Flow cytometric analysis of DNA ploidy and S-phase fraction of Stage IIIB cervical carcinoma

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Summary

Objectives: To evaluate the role of flow cytometry-measured DNA ploidy and S-phase fraction as survival prognostic indicators in women with FIGO Stage IIIB squamous cell carcinoma of cervix.

Methods: We retrospectively reviewed the medical and pathological records of women with Stage IIIB squamous cell cervical carcinoma treated between 1993 and 1996. Flow cytometric analysis of DNA ploidy and S-phase fraction was performed by the modified Hedley technique using paraffin-embedded tissue. Survival was calculated using the Kaplan-Meier life-table analysis.

Results: Of the 75 cases, 66 were analyzable. Diploid tumors were found in 73%. The mean S-phase fraction was 14% (SD = 5.4). The overall 5-year survival rate was 60%. The survival of patients with aneuploidy tumors was significantly worse than that of the diploid tumors ($p = 0.001$). The survival of the patients who had S-phase fraction > 12% was significantly worse than those who had S-phase fraction ≤ 12% ($p = 0.04$).

Conclusions: In this homogeneous study population, we found that aneuploidy and S-phase fraction > 12% correlated with poor survival. Identifying this poor prognostic group would be of benefit in considering additional treatment for a better outcome.

Key words: DNA ploidy; S-phase fraction; Cervical carcinoma.

Introduction

Cervical carcinoma is the leading gynecologic malignancy in the world [1] and also the number one gynecologic malignancy in Thailand [2]. Although treatment of cervical carcinoma is progressing, there is still recurrence of disease that causes problems and leads to poor prognosis, especially in advanced stage. Prognostic factors of cervical carcinoma include stage, cell type, tumor grade, age and performance status [3].

The use of flow cytometry to measure DNA content and proliferation activity of the cancerous tissue has proven to have prognostic significance in terms of risk of recurrence and survival for several human malignancies [4-6]. However, in invasive cervical cancer, the role of these flow cytometric measurements in terms of predicting biologic behavior and hence patient prognosis has been reported with inconsistent conclusion [7-17].

With these conflicting results in mind, we evaluated the role of flow cytometry-measured DNA ploidy and S-phase fraction as survival prognostic indicators in women with squamous cell carcinoma of cervix Stage IIIB who underwent radiotherapy.

Methods

We reviewed the medical records of women with International Federation of Gynecology and Obstetrics (FIGO) Stage IIIB squamous cell cervical carcinoma treated at Ramathibodi Hospital between 1993 and 1996. Seventy-five cases were recruited in the study. All cases received radiotherapy. Radiation treatment was a combination of external whole pelvic irradiation and intracavitary brachytherapy. External whole pelvic irradiation was delivered before intracavitary brachytherapy via a pair of anterior and posterior parallel opposed fields or the four fields "box" technique with anterior, posterior and right, left lateral portals. The treatment machine was a Co 60 machine (MDS Nordian, Ontario, Canada) or linear accelerator (6 MV or 10 MV photon, Varian, Palo Alto, USA) depending on availability of the machine during the treatment. The total dose to the whole pelvis was 45-50.4 Gy in 5-5 1/2 weeks. If there was still definite parametrial disease, an additional boost dose of 5-10 Gy external radiation to one or both sides of the parametrium was considered. The intracavitary radium applicator was employed by afterloading Fletcher's suit. Intracavitary insertion usually followed external irradiation in seven to ten days. The radium source consisted of an intrauterine tandem arranged with three sources, 15, 10, 10 or 10, 10, 10 mg. The insertion provided 22-25 Gy at point A.

The original histopathological slides of each case were reviewed by a gynecologic pathologist (second author). After confirmation of diagnosis and cell type as squamous cell carcinoma, those sections that showed only tumor tissue were selected. Paraffin-embedded tumor tissue was de-waxed in xylene and rehydrated in ethanol using a modified method originally described by Hedley *et al.* [18]. Tissue was digested with pepsin and suspended with Sheath fluid (Becton Dickinson, USA). Cellular DNA was done by using the Cycle TEST™ PLUS DNA Reagent kit containing RNAase and popidium iodide from Becton Dickinson, USA. Tumor cell DNA was measured on a flow cytometer analyzer (FAC Sort, Becton Dickinson, USA). Chicken erythrocytes nuclei were used for instrument setting. Mononuclear cells separated from donor peripheral blood using Ficoll-Hypaque density-gradient centrifugation were used as a DNA diploid control. Nine samples were not analyzable because of excessive background debris. We defined aneuploidy as two distinct GoGI peaks on the histogram [19]. The proliferative activity of the cells and DNA statistical analysis were calculated by Cell Quest™ software

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(Becton Dickinson, USA.) and Mod Fit cell cycle analysis software (Becton Dickinson, USA). Survival was calculated from the date of beginning radiotherapy. Survival curves were plotted using Kaplan-Meier life-table analysis [20] and survival between groups was compared using the log-rank test. Data were analyzed with SPSS 11.5 for windows statistical software, and significance was defined as $p < 0.05$.

Results

There were 66 analyzable cases. The mean age at the diagnosis was 53 years (range 30-70 years) with a mean parity of 5 (range 0-15). All cancers were FIGO Stage IIIB based on accepted clinical diagnostic procedures, and all histology was squamous cell carcinoma. The mean tumor size at cervix was 3.7 cm (range 2.5-6 cm.). The median follow-up time was 61 months (range 7-111 months).

Diploid tumors were found in 48 patients (73%), while aneuploid were found in 18 patients (27%). The mean S-phase fraction of all 66 patients was 14% (range 4.4 - 33.4%, SD = 5.4). The overall survival curve of all patients is shown in Figure 1. The 5-year survival rate was 60%. Figure 2 shows survival curves of patients with diploid tumors in comparison with patients with aneuploid tumors.

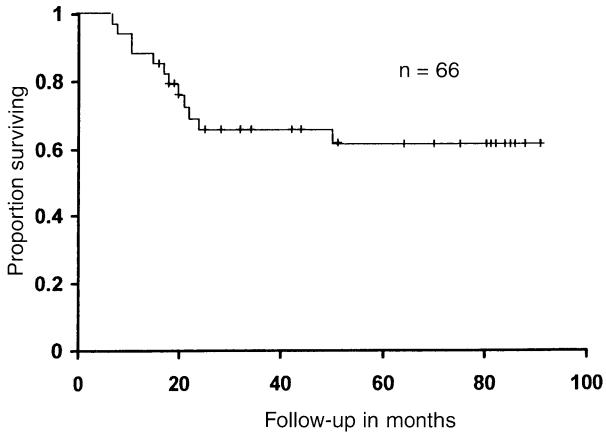

Figure 1. — Survival curve of the entire study patients.

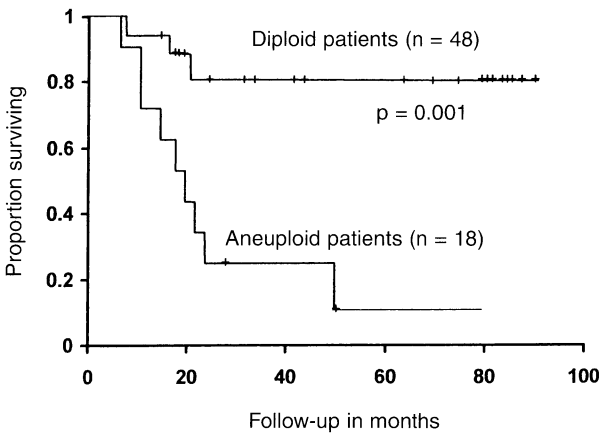

Figure 2. — Survival curve of diploid and aneuploid patients.

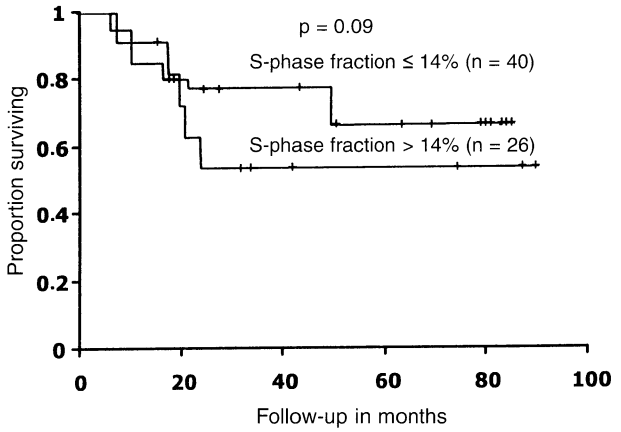

Figure 3. — Survival curve of patients who had S-phase fraction ≤ 14% and > 14%.

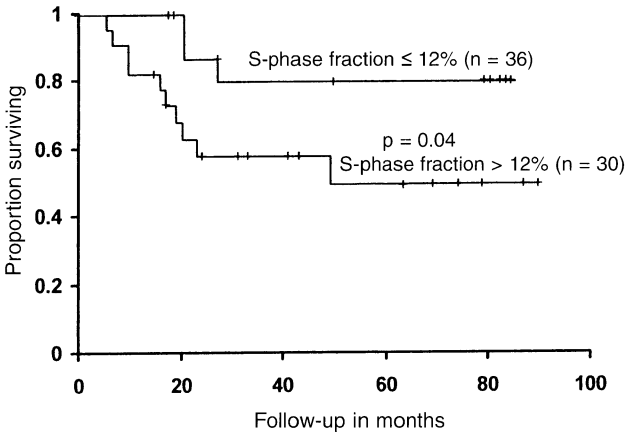

Figure 4. — Survival curve of patients who had S-phase fraction ≤ 12%.

The 5-year survival of the diploid group was 80% while the 5-year survival of the aneuploid group was 10% ($p = 0.001$). When we used a S-phase fraction of 14% (mean) as a cut-off point (> 14% and ≤ 14%), the survival curves of both groups were as shown in Figure 3 with $p = 0.09$. But when we used a S-phase fraction of 12% in Horn's study [16] as a cut-off point (> 12% and ≤ 12%), the survival curve of both groups were as shown in Figure 4. The 5-year survival of the patients who had S-phase fraction of tumors ≤ 12% was 78% while the 5-year survival of those who had S-phase fraction > 12% was 48% ($p = 0.04$).

Discussion

Concerning tumors of the uterine cervix, a variety of reports on the prognostic significance of ploidy and S-phase fraction have shown conflicting results [7-16, 21-23]. However, most of the studied population was in early stage or mixed stages. In early stage cervical cancer, the result of treatment is quite good. According to the report by FIGO, the 5-year survival of Stage I was 80-95% and Stage II was 73-76% [24]. The problem groups are Stage III and IV which have poor prognosis. In these groups the

prognostic factors to indicate high-risk patients are beneficial for considering more aggressive treatment to improve survival. But probably, Stage IV disease is too advanced to expect a good outcome. Thus, in this study population, we recruited only Stage IIIB cervical carcinoma patients. All had squamous cell carcinoma, and all received the same treatment regimen making the studied population homogeneous. This homogeneous population is distinct from other previous studies. The percentage of diploid tumors in this study population was 73% which is rather high compared to 18-73% of the previous reports [4, 7, 13, 23, 25-27]. We found that patients with diploid tumors had statistically significantly better survival rates than those with aneuploid tumors ($p = 0.001$) which could explain the good 5-year survival rate in this study population (60%), while the 5-year survival rate of cervical cancer (mixed cell types) Stage IIIB was 49.4% in the FIGO report [24]. The mean S-phase fraction in this study was 14% which was in the same range with other studies [7, 9, 17]. Using a S-phase fraction of 14% (mean) as a cut-off point, we found no significant difference in survival between the group that had a S-phase fraction > 14 or $\leq 14\%$. When the cut-off point was lowered to 12% according to Horn's study [16], the group that had S-phase fraction > 12 had poorer survival than the group that had S-phase fraction ≤ 12 ($p = 0.04$).

In conclusion, in this study of a homogeneous population with Stage IIIB squamous cell carcinoma of the cervix receiving the same regimen of radiotherapy, we found aneuploidy and S-phase fraction > 12% to be correlated with poor survival. Valid prognostic factors are necessary to estimate the course of the disease, and to define biologically similar subgroups for analysis of therapeutic efficacy. Identifying this poor prognosis group could be of benefit in considering more aggressive treatment for better outcomes in the treatment of Stage IIIB cervical carcinoma.

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