DISCUSSION
INCIDENCE

In this study, highest incidence of cancer cervix was recorded in the age group of 36-45 years (41%) which was followed by the age group 46-45 years with 31% of the cases. Our study is in full agreement with the report of Ray-Choudhury (1997) who found 40% of the cases in the 36-40 years age group. However, the age incidence of this study is at variance with the reports of WHO (1985), Eifel et al. (1987), Robbins et al. (1989) and Samantray (1994) in which the age groups with highest incidence of carcinoma of cervix were 30-35, 48-55, 48-55, 40-45, 51-60 and 41-50 years respectively. However, the mean age of patients of this study coincides with the mean age (45-49 years) in different cancer institutes of India.

Majority of the cancer cervix patients of this study belonged to Hindu community (89%) whereas 11% belonged to Muslim community. Less incidence of cancer cervix in Muslim ladies could be due to practice of circumcision of penis among muslim males. This is also considered universally as the cause of low
incidence of cancer cervix among Jewish and Muslim women throughout the world (Wynder et al., 1954; Terris et al., 1973). This fact was contradicted by Ackerman & del Regato (1977) who found Lebanese women suffering from cancer cervix in more numbers. They postulated genetic factor as a cause of low incidence of cervical cancer in Jewish women. The highest incidence of the disease among Hindus in this study might be due to the fact that Hindus constitute the largest community in Orissa.

Among the patients of this study, cauliflower type of growth was found in highest percentage (45%). Next to this was ulcerative type (35%) and then infiltrative type (20%). Our findings corroborate the figures of Boyd (1991) and Parija et al. (1993).

Microscopically squamous cell carcinoma was found to constitute 96% of all patients of this study. The rest 4% was having adenocarcinoma. The percentage of squamous cell carcinoma was also reported to be highest in different studies. It was found to be 92-93% by Jaffecoute (1960), 97% by Gupta et al. (1987), 78% by Buckley et al. (1988), 90-95% by Coutler & Mason (1990) and 90% by Parija et al. (1993). Zhang (1993) also reported adenocarcinoma cases constituting 4.3% of all carcinomas of the cervix during the period of his study.
Majority of the patients of the present study was of lower socioeconomic status (Wynder & Cornfield, 1954), Christopherson & Parker (1965), Paranjothy (1961), Tyndall (1987) and Samantaray (1994) have also reported the same. Similarly almost all patients were married and have children. Thus our study reestablishes the prevalent view that multiparous women are more prone to develop cervical cancer.

Among the squamous cell carcinoma patients of this study, majority cases (56%) were diagnosed as moderately differentiated squamous cell carcinoma. This study agrees with the findings of Goelner (1976), Anderson (1977), Chung et al. (1981), Tyndall (1987) and Samantaray (1994). However, Boyd (1991), Parija et al. (1993) and Samant (1996) have mentioned highest representation of poorly differentiated squamous cell carcinoma in their reports. We recorded well differentiated squamous cell carcinoma in 14% of the studied patients—the lowest of all squamous cell carcinoma studied by us. The incidence of WDSC were 16.4% and 16.6% in the studies conducted by Chung et al. (1981) and Sakamoto et al. (1986) respectively. Thus our study almost agrees with the studies of latter investigators. The incidence of poorly differentiated squamous cell carcinoma in our study was 26% which was at variance with the findings of Agarwal et al. (1968), Goelnor (1976), Chung et al. (1981) and Marten et al. (1986) who reported 3.8%, 9.7%, 10.2% and 13% respectively.
Carcinoma of cervix in all patients of this study has been diagnosed by cervical cytology employing Papanicolaou’s staining method. The histopathological features of cervical carcinoma could be studied in detail and with accuracy with this technique. Thus Pap-smear technique being easy, quick, sensitive and inexpensive, is considered a very dependable screening method by us.

Cervical carcinoma is the most common cancer in females in India. About 100000 new cases are recorded annually and most of them (80%) come in advanced stages (stage III and stage IV) (Ray Choudhury, 1997). In India many women are in the high risk group who are more likely to get cancer of the cervix. As population screening is not being undertaken uniformly in all parts of the country, cervical cancer continues to kill many women in India. Early detection and diagnosis of this cancer during its pre-invasive stages by Pap-smear screening can make it possible to prevent it. But in India, cervical cytology has still been restricted to the large urban centres as the facilities for cancer diagnosis and screening continue to be institution based. Though cervical and breast cancers in females are included in screening programme, lack of trained personnel, adequate treatment facilities at all places and socio cultural barriers are the problems of cervical cancer control in India and other developing countries (Lunt, 1984). Despite these problems, it is presumed
that detection of cervical cancer (in its pre-invasive stage) by regular pap-smear screening is the best way of controlling this curable cancer in India.

This study reemphasizes the need and urgency of periodic cytological screening in all women who are sexually active. A 70% decrease in mortality rate from cervical cancer in United States has been recorded since 1940 because of cytological screening (Brinton et al., 1987; Koss, 1993; Boring et al., 1994):

Sometimes pap-smear yielded false negative results during our study. However, on repetition the smears could be improved with an increased number of endocervical or metaplastic cells. As the Pap-smears of all the patients of the present study were found to be positive for malignant cells and most were in stage III, they have been chosen for radiotherapy. Pap-smears performed at weekly intervals during the course of radiotherapy revealed the sequential radiation responses in the malignant cells. Thus the cytomorphological changes in response to radiotherapy could be followed up and curability of the disease could be judged basing upon such changes.

PRE-RADIATION CYTOLOGY

Basing on Pap-smear cytology, a meaningful interpretation of cells on the smear from uterine cervix can be made. Cytological study of cervical carcinoma has given rise to terms like dysplasia (CIN I and II), carcinoma in situ (CIN III),
invasive squamous cell carcinoma and adenocarcinoma. In our study, we could mark considerable pleomorphism in the structure of cells of cervical carcinoma. In the pre-radiation smears the common malignant cell was the undifferentiated one. These cells have malignant nucleus of varied shapes and sizes (mostly oval or round) with unequal aggregations of chromatin and empty spaces and scanty cytoplasm. Absence of cytoplasm is a definite criterion for identification of undifferentiated malignant cells. There are some elongated malignant cells in which the nuclei were found to be centrally placed, hyperchromatic, cigar-shaped and appear pyknotic. Cytoplasm of such cells was homogeneous and found to be stretched on either end of the nucleus. Some of the malignant cells were in the shape of tadpoles with disorderly arranged chromatin in the nucleus located centrally in the head of the cell. In the tadpole cells, the thick and homogeneous cytoplasm completely surrounds the nucleus and formed a tail.

In the pre-treatment smears of the studied patients, the malignant cells were found to be variable. This might be due to the fact that squamous cell cancers of the uterine cervix might not have a comparable rate of cellular exfoliation. The degree of decreased mutual adhesiveness in the cancer cells might be reason of the variation in the rate of apartneous exfoliation. The malignant cells of carcinoma of cervix were found to be isolated, in syncytial masses and in sheetlike arrangements. While the number of cell arranged in sheets were few, the number of isolated cells and number of cells arranged in syncytial masses were very high and were of almost
equal proportions. Altered polarity, rapid cell growth and disorganisation of cells in the parent epithelium were presumed to be the causes of syncytia. Similar cellular arrangements were also obtained by Reagan et al. (1957), Feldman et al. (1973) and Bibbo et al. (1975).

In comparison to normal squamous cells, the malignant cells of cervical carcinoma were found to be much smaller. While we recorded a mean cell area of 313.643 ± for malignant cells squamous cell carcinoma, the same has been reported as 229 ± 82.8 μm² and 195 ± 96 μm² by Reagan et al. (1962) and Wied et al. (1962) respectively. As the abnormal cells of the pre-treatment smears have diverse origin, they exhibited wide range of variation in size. Hence the mean cell area computed can't be considered representative of actual size of malignant cells of squamous cell carcinoma.

Nuclei of malignant cells of pre-radiation smears showed variable nuclear area. This was so because the size of the nuclei were commonly variable in the malignant cells of carcinoma of cervix. Hence the mean nuclear area computed by different investigators were found to be different (Reagan et al., 1957; Wied et al., 1962). So far shape was concerned, most of the malignant cells had either round or oval nuclei and very few had either elongated or irregular shaped nuclei. The findings of this study were almost in conformity with the study of Reagan & Patten
The nonisodiametric nuclear form was only due to the nonisodiametric form of the malignant cells of carcinoma of cervix. A small percentage of the malignant cells (15%) was found to be multinucleated which might be due to aberrant mitotic division or endomitosis or failure of cytokinesis.

Hyperchromatinism and pyknosis were the other characteristics of nuclei of malignant cells of cervical cancer. There was irregularity in the distribution of chromatin material in such nuclei which probably occurred, as a cursor to 'nuclear clearing'. Similarly premature senescence might have resulted in the opaque nuclei in the malignant cells.

In the malignant cells of carcinoma of cervix, the nucleoli were both true and false. There were also micro- and macronucleoli. However, the type of nucleoli depended upon the source of the smear. If the smear has been drawn from an active growth region, the nucleoli were distinct and if the smears were from metabolically less active areas they were found to have less number of distinct nucleoli. Our findings were almost identical with those of Reagan & Patten (1961), Love et al. (1973) and Patten (1978).

Depending upon the different reactions in the surface epithelium and differences in the basic cell type, the smears in the studied patients exhibited different
spectra of cells. In well differentiated squamous cell carcinoma, the malignant cell had scanty cytoplasm, and keratin formation, hyperchromatic and opaque nuclei, low mitotic index, uneven distribution of macronucleoli. In this histological type there was a high degree of pleomorphism both in the structure of the malignant cell and their nuclei. Epithelial pearls and intercellular bridges of common sight in this type. In the moderately differentiated squamous cells carcinoma (which was of highest incidence in the present study), the malignant cells had scanty cytoplasm, irregular nuclear structure (behaving atypically), high mitotic activity. But epithelial pearls and intercellular bridges were found to be absent in this type. The malignant cells of the poorly differentiated squamous cell carcinoma lacked cytoplasm totally and had bizarre nuclei. In this type, there was a limited degree of keratinization and no pleomorphism. The cells were either large or small. The nuclei of malignant cells of this type had macronucleoli and were neither hyperchromatic nor coarsely granular. Epithelial pearl formation and isolated cell keratinization were also found to be absent in poorly differentiated squamous cell carcinoma. These, above findings were almost similar to the findings of Poulson (1975), Jackobson et al. (1973), Chung et al. (1981) and Buckley & Fox (1989).

**POST-RADIATION CYTOLOGY**

The pap-smears prepared after first week of radiotherapy started showing the radiation effects which included clumping of malignant cells,
keratinization, tadpoleing and cytoplasmic vacuolation. All these were exhibited by the malignant cells to certain extent in the pre-treatment smears, but as a result of exposure to radiation more and more number of malignant cells showed them to a greater extent. As the radiotherapy continued, the changes like multiple cytoplasmic vacuolation, increase in nuclear size, multinucleation, nuclear pyknosis, giant cell formation, disintegration of nuclei and disappearance of nucleoli were marked in varying degrees in smears at different intervals of treatment. Our post-radiation cytomorphological findings were in conformity with those of Maloney (1950), Graham (1957), Merril (1958), Rayburn & van Nagell (1980), Gupta et al. (1982, 1987) and Shield et al. (1991).

In this piece of work, radiation effect was found to be better in post-menopausal women. In 68.42% of the post-menopausal patients, the smears were found to be negative for malignant cells after full exposure to radiation. Similar results were also obtained previously by Graham & Graham (1953) and Merril (1958) who reported good response in 71% and 75% of post-menopausal patients of their studies respectively. In pre-menopausal younger patients, the response of malignant cells to radiation was less marked and hence the smear negative cases after full exposure of radiation was only 58.33%. Again there was recurrence of the disease in many of these cases. These results suggested that hormonal factors (sex-hormones) might be responsible for unresponsiveness in case of younger patients.
Tissues highly sensitive to ionizing radiation are called radiosensitive. Those intermediate in their response are called radioresponsive and those which are non-responsive, are called radioresistant. Squamous cell carcinoma of epidermal origin is radioresponsive in nature. Among the patients of the study, this response was better marked in the poorly differentiated squamous cell carcinoma. Radiation effects the cells in two-ways i.e., (1) arrests mitosis and (2) degenerates and destroys the cells. Since the malignant cells are in highly dividing stage in poorly differentiated squamous cell carcinoma, they respond well to radiation.

Out of 100 patients, 87 patients responded favourably to radiation therapy. Of these 87 patients, 21 patients remained positive for malignant cells even after full exposure despite the favourable radiation response. All these 87 patients have exhibited vacuolation in the cytoplasm of around 10% of the desquamated non-malignant and epithelial cells in their pre-treatment smears. Thus, this study corroborates the study of Graham & Graham (1953). Again in the post-radiation smears of these 87 patients, infiltration of histiocytes and leucocytes was marked during the course of the treatment and these histiocyte and leucocytes constituted around 50% in their smears. Like Graham (1957), we also recorded an association between small histiocyte count and positive radiation response. Despite the above observation it was marked that positive radiation response did not always lead to total cure of the disease. However, presence of vacuolate cells in the pre-treatment smears
and infiltration of histiocyte and leucocytes during radiotherapy can be considered as positive indices for radio-curability.

Recurrences were recorded from both smear negative and smear positive groups. But while 8% recurrences were from smear negative group, 100% recurrences were from positive group. Thus radioresponsiveness is a direct index of curability. Patients showing radiation changes in their smears from the beginning of radiotherapy, were cleared of malignant cells early in treatment. In the patients of this study, a gradual decline in the number of malignant cells was observed from the first week of radiotherapy to 4th week. Meigs & Parker (1930) have observed complete disappearance of malignant cells from the eleventh day to fortieth day of the treatment. Gupta (1987) also observed a gradual and linear decline in cancer cells until the end of radiotherapy. None of stage IV patients of this study was found to be smear-negative for malignment cell after completion of radiotherapy. Similarly all the four patients with adenocarcinoma also remained smear positive after radiotherapy. Thus it emerges from this study that for post menopausal women having stage III poorly differentiated squamous cell carcinoma with 10% vacuolated cells in their pre-treatment smears, radiotherapy is the ideal method of treatment. This study also proves the effectiveness of pap-smear technique in detection, diagnosis, histopathological gradation and follow up study during and after treatment.
IMMUNOLOGY

The pre-radiation levels immunoglobulins IgG, IgA and IgM of the cervical cancer patients of this study were found to be higher than those of normal controls. The rise in the levels of IgG in the studied patients was found to be significant. Significant rise in the pre-radiation IgG levels was reported by Hughes (1971) and Adelusi & Salimonu (1981). Similarly while the pre-radiation IgM levels of this study were also found to be significantly higher, the rise of IgA in cancer patients was insignificant. Increase of pre-radiation IgM levels was also reported by Plesniear (1972), Gupta et al. (1981) and Adelusi & Salimonu (1981). Similarly Hughes (1971), Gupta et al. (1981) and Chander et al. (1987) recorded higher IgA levels in the cervical cancer patients than the normal controls. While Gupta et al. (1981) reported increased IgA and IgM levels in the cervical cancer patients, Plesnicar (1972) reported increased IgM only. Chander et al. (1987) observed lower pre-radiation IgG and IgM levels and an insignificant rise in IgA levels in 47 patients with cervical cancer. Though our findings were in full agreement with the findings of Adelusi & Salimonu (1981), differs from the findings of other investigators at some point or other.

Many investigators have emphasised on the possibility of production of immunoglobulins specific for tumour antigens on the surface of cancer cells (Levy et al., 1978; Gall et al., 1973; Weintraub, 1973). The increased levels of IgG, IgM and IgA might also be due to severe infections common to cancer cervix patients.
Thus it can be postulated that rise in the levels of immunoglobulins in cancer patients in general and patients with carcinoma of cervix in particular may be attributed either to the presence of cancer antigens or to the antigens arising out of secondary infections.

After the first week of radiotherapy IgG level showed a significant rise, IgM level showed an insignificant rise and the IgA level remained nearly unchanged. There was a decline of IgG level during 2nd and 3rd week of treatment and after 3rd week, it registered a slow rise till the end of 4th week. But the rise of IgM was continuous till the end of 3rd week and then gradually declined. But the IgM levels at the end of 4th week was still higher than its pre-radiation levels. But the increases and decreased of IgM at different week of treatment did not appear statistically significant. The IgA level fluctuates during the course of treatment. However, it showed a fall from the pre-radiation levels at first week, increased during 2nd week and IgA level showed steady decline during 3rd and 4th week of treatment. Radiotherapy might be interfering with the structural aspects of the cancer specific antigens and the incidence of metastatic spread might be reduced during the course of radiotherapy. Possibly these events led to significant fall of IgG levels of the studied cases during first three weeks of radiotherapy. The continuous rise of IgG levels from 3rd week onward can be correlated with the accumulation of cellular necrosis (Strauss et al., 1965). We agree with Chander et al. (1987) in postulating that IgM response was initially evoked by antigenic stimulation from products of
cellular necrosis and in the later phase it also led to increased IgG levels. Products of cellular necrosis stimulating antibody response was also reported by Hencock et al. (1984).

It can be presumed that as the cell mediated immunity declines in patients with carcinoma of cervix, the humoral factor gains importance. Again possibility of infections as the cause of elevation of immunoglobulin levels in cancer cervix patients should also be taken into account.

The gradual and significant increase in the levels of IgG and IgM with increasing stage of the disease can be used as a diagnostic feature of carcinoma of cervix. However, the increased levels of IgA did not have a positive correlation with the clinical stages of the disease. The observed steady pattern of decrease and increase of IgG levels and increase of IgM levels with the progress of radiotherapy may also be correlated with the extent of curability of the disease. However, final assertions on these propositions can only be obtained from a systematic long term follow up study.

CHROMOSOMAL STUDY

A host of spontaneous chromosomal aberrations were recorded in the chromosomal preparations obtained from the bone marrow cells of 50 stage III invasive squamous cell carcinoma patients before radiation. Out of 3274 mitotic metaphases studied, 671 showed one kind of aberration or the other or more than one
aberration. The aberrant metaphases constituted nearly 20.5% of metaphases observed. In comparison to the aberrations observed in the age matched controls (with benign cervical lesions), the cancer patients exhibited a highly significant rate of aberration. Of the 50 patients 36 did show aberrations and mostly the aberrant metaphase had a multitude of aberrations in each. The unstable aberrations (like gaps, ring chromosomes, constrictions, aneuploids, stickiness etc.) are far more than the stable aberrations (like translocation, chromosomes with broken ends etc.). High frequency of spontaneous aberrations have been obtained from the leucocyte cultures of patients with pre-cancerous and cancerous lesions of uterine cervix by Mitra et al. (1982, 1983, 1986). Similarly high frequencies of spontaneous chromosomal aberrations were also recorded in the leucocytes of patients with skin cancer (Taylor et al., 1973), thyroid carcinoma (Hsu et al., 1981; Pathak et al., 1982) and renal cancer (Wanz et al., 1982).

Most of the previous chromosomal studies in cervical cancer have been made from either the leucocyte cultures or from the cancerous cells from the cervix. Again such studies were neither histopathological type specific nor stage-specific. But in the present study, the chromosomal preparations were obtained directly from bone marrow cells of stage III squamous cell carcinoma patients with no metastasis. Thus the aberrations were recorded from in vivo non-cancerous cells.
Most of the aberrant metaphase of the present study were found to possess more than one kind of chromosomal aberration. This was contrary to the report of Mitra et al. (1986) who observed only one aberration per metaphase in most cases. However, they studied the chromosomal spreads from the cultures of leucocytes of cancer patients of different stages while ours was a direct method adopted for stage III patients only. Again in our study, a high frequency of chromosomal aberration (20.49%) has been recorded. This percentage is much higher than the percentages recorded for cervical cancer (17%) by Mitra et al., (1986). The high percentage of aberrations recorded by us might be due to advanced stage of the disease (stage - III) in the patients chosen for this study.

We noticed all kinds of aberrations with 'chromatid gap' outnumbering the rest. Again aberrations like aneuploidy, acentric fragments, constrictions, pycnosis, chromatin extraction, ring chromosomes, stickiness etc. were observed only in the chromosomal preparations of cervical cancer patients but were mostly absent in age matched normal controls. The unusual high frequency of chromosomal aberration and its variety in the studied cases of cancer cervix indicate that constitutional chromosomal instability may precede malignancy and normal women with a frequency of aberration higher than the spontaneous aberrations obtained for normal healthy control women may run the risk of developing cervical cancer. Thus
we agree with Mitra et al. (1986) in presuming chromosomal instability as one of the contributing factors for developing malignancy.

In this study, the chromosomal aberrations were found to occur mostly in combination in a metaphase spread. Thus, it has become very difficult to single out a particular aberration which could be considered specific for carcinoma of cervix. Invariably almost all aberrant metaphases exhibited gaps but they were again found to be present in more than one chromosomes in a metaphase and sometimes in different metaphase. This was also true for other kinds of aberrations. Mitra et al. (1986) have also found gaps to outnumber other aberrations in the leucocyte cultures of cancer cervix patients.

Next to gaps, aneuploids were found to be in high frequency in the bone marrow cells of the cervical cancer patients of this study. These aneuploids had mostly 45 or 44 chromosomes ($2n = 46-1$ or $2n = 46-2$) and one 'C' group chromosome and / or one 'E' group chromosome were found to be absent in them. Though it was difficult to recognise the particular missing chromosome(s) in such complements in the absence of banding pattern data, we could presume them to be chromosome 11 and 17 on the basis of their size (Fig.56). Southern & Herrington (1997) also reported underrepresentation of chromosome 11 and / or 17 in the tissues from invasive squamous cell carcinomas. Their study suggests that since the relevant
tumour - suppressor genes are supposed to be located on chromosomes 11 and 17, relative reduction in the number of these chromosomes is important in the development of invasive cervical neoplasia. Though the present chromosomal study has not been undertaken in the malignant cells of the cervix, we can presume that constitutional numerical reduction of chromosomes 11 and 17 or of any other chromosome supposed to harbour tumour suppressor genes may have an important role in the development of carcinoma of cervix.

There have been quite a number of chromosomal studies undertaken in cervical cancer in the recent past. Of these, the studies of Archimbaud et al. (1987), Kleinerman et al. (1989) and Kao et al. (1990) and those of a few other investigators were restricted either to the treatment period or to post-radiation follow-up period. But Chung et al. (1992), Mitra et al. (1994), Choo et al. (1995), Zimorjic et al. (1995), Rader et al. (1996), Larson et al. (1997 a, b) and Southern & Herrington (1997) have studied the chromosomal aberrations in the cancer cervix patients before treatment. In these studies, mostly the aberrations found to be associated with cervical cancer were translocations (Choo et al., 1995; Zimonjic et al., 1995), deletions (Chung et al., 1992; Mitra et al., 1994; Zimonjic et al., 1995; Rader et al., 1996) and loss of whole chromosomes (Southern & Herrington, 1997). The most common rearrangement so far recorded for cervical cancer is found to involve chromosome 1 (Heim & Mitelman, 1995 a, b; Zimonjic et al., 1995). The
other extensively validated aberration event is the loss of heterozygosity for loci on the chromosomal arms 3p, 4p, 4q and 11q. (Chung et al., 1992; Mitra et al., 1994; Rader et al., 1996; Larson et al., 1997a,b; Green span et al., 1997) However, allelic deletions on other chromosomal arms have also been reported by some investigators recently. The chromosomal investigations in carcinoma of cervix till date reveal that the malignant cells of this cancer present a complex karyotype with a multitude of aberrations/rearrangements-both numerical and structural.

This present piece of chromosomal study clearly indicates that in cervical carcinoma patients, cells other than malignant ones, have also constitutional spontaneous chromosomal aberrations which include both stable and unstable aberrations. This study further establishes that a considerable degree of structural and numerical chromosomal instability is associated with carcinoma of cervix which may predispose an individual to a future potentially life threatening cervical neoplasm. Thus chromosomal data can be used as a parameter for diagnosis, prognosis and curability of this disease.