Original Article

Role of squamous cell carcinoma antigen in monitoring of treatment response of cervical and vaginal malignancies

ABSTRACT

Introduction: As per GLOBOCAN 2012 report Worldwide fourth most common cancer in the female is cervical cancer and approximately 528,000 new cases was found in 2012 in large majority of global burden was found in less developed country. India, the second most populous country in the world, accounts for 27% of the total cervical cancer deaths. The aim of this study was to assess the efficacy of squamous cells carcinoma antigen (SCC-Ag) in monitoring of response to treatment in cervical and vaginal cancer patients.

Materials and Methods: This prospective case–control study was carried out over a period of 1 year in the Department of Obstetrics and Gynecology in collaboration with the Department of Internal Medicine and Pathology. Histopathologically confirmed study group included 8 cases of Stage I, 15 cases of Stage II, 15 cases of Stage III, 8 cases of Stage IV cervical malignancy, and 5 cases of vaginal carcinoma. About 15 healthy cervical cytology-negative women were taken as controls.

Results: Out of 51 cancer cases SCC-Ag level were determined in only Stage I, II, III, and IV cases, assess the response to treatment. The mean SCC-Ag level in all four stage groups decreased significantly after post treatment as compared to pre treatment ($P < 0.001$) and the decrease in post treatment SCC-Ag level increased linearly with stage severity. Similarly, comparing the total or overall (Stage I + Stage II + Stage III) mean change (pre-post) in SCC-Ag level, *t*-test further revealed significant ($P < 0.001$) and decrease of 66.2% at posttreatment as compared to pretreatment.

Conclusion: SCC-Ag might be a useful marker in monitoring the response to treatment.

Keywords: Cervical cancer, International Federation of Gynecology and Obstetrics staging, radiotherapy, squamous cell carcinoma antigen

INTRODUCTION

According to the WHO cervical cancer contributes 12% of all female cancers and it is the most common gynecological malignancy in developing countries.[1,2] Recent reports state that 1.32 lakhs new cases are diagnosed per year and 74,000 annual deaths are reported in India.[3] India, the second most populous country in the world, accounts for 27% of the total cervical cancer deaths.[4] Squamous cells carcinoma antigen (SCC-Ag) is subfraction of TA-4 which is a tumor-associated antigen first described by Kato and Torigoe in 1977.[5] It belongs to the family of serine protease inhibitors. Acidic isoform of SCC-Ag was found in tumor cells, especially those located at the periphery of the tumors and in the serum of cancer patients and it plays an important role in suppressing apoptotic cell death.[6,7] SCC-Ag suppresses the activity of caspase-3 and caspase-9 via downregulation of P38 MAPK and/or MKK3/MKK6, thus SCC-Ag in tumor cells help to protect cancer cells from apoptotic cell deaths.

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In the present era, despite various screening methods such as conventional exfoliative cervicovaginal cytology, liquid-based cytology, and human papillomavirus testing, which is quite effective also in diagnosing the early lesions. However, none of the screening techniques could predict the natural history of disease process or the progressive nature of lesion. Accumulating experimental evidence suggest that the dysregulation of apoptosis may play a role in the pathogenesis of gynecological tumor for this purpose, it is important to identify a reliable biomarker to better characterized the natural history of disease. At present, various immunohistochemical markers are present, but no definite serum biomarker is available; so, it is difficult to monitor the treatment response. Thus, this study was planned to evaluate the role of SCC-Ag as a tumor marker in monitoring the treatment response in patients of cervical and vaginal malignancy. The aim of this study was to assess the efficacy of SCC-Ag in monitoring of response to treatment in cervical and vaginal cancer patients.

MATERIALS AND METHODS

This prospective case–control study was carried out over a period of 1 year in the Department of Obstetrics and Gynecology in collaboration with the Department of Internal Medicine and Pathology at King George's Medical University, Lucknow. Ethical clearance was obtained from the Institutional Ethics Committee of this university. After informed and written consent, 66 subjects were enrolled in this study. According to International Federation of Gynecology and Obstetrics (FIGO) staging, histopathologically confirmed study group included 8 cases of Stage I, 15 cases of Stage II, 15 cases of Stage III, 8 cases of Stage IV cervical malignancy, and 5 cases of vaginal carcinoma. Fifteen healthy cervical cytology-negative women were taken as controls.

FIGO, Stage I cancer patients were treated surgically (Wertheim's hysterectomy), Stage II and Stage III, and 4 cases of vaginal carcinoma were treated by concurrent chemoradiation and Stage IV cervical carcinoma and one case of vaginal carcinoma were treated by palliative chemotherapy and then radiotherapy (RT) was given.

Women suffering from Pemphigus, psoriasis, or eczema were excluded from the study.

Intervention

Five milliliter venous blood samples were drawn into a sterile vial from all patients before treatment. A second sample was drawn, 6 weeks after completion of treatment from Stage I, II, III, and IV patients. Samples were kept at 4°C, centrifuged at 6000 rpm for 15 min, and then immediately frozen at −20°C until assay. Serum SCC-Ag levels (SCC-Ag) were estimated by ELISA technique using enzyme-linked immunosorbent assay kit as per producer protocol (USCN life sciences Inc., Export Processing zone, Economic and development zone, China).

The microtiter plate of kit has been precoated with an antibody specific to SCC-Ag standard and blood sample was then added to the appropriate microtiter plate wells coated with a biotin-conjugated antibody preparation specific for SCC-Ag2. Avidin conjugated to horseradish peroxidase (HRP) is added to each microplate well and incubated.

This is sandwich ELISA kit for *in vitro* quantitative measurement of SCC-Ag2 in human serum (Uscn, Life Science Inc., Export Processing Zone, Economic and Technological Development Zone, China). The microtiter plate of kit has been precoated with an antibody specific to SCC-Ag2. Standard and sample are then added to the appropriate microtiter plate wells coated with a biotin-conjugated antibody preparation specific for SCC-Ag2. Avidin conjugated to HRP is added to each microplate well and incubated. After 3,5,3',5'-tetramethylbenzidine substrate solution is added, only those wells that contain SCC-Ag2, biotin-conjugated antibody, and enzyme-conjugated avidin exhibited a change in color. The enzyme–substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The concentration of SCC-Ag2 in the sample is then determined by comparing the optical density of the sample to the standard curve.

Statistical analysis

SCC-Ag levels in different clinical stages of invasive cervical and vaginal lesions were measured as mean ± standard deviation. Groups were compared by one-way analysis of variance (ANOVA), and the significance of mean difference between the groups was done by Tukey's *post hoc* test. Groups were also compared by factorial two-way ANOVA, and the significance of mean difference within and between the groups was done by Tukey's *post hoc* test. Groups were also compared by repeated measures ANOVA. Discrete (categorical) groups were compared by Chi-square test. A two-sided ($\alpha = 2$) $P < 0.05$ was considered statistically significant. All analyses were performed on STATISTICA software (Windows version 6.0, Dell products, Intel Corporation, US).

RESULTS

Out of 51 cancer cases, pre- and post-treatment SCC-Ag level was determined in only Stage I, II, III, and IV cases, assess

the response to treatment. In this study, we had not assessed posttreatment SCC-Ag level of vaginal carcinoma cases, as we considered this a separate entity. In our study, out of 51 cases, majority of cases were moderately differentiated 42 (82.4%) and mean SCC-Ag level of these patients was 2.88 ± 1.35.

Nine (17.6%) cases were poorly differentiated and mean SCC-Ag level of these patients was 3.36 ± 0.87. There was no statistically significant difference between four groups with respect to age, diet, and tobacco intake ($P > 0.05$) [Table 1]. The cases those received RT had significantly higher premean SCC-Ag levels as compared to those who underwent hysterectomy [Table 2 and Figure 1].

Table 1: Demographic profile of two groups

Characteristics	Control (n=15) (%)	Cases (n=51) (%)	P
Age (years)			
≤45	7 (46.7)	29 (56.9)	0.281
>45	8 (53.3)	22 (43.1)	
Religion			
Hindu	12 (80.0)	51 (100.0)	0.008
Muslim	3 (20.0)	0 (0.0)	
Tobacco intake			
Nonuser	12 (80.0)	26 (51.0)	0.131
User	3 (20.0)	25 (49.0)	
Diet			
Nonvegetarian	7 (46.7)	15 (29.4)	0.304
Vegetarian	8 (53.3)	36 (70.6)	
Age at 1st intercourse (years)			
≤16	11 (73.3)	39 (76.5)	<0.001
>16	4 (26.7)	12 (23.5)	
Contraception used			
Temporary	4 (26.7)	5 (9.8)	0.026
Nonuser	8 (53.3)	41 (80.4)	
Tubal ligation	3 (20.0)	5 (9.8)	

Table 2: Associations of pretreatment squamous cell carcinoma antigen level with histopathological findings, treatment gives response to treatment of cases

Characteristics	n	Pre-SCC-Ag (ng/ml) (mean±SD)	T/F	P
Histopathological examination report				
Moderately differentiated	42	2.88±1.35	1.01	0.316
Poorly differentiated	9	3.36±0.87		
Treatment				
Hysterectomy	8	2.10±0.55	3.75	0.031
Concurrent chemo RT	34	3.28±0.99		
Palliative CT + RT	9	2.52±2.15		
Treatment response				
Complete responder	32	3.04±0.92	4.14	<0.001
Nonresponder	6	4.73±0.90		

RT - Radiotherapy; CT - Chemoradiotherapy; SCC-Ag - Squamous cell carcinoma antigen; SD - Standard deviation

The mean SCC-Ag level in all four stage groups decreased significantly ($P < 0.001$) after posttreatment as compared to pretreatment, and the decrease in posttreatment SCC-Ag level, increased linearly with stage severity [Table 3]. Further, comparing the change/or decrease (pre- to post-treatment) of four stages (FIGO I–IV). ANOVA ($F = 16.83$, $P < 0.001$) followed by Tukey's test revealed significantly ($P < 0.01$) higher decrease in Stage III as compared to both Stage I and Stage II; however, the decrease did not reach statistical significance ($P > 0.05$) when compared between Stage I and Stage II [Table 3].

Similarly, when comparison was done between total pre- and post-treatment values of SCC-Ag levels in Stage I, II and Stage III, t-test further revealed a significant difference ($P < 0.001$) and decrease of 66.7% in SCC-Ag levels posttherapy [Table 3 and Figure 2].

The mean SCC-Ag level in all three groups was compared separately, and significant difference was observed between Stage I versus Stage III pretreatment ($P < 0.001$), posttreatment difference ($P < 0.070$) as compared to other stages [Table 4].

DISCUSSION

In the present study, though the pretreatment mean SCC-Ag levels were higher in poorly differentiated squamous cell carcinoma as compared to moderately differentiated carcinoma but this difference was statistically not significant. Other study reported that close relation of SCC-Ag with squamous epithelium tumor and its raised level was more associated with more differentiated type of carcinoma.[8]

However, the clinical data revealed that there was no significant difference in the incidence of positive serum SCC-Ag concentration between undifferentiated type and more differentiated type of carcinoma.[9]

Authors reported elevated SCC-Ag levels in squamous cell carcinoma cases in terms of different percentage of patients having elevated SCC-Ag level in each stage. Higher SCC-Ag level in Stage III, cervical cancer ($P < 0.001$),[10] but in our study, all cases were had elevated SCC-Ag level and more rise with Stage III malignancy; similarly, other reported significantly higher SCC-Ag levels for Stage III lesions as compared to Stage I and Stage II.

It was higher in patients with higher stages of squamous cell carcinoma, and in the present study, it was observed

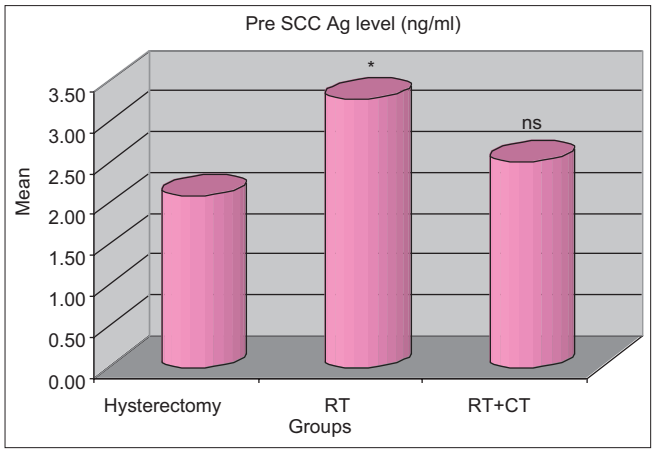

Figure 1: Premean squamous cells carcinoma antigen level according to treatment given to cases. $^{ns}P > 0.05$ or $^{*}P < 0.05$ as compared to hysterectomy

Table 3: Pre- and post-treatment squamous cell carcinoma antigen levels of International Federation of Gynecology and Obstetrics Stage I-IV and pretreatment squamous cell carcinoma antigen level of vaginal carcinoma

Stage groups	*n*	Pre	Post	Mean change (pre-post)	Percentage mean change	*P*
Stage I	8	2.10±0.55	0.50±0.08	1.61±0.55	76.4	<0.001
Stage II	15	3.15±0.84	1.22±0.64	1.93±0.59	61.2	<0.001
Stage III	15	4.12±0.89	1.35±0.57	2.77±0.76	67.3	<0.001
Stage IV	8	3.15±0.87	1.12±0.59	2.03±0.58	62.2	<0.001
Vaginal carcinoma	5	0.72±0.14	-	-	-	-
Total	51	3.31±1.10	1.12±0.62	2.19±0.80	66.7	<0.001

Table 4: Comparison (*P*) of mean squamous cell carcinoma antigen levels between the stages

Comparisons	Pretreatment	Posttreatment
Stage I versus Stage II	0.013	0.175
Stage I versus Stage III	<0.001	0.070
Stage II versus Stage III	0.004	0.996

that SCC-Ag level was increase with the increasing stage of cervical malignancy.

A balance between apoptosis and cell proliferation is crucial features for the maintenance of homeostasis in multicellular organism.[11] It is now well established that apoptosis plays an important role in the regulation of tumor progression.[12,13]

In malignant cell, apoptosis rate was lower as compared to normal cells but in esophageal cancer rate of apoptosis was higher in well-differentiated esophageal tumor than in poorly differentiated tumor.[14]

Other study reported that correlation between SCC-Ag and histological grade in esophageal squamous cell carcinoma.[15]

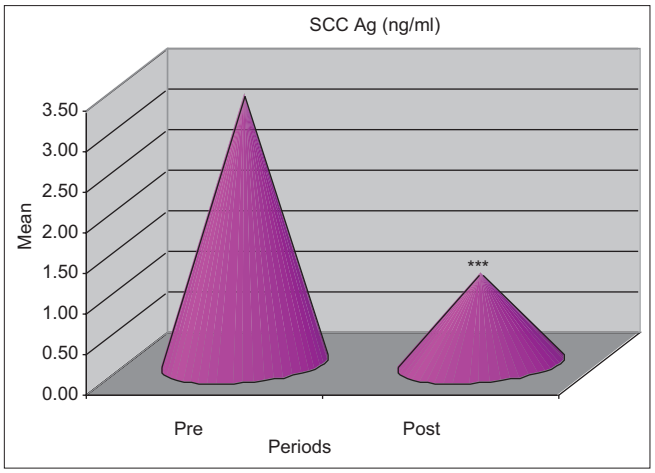

Figure 2: Overall mean change in squamous cells carcinoma antigen level of cases. (pretreatment vs. posttreatment). $^{***}P < 0.001$ as compared to pretreatment

In the present study, hysterectomy was done in 15.7% cases concurrent chemo-RT given in 66.7% cases and neoadjuvant chemotherapy (chemoradiotherapy + RT) was given in 17.6% cases. After treatment response was recorded in Stage I, II, and III in terms of decline in SCC-Ag level, 84.2% cases were responded completely to treatment and 15.8% were not responded to treatment. The cases those received RT had significantly ($P < 0.05$) higher premean SCC-Ag level as compared to patients with Stage I. Nonresponder patient had significantly ($P < 0.001$) elevated premean SCC-Ag level as compared to responder.

Author reported that an elevated pretreatment SCC-Ag can be used to predict the clinical response to neoadjuvant chemotherapy.[16]

Another study reported that patients with residual disease in duration and persistently elevated SCC-Ag level at 2–3 months after RT had a significantly higher incidence of treatment failure.[17]

In our study, SCC-Ag level shows rising trend with tumor size. Reduction of SCC-Ag level more than 50% was observed after treatment. Significant difference was observed in respect to percent change in SCC-Ag level with either microscopic lesion or lesion of more than 4 cm in size. Here, change in SCC-Ag level following treatment was generally universally similar and was less affected by different variables. Hence, prediction of treatment response was difficult and not feasible. Pretreatment SCC-Ag level, along with tumor size was useful in predicting recurrence and the need for postoperative adjuvant therapy.[18]

Few study reported that pre-RT SCC-Ag levels significantly correlated with pre-RT tumor volume and recurrence were

identified in patients with elevated pre-RT SCC-Ag levels.[19-21] In our study, >50% reduction in SCC-Ag level after treatment but difference was statistically not significant. Thus, this tumor marker might also be useful for monitoring the treatment effects and has prognostic value.

In advanced cancers, pretreatment serum SCC-Ag levels are associated with clinical stages, tumor sizes, and lymph node involvement. Furthermore, over 6 ng/ml of serum SCC-Ag level shows a significant independent effect on survival and disease-free survival.[22] Even in the early stage of uterine squamous cell carcinomas, elevated serum SCC-Ag levels predict pelvic lymph node involvement and are associated with a poor prognosis.[23]

Serum SCC-Ag levels are especially useful for monitoring treatment efficacy, disease progression, and recurrence. In general, increased serum SCC-Ag levels reflect disease progression and poor prognosis in squamous cell carcinomas.[24]

CONCLUSION

Thus, the combination of clinical pelvic examination and SCC-Ag levels provides useful information for the further need of treatment. If SCC-Ag increases during follow-up, then these patients were more careful worked up for locoregional recurrence.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
2. Sankaranarayanan R, Black RJ, Swaminathan R, Parkin DM. An overview of cancer survival in developing countries. Lyon, France: IARC Sci Publ; 1998. p. 135-73.
3. Golikeri P. A new vaccine for cervical cancer and some good hope. Mumbai; 2009. Available from: http://www.dnaindia.com/mumbai/report-a-new-vaccine-for-cervical-cancer-and-some-good-hope-1305997. [Last accessed on 2009 Nov 02].
4. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, *et al*. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC cancer base no. 11. Lyon, France: International Agency for Research on Cancer; 2013.
5. Kato H, Torigoe T. Radioimmunoassay for tumor antigen of human cervical squamous cell carcinoma. Cancer 1977;40:1621-8.
6. Nawata S, Murakami A, Hirabayashi K, Sakaguchi Y, Ogata H, Suminami Y, *et al.* Identification of squamous cell carcinoma antigen-2 in tumor tissue by two-dimensional electrophoresis. Electrophoresis 1999;20:614-7.
7. Murakami A, Suminami Y, Sakaguchi Y, Nawata S, Numa F, Kishi F, *et al.* Specific detection and quantitation of SCC antigen 1 and SCC antigen 2 mRNAs by fluorescence-based asymmetric semi-nested reverse transcription PCR. Tumour Biol 2000;21:224-34.
8. Takeuchi S, Nonaka M, Kadokura M, Takaba T. Prognostic significance of serum squamous cell carcinoma antigen in surgically treated lung cancer. Ann Thorac Cardiovasc Surg 2003;9:98-104.
9. Kato H, Suehiro Y, Morioka H, Torigoe T, Myoga A, Sekiguchi K, *et al.* Heterogeneous distribution of acidic TA-4 in cervical squamous cell carcinoma: Immunohistochemical demonstration with monoclonal antibodies. Jpn J Cancer Res 1987;78:1246-50.
10. Rao DN, Ganesh B. Estimate of cancer incidence in India in 1991. Indian J Cancer 1998;35:10-8.
11. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
12. Malaguarnera L. Implications of apoptosis regulators in tumorigenesis. Cancer Metastasis Rev 2004;23:367-87.
13. Townson JL, Naumov GN, Chambers AF. The role of apoptosis in tumor progression and metastasis. Curr Mol Med 2003;3:631-42.
14. Ohbu M, Saegusa M, Okayasu I. Apoptosis and cellular proliferation in oesophageal squamous cell carcinomas: Differences between keratinizing and nonkeratinizing types. Virchows Arch 1995;427:271-6.
15. Matsuda H, Mori M, Tsujitani S, Ohno S, Kuwano H, Sugimachi K. Immunohistochemical evaluation of squamous cell carcinoma antigen and S-100 protein-positive cells in human malignant esophageal tissues. Cancer 1990;65:2261-5.
16. Steller H. Mechanisms and genes of cellular suicide. Science 1995;267:1445-9.
17. Hong JH, Tsai CS, Chang JT, Wang CC, Lai CH, Lee SP, *et al.* The prognostic significance of pre- and posttreatment SCC levels in patients with squamous cell carcinoma of the cervix treated by radiotherapy. Int J Radiat Oncol Biol Phys 1998;41:823-30.
18. Chou CY, Wang ST, Kuo HC, Tzeng CC, Yao BL. Serum level of squamous cell carcinoma antigen and tumor size are useful to identify preoperatively patients at high risk of cervical cancer. Cancer 1994;74:2497-501.
19. National Cancer Registry Programme. Consolidated Report of the Population-based Cancer Registries 2001-2004. New Delhi: Indian Council of Medical Research; 2006.
20. Ohara K, Tanaka Y, Tsunoda H, Nishida M, Sugahara S, Itai Y. Assessment of cervical cancer radioresponse by serum squamous cell carcinoma antigen and magnetic resonance imaging. Obstet Gynecol 2002;100:781-7.
21. Brioschi PA, Bischof P, Delafosse C, Krauer F. Squamous-cell carcinoma antigen (SCC-A) values related to clinical outcome of pre-invasive and invasive cervical carcinoma. Int J Cancer 1991;47:376-9.
22. Ogino I, Nakayama H, Okamoto N, Kitamura T, Inoue T. The role of pretreatment squamous cell carcinoma antigen level in locally advanced squamous cell carcinoma of the uterine cervix treated by radiotherapy. Int J Gynecol Cancer 2006;16:1094-100.
23. van de Lande J, Davelaar EM, von Mensdorff-Pouilly S, Water TJ, Berkhof J, van Baal WM, *et al.* SCC-Ag, lymph node metastases and sentinel node procedure in early stage squamous cell cervical cancer. Gynecol Oncol 2009;112:119-25.
24. Hsu KF, Huang SC, Shiau AL, Cheng YM, Shen MR, Chen YF, *et al.* Increased expression level of squamous cell carcinoma antigen 2 and 1 ratio is associated with poor prognosis in early-stage uterine cervical cancer. Int J Gynecol Cancer 2007;17:174-81.