RESEARCH ARTICLE

Serum Vascular Endothelial Growth Factor-A (VEGF-A) as a Biomarker in Squamous Cell Carcinoma of Head and Neck Patients Undergoing Chemoradiotherapy

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Abstract

Background: To evaluate serum VEGF-A levels in squamous cell carcinoma of head and neck (SCCHN) patients and relationships with response to therapy. Materials and Methods: Serum VEGF-A levels in patients (n=72) treated with radiotherapy (RT) or radio-chemotherapy (RCT) and controls (n=40) were measured by ELISA. Results: Serum VEGF-A levels of the SCCHN cases were significantly higher (p=0.001) than in healthy controls, and in patients with positive as compared to negative lymph node status (p=0.004). Similarly, patients with advanced stage (Stage III-IV) disease had more greatly elevated levels of serum VEGF-A level than their early stage (Stage I-II) counterparts (p=0.001). In contrast, there was no significant difference (p=0.57) in serum level of VEGF-A in patients with advanced T-stage (T3-4) as compared to early stage (T1-2). Similarly, patients with distant metastasis had no significant (p=0.067) elevation in serum VEGF-A level as compared to non-metastatic disease. However, the non-responder patients had significantly higher serum VEGF-A level as compared to responders (p=0.001). Conclusions: Our results suggest that the serum VEGF-A level may be a useful biomarker for the prediction of response to therapy in SCCHN.

Keywords: VEGF-A - squamous cell carcinoma of head and neck - serum levels - prognostic marker

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Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is the 6th most common malignancy world-wide, arising in the upper aerodigestive tract, encompassing the oral cavity, oropharynx, hypopharynx, pharynx and larynx (Syrigos et al., 2009). The prevalence of SCCHN is increasing worldwide (Jefferies et al., 1999). In many developing countries, the prevalence of SCCHN is increasing dramatically and appears as a major threat for public health (Song and Grandis, 2000). Within the United States, the American Cancer Society estimates that there will be 52,140 new cases and 11,460 deaths attributable to these cancers in 2011 (Lu et al., 2011). The common risk factors for HNSCC are tobacco smoking or chewing with pan and alcohol (Elango et al., 2006; Hashibe et al., 2007). HPV infection is also considered as a causal factor for SCCHN (Hocking et al., 2011). In India, the incident rate of SCCHN is much higher than the rest of world (Anuradha et al., 2013). Cancers of the tongue as well as buccal mucosa have been noted to be quite common in India, attributed to the local custom of chewing pan, betel leaf with tobacco (Sellappa et al., 2009).

Patients with SCCHN often present with symptoms at a late stage and high recurrence rate and lower survival rate after treatment, especially in those with neck lymph node metastasis (Ferris et al., 2005; Wang et al., 2013). Despite of improved modern therapeutic interventions, the 5-year survival rate for this disease has improved only marginally over the past decade and recurrent disease is observed in 50% of the patients (Le et al., 2003; Eto et al., 2007; Pulte et al., 2010). The median overall survival for patients with recurrent or metastatic SCCHN remains less than 1 year (Price et al., 2012). Tumour stage and grade are prognostic factors in cancer but do not always distinguish between low risk and high risk patients (Wasif et al., 2010). Efforts are being undertaken for better understanding of the biology of SCCHN which could identify new prognostic and predictive factors allowing tailoring of therapeutic intervention. Treatment failure for SCCHN can be attributed to multiple factors which are difficult to predict for a particular patient. Factors such as age, sex, tumor site, TNM stage, and histological grade may help guide therapy but are not reliable predictors for

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outcome (Rubin Grandis et al., 1998). Although clinical parameters such as nodal status and tumour stage are often employed to guide treatment (Bacci et al., 1998), but their usefulness is limited by the fact that most patients present with stage III or IV tumors (Sobin et al., 1988).

In this endeavour, serum vascular endothelial growth factor (VEGF) level have been evaluated in various well formatted researches all over the world. VEGF is a multifunctional cytokine that exerts a variety of effects on vascular endothelial cells that together promote the formation of new blood vessels, increases vascular endothelial permeability, stimulates the proliferation of endothelial cells and promote cancer cell progression (Dvorak, 2002; Mohammed et al., 2007; Niu et al., 2010; Akdeniz et al., 2013; Huang et al., 2013; Zhang et al., 2013). In particular, serum VEGF-A levels are elevated in patients with HNSCC compared to healthy controls (De Schutter et al., 2005; Hong et al., 2009; Liang et al., 2013). A negative prognostic role for circulating serum VEGF-A levels has been implicated in laryngeal carcinoma (Teknos et al., 2002). A decrease in serum VEGF-A level after cancer surgery has been reported in breast cancer (Tang et al., 2011) and in ovarian cancer (Färkkilä et al., 2011). Jubb et al. (2004), observed that VEGF-A is significantly upregulated in various malignancies including SCCHN. Based on these observations, we have tried to evaluate the predictive significance of serum VEGF-A level in SCCHN undergoing radio-chemotherapy.

The purpose of this study is to investigate the inter-relationship and the predictive significance of serum VEGF-A level as a biological marker along with clinico-pathological parameters in patients of SCCHN treated by radiotherapy and chemotherapy. Serum VEGF-A level may stratify these tumors in favourable and unfavourable groups helping in therapeutic decision making.

Materials and Methods

Clinical specimen

Seventy two patients with SCCHN who were attending the OPD of Radiotherapy, King Georges Medical University, Lucknow were subjected for this study. All patients provided written informed consent. Blood samples were collected from HNSCC patients at baseline. Tumour (T) stage, nodal (N) status and TNM stage were classified according to the 1997 American Joint Committee on Cancer (AJCC) system. Patients with stage I & II tumors were treated with radiotherapy alone and patients with stage III & IV disease received radio-chemotherapy. Control samples ware collected from age and sex matched healthy voluntaries (n=40). Patients samples were evaluated for the level of serum VEGF-A at the baseline of treatment and compared to serum level of healthy controls. The study was approved by Institutional ethics committee, King George's Medical University, Lucknow, INDIA.

Radio-chemotherapy

Early stage patients (Stage I-II, n= 21) were treated with RT alone using a telecobalt machine (Theratron 78^{o}C, AECL, Ottawa, Canada). A dose of 70 Gy of Radiation in 7 weeks by a shrinking field technique was delivered using 2 Gy/ fraction. Patients with advanced stage (Stage III-IV, n=51) received RCT. The dose of radiation was same as mentioned above for early stage patients. Synchronous chemotherapy in the form of injection cisplatinum 30 mg/ m^2 weekly was delivered with adequate hydration, diuresis and anti-emetic prophylaxis. Patients were evaluated one month after the completion of radiotherapy or combination chemo-radiotherapy for response. The response in primary tumour was evaluated using WHO criteria. Complete response (CR) was defined as the disappearance of the tumour; partial response (PR), a reduction of >50% of tumour and rest of the patients with neither CR nor PR were considered as non-responder (NR). CR and PR patients were considered as responders and NR (stable disease SD and progressive disease PD) patients were classified as clinical non-responders.

ELISA

Serum VEGF-A levels were measured with commercially available ELISA kit following manufacturer's instructions (Quantikine VEGF Immunoassay, R&D Systems, Minneapolis, MN, USA). In brief, all samples were analyzed in duplicates at 490 nm with a 96 well plate reader (Fluostar Omega Spectrofluorometer, BMG Technologies, Offenburg, Germany) and mean values were calculated. Serum VEGF- A levels were expressed in nanograms per millilitre.

Statistical analysis

All statistical analysis and graphs were performed with the SPSS 11.5 and graphpad Prism 5 Software. Mann–Whitney tests was conducted to compare between different clinicopathological groups. All data are presented as mean±SEM. Tests were considered significant with p values ≤0.05.

Results

Patient characteristics

Histologically proven SCCHN patients were recruited into this study. Patient characteristics are shown in Table 1.

Serum VEGF-A level in SCCHN

The circulating serum VEGF-A level in SCCHN patients (n=72) as well as from healthy normal controls (n=40) were evaluated. It was found that serum VEGF-A level was significantly (p=0.001) elevated in SCCHN patients (316.51±14.16 pg/μL; Mean±SEM) than those of healthy controls (113.33±10.84 pg/μL; Mean±SEM) Figure 1A and Table 2.

Furthermore, there was a significant elevation in serum VEGF-A level in patients with positive lymph nodes (p=0.004). Serum VEGF-A level in node positive patients was (339.9±16.73 pg/mL; Mean±SEM) in comparison to lymph node negative tumors (260.6±19.11 pg/mL; Mean ±SEM) (Figure 1E and Table 3). Similarly, the significant elevation of serum VEGF-A levels were observed in patients with advanced stage (III-IV) than the patients with early stage I-II (Mean ±SEM; 356.1±13.76 vs 206.0±16.07

pg/mL; respectively; p= 0.001) (Figure 1F and Table 3). However, there was no significant difference (p=0.57) in serum level of VEGF-A in patients with advanced T-stage (T3-4) as compared to early stage of disease (T1-2) (Figure 1D and Table 3). Similarly, the patients with distant metastasis had no significant (p=0.067) elevation in serum VEGF-A level as compared to non-metastatic disease (Figure 1F and Table 3).

Serum VEGF-A level and treatment response

One month after the completion of radiotherapy or radio-chemotherapy, patients were evaluated for response to the treatment. CR was achieved in 20.8% patients (15/72) and PR in 19.4% patients (14/72) resulting in an overall response rate of 40.2% (29/72 patients).

Table 1. General Characteristics of Patients

Characteristics		N (%)
Age (Year)	Mean age	45
	Range	28-62
Sex (No.)	Male	65 (90.3)
	Female	7 (9.7)
Karnofsky performance score (No.)	100	5 (6.8)
	90-100	28 (38.9)
	80-90	31 (43.0)
	<80	8 (11.3)
Clinical stage (T)	1	12 (16.7)
	2	26 (36.1)
	3	18 (25.0)
	4	16 (22.2)
Nodal disease (N)	0	25 (34.7)
	1	19 (26.4)
	2	21 (29.2)
	3	7 (9.7)
Distant metastasis (M)	0	66 (91.7)
	1	6 (8.3)
Stage	I	6 (8.3)
	II	15 (20.9)
	III	23 (31.9)
	IV	28 (38.9)

Table 2. Serum VEGF-A Level in SCCHN and Healthy Control

Group	n	Mean±SEM	p value
Control	40	113.3±10.84	
SCCHN	72	319.3±14.48	<0.001*

Table 3. Correlation between the Clinicopathological Features and Serum VEGF-A Level in SCCHN

Patient characteristics		n	Mean±SEM	p value
Tumour	1-2	38	305.2±19.14	
	3-4	34	320.3±19.07	0.57
Node	ve-	25	260.6±19.11	
	ve+	47	339.9±16.73	<0.004*
Metastasis	0	66	304.91±13.90	
	x	6	394.16±42.24	0.067
Stage	Early stage (I-II)	21	206.0±16.07	
	Advanced Stage (III-IV)	51	356.1±13.76	<0.001*

Table 4. Correlation between the Serum VEGF-A level and Response to Therapy in SCCHN

Group	n	Mean±SEM	p value
Responders (CR+PR)	29	222.1±16.84	
Non-responders (PD+SD)	43	373.2±12.90	<0.001*

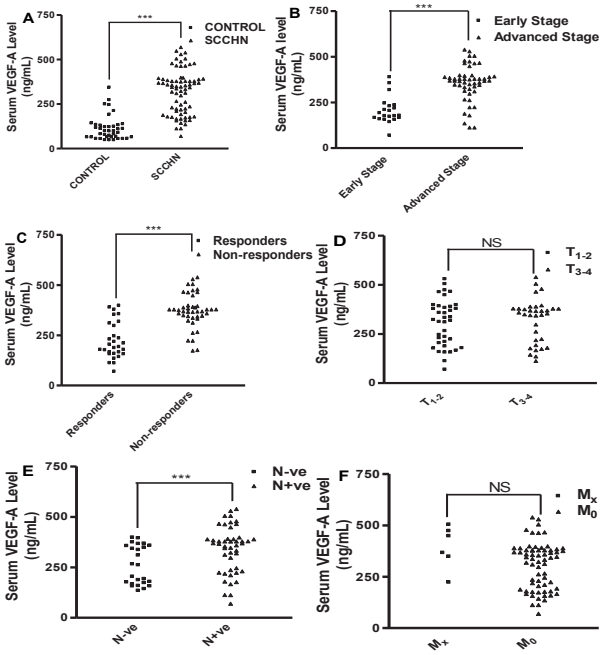

Figure 1. Serum VEGF-A Level in SCCHN. A) Serum VEGF-A level in SCCHN patients in comparison to healthy control; **B)** Advanced stage *vs* early stage; **C)** Responders *vs* Non responders; **D)** Advanced T-stage (T3-4 vs. T1-2); **E)** Node negative *vs* Node positive and; **F)** Distant metastatic *vs* Non metastatic disease. A p value <0.05 was considered statistically significant

On the other hand, NR (Stable disease+Progressive Disease) was observed in 59.8% (43/72 patients). The non-responder patients had significantly elevated level of serum VEGF-A (Mean±SEM; 373.2±12.90 pg/mL) as compared to responders (Mean±SEM; 22.1±16.84 pg/mL) and difference was statistically significant (p=0.001) (Figure 1C and Table 4).

Discussion

VEGF is a member of the platelet-derived growth factor/VEGF family that specifically acts on endothelial cells. It promotes the proliferation of vascular endothelial cells and angiogenesis (Lv et al., 2010). Serum VEGF-A is highly elevated in several types of cancer including colon (De Vita et al., 2004), cervix (Zusterzeel et al., 2009), breast and gynaecological cancer (Koukourakis et al., 2011), non-small cell lung cancer (Liang et al., 2013) and prostate (Singh et al., 2013). In our study, serum VEGF-A level was found to be significantly higher (p=0.001) in SCCHN patients as compared to healthy controls. A similar study previously done by Riedel et al. (2000), De Schutter et al. (2005) and Hong et al. (2009) found that serum VEGF-A level is elevated in head and neck cancer patients as compared to healthy controls.

The correlation of serum VEGF-A with clinicopathological parameters have been evaluated in several study. We also correlated serum VEGF-A levels with clinicopathological parameters in our study. As regard to the relationship between serum VEGF-A levels and clinicopathological parameters in our study, we found that elevation of serum VEGF-A levels were significantly associated with regional lymph node metastasis and

advanced stage (Stage III-IV) of tumour but not with other clinicopathological characteristics. We found that serum VEGF-A level was significantly (p=0.001) elevated in patients with positive lymph node status in comparison to node negative patients. Similarly, the studies in SCCHN (Linder et al., 1998) and breast cancer (Kümmel et al., 2006; Mohammed et al., 2007), revealed that serum VEGF-A expression was significantly associated with lymph node metastasis. They found significantly higher level of serum VEGF-A in node positive vs. node negative patients. Their results were similar to result obtained in our study. We also found that serum VEGF-A level was significantly elevated in advanced stage of tumour (Stage III-IV) in comparison to early stage (Stage I-II) of tumour. Our results are similar to the findings of previous study by Shang et al. (2002) in oral squamous cell carcinoma. However, Bachtiary et al. (2002) and Zusterzeel et al. (2009) did not find any significant correlation between stage of disease and serum VEGF-A level in carcinoma of cervix.

We also correlated the serum VEGF-A level with T-stage and metastatic status in our study. We found no significant correlation between serum VEGF-A level and advanced T-stage (T3-4) as compared to early T-stage (T1-2). It was also not significantly different in metastatic patients vs non-metastatic patients (p=0.067). However, the study in laryngeal cancer by Lv et al. (2011) found that serum VEGF-A level was elevated in advanced T-stage patients (T3-4) in comparison to early T-stage (T1-2); and it was also elevated in patients with metastatic disease in comparison to non-metastatic disease. Similar study by Kemik et al. (2011) in colorectal cancer, observed significantly higher level of serum VEGF-A in (T3-4) vs early T-stage (T1-2); and it was also elevated in patients with metastatic disease as compared to non-metastatic disease. This conflicting result shows necessity of further evaluation of Serum VEGF-A level in large cohort of patients.

We also analysed the serum VEGF-A levels and its association to treatment response. In our study, it was found that higher treatment response rate was achieved in patients with lower serum VEGF-A level group as compared to those with higher serum VEGF-A level. Thus, we found that serum VEGF-A level is a significant (p=0.001) negative predictor of response to radiotherapy or radio-chemotherapy in SCCHN. Similarly, the study done by Song et al. (2013) in non-small cell lung cancer, revealed that elevated serum VEGF-A significantly correlated with treatment response. In contrast, previous study done by Caballero et al. (2007) in cervix cancer (n=33), they evaluated a small group of patients (n=33), found that no significance difference in serum VEGF-A level between responders and non-responders. The similar study done by Katanyoo1 et al. (2011) in 40 cervix cancer patients, they did not found any significant correlation between serum VEGF-A level in responders as compared to non-responders. The incongruity between the previous study and present study results may be because of small sample size in previous study.

In conclusion, serum VEGF-A level was found to be significantly elevated in SCCHN patients compared to with healthy controls in our study. Serum VEGF-A level was significantly higher in patients with lymph node positivity and advanced stage of disease. Patients with higher serum VEGF-A levels had poorer response to RT/RCT. It is interesting to speculate that higher serum VEGF-A level may be useful in differentiating a subset of SCCHN patients who are less likely to respond to RT/RCT and who may be suitable for some other therapeutic strategy or more aggressive treatment. The ultimate utility of serum VEGF-A as a predictive biomarker for poor response of therapy in SCCHN needs to be evaluated on a larger patient population of SCCHN.

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