Soluble E-cadherin and IL-6 serum levels in patients affected by prostate cancer before and after prostatectomy

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Received January 31, 2012; Accepted March 27, 2012

DOI: 10.3892/or.2012.1785

Abstract. Prostate specific antigen (PSA) is still the best available tumour marker in prostate cancer (PCa), but presents some limits. Therefore, there is a need for novel markers in the detection and management of PCa. The 80-kDa soluble form of E-cadherin (sE-cad) and the cytokine IL-6 are being discussed as supplemental serum markers for PCa. In this study, sE-cad and IL-6 serum levels were determined in patients with pathological localized or locally advanced PCa without any previous treatment. These patients underwent radical retropubic prostatectomy (RRP) in accordance with the EAU Guidelines on Prostate Cancer. The molecules were determined via immunoenzymatic assays in samples collected before and after surgery. Statistical analysis was performed by Student's t-test and Pearson's correlation test. sE-cad levels were 6.0±2.7 and 4.6±2.3 μg/ml, before and after RRP, respectively. A highly statistically significant decrease in sE-cad concentrations after RRP was observed (P<0.0001), in 50/61 patients (82%). sE-cad levels before and after surgery were correlated (Pearson's correlation coefficient, r=0.6993, P<0.0001). sE-cad values detected after surgery were higher in patients with PSA levels >10 ng/ml (P<0.05). sE-cad levels before RRP were significantly higher in patients with G3 tumours compared to those with G2 tumours (P<0.02). Finally, sE-cad concentrations both before and after surgery were higher in tumours with Gleason score =7 compared to those with Gleason score <7 (P<0.002 and P<0.05, respectively). Preliminary data from 20 patients indicated a statistically significant increase in IL-6 levels after RRP (11.2 vs. 7.2 pg/ml, P<0.001). This is the first study on the reduction in sE-cad levels after RRP in PCa patients. Moreover, it shows that preoperative sE-cad concentrations are higher in patients with less differentiated PCa. Promising findings of this pilot study may lead to investigation of sE-cad in a larger study with follow-up.

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Key words: prostate cancer, serum markers, soluble E-cadherin, IL-6

Introduction

Prostate-specific antigen (PSA) represents the most reliable tumour marker for the diagnosis and management of prostate cancer (PCa) established to date. Nevertheless, PSA presents some limitations including the fact that it does not distinguish indolent from aggressive cancers. This substantially results in the risk of overdiagnosis and overtreatment. Moreover, PSA does not provide prognostic information (1,2). For these reasons, there is an urgent need for new markers to enhance the clinical management of PCa (3). Among biomarkers measured in the serum, adhesion molecules and inflammatory cytokines and their receptors are interesting candidates from a clinical point of view (4-7).

E-cadherin (E-cad) is a calcium-dependent transmembrane protein involved in maintaining cell polarity and normal epithelial structure (8). The loss of E-cad has been recognized as an important mechanism for cancer progression (9). Aberrant expression of the E-cad/catenin complex has been found in PCa and disruption of E-cad is associated with reduced survival (10,11). E-cad ectodomain shedding determines the release into the body fluids of a soluble 80 kDa E-cad fragment (sE-cad) (12). High sE-cad serum levels are present in cancer patients when compared with those of healthy individuals and they are significantly associated with metastasis, recurrence, and prognosis in some malignancies, including PCa (13-18). It has been reported that sE-cad is involved in stimulation of invasion in a paracrine manner, in promotion of cell junction disruption and of cell dissemination thereby inducing the migratory phenotype (19). Moreover, the highest expression levels of sE-cad have been found in patients with metastatic cancers, and high levels at the time of diagnosis are associated with a significant risk of biochemical failure (16-18). Nevertheless, there is no agreement among authors about correlation between sE-cad and clinical or pathological para-meters. Ahmed *et al* (18) found a relationship between

sE-cad and PSA or poor Gleason grade, whereas other authors did not report any correlation between any clinical parameters and sE-cad levels (17).

Regarding interleukins (IL), they are pleiotropic cytokines with a wide range of biological activities including immune regulation, hematopoiesis, inflammation and oncogenesis (20). High levels of IL-6 in serum are associated with more voluminous tumours, higher Gleason score and the presence of lymph node metastases (21,22). These levels are dramatically elevated in patients with PCa metastatic to bone (23). Plasma levels of IL-6 and its soluble receptor in patients with clinically localized PCa are independent predictors of biochemical progression after surgery. The incorporation of the soluble receptor for IL-6 in standard predictive nomograms together with other biomarkers facilitates the identification of patients for adjuvant therapy after radical retropubic prostatectomy (RRP) (24).

In the current study, for the first time the evaluation of E-cad levels was performed in serum samples collected before and after RRP of patients affected by primary PCa. In a small cohort of the same group of patients, IL-6 values were determined. The potential use of these molecules as serum markers for PCa is discussed.

Patients and methods

Patients. Sixty-one patients with clinically localized PCa, who underwent RRP, in accordance with the EAU Guidelines on Prostate Cancer, were enrolled into the study. None of the patients received preoperative hormonal treatment or radiotherapy. On the basis of the current pathological TNM classification, tumours were classified as local or locally advanced PCa. Patients underwent standard pelvic lymphadenectomy (external iliac and obturatory lymph nodes removal) if PSA ≥10 ng/ml and/or biopsy Gleason score ≥7. Serum samples were collected with standard procedure before and after surgical treatment, at least three days after i.v. infusion of fluids in the post-operative period, and kept at -80˚C until the time of sE-cad and IL-6 determinations. For the present analyses, samples were collected at a session between 7:00 and 9:00 a.m. All patients gave oral consent to use their serum samples for research purpose.

Serum analysis of sE-cad and IL-6. Serum concentrations of the 80-kDa fragment were analysed using the Human E-cadherin EIA kit from Zymed Laboratories (San Francisco, CA, USA). In this commercially available kit, HECD-1 is the E-cad-specific antibody known to map to the extracellular domain of E-cad, from where the 80-kDa fragment derives. The absorbance was measured at 450 nm using a standard 96-well microtiter plate reader.

IL-6 was determined by using a human IL-6 ELISA kit (Thermo Scientific, Rockford, IL, USA). The absorbance was measured at 450 and 550 nm using a standard 96-well microtiter plate reader. All samples were run in duplicate and the mean value was used for statistical analysis.

Serum samples collected from healthy donors were tested in each kit as internal control. The control group consisted of 11 healthy men under 30 years of age with no history of previous prostatic disease.

Table I. Patients and tumour characteristics.

Number of Patients	61
Age (years), median	67
Total PSA (ng/ml), mean ± SD	8.98±5.7
Pathological T-stage (N)	
T2	42
T3	15
Unknown	4
Gleason score (N)	
<7	25
=7	31
>7	4
Unknown	1
Tumour grade (N)	
G2	16
G3	27
G4	3
Unknown	15

Statistical analysis. Data analysis was performed by using the GraphPad Prism version 3. Normality test was used to determine whether data are modelled by a normal distribution. The differences between two groups were explored by paired Student's t-test. The relationship between sE-cad and IL-6 serum content and pathological variables was evaluated by linear regression analysis and Pearson's correlation test.

Patients were stratified into four groups according to the major prognostic factors: PSA, T-stage, grade and Gleason score. sE-cad and IL-6 levels in patient subgroups were analyzed using the unpaired Student's t-test. $P<0.05$ was considered as statistically significant.

Results

sE-cad

Patients. The median age of the 61 patients was 67 years (range, 55-74). The mean preoperative PSA for the 61 patients was 8.98±5.7 ng/ml (range, 2.38-28.6). The pathological T-stage was T2 in 42 patients, T3 in 15 patients and unknown in 4 patients. Pathological Gleason score was <7 in 25 cases, =7 in 31 cases, >7 in 4 cases and unknown in 1 case. Tumour grade was G2 in 16 cases, G3 in 27 cases, G4 in 3 cases, unknown in 15 cases (Table I).

Of the 61 patients, 44 had a bilateral neoplasia, 17 had positive surgical margin status, 30 had perineural and 2 seminal vesicle infiltration. Mean wet weight of prostate was 53.3±33.5 g.

sE-cad serum levels. The sE-cad was detectable and measured in all serum samples of the 61 PCa patients. The mean ± SD of sE-cad value was 6.01±2.7 μg/ml (95% CI, 5.32-6.70) in the samples collected before RRP (pre-surgery) and 4.62±2.3 μg/ml (95% CI, 4.01-5.21) in those obtained after RRP (post-surgery) (Fig. 1). Serum E-cad levels were signifi-

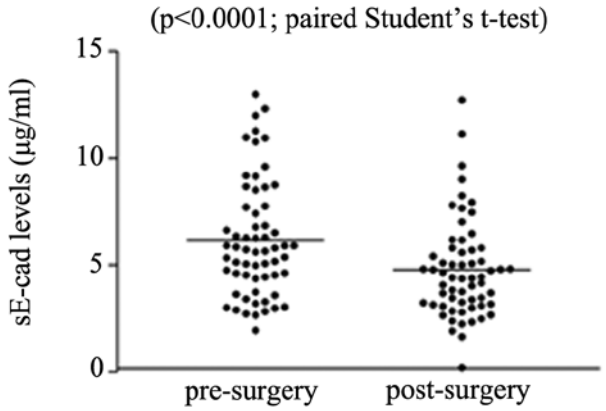

Figure 1. Soluble E-cadherin (sE-cad) levels determined in serum samples collected before and after prostatectomy. Mean values are indicated by horizontal bars.

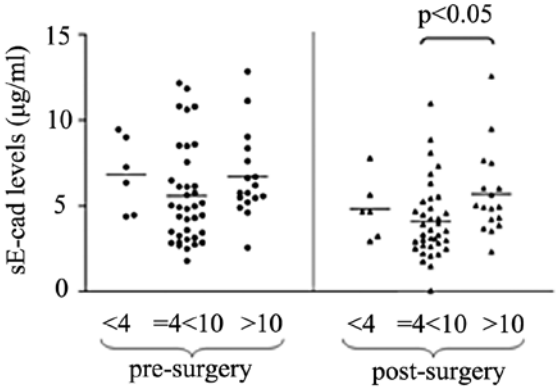

Figure 3. Soluble E-cadherin (sE-cad) levels determined in serum samples collected before and after prostatectomy. Values were stratified into 3 groups based upon PSA levels (<4, =4 <10, >10 ng/ml). Mean values are indicated by horizontal bars.

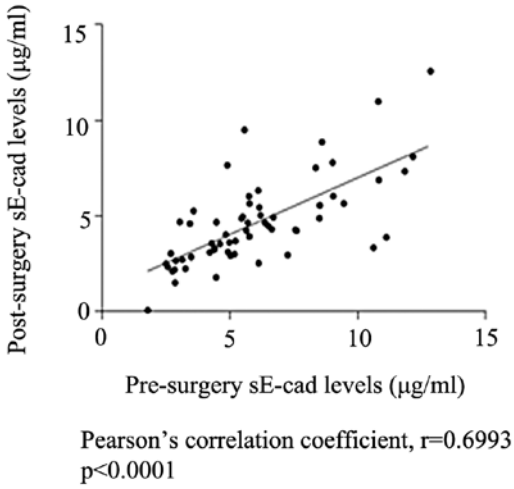

Figure 2. Relationship between soluble E-cadherin (sE-cad) levels determined in serum samples collected before and after prostatectomy determined by using Pearson's Correlation test.

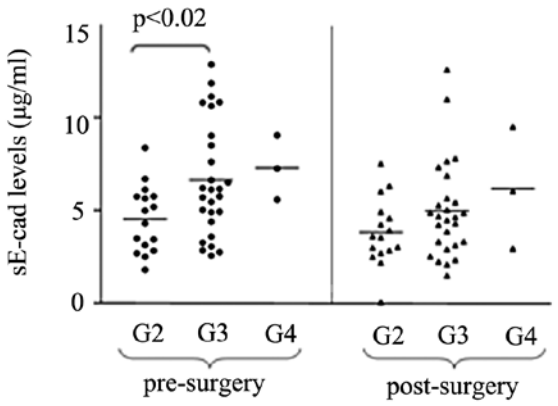

Figure 4. Soluble E-cadherin (sE-cad) levels determined in serum samples collected before and after prostatectomy. Values were stratified into 3 groups based upon tumour grade (G2, G3 and G4). Mean values are indicated by horizontal bars.

cantly higher than those of healthy subjects (4.09±0.45 μg/ml, 95% CI, 3.09-5.09, n=11, P=0.025).

A statistically significant (P<0.0001) reduction in sE-cad levels was observed between the samples collected after surgery and those collected before surgery. It should be noted that sE-cad values were lower in 50/61 cases (82%) after RRP. In 34/50 cases (68%), the decrease was >20%. In 11/61 (18%) patients, an increase in sE-cad levels was found and in 5/11 cases (45%) this increase was >20%. There was no statistically significant difference in sE-cad values detected in serum samples taken after surgery and those obtained from healthy donors.

A positive correlation between sE-cad values before and after RRP (Pearson correlation coefficient, r=0.67, P<0.0001) was found (Fig. 2). On the other hand, no correlation was observed between sE-cad levels and any of the following parameters: preoperative PSA level, tumour stage (TNM), Gleason score, grade, surgical margin status, perineural invasion and weight of prostate gland (data not shown). sE-cad values were retrospectively stratified into three groups based upon PSA levels, Gleason score, grade and T-stage. This analysis revealed that sE-cad values detected after surgery were significantly higher in patients with PSA levels >10 ng/ml (P<0.05, Student's t-test, Fig. 3). sE-cad levels obtained before RRP were significantly higher in patients with G3 tumours than in those with G2 tumours (P<0.02, Student's t-test, Fig. 4) and sE-cad concentrations both before and after surgery were higher in tumours with Gleason score =7 than in those with Gleason score <7 (P<0.002 and P<0.05, respectively, Student's t-test, Fig. 5). No statistically significant difference in the levels of sE-cad was found in groups of patients with different T-stage.

IL-6

Patients. IL-6 levels were determined in 20 patients randomly chosen. The median age of these patients was 67 years (range, 57-72). The mean preoperative PSA for all 20 patients was 8.02±5.8 ng/ml (range, 2.8-28.6). The pathological T-stage was T2 in 11 patients, T3 in 5 patients and unknown in 4 patients. Gleason score was <7 in 4 cases; equal to 7 in 14 cases and >7 in 2 cases. Tumour grade was G2 in 2 cases, G3 in 10 cases, and unknown in 8 cases (Table II).

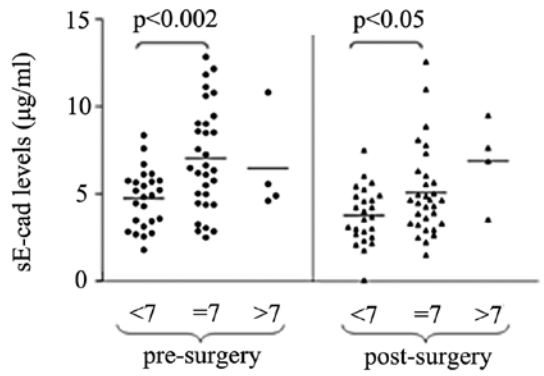

Figure 5. Soluble E-cadherin (sE-cad) levels determined in serum samples collected before and after prostatectomy. Values were stratified into 3 groups based upon Gleason score (≤6, =7, ≥8). Mean values are indicated by horizontal bars.

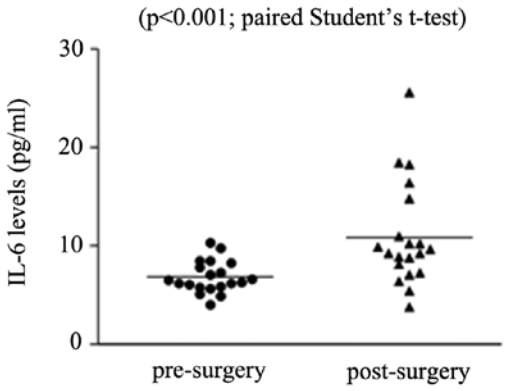

Figure 6. IL-6 levels determined in serum samples collected before and after prostatectomy. Mean values are indicated by horizontal bars.

Table II. Patients and tumour characteristics of the cases in which IL-6 was evaluated.

Number of Patients	20
Age (years), median	67
Total PSA (ng/ml), mean ± SD	8.02±5.8
Pathological T-stage (N)	
T2	11
T3	5
Unknown	4
Gleason score (N)	
<7	4
=7	14
>7	2
Tumour grade (N)	
G2	2
G3	10
Unknown	8

Of the 20 patients, 16 had a bilateral neoplasia, 7 had positive surgical margin status, 13 had perineural and 1 seminal vesicle infiltration. The mean wet weight of prostate was 57.8±32.49 g.

IL-6 serum levels. The mean ± SD of IL-6 value was 7.19±2.29 pg/ml (95% CI, 6.07-7.58) and 11.22±5.08 pg/ml (95% CI, 8.49-13.23) before and after RRP, respectively (Fig. 6). A statistically significant (P<0.001) increase in IL-6 levels was observed in the samples collected after surgery with respect to those collected before.

No correlation was found between IL-6 values and all the clinical and pathological parameters, or between IL-6 and sE-cad levels (data not shown).

Discussion

In the present study, the serum levels of sE-cad and IL-6 were determined in samples collected before and after RRP from a strictly selected group of patients affected by primary PCa. Our patients were homogeneous in age and had not received any previous treatment. This is quite important because it is reported in the literature that sE-cad and/or IL-6 values can be affected by age, treatment or circadian rhythm (25-27), even if there is no agreement among all authors particularly regarding sE-cad (25,28). Moreover, as described in Patients and methods, blood samples were taken at least three days after i.v. infusion of fluids in the postoperative period to avoid less concentrations of blood solutes resulting from plasma dilution.

sE-cad levels found in preoperative serum samples are in the range reported in the literature in patients affected by this or other types of cancers (5,13,15,16,28). Values determined in patients were significantly higher than those observed in healthy subjects according to reports by other authors (5,15,16,18,28). After RRP, a significant decrease in E-cad levels was observed. It should be pointed out that this was evident in 50/61 cases (82%). To our knowledge, this is the first time that the variation in sE-cad values after RRP has been evaluated. For the first time, we have demonstrated that surgery diminishes the circulating form of E-cad in patients affected by PCa. The results from linear regression analysis and the Pearson's correlation coefficient indicate a relationship between E-cad levels in preoperative and postoperative samples. No correlation exists between levels of sE-cad and biochemical and histopathological findings. However, some interesting results were observed when patients were stratified into subgroups even if it should be pointed out that the limit of these subgroups is represented by the small number of subjects included in each of them. Despite this problem, it should be remarked that sE-cad levels were higher in patients with high grade tumours (G3 vs. G2 and Gleason score =7 vs. <7). This is in agreement with data from other authors (16-18), although we did not find any statistically significant correlation.

Moreover, in patients with PSA =4 <10 ng/ml the reduction in sE-cad after surgery was higher than that of patients with PSA >10 ng/ml.

Concerning IL-6, values before surgery were in the range reported in the literature (22,24) and a statistically significant increase in IL-6 levels was observed after surgery. This could be consistent with the existence of inflammatory and regenerative phenomena intervening in the postoperative period. No

correlation was found between the levels of IL-6 and sE-cad detected before and after surgery in the same patients. As far as we know, there is no agreement in the literature concerning variation of serum IL-6 levels after surgery. This may be due to the differences in the time in which serum samples were collected. Shariat *et al* (22) found a decrease while Jurczok *et al (*29) observed an increase in IL-6 levels after RRP but in the first study samples were collected after 6-8 weeks while in the second after 0-72 h from the surgery.

In our study, there was no association between IL-6 serum levels and any of the clinical and pathological parameters considered.

Our findings indicate that surgery results in a decrease of sE-cad and in an increase in serum levels of IL-6, in patients affected by PCa. The same biological responses have been reported by Lin *et al* in patients affected by colon cancer who underwent surgery (30). Demonstration that the levels of these molecules are modified significantly in the post-surgery period must be considered important, particularly in view of their use as follow-up markers. It would be useful to compare the values of these markers measured during follow-up with the values determined before surgery or after surgery and this is what we are doing with a new cohort of patients.

At present, the small number of cases in which the cytokine IL-6 was determined does not allow us to draw any conclusion. As far as sE-cad is concerned, the overall results obtained so far, even if preliminary, are encouraging in the use of this molecule as a valuable marker associated with PSA in PCa diagnosis and as a prognostic marker at diagnosis. Further investigations with a larger number of subjects and a follow-up may better define the role of these molecules in the clinical management of patients with PCa.

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