Locally advanced cervical cancer – neoadjuvant chemotherapy followed by concurrent chemoradiation and targeted therapy as maintenance: A phase II study

ABSTRACT
Aim: The survival in locally advanced cervical cancer remains low. We evaluated the role of neoadjuvant chemotherapy (NACT), chemoradiotherapy (CRT), followed by gefitinib maintenance in locally advanced cervical cancer.

Materials and Methods: Twenty-five patients with locally advanced carcinoma cervix were enrolled between July 2012 and May 2013. Patients received 6 weekly doses of NACT Paclitaxel (60 mg/m²) and carboplatin (AUC 2), followed by CRT and brachytherapy. The analysis of epidermal growth factor receptor (EGFR) expression was carried out by immunohistochemistry. Gefitinib (250 mg daily) was given as maintenance therapy for 1 year after completion of chemoradiation. Comparison of EGFR expression and survival outcomes was done.

Results: Twenty-four of 25 patients completed the neoadjuvant chemotherapy and concurrent chemoradiotherapy. Post-CRT, all patients were started on gefitinib maintenance, and twenty patients completed the intended 1 year of gefitinib maintenance. Nineteen (76%) patients had a radiological complete response to NACT. EGFR was moderately or strongly expressed in 86.3% of the patients. The 3-year overall survival was 69.8%, and 3-year progression-free survival was 51.4%. Expression of EGFR was not found to be a significant factor affecting overall survival or progression-free survival.

Conclusions: Weekly neoadjuvant chemotherapy is associated with a good response rate in locally advanced cervical cancer. Neoadjuvant chemotherapy, chemoradiation, followed by gefitinib maintenance gives good survival outcome in patients with locally advanced cervical cancer.

KEY WORDS: Chemoradiotherapy, epidermal growth factor, induction chemotherapy, molecular targeted therapy, receptor, uterine cervical neoplasms

INTRODUCTION
Concurrent chemoradiation is the standard treatment for advanced cervical cancer.[1] Despite the advances in treatment and practice of concurrent chemoradiotherapy (CRT), the 5-year survival rate remains low; stage IIB 50%–60% and stage IIIB 30%–40%. Neoadjuvant chemotherapy (NACT) before concurrent chemoradiation (CRT) is an attractive approach.[2] The possible advantages include a reduction in primary tumor volume and control of micrometastasis. Although many studies have demonstrated favorable outcome with neoadjuvant chemotherapy, its impact of NACT on overall survival remains unproven.[3,4]

Two studies have explored the use of neoadjuvant chemotherapy with paclitaxel and carboplatin (6–8 weeks) before concurrent chemoradiation with promising results. Recently, the use of targeted therapy is being explored in several tumors including squamous cell carcinoma of head and neck (SCCHN). Similar to SCCHN, epidermal
growth factor receptor (EGFR) is overexpressed in squamous cell carcinoma of the cervix in more than 80% of cases. Gefitinib (EGFR inhibitor) has been used in patients with relapsed and refractory cervical cancer. With this goal, we prospectively studied the efficacy and feasibility of targeted therapy (gefitinib) after NACT and CRT as maintenance in locally advanced cervical cancer. Gefitinib maintenance was planned for 1 year as most of the relapses occur in first 2 years and gefitinib may eradicate-resistant clones, responsible for relapses. This report describes the results of this trial.

MATERIALS AND METHODS

Between July 2012 and May 2013, 25 patients with locally advanced cervical cancer (International Federation of Gynecology and Obstetrics IIB–IIIB) were included. Eligibility criteria included newly diagnosed patients with squamous cell carcinoma of the cervix with the Eastern Cooperative Oncology Group (ECOG) performance status 0–2, without significant comorbidities. The primary endpoint was the achievement of response rate after NACT. Secondary endpoints were the relationship between the expression of EGFR and response to therapy and toxicity to treatment. The study was approved by Institute Ethics Committee and informed written consent was obtained from all patients before starting treatment.

Pretreatment assessment

All patients underwent detailed clinical evaluation including complete history and gynecological examination in gynecology tumor clinic by a team comprising of a gynecologic oncologist, radiation, and medical oncologist. Pretreatment evaluation included complete blood count, liver and kidney function test, histopathological examination of cervical mass, chest radiograph, cystoscopy, and sigmoidoscopy. A contrast-enhanced computed tomography scan of abdomen and pelvis was done for imaging of the primary disease and nodes.

Neoadjuvant chemotherapy

Patients received 6 weekly doses of paclitaxel (60 mg/m² intravenous over 1 h on day 1) and carboplatin (AUC 2 intravenous over 60 min on day 1) and were administered in daycare. The dose of paclitaxel and carboplatin was reduced by 50% when absolute neutrophil count (ANC) was 1000–1500/mm³ and platelet 75,000–100,000/mm³. Chemotherapy was withheld if ANC was <1000 and/or platelets were <75,000/mm³.

Radiation therapy

External beam radiotherapy (EBRT) to a dose of 50.4 Gy in 28 fractions was delivered using four-field box technique after simulation by 6/15 MV photon in linear accelerator over a period of 5½ weeks. Cisplatin (40 mg/m²) was administered concurrently once a week as per standard guidelines during EBRT. Brachytherapy was given within 1 week of completion of EBRT by Microselectron HDR unit (Elekta AB, Stockholm, Sweden). A dose of 21 Gy in 3 fractions was delivered to point A at weekly intervals.

Gefitinib maintenance

The patients were assessed 4 weeks after the completion of intracavity brachytherapy (ICBT) and were started on gefitinib 250 mg once daily if there was no residual toxicity of CRT. Gefitinib maintenance was planned for 1 year. Patients were monitored for toxicity and drug was withheld in the presence of grade III–IV skin reaction/diarrhea.

Epidermal growth factor receptor estimation

EGFR estimation was carried out by immunohistochemistry on formalin-fixed paraffin-embedded biopsy tissues. Paraffin-embedded tissue sections were cut (4–5 µ thick sections) and taken on slides coated with 3-aminopropyltriethoxysilane (Sigma-Aldrich, USA). The sections were deparaffinized. Antigen retrieval was done by boiling in a domestic oven for 30 min in 10 mm citrate, pH 6.0. For EGFR proteinase K was used for antigen retrieval. After cooling to room temperature, blocking for endogenous peroxidase activity was carried out by treating the sections with commercially available "hydrogen peroxide block," that is, 4% H₂O₂ in methanol for 30 min, followed by treatment with commercially available Ultra V Block solution for 5 min. In between the two steps, slides were washed in running water followed by two washes in Tris-buffered solution (pH 7.4–7.6). The slides were then washed in Tris-buffered solution and incubated overnight at 4°C with monoclonal antibodies against EGFR (anti-EGFR [SP9] MAb, Spring biosciences, CA USA, 1:800 dilution). A secondary antibody formulation conjugated to an enzyme-labeled polymer was used for the detection of the primary antibody linked to the antigen. The polymer complex was visualized with diaminobenzidine as the chromogen. Counterstaining was done with hematoxylin. Appropriate positive and negative controls were run with each batch.
Evaluation during treatment
Patients were examined weekly during NACT and EBRT; blood counts were done and acute treatment-related morbidities if any were recorded. Toxicity grading was recorded according to Radiation Therapy Oncology Group and Common terminology criteria for adverse events version 4.0.

Response evaluation
The response assessment was done at the end of NACT by contrast-enhanced computed tomography (CT) scan of abdomen and pelvis along with a clinical examination by the same team. Tumor response to EBRT was recorded by pelvic examination before the first session of brachytherapy. At the completion of concurrent chemoradiation and brachytherapy, patients were evaluated clinically and radiologically using a contrast-enhanced CT scan of abdomen and pelvis. Patients were followed on an outpatient basis at 3 months’ interval during first 3 years and then at 6 monthly intervals. At each visit and subsequently, history and physical examination including pelvic examination was carried out.

Statistical analysis
Data were recorded on a predesigned pro forma and entered into an excel spreadsheet. Categorical variables were summarized by frequency (%) and quantitative variables were summarized as median and range. Kaplan–Meier test was used for survival analysis and log-rank test was used for univariate analysis. Overall survival was calculated from the date of diagnosis to date of death due to any cause and progression-free survival was calculated from date of diagnosis to date of death or progression. Data analysis was done using SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. (Chicago, USA, SPSS Inc.) statistical software.

RESULTS
A total of 25 patients were included in this study. Patient’s median age was 47 years (range 32–60 years). The median duration of symptoms was 9 months. About 56% of the patients had stage IIIB disease and 62% of the patients had a tumor size more than 4 cm. The median hemoglobin levels at presentation were 11 g/dl (range 8–12.3 g/dl). About 16% of the patients had evidence of pelvic lymph nodes enlargement on baseline CT scan. The patient’s characteristics are shown in Table 1.

All 25 patients completed 6 weeks of NACT. The median time to complete the NACT was 7 weeks. One patient had progressive disease and succumbed to disease while on NACT. Twenty-four patients received concurrent chemoradiation. All 24 patients underwent ICBT after completion of EBRT. Twenty-four patients were started on gefitinib maintenance therapy and twenty patients completed the intended 1-year gefitinib therapy with a good compliance rate of 91%.

Toxicity
During NACT 10 (40%) patients developed ≥grade III hematological toxicity (thrombocytopenia in 3 patients, neutropenia in 6 patients, and anemia in 1 patient). Five (20%) patients developed febrile neutropenia and received oral antibiotics and granulocyte colony-stimulating factor. Three patients (12%) developed ≥grade III hematological toxicity during CRT and two had febrile neutropenia (8.4%). There was no treatment-related deaths. There was no grade III skin, lower genitourinary, or gastrointestinal toxicity during CRT. During gefitinib maintenance, diarrhea was the most common side effect; 12 patients (50%) developed grade ≥II diarrhea, 3 had (12%) ≥III diarrhea needing treatment break. Diarrhea was managed by loperamide and oral rehydration solution. Grade II skin reaction mainly involving the face and trunk was observed in ten patients (41.6%) [Table 2].

Epidermal growth factor receptor analysis
Tissue blocks were evaluable for 22 patients. Of these, EGFR expression was evaluable for 20 patients. Of these, 16 (80%) patients were EGFR positive and 4 (20%) were EGFR negative. Tissue blocks were evaluable for 22 patients. Of these, EGFR analysis was evaluable for 20 patients. Of these, 16 (80%) patients were EGFR positive and 4 (20%) were EGFR negative. Tissue blocks were evaluable for 22 patients. Of these, EGFR expression was evaluable for 20 patients. Of these, 16 (80%) patients were EGFR positive and 4 (20%) were EGFR negative.
expression was seen in 21 cases and 1 case did not express EGFR at all. It was graded as mild+ \((n = 2)\), moderate++ \((n = 12)\), and strong+++ \((n = 7)\). It was moderate or strongly positive in 19 patients \((86\%)\) [Figure 2].

**Treatment outcomes**

Nineteen \((76\%)\) patients had a radiological complete response after NACT. Five patients had a radiological partial response, and one patient had progressive disease. At the end of CRT, 21 \((87.5\%)\) patients had a complete response to therapy, 3 \((12.5\%)\) patients had a partial response, and one patient had progressive disease. Of the 24 patients on maintenance gefitinib therapy, 8 patients had progressive disease at a median interval of 6.5 months after completion of brachytherapy \((range 5–16)\) after completion of CRT. Currently, 16 \((64\%)\) patients continue to be in complete remission at a median follow-up of 27 months. Of the 8 patients who developed progressive disease while on follow-up, 4 patients had a local recurrence and 4 had distant metastasis. Three of the eight patients received palliative chemotherapy; rest were offered supportive care only.

The median overall survival was not reached and 3-year overall survival was 69.8%. The median progression-free survival was not reached and 3-year progression-free survival was 51.4% [Figure 3]. Expression of EGFR, hemoglobin, lymph node status, and tumor size were not found to be significant factors affecting overall survival or progression-free survival.

**DISCUSSION**

The present study was carried out to study the response to neoadjuvant chemotherapy, and role of gefitinib maintenance on the outcome of locally advanced carcinoma of the uterine cervix. Several studies have evaluated the role of NACT in locally advanced cervical cancer. A meta-analysis of 21 randomized trials demonstrated an association between treatment outcome with chemotherapy dose and cycle duration. The short course of weekly dose-dense paclitaxel and carboplatin before concurrent chemoradiation could reduce tumor size and address systemic micrometastasis. The chemotherapy regimen in the current study was based on our earlier study that demonstrated good response rate and high compliance with dose-dense weekly NACT with carboplatin and paclitaxel followed by CRT. The findings in our study are comparable to observations. The major hematological toxicity was grade III neutropenia observed in 40% and 12% of patients with NACT and CRT, respectively, similar to earlier studies. The relatively higher incidence of hematological toxicity also been reported with the weekly schedule of paclitaxel for head and neck squamous cell carcinoma, breast, and ovarian cancer.

Several studies have reported patient age, baseline hemoglobin, lymph node status, and tumor size to be significant prognostic factors in patients with carcinoma cervix. However, in our study factors, for example, hemoglobin, lymph node status, and tumor size did not demonstrate any association with response to treatment. This could be attributed to small sample size in our study.

Evaluation EGFR expression in cervical cancer has ushered in a new era of diagnosis and management of cervical cancer. EGFR stimulation activates tyrosine kinase domain that controls cell proliferation, angiogenesis, and metastasis. Evaluation of EGFR expression status and potential pathway targets may be of value in choosing a therapeutic option in cervical cancer. The role of gefitinib in cervical cancer has...
been inadequately addressed and studies are limited to the metastatic or recurrent setting. Goncalves et al. evaluated the role of gefitinib as second- or third-line treatment in recurrent or metastatic cervical cancer and found therapeutic benefit with gefitinib.[17] In the present study, EGFR was moderate or strongly positive in 86% of the study cohort but did not depict any statistical correlation with outcome. In an earlier study by Pérez-Regadera et al., moderate-to-strong expression of EGFR was associated with higher risk of pelvic recurrence and inferior disease-free survival.[19] However, similar to our findings other studies have not demonstrated the relationship between EGFR expression and survival.[18]

In our study, gefitinib was administered as maintenance after chemoradiation and 67% of patients remained disease free at a median follow-up of 27 months. The gefitinib maintenance therapy was planned for 1 year as most of the recurrences in locally advanced carcinoma cervix occur within the first 2 years of completion of radical CRT. In our study, gefitinib was well tolerated with the incidence of grade III or higher toxicity only in three (12%) patients and was managed conservatively. The toxicity in our study was much lower than a similar study by Goncalves et al. (grade III toxicity 12% vs. 26.7%) and this could be attributed to the lower dose of gefitinib administered in our study (250 mg vs. 500 mg).[17]

Prospective study and a uniform treatment protocol are the strengths of the current study. The limitations of the present study include small sample size and short follow-up. This study clearly demonstrates the feasibility of giving weekly neoadjuvant chemotherapy before concurrent chemoradiation. Whether such an approach will lead to a survival benefit in this poor risk group needs to be confirmed in a randomized trial. Such a study is currently undergoing, and results are expected next year.[19]

CONCLUSIONS

Neoadjuvant chemotherapy followed by concomitant chemoradiation and maintenance therapy with gefitinib is a feasible option for management of locally advanced carcinoma of cervix. We observed high response rate with neoadjuvant chemotherapy followed by chemoradiation with acceptable toxicity. Gefitinib maintenance was well tolerated and may be of help in continuing maintaining response and improved outcome in locally advanced carcinoma cervix. Given the limitation of the small sample size a larger phase III randomized controlled study with adequate sample size and longer follow-up is warranted.

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Conflicts of interest
There are no conflicts of interest.

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