RESEARCH ARTICLE

Application of Tumor Markers SCC-Ag, CEA, and TPA in Patients with Cervical Precancerous Lesions

Farah Farzaneh[1]*, Shapour Shahghassempour[2], Bahram Noshine[2], Maliheh Arab[1], Mehdi Yaseri[3], Mitra Rafizadeh[4], Kamyab Alizadeh[1]

Abstract

Background: To determine the potential clinical utility of tumor markers CEA, TPA, and SCC-Ag for early detection of cervical precancerous lesions. Materials and Methods: A case-control study was carried out on 120 women (46 patients with histologically confirmed cervical precancerous lesions and 74 healthy controls). The significance of serum selected tumor markers in early detection of cervical intraepithelial neoplasia (CIN) were assessed. Results: Of the case group, the rates of CIN I, II, III, was 69.6%, 23.9%, and 6.5%, respectively. According to the manufacturer's cut-off values of 2ng/ml, 5ng/ml, and 70 U/ml for SCC-Ag, CEA and TPA tests, in that order, SCC-Ag test had a sensitivity of 13%, but CEA and TPA tests could not distinguish between case and control groups. The diagnostic sensitivities were highest at cut-off values of 0.55 ng/ml for SCC-Ag, 2.6ng/ml for CEA, and 25.5 U/ml for TPA which were 93%, 61%, and 50%, respectively. However, the area under the receiver operating characteristic curve was the largest for SCC-Ag (0.95 vs. 0.61 and 0.60 for CEA and TPA, respectively). Moreover, there was a highly significant direct correlation between SCC-Ag concentration and the degree of cervical precancerous lesions (r=0.847, p<0.001). Conclusions: The new cutoff of 0.5 for SCC-Ag test might be useful as a tumor marker in Iranian patients with CIN and it needs to be more evaluated by studies with larger populationa.

Keywords: Cervical cancer - CIN- SCC antigen - TPA antigen - carcinoembryonic antigen - tumour markers

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Introduction

Cancer of cervix is the third most common cancer and the fourth leading cause of cancer death of women worldwide. It is estimated that cervical cancer contributes to approximately 9% of the total new cancer cases and 8% of the total cancer deaths among women in 2008. However, over 85% of the world's cervical cancer burden is in developing countries where screening programs are not well established or minimally effective (Jemal et al., 2011). Although, screening programs for detection of cervical cancer precursors using Papanicolaou cytological testing (Pap smear) has shown to be very successful in developed countries, so that effective reduction in the incidence of cervical cancer by 75% to 90% as well as decrease in its related mortality by 50% occurred in industrial world (Wallace et al., 2007; Cuzick et al., 2008). The lack of such appropriate screening programs has led to high burden of cervical cancer in low resource countries (Parkin et al., 2008; Mathew and George 2009) where about 83% of new cases and 85% of cervical cancer deaths are reported from developing world (Porika et al., 2010).

The Pap smear results may be influenced by several factors including quality of specimens, technique of specimen collection by the clinician, and the skill of the cytologist/pathologist which all have led to high false-negative rates (Jesdapatarakul et al., 2011). A meta-analysis study of reported a sensitivity of 59% for conventional Pap test and while it was the least among other screening strategies including visual inspection with acetic acid (77%), magnified visual inspection with acetic acid (64%), visual inspection with Lugol iodine (91%), human papillomavirus testing with Hybrid Capture 2 assay (74%), and thin liquid-based cytology (88%) (Chen et al., 2012). Indarti et al. (2013) indicated that high survivin expression by immunocytochemistry or immunohistochemistry staining along with other risk factors including age, number of sexual partners, level of education, use of oral contraceptives, and positive high-risk HPV DNA can be useful for prediction of developing precancerous cervical lesions and the progressivity of CIN lesions. Therefore, finding new method of early detection of CIN and cervical cancer is still one important subject in medical research area.

Tumor markers are proteins or enzymes generated by tumor cells or host cells in response to tumorigenesis and during the past decade they were frequently used for screening and monitoring in different cancers. There are

*[1]Preventive Gynecology Research Center, Department of Obstetrics and Gynecology, [3]Clinical Research and Development Center, [4]Department of Pathology, Imam Hossein Hospital, [2]Tissue Engineering and Nanomedicine Research Center, Taleghani Teaching Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran *For correspondence: farahzaneh@yahoo.com*

many identified tumor markers associated with cervical cancer and precancerous lesions in their diagnosis, monitoring of response to therapy and detection of recurrence during follow-up. Kato and Torigoe first described the squamous cell carcinoma antigen (SCC-Ag) in 1977, a glycoprotein of 48 kDa which was isolated from SCC of the uterine cervix (Kato and Torigoe, 1977). The serum level of SCC-Ag elevated in about 30% to 90% of patients with cervical cancer according to stage (Jeong et al., 2011). Carcinoembryonic antigen (CEA) is another glycoprotein of 180 kDa which is expressed during embryonic development. It is overexpressed in a wide variety of tumor types and several studies reported its expression in cervical precancerous lesion, particularly in cervical adenocarcinomas (Tendler et al., 2000). Tissue polypeptide antigen (TPA) is a single-chain polypeptide with a molecular weight of 45 kDa which was first isolated from membranes of various human carcinomas in 1975 and can be detected in normal and malignant epithelium of the cervix (Volgger et al., 2008). It is considered to reflect tumor proliferation and reported to have a sensitivity of 28-34% for cervical cancer (Juang et al., 2000).

Based on the National Cancer Registry reports in Iran, cervical cancer is the 13th cancer among Iranian women and the age-standard incidence rate of 1.9 per 100,000 population is estimated in this area (Mousavi et al., 2009). The aim of this study was to determine the potential clinical utility of tumor markers SCC-Ag, CEA, and TPA for detection of cervical precancerous lesions in Iranian women referring to clinical colposcopy in two university hospitals.

Materials and Methods

A case-control study was conducted between September 2008 and January 2011 on all women referred to gynecology clinic of two tertiary university hospitals in Tehran, Iran. Patient group were women with abnormal Pap smears or any other complaints (post coital bleeding, abnormal cervical appearance, chronic vaginal discharge) who underwent colposcopy and had a cervical biopsy of any colposcopically identified abnormalities. Patients with histologically confirmed cervical precancerous lesions who had no previous treatment were enrolled in the study as case group. The control group consisted of women attending for routine Pap smear screening with no complaint necessitating the colposcopy examination and whose pelvic examination and Pap smear results were normal. If these women were volunteered to participate in this research, they asked to sign informed consent form for colposcopic evaluation and if the colposcopy evaluation was normal done by an expert colposcopist, they considered as control group. Therefore, the exclusion criteria for control group were: i) an abnormal cervical appearance; ii) a history of postcoital bleeding; iii) refractory vaginal discharge; iv) exposure to DES (diethylstilbestrol) in utero; v) a history of vaginal or vulvar neoplasia; vi) the presence of genital condyloma acuminatum; vii) partner with genital condyloma or genital neoplasia; viii) abnormal cervical appearance in colposcopy examination; and viiii) the eligible women who refused to participate in the study. The study protocol was approved by the Institutional Review Boards (IRB) of the Shahid Beheshti University of Medical Sciences as well as local research Ethics Committee of the university. All potential women who were admitted to participate in the study signed an informed consent form as well as a demographic questionnaire.

Blood samples were collected from control groups as well as patients (cases) before initiation of any treatment and sera were stored at -80°C until analysis. Serum antigen concentrations including SCC-Ag, CEA, and TPA were determined with a commercial quantitative colorimetric sandwich ELISA kit (Antibodies-online GmbH, Atlanta, Georgia, USA) according to the manufacturer's protocols. Assays for SCC-Ag, CEA, and TPA were carried out at the clinical chemistry laboratory of Taleghani University Hospital. Upper limits of normal according to the manufacturer were 2ng/ml for SCC-Ag, 5ng/ml for CEA, and 70 U/ml for TPA.

Values are presented as means±standard deviation (SD) or medians for quantitative variables and frequency (percentage) for qualitative variables. A 95% confidence interval (CI) for the ratio of the means between two groups was calculated. The values were compared between two groups using the unpaired t-test or among three or more groups using one-way analysis of variance. A chi-square or Fisher's exact test was used to compare categorical variables. Statistical analysis comparing area under the receiver operating characteristic (ROC) curve analysis using area under the curve (AUC) was performed for discrimination between groups, as an index of global tests performance. The Youden's Index was used to identify the optimal cut-off point for each tumor markers. The statistical comparison of the methods was performed using the McNemar's test. Adjusted odds ratios (ORs) and their 95% CIs were estimated with logistic regression models. Statistical analysis was carried out using (SPSS, Chicago, Illinois, USA, version 17) statistical software and the statistical significant level was defined as $p < 0.05$.

Results

A total of 120 women were included in the study. A series of 46 patients with histologically confirmed cervical precancerous lesions were enrolled as case group. The control group consisted of 74 women attending for routine Pap smear screening with no previous cervical pathology whose pelvic exam, result of Pap smear test and colposcopic exam were normal. The mean age of all participants was 40.9±10.9 years and there was no significant difference between two groups. Of the patient group, CIN I, CIN II, and CIN III lesions were seen in 32 (69.6%), 11 (23.9%), and 3 (6.5%) women, respectively. Table 1 shows the demographic characteristics and the comparison between cases and controls. There was a significant difference between two groups in the number of women with history of sexual intercourse before the age of 16 years which was reported in 25 (54.3%) and 6 (8.1%) women in cases and controls, respectively (OR=3.4, 95%CI 2.26-5.16, $p<0.001$). Surprisingly, the rate of vaginal discharge was significantly more in

controls [34 (45.9%)] compared with cases [11 (23.9%)] (OR=0.70, 95%CI 0.53-0.92, p=0.02). All other compared variables including gravidity, parity, history of post-coital bleeding, menopause, and using hormonal contraception demonstrated no differences between two groups. One and two women in case and control groups, respectively, had a positive history of smoking and one participant in control group was infertile.

The serum level of tumor markers including SCC-Ag, CEA, and TPA was compared between two groups. Patients with cervical pre-cancer lesions had significantly higher SCC Ag levels than those in control group (p<0.001). However, there was no difference between two groups in the mean of CEA and TPA levels (Table 2). The cut-off values for serum SCC-Ag, CEA, and TPA concentrations were 2ng/ml, 5ng/ml and 70U/ml, respectively, according to the manufacturer's instructions. These values provided an overall sensitivity of 13% for SCC-Ag test, but CEA and TPA tests could not distinguish between the patients and healthy individuals at these cut-off points. Furthermore, McNemar's test showed a significant difference between the result of all serologic tests at the given cut-off values and the cytology as the gold standard (p<0.001). However, the diagnostic sensitivity and specificity for detection of cervical precancerous lesions were highest at the following cut-off values: 0.55 ng/ml for SCC-Ag, 2.6ng/ml for CEA, and 25.5 U/ml for TPA. The sensitivity/specificity percentages for the new values were 93%/96%, 61%/62%, and 50%/70% for SCC-Ag, CEA, and TPA, respectively. Also, statistical analysis by McNemar's test determined no significant differences between the new cut-off points and the cytology results (Table 3). However, the AUC was the largest for SCC-Ag (0.95 *vs* 0.61 and 0.60 for CEA and TPA, respectively). With the cut-off of 0.55 for SCC-Ag, 43 (93.5%) women in case group versus 3 (6.5%) in control group had a positive result (OR=14.71, 95%CI 4.92-43.98, p<0.001). Moreover, there was a highly significant direct correlation between SCC-Ag concentration and the degree of cervical precancerous lesions (r=0.847, p<0.001).

Table 1. Comparison of Demographic Information, Gynecologic and Obstetric Histories in Case and Control Participants

	Cases (n=46)	Control (n=74)	OR*	95% CI	p value
Age (years)	40.9±10.9	39.7±11.3			0.561
Abnormal Cytology					
CIN I	32(69.6%)				
CIN II	11(23.9%)				
CIN III	3 (6.5%)				
1st coitus<16 years	25(54.3%)	6(8.1%)	3.42	2.26-5.16	<0.001
Gravid <3	15(34.1%)	33(46.5%)	0.72	0.44-1.19	0.24
Para <3	19(43.2%)	39(54.9%)	0.75	0.47-1.19	0.25
Post-coital bleeding	24(52.2%)	32(43.2%)	0.8	0.50-1.26	0.35
Vaginal discharge	11(23.9%)	34(45.9%)	0.7	0.53-0.92	0.02
Menopause	12(26.1%)	17(23%)	0.9	0.54-1.50	0.83
Hormonal contraception	6(13.0%)	11(14.9%)	1.1	0.55-2.19	>0.999

*CIN; cervical intraepithelial neoplasia

Table 2. The Mean Serum Levels of Tumor Marker

Tumor marker	Cases (n=46)	Control (n=74)	p value
SCC-Ag (ng/ml)	1.29±0.61	0.24±0.17	<0.001
CEA (ng/ml)	2.78±0.74	2.89±3.4	0.83
TPA (U/ml)	24.43±10.65	22.81±6.08	0.29

*SCC-Ag, squamous cell carcinoma antigen; TPA, tissue polypeptide antigen; CEA, carcinoembryonic antigen

Table 3. Tumor Markers Test Characteristics for the Presence or Absence of Cervical Cancer/Precancerous Lesions

Tumor marker	Cut-off values	Sensitivity	Specificity	PPV	NPV	McNemar's p value
SCC-Ag	>2 ng/ml	13%	100%	100%	65%	<0.001
	>0.55 ng/ml	93%	96%	93%	96%	>0.999
CEA	>5 ng/ml	-	99%	-	61%	<0.001
	>2.6 ng/ml	61%	62%	50%	72%	0.184
TPA	>70 U/ml	-	100%	50%	62%	-
	>25.5 U/ml	50%	70%	51%	69%	>0.999

*SCC-Ag, squamous cell carcinoma antigen; TPA, tissue polypeptide antigen; CEA, carcinoembryonic antigen

Discussion

Our study found that SCC-Ag can detect the presence of cervical precursors among Iranian women with a high sensitivity of 93% and an acceptable specificity of 96% when its upper limit was 0.55ng/ml.

Previous studies have shown that in early stage of cervical cancer, SCC-Ag can preoperatively predict the likelihood for adjuvant radiotherapy and detect high risk patients for recurrence as well (Reesink-Peters et al., 2005; Jeong et al., 2011). Also, its pretreatment levels was related to tumor stage, tumor size, depth of invasion, lymph-vascular space involvement, lymph node status and clinical outcome (Gadducci et al., 2004). Furthermore, it was the most accurate serologic tumor marker in cervical cancer staging and a cost-effective tool for monitoring of treatments (Esajas et al., 2001; Forni et al., 2007). The studies on relation of tumor markers and cervical cancer precursors are limited. However, we found new values for upper limit of tumor markers, especially SCC-Ag, to detect precancerous cervical lesions. In 1998, Hong et al found that cervical cancer patients with pretreatment SCC-Ag levels higher than 10ng/ml had a poorer prognosis (Hong et al., 1998). In the same year, Takeshima et al stated that SCC-Ag levels higher than 4ng/ml increased the risk of nodal metastasis by eight times (Takeshima et al., 1998). Although, there are inter-study variations in cut-off values, consistent conclusions include that the degree of elevation of SCC-Ag levels in patients with cervical lesions corresponding to extent of disease.

Further research with a larger sample size is required for a more definitive conclusion. The follow-up of patients with cervical precancerous lesions is recommended to evaluate the prognostic value of different tumor markers in the possibility of transition from precursor lesions to invasive carcinoma. The significant correlation between serum SCC-Ag and the degree of cervical neoplasia and especially the new value of 0.55ng/ml as a cut-off point for detection of cervical precursor lesions indicates that it can be useful in screening programs along with other methods to detect cervical cancer in the neoplastic process which leads to early treatment without operative intervention and with preservation of the patient's fertility.

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