INTRODUCTION
Cancer ranks fifth in order of all causes leading to human death. Cancer of the cervix is the fifth most common of all cancers preceded by cancer of stomach, lung, breast and large bowel. It is the second most common cancer in females after breast cancer and is the third most frequent of female genital cancers (Boring et al., 1991). It now ranks 8th among the killer cancers of females constituting 10-15% of all cancers. Annually 200,000 deaths were caused by carcinoma of cervix and more than 465,000 new cases of invasive cancer of the cervix are reported every year world wide (Ray-Chowdhury, 1997). The peak age of incidence of carcinoma of cervix falls between 40 and 50 years. Most patients are of low socio-economic status and are married.

Carcinoma of uterine cervix is the most frequent neoplasm in India constituting 20% to 50% of all neoplasms (Devi & Pravabati, 1961; WHO Bulletin, 1986; Murthy et al., 1990) and 85% of all female genital tract cancer. Annually approximately 100,000 new cases of cancer of the cervix are recorded in India (Luthra et al., 1987; Ray-Chaudhury, 1997). About 30% of all types of malignant diseases attending Gynaecology and Radiotherapy clinics comprise the cancer cervix.
It is also the most common type of cancer among women in Iran, Africa, Latin America, China and other Asian countries (Parkin et al., 1984). High frequencies of cervical cancer are reported from Columbia (52.9%) and Brazil (37.5%). Cancer of cervix is infrequent in Jewish and Moslem women and circumcision of Jewish and Moslem men was postulated as a cause (Terris et al., 1973). However, Ackerman & del Regato (1977) postulated genetic factors as a cause of low incidence of cervical cancer in Jewish women. Jewesses born either in Israel, Europe or America have the lowest frequencies. In North America and Europe, it is the fourth most common cancer in women. Three quarters of all the reported cases of cervical cancers are from developed countries.

Cervical cancer constitutes approximately 45% of all cancers in each of Bangalore, Chandigarh and Chennai, 30% in Mumbai, 25% in Tiruvananthapuram and 22% in Dibrugarh (Annual Report 1986). In AHRCC, Cuttack where the present study has been undertaken, the percentage of cancer cervix patients is 22.2% of all female admission.

The risk factors of incidence of carcinoma of cervix are known to be low socio-economic status, poor dietary quality, low age at first intercourse, large number of sexual partners, pregnancy at a young age, immuno deficiency, vitamin A and C deficiency, infection of HPV, HSV-2 and HIV virus, smoking, oral contraceptive pills, racial factors, multiple marriage, and prostitution (Christopherson & Parker, 1965; Rotkin, 1967; Keighley, 1968; Barber, 1975; Wright et al., 1980;
Skegg et al., 1982; Dallenbach-Hellweg, 1984; Stubblefield, 1984; Crum et al., 1985; Peters et al., 1986; Brinton et al., 1987; Winklar & Richart, 1987; Wilezynski et al., 1988; Robbins et al., 1989; Whelan et al., 1990; Parazzini & La Vacchia, 1990; Kessler, 1990; Howley, 1991; Anderson, 1991; Meanwell, 1991; Lorincz et al., 1992; Lancaster 1992; Spinillo et al., 1992; Maiman et al., 1993, Ray-Chowdhury, 1997). Contrary to the above, the frequency of cervical cancer is much less in women with inactive sexual lives and in women who have a mutually monogamous relationship and in women with no children (Taylor et al., 1959; Rotkin, 1967; Barber, 1975).

It has also been demonstrated that the risk for cervical cancer may be increased in the wives of men who have previously been married to women who developed cervical cancer (Kessler, 1976). Women married before the age of 20 years are found to have higher frequencies of cervical carcinoma (Christopherson & Parker, 1965). Invasive carcinoma is twenty times more among wives of unskilled labourers than professional men. Smoking is reported to double the risk of developing cervical cancer.

Classification

Pathologically carcinoma of cervix has been classified on the basis of macroscopic pathology, microscopic pathology and histologic pathology.
Macroscopic Pathology

On the basis of gross examination carcinoma of cervix can be divided into the following types:

i. Exophytic tumours: These are often papillary and may form a bulky mass of considerable size while still confined to the superficial portion of the cervix.

ii. Nodular tumours: They usually originate typically in the endocervix, forming multiple firm masses that expand the cervix and isthmus. The mass may be large and when it is disturbed circumstantially, it has been called barrel shaped.

iii. Ulcerative tumours: These have an infiltrative pattern of growth and eventually become necrotic in centre and sloughs, leaving a cavity surrounded by invasive cancer.

Microscopic Pathology

Microscopically all the carcinoma are broadly divided into three groups though rarer varieties are also present. They are: 1) Squamous carcinoma, 2) adenocarcinoma and 3) adenosquamous carcinoma.
Histological pathology

Histologically the squamous carcinoma of uterine cervix can be divided into (1) dysplasia of uterine cervix (CIN - I, CIN - II), (2) Carcinoma in situ (CIN - III) and (3) invasive squamous cell carcinoma. Dysplasia may again be divided into mild dysplasia (CIN-I), (2) moderate dysplasia (CIN - II) and severe dysplasia (CIN - III). Similarly invasive squamous cell carcinoma can be classified into ten subtypes i.e., invasive squamous carcinoma, invasive adenocarcinoma, adenosquamous carcinoma, clear cell adenocarcinoma, glassy cell carcinoma, verrucous carcinoma, adenocystic carcinoma, mucinous adenocarcinoma, carcinoid tumours and malignant melanoma.

For prognosis and treatment point of view all the epidermoid carcinoma of cervix are graded according to their degree of differentiation. They are (1) well differentiated, (2) moderately differentiated, (3) poorly differentiated. Higher the grade worse is the prognosis. A moderately differentiating non-keratinising large cell epidermoid carcinoma is the most common pattern (70%) and carries the best prognosis (Anderson’s Pathology, 1991).

Histopathological features of cervical carcinoma:

1. Squamous Cell Carcinoma
   a. Well differentiated squamous cell carcinoma - Well differentiated squamous cell carcinoma forms bands or islands of tumour cells and characteristically have well formed epithelial pearls and intercellular bridges.
Nuclei are large, pleomorphic and hyperchromatic. Mitotic figures are sparse or abundant. Vascular and lymphatic permeation are frequently seen (Paulsen, 1975; Chung et al., 1981; Buckley & Fox, 1989).

b. Moderately differentiated squamous cell carcinoma- This type is characterised by greater degree of pleomorphism, marked nuclear irregularity, higher cytoplasmic ratios and frequent mitotic activity. Epithelial pearls and intercellular bridges are absent, rather individually keratinised, dyskeratotic cells occur singly or clumps (Paulsen, 1975; Buckley & Fox, 1989).

c. Poorly differentiated squamous cell carcinomas - They exhibit smaller growth patterns and squamous cell neoplasm may be small or large cell type. Some evidences of keratinization may be seen. May be adenosquamous or adenocarcinomas in reality that are excluded by mucin stains. May show pseudoglandular formation without mucin (Buckley & Fox, 1989).

d. Verrucous carcinomas - These carcinomas have bland appearance. Beneath the hyperkeratotic or parakeratotic epithelium papillary trends are seen without connective tissue core. Cytologic atypia and mitotic activity are insignificant. Characteristically there is chronic inflammatory cell in filtrate (Buckley & Fox, 1989).
II. Adenocarcinoma

a. Endocervical adenocarcinoma: There is little or no cytological atypia in this type and it resembles the endocervical glands. Definite positive staining for carcinoma embryonic antigen is seen. Tissue contains both intracytoplasmic and intraglandular mucus which may be found to spill over. The least differentiated adenocarcinomas have no morphological picture indicating their origin, but can be identified by special stains only (Silverberg & Hurt, 1975; Buckley & Fox, 1989).

b. Papillary serous adenocarcinoma: Adenocarcinoma of cervix growing in a papillary pattern, some show differentiation along tubal lines. Resemble serous papillary adenocarcinoma of ovary (Buckley & Fox, 1989).

c. Endometrioid adenocarcinoma: Simulate endometrioid adenocarcinoma of endometrium. It can arise in foci of cervical endometriosis or undifferentiated endocervical reserve cells (Buckley & Fox, 1989).

d. Clear cell adenocarcinoma: These show a complex or mixed solid, tubular and papillary or microcystic pattern. Cells have vesicular nuclei, eosinophilic or granular cytoplasm, may have hobnail nuclei (Buckley & Fox, 1989).
e. **Mesonephric adenocarcinoma**: It arises deep in the cervix, in the lateral wall which is the site of mesonephric duct remnants. Histologically these carcinomas simulate clear cell carcinoma but contain neither mucin nor glycogen (Hart & Norris, 1972; Buckley & Fox, 1989).

f. **Enteric adenocarcinoma**: Usually these are well differentiated with acini lined by columnar cells with ovoid nuclei and prominent brush border, goblet cells and panneth cells may be present. (Abell, 1973; Buckley & Fox, 1989).

III. **Mixed Tumours**

Mixed tumours include (a) adenosquamous carcinoma showing evidences of squamous differentiation and mucus secretion, (b) Glassy cell carcinoma comprising large polygonal cells with distinct limiting margin, abundant finely granular cytoplasm, large vesicular nuclei and prominent nuclei and (c) adenoid cystic carcinoma which are formed of small uniform balaloid cells with scanty cytoplasm and irregularly dark staining nuclei arranged in sheets, nests and islands (Buckley & Fox, 1989).

IV. **Small Cell Carcinoma**

There are three types of small cell carcinomas:
a. **Neuroendocrine Tumours**: These tumours range from carcinoids to poorly differentiated neoplasms showing neuroendocrine granules and peptide substances (Jones 1976; Silva, 1984; Buckley & Fox, 1989).

b. **Basaloid carcinoma**: This type of small cell carcinoma simulates basal cell carcinoma and has infiltrative pattern of growth. This is also characterised by nests or cords of basaloid cell with peripheral palisade cells and absence of desmoplastic stromal response (Buckley & Fox, 1989).

c. **Sub-columnar reserve cell carcinoma**: The lesions are formed of uniform cells with hyperchromatic nuclei and scanty cytoplasm cells are arranged in sharply defined nests that replicate an endocervical crypt like pattern. No stromal response or cellular moulding is seen (Buckley & Fox, 1989).

There are still other types of cervical tumours which include: (a) undifferentiated carcinoma (Paulsen *et al.*, 1975; WHO classification, (b) malignant melanoma (Evers, 1950; Willis, 1967), (c) cervical sarcoma (Paulsen *et al.*, 1975; WHO classification), (d) primary lymphoma of the cervix (Symmer, 1991).

Some investigators have also attempted to classify cervical carcinoma basing on the criteria like presence or absence of tumour marker like CEA (Steeper
& Wick, 1986), oestrogen or progesterone receptor status (Martin et al., 1986; Twiggs et al., 1987), blood group antigen A, B and H (To & Singleton, 1986; Sakamoto et al, 1986) and oncogene expression (Hendy-Ibbs et al., 1987). However, these classifications did not get wide acceptance as they did not provide significant prognostic value.

The staging of cervical carcinoma is a clinical staging classification that comprises physical examination (like inspection, palpation and biopsy), laboratory studies and rectogenographic evaluation.

In 1985, the Oncology Committee of the International Federation of Gynaecology & Obstetrics (FIGO) made changes in the FIGO classification of cervical carcinoma. This new classification of FIGO is accepted worldwide and is as follows:

Stage O: Carcinoma in situ.

Stage I: The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded).

Stage IA1: Pre-clinical carcinoma of the cervix i.e., those diagnosed only by microscopy.
Stage IA2: Lesions detected microscopically that can be measured. The upper limit of the measurement should not show a depth of invasion of more than 3 mm taken from the base of the epithelium, either surface or glandular from which it originates, and a second dimension, the horizontal spread must not exceed 7 mm.

Stage IB: Lesions of greater dimensions than stage IA2 whether seen clinically or not.

Stage II: The carcinoma extends beyond the cervix, but has not exceeded on to the pelvic wall. The carcinoma involves the vagina but not the lower third.

Stage IIA: No obvious parametrial involvement.

Stage III: The carcinoma has extended to the pelvic wall. On rectal examination there is no cancer free space between the tumour and the pelvic wall. The tumour involves the lower third of the vagina. All cases with hydrenephrosis or nonfunctioning kidney should be included unless of other cause.

Stage IIIA: Extension to the lower third of the vagina. No extension on to the pelvic wall.

Stage IIIB: Extension on to the pelvic wall or causes hydrenephrosis or nonfunctioning kidney.
Stage IV: The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder or rectum.

Stage IV A: Spread of the growth to adjacent organs.

Stage IV B: Spread to the distant organs.

DIAGNOSIS AND PROGNOSIS

Diagnosis of cancer of cervix is done by cytological study, colposcope, biopsy, cervical conization, multiple punch biopsy, urettage, radiology, cytoscopy, rectosigmoidoscopy surgical staging etc. Of the above methods exfoliate cytology is comparatively very simple and sensitive method. Periodic cytological screening with the Pap smear is thought to be essential for preventing the development of invasive cervical cancer. Pap smears are performed at the time of routine pelvic examination. Screening with Pap smears has been reported to have reduced mortality from cervical cancer (Brinton et al., 1987, Boring et al., 1994; Koss, 1993; Mark et al., 1997).

Though diagnosis of cervical cancer through cytological screening has been in operation now for more than thirty years in U.S.A., the same has not been undertaken uniformly in other countries of the world in general and in developing countries in particular (Lunt, 1984). In India screening for cervical cancer is restricted to some specific centres located at larger urban areas only.
As per the recommendations of the American Cancer society, asymptomatic women of 20 years of age and older and sexually active women under 20 years of age should have cytological screening for 2 consecutive years and at least one screening every 3 years until age 65. They further recommended that women who are at high risk for developing cervical carcinoma because of early age at time of coitus, multiple sexual partner and multiparity should have a yearly cytological screening. In 1988, the American College of Obstetricians and Gynaecologists and the American Cancer Society have recommended that "All women who are or have been, sexually active, or have reached the age of 18 years, should have an annual Pap test and pelvic examination. After a woman has had three or more consecutive satisfactory normal annual examinations, the Pap test may be performed less frequently at the discretion of her physician".

In late 18th century and early 19th century, cytology and its significance in the prognosis of cervical cancer have been established. In 1838, Donne & Mueller made their earliest studies on microscopic examination of exfoliate cells. Donne described the examination of fresh smear prepared from human colostrum. Mueller described in detail the appearance of cancer cells. But it was only in 1943 when Papanicolaou's famous monograph "The diagnosis of uterine cancer by exfoliate cytology" ushered in the modern era of cytological diagnosis. It was only 6 years after the publication of this monograph, Papanicolaou's method received general
acceptance as a screening and diagnostic technique. Ayre (1947) also reported the reliability in the diagnosis of cancer by this method.

The prognosis of the cervical cancer is reported to be influenced not only by FIGO staging but also by a number of tumour characteristics like clinical tumour diameter, lymph node metastasis, lymph-vascular space invasion, deep stromal invasion, parametrial extension, strong inflammatory response, uterine body involvement, histologic grade, hemoglobin level, squamous cell carcinoma antigen, patient age, platelet count, tumour vascularity, DNA ploidy and HPV infection. According to Abu-Ghazalah (1984) the prognoses of carcinoma of cervix do not seem to be influenced by peritonial cytology status when other poor prognostic factors were considered. Peters et al. (1985) used the micronucleus assay and flow cytometric analysis, tumour cell proliferation kinetics and ploidy to illustrate potential methods of selecting patients for fast neutron radiotherapy. Nakano et al. (1989) studied the prognostic significance of Langerhans Cell (LC) infiltration in cancer nests in 391 patients with squamous cell carcinoma. Their results suggested that LCs in cancer nests may play a significant role in the immunologic defense against cancer in advanced stage of the cancer.

Zhang (1993) studied adenocarcinoma of the uterine cervix in 121 cases. Their findings revealed that the prognosis for adenocarcinoma of the cervix is closely related to the poor sensitivity to radiotherapy, clinical staging and tumour size.
Begum (1993) undertook a cytopathological and immunohistochemical study for estimating radiotherapeutie effects in uterine cervical cancer. From her findings it is concluded that Brd U L. I., revealed by immunohistochemical study, is the most suitable indicator to estimate the response of radiation in the cancer cells of uterine cervix and that C-myc oncogene product has the potentiality to be used as a prognostic factor in uterine cervical cancer treated by radiation therapy.

Stock et al. (1994) evaluated and compared the histopathological grading systems of epithelial carcinoma of the uterine cervix through gynecologic oncology group studies. According to these investigators, histologic grade, irrespective of the pathologists making the diagnosis, had no correlation to prognosis.

Rutgers et al. (1995) studied the angiogenesis in uterine cervical squamous cell carcinoma. Their study tested the hypothesis that increased angiogenesis in squamous cell carcinoma is an indicator of poor prognosis. But they found no correlation between the mean vessel count and stage or between mean vessel count and disease status on an average follow up of 21 months.

Lehuncher-Michel et al. (1997) conducted an investigation on the effect of smoking on the micronucleated epithelial cells in the smears from uterine cervix. They did not notice any association between number of cigarretes smoked and
micronucleated cell levels. Their results also suggested that consuming 5-20 cigarettes per day was not enough to show a smoking effect on cervical micronucleated cells.

TREATMENT

Cancer of cervix can be treated by either surgery or radiotherapy or combination of both. The primary method of therapy for preinvasive (Stage O) and microinvasive (Stage IA1 and IA2) carcinoma of cervix is surgery. Radiation therapy is rarely used in these stages except where there is contraindication to surgery Stages IB and IIA carcinoma of cervix can be managed equally effectively by either radical surgery or irradiation therapy. In stages IIB, III and IV, radiation therapy is only method of choice.

The treatment of cervical cancer more advanced than stage IIA is irradiation. In stage IIB the patients are treated with irradiation, but a conservative hysterectomy is performed after high pre-operative irradiation in patients with a barrel shaped cervix and limited parametrial infiltration that regress completely in 4 to 6 weeks after completion of irradiation. In stage III, the treatment is external radiotherapy.

Stage I and stage IIA small volume tumours are treated by brachy therapy alone with 3 intracavitary treatments at weekly intervals to a total tumour dose of 8000 cGY.
Stage IB and IIA bulky tumours are treated by giving a dose of 4000 - 5000 cGy by external beam therapy in 20-25 fractions over 4 to 5 weeks and this is followed by a single caesium insertion of 2500 cGy or 3000 cGy to point A depending on external beam dose. Some radiotherapists use central lead shielding during external beam therapy in the early disease. They give 8000 cGy to point A with three caesium insertions followed by 4000 - 4500 cGy to the pelvis with opposing fields and central lead shield.

Patients with Stage IIB, IIIA -B, and IVA are treated with a dose of 5000 cGy given to the whole pelvis in 5 weeks in 25 fractions. If bulky disease is present in one parametrium, a further 500 -1000 cGy may be given in three to five fractions to this area. Following this, a caesium insertion is performed and a further 2500 cGy is given to point A. If an insertion is not possible, the central disease may be taken to a dose of 6000 - 7000 cGy using external beam therapy alone.

Patients with Stage IVB are subjected to palliative treatment by opposed field giving 3000 cGy midline dose in six treatments over 3 weeks.

There are two recent techniques in radiation therapy for cervical cancer i.e., (1) interstitial brachytherapy and (2) altered fractionation schedule. Interstitial irradiation allows delivery of locally high dose of radiation with rapid fall off in
surrounding tissue. External beam radiation therapy has been administered with close of 180 to 200 cGy per traction, 5 days per week.

POST-RADIATION CYTOLOGY:

Many early investigators have studied the effect of radiation on malignant cells by serial biopsies. They observed marked swelling of cells, vacuolization of the malignant cells, pyknosis of the nuclei, decrease in number of mitosis, increase in the number of atypical mitosis, increase in atypical mitosis with no normal mitotic figures, a rise in the normal division of cells and complete disappearance of malignant cells from eleventh to fortieth day of the treatment.

Graham (1947) made a detailed study of normal vaginal cells and the changes seen during radiation in normal and malignant cells. She observed that there were more number of leucocyte, clumping of leucocytes in tight groups and polymorphonuclear leucocytes, lymphocytes, histocytes, foreign body giant cells in the vaginal secretion in cancer cases. Graham also divided smears into four groups i.e., (1) smears during X-rays and radium therapy, (2) follow up from 1 to 6 months after treatment, (3) follow up after 6 months to 1 year and (4) after one year to 15 years.

When normal vagina is exposed to radiation, changes are seen at 3 days over basal cells. The radiation changes of the normal vagina are (1) change in
staining of basal cells from basophilic to brownish (early sign of degeneration) seen from 2nd to 10th days, (2) karyorrhexis of the nucleus with increase in its size and pycnosis from 12th day, (3) great increase in size of basal layer cells, from 12th day, (4) abnormal vacuolization of cells of basal layer from 15th day, (5) appearance of basal cells in different shapes like dumb bell, elongated and tadpole forms and (6) multinucleation of cells.

Radiation changes in malignant cells occur from 11th day of treatment. These changes include (1) increase in size of malignant cells to four times, (2) cytoplasm vacuolation (3) multinucleation, (4) appearance of giant cells with 6-7 nuclei, (5) nuclear pycnosis, (6) absence of nucleolus. Twenty four days after treatment, the malignant cells are found to disappear.

One to six months after radiation treatment, two types of changes are seen i.e., (1) moderate - half the epithelial cells showed radiation change and the malignant cells disappear, and (2) marked changes - all cells showing radiation changes. After a duration of 6 months to one year of treatment there are evidences of no reaction, evidences of foreign body, giant cell formation, small histocytes and leucocytes and evidences of malignant cells.

One to fifteen years after radiation, one-third cases show basal cells degenerate, aberrant or in clumps, one third cases show precorrified basal cells,
normal or degenerate and the other one third with cornified, precornified and basal cells with leucocytes and histiocytes, or no evidence of any foreign body giant cells.

Graham & Graham (1953) subsequently carried out study with pretreatment vaginal smears in patients with carcinoma cervix and found basal cells counts associated with particular changes and prognostic significance. They found that some patients with cancer cervix have basal cells with dense basophilic, finely vacuolated cytoplasm in the vaginal smear before any treatment. When 10% or more of the desquamated nonmalignant and epithelial cells are of this type, the patient and the cancer usually respond favourably to radiotherapy. If less than 10%, the cancer responds poorly to radiation therapy.

Graham (1957) also found an association of histiocyte count and the prognosis in the cancer cervix patient. If small histiocyte count was more than 50%, favourable response was seen in patient treated with radiation therapy than in those who have small histiocyte count less than 50%.

Maloney (1950) studied radiation changes in normal vaginal cellular elements and in the malignant cells. When the changes were marked, the smears usually cleared of malignant cells early in treatment, and the response was judged to be good. While little radiation effect was noted and the smears remain positive, the response was poor. A relationship in clinical course and prognosis was found in
patients in those smears cleared of malignant cells before the 20th high voltage treatment and remained positive.

Graham (1957) recorded percentage as zero if no malignant cells were found on extensive search either healthy or irradiated. And if it was difficult to find any normal cells not showing advanced radiation changes the percentage was 100. If radiation response is more than 60%, there is good prognosis and if less than 60%, there is poor prognosis.

Glucksman & Stanley (1948) devised a system of weekly biopsies from the growing edge of tumour after radium application (given in three sitting one week apart) and they noted that the response was good when the differentiating cells and degenerating cells increased by second week of treatment and poor when the viable types of tumour cells, resting and mitotic, persist. Also according to them, tumours of high differentiation are more radiocurable than those that are anaplastic.

Gusberg et al. (1954) enlisted various factors responsible for radiation resistance. These factors are different radiation techniques, tumour differentiation, type of tumour (exophytic & endophytic ulcerative), quality of tumour bed or strand, the general health, habits, age and endocrine status, local cauterization effects of radium, variation in sensitivity of tumour cells and effect of over radiation.
According to Jones & Davis (1959) the susceptibility of a patient and of her tumour to radiation should be assessed to ascertain the best therapeutic modality. According to Davis et al., (1960), the exposure of a cell population to appropriate radiation produces a variety of well known mitotic derangements like mitotic arrest, aberrant mitosis and mitotic delay and the radiation damaged cells develop macrocytosis and multinucleation. Merril (1958) advocated that cytological prognosis in cancer depends on many extrinsic factors like age, hormones, trauma and inflammation that modify cell population. They also found that radiation response is more frequent in post menopausal than in premenopausal women and increases with advancing age. Graham & Graham (1953) reported that the fewer the malignant cells found in vaginal cytology, the greater the possibility of survival, if the treatment were surgical and the reverse is true in the patients treated by radiation. It has also been reported that there were numerous basal and parabasal cells in the smears of women near or after menopause, protoplasmic vacuolisation or tinctorial changes in women of advanced age, regardless of whether or not the women had cancer of cervix and there was no correlation between SR and prognosis. According to Shier (1954), persistence of malignant cells in smears beyond the third week of external pelvis therapy carries a poor prognosis.

There were published evidence that cervical carcinoma with large nuclei have a significantly better prognosis following radiotherapy than tumour with
small nuclei. Some early reports suggested adenocarcinoma of cervix was as likely to respond to radiotherapy as squamous cell carcinoma (Gusberg & Corscaden, 1951), but more recent investigations indicate that adenocarcinoma with or without a squamous component is less likely to respond to this form of therapy (Kogan et al., 1973). Patients in whom tumour regressed completely by the end of external radiation were reported to show an excellent prognosis.

Rayburn & van Nagell (1980) have studied the value of cervicovaginal cytologic screening in patients with biopsy proved recurrences. They found that fifty six of 110 patients with a known recurrence had changes on a Papanicolaous smear consistent with suspected or definite malignant cells. According to them screening by frequent clinical examination and sampling of exfoliative carcinovaginal cells remain paramount after treatment of carcinoma of the cervix uteri. While studying one hundred sixty-seven patients with clinical Stage 1 carcinoma of the endometrium, Creasman et al., (1981) have emphasised the importance of peritoneal cytologic examination in the prognosis of endometrial cancer. Gupta et al. (1982) observed radiation changes in all postirradiated smears of squamous cell carcinoma of cervix from 56 females, although to a variable degree. According to them a high classification index is a good guide for recurrence and the presence of malignant cells at any stage was of great significance indicating poor radiation response or recurrence.
On examining the vaginal smear of specimens of the patients who received operative therapy, irradiation or chemotherapy, Shibata (1982) has suggested the effectiveness of long-term follow-up vaginal cytology following treatment of cervical carcinoma and usefulness of serial cytology in evaluation of the effects of radiation and chemotherapy for cervical carcinoma.

Gupta et al. (1987) had undertaken a study of acute/immediate radiation changes in 2020 sequential vaginal smears in 101 patients of carcinoma of the cervix uteri. They calculated the percentage of cancer cells in pretreatment vaginal smears and studied the radiation changes in benign and malignant cells, such as cell size, vacuolation of cytoplasm, multinucleation and nuclear changes. They observed a gradual and linear decline in cancer cells until the end of the therapy.

Hopkins et al. (1990) have observed that radiation therapy in the immediate post operative period produced a survival of 88% among the ninety-two patients with invasive cervical cancer initially treated by hysterectomy. According to their findings, survival was significantly influenced by tumour grade and the amount of post-operative radiation therapy, while age, amount of residual tumour, and presence of tumour at surgical margin did not influence survival.
Hiura et al., (1990) examined the effect of radiotherapy on 136 patients with cervical carcinoma (Stages I-IV) by cytology and biopsies obtained by colposcopy. They advocated that effective serial plasma determinations of CEA, SCC and TPA in patients with cervical carcinoma following therapy may often be useful in the evaluation of therapy as well as in the earlier detection of recurrent disease.

Santos et al. (1989) found evidences of Chlamydia infection in a series cervicovaginal smears of women with uterine carcinoma treated with radiotherapy with the Papanicolaou stain. The presence of cells with radiotherapeutic changes infected by Chlamydia is a new finding in cytology. According to Sutton (1989) a prospective randomized study of radioactive chromic phosphate, whole abdomen radiotherapy, or adjunctive chemotherapy versus no treatment in patients with malignant peritoneal cytology is clearly needed. With the help of cervical cytology Whitekar et al. (1990) could detect recurrent squamous carcinoma post radiotherapy. They opined that in experienced hands routine smear cytology post radiotherapy is a reliable and useful addition to surveillance. Marrow et al. (1991) have also used cervical cytology to find out relationship between surgical pathological risk factors and outcome in clinical stage I and II carcinoma of the endometrium.

Shield et al. (1991) studied the accuracy of cervicovaginal cytology in the detection of recurrent cervical carcinoma following radiotherapy. They found
a cytologic diagnosis of recurrent carcinoma for 48.9% (23/47) of cases with local recurrence. Their study also revealed that positive predictive value for a histologic diagnosis of recurrent cervical carcinoma after a positive cytology report for a group of 61 patients was 98.4%. They reported that a cytologic diagnosis of locally recurrent carcinoma preceded clinical signs in 15/61 (24.6%) of cases. The results of their study indicate that although cervicovaginal cytology after radiotherapy for cervical cancer does not have high sensitivity, it is reliable test for the early diagnosis of local recurrence prior to onset of clinical signs.

Gibbons et al. (1991) studied cases of fifty six patients with surgical stage III or IV endometrial carcinoma or earlier stage disease with two or more risk factors for peritoneal recurrence who were given post operative whole abdomen in pelvic irradiation (WAPI) with nodal and vaginal boosts. Using multivariate analysis, they demonstrated that only surgical stage and histologic grade had prognosis significance for survival and disease free survival. Their study suggests that post operative WAPI is a safe and efficacious treatment alternative for patients with surgical stage I through III high risk endometrial carcinoma.

Shield et al. (1992) consider cervicovaginal cytology as a valuable tool for the detection of locally recurrent cervical cancer despite the difficulties in collection of representative samples, benign radiation changes, post-irradiation
dysplasia and the frequent occurrence of repair cells and active stromal cells in post-irradiation smears causing diagnostic problems. According to them awareness of the cellular changes resulting from irradiation, and the varied composition of post irradiation smears may lead to more accurate interpretation of the cytological findings. Kristiensen et al. (1992) reported the cytology, histology and electron microscopy of the malignant melanoma of the cervix in a patient presenting with postmenopausal bleeding.

Zhang (1993) has analysed 121 patients with adenocarcinoma of the uterine cervix of which ninety-eight patients were treated by radiation alone. He observed a local recurrence rate of 38.8% after radiation in 98 cases. According to him, the prognosis for adenocarcinoma of the cervix is closely related to the poor sensitivity to radiotherapy, clinical staging and tumour size.

In order to estimate the effects of radiation on cancer tissue, Begum (1993) examined the cytopathological findings, Brdu labelling index, tumour markers and C-myc oncogene products in 30 patients of uterine cervical cancer before radiation, at around 10 Gy, 30 Gy, 50 Gy and at the end of the therapy. In the cytology, she found that there were radiation effects, such as the enlargement and vacuolation of the nuclei and cytoplasm at around 30 Gy of radiation.
Angeles & Saigo (1994) evaluated the cytologic components in vaginal smears of 23 women with rectovaginal fistulae. They could document rectovaginal fistulae in vaginal cytology in 65% of patients with the condition.

Since invasive cervical cancer is the most common malignancy among Indian women and since it is one of the more curable human malignancies, it needs early diagnosis. For control of cancer cervix, population screening is highly essential and Papsmear cytological screening is a well proven strategy. The present study aims at testing the reliability of Papsmear technique in the diagnosis of cervical cancer and in studying the cytological changes in the malignant cells during the course of radiotherapy in some patients attending AHRCC, Cuttack (Orissa).

CYTOGENETICS

A variety of chromosomal aberration has been observed in the neoplastic cells. As such aberrations often occur in combination, it becomes very difficult to correlate a particular type of aberration to a particular cancer. Again unless the chromosomes are obtained from tumour cells, they can not be considered relevant to the malignant disease.

The first consistent chromosome abnormality in any cancer was Philadelphia or Ph’ chromosome identified in chronic myeloid leukaemia (CML). This aberration is a translocation involving chromosome No. 9 and 22 : [t (9 : 22)
Over the past few years, it is reported that specific acquired clonal chromosomal aberrations are also associated with other malignant myeloid diseases, malignant lymphoproliferative diseases, acute lymphocytic leukaemia, solid tumours, malignant tumours, embryonic tumours and germ cell tumours. (Le Beau, 1986; Sandberg, 1990; Tycko & Sklar, 1990; Mandahl, 1991; Mitelman et al., 1993; Mitelman, 1994; Rabbits, 1994; Le Beau & Larson, 1994; Heim & Mitelman, 1995 a, b). For all these diseases, chromosomal preparations have been obtained from tumour cells. But there are also reports of occurrence of spontaneous chromosomal aberrations in leucocytes or other tissues of patients suffering from different types of cancer. For example, high frequency of spontaneous chromosomal aberrations have been obtained from the leucocytes of patients with skin cancer (Taylor et al., 1973), thyroid carcinoma (Hsu et al., 1981; Pathak et al., 1982), renal cancer (Wanz et al., 1982) and precancerous and cancerous lesions of uterine cervix (Mitra et al., 1982, 1983, 1986).

Although carcinomas account for the greatest proportion of malignant disease, they represent only about 20% of karyotypic data, whereas most information is available for leukaemia and lymphoma. It has become clear from the beginning of the cytogenetic analysis of human malignant disease, that virtually all solid tumours including the non-Hodgkins lymphomas, had an abnormal karyotype and that some of these abnormalities were limited to a given tumour.
Chromosomal abnormalities have been observed in the leucocyte cultures of women with precancerous and cancerous lesions (Mitra et al., 1982, 1983, 1986). Mitra et al. (1986) have studied spontaneous chromosomal aberrations in 89 women with precancerous lesions and 53 woman with cancerous lesions of uterine cervix along with 87 control women. They found that the frequency of metaphases with aberrations including both gaps and breaks were found to be 6-39, 10-41, and 17-24 per cent in controls, precancerous group and cancerous group respectively. Their data indicate the existence of the chromosomal instability in the majority of the cervical cancer patients and in some women with precancerous lesions. According to them if chromosomal instability is presumed to be one of the contributing factors for developing malignancy, the precancerous cases with higher frequency of chromosomal aberrations may have higher risk for developing cancer.

Archimbaud et al. (1987) have reported the case of a 70 years old women in whom Philadelphia chromosome positive acute leukaemia occurred 12 years after radiation therapy for a carcinoma of cervix. Leonard et al. (1987) examined the peripheral blood lymphocytes of patients undergoing radiation therapy for pelvic tumours for dicentric and centric ring chromosome. They reported that the dose inducing ten dicentrics or rings is 5-62 Gy at the target volume and thus, is intermediate between the doses at the target volumes displaying the same effects in patients treated for mammary carcinoma (15 Gy) or for ankylosing spondylitis (2 Gy).
Ghosh & Ghosh (1988) have reported that at 37°C the mean frequency of SCE was found to be $8.26 \pm 1.91$ in untreated patients with cervical cancer and $7.91 \pm 1.68$ in cancer patients treated with radiotherapy and these values were significantly higher than the control value of $5.34 \pm 1.28$ exchanges.

Kleinerman et al. (1989) examined approximately 200 metaphases from each of 96 irradiated and 26 non-irradiated cervical cancer patients treated more than 17 years ago to evaluate the extent of residual chromosome damage in circulating T-cell lymphocytes. They observed that unstable aberrations did not differ significantly between irradiated and non-irradiated patients and however, stable aberrations (i.e. translocation, inversions, or chromosomes with deleted segments) were significantly higher among irradiated compared to non-irradiated women. They also found a significant increase in frequency of these stable aberrations with increasing dose to the bone marrow. According to them these data are indicative of a direct relationship between radiation dose and extent of damage to somatic cells persisting in populations and can be detected many years after partial body radiation exposure. They also observed that a very high dose delivered to the pelviscavity in fractionated doses resulted in far fewer persistent stable aberrations than lower doses delivered either in acute whole body exposure or in fractionated doses to the spinal column and sacroiliac joints.
Kao *et al.* (1990) reported a trisomy 4 in a 61 year old woman who developed leukaemia 4½ years after receiving radiation therapy for uterine carcinoma. Ammenheuser *et al.* (1991) observed a significant increase in frequencies of dicentric chromosomes in lymphocytes after 1 week of radiotherapy which continued to increase during therapy and remain elevated during treatment. To examine whether chromosome aberration rates in lymphocytes from women irradiated for benign and malignant gynaecological disease many years after exposure might serve as population markers of cancer risk, Kleinerman *et al.* (1994) collected blood samples from 60 woman treated for BGD (34 with radiation), and they compared cytogenetic data with previous results from 96 women irradiated for cervical cancer. They found that remarkably, the rate of stable aberrations which reflects nonlethal damage in surviving stem cells, was only slightly higher among the cancer patients. They also observed that the lower dose regimens to treat benign disorders resulted in much higher aberration yields per unit dose than those for cervical cancer. From these observations, they concluded that for patient populations given partial body radiotherapy, stable aberrations at a long time after exposure appear to serve as biomarkers of effective risk rather than as biomarkers of radiation dose received.

The above cytogenetic studies in cervical carcinoma are mostly restricted either to the treatment period or to post-radiation follow up period. Pre-
radiation chromosomal studies in cancer cervix cases till date reveal that the malignant cells of this cancer have typical complex karyotypes.

Carcinoma cervix cell lines were reported to exhibit translocation, loss of chromosomes and allelic deletions.

Chung et al. (1992) suggested that the deletion events occurring on the short arm of chromosome 3 at 3,25 and 3,14 are the early events in the development of carcinoma of cervix. Mitra et al. (1994) reported loss of heterozygosity at sites of 11 chromosomal arms (1q, 3p, 3q, 4q, 5p, 5q, 6p, 10q, 11p, 18p and Xq) through an allotype analysis of cervical carcinoma. Loss of heterozygosity in 6 chromosomal regions (3p, 14, 1-12, 11q, 23.3, 6p, 22-21.3, 19q, 13.4, 6q, 21-23.33 and 2q, 33-37) was reported by Rader et al. (1996). According to these investigators loss of specific chromosomal regions in a significant number of invasive cervical cancers suggests the elimination of genes involved in the cell cycle regulation or the suppression of tumour development. Of the chromosomal arms so far identified for loss of heterozygosity, regions on 3p, 4p, 4q and 11q have been validated extensively for harbouring tumour suppressor genes (Larson et al., 1997 a).

Choo et al. (1995) have reported that the loss of one of the two altered jun-B alleles was a consequence of a chromosomal translocation involving
Chromosome 19 and chromosome 16 in a cervical cancer cell line (CC 7T). Zimonjic et al. (1995) have found translocations of chromosome 1, involving a specific site on the short arm and partial a complete loss of the short arm of chromosome 9, as well as loss of chromosome 13 in two HPV-negative cervical carcinoma cell lines (C-33A and HT3). In addition, they observed involvement of chromosome 1 in translocations with chromosomes 9, 18 and 21 in the C-33A cell line and complex rearrangements and deletions of chromosomes 1p, 3p, 9p, 10q/p, 11p/q and 17p in HT-3 cells.

Southern & Herrington (1997) reported numerical differences between chromosomes in 76% of the studied cases with underrepresentation of chromosomes 11 and/or 17 relative to X in 64% cases. Their findings suggest that relative reduction in the number of chromosomes 11 and 17 is important in the development of invasive cervical neoplasia and are consistent with the putative presence of relevant tumour-suppressor genes on their chromosomes.

As a considerable degree of chromosomal instability is found to be associated with many types of cancer including cancer cervix uteri, such high frequency chromosomal instability can be used as an indicator for diagnosis, prognosis and curability of cancer cervix. One of the aims of the present piece of investigation is to record the frequency of different types of chromosomal aberrations obtained from chromosomal spreads prepared from bone narrow cells of patients with
cervical cancer and to study the possibility of use of chromosomal parameters in the assessment of curability, prognosis and recurrence of cancer cervix.

IMMUNOLOGY

A host of disorders affecting all organs systems are clearly attributable to immunological reactions to exogenous agents or to the abnormal emergence of immunity against one's own tissue and cells. The humoral basis of immunity was established towards the end of 19th century. Tiselius (1937) separated serum proteins into albumin, alpha, beta and gamma globulins based on their electrophoretic mobilities. It was resolved in 1964, that the generic term 'immunoglobulin' be used and the term was internationally accepted for proteins of animal origin with known antibody activity and for certain other proteins related to them by chemical structure. The symbols are used 'γ' for gamma globulin and 'Ig' for immunoglobulin. Immunoglobulins are synthesised by differentiated 'B' cells (plasma cells and to some extent by lymphocytes also). All antibodies are immunoglobulins, but all immunoglobulins may not be antibodies. The immunoglobulins are classified into various classes i.e., IgG, IgA, IgM, IgD and IgE.

Immunoglobulins may be quantified by several immunochemical techniques such as:

1. Quadin's single diffusion method;
2. Quchterlony's double diffusion method;
3. Immunoprecipitation of Miede burgors method;
4. Immunoprecipitation of photometric assay method;
5. Single radial immunodiffusion of Mancipi method;

Frequency of neoplasm is far greater than would be expected from clinically detectable cancer. Thus it seems likely that many transformed cells do not progress to become life threatening cancers and, therefore, must have been destroyed by the body early after their transformation. Theories of immunosurveillance suggest that cancer cells can be perceived as foreign and be subsequently destroyed by cytotoxic immune mechanisms. One function of the immune system is to continually "Survey" the body for such malignant transformations. The immune system responds to cancer cells displaying new or foreign tumour antigens with specific T or B cell responses.

The escape mechanism that different tumours have developed to circumvent specific immune mechanisms include: (1) "sneaking through", (2) modulating tumour Ags, (3) masking tumour Ags, (4) inducing tolerance, (5) producing blocking Ags, and (6) producing or expressing immunosuppressants.

Immune diagnosis of tumour can be used to either detect tumour markers or evaluate the antitumour response of the host. Tumour markers or
antibodies to oncofetal proteins like AFP and CEA can be used to detect the presence of cancer in the host.

Detection of a tumour-specific immune response can also be important as a means of demonstrating the presence of a tumour in the host. Humoral responses can be assessed by testing for the presence of certain antibodies in serum which are diagnostic for certain tumours. Cell mediated responses can be measured after Ag stimulation of host T cells.

Tumour markers and antitumour immune responses have proved useful in evaluating the progression or regression of the disease, a patient's responses to therapy, and in determining the recurrence of the disease. For example, it can be used to monitor AFP and CEA levels in patients with certain cancers following primary tumour removal. A rise in the levels of the oncofetal Ags postsurgery usually indicates a relapse, whereas low serum levels indicate continued remission.

It was first observed that the host might exert some form of specific immunological control. This initial observation formed a groundwork for future immunobiologists. Then Brent et al. (1958) noticed that guinea pigs which were actively rejecting foreign grafts showed a delayed hypersensitive reaction to a cellular extract prepared from the graft cells. Hughes & Lytton (1964) observed that
27% of their patients showed a positive delayed hypersensitive reaction to a cellular extract made out of the malignant tumour tissue obtained at the time of operation and injected thereafter.

Burnet (1970) pointed out that the immune system constantly and consistently surveys the tissue cells of individuals and eliminates any mutant cell or neoplastic cell in the process of such vigilance. It has been suggested that isoantigenic modification on the surface of cancer cells causes the appearance of blocking antibody which crossreacts with the antigen on the surface of responding and/or target cells. It is also suggested that the B cellarch is directed towards the production of antibodies in response to foreign antigen (Sengupta et al., 1978). According to Adler et al. (1980) and several other workers it is presumed that the influence of patient’s immune capacity on the mode of evaluation of the neoplastic diseases is likely to be reflected in terms of prognosis.

As against cell mediated immune system, the humoral immune system plays almost negligible role in cancer evolution. However reports of Keller (1976) and Sengupta et al. (1978) reveal a B cell hyperplasia in carcinoma oesophagus, head and neck cancers and lymphomas.
It is observed from the vast body of literature that immune system plays an important role in the evolution of cancerous process. Cell mediated immune system is evidently responsible for the immune surveillance for the cancer development.

Extensive studies on the immune status of the patients suffering from different neoplasms were done by several workers using variety of assay systems (Krant et al., 1968; Eilber & Morton, 1970; Gorrioch et al., 1970; Ducos et al., 1970; Whittaker et al., 1971; Catalona et al., 1974; Gross et al., 1975). These investigators have shown a depression of immune system of cancer patients to a variety of antigenic stimuli.

Importance of humoral immune status in patients with breast cancer has been well documented (Wang et al., 1977; 1979; Howard & Taylor, 1979; Hsu et al., 1981; Strender et al., 1981; Nayak, 1987).

Weintraub et al., (1973) have demonstrated tumour specific antibodies in patients with carcinoma of cervix. Dorsett et al. (1975) have shown evidence of a tumour specific antibody response in human gynaecologic malignancies. While Gupta et al., (1981) reported increased IgA and IgM levels in patients with cervical cancer, Plesnicar (1972) reported increased IgM levels in such patients.
Significant role of host immunity in the biologic behaviour of carcinoma of cervix has been emphasized by a host of investigators (Gatti & Good, 1971; Krueger et al., 1985). Many investigators have demonstrated a depression of cell mediated immune system and elevation of humoral immune system with increased immunoglobulin levels in cervical cancer patients (Chiang et al., 1976; Lewis, 1972; Ito et al., 1976). It is also believed that the increased levels of IgG and IgA might be due to severe infections common to cancer cervix patients (Chiang et al., 1976).

Onsrud (1982) observed reduced generation of suppressor T cells in human mixed lymphocyte culture after radiotherapy in 11 patients with endometrial cancer. The investigations of Nakano et al., (1989) suggest that Langerhan cells in cancer nests may play a significant role in the immunologic defence against cancer in advanced stage of cervical cancer. In order to evaluate the clinical significance of multiple tumour markers, Hiura et al., (1990) measured plasma levels of carcinoembryonic antigen (CEA), squamous cell carcinoma related antigen (SCC), tissue polypeptide antigen (TPP) and immunosuppressive acidic protein (IAP) before and after treatment in 136 patients with invasive cervical carcinoma (Stages 1 - IV). They found that for CEA, SCC and TPA, there was a significant reduction in values between the pretreatment and post treatment periods, but plasma IAP was transiently increased after operation. The authors emphasized the importance of effective serial
plasma determinations of CEA, SCC and TPA in patients with cervical carcinoma following therapy in the evaluation of therapy and early detection of recurrence.

Chander et al. (1987) observed lower IgG and IgM levels and an insignificant rise in IgA levels in 47 patients with cervical cancer. They postulated 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 the antibody response was also reported by Hencock et al. (1984).

Begum (1993) undertook a cytopathological and immunohistochemical study in 30 patients for estimating radiotherapeutic effects in uterine cervical cancer. They examined the tumour markers in these patients before radiation at around 10 Gy 30 Gy, 50 Gy and at the end of the therapy. They found the location of SCC and SLX in the cytoplasm.

Of late, there were quite a few immunohistochemical studies in carcinoma of cervix and other cervical lesions.

Raju (1994) examined the expression of the proliferating cell nuclear antigen (PCNA) to determine the proliferative activity of the cells in non neoplastic
and neoplastic lesions of the uterine ectocervix. They found significantly higher percentage of PCNA-positive cells in premalignant and malignant lesions of the uterine cervix than in non neoplastic lesions. Their immunohistochemical study suggested that the cell proliferation index obtained by using PCNA may be useful adjunct to histological diagnosis of various grades of dysplasia.

Harmsel et al. (1995) immunohistochemically showed that p53 accumulation in premalignant cervical lesions is almost identical to the low levels detected in normal endo- and ectocervical epithelia reserve cells, immature and mature squamous metaplastic epithelium. They also observed that p53 levels were low and seem to be independent of the grade in cervical intraepithelial neoplasia (CIN) while carcinomas of cervix contained high levels of immunohistochemically detectable p53. These studies also demonstrated that alterations in p53 levels and presence of HPV 16 are not mutually exclusive markers of cervical tumorigenesis and hence p53 expression is probably an inadequate prognosticator for estimating progression or regression of CIN lesions.

The study of Mc Cluggage (1995) indicated that immunohistochemical staining with K-67 and MIBI may be of use in the often difficult histological distinction of tuboendometrial metaplasia from malignant endocervical glandular lesions. Komatsu et al. (1996) demonstrated M-proteinemia at the position of beta-
globulin with a high level of IgG and low levels of IgA and IgM in the blood of a 77-year-old man with cervical Castleman’s disease. The investigators speculated that the complication of benign M-proteinemia in this patient was not incidental, but caused by an underlying immunological abnormality of the B cells. While studying the immune activation in cervical neoplasia, Hildesheim et al. (1997) observed a cross-sectional association between plasma soluble interleukin 2 receptor levels and disease.

The present investigation deals with the study of pre and post radiation levels of immunoglobulin (IgG, IgM and IgA) in 50 patients with cervical cancer (stages III & IV) to assess the importance of these parameters in relation to prognosis, curability and recurrence of the disease.