RESEARCH ARTICLE

Dickkopf-1 Levels in Turkish Patients with Bladder Cancer and its Association with Clinicopathological Features

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Abstract

Background: Evidence indicates that Dickkopf-1 (DKK-1) levels may be a biomarker for cancer risk. The aim of this study was to assess DKK-1 and its correlation with clinic-pathological features in patients with bladder cancer. Materials and Methods: DKK-1 levels were determined in serum samples from 90 patients with bladder cancer before transurethral tumor resection. The concentrations of DKK-1 were determined by using enzyme linked immune-sorbent assay (ELISA). Results: Elevated preoperative DKK-1 levels were associated with tumor stage (p<0.001), grade (p<0.001) and histological grade (p<0.001). Conclusions: The results of our study demonstrated that the level of serum DKK-1 is correlated with both disease progression and increase in the tumor grade. Preoperative serum DKK-1 elevation may thus represent a novel marker for the determination of bladder cancer and the detection of patients with a likely poor clinical outcome.

Keywords: Bladder cancer - DKK-1 - clinic-pathological features - prognosis - Turkey.

Asian Pac J Cancer Prev, **15 (1)**, 381-384

Introduction

Bladder cancer is the fourth most common cancer and cause of cancer-related deaths in men and the eight in women; the 5-year survival rate of bladder cancer ranges from 30%-90% (Jemal et al., 2003). The main reasons for the poor survival despite feasibility of effective treatment are the high relapse rate (50-80% depending on the initial stage and grade) and lack of a sensitive method for early detection, especially carcinoma in situ tumors. Bladder cancer is generally associated with hematuria, dysuria, and urination, but more specific screening tests for bladder cancer have not been until lately.

The Dickkopf family encodes secreted proteins and comprises of four primary members in vertebrates (DKK 1, 2, 3, 4). DKK-1 was originally identified as embryonic head stimulant and Wnt antagonist in Xenopus (Glinka, Wu, Onichtchouk, Blumenstock, Niehrs, 1997). DKK-1 is its aptness to modulate Wnt signaling. The DKK-1 has function mediated by Wnt/β-catenin signaling, but it has been demonstrated that it may have also β-catenin-independent functions (Niehrs, 2006).

DKK-1 inhibits Wnt-mediated signaling in Xenopus (Glinka et al., 1998). Besides, by inhibiting the Wnt signaling pathway, DKK-1 is adequate and essential for head induction (Glinka et al., 1998). Additionally, DKK-1 inhibits the Wnt signaling pathway and how DKK-1 is regulated are still not clear.

Some studies have demonstrated over-expression of DKK-1 in patients with Wilms tumor, hepatoblastoma, hepatocellular carcinoma, gastric cancer, rectal cancer, colon cancer, gynecological cancers, indicating that DKK-1 has a latent oncogenic role in these tumors (Wirths et al., 2003; Patil et al., 2005; Jianget al., 2009; Kemik et al., 2011; Soydinc et al., 2011; Tung et al., 2011; Gomceli et al., 2012; Lee et al., 2012).

In this study, we examined preoperative serum DKK-1 levels with regard to clinic-pathologic significance and co-relation of each factor.

Materials and Methods

Patients

Patients (n=90) were recruited at the Department of Urology, YuzuncuYıl University Medical Faculty, and archived data on patients newly diagnosed between March 2010 and June 2013 were reviewed. All of the patients were ethnically Turkish. The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000. The study protocol was approved by the local ethic committee. All subjects gave informed consent.

All patients were primarily reviewed by history-taking,

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clinical examination, standard laboratory investigations, chest radiography, excretory urography and/or abdominal ultrasonography. Abdominal and pelvic computed tomography and radioisotope bone scans were performed for patients with evidence of advanced disease. The pathologic stage was assigned according to the 2002 American Joint Committee on Cancer TNM staging system. The pathologic grade was classified according to the 1998 WHO International Society of Urological Pathology classification system.

The control group consisted of 70 healthy subjects that were asymptomatic and had an insignificant medical history and physical examination. None of the control subjects were taking antioxidant supplementation, such as vitamin E or C. In addition, the subjects were not consuming alcohol, tobacco, or any other drugs. Additionally, the subjects had no known acute or chronic diseases.

Methods

Blood samples were drawn from all patients before surgical treatment. None of the bladder cancer patients had received chemo- and radiotherapy before the blood samples were collected. To standardize the clotting conditions, all sera were separated within 1h after blood collection, separated into aliquots and stored at -80°C until assayed.

Serum levels of DKK-1 were measured by commercially available ELISA kits (R and D Systems, Minneapolis, MN). 96-well plates were coated overnight at room temperature with monoclonal mouse antihuman DKK-1 capture antibodies in phosphate-buffered saline (PBS). The plates were washed with PBS/Tween and blocked with 1% bovine serum albumin in PBS for 1 hour at room temperature. The samples were added to the plates and incubated for 2 hours. Goat antihuman detection antibodies were added, and the plates were incubated for another 2 hours. Streptavidin-horseradish peroxidase was added and incubated for 20 minutes. After the plates were washed with PBS, the substrate reagent was added for another 20 minutes. The substrate reaction was stopped upon addition of 1 mol/l sulfuric acid, and extinction was measured at 450 nm wavelength using a multiple ELISA reader (Anthos Microsystems, Germany) All measurements were performed in duplicate for each sample, and the mean value was calculated (Kemik et al., 2011).

Statistical analyses

One-sample Kolmogorov-Smirnov and Shapiro Wilk normality test control charts were drawn with the histogram. Data are expressed as mean, standard deviation, median, minimum, maximum, presented as frequencies and percentages. In variables with normal distribution, independent samples t test, one-way ANOVA and Tukey HSD tests were performed with binary comparisons. In variables with normal distribution, Mann-Whitney U, Kruskal-Wallis one-way analysis of variance and Bonferroni-corrected Mann-Whitney U tests were performed with binary comparisons. Yates-corrected chi-square and chi-square tests were used for nominal variables. Significance of $p < 0.05$ were taken and duplex. SPSS 21.0 statistical software was used for all analyses.

Results

Table 1 provides demographics and clinic-pathologic data for all the patients. The serum DKK-1 levels were measured in 70 healthy subjects (35 men and 35 women) with a median age of 59.53±8.19 (range, 41-71years) years. The study group included 90 patients with bladder cancer (47 men and 43 women) with a median age of 59.47±6.35 (range, 40-72) years. Twenty one patients (23.4%) had tumors smaller than 3cm in size. Seventy five (83.3%) patients had distant metastasis. The postoperative T stages of patients were T1, T2, T3, and T4 in 17, 21, 25, and 27 patients, respectively.

Table 2 provides the serum DKK-1 levels of all patients and control group. The two groups did not differ in age

Table 1. Clinic-Pathologic Variables

Clinic-pathologic Variables		Controls(n=70)	Patients(n=90)	p
Age (y)		59.53±8.19	59.47±6.35	>0.05
Gender (F/M)		35/35	43/47	
Tumor size	<3 cm		21 (23.4%)	
	≥3 cm		69 (76.6%)	
Lymph Node,	N0		9 (10%)	
Metastasis	N1		13 (14.4%)	
	N2		68 (75.5%)	
Distant Metastasis	M0		25 (16.7%)	
	M1		75 (83.3%)	
Lymphvascular	Absen		27 (30%)	
involvement	Present		63 (70%)	
T stage	T1		17	
	T2		21	
	T3		25	
	T4		27	

Table 2. Serum DKK-1 Levels of Control Groups and All Patients (Mean±SD)

	Controls	Patients	p
DKK-1 (ng/mL)	10.44±2.08	72.83±34.90	0.001

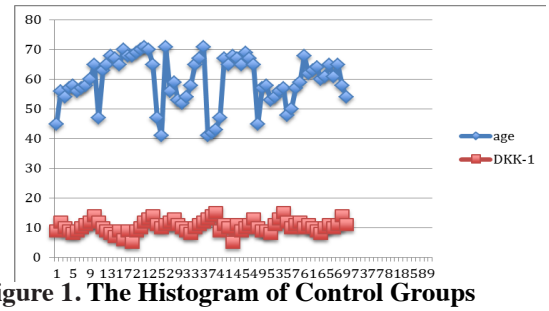

Figure 1. The Histogram of Control Groups

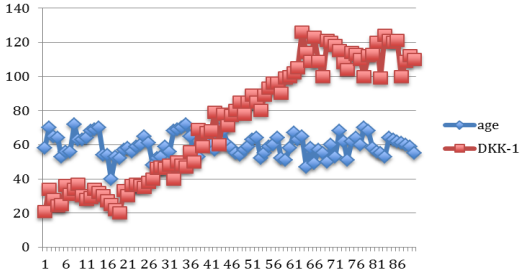

Figure 2. The Histogram of All Patients

(p>0.05) (Figure 1 and 2). In the healthy group, the mean serum DKK-1 level was 10.44±2.08 ng/mL. The mean serum DKK-1 levels for the 90 patients with bladder cancer was 72.83±34.9 ng/mL. The serum DKK-1 levels were significantly higher in the patients with bladder cancer than those in the control group (p<0.001).

Table 3 provides the serum DKK-1 levels of patients with bladder cancer according to clinic-pathologic variables.

The serum DKK-1 levels were increased from T1 tumors to T4 tumors, and this difference was statistically significant (p<0.001) (Figure 3). Additionally, serum DKK-1 levels were significantly higher in patients with bladder cancer with increased tumor burden, lymph vascular involvement, distant metastasis, and lymph node metastasis (p<0.001).

Tumor size, lymph vascular involvement, and distant metastasis were similar to the age variable between the groups (respectively; p=1, p=0.041, p= 0.632, p=0.702). Lymph node metastases, distant metastases were similar to the gender variable between the groups (respectively; p=0.922, p=0.932). Gender is distributed homogenously between T stages (p=0.816).

We showed a significant correlation between serum DKK-1 levels and tumor size with higher DKK-1 levels detected at the >3cm tumor size (p=0.003), and between serum DKK-1 levels and T stages (p=0.002). The serum DKK-1 levels were significantly correlated with distant metastasis and lymph node metastasis and lymph vascular involvement (p=0.004, p=0.005, p=0.003).

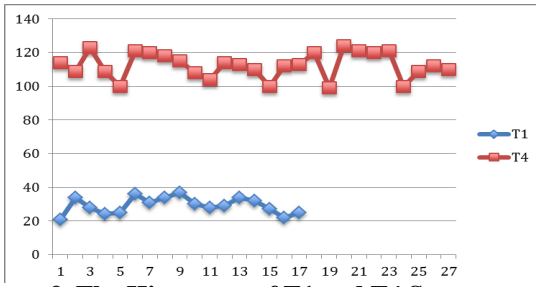

Figure 3. The Histogram of T1 and T4 Stages

Table 3. Serum Dkk-1 Levels of Clinic-Pathologic Variables (Mean±SD) (Minimum level-Maximum level)

Clinicopathologic variables		DKK-1 (ng/mL)
Tumor size	<3 cm	30.0±4.88 (20-37)
	≥3 cm	86.16±28.55 (35-126)
	p	<0.001
Lymph Node -Metastasis	N0	32.5±6.91 (22-40)
	N1	43.2±7.83 (35-55)
	N2	97.51±20.4 (50-126)
	p	<0.001
Distant Metastasis	M0	23.56±7.9 (25-51)
	M1	99.27±21.5 (51-126)
	p	< 0.001
Lymphvascular -involvement	Absent	29.0±5.0 (20-37)
	Present	85.36±29.13 (30-126)
	p	< 0.001
T stage	T1	29.71±4.48 (21-37)
	T2	41.62±11.14 (20-69)
	T3	85.48±15.14 (59-126)
	T4	112.56±7.5 (99-124)
	p	< 0.001

Discussion

Bladder cancer is the leading cause of death from other malignant tumors. It is widely realized that early diagnosis and treatment are key for better clinical outcome in patients with bladder cancer. Using biomarkers to define patients with a highest risk of developing vicious prognosis may thus decrease mortality and medical costs.

Preoperative serum DKK-1 is substantially up-regulated in diverse stages of various cancer and tissue microenvironment surrounding cancer. (Sato et al., 2010; Kemik et al., 2011; Gomceli et al., 2012; Jiang, Huang, Zhang, 2013; Yang et al., 2013).

DKK-1 expression was considered as a significant marker of several cancers clinic-pathological variables in various cancers (Jiang et al., 2009; Kemik et al., 2011; Yang et al., 2013). For the first, it was found that DKK-1 are expressed by human bladder cancer cells and higher serum levels are associated with the distant metastasis, tumor burden, advanced tumor grade, lymph node metastasis and lymph vascular involvement in patients with bladder cancer in this study.

This study showed that serum DKK-1 levels are concern to tumor size, tumor grade, and tumor progression. It is recognized that DKK-1 is a multifunctional protein that is dependent to Wnt pathway, comprising the content to stimulate the growth of bladder cancer cells when DKK-1 is propound in surplus.

Even the development of modern cancer therapy, cancer is still one of the major reasons of death in the world. Over the last decades various molecular targets and biomarkers of cancer therapy have been showed. Besides suppression of some of the target molecules can procure survival profits to a limited subset of the patients, and a few helpful biomarkers are presently forthcoming. In order that definitive molecules contained in bladder cancer and those helpful as therapeutic targets and biomarkers for cancer, it has constituted effective screening system and identified DKK-1 as an onco-protein in which up-regulation is a notable remark of the malignant human tumors.

If notified by larger studies, the determination of DKK-1 would be of limited appreciated to procure existence of the angiogenesis.

DKK-1 may be a less toxic therapeutic agent to inhibit cancer cell proliferation in certain human cancers overexpressing DKK-1, although further preclinical studies are needed to demonstrate its capability and reliable. Besides, DKK-1 could be a safe and less invasive biomarker for the selection of patients (Sato et al., 2010).

Over expression of DKK-1 may overdrive the receptor ligand signals and change the imbalance of cell signal, which can induce cell death. DKK-1 is known to enhance c-Jun-NH_2-kinase phosphorylation that induces apoptosis in animal cap explants (Niehrs, 2006). The presence of DKK-1 expression alters clinical stage and pathology of the cancer.

In summary, our data show that DKK-1 was associated with tumor stages, invasion, and metastasis. Taken

together, although the mechanism remains unclear, DKK-1 functions as a tumor stimulator in bladder cancer; thus it is believed that it can be uses as a marker.

References

Glinka A, Wu W, Onichtchouk D, Blumenstock C, Niehrs C. (1997) Head induction by simultaneous repression of Bmp and Wnt signaling in Xenopus. *Nature*, **389**, 517-19.

Glinka A, Wu W, Delius H, et al (1998). Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature*, **391**, 357-62.

Gomceli I, Bostancı EB, Ozer I, et al (2012). A novel screening biomarker in gastric cancer:serum Dickkopf-1. *Hepatogastroenterology*, **59**, 1661-64.

Jemal A, Murray T, Samuels A, et al (2003). Cancer statistics. *CA Cancer J Clin*, **53**, 5-26.

Jiang T, Wang S, Huang L, Zhang S (2009). Clinical significance of serum DKK-1 in patients with gynecological cancer. *Int J Gynecol Cancer*, **19**, 1177-81.

Jiang T, Huang L, Zhang S (2013). Jun DKK-1 is a serum as a clinical and prognostic factor in patients with cervical cancer. *Int J Biol Markers*, **28**, 221-25.

Kemik O, Kemik AS, Sumer A, et al (2011 Aug). Relationship between clinicopathologic variables and serum and tissue levels of Dickkopf-1 in patients with rectal cancer. *J Investig Med*, **59**, 947-50.

Lee HS, Lee HE, Park do J, et al (2012). Clinical significance of serum and tissue Dickkopf-1 levels in patients with gastric cancer. *Clin Chim Acta*, **413**, 1753-60.

Niehrs C (2006). Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene*, **25**, 7469-81.

Patil MA, Cua MS, Pan KH, et al (2005). An integrated data analysis approach to characterize genes highly expressed in hepatocellular carcinoma. *Oncogene*, **24**, 3737-47.

Sato N, Yamabuki T, Takano A, et al (2010). Wnt inhibitor Dickopf-1 as a target for passive cancer immunotherapy. *Cancer Research*, **70**, 5326-36.

Soydinc HO, Duranyıldız D, Camlıca H, Oral EN, Yasasever V (2011). Lack of diagnostic potential f Dickkopf-1 in colon and rectum cancers. *Asian Pac J Cancer Prev*, **12**, 3187-9.

Tung EK, Mak CK, Fatima S, et al (2011). Clinicopathological and prognostic significance of serum and tissue Dicckopf-1 levels in human hepatocellular carcinoma. *Liver Int*, **31**, 1494-504.

Wirths O, Waha A, Weggen S, et al (2003). Overexpression of human Dickkopf-1, an antagonist of wingless/Wnt signaling, in human hepatoblastomas and Wilms' tumors. *Lab Invest*, **83**, 429-34.

Yang S, Chen GD, Fang F, et al (2013). Dickkopf-1: as a diagnostic and prognostic serum marker for early hepatocellular carcinoma. *Int J Biol Markers*, **8**, 286-97.