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The Correlation Between Serum Chemokines and Clinical Outcome in Patients with Advanced Biliary Tract Cancer

Su Jin Lee, Jung Eun Kim, Seung Tae Kim, Jeeyun Lee, Se Hoon Park, Joon Oh Park, Won Ki Kang, Young Suk Park and Ho Yeong Lim

Division of Hematology and Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Abstract

BACKGROUND: Biliary tract cancers (BTCs) are known to have a dismal prognosis. A number of chemokines play important roles in the progress of BTCs. However, the serum levels of chemokines in BTCs have not yet been explored. *METHODS:* The sera of healthy donors (n = 8) and patients with BTCs who were enrolled in second line sunitinib trials (n = 27) were collected. The concentrations of three kinds of chemokines (CXCL5, CXCL8 and CXCL12) were measured using ELISA assay. The median concentrations of chemokines were compared between healthy donors and BTC patients and the role of chemokines as a prognostic biomarker was examined. *RESULTS:* BTC patients generally had higher serum levels of CXCL5 and CXCL12 compared to healthy donors. Patients with cholangiocarcinoma showed significantly higher levels of serum CXCL12 than patients with gallbladder cancer. In survival analysis, only CXCL12 level showed a prognostic impact on overall survival (median OS: 6.9 vs. 0.9 months in low CXCL12 vs. high CXCL12, respectively; $P = .008$). High CXCL5 levels were also correlated with poor survival without statistical insignificance (median OS: 6.2 vs. 2.0 months in low CXCL5 vs. high CXCL5, respectively; $P = .070$). *CONCLUSIONS:* There was a significant difference in OS according to the level of CXCL12, suggesting that serum CXCL12 levels may be a useful surrogate marker for clinical outcome in advanced BTCs.

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Introduction

Biliary tract cancers (BTCs), including cholangiocarcinoma and gallbladder cancer, are low-incidence cancers [1], but relatively more common in Asia and Latin America [2]. Most patients (>65%) are diagnosed with unresectable disease and there is a high relapse rate in the minority of patients who undergo potentially curative surgery [3]. Combination chemotherapy with gemcitabine and platinum agents seems to be a reasonable treatment option as first-line treatment based on randomized phase III trial (ABC-02) [4]. However, prognosis of advanced and metastatic BTCs is poor with a five-year survival rate of about 2% for stage IV BTCs [5]. Therefore, it is urgent to uncover the molecular mechanisms of BTCs and identify potential therapeutic targets to improve prognosis.

Chemokines, small molecular weight proteins (approximately 8-13 kDa), are chemotactic cytokines specialized in regulating the migration of immune cells into damaged or diseased organs in response to pro-inflammatory stimuli [6]. Together with their corresponding receptors, chemokines promote the extravasation of immune cells from the circulation into injured tissue and regulate the migration of immune cells through the tissue. To date, about 50 different chemokines and 20 difference chemokine receptors have been identified [7,8]. Over the past few years, studies have increasingly shown that chemokines play an important role in several aspects of tumor progression [9–12]. Chemokines released by tumor and stromal cells can induce the expression and distribution of tumor-associated leukocytes, trigger angiogenesis and generate fiber keratinocytes [13,14]. Chemokines released into the matrix can also directly contribute to the growth of malignant cells [9,14]. C-X-C chemokine receptor 4 (CXCR4) is frequently overexpressed in cancer

Address all correspondence to: Ho Yeong Lim, MD, Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea.
E-mail: hoylim@skku.edu
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Table 1. Baseline Characteristics of Patients (N = 27)

Variables	No. of Patients (N = 27)	% of Patients
Age, median (range), years	55 (38-75)	
≤65	23	85.2
>65	4	14.8
Sex		
Male	17	63.0
Female	10	37.0
Primary site		
Intrahepatic duct	12	44.4
Extrahepatic duct	4	14.8
Gallbladder	11	40.7
Disease status		
Recurrent	4	14.8
Primarily metastatic	23	85.2
First-line chemotherapy		
Gemcitabine/platinum combination	19	70.4
5FU/platinum combination	8	29.6
Site of metastasis		
Liver	22	81.5
Lymph node	22	81.5
Lung	9	33.3
Peritoneum	8	29.6
Pleural	3	11.1
Bone	2	7.4
CA 19-9		
≤37 IU/mL	9	33.3
>37 IU/mL	18	66.7

cells, and chemokine ligand 12 (CXCL12)-CXCR4 interactions underlie invasiveness in a variety of cancers [11,12].

There are a few reports about the effects of chemokines and chemokine reception interaction in BTCs, such as CXCL12-CXCR4 or chemokine ligand 5 (CXCL5)-C-X-C chemokine receptor 2 (CXCR2) [15–17]. However, these have all been examined in tumor tissues. Therefore, we explored whether circulating chemokines are detectable in enzyme-linked immunosorbent assay (ELISA) and if they have prognostic impact on survival in patients with BTCs.

Materials and Methods

Patients

From May 2009 to October 2010, a total of 56 patients were enrolled in sunitinib BTC trials and blood samples from 27 patients were collected for biomarker analysis. The eligibility criteria and design of this study were previously described [18]. Informed consent was signed and obtained from all patients before being involved in the study. The Ethics Committee of Samsung Medical Center approved and supervised this study.

Blood Samples and ELISA Assay

Blood samples (5ml) were drawn from BTC patients or healthy donors before chemotherapy or after surgery, respectively. After collection, the samples were kept at room temperature for 2 hours to allow clotting and then were immediately centrifuged at 2200 rpm for 15 minutes at 4°C and were the cryopreserved at −80°C until ELISA assays were run. The level of serum chemokine was quantified using a commercially available ELISA kit (R&D) according to the manufacturer's instructions.

Selection of Cut-Off Value for Chemokines. Since there was no reference range available for serum chemokine levels, the "minimum P value" approach [19–21] was applied to estimate an optimal cut-off of chemokines for the best separation of patients' PFS/OS by X-tile software [22], version 3.6.1 (Yale University, New Haven, CT). X-tile plots can be used to divide a population into two levels (low and high level) and provide an "on-the-fly" histogram with an associated Kaplan-Meier curve, and the best P value is available after rigorous statistical evaluation by X-tile.

Statistical Analyses

Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). The independent *t*-test or Mann-Whitney *U*-test were used for testing statistical significance of mean differences between the two groups. The progression-free survival (PFS) and overall survival (OS) were estimated by the Kaplan-Meier estimates method. The log-rank test was applied to compare survival between the two groups. Univariate and multivariate analyses were based on the Cox proportional hazards regression model. A *P*-value of less than 0.05 was regarded as statistically significant.

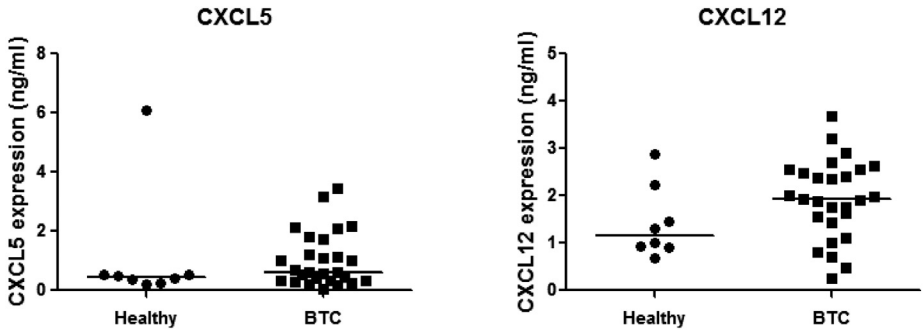
Results

Patients' Characteristics

This analysis included 27 patients who received sunitinib as second-line treatment for advanced BTCs. Baseline characteristics are presented in Table 1. The median age was 55 years (range, 38-75 years) and patients were predominantly male (63.0%). Twelve patients (44.4%) had intrahepatic duct cancer, 4 (14.8%) had extrahepatic duct cancer and 11 (40.7%) had gallbladder cancer. Two-thirds of patients (66.7%) had high CA19-9 level at baseline.

Chemokine Level

The median serum CXCL5 levels were 0.4325 ng/mL and 0.601ng/mL in healthy donors and patients with BTCs, respectively.



Median: 0.4325 vs 0.601, *P*= 0.922

1.1535 vs. 1.939, *P* =0.131

Figure 1. Distribution of serum chemokine level in healthy donors and patients with BTC