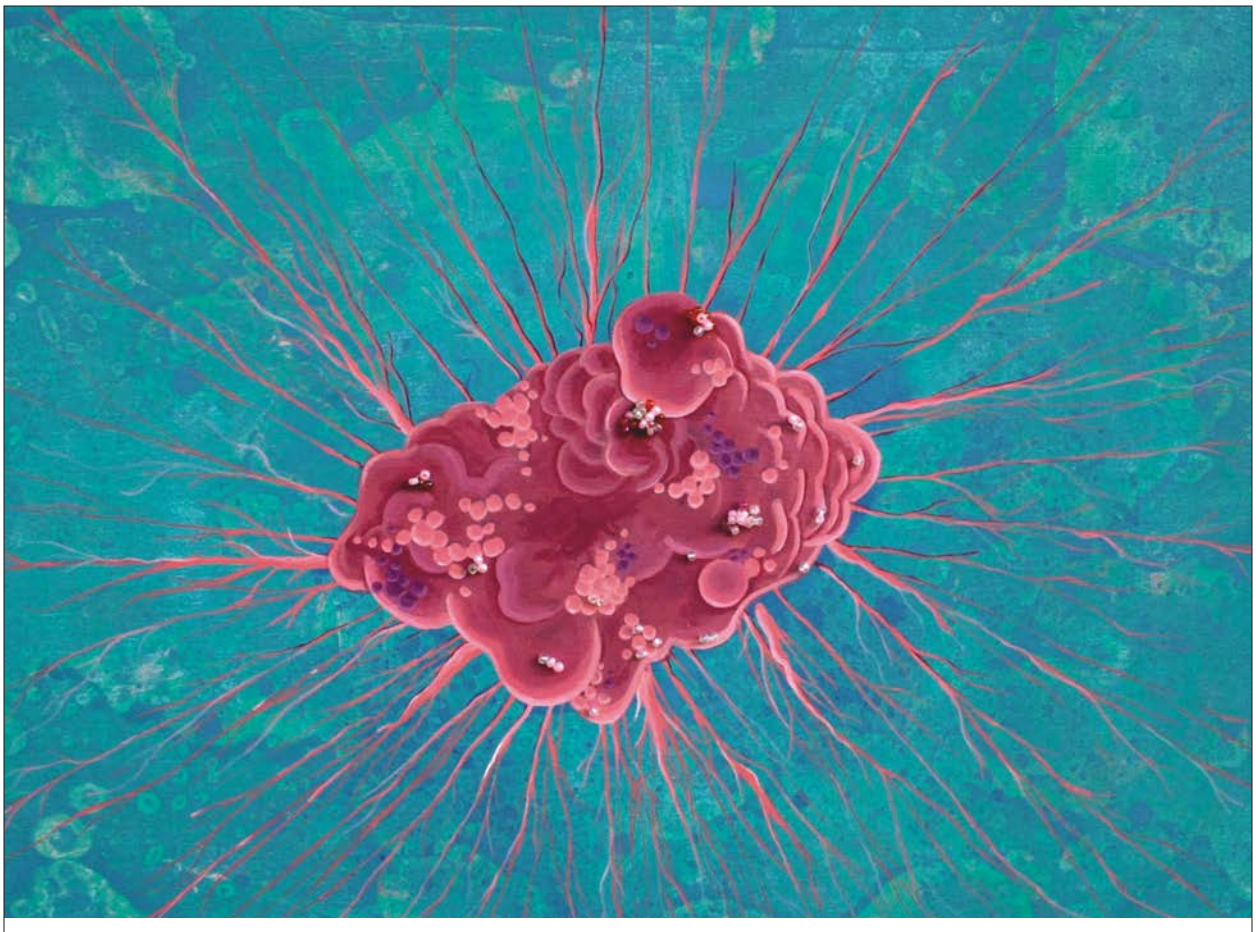

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Serum Levels of Squamous Cell Carcinoma Antigen and CA 125 in Cervical Intraepithelial Neoplasia and Invasive Squamous Cell Carcinoma of the Uterine Cervix

[1]M.L. Looi, [2]M.D. Ahmad Zailani Hatta, [3]M.A. Siti Aishah,
[4]M.H. Baizurah, [1]W.N. Wan Zurinah and [1]M.Y. Yasmin Anum
[1]Department of Biochemistry, Faculty of Medicine,
Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz,
50300 Kuala Lumpur, Malaysia
[2]Department of Obstetric and Gynecology, Faculty of Medicine,
Hospital Universiti Kebangsaan Malaysia, Jalan Yaacob Latif,
Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia
[3]Department of Pathology, Faculty of Medicine,
Hospital Universiti Kebangsaan Malaysia, Jalan Yaacob Latif,
Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia
[4]Unit of Chemical Pathology, Hospital Kuala Lumpur,
Jalan Pahang, 50300 Kuala Lumpur, Malaysia

Abstract: The aim of this study is to determine whether Squamous Cell Carcinoma Antigen (SCC-Ag) and (Cancer Antigen) CA 125 are helpful in determining the stage of cervical cancer. The Serum levels of (SCC-Ag) and CA 125 were determined by enzyme immunoassay in 26 patients with Squamous Cell Carcinoma (SCC) of the uterine cervix, 30 patients with Cervical Intraepithelial Neoplasia (CIN) and in 35 healthy women (normal control). The cut-off values for SCC-Ag and CA 125 were chosen at 1.80 μg L^{-1} and 35.0 U mL^{-1}, respectively. Elevated SCC-Ag levels were found in 69% of patients with SCC with a specificity of 97%. Sensitivity and specificity of serum CA 125 were 23 and 97%, respectively. Statistical analysis showed that advanced SCC (FIGO stage III and IV) had significantly higher levels of serum SCC-Ag than CIN and normal control ($p = 0.0001$). However, increased levels of CA 125 were only found in SCC stage IV. There were no significant differences in serum SCC-Ag and CA 125 levels between CIN and normal control. Using chi-square test, we found a strong correlation ($p = 0.0140$) between the staging of SCC and the elevation of serum SCC-Ag but not for CA 125 ($p = 0.5080$). We concluded that SCC-Ag is a better tumor marker than CA 125 in detecting squamous cell carcinoma of the cervix and its level in serum correlated with staging of cervical SCC.

Key words: Cervical cancer, Cervical Intraepithelial Neoplasia, Squamous cell carcinoma, serum markers, SCC-Ag, CA 125

Introduction

Cervical cancer ranks second in the top ten cancers in Malaysia (National Cancer Registry of Malaysia, 2002). The predominant histological type of cervical cancer, squamous cell carcinomas

Corresponding Author: M.Y. Yasmin-Anum, Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia
Tel: 603-4040 5297 Fax: 603-2693 8037

(Smith *et al*., 2000) arise from reversible precursor lesions called Cervical Intraepithelial Neoplasia (CIN). Tumor markers may help in assessing the stage of the disease in newly diagnosed patients as well as in the monitoring of its course after treatment (Gaarenstroom *et al*., 1995; Pras *et al*., 2002). Therefore, many efforts have been made to identify the most sensitive serum marker(s) for the follow-up care and therapy control of the tumor.

Squamous Cell Carcinoma Antigen (SCC-Ag) is a subfraction of TA-4, a tumor associated antigen first described by Kato and Torigoe (1977). It has been characterized as a glycoprotein with a molecular weight of 48,000 Daltons. The clinical value of SCC-Ag as serum tumor marker has been demonstrated in numerous studies on Squamous Cell Carcinoma (SCC) of the uterine cervix (de Bruijin *et al*., 1998), lung cancer (Vassiliakopoulos *et al*., 2001) and neck and esophagus cancer (Snyderman *et al*., 1995). In SCC of the cervix, many studies have shown that serum SCC-Ag is an important new prognostic marker (Duk *et al*., 1996; Takeshima *et al*., 1998; Juang *et al*., 2000; Strauss *et al*., 2002), a valuable diagnostic tool in the staging of squamous cell carcinoma (de Bruijin *et al*., 1998; Esajas *et al*., 2001), useful in monitoring of therapy given to patients (Hong *et al*., 1998) and in follow-up of patients (Bonfrer *et al*., 1997; Pras *et al*., 2002).

CA 125 is a glycoprotein normally expressed in coelomic epithelium during fetal development (Alagoz *et al*., 1994). This epithelium lines body cavities and envelopes the ovaries (Perkins *et al*., 2003). Elevated CA 125 values most often are associated with epithelial ovarian cancer (Niloff *et al*., 1988), although increased levels have also been found in other malignancies such as adenocarcinoma of the cervix, endocervix and endometrial carcinomas (Borras *et al*., 1995). In cervical cancer, elevated values of CA 125 has been reported to be associated with advanced International Federation of Gynecology and Obstetric (FIGO) stage, disease progression and survival (Duk *et al*., 1990).

The main aim of the present study was to evaluate the serum levels of SCC-Ag and CA 125 in cervical cancer patients with Cervical Intraepithelial Neoplasia (CIN) and SCC of the cervix.

Materials and Methods

A total of 91 subjects (26 SCC, 30 CIN and 35 healthy women as control group) were recruited between January 2004 and June 2005 from Obstetric and Gynecology Clinic of Hospital Universiti Kebangsaan Malaysia (HUKM). This research was approved by the institutional ethics review board in the HUKM. Consent was obtained from all subjects prior to sample collection.

Patients with newly diagnosed SCC of the cervix were clinically staged in accordance with the FIGO criteria. Of the 26 patients with SCC of the cervix, 9 patients were SCC stage I, 7 patients with stage II, 5 patients with stage III and 5 patients with stage IV. However, patients with newly diagnosed CIN of the cervix were classified by their abnormal Pap smear results and confirmation by colposcopy observation. Sixteen patients presented with CIN 1, 7 had CIN 2 and 7 had CIN 3. All normal subjects were seen for routine annual vaginal examination and all of them had a normal Pap smear result and normal biochemical profile.

Blood samples were collected prior to treatment. The samples were separated and sera were stored at -80°C until assayed. All the serum specimens were assayed for SCC-Ag and CA 125. The SCC-Ag was measured by Enzyme Immuno Assay (EIA) using CanAg SCC EIA kit from CanAg Diagnostics (Gothenburg, Sweden). A value of 1.80 μg L^{-1} was chosen as the upper limit of normal for SCC-Ag. CA 125 measurements were performed with the Axsim CA 125 Microparticle Enzyme Immunoassay (MEIA) kit from Abbott Laboratories, Diagnostic division. For this assay the internationally used cut-off level of 35 U mL^{-1} was chosen.

Data were analyzed using the SPSS statistical package. Mean and standard error of antigen levels for each group were calculated. The χ^2 test was used to investigate differences in the number of patients with elevated marker levels between subgroups. The ANOVA was used for comparison of the means. Sensitivity and specificity of tumor antigens were also calculated using the following formulae; sensitivity = true positive/(true positive + false negative); specificity = true negative/(true negative+false positive).

Results

In this study, the cut-off values for SCC-Ag and CA 125 were chosen at 1.80 μg L^{-1} and 35.0 U mL^{-1}, respectively. Overall, elevated serum levels of SCC-Ag and CA 125 were found in 69 and 23% of all SCC patients (N = 26), respectively, with specificity of 97% for both cases (Table 1).

We demonstrated a strong relationship (χ^2 test: p = 0.0001) between the positivity of serum SCC-Ag values (>1.80 μg L^{-1}) and the presentation of cancer (Table 2).

We found a strong correlation (p = 0.0140) between the staging of SCC and the elevation of serum SCC-Ag (Table 3).

Mean values of SCC-Ag for CIN samples were found within the normal range (0.20 -1.80 μg L^{-1}). However, mean values of SCC-Ag for all stages of SCC (I - IV) were significantly different when compared to normal (Table 4). Mean values of SCC-Ag for advanced cervical SCC (stage III and IV) were also found to be significantly higher when compared to control, CIN (1, 2 and 3) and early cervical SCC (stage I and II) (ANOVA test: p = 0.0001) (Table 4). However as for CA 125, there were no significant differences of serum CA 125 levels among CIN patients and patients with SCC stage I, II and III when compared to normal values. Mean values of CA 125 for normal, CIN and SCC I, II and III were found to be less than 35 U mL^{-1} (Table 4).

There was no relationship between the positivity of serum level of CA 125 and presentation of cancer observed in this study (χ^2 test: p = 0.0800) (Table 5).

Table 1: Cut-off values, sensitivity and specificity of serum SCC Ag and CA 125 in cervical cancer

	SCC-Ag	CA 125
Cut-off value	1.80 μg L^{-1}	35 U L^{-1}
Sensitivity	69%	23%
Specificity	97%	97%

Table 2: Contingency table shows the relationshiph between the status of the disease (CIN or SCC) and the levels of serum SCC-Ag

	SCC-Ag		
Status	<1.80 μg L^{-1}	>1.80 μg L^{-1}	Total
CIN	28	2	30
SCC	8	18	26
Total	36	20	56

x^2=23.747, p=0.0001

Table 3: Contingency table shows the relationship between the staging of cervical SCC and the levels of serum SCC-Ag

	SCC-Ag		
Stages	<1.80 μg L^{-1}	>1.80 μg L^{-1}	Total
Early stages (SCC I and II)	7	9	16
Advanced stages (SCC III and IV)	0	10	10
Total	7	19	26

χ^2 = 5.987, p = 0.0140

Table 4: Mean (±SEM) serum tumor antigen levels in normal control, CIN patients and SCC patients. [a]Significantly ($p<0.05$) different when compared with normal subjects. [b]Significantly ($p<0.05$) different when compared with CIN 1, 2, 3 and SCC stage I and II. [c]Significantly ($p<0.05$) different when compared to other groups

	No. of patients (N)	SCC-Ag (μg L^{-1}) Mean±SEM	CA 125 (U mL^{-1}) Mean±SEM
Normal	35	0.74±0.06	9.57±0.84
CIN11	16	1.01±0.12	19.15±6.54
2	7	0.83±0.14	14.00±2.69
3	7	1.07±0.23	11.47±2.93
SCC I	9	3.76±1.71[a]	28.72±12.84
II	7	3.94±1.02[a]	21.81±9.05
III	5	17.42±6.35[ab]	12.18±0.43
IV	5	18.64±9.62[ab]	77.42±43.13[c]

Table 5: Contingency table shows the relationship between the status of the disease (CIN or SCC) and the levels of serum CA 125

	CA 125		
Status	<35 U mL^{-1}	>35 U mL^{-1}	Total
CIN	28	2	30
SCC	20	6	26
Total	48	8	56

$\chi^2 = 3.063$, $p = 0.0800$

Table 6: Contingency table shows the relationship between the staging of cervical SCC and the levels of serum CA 125

	CA 125		
Stages	<35 U mL^{-1}	>35 U mL^{-1}	Total
Early stages (SCC I and II)	13	3	16
Advanced stages (SCC III and IV)	7	3	10
Total	20	6	26

$\chi^2 = 0.439$, $p = 0.5080$

There was no correlation between staging of SCC and elevation of serum CA 125 (χ^2 test: $p = 0.5080$) (Table 6). Serum CA 125 (>35 U mL^{-1}) was only significantly elevated in patients with SCC stage IV (ANOVA: $p = 0.002$) when compared with normal, CIN and SCC stage I, II and III.

Discussion

SCC-Ag assay has been widely investigated in the management of patients with cervical cancers. For instance, Gaarenstrom *et al*. (2000) reported that serum SCC-Ag was elevated (cut-off level, >1.50 μg L^{-1}) in 51% of patients with SCC stage I and II. With the same cut-off level, Juang *et al*. (2000) found that this marker has a role in predicting survival rate of patients diagnosed with cervical SCC, indicating the need for pretreatment evaluation of SCC-Ag. Pras *et al*. (2002) also detected elevated level of SCC-Ag (>1.90 μg L^{-1}) in 60% of cervical cancer patients. Their study also showed that SCC-Ag was raised in 70 % of patients with recurrent cervical cancer. However, Strauss *et al*. (2002) were using a higher cut-off value (3.00 μg L^{-1}) of SCC-Ag to detect the recurrence and overall survival of the cancer.

Researchers have studied the correlation of CA 125 levels with the presence of tumor in cervical carcinoma, at the reference values varying from 6.0 to 35.0 U mL^{-1} (Schwartz *et al*., 1987; Duk *et al*., 1989; Borras *et al*., 1995; Bender *et al*., 2003). Some studies have also reported on the sensitivity of this marker ranging from 23 to 52% (Duk *et al*., 1989; Borras *et al*., 1995; Massuger *et al*., 1997).

The comparison of several tumor antigens needs the choice of cut-off levels selected according to well established criteria. In this study we compared the sensitivity of different tumor markers with respect to cut-off values corresponding to a prefixed specificity of value 97% in respective control population.

The data from this study demonstrated that in SCC of the cervix, SCC-Ag is a better tumor marker with 69% true-positive test results versus 23% for CA 125. The sensitivity and specificity of SCC-Ag in the current study is almost similar to that reported by Pras *et al*. (2002) and Juang *et al*. (2000) for SCC of the cervix.

Mean serum levels of SCC-Ag in CIN patients were within the normal range (0.20-1.80 μg L^{-1}) and the antigen levels were almost the same for stage I (3.76±1.71 μg L^{-1}) and stage II (3.94±1.02 μg L^{-1}) patients. However significantly higher SCC-Ag levels were found for stage III (17.42±6.35 μg L^{-1}) and IV (18.64±9.62 μg L^{-1}). This is in agreement with previous reports (Crombach *et al*., 1989; Åvall-Lundqvist *et al*., 1992; Massuger *et al*., 1997). Crombach *et al*. (1989) suggested that the release of SCC-Ag into circulation depends on infiltration of tumor growth and on tumor mass, suggesting that SCC-Ag may be useful at predicting advanced stage disease. Such findings may imply a clinical application of SCC-Ag levels as an adjunct to staging, especially when surgical staging is not performed (Åvall-Lundqvist *et al*., 1992). Bonfrer *et al*. (1997) suggested that, SCC-Ag should be considered as the most accurate and valuable serum marker for SCC of the cervix. In their study, SCC-Ag appeared to be a better parameter than the cytokeratins, Cyfra 21-1 and TPA in predicting the presence of tumor during follow-up and survival of patients with cervical cancer.

Studies have found that CA 125 levels were only significantly higher in patients with adenocarcinoma than in those with SCC of the cervix (Duk *et al*., 1990; Borras *et al*., 1995; Tabata *et al*., 2000; Bender *et al*., 2003). Previous reports indicated that approximately 95% of all invasive cervical cancer was of squamous cell histology; however, the second most histologic cell type was adenocarcinoma (Smith *et al*., 2000). All of our cancer patients recruited were diagnosed as squamous cell type. In this present study, we found CA 125 was only elevated in SCC stage IV and its level was not related to the presentation of cancer and clinical staging. CA 125 has been reported to be elevated in several other malignancies including ovarian cancer and endometrial cancer (Gadducci *et al*., 2004). In the adult, CA 125 can also be detected in almost all normal or benign and malignant neoplastic tissues derived from coelomic epithelium, such as normal and malignant glandular epithelium of the endocervix (Duk *et al*., 1989). Because of the lack of tumor specificity and organ specificity, the determination of this antigen is not suitable for the screening or monitoring of patients with cervical SCC. Thus the clinical value of CA 125 as a tumor marker for cervical cancer appears to be limited.

In conclusion, we suggest that serum levels of SCC-Ag may be of value as an adjunct in assessing clinical stage of cervical cancer. This is in agreement with the recommendation given by the National Academy of Clinical Biochemistry (NACB) (Gaarenstroom and Bonfrer, 2005) guidelines that stated although SCC-Ag is not suitable for screening or diagnosis of cervical cancer, serum SCC levels correlate with tumour stage, tumour size, residual tumour after treatment, recurrent or progressive disease and survival. CA 125 however is not a sensitive tumor marker when compared to SCC-Ag and should not be used for the screening and monitoring of patients with SCC of the cervix.

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