

Hepatocyte Growth Factor as a Tumor Marker in the Serum of Patients with Prostate Cancer

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ABSTRACT

Background and Aim: Prostate cancer is a leading cause of death among men worldwide; it is invasive and metastasizes to different organs. Metastatic spread of this type of cancer is the greatest barrier to achieve cure. The present study is carried out to study the serum levels of hepatocyte growth factor (HGF) in patients with prostate cancer in relation to stage and grade and to evaluate its diagnostic and prognostic clinical validity as a tumor marker.

Patients and Methods: The study included 47 patients with prostate cancer and 15 apparently healthy men as a control group. The patients were divided into two groups, including 27 patients with localized prostate cancer (group I) and 20 patients with metastatic prostate cancer (group II). Detection of serum levels of HGF and prostate specific antigen (PSA) was carried out by an enzyme immunoassay.

Results: The serum levels of HGF and PSA were significantly increased in groups I and II as compared to the control group and were highest in group II. The best cut-off value for HGF was 663.8pg/ml with 83% and 93.3% sensitivity and specificity, and was 4.4ng/ml for PSA with a sensitivity and specificity of 85.1% and 100%; respectively; with positive and negative predictive values of 97.5%, 63.6% and 100%, 68.2%; respectively. Combining PSA and HGF was more accurate in distinguishing between patients with metastatic disease and those with localized disease than either marker alone with a sensitivity of 98.1% ($p < 0.05$).

Conclusions: HGF is elevated in the serum of patients with carcinoma of the prostate and this elevation is related to the stage of malignancy and is independent of age. These results imply that HGF may be an important serum marker for prostate cancer.

Key Words: Prostate - Adenocarcinoma - Hepatocyte growth factor (HGF) - Prostate specific antigen (PSA).

INTRODUCTION

Prostate cancer is one of the most common non-cutaneous cancers in men, with a risk increasing after the age of 50 [1]. Because local

extension beyond the capsule of the prostate rarely produce symptoms, about one to two thirds of patients have local extra-capsular extension or distant metastases at diagnosis [2]. The ten years survival rates are 75% in cancer confined to prostate, 55% with regional extension, and 15% with distant metastases [3]. So, early diagnosis is crucial to successful management of prostate cancer patients [4].

Prostate-specific antigen (PSA) is a protein produced by the cells of the prostate gland [5]. It is a tissue-specific serine protease. As men age, both benign and malignant prostate lesions become more frequent. While PSA does not allow doctors to distinguish between a benign prostatic condition and cancer, an elevated PSA level may indicate that other tests are necessary to determine whether cancer is present.

Polypeptide growth factors have been implicated in stromal-epithelial interactions in prostatic carcinoma as well as in a multitude of biological behaviors of prostate cancer cells [6]. Over expression of these protein factors may be a critical mechanism in prostate cancer progression, particularly in the progression of androgen independent disease [7]. Scatter factor (SF), also known as hepatocyte growth factor (HGF), is a pleiotropic polypeptide growth factor with a number of biological activities, including cell scattering, stimulation of cell motility, mitogenesis, morphogenesis, angiogenesis, and cellular invasiveness. HGF complementary DNA has a transforming activity and a broad array of tumors develops in HGF transgenic mice. HGF may induce cell dissociation and scattering of prostate cancer cells, act as a mitogen for DU 145 and PC-3 prostatic

carcinoma cells, and stimulate urokinase-type plasminogen activator and the invasiveness of prostatic carcinoma cells [8]. The membrane receptor for HGF (c-MET protooncogene product) is over expressed in prostatic carcinoma [9]. Recently, over expression of HGF in prostatic carcinoma tissues was also reported [10].

Thus, the aim of this study was to determine the serum level of HGF in men with metastatic prostate carcinoma compared to those with localized cancer and controls in order to assess its clinical value as a serum tumor marker and its relationship with the outcome.

PATIENTS AND METHODS

This study was carried on 47 patients, who were newly admitted to the surgery department of National Cancer Institute, Cairo University from May 2002 to December 2003. Their age ranged from 54 to 75 years. The diagnosis of prostate cancer was confirmed by needle biopsy or after prostatectomy specimen examination. The staging procedures included digital rectal examination (DRE), i.v. pyelography, bone scanning, computed tomography (CT), magnetic resonance imaging (MRI) and/or ultrasonography of the abdomen and pelvic cavity. Besides, 15 apparently healthy, age matched men served as controls.

Patients were Divided Into Two Groups:

Group I: 27 patients with localized prostate carcinoma (stages pT1, pT2 or pT3).

Group II: 20 patients with metastatic prostate carcinoma (pM1).

Blood samples (5ml) were collected from patients by venipuncture before the start of treatment and sera were obtained after clotting and centrifugation for 10min at 3000rpm. Sera were stored at -80°C until assay.

Determination of serum hepatocyte growth factor by quantitative sandwich enzyme immunoassay (ELISA) technique using a kit supplied by R and D systems, Minneapolis MN 55413, USA (catalog no. DHG00): The test procedure began by the addition of samples and standards to microassay wells precoated with the captured antibody. After washing any unbound substances, an enzyme-linked polyclonal antibody for HGF was added. Following a wash to remove

any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of HGF bound in the initial step. The color development was stopped and the intensity of the color was measured [11].

Determination of serum prostate specific antigen by ELISA which is a quantitative sandwich enzyme immunoassay using commercially available kits supplied by Abazyme, (LC, Needham, MA, USA) [12]: Colored reaction products were measured with an ELISA reader. Results are expressed as ng PSA/ml of serum based on a standard curve.

Statistical Analysis: Statistical Package for Social Science (SPSS) program version 9.0 was used for analysis of data. Data was summarized as mean and SD. A non parametric test was used for the analysis of two independent variables. Pearson's correlation was also done, r value was considered weak if <0.25 , mild if ≥ 0.25 - <0.5 , moderate if ≥ 0.5 - <0.75 and strong if ≥ 0.75 . p -value was considered significant if <0.05 . Cut-off value was calculated according to the mean ± 2 SD of the controls. Sensitivity, specificity, positive predictive value, negative predictive value, and over all accuracy of HGF and PSA were calculated.

RESULTS

Table (1) presents the clinical features of the patients and controls.

Table (2) and figures. (1,2) show the descriptive statistics of serum levels of HGF and PSA. HGF was significantly increased in prostate cancer patients when compared to the control group as shown in table (3) ($p < 0.0001$).

A significantly increased serum HGF was found in prostate cancer patients with metastatic disease when compared to patients with a localized disease ($p < 0.002$). The serum levels of PSA showed a significant increase in all prostate cancer patients compared to the controls ($p < 0.0001$), and a significant increase was found in group II when compared to group I ($p < 0.0001$). The elevation in levels of HGF was not related to age ($p > 0.05$) and ($r = 0.2$).

Fig. (3) shows the relation of serum HGF with PSA in the different studied groups. There was a significant positive relation ($p < 0.05$)

between HGF and PSA ($r=0.4$). Combining PSA and HGF was more accurate in distinguishing between patients with metastatic disease and those with localized disease than either marker alone, where the sensitivity increased from 83% for HGF and 85.1% for PSA to 98.1% for the combined markers.

Table (4) shows the sensitivity, specificity, positive predictive value, negative predictive value and over all accuracy of HGF and PSA. The cut-off point of HGF was 663.8pg/ml. Two of the control group showed a HGF level above the cut-off point. On the other hand, 8 of the prostate cancer patients had HGF levels below the cut-off point, whereas none of the patients with metastasizing disease had a HGF level

below the cut-off point. The cut-off point of PSA was 4.4ng/ml. None of the control group had a PSA level above the cut-off value and none of group II patients had a PSA level below the cut-off. Whereas, 7 of group I patients showed levels less than the cut-off point.

Fig. (4) illustrates that serum HGF levels were significantly related to stage of prostate cancer when group I was compared with group II. There was a significant increase in serum levels of HGF in stage pM1 compared to stages pT1 and pT2 ($p<0.005$), but there was no difference between stage pM1 and pT3. Fig. (5) demonstrates the significant relation between serum PSA levels and the stage of prostate cancer ($p<0.0001$).

Table (1): Clinical features of the different studied groups.

Variables	Controls	Group I	Group II
Number	15	27	20
Age	63.6±6.1	61.3±5.6	64.3±6.1
Stage	–	PT1 or pT2 or pT3	pM1

Age expressed as mean±SD.

Group (I) Patients with localized prostate cancer.

Group (II) Patients with metastatic prostate cancer.

Table (2): Descriptive statistics of HGF and PSA levels in the different studied groups.

Variables	HGF (pg/ml)	PSA (ng/ml)
<i>Control group:</i>		
Range	224-794	0.5-3.8
Mean±SD	405±129.4	1.8±1.3
<i>Group I:</i>		
Range	411.2-1450	2.2-77.3
Mean±SD	823.5±262.8	17.4±18.8
<i>Group II:</i>		
Range	738.4-3212	14.1-224.1
Mean±SD	1260.0±641.8	96.0±70.4

HGF = Hepatocyte growth factor.

PSA = Prostate specific antigen.

Table (3): Statistical significance of HGF and PSA between the different studied groups (p value).

	HGF	PSA
C vs GI	0.0001**	0.0001**
C vs GII	0.0001**	0.0001**
GI vs GII	0.002*	0.0001**

Non-significant ($p>0.05$).

* Significant ($p<0.05$).

** Highly significant ($p<0.01$).

Table (4): The sensitivity and the specificity of HGF (pg/ml) and PSA (ng/ml) levels in the different studied groups.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPP (%)
<i>Group I:</i>				
HGF	70.4	93.3	95	63.6
PSA	74.1	93.3	95	63.6
<i>Group II:</i>				
HGF	100	93.3	95.2	100
PSA	100	100	100	100
<i>Groups I & II:</i>				
HGF	83	93.3	97.5	63.6
PSA	85.1	100	100	68.2

PPV = Positive predictive value.

NPN = Negative predictive value.

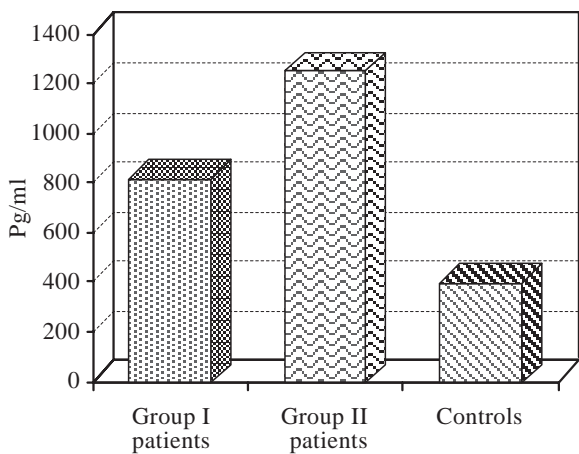


Fig. (1): Hepatocyte growth factor levels in the studied groups.

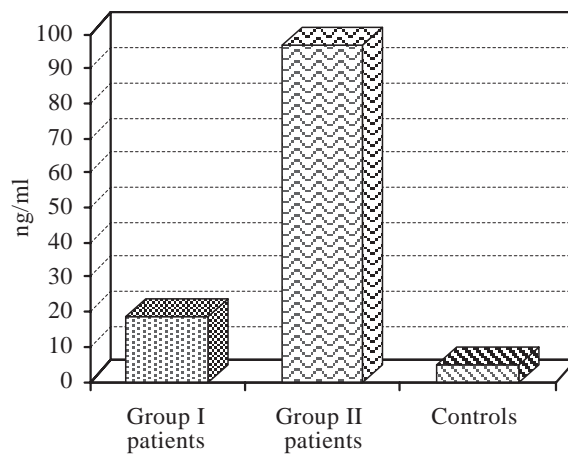


Fig. (2): Prostate specific antigen in the different studied groups.

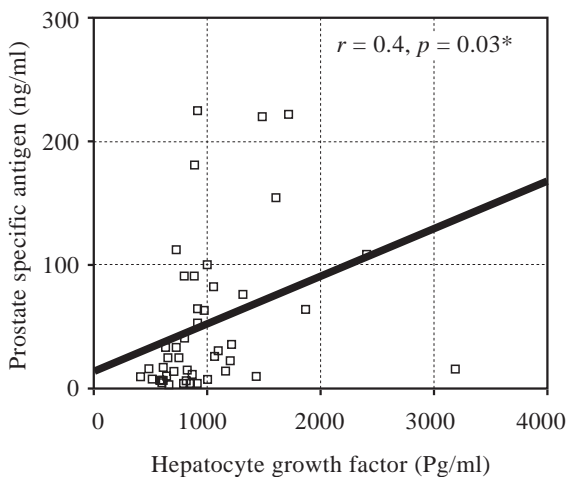


Fig. (3): Correlation between prostate specific antigen and hepatocyte growth factor in patients studied.

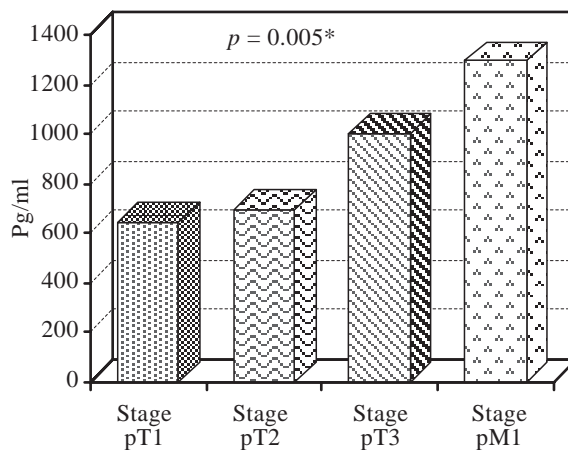


Fig. (4): Hepatocyte growth factor in the studied patients in relation to staging of disease.

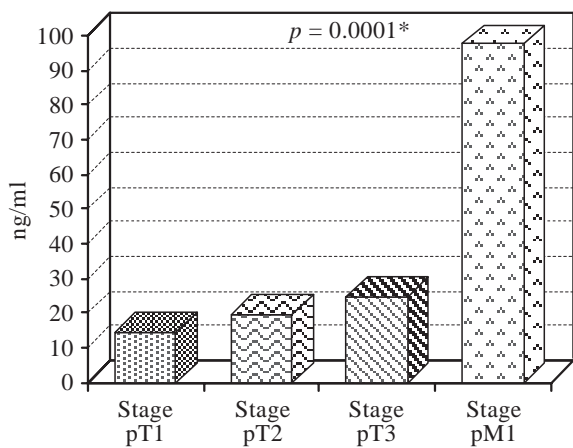


Fig. (5): Prostate specific antigen in the studied patients in relation to staging of disease.

DISCUSSION

HGF has previously been reported to be elevated in the serum of patients with malignancy, including breast cancer [13], colorectal carcinoma [14], non-small cell lung cancer [15], multiple myeloma [16], acute myelocytic leukemia [17] and gastric carcinoma [18]. *In-vitro* HGF may induce prostatic carcinoma cell activities that are associated with the progression and spread of prostate cancer. HGF has been shown *in-vitro* to induce cellular proliferation and scattering of the DU145 prostatic carcinoma cell line [19], disaggregation of LNCaPGC and PC-3 carcinoma cells and invasiveness of the DU145 and PC-3 prostatic carcinoma cell lines

[19]. In bone marrow environment, HGF appears to be important for prostatic carcinoma growth as demonstrated in co-culture experiments. HGF promotes the proliferation, differentiation, motility and invasion of epithelial cells by binding to its cell surface receptor, the *Met* tyrosine kinase. In the prostate, *Met* is expressed predominantly by prostate epithelial cells (PrEC), whereas, HGF is synthesized by prostate stromal cells (PrSC). *Met* is also expressed in localized and metastatic prostate cancers [20]. HGF secreted by PrSC stimulates tyrosine phosphorylation of the *Met* receptor. HGF depletion reduces cell migration by approximately 50%. The response of PrEC is specific for HGF since the other growth factors tested did not significantly affect growth or migration of PrECs [20]. The latter study also reported that neutralizing antibodies against HGF completely abrogated the stimulatory effect of PrSC cells.

The present study showed significantly elevated levels of serum HGF in patients with prostate cancer when compared with controls and in patients with metastatic disease (group II) compared to those with localized disease (group I). The cut-off point of HGF was 663.8pg/ml with a sensitivity of 70.4% in group I and of 100% in group II and a specificity of 93.3% for both groups. So, serum HGF could be used as a noninvasive marker for diagnosis of prostate cancer especially in metastatic patients. Also, HGF may serve as an indicator of tumor progression and future recurrence in patients with prostate cancer. These results are in agreement with Naughton and co-workers who reported that HGF was elevated in men with prostate cancer compared with those without prostate cancer, and the levels were higher in men with metastatic disease than in those with localized disease [21]. They also concluded that HGF is elevated in the serum of patients with prostate cancer and this elevation is related to the stage and grade of disease, implying that HGF may be useful as a serum marker and may also be functionally important in predicting the progression and metastatic spread of carcinoma of the prostate.

Tsuka and colleagues suggested that the basis of increased HGF in the serum of men with prostate cancer may be related to autocrine and/or paracrine mechanisms of regulation of HGF synthesis [22]. Naughton and coworkers

stated that prostatic carcinoma cells express increased HGF compared with benign prostatic epithelial cells but it is not known whether an autocrine loop of regulation of HGF expression exists [21]. Another study illustrated that a paracrine loop appears to be operating since it has been demonstrated that *in-vitro* prostatic carcinoma cells secrete factors that stimulate HGF production by benign prostatic stromal myofibroblasts [10].

Naughton and coworkers further added that it is tempting to speculate that in patients with metastatic prostate cancer to bone, increased serum HGF may be partially related to enhanced HGF secretion by bone marrow stromal cells that is induced by metastatic prostatic carcinoma cells. This proposal represents an avenue for future investigation [21].

In addition, the results of this study showed that the serum PSA level was significantly increased in prostate cancer patients compared to controls, and a significant increase was found in patients with metastatic disease (group II) when compared to patients with localized disease (group I). These results are in agreement with Naughton and co-workers [21]. The cut-off point of PSA in the present study was 4.4ng/ml with a sensitivity of 74.1% and a specificity of 93.3% in group I and both sensitivity and specificity of 100% in group II. The positive predictive value was 100% and the negative predictive value was 68.2%. The sensitivity of PSA in both groups was 85.1%. This is in agreement with Catalona and co-workers who stated that in screening studies; a PSA value greater than 4 had a reported sensitivity of over 80% in detecting prostate cancer in asymptomatic men [23]. On the other hand a sensitivity as low as 27.48% was reported by Gilbert and colleagues for a PSA level between 2.5 and 4.0ng/ml and 30.08% for a range of 4.0 to 10.0ng/ml [24].

After initially using a point cut-off of <4.0ng/ml in 1994, Catalona and colleagues now recommend a cut-off point of 2.6ng/ml as an indicator for biopsy [23]. They found a prostate cancer incidence rate of 22% in the 2.6-4.0ng/ml PSA range (in biopsies). Krumholtz and coworkers found that tumors in this PSA range had favorable characteristics, they were significantly smaller and were more often organ-confined (88% vs. 63%) compared with tumors detected

in the PSA range of ≥ 4.0 ng/ml [25]. In the prostate cancer prevention trial by Thompson and colleagues every man in the placebo group was offered a prostate biopsy after the 7-year study period [5]. For those with PSA values 4.0 ng/ml, the prostate cancer incidence rate was 15% and of these tumors, 15% contained Gleason pattern 4, which indicates that high grade cancer in the low PSA range is not a rare finding. Similarly, a sub-study performed by other investigators in the screening arm of the ESRPC trial for patients with PSA levels in the range of 2.0-3.9 ng/ml, prostate cancer was observed in 17% of the sextant biopsies, and the detection rate was 14% four years after the initial screen [26].

The results of the present study also showed significant positive relation between HGF level and PSA in prostate cancer patients. Combining PSA and HGF was more accurate in distinguishing between patients with metastatic disease and those with localized disease than either marker alone where the sensitivity increased from 83% for HGF and 85.1% for PSA to 98.1% for the combined markers. Similarly, it was found that combining PSA and HGF was more accurate for discriminating metastatic disease from local disease than either marker alone [21]. It was suggested that the serum HGF level may be useful for staging and following the course of treatment in prostate cancer although this use requires validation in a large prospective study. In addition, Kattan and colleagues concluded that HGF level might be additive to existing model for predicting recurrence in men with localized disease [27].

Moreover, Lamszus and coworkers reported that polypeptide growth factors and their receptors may also serve as targets for therapy. There is already an example of the successful application of this approach in the antigrowth factor receptor antibody trastuzumab. This humanized monoclonal antibody is directed against the *Her-2* receptor and has been observed to confer a survival advantage in women with metastatic breast cancer when given with chemotherapy. The *c-MET* receptor for HGF may be targeted similarly.

In conclusion, HGF is significantly elevated in the serum of patients with prostate cancer and this elevation is related to the stage of malignancy and is independent of age. These results imply that HGF could be used as a diag-

nostic marker for prostate cancer. Moreover, HGF can discriminate between localized and advanced prostate cancer. Combining HGF and PSA increases the diagnostic value of these markers in separating localized prostate cancer from metastatic disease.

REFERENCES

- 1- Wingo PA, Tong T, Boldn S. Cancer statistics. CA Cancer J Clin. 1995, 45: 8-30.
- 2- Mettlin C, Jones GW, Murphy GP. Trends in prostate cancer care in the United States, 1974-1990: observation from the patient care evaluation studies of the American College of Surgeons Commission on Cancer. CA Cancer J Clin. 1993, 43: 83-91.
- 3- Kramer BS, Brown ML, Prorok PC, Potosky AL, Gohagan JK. Prostate cancer screening: What we know and what we need to know. Ann Intern Med. 1993, 119: 914-23.
- 4- Fidler IJ. Critical factors in the biology of human cancer metastasis: Twenty-eighth G.H.A. Clowes Memorial Award Lecture. Cancer Res. 1990, 50: 6130-8.
- 5- Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL. Prevalence of prostate cancer among men with a prostate-specific antigen level ≤ 4.0 ng per milliliter. N Engl J Med. 2004, 350: 2239-46.
- 6- Koenenman KS, Yeung F, Chung LW. Osteomimetic properties of prostate cancer cells: A hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. Prostate. 1999, 39: 46-53.
- 7- Netto GJ, Humphrey PA. Molecular biologic aspects of human prostate carcinoma. Am J Clin Pathol. 1994, 102 (4 Suppl 1): S57-64.
- 8- Nakashiro KL, Okamoto M, Hayashi Y, Oyasu R. Hepatocyte growth factor secreted by prostate-derived stromal cells stimulates growth of androgen-independent human prostatic carcinoma cells. Am J Pathol. 2000, 157: 795-804.
- 9- Humphrey PA, Zhu X, Zarnegar R, Swanson PE, Ratliff TL, Vollmer RT. Hepatocyte growth factor and its receptor (c-MET) in prostate carcinoma. Am J Pathol. 1995, 147: 386-91.
- 10- Zhu X, Humphrey PA. Over expression and regulation of expression of scatter/hepatocyte growth factor in prostatic carcinoma. Urology. 2000, 56: 1071-4.
- 11- Nakamura T, Nawa K, Ichihara A. Partial purification and characteristic of hepatocyte growth factor from serum of hepatectomized rats. Bioch Biophys Res Commun. 1984, 122: 1450-59.
- 12- Oesterling JE, Martin SK, Bergstralh EJ, Lowe FC. The use of prostate specific antigen in staging patients with newly diagnosed prostate cancer JAMA. 1993, 269: 57-60.

- 13- Toi M, Taniguchi T, Ueno T, Asano M, Funata N, Sekiguchi I. Significance of circulating hepatocyte growth factor levels as a prognostic indicator in primary breast cancer. *Clin Cancer Res.* 1998, 11: 147-7.
- 14- Fukuura T, Miki C, Inoue T, Matsumoto K, Suzuki H. Serum hepatocyte growth factor as an index of disease status of patients with colorectal carcinoma. *Br J Cancer.* 1998, 78: 454-5.
- 15- Siegfried JM, Weissfeld LA, Luketich JD, Weyant RT, Gubish CT, Landreneau RJ. The clinical significance of hepatocyte growth factor for non-small cell lung cancer. *Ann Thorac Surg.* 1998, 66: 1915-8.
- 16- Seidel C, Borset M, Turesson I, Abildgaard N, Sundan A, Waage A. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. *Blood.* 1998, 91: 806-8.
- 17- Hjorth-Hansen H, Seidel C, Lamvik J, Borset M, Sudan A, Waage A. Elevated serum concentrations of hepatocyte growth factor in acute myelocytic leukemia. *Eur J Haematol.* 1999, 62: 129-7.
- 18- Wu CW, Chi CW, Su TL, Liu WY, Peng FK. Serum hepatocyte growth factor level associated with gastric cancer progression. *Anti-cancer Res.* 1998, 18:36-57.
- 19- Davies G, Jiang WG, Mason MD. Cell-cell adhesion molecules and signaling intermediates and their role in the invasive potential of prostate cancer cells. *J Urol.* 2000, 163: 985-7.
- 20- Gmyrek GA, Walburg M, Webb CP, Yu H, You X, Vaughan ED. Normal and malignant prostate epithelial differ in their response to hepatocyte growth factor/scatter factor. *Am J Pathol.* 2001, 159: 579-90.
- 21- Naughton M, Picus J, Zhu X, Catalona WJ, Vollmer RT, Humphrey PA. Scatter factor-hepatocyte growth factor elevation in the serum of patients with prostate cancer. *J Urol.* 2000, 165 (4): 1325-8.
- 22- Tsuka H, Mori MH, Li B, Kanamaru H, Matsukawa S, Okada K. Enhanced hepatocyte growth factor level in human prostate cancer treated with endocrine therapy. *Int J Oncol.* 1998, 13: 169-6.
- 23- Catalona WJ, Hudson MA, Scardino PT, Richie JP, Ahmann FR, Flaigan RC. Selection of optimal specific antigen cut-offs for early detection of prostate cancer: receiver operating characteristic curves. *J Urol.* 1994, 152: 2037-42.
- 24- Gilbert SM, Cavallo CB, Kahane H, Lowe FC. Evidence suggesting PSA cut point of 2.5ng/mL for prompting prostate biopsy: Review of 36, 316 biopsies. *Urology.* 2005, 65: 549-53.
- 25- Krumholtz JS, Carvalhal GF, Ramos CG, Smith DS, Thorson P, Yan Y. Prostate-specific antigen cut-off of 2.6ng/ml for prostate cancer screening is associated with favorable pathologic tumor features. *Urology.* 2002, 60: 467-73.
- 26- Raaijmakers R, Blijenberg BG, Finlay JA, Rittenhouse HG, Wildhagen MF, Roobol MJ. Prostate cancer detection in the prostate-specific antigen range of 2.0 to 3.9ng/ml: Value of percent free prostate-specific antigen on tumor detection and tumor aggressiveness. *J Urol.* 2004, 171: 2245-49.
- 27- Kattan MW, Eastham JA, Stapietion AMF, Wheeler TM, Scardino PT. A preoperative nomogram for disease recurrence following radical prostatectomy. *J Natl Cancer Inst* 1998, 9: 766-5.