RESEARCH ARTICLE

Prognostic Value of Pretreatment Serum Alkaline Phosphatase in Nasopharyngeal Carcinoma

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Abstract

Background: The prognostic value of serum alkaline phosphatase (S-ALP) has not been fully validated for nasopharyngeal carcinoma (NPC). Materials and Methods: S-ALP levels were measured in 601 patients newly diagnosed with NPC before radical treatment, and possible associations of these levels with 5-year overall survival (OS) and tumor-free survival (TFS) were explored using univariate and multivariate analyses. Results: Elevated pretreatment S-ALP (>85 U/L) was significantly less frequent among patients classified as T1+2 or stage I+II than among those classified as T3+4 or stage III+IV. Multivariate analysis showed that elevated pretreatment S-ALP (>85 U/L), age, T classification and N stage were independent predictors of poor OS and TFS. Conclusions: Pretreatment S-ALP may be a reliable biomarker to evaluate the long-term prognosis of patients with NPC.

Keywords: Nasopharyngeal carcinoma - pretreatment - serum - alkaline phosphatase - prognosis

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Introduction

Nasopharyngeal carcinoma (NPC) is common in southern China, and Guangdong and Guangxi province are the largest continuous endemic areas. In these areas, NPC occurs with an incidence up to 20-30/100, 000 per year (Yu et al., 2002), accounting for approximately 50% of the 80, 000 cases newly diagnosed each year around the world (Wildeman et al., 2012).

The primary treatment for NPC is radical radiotherapy with or without chemotherapy. Although the survival rate has significantly improved as these procedures have advanced, approximately 50% of NPC patients experience recurrence, especially patients with late-stage disease (Huncharek et al., 2002; Teo et al., 2002). This highlights the need for accurate evaluation of NPC prognosis. The TNM classification is widely used to predict prognosis (Tang et al., 2010), but this scheme fails to capture the heterogeneous prognoses among patients sharing the T classification or the variability in survival and response to chemotherapy among NPC patients with distant metastasis (Fandi et al., 2000; Au et al., 2003). Thus, defining novel prognostic indicators is of great importance.

In many malignant tumors, serum markers have been shown to be useful prognostic factors (Saif et al., 2005; Li et al., 2012; Fei et al., 2013). Such markers can normally be assayed easily, allowing rapid, inexpensive and noninvasive evaluation of disease development and prognosis (Ma et al., 2004). Among serum markers, serum enzymes are increasingly under investigation as potential diagnostic and prognostic markers in malignant tumors (Li et al., 2012; Wan et al., 2013). Elevated levels of serum alkaline phosphatase (S-ALP) have been reported to predict poor outcomes in various malignant cancers, including prostate carcinoma, colorectal carcinoma, and malignant tumors in soft tissues of the extremities (Bacci et al., 2002; Saif et al., 2005; Sonpavde et al., 2012). Whether S-ALP has prognostic value in NPC is unclear. To the best of our knowledge, only one study in English or Chinese has been published on this question (Li et al., 2012). The authors of that study analyzed 533 cases from a single institute in Guangdong province and suggested that S-ALP levels above the normal upper limit (>110 U/L in their work) predicted poor overall survival (OS) and local recurrence-free survival. However, they failed to identify this marker as an independent prognostic factor in multivariate analysis, raising doubts about its prognostic value. In addition, these patients were from a single endemic area of southern China, raising the question of whether the results are valid for other populations.

To address these questions, we retrospectively analyzed patient data for a large cohort of NPC patients treated at our hospital in Guangxi province, the second large endemic area of NPC in southern China. These patients are likely to be representative of the entire province, as ours is the only medical center in Guangxi focusing on NPC prophylaxis and treatment. We analyzed possible correlations of pretreatment S-ALP with OS and tumor-free TFS. This study aimed to provide more extensive evidence for, and deeper insights into, the prognostic value of pretreatment S-ALP for predicting the long-term outcome of NPC patients in endemic areas.

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Materials and Methods

Patients

This study retrospectively analyzed data from patients newly diagnosed with NPC and treated in the radiotherapy department at the Affiliated Tumor Hospital of Guangxi Medical University in Guangxi, China from July 2003 to March 2006. This study was approved by the Ethics Committee of Guangxi Medical University. The patients analyzed have been studied in previous work examining the association between lactate dehydrogenase levels and prognosis (Wei et al., 2014). The inclusion criteria for patients in this study were as follows: (a) histological diagnosis with type II or III NPC based on the World Health Organization typing system; (b) analysis of the nasopharynx and head and neck using computed tomography or magnetic resonance imaging before radical treatment; (c) no radiotherapy or chemotherapy before collection of blood for S-ALP measurements; (d) no evidence of distant metastasis or secondary carcinoma at diagnosis; (e) complete radical radiotherapy, with or without chemotherapy; and (f) absence of serious heart, lung, kidney, liver and bone problems that would disqualify patients from receiving radiotherapy and chemotherapy.

For all patients, the following data sources were consulted: complete medical history, physical examination, full blood count, baseline serum biochemistry analysis, nasopharyngo-fiberscope inspection and nasopharyngeal tumor biopsy. All patients were classified according to the American Joint Committee on Cancer (AJCC) staging system (6th edition). Patients were classified as positive for smoking if they had smoked at least 100 cigarettes in their lifetime (Qin et al., 2011).

S-ALP measurements

Fasting blood samples were obtained from all patients through venipuncture before radical treatment. S-ALP levels were assayed using a commercial kit according to the manufacturer's instructions (Beijing Strong Biotechnologies, Beijing, China). Measurements were carried out on a Hitachi 7170A automated analyzer. The manufacturer-specified normal range of S-ALP using this kit is 45-135 U/L.

Treatment

All patients received radical radiotherapy using conventional or three-dimensional conformal radiotherapy (3DCRT). The following cumulative radiation doses were delivered: nasopharyngeal region, 64-89 Gy; neck (N1, N2, or N3), 70-80 Gy; and preventive dosing to the neck without lymph node involvement (N0), 50-64 Gy. In addition, 338 of 601 patients (56.2%) also received cisplatin-based induced, concurrent or adjuvant chemotherapy.

Patient follow-up and determination of OS and TFS

After treatment, patients were followed-up with routine checkups in our hospital or at a local hospital every 3-6 months during the first 3 years after treatment, and subsequently every 3-12 months. Follow-up data from checkups in local hospitals were obtained by telephone. OS was defined from the time of diagnosis to the time of death or until the last recorded follow-up visit if the patient was still alive at the end of the study. TFS was defined from the time of diagnosis to the time of tumor progression, death, or last follow-up if the patient was still alive.

Statistical analysis

Data were analyzed statistically using SPSS 15.0 software (IBM, USA). OS and TFS were calculated using the Kaplan-Meier method. To identify the proper cutoff value of S-ALP, we used the minimum p value approach (Altman et al., 1994; Mazumdar et al., 2000). In this approach, we first varied the cut-off value systematically from 45 to 165 U/L in steps of 10 U/L and used each value to stratify the patients into low and high groups. For each cut-off value, we generated OS and TFS curves for the low and high groups and compared them using the log-rank test. We chose the smallest p values associated with OS and TFS given by the log-rank test and adjusted them as described previously (Altman et al., 1994; Mazumdar, et al., 2000). If the adjusted p values were below the significance threshold of 0.05, the cut-off associated with these p values was then chosen for subsequent univariate and multivariate analysis.

The following binary variables were used in univariate and multivariate analysis: pretreatment S-ALP (low or high), sex, age (≤50 or >50 years), smoking history (yes or no), and chemotherapy (yes or no). The following continuous variables were used: T classification, N classification, and TNM stage. Univariate analysis was used to identify possible correlations of each of these variables with OS and TFS in the following samples: the entire cohort, male and female subgroups, smoking and non-smoking subgroups, and subgroups with S-ALP level ≤135 U/L or >135 U/L. Variables showing a correlation with $p<.10$ were then used in multivariate analysis based on the Cox proportional hazards regression model. In this analysis, the correlations of various combinations of prognostic factors with OS and TFS were calculated.

Chi-squared tests were conducted to determine whether S-ALP (high or low) correlated with clinical characteristics. Differences with a two-sided $p<.05$ were considered significant.

Results

Patient characteristics

During the enrollment period, 696 patients newly diagnosed with NPC were treated in the inpatient radiotherapy department of our hospital; S-ALP levels prior to radical treatment were available for 693. Of these 693 patients, 92 (13.3%) were excluded for failing to fulfill the inclusion criteria, comprising 30 (4.3%) who showed evidence of distant metastasis, 29 (4.2%) who did not receive complete radical treatment, 14 (2.0%) who received radiotherapy or chemotherapy before blood was drawn for S-ALP measurement, 4 (0.58%) who had secondary malignant tumors at the time of diagnosis, 2 (0.3%) who were histologically diagnosed with type I

Table 1. Patient Characteristics

Characteristic	No. (%)	Pretreatment serum ALP level, n (%)		*p*
		≤85 U/L	>85 U/L	
Age (y)				
≤50	436 (72.6)	293 (48.8)	143 (23.8)	0.054
>50	165 (27.4)	97 (16.1)	68 (11.3)	
Gender				
Male	440 (73.2)	262 (43.6)	178 (29.6)	<0.001
Female	161 (26.8)	128 (21.3)	33 (5.5)	
Tumor classification				
T_{1+2}	379 (63.1)	260 (43.3)	119 (19.8)	0.013
T_{3+4}	222 (36.9)	130 (21.6)	92 (15.3)	
Lymph node classification				
N_{0+1}	421 (70.0)	276 (45.9)	145 (24.1)	0.601
N_{2+3}	180 (30.0)	114 (19.0)	66 (11.0)	
TNM stage (AJCC)				
I+II	262 (43.6)	182 (30.3)	80 (13.3)	0.039
III+IV	339 (56.4)	208 (34.6)	131 (21.8)	
Smoking history				
Yes	136 (22.6)	76 (12.6)	60 (10.0)	0.012
No	465 (77.4)	314 (52.3)	151 (25.1)	
Alcohol consumption				
Yes	78 (13.0)	46 (7.7)	32 (5.3)	0.24
No	523 (87.0)	344 (57.2)	179 (29.8)	
Chemotherapy				
Yes	338 (56.2)	213 (35.4)	125 (20.8)	0.321
No	263 (43.8)	176 (29.3)	87 (14.5)	
Family history of NPC				
Yes	35 (5.8)	21 (3.5)	14 (2.4)	0.591
No	566 (94.2)	365 (60.7)	201 (33.4)	

*AJCC, American Joint Committee on Cancer; ALP, alkaline phosphatase; NPC, nasopharyngeal carcinoma

NPC, and 13 (1.9%) who were lost to follow-up. The remaining 601 cases (86.7%) met the inclusion criteria and were included in the analysis.

The ratio of males to females was 2.7:1, and the mean age was 44.3 years (range, 11-82 years). Of the 601 patients, 573 (95.3%) showed normal S-ALP levels (defined as 45-135 U/L) and only 4 (0.7%) showed abnormally low S-ALP levels (defined as <45 U/L). Seven patients were younger than 18 years, and only 3 of them had S-ALP levels >135 U/L. Four patients (0.7%) were pregnant when diagnosed with NPC; 3 of these 4 women underwent voluntary abortions and one had a spontaneous vaginal delivery of a healthy baby. All 4 women had normal S-ALP values by the time measurements were taken and before radical treatment (69, 70, 116 and 70 U/L).

The median follow-up time was 51.5 months (range, 5-116 months). During follow-up, 216 patients (35.9%) experienced tumor progression after treatment, including those who developed local or regional recurrence or distant metastasis. By the end of follow-up, 169 patients (28.1%) had died, and all but 2 deaths were due to local recurrence or distant metastasis. The 2 exceptions were deaths due to diseases unrelated to NPC.

Identification of the cut-off value for classifying pretreatment S-ALP as high or low

Cut-off values for classifying patients as having high or low S-ALP were varied systematically, and 5-year OS and 5-year TFS curves were generated for the high and low subgroups (Table 1). P values were determined for the differences between the OS and TFS curves for the high and low subgroups for each cut-off value, and the cut-off giving the smallest *p* values was selected (85 U/L). These *p* values were adjusted using the minimum *p* value approach (Altman et al., 1994; Mazumdar, et al., 2000), which gave adjusted values below the significance threshold of 0.05 for OS differences and TFS between the low and high groups. And we confirmed 85 U/L as the cut-off value to use in subsequent uni- and multivariate analyses.

Table 2. Differences in 5-year Overall Survival (OS) and 5-year Tumor-free Survival (TFS) for 601 Patients with NPC After Stratification into Groups with Low or High S-ALP Levels Based on the Indicated Cut-off Values

Cut-off (U/L)	No. low/high	5-year OS		5-year TFS	
		χ^2	*p*	χ^2	*p*
45	25/597	0.0004	0.9822	1.891	0.1691
55	98/503	1.401	0.2365	4.226	0.0398
65	178/423	2.941	0.0864	5.008	0.0252
75	290/311	5.901	0.015	10.857	0.001
85	390/211	11.327	0.0008	15.336	0.00009
95	468/133	9.03	0.0027	12.208	0.0005
105	515/86	3.781	0.0518	6.495	0.0108
115	544/57	5.978	0.0145	12.057	0.0005
125	564/37	11.187	0.0008	13.532	0.0002
135	577/24	4.855	0.0276	7.207	0.0073
145	583/18	6.351	0.0117	8.324	0.0039
155	589/12	1.931	0.1646	3.024	0.0821
165	591/10	0.086	0.7692	0.74	0.3895

*Abbreviations: AJCC, American Joint Committee on Cancer; S-ALP, serum alkaline phosphatase; OS, overall survival; TFS, tumor-free survival

Association between elevated S-ALP and clinical characteristics of patients with NPC

Mean S-ALP in our patient population was 80.82 U/L, with an SD of 32.9 U/L. Of the 601 patients, 390 (64.9%) had elevated pretreatment S-ALP, defined as >85 U/L based on our scan of cut-off values described above. Patients classified as T3+4 or stage III+IV, smokers and males were more likely to have elevated S-ALP than were patients classified as T1+2 (p=0.013) or stage I+II (p=0.039), non-smokers (p=0.012) and females (p<0.001). In contrast, the frequency of elevated S-ALP was similar between the following subgroups of patients: those classified as N0+1 and those classified as N2+3 (p=0.601), those ≤50 and >50 years old (p=0.054), those with and without family history of NPC (p=0.591), those who consumed alcohol or not (p=0.240), and those on chemotherapy or not (p=0.321) (Table 1).

Association between elevated S-ALP (>85 U/L) and OS and TFS in the entire cohort

In univariate analysis, the following clinical characteristics correlated significantly with 5-year OS: pretreatment S-ALP (p<0.001), age (p=0.005), T classification (p<0.001) and N classification (p=0.002). In contrast, the following characteristics did not show a significant association with OS: sex (p=0.210), smoking history (p=0.075), drinking history (p=0.275), family history of NPC (p=0.588), and chemotherapy (p=0.888).

The following variables correlated significantly with TFS: pretreatment S-ALP (p<0.001), age (p=0.002), sex (p=0.04), smoking history (p=0.017), T classification (p=0.005) and N classification (p<0.001). However, drinking history (p=0.093), family history of NPC (p=0.860), and chemotherapy (p=0.151) did not show a significant association with TFS.

In multivariate analysis, the following emerged as independent prognostic factors for poor OS (Table 3): elevated pretreatment S-ALP [hazard ratio (HR) 1.522, 95% confidence interval (CI) 1.119 to 2.071], age (HR 1.518, 95%CI 1.104 to 2.088), T classification (HR 1.392, 95%CI 1.186 to 1.633) and N classification (HR 1.245, 95%CI 1.072 to 1.446). The following factors emerged as independent predictors of poor TFS: elevated pretreatment S-ALP (HR 1.529, 95%CI 1.164 to 2.010), age (HR 1.478, 95%CI 1.115 to 1.961), T classification (HR 1.223, 95%CI 1.060 to 1.410) and N classification (HR 1.240, 95%CI 1.085 to 1.416). In contrast, sex (HR 1.181, 95%CI 0.839 to 1.663) and smoking history (HR 1.242, 95%CI 0.903 to 1.709) did not show any significant association with TFS.

We also performed a log-rank test to compare OS and TFS for the entire cohort of 601 patients depending on whether they had low or high S-ALP values. Those with lower pretreatment S-ALP (≤85 U/L) showed significantly higher 5-year OS and 5-year TFS than did those with high S-ALP levels (>85 U/L): OS, 76.5% *vs* 63.0% (p=0.0008); TFS, 68.8% *vs* 54.9% (p=.00009) (Figures 1-2).

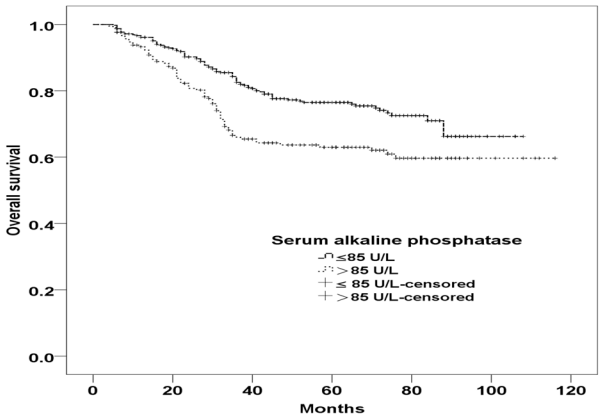

Figure 1. Overall Survival of 601 Patients with NPC Stratified by Pretreatment Serum Levels of Alkaline Phosphatase (≤ or >85 U/L) (p=0.0008)

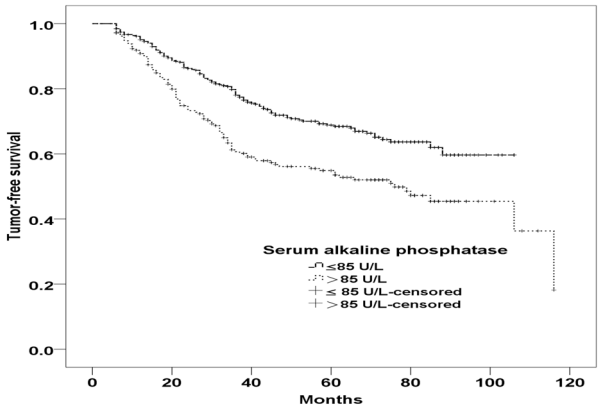

Figure 2. Tumor-Free Survival of 601 Patients with NPC Stratified by Pretreatment Serum Levels of Alkaline Phosphatase (≤ or >85 U/L) (p <0.0001).

Association between elevated S-ALP (>85 U/L) and the OS and TFS of subgroups stratified by sex, smoking and an S-ALP cut-off of ≤135 U/L

To study the possible effect of sex on the prognostic value of S-ALP before treatment, we repeated the univariate and multivariate analyses separately for male and female patients (Table 4). Among males, elevated S-ALP remained an independent prognostic indicator of both poor OS (HR 1.509, 95%CI 1.065 to 2.136) and poor TFS (HR 1.506, 95%CI 1.112 to 2.040). Among females, elevated S-ALP did not show significant correlation with OS or TFS.

To determine whether smoking influenced the

Table 3. Cox Multivariate Analysis to Identify Prognostic Factors in 601 Patients with NPC Using a Cut-off of 85 U/L to Define Low and High S-ALP Levels

Prognostic factor	p	HR	95%CI for HR
Overall survival			
Pretreatment S-ALP	0.007	1.522	1.119 to 2.071
Age	0.01	1.518	1.104 to 2.088
T classification	<0.001	1.392	1.186 to 1.633
N classification	0.004	1.245	1.072 to 1.446
Tumor-free survival			
Pretreatment S-ALP	0.002	1.529	1.164 to 2.010
Age	0.007	1.478	1.115 to 1.961
T classification	0.006	1.223	1.060 to 1.410
N classification	0.002	1.24	1.085 to 1.416

*Abbreviations: HR, hazard ratio; 95%CI, 95% confidence interval; S-ALP, serum alkaline phosphatase

Table 4. Cox Multivariate Analysis of Subgroups of NPC Patients to Identify Correlations of Pretreatment S-ALP with 5-year OS and 5-year TFS Based on a Cut-Off of 85 U/L to Define Low and High S-ALP Values

Subgroup	No. (%)	5-year OS			5-year TFS		
		HR	95% CI	p	HR	95% CI	p
Gender							
Male	440 (73.2)	1.509	1.065 to 2.136	0.021	1.506	1.112 to 2.040	0.008
Female	161 (26.8)	-	-	ns			ns
Smoking history							
Yes	136 (22.6)	-	-	ns	-	-	ns
No	465 (77.4)	1.675	1.165 to 2.407	0.005	1.579	1.147 to 2.174	0.005
S-ALP							
≤135 U/L	577 (96.0)	1.467	1.064 to 2.023	0.02	1.507	1.137 to 1.999	0.004
>135 U/L	24 (4.0)	-	-	ns	-	-	ns

*Abbreviations: HR, hazard ratio; 95%CI, 95% confidence interval; S-ALP, serum alkaline phosphatase

prognostic value of S-ALP, we also repeated the univariate and multivariate analyses separately with the patients with and without smoking history. Elevated pretreatment S-ALP (>85 U/L) retained its prognostic power for predicting poor OS (HR 1.675, 95%CI 1.165 to 2.407) and poor TFS (HR 1.579, 95%CI 1.147 to 2.174) of patients without smoking history, but did not for predicting poor OS and poor TFS of those with (Table 4).

To determine whether the prognostic value of S-ALP depended on our choice of cut-off for classifying patients as having low or high S-ALP, we repeated the multivariate analysis with only patients with S-ALP within the upper limit of the normal range (≤135 U/L) as defined by others (Li et al., 2012) and by the manufacturers of our S-ALP assay kit. We found that elevated S-ALP remained an independent prognostic indicator of both poor OS (HR 1.467, 95%CI 1.064 to 2.023) and poor TFS (HR 1.507, 95%CI 1.137 to 1.999) (Table 4).

Discussion

S-ALP has been reported to be a sensitive indicator for diagnosis and recurrence monitoring of some malignant cancers (Wei et al., 1993; Crivellari et al., 1995), but one study suggested it would be inappropriate for NPC diagnosis because of the low percentage of patients with abnormally high S-ALP (>110 U/L) (Li et al., 2012). Similarly, our results showed that only 24 of 601 patients (4.0%) had abnormally high S-ALP with a diagnostic accuracy for NPC much less than other useful serum biomarkers (Chen et al., 2013) , confirming that S-ALP is unsuitable for diagnosis or screening of this tumor. Instead, our results show that the value of S-ALP lies in predicting prognosis. Our multivariate analysis showed that elevated S-ALP (>85 U/L) independently predicted poor 5-year OS (HR 1.522, 95%CI 1.119 to 2.071) and poor 5-year TFS (HR 1.529, 95%CI 1.164 to 2.010). To the best of our knowledge, this is the first report of pretreatment S-ALP as an independent prognostic factor for evaluating the long-term outcome of NPC.

In contrast to our findings, Li et al. found abnormally high S-ALP (>110 U/L) to be associated with shorter time to death and local recurrence, but they did not observe an independent prognostic value for NPC (Li et al., 2012). Two factors may explain this discrepancy: (1) only 7.7% of the patients in the study by Li et al. had elevated S-ALP (>110 U/L), which may have led to insufficient statistical power and selection bias; and (2) it may be inappropriate to use the normal upper limit (110 U/L in the work of Li et al.) as the cut-off for classifying patients as having low or high S-ALP. Based on the results with our cohort, it may be that 85 U/L, which falls within the normal range, may be a more suitable cut-off value for classifying low and high S-ALP for prognostic purposes.

Whether and how elevated S-ALP may affect NPC outcomes remains unclear. In previous work, NPC patients classified as T3+4 were reported as more likely to have elevated S-ALP levels (>110 U/L) than those classified as T1+2 (Li et al., 2012). Our results confirm and extend those findings by showing that patients classified as T3+4 or stage III+IV were more likely to have S-ALP >85 U/L than were patients classified as T1+2 or stage I+II. Since only the T3+4 classification involves invasion of the bony structure in the skull base and paranasal sinus (according to AJCC guidelines), elevated S-ALP may indicate the involvement of this bony structure. Consistent with this possibility, the skull base is the most frequent site of recurrence because the radiation dose is so attenuated there (Cheng et al., 2006). S-ALP reflects the combined activity of several phosphate monoester hydrolase isoenzymes found in several organs, including bone (Turan et al., 2011). The involvement of the bony skull base and sinus in NPC patients may increase the release of the skeletal ALP isoenzyme from osteoblasts, contributing to S-ALP elevation (Li et al., 2012).

We also analyzed the likelihood that patients at different NPC stages would show S-ALP that was elevated but still within the normal range (>85 but ≤135 U/L). Patients classified as T3+4 were more likely to have S-ALP in this range than were patients classified as T1+2 (data not shown). This suggests that NPC patients are at higher risk of bone structure invasion even when S-ALP is at the upper end of the normal range.

While our results indicate a significant association between elevated S-ALP before treatment and higher T classification, our multivariate analysis shows that elevated S-ALP predicts NPC prognosis independently of T classification, raising the possibility that it affects prognosis through other mechanisms. How S-ALP may affect the outcome of NPC has not been clearly investigated. One possibility is that it acts through matrix metalloproteinase-9 (MMP-9), since it has already been shown to correlate directly with MMP-9 expression in another malignant tumor, primary osteosarcoma (Han et al., 2012). MMP-9, which has been positively associated with poor response to chemotherapy in patients with metastatic gastric carcinoma (Al-Batran et al., 2008), may also play a role in chemoresistance in NPC. In addition, previous studies have shown that increased levels of bone ALP isoenzyme, which is a major part of S-ALP, may correlate with early distant metastasis in malignant bone tumors (Van Hoof et al., 1992; Akimoto et al., 1998; Lorente et al., 1999; Saif et al., 2005). Hence, we speculate that elevated S-ALP may reflect distant micrometastases in other organs such as bone; distant micrometastases are frequently detected at NPC diagnosis, and these undoubtedly contribute to poor OS and TFS (Ma et al., 2010).

One study suggested that elevated S-ALP (>165 U/L) may be a useful indicator of lymph involvement in esophageal cancer (Aminian et al., 2011). In our case, however, we did not observe a significant association between S-ALP and the N classification of NPC, consistent with a previous report (Li et al., 2012). This discrepancy may reflect the different cut-off values used to classify S-ALP as high or low or differences between malignant cancer types.

We found that males were slightly more likely than females to have elevated S-ALP (Table 1), suggesting an association between elevated S-ALP and gender. This tendency may also reflect the fact that many more males

than females in our cohort had a smoking history (30.5% *vs* 1.2%), given that we found elevated S-ALP (>85 U/L) to be significantly more frequent in those with a smoking history than in those without. This result is consistent with a previous study showing that S-ALP levels were significantly higher in cigarette smokers than in non-smokers (Sripanidkulchai et al., 2004). To investigate whether sex and smoking affected the prognostic value of S-ALP in NPC, we stratified our cohort into male and female subgroups, as well as smoking and non-smoking subgroups. Univariate and multivariate analyses were repeated for each subgroup. Pretreatment S-ALP retained its independent power for predicting 5-year OS and TFS for the non-smoker subgroup and the male subgroup, but not for the smoker subgroup or female subgroup. Given that our cohort contained relatively small numbers of smokers and women (136 and 161), it would be premature to conclude that S-ALP lacks prognostic power in such patients. Future large studies are needed to address this question.

The fact that S-ALP isozymes can originate in several organs, including bone, liver, placenta, bowel and kidney, means that physiological and pathological factors involving these organs may affect the S-ALP level independent of NPC progression, confounding our univariate and multivariate analysis. We suggest that this possibility is less likely because none of our patients showed evidence of severe diseases known to cause abnormal S-ALP levels (Limdi et al., 2003), including bile duct obstruction, primary biliary cirrhosis, drug-induced cholestasis, primary sclerosing cholangitis, adult bile ductopenia, bone disease, or distant metastasis. In fact, only 4% of our patients presented with S-ALP >135 U/L, and eliminating these cases did not alter our finding that elevated S-ALP (>85 U/L) is an independent prognostic indicator for poor OS and TFS (Table 4).

Another source of elevated S-ALP unrelated to NPC can be young age, since S-ALP can rise to very high levels during childhood and adolescence (Limdi et al., 2003). We feel that this is less likely to interfere with our results, since only 7 of our patients were under 18 at the time of diagnosis, and only 3 of them presented abnormally high S-ALP. Similarly, pregnancy has been associated with abnormally high S-ALP due to the large amount of ALP isoenzyme released from the placenta (Limdi et al., 2003). Only 4 of our patients were pregnant at the time of diagnosis; all underwent voluntary abortion or had spontaneous vaginal delivery, and S-ALP levels were within the normal range by the time measurements were taken and radical treatment was begun.

These arguments strongly suggest that the elevated S-ALP in our cohort reflected primarily NPC progression rather than unrelated confounders.

In conclusion, our data suggest that elevated pretreatment S-ALP is an independent prognostic factor for poor outcome in NPC patients.

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