Clinical Oncology 25 (2013) 308–317

Contents lists available at SciVerse ScienceDirect

Clinical Oncology

journal homepage: www.clinicaloncologyonline.net

Original Article

Elevated Serum Endostatin Levels are Associated with Poor Survival in Patients with Advanced-stage Nasopharyngeal Carcinoma

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Received 12 January 2012; received in revised form 22 October 2012; accepted 29 October 2012

Abstract

Aims: To evaluate the prognostic value of serum endostatin levels in patients with advanced-stage nasopharyngeal carcinoma (NPC).
Materials and methods: Between August 2003 and March 2005, 218 patients with advanced-stage NPC were enrolled in this study, including 70 patients in the training cohort and 148 in the validation cohort. The pre-treatment serum endostatin and vascular endothelial growth factor (VEGF) levels were measured using competitive enzyme immunoassays. For the normal control, serums samples from 20 healthy individuals were also collected.
Results: Serum endostatin levels in the patients with advanced-stage NPC were significantly higher than those of controls, but VEGF levels were similar in the two groups. Univariate analysis revealed significant differences between the high and low endostatin level groups regarding 5 year overall survival (63.9% versus 90.5%; $P = 0.003$), progression-free survival (PFS) (50.2% versus 79.3%; $P = 0.003$) and distant metastasis-free survival (DMFS) (59.1% versus 85.3%; $P = 0.01$) in the training cohort. Using the same cut-off value generated from the training cohort, there were also significant unfavourable correlations between serum endostatin levels and overall survival ($P = 0.001$), PFS ($P = 0.001$) and DMFS ($P = 0.002$) in the second independent validation cohort. Multivariate analysis using the entire group ($n = 218$) revealed that the serum endostatin level was an independent unfavourable prognostic factor for overall survival (hazard ratio 4.8; 95% confidence interval 2.48–9.23; $P < 0.0001$), PFS (hazard ratio 3.44; 95% confidence interval 2.06–5.74; $P < 0.0001$) and DMFS (hazard ratio 3.65; 95% confidence interval 1.92–6.94; $P < 0.0001$) in patients with advanced-stage NPC. No associations were observed between the outcomes and the serum VEGF levels in patients with advanced-stage NPC.
Conclusions: High endostatin levels are associated with poor survival and this knowledge may improve the risk stratification of patients with advanced-stage NPC.

Key words: Distant metastasis; endostatin; nasopharyngeal carcinoma; prognosis; vascular endothelial growth factor

Introduction

Nasopharyngeal carcinoma (NPC) is a common malignancy in southern China, with an annual incidence of 15–50 cases per 100 000 people [1]. NPC is radiosensitive and is traditionally treated using radiotherapy. More than 50% of patients with advanced-stage NPC eventually develop post-treatment distant metastasis after radiotherapy alone [2,3]. Although a combination of chemotherapy with standard radiotherapy has improved treatment outcome in patients with advanced-stage NPC, the incidence of metastasis remains high [4–9]. Clinical parameters such as nodal and tumour stage are often used to guide treatments [10]. More accurate prognostic stratification at the time of diagnosis is essential for further improving treatment outcome. The use of circulating prognostic biomarkers has been considered to be worthy of exploration.

Angiogenesis-related cytokines may play an important role in determining the outcome of cancer patients because tumour angiogenesis is necessary for the growth and

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0936-6555/$36.00
http://dx.doi.org/10.1016/j.clon.2012.11.009

dissemination of solid tumours [11]. It has been proposed that the angiogenic phenotype of tumours is the result of a net balance between positive and negative regulators of neovascularisation. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor are major positive regulators of tumour-related angiogenesis [12–14]. Endostatin, a naturally occurring 20 kDa carboxy-terminal proteolytic fragment derived from type XVIII collagen [15], is one of the better-characterised endogenously produced angiogenesis inhibitors that interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor and VEGF [16]. Elevated serum endostatin levels have been reported to be associated with poor outcome or an aggressive phenotype in a variety of malignancies, including renal cell carcinoma, non-small cell lung cancer, non-Hodgkin lymphoma, soft tissue sarcoma, metastatic gastric carcinoma and breast cancer [17–23]. The clinical significance of the serum endostatin level in the patients with NPC is unknown. In the present study, we evaluated the serum levels of endostatin and VEGF and investigated their relationship with survival in two independent cohorts of patients with advanced-stage NPC.

Materials and Methods

Patients and Serum Samples

Patients with biopsy-confirmed, previously untreated NPC with stage III and IV (based on the criteria of the 2002 American Joint Committee on Cancer) were eligible for this study [10]. Other criteria included age older than 18 years and an Eastern Cooperative Oncology Group performance status of 0 or 1. The exclusion criteria included the presence of a distant metastasis or other concomitant malignant disease. The testing cohort of patients comprised 70 consecutive patients who were recruited between August 2003 and December 2004, with most of them receiving induction chemotherapy followed by radiotherapy. The validation cohort comprised 148 consecutive patients recruited between January 2004 and April 2005, with most of them receiving concurrent chemoradiotherapy with or without induction chemotherapy. This study was approved by the Clinical Research Ethics Committee of the Sun Yat-sen University Cancer Center, and written informed consent was obtained from all patients. All patients were evaluated with a complete physical examination, fibre optic nasopharyngoscopy and biopsy, magnetic resonance imaging (MRI) of the head and neck, chest X-ray, abdominal sonography, bone scan, complete blood count and biochemical profiles. The characteristics of the two cohorts were comparable (Table 1). The serum samples of 20 healthy individuals (15 men; five women) without any evidence of disease were also collected for controls, with a median age of 44 years (range 20–70 years).

Treatment

All patients were treated with a uniform radiotherapy protocol, as previously described [2]. Megavoltage photons (6–8 MV) were used to treat the primary tumour and neck area with conventional fractionation (radiotherapy was given five times per week at 2 Gy/day). The accumulated radiation dose to the primary tumour was 68–70 Gy. The accumulated doses to the involved and uninvolved areas of the neck were 60–62 and 50 Gy, respectively.

Before January 2004, induction chemotherapy followed by radiotherapy was typically used to treat advanced-stage NPC in our department. Since January 2004, concurrent chemoradiotherapy with or without induction chemotherapy has been recommended to treat advanced-stage NPC [5,7,24]. Induction chemotherapy was used to treat 62 patients (88.5%) in the testing cohort. Concurrent chemoradiotherapy with or without induction chemotherapy was used to treat 129 patients in the validation cohort (87.2%). For induction chemotherapy, two to three cycles of PF chemotherapy (100 mg/m^2 cisplatin intravenous drip on day 1 and 1000 mg/m^2/day 5-fluorouracil [5-FU] continuous intravenous drip for 120 h) repeated every 3 weeks were administered. For concurrent chemoradiotherapy, 100 mg/m^2 DDP on days 1, 22 and 43 during radiotherapy or 40 mg/m^2 DDP weekly during radiotherapy was provided.

Salvage treatments were used after documented relapse or when the disease was persistent, whenever possible. These treatments included re-irradiation, chemotherapy and/or surgery.

Assessment of Serum Endostatin and Vascular Endothelial Growth Factor Concentrations

The serum levels of endostatin and VEGF were measured using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). All samples were prepared and tested in duplicate according to the manufacturer's instructions, and the mean values were determined. All serum samples were measured by an investigator who was blind to the clinical data.

Follow-up

After the completion of treatment, the patients were followed-up at least every 3 months during the first 3 years and every 6 months thereafter until death. All events were measured from the date of enrolment; 94.0% (205/218) of the patients started treatment within 7 days from the date of enrolment. All local recurrences were diagnosed using fibre optic endoscopy and biopsy and/or MRI of the nasopharynx and the skull base showing progressive bone erosion and/or soft tissue swelling. Regional recurrences were diagnosed based on a clinical examination of the neck and, in suspected cases, by fine needle aspiration or MRI of the neck. Distant metastases were diagnosed based on clinical symptoms, a physical examination and imaging methods, including chest radiography, abdominal sonography, whole-body bone scans, computed tomography and MRI. In the event of death, the reasons for death were verified using the medical records or death certificates or by either relatives or the primary physicians who had witnessed the death. The follow-up ended on 31 May 2010,