriate activity of the enzyme telomerase, which enables the synthesis of the telomeric repeat, thereby the cancer cells acquire the capacity to undergo infinite divisions, without the restriction of the shortening telomere. Hence, detection of telomerase activity can help identify cells which are more likely to be dysplastic with a tendency to progress to frankly malignant cell.

Methodology

Several methodologies are available for detection of telomerase activity, such as the PCR-based

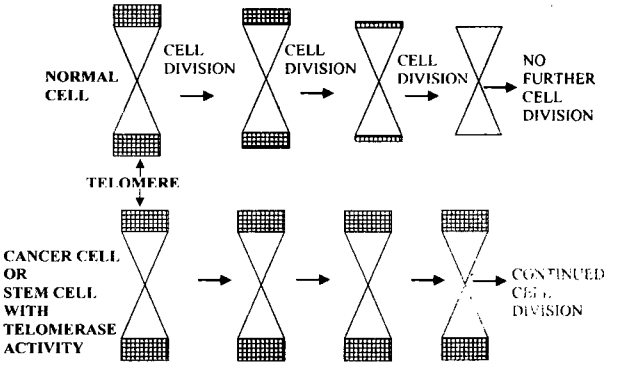

Figure 3 Role of telomere in cell division.

Telomeric Repeat Amplification Protocol (TRAP) (Kim et. al 1994) and the Stretch PCR Assay (Tatematsu et al. 1996), which can help quantitate the telomerase activity.

Using the Stretch PCR assay and the TRAP method, Kyo et al. (1998) examined normal, dysplastic and malignant cervical epithelial cells for telomerase activity. Their results showed that telomerase activity is quantitatively different between the three groups, with cancers having telomerase activity above 30 Units. In the case of normals, it was less than 10 Units and in dysplasias, less than 30 Units.

Studies have also been done for assessing the expression of the protein catalytic subunit of the telomerase (hTERT) using immuno-histochemistry and *in situ*-hybridisation was used to detect the mRNA of the telomerase (hTR) in normal, dysplastic and malignant cervical epithelium (Frost et al. 2000). Their results showed that in normal cervical epithelium, expression of hTERT, was confined to the basal and suprabasal cells. In contrast, in LSIL, HSIL and in invasive cervical cancer, the expression was detected in all layers of the epithelium. hTR was localised to the basal and suprabasal cell layers of normal and LSIL, whereas in HSIL and invasive cervical cancer, the distribution involved all levels of the pathologic epithelium.

Limitations

1. The methodology used for determining telomerase activity is complex (especially TRAP and Stretch PCR) and may require specialized personnel for processing and analysis.
2. Validation of the results will be required by studying larger numbers.
3. Cost would be in the range of Rs.1000 -1500.

Cyclin E

Rationale

Cyclin E is one of the cell cycle regulatory molecule which is needed for progression through the G1-S phase transition. Cyclin E is essential for the cells to enter S phase. When Cyclin E is overexpressed, the cells progress through G1 into S phase at a faster rate (Ohtsubo et al. 1993). Such cells also have a diminished requirement for the growth factors, indicating that Cyclin E may overlap with the D type Cyclins in integrating growth factor signal transduction into the cell cycle. Cyclin E shares some of the characteristics with Cyclin A which regulates cells once they have entered the S phase. Both Cyclin E and A in conjunction with CDK2 associate with pRB related proteins p107 and transcription factor E2F. Cyclin E differs from Cyclin D in the aspect that it is required for S phase progression in cells that lack a functional Rb (Ohtsubo et al. 1995).

In our study on expression of Cyclin E in normal, dysplastic and malignant cervical epithelium, 30 normal cervical epithelial samples, 56 dysplasias (CIN I =12; CIN 2 =8; CIN 3 =36) and 141 Invasive cervical cancers were studied. A 3 layered ABC Immuno-histochemical technique was used. The primary antibody, HE12, (Santa Cruz) was used at a dilution of 1:200, with antigen retrieval using wet autoclaving. DAB was used as the chromogen.

Normal cervical epithelium showed only sporadic and occasional cell positivity for Cyclin E. The positive nuclear immuno-expression was observed in the para-basal cells.

CIN-I:- Of the 12 cases histologically diagnosed as CIN-I, nuclear positive immunoexpression was found in 8 (66.6%) cases. The intensity was mild with focal nuclear expression.

CIN- II:- Of the 8 cases histologically diagnosed as CIN- II, mild with focal nuclear positive immunoexpression was observed in 4 (50%) cases. None of the cases showed diffuse staining for Cyclin E.

CIN –III :- Of the 36 cases diagnosed as CIN-III, 32 (88.8%) cases showed diffuse and intense nuclear staining for Cyclin E. There was a significant association in the staining of Cyclin E and grade of CIN with a diffuse and intense staining observed in CIN-III cases compared to CIN-I and CIN- II. ($p<0.03$) (table 4).

Invasive cervical cancers:- Out of 141 cases with invasive cervical carcinoma, positive nuclear expression for Cyclin E was found in 123 (87.2%) cases. The expression was diffuse and intense compared to normal and low grade CINs. Association between nuclear expression of Cyclin E with respect to different grades of CINs and invasive tumours was found to be statistically significant. ($p<0.009$).

Table 4 *Nuclear Expression of Cyclin E in Cins and in Invasive Tumours*

	Cyclin E +ve	Cyclin E –ve
CIN I (n=12)	8 [66.6] (mild, focal)	4 [33.3]
CIN II (n=8)	4 [50] (mild, focal)	4 [50]
CIN III/CIS (n=36)	32 [88.8] (intense, diffuse)	4 [11.1] ♣
Invasive Tumours (n=141)	123 [87.2] (intense, diffuse)	18 [12.7] ♦

Numbers within brackets denote percentages. Statistical significance assessed by chi square test. ♣ -p<0.03; ♦ p=0.009

Our study also showed down-regulation of Cyclin D1 (data not shown) along with over-expression of Cyclin E in cervical tumours. Cyclin E therefore appears to have an important role to play in cervical tumourigenesis than Cyclin D1. In the present study, CIN III lesions over-expressed Cyclin E compared to low grade CINs and normals. This has been reported earlier by Kanai et al. (1998) and Kim et al. (2000). Our reports confirm their finding of over-expression of Cyclin E in invasive cancers and high grade pre-cancers. This phenomenon is also seen in colorectal cancers. Yasui et al. (1996), have reported a gradual accumulation of Cyclin E in the evolution of an adenoma to adenocarcinoma.

Limitations

1. Needs trained personnel for interpretation of the Immuno-histochemical slides.
2. Cost will be around Rs.500 - 750.
3. Larger numbers of samples to be studied to validate the results.

Mini-Chromosome Maintenance (MCM) Proteins

Rationale

MCM proteins are highly conserved proteins involved in cell proliferation (Kearsey et al. 1998). They form a prereplicative complex that is required for DNA replication (Newlon 1997). There are several members of the MCM proteins - MCM 2, 3, 4, 5, 6 and 7. The prereplicative complex consists of origin recognition complex (ORC) proteins, cdc6 and MCM proteins. Although the ORC protein levels are constant during cell cycle and during quiescence, cdc6 and MCM protein are detected only during cell cycle, thus making them specific markers of cycling cells (Freeman et al. 1999).

Freeman's study (1999) had shown that the MCM proteins can be detected by immuno-histochemistry in the nucleus of the basal cell of normal cervical epithelium which extends to 50% of cells in the superficial layers in CIN I lesions and upto 90% of cells in superficial layers in CIN III and in more than 70% of invasive cervical cancer cells. MCM 2, 5, 7 and cdc6 gave similar pattern of expression.

Methodology

Immuno-histochemistry using 3 layer ABC technique.

Limitations

1. The study describes small number of samples (Freeman et al. 1999). Larger series will be needed to confirm and apply the findings.
2. Cost will be around Rs.500 to Rs.750, for each molecule.

Ki67

Rationale

Ki67 has been associated with the growth fraction of proliferating cells (Brown et al. 1990). Studies have associated Ki67 expression with increasing grades of dysplasia (Mittal 1999). MIB a monoclonal antibody to the Ki67 antigen, is now the preferred antibody for use in Immuno-histochemistry. The Ki67 antigen is considered to be nuclear matrix protein proliferation antigen (Verheijen et al. 1989).

Methodology

A three layered ABC immuno-histochemical approach with antigen retrieval is used.

Limitations

1. Levels of expression of Ki67 have been found to vary with nutrient deprivation (Baisch et al. 1987).
2. The Ki67 is not considered essential for cell proliferation (Verheijen et al. 1989).
3. Cost will be around Rs.500 to Rs.700.
4. Data from large series showing the utility of the marker, is needed.

Others

MN/CA9 Protein

Rationale

MN/CA9 is a novel transmembrane glycoprotein (Liao et al. 1996), found to be expressed in all Adenocarcinoma in-situ (AIS) of cervix and in 90% of squamous cancers. In the study reported by Liao et al. 2000, MN/CA9 was found to distinguish

between normal and benign (both of which were negative) versus atypical and neoplastic (staining present). However, it did not help in distinguishing CIN I from atypia.

Methodology

A 3 layered ABC immuno-histochemical technique was used.

Limitations

1. Larger series required for confirmation of the findings.
2. As with other techniques which use IHC, trained personnel required for processing and analysis.
3. Cost will be around Rs.500 to Rs.700.
4. Unable to distinguish between CIN I and atypia. More useful in Adenocarcinoma in situ.

NMP179

Rationale

NMP179 is a monoclonal antibody which detects a cervical tumour associated nuclear matrix protein (Keesee et al. 1998). Using IHC on thin prep smears from normal, LGSIL and HGSIL, it was found that NMP179 assay detected low and high grade SIL with a sensitivity of 79.3% and a specificity of 70.4% (Keesee et al. 1999).

Limitations

1. A larger series will be required to evaluate the usefulness of the NMP179 assay.
2. As with other IHC techniques trained personnel required.
3. Cost will be around Rs.500 to Rs.700.

Can we use a Surrogate Marker for Cervical Cancer Screening?

At the moment as discussed above, although there are some promising approaches, they need to be studied in large populations preferably in a field setting to confirm the efficacy as well as the reliability. One of the promising approaches appears to be the use of p16, but here again follow-up studies will be required to assess the positive and negative predictive value.

Hence at the moment, although several approaches and targets are available, none can be used on a national scale.

What do we do NOW?

Until we have a reliable diagnostic approach that could circumvent the inter-observer variation and be reliably able to identify the high-risk women who are likely to develop cervical cancer, we need to evolve a National Strategy that could be put in place with a minimum of effort and with the existing infrastructure. At the Cancer Institute (WIA), under an ICMR project covering the Tindivanam taluk, Village Health Nurses (VHN's) were trained to visually examine the cervix and to take a Pap smear. The VHN's were found to be able to distinguish between a normal cervix and an abnormal one (95% accuracy) and were found to be able to take a good quality Pap smear (80%) (Gajalakshmi et al. 1996). Based on the success of the project, the study was extended to Cuddalore and Villupuram districts. Under the District Cancer Control Programme, 672 VHN were trained and more than 4.3 lakh individuals were screened between 1.6.92 to 31.3.98. The unaided Visual Inspection (VI) has been found to be having a lower sensitivity and specificity to detect precursor dysplastic lesions (Sankarnarayanan et al. 1997). Hence, although unaided VI is not the method to be used, it demonstrated that the VHNS could be trained satisfactorily.

The WHO has stated that the use of Visual inspection after painting the cervix with acetic acid is a promising approach. Again studies being done at, in Osmanabad district of Maharashtra, in Mumbai and in Ambillikai in Dindigul district, under support from IARC, Lyon will serve to address the question as to whether Visual inspection with acetic acid (VIA) can help reduce the cervical cancer incidence and its mortality (Sankarnarayanan et al. 2001). VIA and cytology were found to have similar performance in detecting moderate dysplasia or more severe lesions (specificity for VIA and cytology were 92.2% and 91.3%, respectively. The positive predictive value [PPV] was 17% for VIA and 17.2% for cytology) (Sankarnaryanan et al. 1998). These low cost approaches depend on the will and the motivation of the field staff. A Government initiative on the lines of family planning programme, should help in getting the programme take root all over the country, without waiting for the high tech revolution.

The final and the best approach may still be a HPV vaccine which when made available, should help prevent the critical HPV infection in all sexually active men and women. The first generation vaccines are being evaluated in clinical trials and the early results are promising. The HPV16 L1 Viral Like Particle (VLP) vaccine has been shown to be well tolerated and highly immunogenic even without adjuvant (Harro et al. 2001). HPV16 VLP vaccine has also been shown to reduce the incidence of infection of both HPV16 infection and HPV16 related CIN (Koutsky et al. 2002). Vaccine trials using bivalent (HPV16 and 18) and tetravalent (HPV6,11,16 and 18) VLP's are underway. When validated and proven to be effective, these vaccine strategies could help greatly in controlling the incidence of cervical cancer.

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References

ALTS Group 2000 Human Papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: basline data from a randomized trial. The Atypical Squamous Cell of Undetermined Siginificance/Low-Grade Squamous intraepithelial Lesions Triage Study (ALTS) Group; *J. Natl. Cancer Inst.* 92 397-402

Baisch H and Gerdes J 1987 Simultaneous staining of exponentially growing versus plateau phase cells with the proliferation-associated antibody Ki67 and propidium iodide: analysis by flow cytomertry; *Cell Tissue Kinet.* 20 387-391

Brown D C and Gatter K C 1990 Monoclonal antibody Ki67; its use in histopathology; *Histopathol.* 17 489-503

Freeman A, Morris L S, Mills A D et al. 1999 Minichromosome Maintenance proteins as Biological markers of Dysplasia and Malignancy; *Clin. Cancer Res.* 5 212-2132

Frost M, Bobak J B and Gianani R et al. 2000 Localization of telomerase hTERT protein and hTR in benign mucosa, dysplasia and squamous cell carcinoma of the cervix; *Am. J. Clin. Pathol.* 114(5) 726-34

Gajalakshmi C K, Krishnamurthi S, Revathy A, Shanta V 1996 Cervical Cancer screening in Tamilnadu, India: a feasibility study of training the village health nurse; *Cancer Causes and Control* 7 520-524

Harro C D, Pang Y S, Roden R B S, et al. 2001 Safety and Immunogenicity Trial in Adult Volunteers of a Human Papillomavirus 16 L1 Virus-Like Particle Vaccine; *J Natl. Cancer Inst.* 93(4) 284-292

Ho G Y F, Bierman R, Beardsley L, Chang CJ and Burk RD 1995 Natural history of cervico-vaginal papilloma virus infection in young women; *J. Natl. Cancer Inst.* 87 1365-1371

Jarboe E A Liaw K L and Thompson L C et al. 2002 Analysis of telomerase as a diagnostic biomarker of cervical dysplasia and carcinoma; *Oncogene* 21(4) 664-73

Kanai M, Shiozawa T, Xin L, Nikaido T and Fujii S 1998 Immunohistochemical detection of sex steroid receptors, cyclins and cyclin dependent kinases in the normal and neoplastic squamous epithelia of the uterine cervix; *Cancer*, 82 1709-1719

Kavita N, McCrory D C, Myers E R and Bastian L A, et al. 2000 Accuracy of the Papanicolaou Test in Screening for Follow-up of Cervical Cytologic Abnormalities: A Systematic Review; *Ann. Intern. Med.* 132 810-819

Kearsey and Labib K 1998 MCM proteins: evolution, properties and role in DNA replication; *Biochem. Biophys. Acta* 1398 113-136

Keesee S K, Marchese J M and Meneses A et al. 1998 Human cervical cancer-associated nuclear matrix proteins; *Exp. Cell Res.* 244 14-25

_____, Meyer J L, Hutchinson M L et al. 1999 Preclinical feasibility study of NMP179, a nuclear matrix protein marker for cervical dysplasia; *Acta Cytol.* 43(6) 1015-22

Kim N W, Piatyszek M A and Prowse K R et al. 1994 Specific association of human telomerase activity with immortal cells and cancer; *Science* 266 2011-2015

Klaes R, Friedrich T and Spitkovsky D et al. 2001 Overexpression of p16 (INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri; *Int. J. Cancer* 92(2) 276-284

Koutsky L A, Ault K A and Wheeler C M et al. 2002 A controlled Trial of a Human Papillomavirus Type 16 Vaccine; *N. Engl. J. Med.* 347 1645-1651

Kyo S, Takakura M, Ishikawa H and Sasagawa T et al. 1997 Application of telomerase assay for the screening of cervical lesions; *Cancer Res.* 57(10) 1863-1867

Kyo S, Takakura M, Tanaka M, Kanaya T and Inoue M 1998 Telomerase activity in cervical cancer is quantitatively distinct from that in its precursor lesions; *Int. J. Cancer* 79(1) 66-70

Liao S Y and Stanbridge E J 1996 Expression of the MN antigen in cervical Papanicolaou smears is an early diagnostic biomarker of cervical dysplasia; *Cancer Epidemiol. Biomarkers Prev.* 5(7) 549-557

_____ and _____ 2000 Expression of MN/CA9 protein in Papanicolaou smears containing a typical glandular cells of undetermined significance is a diagnostic biomarker of cervical dysplasia and neoplasia; *Cancer* 88(5) 1108-1121

Mittal K 1999 Utility of proliferation-associated marker in MIB-1 in evaluating lesions of the uterine cervix; *Adv. Anat. Pathol.* 6(4) 177-85

Murthy N S, Agarwal S S and Prabhakar A K et al. 1993 Estimation of reduction of life-time risk of cervical cancer through one life-time screening; *Neoplasma* 40(4) 255-258

Newlon C S 1997 Putting it all together: building a prereplicative complex; *Cell* 91 717-720

Ohstubo M and Roberts J M 1993 Cyclin dependent kinase regulation in GI in mammalian fibroblasts; *Science* 259 1908

_____, Theodoras A M and Schumacher J et al. 1995 Cyclin E, a nuclear protein essential for the GI-S phase transition. *Mol. Cell Biol.* 15 2612-2624

Prabhakar A K 1992 Cervical Cancer in India strategy for control; *Ind. J. Cancer* 104 29-32.

Quade B J, Park J J, Crum C P, Sun D and Dutta A 1998 *In vivo* cyclin E expression as a marker for early cervical neoplasia; *Mod. Pathol.* 11(12) 1238-46

Ries L A, Kosary C L and Hankey B F et al. eds. 1998 SEER cancer statistics review 1973-1995 (Bethesda, MD: *National Cancer Institute)*

Sankaranarayanan R 1997 Visual inspection as a screening test for cervical cancer control in developing countries; in *New Developments in Cervical Cancer Screening and Prevention* 411-421 eds E Franco and J Monsonego (Oxford: Blackwell Science)

_____ Budukh A M and Rajkumar R 2001 Effective Screening programmes for cervical cancer in low and middle-income developing countries; *Bulletin of the World Health Organization* 79(10) 954-962

_____, Wesley R and Somanathan R et al. 1998 Visual Inspection of the Uterine Cervix after the Application of Acetic Acid in the Detection of Cervical Carcinoma and its precursors; *Cancer* 83(10) 2150-2156

Seilles E, Geslin I and Mougin C 1999 Biology of papillomavirus infections IV Sero epidemiological data; *Ann. Biol. Clin.* 57(4) 393-400

Tatematsu K, Nakanishi T and Kitamoto M et al. 1996 A novel quantitative "stretch PCR assay", that detects a dramatic increase in telomerase activity during the progression of myeloid leukemias; *Oncogene* 13 2265-2274

Verheijen R, Kuijpers H J and van-Driel R et al. 1989 Ki67 detects a nuclear matrix proliferation-related antigen; *J. Cell Sci.* 92 531-540

von Knebel Doeberitz M 2001 New molecular tools for efficient screening of cervical cancer; *Dis. Markers* 17(3) 123-128

Walboomers M J, Jacobs M V and Manos M M et al. 1999 Human papilloma virus is a necessary cause of invasive cervical cancer worldwide; *J. Pathol.* 189(1) 12-19

Yasui W, Kuniyasu H and Yokozaki H et al. 1996 Expression of cyclin E in colorectal adenomas and adenocarcinomas: correlation with expression of Ki-67 antigen and p53 protein; *Virchows Arch.* 429 13-19