SUMMARY
This piece of work deals with cytomorphological, chromosomal and immunological investigations in 100 carcinoma of cervix patients in state III and stage IV attending Acharya Harihar Regional Cancer Centre, Cuttack during a period of 5 years (1992 to 1997). None of these patients has been operated and all cases have been assessed by the radiotherapists and gynaecologists of the institute before, during and after treatment by radiotherapy.

For cytological diagnosis, smears were collected from growth area with the help of Ayer's spatula and stained by Papanicolous staining procedure. To each of the hundred selected cases 28 exposures of radiation were given. All cases were treated with Co 60 teletherapy of a dose of approximately 5500-6000 cGy. Brachytherapy of 500 to 2000 cGy to point A was given in selected cases. The patients were examined at weekly intervals during treatment to assess the radiation response clinically and through serial PAP smear from the growth area. Nuclear area and cellular area of malignant cells were computed before radiation and after each seven
doses. Number of malignant cells present in the smears before treatment and after each week of radiotherapy were determined by counting them in at 10 microscope fields per slide per week of treatment. Chromosomal preparation of 50 pap-smear confirmed cases of squamous cell carcinoma belonging to stage III were obtained from bone marrow cells before radiotherapy by employing the air-dry technique. For studying the changes of humoral factor of immune system of 50 patients with stage III and stage IV cervical cancer and the effect of radiation on the parameters like IgG, IgM and IgA, blood samples were collected and the immunoglobulin estimation was done using Tripartigen plates supplied by Hoechst India Ltd. by immune diffusion method.

Out of 100 studied patients, 95 were in stage III and 5 were in stage IV of the disease. The highest age incidence was recorded from 36-45 age group. Cervical carcinoma was ulcerative type in 35 patients, infiltrative type in 20 patients and cauliflower type in 45 patients, Squamous cell carcinoma was detected in 96 patients and adenocarcinoma in 4 patients. Among squamous cell carcinoma patients, poorly differentiated squamous cell carcinoma was found in 26 cases, moderately differentiated squamous cell carcinoma in 56 cases and well differentiated squamous cell carcinoma in 14 cases.
The malignant cells of pre-treatment pap-smears appeared irregular, polygonal, oval, elongated and caudate. The nucleus in them was oval, elongate or irregular. Some were tadpole shaped. The cytoplasm was opaque in most malignant cells and vacuolated in a few. Majority malignant cells were mononucleated and a few were multinucleated. Both false and true nucleoli were found in nuclei of malignant cells. Slightly or highly degenerated erythrocytes were seen in some malignant cells. In some cases infections of Trichomonas and Bacillus vaginalis were detected.

The papsmears obtained from the growth area of the radiation treated patients at weekly intervals revealed the radiation responses in both normal cells and malignant cells. After 1st week of radiotherapy, the morphological changes marked in the malignant cells were increase in cell size, decrease in the size of nuclear area, polymorphism, cytoplasmic vacuolation, keratinization and tadpoling. At the end of 2nd week, there was further increase in cell size, multiple vacuolation of cytoplasm, giant cell formation, nuclear pycnosis, multinucleation, polymorphism and clumping of malignant cells. After completion of 3rd week of radiation treatment, the malignant cells were found to increase still further in size and exhibited multiple vacuolation of cytoplasm, multinucleation, disintegration of cell membrane, multiple giant cell formation, keratinization, infiltration of inflammatory cells and histiocytes. At the end of 4th week, either the smears were totally cleared of malignant cells or possess a very few of them with multinucleation, multiple vacuolation and
polymorphism. Radiation response was better marked towards 14th day of radiotherapy. Of the 95 stage III patients, 66 were found to be smear negative for malignant cells after full exposure to radiation. The rest 34 cases (including 5 stage IV cases) were smear positive. Radiation effect was better marked in post-menopausal women. Similarly the radiation response was observed to be best in poorly differentiated squamous cell carcinoma patients. Patients in whose smears small histiocyte and leucocytes appeared during radiotherapy (more than 50%) were observed to respond better to this treatment. Again in patients having more than 10% vacuolated cells in their pre-treatment smears, the radiation response was found to be good.

In 50 patients (48 in stage III and 2 in stage IV) of this study, the humoral immune response has been evaluated before and during radiotherapy. The IgG levels in patients before treatment were found to be significantly higher than those of the normal controls. A much higher IgG level was recorded after 1st week of radiotherapy. After 1st week, the IgG level showed a steady decline till the end of the 3rd week of radiotherapy. But the IgG level at the end of fourth week of treatment was observed to be a little higher than that at the end of 3rd week and was almost similar to that of the pretreatment patients. The IgM levels in patients before treatment was observed to be significantly higher than those of normal controls. From the beginning of radiotherapy it showed a trend of rise till the end of 3rd week of treatment after which it declined almost to the level of that after 1st week of
treatment. Pre-treatment level of IgA was slightly higher than the level of controls and it fluctuated throughout the period of radiotherapy.

A total of 3274 metaphase spreads from bone marrow chromosomal preparations of 50 patients were analyzed. About 20% of the studied metaphase exhibited chromosomal aberrations which included gaps, breaks, aneuploids, fragments of unknown origin, pycnosis, chromatin extraction, ring chromosomes, terminal fusions, translocations and stickiness. Almost all abnormal metaphases have more than one kind of aberration each. The highest frequency of metaphase with aberrations was 65.3% recorded in 2 patients. Fourteen out of fifty patients did not exhibit aberrations in their metaphase spreads. Aneuploids constitute the highest percent (95.5%) of all metaphases with aberrations and a majority of them were monosomics, with 2n = 45 chromosomes in which one of the ‘C’ or ‘E’ group chromosomes was found to be missing. Aberrations like gaps, aneuploids and breaks were found to be much higher than those encountered in the controls and thus were statistically significant. But other aberrations like acentric fragments of unknown origin, terminal fusions, translocations and stickiness etc. were only found in patients with carcinoma of cervix.

The age incidence (36-45 years), community incidence (89% Hindus), clinical type incidence (45% Cauliflower type), stage incidence (95% stage III), cytological type incidence (56% M.D.S.C.) etc. were in conformity with many
previous studies. This study reemphasizes the need and urgency of periodic pap-smear screening in all women who are sexually active in the developing countries in general and in India in particular. Serial pap-smears could show the progression of radio-responses in the malignant cells very clearly and accurately and also helped in checking recurrences.

The radiation effect was found to be better in post-menopausal patients. Better radiation response was also marked in the patients with poorly differentiated squamous cell carcinoma. Out of 100 patients, 87 patients responded favourably to radiation. In the post-radiation smears of these 87 patients, infiltration of histiocytes and leucocytes was marked during treatment. None of the stage IV patients and none of the adenocarcinoma patients were found to be smear negative for malignant cells after completion of 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.

The pre-radiation levels of immunoglobulins IgG, IgA and IgM of the cervical cancer patients of this study were found to be higher than those of normal
controls. This study postulates 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. It also emerges from this study that radiotherapy might be interfering with 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 was correlated with the accumulation of cellular necrosis. It was also presumed that as the cell mediated immunity declines in patients with carcinoma of cervix, the humoral factor gains importance. 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.

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 women may run the risk of developing cervical cancer. This study agrees with other investigators in presuming that chromosomal instability is one of the contributing factors for developing malignancy.
The 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 pre-dispose 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.