

RESEARCH ARTICLE

Haptoglobin Levels in Turkish Patients with Bladder Cancer and its Association with Clinicopathological Features

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Abstract

Although alteration in the haptoglobin phenotype has been reported in patients with bladder cancer, serum haptoglobin levels have not been evaluated. We hypothesized that serum haptoglobin can be used as a biomarker. The aim of this study was to evaluate the expression of haptoglobin in bladder cancer and to determine the relationship with clinicopathological features. A total of 68 serum specimens obtained before surgery were used to investigate haptoglobin expression using the sandwich ELISA technique. Serum haptoglobin levels were higher in the patients with bladder cancer compared to healthy controls ($p < 0.0001$). Additionally, the levels of haptoglobin protein increased with increasing tumor grades ($p < 0.001$) and were significantly higher in patients with metastatic disease and the presence of lymphovascular involvement, lymph node metastases and increasing tumor burden ($p < 0.0001$). This study suggests that elevated haptoglobin levels are associated with a higher stage, grade, and extent of distant metastasis and larger tumor size. Haptoglobin may therefore provide a useful diagnostic and treatment biomarker for patients with bladder cancer.

Keywords: Haptoglobin - biomarker - bladder cancer - Turkey

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Introduction

Bladder cancer includes several types of malignancy that arise in the urinary epithelial lining of the bladder. Non-epithelial cancers (such as lymphoma or sarcoma) are rarely observed in the bladder, and these cancers are not properly included in the colloquial term 'bladder cancer.' Bladder cancer is a disease in which abnormal cells multiply without control in the bladder. The most common type of bladder cancer develops from the urothelium and is known as transitional cell carcinoma (Lapham et al., 1997; Sharma et al., 2009).

Haptoglobin is a protein that is encoded by the HP gene in humans (Wassell, 2000). Haptoglobin is an acute phase protein that is capable of binding hemoglobin. Haptoglobin also acts as an antioxidant and modulates many aspects of the acute phase response. There are 3 major haptoglobin phenotypes; Haptoglobin 1-1, haptoglobin 2-1, and haptoglobin 2-2 (Wassell, 2000). Native haptoglobin is a heterotetramer composed of two α and two β subunits attached to each other with disulfide bonds (Kurosky et al., 1980). The human β subunit is a 38 kDa polypeptide that is linked to α isoforms with disulfide bonds. There is only one type of β subunit, whereas the α chain is represented by two isoforms, α -1 and α -2. The

amino acid sequences in the α isoforms are similar to the α -1 isoform that has 83 amino acids (9-kDa polypeptides). The alpha-2 isoform contains 2 chains. The alpha-1 chain contains 12-70 amino acids, and the alpha-2 chain contains 142 amino acids.

In general, the development of cancer involves a series of changes in protein expression in the serum and cancerous tissues (Serrano et al., 2005; Gkialas et al., 2008; Varela et al., 2008; Hyrsil et al., 2009; Kim et al., 2011). However, the only way to monitor disease progression is to analyze bladder specimens. Only one protein expression investigation has focused on bladder carcinogenesis, and that study identified several proteins. However, biomarker candidates found within the bladder samples need to be assessed in body fluids (e.g., blood, urine and tissue), which can be used more practically for diagnosis. Recent studies have identified several promising candidate serum biomarkers that successfully discriminate patients with bladder cancer from healthy control subjects (Glybchenko et al., 2011; Safyam et al., 2011). Therefore, we hypothesize that serum haptoglobin levels can accurately be used as a single prognostic marker for bladder cancer.

Haptoglobin levels have been investigated in various malignant tumors, including lung cancer (Beckman

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et al., 1986), breast cancer (Awadallah et al., 2004), urogenital tumors (Dunzendorfer et al., 1980) and esophageal squamous cell carcinoma (An et al., 2004). The haptoglobin phenotype in patients with bladder cancer was reported in a study by Benkmann et al. (1987). However, to the best of our knowledge, serum haptoglobin levels have not been evaluated in patients with bladder cancer.

In this study, we have examined the correlation between serum haptoglobin levels and clinicopathological variables of bladder cancer. New tumor biomarkers are needed to improve bladder cancer diagnosis.

Materials and Methods

Patients

In this study, 68 newly diagnosed patients with bladder cancer and 62 healthy subjects were enrolled.

Initially, we performed a history, a clinical examination, standard laboratory investigations, chest radiographs, and abdominal ultrasonography for all patients. Abdominal and pelvic computed tomographies were performed for patients with evidence of advanced disease. None of the patients with bladder cancer had diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, tobacco abuse, or psychiatric, metabolic, hepatic or renal disease. None of the patients used supplemental vitamins.

Pathologic stage was assigned according to the 2002 American Joint Committee on Cancer TNM staging system. Pathologic grade was classified according to the 1998 WHO/International Society of Urological Pathology classification system. The Urological Pathological Classification system was used depending on the criteria met from the 1998 WHO system.

The control group consisted of 62 healthy subjects that were asymptomatic and had an unremarkable medical history and normal physical examination. None of the control subjects were taking antioxidant vitamin supplementation, such as vitamins E or C. In addition, the subjects were not consuming alcohol, tobacco, or any other drugs. Furthermore, the subjects had no known acute or chronic diseases.

The study protocol was carried out in accordance with the Helsinki Declaration as revised in 2000. The study protocol was approved by the local ethics committee, and informed consent was obtained from each subject.

Haptoglobin measurement

All blood samples were collected in 5 mL Vacuette serum tubes containing clot-activating factor. All samples were transported to the laboratory, centrifuged immediately at 4°C for 10 minutes at 1,500g, and stored at -80°C until analysis.

Haptoglobin levels were measured using the sandwich ELISA technique, using two polyclonal antibodies against this protein. Anti-human haptoglobin antibody in dilution (1:100) with 0.5% bovine serum albumin in PBS buffer (0.05% Tween-20 in phosphate-buffered saline) was coated onto plates overnight at 4°C. The blockade was made by adding 200 µL of 3% of non-fat dry milk in PBS at room temperature for 2 hours. Then, the serum samples were mixed at room temperature for 2 hours by adding 200

µL of PBS. Purified human haptoglobin (Sigma-Aldrich) was used as a standard at concentrations ranging from 10 ng/mL to 10 mg/mL. To measure the concentration of haptoglobin, the plate was read at 405 nm.

Statistical analysis

The Shapiro Wilk test of normality control charts and histograms were drawn. The median values for the descriptive variables are normally distributed, and the minimum and maximum values were given. The groups were compared with the Kruskal-Wallis one-way analysis of variance. Post hoc comparisons were evaluated with the Bonferroni-corrected Mann-Whitney U test. Analyses were performed using the SPSS 17.0 statistical package program. The significance level was set at $p < 0.05$.

Results

Table 1 provides demographic and clinicopathological data for all of the patients. The serum haptoglobin levels were measured in 62 healthy controls (30 men and 22 women) with a median age of 49 (30-68) years. The study group included 68 patients (34 men and 34 women) with a median age of (34-67) years.

As shown in Table 2, the haptoglobin levels correlated with distant metastasis and clinical stage of disease. The two groups did not differ in age ($p > 0.05$). The serum haptoglobin levels of all study participants are given in Table 3. In the healthy control group, the mean±SD serum haptoglobin level was 25±4 ng/mL. The mean

Table 1. Demographic and Clinicopathological Variables of All Patients

Clinico-pathologic variables	All Patients (n=68)
Age (years)	55.9±11.2
Gender (F/M)	34/34
Lymphvascular involvement (A/P)	36/62
T Stage:	
T1/T2	14/23
T3/T4	29/32
Distant metastasis (M0/M1)	41/57
Lymph node metastasis (N0/N1/N2)	29/31/38
Tumor diameter: <3cm/≥3cm	39/59

Table 2. Serum Haptoglobin Levels of Patients with Bladder Cancer According to Clinicopathological Variables (Mean±SD)

Variables	Haptoglobin levels (ng/mL)	
Lymphvascular involvement:	Absent	43±12
	Present	315±96
T Stage:	T1	40±10
	T2	109±38
	T3	278±79
	T4	386±41
Distant metastasis:	M0	42±15
	M1	347±63
Lymph node metastasis:	N0	50±10
	N1	198±63
	N2	336±55
Tumor diameter:	<3cm	44±17
	≥3cm	320±43

Table 3. Serum Haptoglobin Levels of Patients and Healthy Controls (Mean±SD)

	Healthy control group	All Patients	p
Age (years)	54.7±10.3	55.9±11.2	>0.05
Haptoglobin(ng/mL)	25.0± 4.0	315.0±98.0	<0.0001

preoperative haptoglobin level for the 68 patients with bladder cancer was 315±98 ng/mL. The serum haptoglobin levels were significantly higher in the patients with bladder cancer than in the healthy controls (p<0.0001).

The haptoglobin levels increased from grade 1 tumors to grade 4 tumors, and this difference was statistically significant (p<0.001). Additionally, serum haptoglobin levels were significantly higher in patients with bladder cancer with metastatic disease, lymphovascular involvement, lymph node metastasis and increasing tumor burden (p<0.0001).

Discussion

Acute phase reactant proteins, such as haptoglobin, have been shown to circulate at higher levels in patients with inflammatory diseases and cancer (Thompson et al., 1993; 1987; Shah et al., 2010). Haptoglobin is synthesized in the liver, and higher concentrations of haptoglobin have been observed in tumor tissues (Ahmed et al., 2004; Takeda et al., 2011). This protein concentration mimics the epithelial-mesenchymal transition phenomenon; however, the response of tumor tissues resembles the inflammatory response in cells, especially fibroblasts (Lee et al., 2006).

The haptoglobin is potentially secreted by 3 different sources: the cancer cells themselves, the surrounding tissue, and/or the liver (Miyoshi et al., 2010). Some haptoglobin proteins are released from normal hepatocytes from the apical region of the liver into the bile duct (Nakagawa et al., 2006). Although cancer cells in the bladder produce prostate specific antigen (PSA) (Glybchenko et al., 2011), this polypeptide is secreted into bile but not blood. Micro- and macrometastases in the liver, however, may ravage the cellular content of the hepatocytes and release this polypeptide into the blood.

Several studies have associated serum haptoglobin concentration with many cancers, including ovarian cancer, breast cancer, small cell lung cancer, esophageal squamous cell carcinoma, pancreatic cancer, liver cancer and prostate cancer (Ahmed et al., 2004; Awadallah et al., 2004; An et al., 2005; Ang et al., 2006; Okuyama et al., 2006; Fujimura et al., 2008; Shah et al., 2010). Furthermore, Dunzendorfer et al. (1980) found increased serum haptoglobin levels in patients with urogenital tumors. Only haptoglobin phenotype has been examined in patients with bladder cancer, as observed in a study by Benkmann et al. (1987). They discovered that a certain haptoglobin phenotype was found at significantly lower levels in patients with bladder cancer.

In the present study, we observed that serum haptoglobin levels were significantly higher in patients with bladder cancer than in healthy controls. Furthermore, we demonstrated the relationship between haptoglobin concentration and tumor stage and distant metastasis.

The serum expression of this protein was increased in bladder cancer, and high levels have been positively correlated with distant metastasis and poor prognosis. Accordingly, haptoglobin is an informative prognostic marker for bladder cancer. This is the first study to report serum haptoglobin levels in patients with bladder cancer.

Haptoglobin plays a fundamental role in cell migration. This role suggests the influence of this protein in cancer and may be used to evaluate the success of treatment of bladder cancer. We demonstrated that haptoglobin levels were associated with tumor progression in bladder cancer.

Haptoglobin could be a useful marker for investigating angiogenesis in bladder cancer. Haptoglobin could be used to estimate the value of a serum cell growth marker and may be used to assess the response to treatment in bladder cancer patients. Serum haptoglobin concentration was investigated in patients over the course of one cycle of anti-cancer treatment.

Our studies showed that serum haptoglobin levels could be a useful independent biomarker in patients with bladder cancer. Overall, our data suggest that high serum haptoglobin levels are associated with bladder cancer metastasis. We believe that more work is necessary; however, this study will be able to benefit anticancer therapies.

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References

- Ahmed N, Barker G, Oliva KT, et al (2004). Proteomic-based identification of haptoglobin precursor as a novel circulating biomarker of ovarian cancer. *Br J Cancer*, **91**, 129-40.
- An JY, Fan ZM, Zhuang ZH, et al (2004). Proteomic analysis of blood level of proteins before and after cooperation in patients with esophageal squamous cell carcinoma at high-incidence area in Henan Province. *World J Gastroenterol*, **10**, 3365-8.
- Ang IL, Poon TC, Lai PB, et al (2006). Study of serum haptoglobin and its glycoforms in the diagnosis of hepatocellular carcinoma: a glycoproteomic approach. *J Proteome Res*, **5**, 2691-700.
- Awadallah SM, Atourn MF (2004). Haptoglobin polymorphism in breast cancer patients from Jordan. *Clin Chim Acta*, **341**, 17-21.
- Beckman G, Eklund A, Frohlander N, Stjernberg N (1986). Haptoglobin groups and lung cancer. *Hum Hered*, **36**, 258-60.
- Benkmann HG, Hanssen HP, Ovenbeck R, Goedde HW (1987). Distribution of alpha-1 antitrypsin and haptoglobin phenotypes in bladder cancer patients. *Hum Hered*, **37**, 290-3.
- Dunzendorfer U, Jung K, Ohlenschlager G (1980). Transferrin, C3 complement, haptoglobin, plasminogen and alpha 2-microglobulin in patients with urogenital tumors. *Eur Urol*, **6**, 232-6.
- Fujimura T, Shinohara Y, Tissot B, et al (2008). Glycosylation status of haptoglobin in sera of patients with prostate cancer benign prostate disease or normal subjects. *Int J Cancer*, **122**, 39-49.

- Gkialas I, Papadopoulos G, Iordanidou L, et al (2008). Evaluation of urine-tumor-associated trypsin inhibitor, CYFRA 21-1, and urinary bladder cancer antigen for detection of high-grade bladder carcinoma. *Urology*, **72**, 1159-63.
- Glybchenko PV, Shakhpazian NK, Ponukalin AN, Zakharova NB (2011). Molecular markers for the diagnosis of non-muscle invasive bladder cancer. *Klin Lab Diagn*, **5**, 16-20.
- Hyrsl L, Zavada J, Zavadova Z, et al (2009). Soluble form of carbonic anhydrase IX in transitional cell carcinoma of urinary tract. *Neoplasma*, **56**, 298-302.
- Kim TS, Rhew HY, Hwang HY (2011). Pilot study of the clinical significance of serum and urinary her-2/neu protein in bladder cancer patients. *Korean J Urol*, **52**, 815-8.
- Kurosky A, Barnett DR, Lee TH, et al (1980). Covalent structure of human haptoglobin: a serine protease homolog. *Proc Natl Acad Sci USA*, **77**, 3388-92.
- Lapham RL, Ro JY, Staerckel GA, Ayala AG (1997). Pathology of transitional cell carcinoma of the bladder and its clinical implications. *Semin Surg Oncol*, **13**, 307-18.
- Lee JW, Kim HS (2006). Endogenous retrovirus HERV-I LTR family in primates: sequences, phylogeny, and evolution. *Arch Virol*, **151**, 1651-5.
- Miyoshi E, Shinzaki S, Moriwaki K, Matsumoto H (2010). Identification of fucosylated haptoglobin as a novel tumor marker for pancreatic cancer and its possible application for a clinical diagnostic test. *Methods Enzymol*, **478**, 153-64.
- Nakagawa T, Uozumi N, Nakano M, et al (2006). Fucosylation of N-glycans regulates the secretion of hepatic glycoproteins into bile ducts. *J Biol Chem*, **281**, 29797-806.
- Okuyama N, Ide Y, Nakano M, et al (2006). Fucosylated haptoglobin is a novel biomarker for pancreatic cancer: a detailed analysis of the oligosaccharide and a possible mechanism for fucosylation. *Int J Cancer*, **118**, 2803-8.
- Safyam A, Singh P, Sharma M, Seth A, Sharma A (2011). CYFRA21-1: a potential molecular marker for noninvasive differential diagnosis of urothelial carcinoma of bladder. *Biomarkers*, **16**, 413-21.
- Shah A, Singh H, Sachdev V, et al (2010). Differential serum level of specific haptoglobin isoforms in small cell lung cancer. *Curr Proteomics*, **7**, 49-65.
- Serrano D, Baglietto L, Johansen H, et al (2005). Effect of the synthetic retinoid on circulating free prostate-specific antigen, IGF-1, and insulin like growth factor binding protein-3 levels in men with superficial bladder cancer. *Clin Cancer Res*, **11**, 2083-8.
- Sharma S, Ksheersagar P, Sharma P (2009). Diagnosis and treatment of bladder cancer. *Am Family Physician*, **80**, 717-23.
- Takeda Y, Shinzaki S, Okudo K, et al (2011). Fucosylated haptoglobin is a novel type of cancer biomarker linked to the prognosis after an operation in colorectal cancer. *Cancer*, **10**, 1002.
- Thompson S, Dargan E, Griffiths ID, Kelly CA, Turner GA (1993). The glycosylation of haptoglobin in rheumatoid arthritis. *Clin Chim Acta*, **220**, 107-14.
- Thompson S, Turner GA (1987). Elevated levels of abnormally-fucosylated haptoglobin in cancer sera. *Br J Cancer*, **56**, 605-10.
- Varela JC, Atkinson C, Woolson R, Keane TE, Tomlinson S (2008). Upregulated expression of complement inhibitory proteins on bladder cancer cells and anti-MUC1 antibody immune selection. *Int J Cancer*, **123**, 1357-63.
- Wassell L (2000). Haptoglobin: function and polymorphism. *Clin Lab*, **46**, 547-52.