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Clinical Usefulness of Tumor-Associated Antigen TA-4 in Cancer of the Uterine Cervix

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= Abstract = To evaluate the clinical usefulness of tumor antigen TA-4 as a tumor marker in cervical cancer, serum TA-4 level was measured in 62 normal female subjects, 70 patients with cervical cancer and 49 patients with various benign or malignant gynecologic diseases. Mean serum TA-4 level in normal subjects was 0.42 ± 0.22 ng/ml, and postive value was considered to be greater than 1.5 ng/ml. The pretreatment positive rate of serum TA-4 was 42% in cervical squamous cell carcinoma, none in cervical adenocarcinoma, 33% in ovarian cancer, 10% in cervical dysplasia and none in other diseases. In cervical squamous cell carcinoma, the mean value of individual serum TA-4 as well as the percentage of patients with positive TA-4 were positively related to clinical stage based on the FIGO system and to the extent of tumor based on the Meigs-Brunschwing surgical staging, and they were significantly higher in keratinizing type and large cell non-keratinizing type than those in small cell type ($p<0.05$). Elevated serum TA-4 level returned to normal after complete tumor resection so rapidly that 90% of cases showed normal values on second postoperative day and all showed normal values on sixth postoperative day. These results indicate that (1) the specificity of TA-4 is high, (2) there is some sensitivity of this antigen to cervical squamous cell carcinoma, (3) it is of limited value to use this antigen for screening the disease, (4) but it would be a useful tool in the detection of recurrence, in predicting the extent of disease and in monitoring the response of tumor to therapy in patients with squamous cell carcinoma of the uterine cervix.

Key Words: ***TA-4, Squamous cell carcinoma, Uterine cervix***

INTRODUCTION

It is well accepted that recent progress in cytologic screening system has provided the easy performance of the initial diagnosis of cervical cancer. However, cervical cancer is often associated with central recurrence where regression or progression is difficult to evaluate by conventional diagnostic methods. So some reliable method is required for monitoring the disease to determine the effectiveness of treatment and to predict the prognosis and to detect the recurrence. Recently, much attention has been focused on tumor-marker in this disease. Several investigators reported the isolation of tumor-antigens of cervical carcinoma (Levi *et al.* 1971; Hollinshead *et al.* 1972; Aurelian *et al.* 1973). However, it had been difficult to develop conventional method for measuring these antigens. A large number of tumor related substances have been studied (van Nagell *et al.* 1981; Nadkarn *et al.* 1982; Niloff *et al.* 1984; Sawada *et al.* 1984; Inoue *et al.* 1985). Among them, carcinoembryonic antigen (CEA) is one of the useful and widely available tumor markers in cervical cancer (van Nagell *et al.* 1978). But the disadvantages of this test are its long half-life (van Nagell *et al.* 1978) and problems with interpretation due to the lack of tumor cell type specificity. Tumor antigen TA-4 is a protein with a molecular weight of approximately 48,000 which was originally purified from squamous cell carcinoma of the uterine cervix and named by Kato and Torigoe (1977).

Using radioimmunoassay (RIA) method, they reported that TA-4 appeared specifically in the circulation of patients with squamous cell carcinoma

and the serum TA-4 levels reflected some tumor behaviors in cervical squamous cell carcinoma (Kato *et al.* 1979, 1982, 1983 & 1984). By recent development of TA-4 RIA KIT (Dainabot Ltd., Tokyo, Japan), the practical application of this antigen has become easy. Present study was performed to evaluate the clinical usefulness of TA-4 as a tumor-marker in cervical squamous cell carcinoma using this RIA kit.

MATERIALS AND METHODS

The patient population for this investigation consisted of 62 normal nonpregnant women, 70 patients with cervical cancer, 16 patients with other gynecologic malignancies, 2 patients with hydatidiform mole, 10 patients with uterine cervical dysplasia, 10 patients with myoma of the uterus and 11 patients with benign ovarian tumor (Fig. 1).

All cases were dignosed and/or treated at Seoul National University Hospital from March to July, 1985 and the diagnosis was verified by histologic examination of biopsy specimens. The clinical stage of each case was determined according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. Blood samples were collected before treatment. In whom complete tumor resection was performed, blood samples were collected on postoperative 2nd and 6th day to evaluate the change of serum TA-4 level. All serum samples were stored at −20°C until assayed. Determinations of serum TA-4 level were performed quantitatively by double antibody radioimmunoas-

		No. of case	POS (%)	Mean ± S.D.	TA-4 (ng/ml) 1.0 1.5 2 3 5 10 15 20 50
Normal		62	1.6	0.42±0.13	n=61
Myoma		10	0	0.62±0.22	
Benign ovarian tumor		11	0	0.71±0.20	
Dysplasia of cervix		10	10	1.39±1.12	
Cervix cancer	SCC*	62	37	5.42±11.72	68
	SCC Recurrent	6	83	36.12±43.47	70 120
	Adeno CA	2	0	0.85±0.25	
Endometrial cancer		3	0	0.66±0.27	
Ovarian cancer		9	33	1.06±0.80	
Choriocarcinoma		2	0	0.55±0.15	
H-mole		2	0	0.35±0.25	
Vagina cancer		1	0	1.10	
Leomyosarcoma		1	0	1.20	

* SCC : Squamous cell carcinoma

Fig. 1. Pretreatment serum TA-4 level.

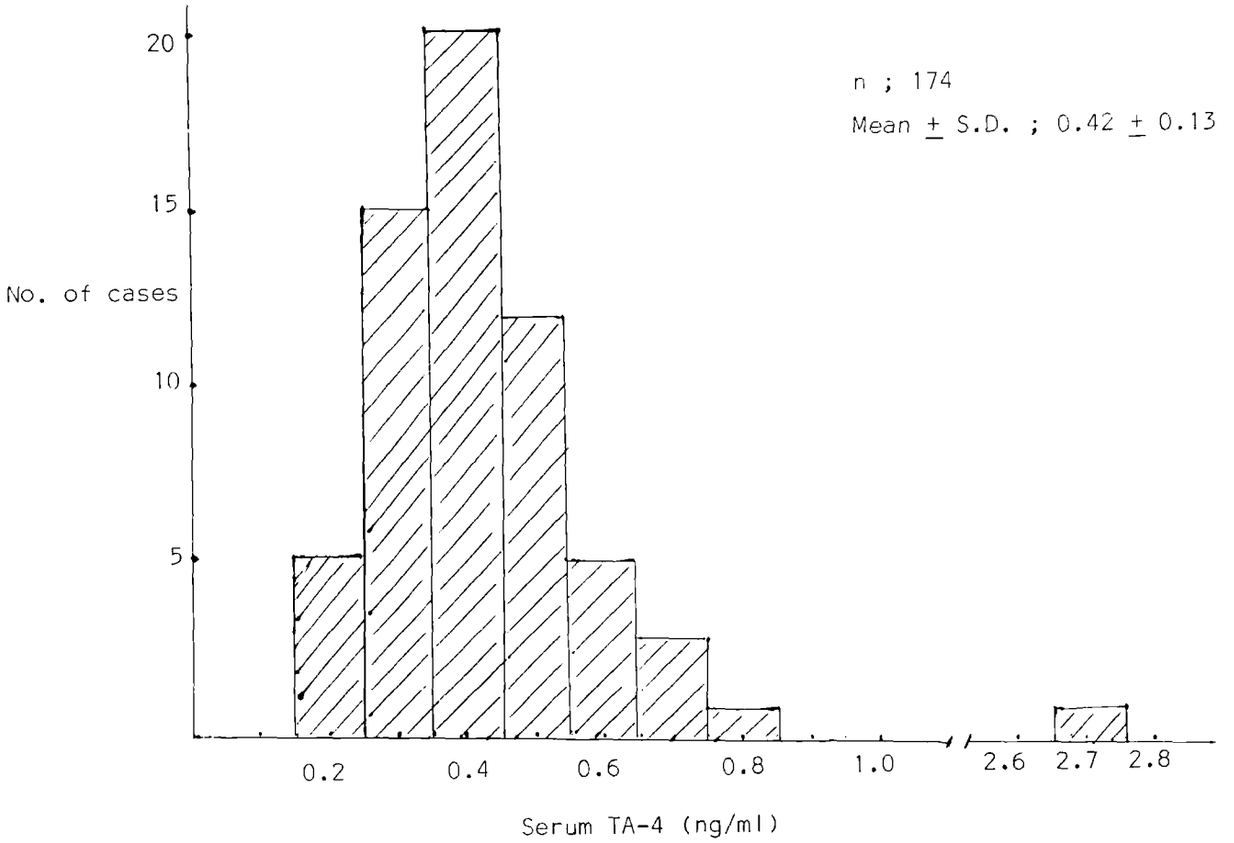

Fig. 2. Histogram of serum TA-4 levels of 62 healthy female subjects.

say using TA-4 RIA kit (Dainabot Ltd., Tokyo, Japan). The sensitivity of this kit was from 1.5 ng/ml to 150 ng/ml. In cases whose values were below 1.5 ng/ml, TA-4 level was determined by extrapolation of standard curve and in cases over 150 ng/ml, it was determined by dilution with normal saline.

The intraassay and interassay coefficients of variation were 5.1% and 7.8%, respectively.

RESULTS

1. Serum TA-4 values in normal healthy female subjects:

Mean value of the serum TA-4 of 62 normal healthy subjects was 0.42 ± 0.22 ng/ml and the value showed the feature of normal distribution except 1 case who showed the value of 2.70 ng/ml (Fig. 2). There was no significant difference between each age group. Based on the mean value plus three standard deviations (SD), normal range of the serum TA-4 level was arbitrarily taken to be less than 1.5 ng/ml (the minimal sensitivity of this kit) and values above 1.5 ng/ml were considered to be "positive". Only 1 case (1.6%) among 62 normal subjects studied showed a positive TA-4 value.

2. Pretreatment serum TA-4 values:

Individual pretreatment serum TA-4 values in cases stuided are shown in Fig. 1. All cases of benign gynecologic tumors showed normal level. But 10% (1/10) of patients with uterine cervical dysplasia showed a positive value. The proportion of positive cases in patients with squamous cell carcinoma was 37% (23/62), which was significantly higher than that (0%, 0/2) with adenocarcinoma ($p<0.001$). And 87% (5/6) of the patients with recurrent cervical cancer showed a positive TA-4 value. In 9 patients with carcinoma of the ovary, 3 had a slightly elevated TA-4 value, but there was no significant difference in mean value compared with that of normal subjects. All patients with other gynecologic malignancies had serum TA-4 values below 1.5 ng/ml.

3. Pretreatment serum TA-4 values in patients with cervical squamous cell carcinoma by clinical stage:

The relationship of the stage of disease based on the FIGO system to serum TA-4 value is summarized in Fig. 3. The values were elevated in a greater percentage of patients with advanced disease than with early disease. The positive values were observed in none of the patients with stage 0 and stage Ia, 20% with stage Ib, 56% with stage

Clinical stage	No. of case	Pos (%)	Mean ± S.D.	TA-4 (ng/ml) 1.0 1.5 2 3 5 10 15 20 50
0	7	0	0.94± 0.25	
Ia	11	0	0.80± 0.24	
Ib	15	20	1.11± 0.83	
IIa	9	56	4.37± 6.61	
IIb	9	56	6.96±13.26	
IIIa	1	100	1.90	
IIIb	8	88	15.34±21.25	68
IVa	2	100	24.30±22.70	
Rec	6	83	38.12±43.47	70 120

Fig. 3. Pretreatment serum TA-4 values in squamous cell carcinoma of the uterine cervix by clinical stage based on the FIGO system.

IIa and stage IIb, 100% with stage IIIa and stage IVa, 88% with stage IIIb, respectively. Mean value before therapy was 0.94±0.25 ng/ml in stage 0, 0.80±0.24 ng/ml in stage Ia, 1.11±0.83 ng/ml in stage Ib, 4.37±6.61 ng/ml in stage IIa, 6.96± 13.26 ng/ml in stage IIb, 1.90 ng/ml in stage IIIa, 15.34±21.25 ng/ml in stage IIIb, 24.30±22.70 ng/ml in stage IVa, respectively. In addition, the patients with recurrent cancer showed high percentage (83%) of positive value and highest mean TA-4 value (38.12±43.47 ng/ml).

4. Pretreatment serum TA-4 values in patients with cervical squamous cell carcinoma by the histologic type:

The relationship of the histologic type of disease to serum TA-4 value before therapy is shown in Fig. 4. In keratinizing type and large cell non-keratinizing type, the proportion of positive cases were 67% and 41% respectively, and mean value was 14.60±28.54 ng/ml and 7.90±15.20 ng/ml, respectively, which were significantly higher than those of small cell type (25% and 1.10±0.60 ng/ml) ($p<0.05$). But there was no significant difference between keratinizing type and large cell non-keratinizing type.

5. Pretreatment serum TA-4 values in patients with cervical cancer by postoperative surgical staging:

Fig. 5 illustrates the relationship between the serum TA-4 values in patients with cervical cancer and the extent of the tumor based on the classification of the Meigs-Brunschwing surgical staging (Meigs and Brunschwing 1952) in whom radical operation was performed. The proportion of positive cases and the mean value of serum TA-4 increased significantly with extending disease class. Serum TA-4 values above 1.5 ng/ml were observed in none of patients with Class 0 and Class Ao, 7% in Class A, 56% in Class B, 33% in Class C, and 100% in Class D, respectively. Mean value of serum TA-4 before therapy was 0.90±0.37 ng/ml in Class 0, 1.00±0.62 ng/ml in Class A, 1.10 ng/ml in Class Ao, 5.70±5.48 ng/ml in Class B, 3.30 ±3.20 ng/ml in Class C, and 13.70±11.29 ng/ml in Class D, respectively. The mean values in Class B ($p<0.05$) and Class D ($p<0.01$) were significantly higher than those in Class 0, Class A and Class Ao.

6. Post-treatment serum TA-4 values:

Fig. 6 illustrates the post-treatment serum

Histologic Type	No. of case	Pos. (%)	Mean ± S.D.	TA-4 (ng/ml) 1.0 1.5 2 3 5 10 15 20 50
Ke	21	67	14.6±28.54*	68 120
L	29	41	7.0±15.20*	70
S	4	25	1.1± 0.62	

Ke : Squamous cell carcinoma, keratinizing type
L : Squamous cell carcinoma, large cell non-keratinizing type
S : Squamous cell carcinoma, small cell type
* $p < 0.05$ compared to small cell type

Fig. 4. Pretreatment serum TA-4 values in squamous cell carcinoma of the uterine cervix by the histologic type

Class	No. of case	Pos (%)	Mean ± S.D.	TA-4 (ng/ml) 1.0 1.5 2 3 5 10 15 20 50
0	7	0	0.9± 0.37	
A	23	7	1.0± 0.62	
Ao	1	0	1.1	
B	9	56	5.7± 5.48*	
C	3	33	3.3± 3.20	
D	3	100	13.7±11.29**	

* $p < 0.05$ compared to class 0, A, Ao
** $p < 0.01$ compared to class 0, A, Ao

Fig. 5. Pretreatment serum TA-4 values in cancer of the uterine cervix by the extent of tumor based on the classification of the Meigs-Brunschwing surgical staging.

TA-4 values of 9 patients who received complete tumor resection among 28 patients with cervical cancer whose pretreatment serum values showed "positive" and also illustrates that of 1 patient with cervical dysplasia who received total abdominal hysterectomy and whose pretreatment serum TA-4 value showed "positive." The fall of the serum TA-4 value was so rapid that 90% of the cases showed normal serum values on postoperative second day and all cases showed normal serum values on postoperative sixth day.

DISCUSSION

TA-4 is a protein with a molecular weight of approximately 48,000, which was originally purified from human squamous cell carcinoma of the uterine cervix by Kato and Torigoe (1977). As this antigen has been found not only in cervical squamous

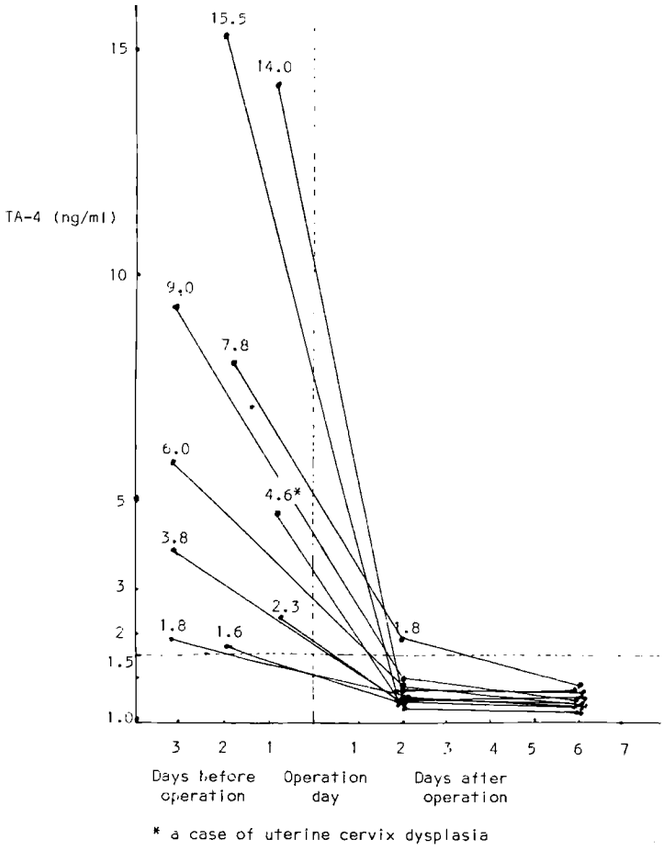

Fig. 6. Changes in serum TA-4 values after complete removal of cervical cancer tissue or dysplasia tissue.

cell carcinoma but also in a variety of malignant or benign disease and normal squamous cellular tissue (Morioka 1980; Aramaki *et al.* 1981), it would not be a specific antigen to cervical squamous cell carcinoma. It was reported that determination of serum TA-4 level reflected the extent of the diesase (Kato *et al.* 1982), had some prognostic significance (kato *et al.* 1983), and would be a useful aid for the detection of cervical squamous cell carcinoma (Kato *et al.* 1984). But there has been some difficulty in practical application of this antigen because primary tumor tissue of cervical cancer is too small to extract large amount of TA-4. TA-4 RIA kit used in this study was developed with the use of TA-4 preparation extracted and purified from liver metastasis of cervical squamous cell carcinoma and anti-TA-4 serum prepared by immunizing rabbits with the purified TA-4.

Clinically useful tumor marker should have high sensitivity and specificity, so it is important to establish precise normal range. In this study, mean TA-4 value in 62 normal women was 0.42 ± 0.22 ng/ ml. Based on the mean value plus three standard deviation(SD), the normal limit of serum TA-4 level in our study was considered to be less than 1.5 ng/ml (the minimal sensitivity of this kit). Previous investigators in Japan with this kit defined the upper limit of normal level of serum TA-4 as 2.0 ng/ml (Hirohashi *et al.* 1984; Torigoe *et al.* 1984; Mochizuki and Maruo 1985), and 4.0 ng/ml (Kuzuya *et al.* 1984), somewhat higher than that of our study. This slight discrepancy may be contributed to the differences in the race and/or population studied. And in their studies, the proportion of positive cases in normal subjects was less than 5.5%. Our result showed that 1 case among 62 normal women (1.6%) and no case among 21 patients with benign gynecologic tumors have a positive TA-4 value, indicating that the specificity of this antigen is high.

Some investigators reported that the proportion of positive cases in patients with ovarian cancer and endometrial cancer was 0% (Hirohashi *et al.* 1984; Shibata *et al.* 1984; Kujuy *et al.* 1984), but Torigoe *et al.* (1984) reported that it was 26% in ovarian cancer and 14% in endometrial cancer. In our study, positive value of TA-4 was found in 33% of 9 patients with ovarian cancer, similar to that of Torigoe *et al.* (1984) (26%), but it was not found in all patients with endometrial cancer, vaginal cancer, choriocarcinoma, and hydatidiform-mole. In addition, in spite of high proportion of positive cases (33%) in patients with ovarian cancer, mean TA-4 value was not significantly different from that of normal subjects.

Accordingly, further determinations of TA-4 in a large number of patients with other gynecologic malignancies will be necessary to confirm the proportion of positive cases.

The proportion of positive cases in patients with cervical squamous cell carcinoma was 42% and it increased progressively as stage advanced, but the proportion in patients with cervical adenocarcinoma was 0%. This finding is similar to that of other investigators (Hirohashi *et al.* 1984; Torigoe *et al,* 1984; Kuzuya *et al.* 1984; Mochizuki and Maruo 1985). This result suggests that serum TA-4 level is mainly elevated in patients with squamous cell carcinoma of the uterine cervix and this test has some sensitivity for this disease.

However, among patients with stage 0 and stage Ia, no positive TA-4 value was obtained. These findings indicate that, although the detection of TA-4 in the circulation is highly indicative of the presence of cervical squamous cell carcinoma, the

low positive rate particularly in the early stage of disease limits the use of this method for screening this disease. But the finding that high percentage (83%) of positive value of TA-4 was observed in patients with recurrent cervical cancer suggests that serum TA-4 level may increase when the disease recurs in spite of undetectable level at pretreatment period and, for that reason, serial determination of this tumor antigen will be useful for the early diagnosis of recurrent disease in cervical cancer. In this study, one patient with recurrent disease showed undetectable TA-4 level. This finding indicates that some patient might show undetectable serum TA-4 level throughout the course of their disease. It is unlikely that some tumor tissues do no produce TA-4 at all, because Morioka (1980) studied the tissue distribution of TA-4 in 13 patients with cervical squamous. cell carcinoma and found that all tumor tissues tested contained TA-4 activity. So further extensive efforts should be undertaken to develop a more sensitive method for the detection of TA-4.

Previous studies on the serum TA-4 levels in patients with cervical squamous cell carcinoma according to the histologic type showed conflicting results (Torigoe *et al.* 1984; Shibata *et al.* 1984; Inoue *et al.* 1985). Our result shows significantly higher TA-4 concentration in keratinizing type and large cell non-keratinizing type than that in small cell type, which is consistent with the observation of Shibata *et al.* (1984) who tested TA-4 concentration in cancer tissue by immunohistochemical method.

Kato *et al.* (1982) reported that serum TA-4 levels reflected the extent of disease, and that high pretreatment TA-4 levels indicated the presence of the widespread tumor and high possibility of recurrence after surgery, although they had been unable to find any direct relationship between serum TA-4 levels and pretreatment clinical stages (Kato *et al.* 1979). In this study, similar result was obtained by comparing the pretreatment serum TA-4 value with the extent of tumor based on the classification of the Meigs-Brunschwing surgical staging.

Shibata *et al.* (1984) reported that the plasma half-life of TA-4 in circulation was about 20 minutes. In this study, elevated serum TA-4 levels fell rapidly to normal levels within 6 days after complete tumor resection. The rapid disappearance of TA-4 in the circulation after complete tumor resection indicates the usefulness of this antigen as a tumor marker for the determination of efficacy of therapy particularly in patients whose pretreatment serum values were positive.

From these findings, although the determination of serum TA-4 is of limited value for screening cervical cancer, it would be a useful aid in the detection of recurrence, in predicting the extent of disease and in monitoring the response of tumor to therapy in patients with cervical squamous cell carcinoma.

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= 국문초록 =

子宮頸部癌 患者에 있어서 腫瘍關連 抗原 TA-4에 關한 硏究

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子宮頸部癌 患者에 있어서 腫瘍關連抗原 TA-4의 腫瘍標識物質로서의 有用性을 檢討하기 위하여 子宮頸部癌 患者 10例와 正常女性 62例, 各種 婦人科 良性 또는 惡性疾患 患者 49例의 血淸 TA-4値를 測定하여 다음과 같은 結果를 얻었다.

1. 正常女性의 血淸 TA-4値는 0.42±0.22 ng/ml로 그 正常範圍를 1.5 ng/ml 以下로 設定하였다. 正常女性 中 1例가 陽性(1.6%)이었다.

2. 各種疾患의 治療前 血淸 TA-4値의 陽性率은 子宮頸部癌은 40%였고 그 중 子宮頸部 扁平上皮癌은 42%, 腺癌은 0%이었으며 卵巢癌은 33%, 子宮頸部異形症은 10%이었으나 그 외의 婦人科疾患에 있어서는 全例가 陰性이었다.

3. 子宮頸部 扁平上皮癌의 治療前 血淸 TA-4値는 FIGO分類 및 Meigs-Brunschwing分類에 따른 臨床進行期가 增加함에 따라 높은 陽性率 및 平均値를 보였으며, keratinizing type과 large cell non-keratinizing type이 small cell type에 비해 높은 陽性率 및 平均値를 보였다($p<0.05$).

4. 治療前 血淸 TA-4値가 陽性이었던 子宮頸部癌 28例中 根治手術을 行한 10例에서 手述後 2日째에 90%에서, 6日째에는 全例에서 TA-4値가 正常범위로 떨어졌다.

以上의 結果로 보아 TA-4는 子宮頸部 扁平上皮癌에 높은 特異性 및 어느 정도의 敏感性을 가졌으며, 再發癌의 早期診斷과 癌의 進行程度의 豫測 및 治療效果의 判定에 有用性이 있다고 생각된다.