PRINT: ISSN 0970-9274 ONLINE: 2456-6608
J. Hum. Ecol., 17(2): 129-135 (2005)
DOI: 10.31901/24566608.2005/17.02.07

A Comparison of the Urothelial Cells and Cervix Scraping Techniques in the Screening Process for Cancer of the Cervix

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KEYWORDS Bladder Cells. Cervix Smears. Micronuclei

ABSTRACT Earlier detection of carcinoma of the cervix can achieve a goal of total or near total eradication of invasive carcinoma of the cervix bringing about sharp reductions in its incidence and mortality due to it. This has significance for Indian women where the incidence of the cancer and recognized risk factors are high. The Micronucleus assays in exfoliated bladder and cervical cells of women, coming to attention for gynaecological complications and subsequently diagnosed with cervix cancer, have been compared for specificity, sensitivity and efficiency. The test in cervix smears has better specificity and efficiency while the assay in urothelial cells has an edge over sensitivity. These tests, after validation, probably can assist as screening measures for cervix cancer in view of their simplicity, rapidity and cost-effectiveness as desired for population screening of risk groups.

INTRODUCTION

In all developing countries as in India, maternal morbidity and mortality are points of cynosure. However besides direct gynaecologic and obstetric complications, another important issue leading to these outcomes is the high incidence of cervix cancer especially among the economically - disadvantaged. In fact the total incidence of mortality from cancer at all sites is greater in the lower socio-economic groups and is due mainly to increased incidence and mortality at certain sites including the cervix uteri (Tomatis, 1995). Poor personal hygiene, inadequate pre- and post-natal care are some of the reasons leading to the repeated infections of the cervix (Gawande et al., 1998). Risk for cervical cancer has been reported to be variable from one population group to another because of its multifactorial etiology (Shah et al., 1985). It appears that a larger proportion of the Indian female population is more vulnerable to cervical neoplasia, since the recognised risk factors for cancer of the cervix like illiteracy, low socio-economic status, early menarche, early marriage, multiparity, first child birth at an early age, poor genital hygiene and genital infections are widely prevalent in this population (Dutta et al., 1990; Thompson, 1992). Almost 20 per 100,000 Indian women have cervix cancer and it has been estimated (Chauhan et al., 1998) that one in 63 is likely to suffer from it in her lifetime. The figures from central India are more alarming where the prevalence of cervical cancer had been reported to be 21.3 per100,000 population (Gawande et al., 1998).

However, cancer of the cervix responds favourably to secondary prevention measures as it has a long pre-clinical phase that usually requires 2 to 10 years to penetrate the basement membrane and invade tissues (Mackay and Evans, 1997). Statistics reveal that about 4 cases of every 5 cervical carcinoma patients actually occur in those countries that are without screening programmes (Richart, 1995). Reductions in the U.S. (~70%), Finland (~50%), Sweden (~34%) and Iceland (~80%) correlate to the intensity of the screening effort (National Cancer Screening Institute, 1997). In fact in developed countries, 80% of cases are curable because of early detection while in developing countries, 80% of cases are incurable at the time of detection if they are detected at all (Arora, 1999). Rather, women do not come forward for routine gynaecological examination due to lack of knowledge about its early symptoms, fear of cancer (fatalistic attitude) and lack of awareness about the possibility of a cure. Regular screening can be achieved by imparting appropriate education to the masses regarding the early signs and symptoms of cervix cancer and utilizing rapid screening/ diagnostic measures.

Different nations recommend different ages to be covered by mass screening programmes for cervix cancer. WHO had recommended that for

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countries where resources are limited, every woman at least once in her lifetime at the age of 45 should participate in mass screening programmes (WHO, 1986). Routine protocol empathizes that every female above 25 years should annually get a Pap smear examination as a screening procedure (c.f. Bedi, 1999) since marked reductions in morbidity and mortality from cervix cancer have been achieved among screened persons (Hodge et al., 1998). Mass screening by cytology, to detect precursors of cancer of uterine cervix, is among the most successful of health measures. For stage IA1 lesions, cure rates of almost 100% have been achieved after simple hysterectomy or cone biopsy and of 70-85% for stages I or II A by surgery or radiation (NIH, 1996). Therefore, survival of cervix cancer patients is most directly related to the stage of disease at diagnosis, and the best way to increase detection of cervical neoplasms in pre-cancerous or localized stage is to improve the scope and quality of cytology screening.

Though the Pap smear is the most frequently used test in mass screening programmes, yet it is not totally reliable. The occurrence of genetic instability, either as a result of, or leading to, an increase in chromosomal rearrangements in most cancer types merits cytogenetic investigations. Such clastogenic effects can be also used as an effective screening measure for detection of cervical cancer in its pre-clinical stage. Ahuja et al. (1996) had suggested that in combination with morphological, biochemical and cytogenetic parameters, the Comet Assay along with the Micronucleus Test (MNT) may serve as novel tools to detect and predict the stage of cervical dysplasia.

Micronuclei can be detected in exfoliated cells of the buccal mucosa, urinary baldder, cervix or bronchi and seem to reflect chromatid and chromosome aberrations which occurred in the proliferating basal layers (Stich et al., 1982; Stich and Rosin, 1984; Fenech et al., 1999). As the sampling of such exfoliated cells is generally fast and highly economical, the MN assay has been the method of choice for survey of large population groups especially as a preliminary indicator for pre-cancerous lesions (Fenech et al., 1999). It was hence conceived to survey local hospitals for women requiring consultations for gynaecological complaints and to screen for micronuclei in the bladder exfoliated and cervix epithelial cells of just-diagnosed cervix cancer patients. The study was aimed to assess the two sources as to the better indicator for cervix cancer and suggest it for screening in order to assist in decreasing cervix cancer morbidity and mortality.

MATERIALS AND METHODS

The study sample comprised patients (n=25) visiting the local hospitals (Guru Ram Das Medical College and Hospital, Guru Teg Bahadur Hospital and Karam Singh ward of Radiotherapy) initially for gynaecological complications, like inter-menstrual bleeding, post-coital bleeding, leuchorrea and prolongation of the menstrual cycle and subsequently diagnosed with cancer of the cervix. An age- and socio-economic status (SES)-matched control group included women (n=25) also with gynaecological complaints but not testing positive for the cancer in the absence of healthy women taking a routine cervix examination.

Anamnesic data obtained from each subject during confidential interviews was recorded on a pre-designed questionnaire. Special emphasis was placed on queries relating to the risk factors for cervix cancer, viz., age-at-marriage, age-at-first childbirth, reproductive performance, gynaecological complaints, economic status, dietary habits and life-styles. Medical anomalies and any familial incidences of cervix cancer were also noted. The nature of the research work was explained to the subjects and written, voluntary consent was obtained for both, the collection of cervix smears (by the gynaecologist) and mid-stream urine samples (~5ml).

The MN Test

Both cervix smears and urine were processed for the MN test (Chakrabarti and Dutta, 1988) within 3-4 hrs of collection. Briefly, the samples were washed in phosphate buffered saline with alternate centrifiguation at 1200rpm for 10 min. This was repeated thrice and 2-3 slides were prepared per sample. The cells were fixed in methanol for 20 min, stained in May – Gruenwald's stain (0.25%) for 5 min, counter-stained with Giemsa (1%) for 3 min and mounted in DPX. Blind scoring of the slides was carried out. As the sample amount was variable, average scorable cells were 786 from smears and 302 from urine. These cells were scored initially at 40x

while micronuclei were confirmed at 100x. In order to rule-out bias, two observers randomly cross-checked the MNd cells. The data obtained were subjected to statistical analysis using Student's t-test as has also been done for MN data by others (Reali et al., 1987; Sarto et al., 1990). Since different tissues were sampled from the same subject for scoring cytogenetic damage, the specificity, sensitivity and efficiency of the two assays was also compared (Wisweswara Rao, 1996).

RESULTS AND DISCUSSION

The break-up of information in Table 1 (for cancer patients) and in Table 2 (for control individuals) reveals at a glance the data on various etiological factors which have been implicated in inducing cancer of the cervix, primarily age-at-marriage, reproductive history and socio-economic status (Schiffman and Brinton, 1995; Gawande et.,al. 1998; Caplash and Sobti, 1999). The pedigrees of the patients revealed no cervix cancer cases among their relatives.

The age distribution of the cervix cancer patients was in the range of 28-75 years while the age of control individuals varied form 25-70 years. Age has an impact on the incidence of this cancer (Sadeghi et al., 1988). Mild to moderate dysplasia was observed to be the most frequent type in women 25 - 29 years of age (prevalence rate - 25.7/1000); severe dysplasia and carcinoma *in situ* were reported in women 35 to 39 years of age (prevalence rate - 4.6/1000), while invasive

Table 1: Percent frequency of micronucleated cells in cervix cancer patients

Subject code	*Cancer stage*	*Age (yrs)*	*Age -at-marriage (yrs)*	*Total conce-ptions*	*SES Status*	*MNd cells /total urothelial cells scored*	*% frequ-ency MNd cells*	*MNd cells /total cervix cells scored*	*% frequ-ency MNd cells*
018	Ib	45	16	6	L	0/77	-	2/1000	0.200
019	Ia	40	16	8	L	1/267	0.374	2/980	0.204
020	Ib	30	20	3	L	0/257	-	4/1010	0.396
021	IIa	40	16	3	L	0/349	-	2/750	0.267
022	IIIa	35	18	4	L	2/226	0.884	3/980	0.306
023	IIb	30	16	8	L	1/282	0.354	9/1190	0.756
026	IIb	58	20	8	L	1/410	0.243	6/1070	0.588
028	IIIa	45	20	4	L	2/395	0.506	0/128	-
031	Ia	50	17	9	M	1/260	0.384	0/380	-
032	IIIa	48	15	7	L	1/323	0.309	6/1100	0.545
033	Ib	39	17	5	L	1/334	0.299	1/500	0.200
034	IIIb	65	20	5	M	1/232	0.431	0/800	-
035	IIb	50	20	4	L	0/456	-	0/540	-
036	IIIb	40	17	9	L	0/204	-	3/705	0.426
037	IIb	60	17	4	L	0/364	-	1/400	0.250
038	Ib	45	21	4	M	0/274	-	4/1020	0.392
039	IIIa	28	19	5	M	2/370	0.541	3/810	0.370
040	IIIb	60	15	5	L	3/319	0.928	3/720	0.274
041	IIa	55	16	2	L	1/327	0.305	4/722	0.487
042	IIIa	55	16	6	L	2/227	0.881	5/910	0.549
043	Ia	47	18	3	M	2/419	0.477	3/900	0.333
046	Ib	32	26	3	M	1/329	0.303	1/990	0.101
047	Iia	40	19	7	L	1/293	0.341	5/820	0.609
050	IIIb	75	16	3	L	2/270	0.740	2/500	0.400
051	IIa	60	16	3	L	2/288	0.694	3/670	0.448
Total				127		27/7552	0.359** ± 0.058	71/19655	0.324** 0.024±
Control(n=25)*				95*		4/7143	0.058 ±0.066	7/18446	0.031± 0.023

**- Highly significant compared to respective control group data, p≤0.05 and p≤0.01 (Student's t-test).
* - n=24 for urine sample data as one sample failed to yield cells; no. of pregnancies =93 for the total control in this case.
SES- socioeconomic status
a-Cancer stages after FIGO

Table 2: Percent frequency of micronucleated cells in control group

Subject code	*Gynaecological complaints*	*Age (yrs)*	*Age-at-marriage*	*Total conceptions*	*SES status*	*MNd cells/ total urothelial cells scored*	*% frequency MNd cells*	*MNd cells/ total cervix cells scored*	*% frequency MNd cells*
008	Pain in lower abdomen,leucorrhea	35	18	4	M	0/233	-	1/867	0.110
009	Cervix eroded	30	20	6	L	0/268	-	0/980	-
010	Irregular periods, post-coital pain	26	13	2	L	1/224	0.446	0/1000	-
011	Pain in lower abdomen	60	16	8	M	0/213	-	0/954	-
012	Cervix bleeding on touch; blood clot during mensuration	30	24	3	L	0/161	-	0/988	-
013*	Pain in lower abdomen	28	17	2	L	-	-	0/160	-
014	Leucorrhoea, irregular bleeding	25	19	6	L	0/303	-	2/890	0.220
015	Foul smelling discharge	32	16	6	L	0/316	-	0/560	-
016	Pain in lower abdomen	30	22	1	L	0/314	-	0/360	-
017	Prolonged menstrual cycle	45	19	2	M	0/194	-	0/550	-
024	Pain in lower abdomen	70	15	5	L	0/306	-	0/950	-
025	Irregular mensuration	29	20	2	M	0/438	-	0/890	-
027	Pain in lower abdomen	55	17	3	M	0/399	-	1/991	0.100
029	General weakness, body aches	35	22	3	M	0/238	-	1/1090	0.090
030	Pain in lower abdomen	45	18	6	M	0/260	-	0/520	-
044	Inter-menstrual cycle	34	16	3	M	1/339	0.295	0/998	-
045	Disturbed menstrual cycle	38	21	2	M	0/362	-	0/450	-
048	Irregular menstrual cycle	26	22	2	M	0/306	-	0/520	-
049	Bleeding, pain in lower abdomen	68	16	4	L	1/310	0.323	0/790	-
052	Leucorrhoea	40	19	3	M	1/285	0.350	1/710	0.140
053	Bleeding, pain in legs, backache	45	19	5	L	0/331	-	0/556	-
054	Pain in lower abdomen	28	19	4	M	0/290	-	0/682	-
055	Leucorrhoea	42	19	6	L	0/340	-	1/800	0.130
056	Pain in lower abdomen; leucorrhoea	35	20	4	M	0/379	-	0/650	-
057	Irregular menstrual cycle	28	18	3	M	0/234	-	0/540	-
Total				95*		4/7143	0.058 ±0.066	7/18446	0.031 ±0.023

• * 93 in urine sample data.

carcinoma was commoner in women older than 50 years with a prevalence rate of 0.47 per 1000. All these groups are covered in this sampled population.

It was generally observed that there were maximum number of individuals in both sample groups who had married young, i.e. between 15-20 years. It may be recalled that women less than 16 years of age at the start of sexual intercourse, have a relative risk of 1.7 for cervix cancer (Zhang et al., 1989; Brinton et al., 1990; Edebiri. 1990).

Most of the cervix cancer patients belonged to families with low SES. The reason probably is that individuals from well-off families prefer private treatment rather than from government or charitable hospitals. It has in fact been observed that uneducated women, due to lack of the knowledge of proper hygiene and preventive measures, are more prone to cervix cancer (Arora, 1999). Besides this, low socio-economic status in combination with malnutrition, vitamin deficiency and personal unhygienic life-styles coupled with illiteracy, makes the individuals also prone to infections (Brinton et al., 1990; Tomatis, 1995).

Among the patients was a young one (28 yrs; 039) suffering from stage IIIa of cervix cancer as well as the oldest one (75 yrs; 050) who had stage IIIb. With regard to the reproductive histories of the patients, more numbers of abortions (n=22) and total conceptions (n=127) were noted for cancer patients as compared to that in controls (n=16, n=95, respectively). Upto six spontaneous abortions were recorded for one patient (031). In literature also multiparous women have been reported to be more prone to develop cervical cancer than nulliparous, because of the trauma associated with multiple childbirths (Krul et al.,

1996; Gawande et al., 1998; Sobti et al., 1999). The relative risk of cervical adeno-carcinoma also increases with more number of induced abortions (Zhang et al., 1989; Parazzini et al., 1992).

For the stage type (FIGO, after Hatch, 1992) of cervix cancer, 8 cases out of 25 cancer patients were with stage 1 (3 cases with stage 1a and 5 were with stage 1b) an equal number were with stage II (4 with IIa and 4 with IIb) and 9 patients were with stage III (5 with IIIa and 4 with IIIb). Except for one subject (050) who was a daily smoker of 2-3 cigarettes, no other patient or control individual had this or alcohol drinking habit. Smoking among other potential risk factors (Brock et al., 1989; Simons et al., 1995) also seems to be associated with the development of cervix cancer.

The MN Test in Urothelial Cells: Cytogenetic damage was indexed by the number of micronucleated cells among the cells scored. This was observed in 17 cancer patients (72% of patients). Possibilities for their absence in others could be due to the lesser number of exfoliated cells available in urine samples and the cell population screened not being target sites. However, the overall genetic damage in cervix cancer patients (0.359 ±0.058 frequency of MNd cells) was significantly high as compared to that in (0.058±0.066) controls (t_{cal} =6.244, t_{tab} 5% =2.014, 1% = 2.690, df = 47). The number of micronucleated cells varied from 1 to 3 per individual with the cells scored varying from 204 to 456 except in one case (018) where the cells obtained and scored were only 77. The highest frequency of MNd cells i.e. 0.928 was observed in a patient (040) with stage IIIb cancer. She also had an early marriage (15 years) though her age at detection was 60 years. She belonged to low socio-economic status and her reproductive history included four children and one spontaneous abortion.

In the control individuals the micronucleated cells in urine samples were seen in only four out of the 24 individuals (16.7 %; MNd cells freq.= 0.058±0.066). The gynaecological problems with these individuals (010, 044, 049, 052) were irregular periods, post-coital pain, inter-menstrual bleeding and pain in lower abdomen. The absence of micronucleated cells in others with these symptoms however cannot be explained and in fact an historic base line on such control samples does not exist.

The MN Test in Cervix Smears: There were 21 individuals with cervix cancer (84%) in whom MNd cells were observed. The percent frequency of MN ranged from 0.101 to 0.756 while the cells scored varied from a low of 128 (with no MN) to as many as 1190 (with 9 MN) depending on the sample size available. The over all frequency of MNd cells (0.324 ± 0.024) in the patients' group was statistically significant (0.031 ± 0.023) from that observed in control (MN present in 24% of subjects) individuals (t_{cal}= 16.280, t_{tab}(5%)=2.008; (1%) δ = 2.678; df 48). The highest frequency of MN (0.756) was found in a young patient with IIb stage of cancer (δ 023), age-at-detection or age at sample collection was 30 years; she had a total number of 8 pregnancies and belonged to a low SES family.

In the control group individuals, the highest frequency of MN observed was 0.220; the individual (δ 014) was only 25 years old but she had 6 pregnancies. She had presented with irregular bleeding and leucorrhoea. There were five other control individuals (008,027, 029, 052, 055) with percent frequencies of micronuclei ranging from 0.090 to 0.140; their common symptoms were leucorrhoea, pain in lower abdomen and general weakness though in cells of other control individuals with same symptoms, no MNd cells were observed at all. Only one subject (052) had MN in smears and urothelial cells.

In literature also chromosomal instabilities have been reported for cervix cancer primarily including those for chromosomes 1,4,5,11,14,15 and 17 (Murty et al., 1988; Sreekantaiah et al., 1988; Atkin et al., 1990 ; Mitra et al., 1994). Other anomalies associated with cervix cancer are double minutes (Gebhart et al., 1984), modal chromosome counts of 47,48 (Katayama et al., 1990) as well as a trisomic condition of chromosome 8 (Mark et al., 1999). DNA damage in lympho-cytes and epithelial cells of the cervix (Jaiswal et al., 1994; Udumudi et al., 1998; Desai et al., 1998) as well as MN in uterine smears (Chakrabarti and Datta,1988) of cervix cancer patients have also been documented lending support to this study.

In order to determine which of the two assays has better sensitivity, specificity and efficiency (Tables 3, 4), the data obtained using these assays was subjected to analysis (Wisweswara Rao, 1996).

Sensitivity is defined as the proportion of disease cases rightly detected as diseased by the

test. Specificity is the proportion of non-diseased cases rightly detected as non-diseased by the test.

The sensitivity (=positive predictivity

Table 3: Patient and control data for MN test in urothelial cells

MN test in urothelial cells	*Test Results (No.of individuals)*		
	Positive	*Negative*	*Total*
MN present	18	4	22
MN absent	7	20	27
Total	25	24	49

Sensitivity= 18/25 =0.7 =72%; Specificity=20/24=0.833=83.3%;
Efficiency= 38/49=0.7755=77.55%

Table 4: Patient and control data for MN test in cervix smears

MN test in cervix smears	*Test Results (No. of individuals)*		*Total*
	Positive	*Negative*	
MN present	21	6	27
MN absent	4	19	23
Total	25	25	50

Sensitivity=21/25=0.84=84%; Specificity=19/25=0.76=76%; Efficiency=40/50=0.80=80%

=P(Total +ve/Diseased) was calculated as 72% and 84% for MN tests in urine and cervix smear samples, respectively. The specificity (=negative predictivity= P(Test –ve/Non-diseased) was 83.3% and 76.0% while efficiency was found to be 77.55% and 80.00% for the MN assays in urine and smear samples, respectively.

The analysis indicates that the MN test in cervix smears has better efficiency and better sensitivity, i.e. positive predictivity than the test in urothelial cells. However, the negative predictivity is better for the latter test. Since the cervix is the target for cervix cancer, MN test in the target tissue should definitely have an advantage over testing non-target cells. Nonetheless, the sensitivity of the MN test in urothelial cells can not be ignored as it too has a positive predictivity of 72% and could perform well in those situations where a non-invasive cell population is advanatageous for screening in view of bleeding and other gynaecological complications associated with cancer of the cervix. These assays need to be validated however, before they can find wide applications in screening programmes. Their simplicity, least cost-effectiveness and rapidity of results' retrieval seem to be optimal choices for the developing nations to bring down morbidity and mortality associated with this cancer.

ACKNOWLEDGEMENTS

We are grateful to Dr. Badaruddoza for statistical analysis.

REFERENCES

Ahuja, Y.R., Rajeswari, N., Anuradha, G.and Jaiswal, M.: Molecular epidemiology of individual risk to cancer. pp. 165-170. In: *Current concepts in Human Genetics*. J R Singh (Ed.). Guru Nanak Dev University Press, Amritsar (1996).

Arora, R.: Cervical Cancer : A clinical approach. *Obs. and Gyane.,* **4(11):** 658-666 (1999).

Atkin, N.B., Baker, M.C. and Fox, M.F.: Chromosomal changes in 43 carcinomas of the cervix uteri. *Cancer Genet. Cytogenet.,* **44:** 229-241 (1990).

Bedi, S.: Pap smear examination once every year. *Amritsar Newsline,* Sept. 7 (1999).

Brinton, K.A., Reeves, W.C., Brenes, M.M., Herrero, R., Britton, R. C., Garcia, M., and Rawls, W.E.: Oral contraceptive use and risk of invasive cervical cancer. *Int. J. Epidemiol.,* **19(1):** 4-11 (1990).

Brock, K.E., Maclennan, R., Brinton, L.A., Melnick, J.L., Adam. E., Mock, P.A. and Berry, G.: Smoking and infectious agents and risk of in-situ cervical cancer in Sydney, Australia. *Cancer Res.,* **49:** 4925-4298 (1989).

Capalash, N. and Sobti, R.C.: Epidemiology of cervical cancer. A Case control study on North Indian population. *Indian J. Cancer*, **36:** 179-185 (1999).

Chakarabarti, R.N. and Dutta, K.: Micronuclei test in routine smears from uterine cervix. *Eur. J. Gyanecol. Oncol.,* **9(5):** 370-372 (1988).

Chauhan, A.K. Dass, B.P. Hooda, H.S., Kaushal, V. and Manocha, K.K.: Radiotherapy for early cervical cancer. *Obs. and Gynae.,* **3(10)** : 605-606 (1998).

Desai, N., Rajeshwari, N., Jaiswal, M., Anuradha,U. and Ahuja, Y.R.: Comet assay as an aid to Pap smear test. *The Journal of Obstetrics and Gynaecology of India,* **147:** 81-84 (1997).

Dutta, P.K., Upadhya, A., Dutta, M., Urmil, A.C., Thergoakar, M.P. and Ganguly, S.S. : A case control study of cancer cervix patients attending Command Hospital, Pune, *Ind. J. of Cancer.,* **27(2) :** 101-108 (1990).

Edebiri, A.A.: Cervical intraepithelial neoplasia. The role of age at first coitus in its eitology. *J. Reprod. Med.,* **35(3):** 256-259 (1990).

Fenech, M., Holland, N., Chang, W.P., Zeiger, E. and Bonassi, S. : The Human Micronucleus Project – An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat. Res.,* **428:** 271-283 (1999).

Gawande, V.V., Wahab, J.N., Zodpey, S.P. and Vasudeo, N.D.: Risk factors for cancer cervix: A case control study. *Indian Journal of Cancer,* **35:** 164-170 (1998).

Gebhart, E., Bruderlein, S., Tulusan, A.H., Von Maillot, K. and Birkmann, J.: Incidence of double minutes, cytogenetic equivalents of gene amplification, in human carcinoma cells. *Int. J. Cancer,* **34(3):** 369-373 (1984).

Hatch, K.D.: *FIGO Staging of Cancer Cervix.* Williams and Wilkins Company, NAME OF PLACE (1992).

Hodge, F. S., Harrison, A.S., Gurgin, V. and Fredricks, L.: Cervical cancer screening. *Cancer,* **83:** 1799-1804 (1998).

Jaiswal, M., Anuradha, G., Rajeshwari, N., Raju, K.N., Balakrishna, N., Rao, K.V., Prasad, S., Jain, S.N. and Ahuja, Y.R.: Comet assay on cervical epithelial cells and leucocytes of patients with pre-cancerous and cancerous lessions of the cervix. *Med. Sci. Res.,* **22:** 879-881 (1994).

Katayama, K.P., Stafl, A., Woodruff, J.D., Masukawa, T. and Johnes, H.W. Jr.: Analysis of false negative cytology by a chromosome study. *Asia Oceania J. Obstet. Gynaecol.,* **16(1):** 85-87 (1990)

Krul, E.J.T., Peters, L.A.W., Vandenbroucke, J.P., Vrede, M.A., Kanten, R.W.V. and Fleuren, G.J.: Cervical carcinoma in Surinam. *Cancer,* **77(7):** 1329-1333 (1996).

Mackay, H.T. and Evans, A. T.: Gynecology and Obstetrics, pp. 668-700. In: *Current Medical Diagnosis and Treatment*. L.M. Tierney, S.T. McPhee and M.A. Papadakis (Eds.). Appleton and Lange, Stamford, C.T. (1997).

Mark, H.F., Feldman, D., Samy, M. Sun, C., Das, S. Mark, S. and Lathrop, J.: Assessment of chromosome 8 copy number in cervical cancer by fluorescent in situ hybridization. *Exp. Mol. Pathol.,* **66(2) :** 157-162 (1999).

Mitra, A.B., Murthy, V.V.S., Singh, V., Rong, G., Mahendra, P., Sodhani, P., Luthra, V.K. and Chagants, R.S.K.: Cytogenetical analysis of the cervix uteri- A molecular approach, *J. Natl. Cancer Inst.,* **87:** 742-745 (1994).

Murthy, V.V., Mitra, A.B., Das, B.C., Murthy, N.S.,and Luthra, U.K.: Chromosmal phenotyeps in patients with precancerous lesions of the uterine cervix which progressed to cancer during follow-up. *Oncology,* **45(5):** 384-388 (1988).

National Cancer Screening Institute. Screening for cervical cancer. *Cancer Net,* **208** (1997).

National Institutes of Health. Cervical cancer. *NIH consensus statement*. **14:** 1-38 (1996).

Parazzini, F., La, Vecchia., Negri, E., Fedele. E., Franceschi S., and Gallotti, L.: Risk factors for cervical intraepithelial neoplasia. *Cancer,* **69:** 2276-2282 (1992).

Reali, D., Di Marino, F., Bahramandpour, S., Carducci, A. and Barale, R.: Micronuclei in exfoliated urothelial cells and urine mutagenicity in smokers. *Mutat. Res,*. **192:**145-149 (1987).

Richart, R.M. : Screening: the next century. *Cancer,* **76:** 1919-1927 (1995).

Sadeghi, S.B., Sadeghi A. and Stanley J. R.: Prevalence of dysplasia and cancer of the cervix in a nationwide, planned parenthood population. *Cancer,* **61 :** 2359-2361 (1988).

Sarto, F., Tomannin, R., Giacomelli, L., Canova, A., Raimondi, F., Ghiotto, C., and Fiorentino, M.V.: Evaluation of chromosomal abberations in lymphocytes and micronuclei in lymphocytes, Oral mucosa and hair root cells of patients under antiblastic therapy. *Mutat. Res.,***228:** 157-169 (1990).

Schiffman, M.H. and Brinton, L.A.: The epidemiology of cervical carcinogenesis. *Cancer,* **76:** 1888-1901 (1995).

Shah, M., Parikh, B., Shah, C.A. and Jani, D.: Epidemiological study of the cancer of the uterine cervix in adivasi people of five different states of India. *J. Obst. Gynaec of India,* **335:** 355-360 (1985).

Simons, A.M., Mugica, C., Rodriguez, T.A., Mait land, N., Anderson, M., Phillips, D.H. and Coteman, D.V.: Demonstration of smoking related DNA damage in cervical epithelium and correlation with human papilloma virus type 16, using exfoliated cervical cells. *Br. J. Cancer,* **71:** 246-247 (1995).

Sobti, R.C., Capalash, N., Kochar, J., Sharma, S., Karn, B.K., Singh, K. and Bhasin, D.: Molecular genetics of cancer with special reference to cancers of G.I. tract, lung, head, neck and cervix. *J. Punjab Acad. Sci.,* **1:** 63-67 (1999).

Sreekantaiah, C., Bhargava, M.K. and Shetty, N.J.: Chromosome 1 abnormalities in cervical carcinoma. *Cancer,* **62(7):** 1317-1324 (1988).

Stich, H.F., Curtis, J.R. and Parida, B.B.: Application of the MNT to exfoliated cells of high cancer risk groups : Tobacco chewers. *Int. J. Cancer,* **30:** 553-559 (1982).

Stich, H.F. and Rosin, M.P.: Micronuclei in exfoliated cells as a tool for studies in cancer risk and cancer intervention. *Cancer Letter,* **22:** 241-253 (1984).

Thompson, J.D.: Cancer of cervix , pp.1162-1163. In : *Te Linde's Operative Gynecology.* R. W.Te Linde , J. A. Rock and J. D. Thompson (Eds.). J B Lippincott Company, Philadelphia (1992).

Tomatis, L.: Socio-economic factors and human cancer. *Int. J. Cancer,* **62:** 121-125 (1995).

Udumudi, A., Jaiswal, M ., Rajeshwari, N., Desai, N., Jain, S., Balakrishna, N., Rao, K.V. and Ahuja Y.R . : Risk assessment in cervical dysplasia patients by single cell gel electrophoresis assay: Study of DNA damage and repair. *Mut. Res.,* **412 (2) :** 195-205 (1998).

Wisweswara Rao, K.: *Biostatistics.* Jaypee Brothers Med Pub. Ltd., New Delhi (1996).

WHO.: Control of cancer cervix, a WHO meeting. *Bull WHO,* **64:** 607-618 (1986).

Zhang, Z.E., Parkin, D.M., Yu, S.Z., Estene, J. and Yang, X.Z.: Risk factors for cancer of the cervix in a rural Chinese population. *Int. J. Cancer,* **43:** 762-767 (1989).