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CHROMOGRANIN A EXPRESSION IN FAMILIAL VERSUS SPORADIC PROSTATE CANCER

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ABSTRACT

Objectives. To evaluate whether a significant difference in chromogranin A (CgA) levels exist between patients with familial and sporadic cancer.

Methods. The study included 146 patients with clinically localized prostate adenocarcinoma (Stage T2N0M0), who underwent radical prostatectomy between June 1999 and June 2004. Patients were considered to have a positive family history for prostate cancer when the index patient confirmed the diagnosis of prostate cancer in a first-degree relative (brother or father). On the day of surgery, a blood sample for the determination of serum prostate-specific antigen and CgA levels (radioimmunoassay) was obtained from all patients. In a subgroup of 20 patients, CgA mRNA expression was also evaluated by reverse transcriptase-polymerase chain reaction at the prostatic tissue level.

Results. A positive familial history was found in 28 (19.2%) of the 146 patients. The mean patient age in the familial group was significantly ($P < 0.0001$) lower than that in the sporadic group. No significant difference between the familial and sporadic groups was found in terms of prostate-specific antigen level ($P = 0.9625$) or Gleason score distribution ($P = 0.4891$). The familial group had significantly ($P = 0.0013$) lower serum CgA levels (43.3 ± 12.7 ng/mL, median 39.9) compared with the sporadic group (55.9 ± 19.4 ng/mL, median 54.1). The familial group also had significantly ($P = 0.0432$) lower expression of tissue CgA mRNA compared with the sporadic group.

Conclusions. The result of significantly lower CgA expression in familial compared with sporadic prostate cancer cases suggests that neuroendocrine activity is not increased in familial cases and also confirms that familial cancer is not a more aggressive disease. UROLOGY **66:** 1010–1014, 2005.

A family history of prostate cancer has been established as one of the strongest risk factors for prostate cancer.[1] Men with a first-degree relative (father or brother) affected were twice as likely to have prostate cancer as men with no relatives affected.[2,3] Familial aggregation of prostate cancer is observed in about 15% to 25% of cases.[4] The clinical presentation of familial prostate cancer remains controversial. No specific clinical or pathologic patterns associated with familial prostate cancer have been observed, except for early age at diagnosis.[1,5] Whether familial prostate cancer has a different prognosis than sporadic prostate cancer is also still controversial.

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Recently, increasing attention has been focused on the neuroendocrine (NE) differentiation of prostate adenocarcinoma and, in particular, on its possible clinical significance. Some investigators have stressed that prostate adenocarcinoma with NE differentiation tends to be more aggressive.[6]

Serum levels of NE markers, particularly chromogranin A (CgA), could reflect the NE activity of prostate carcinoma and could be used during clinical evaluation. At present, CgA appears to be the best marker of NE activity in the prostate gland.[7] The clinical relevance of NE activity in advanced prostate cancer after androgen deprivation therapy is better recognized.[6,7] Moreover, we and others, have stressed that in nonmetastatic prostate cancer considered for radical prostatectomy, serum CgA could also be a significant predictor of locally advanced (pT3) and poorly differentiated (Gleason score 7 [4 + 3] or greater) prostate adenocarcinoma.[6,8] Therefore, if NE activity influences prostate cancer growth, one might expect NE markers, such as CgA, to

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correlate with a more aggressive disease. The aim of the present study was to evaluate whether in patients with newly diagnosed clinically localized prostate adenocarcinoma, a significant difference in CgA levels exists between familial and sporadic prostate cancer. Our analysis could also confirm whether familial disease is a different and more aggressive disease. However, in this study we did not evaluate any correlations with progression or patient survival.

MATERIAL AND METHODS

This was a prospective, single-center study. Between June 1999 and June 2004, 184 consecutive men with newly diagnosed clinically localized prostate cancer underwent radical retropubic prostatectomy (RRP) and staging lymphadenectomy at our clinic. Inclusion in this study was based on the following criteria: clinically localized T2N0M0 prostate adenocarcinoma, no previous hormonal or radiotherapy, no previous surgery of the prostate gland, and histologically proven adenocarcinoma of the prostate at RRP. Of the 184 men, 146 met the inclusion criteria and agreed to inclusion in this analysis. All men were white. All 146 patients had newly diagnosed, biopsy proven, clinical Stage T2N0M0 prostate adenocarcinoma, as determined by digital rectal examination, transrectal ultrasonography, bone scan, and computed tomography. None of these patients presented with a history of other disorders or therapies or conditions known to interfere with the CgA level (ie, NE malignancies, previous or concomitant other neoplastic history, adrenal incidentaloma, endocrine manipulation therapies, or uncontrolled hypertension). For all patients, the family history was accurately evaluated at presentation as a part of the initial and preoperative studies by the physician. A positive family history was noted when the index patient confirmed the diagnosis of prostate cancer in a first-degree relative (brother or father). Confirmation of a positive family history was done by the patient and, as in other studies,[9] for most of the patients we did not have access to the pathology reports for the prostate cancer of the relative. A family history was considered positive if the index patient knew that the relative had had prostate cancer. A report of "bone metastasis" or "prostate troubles" was not considered positive. The 146 patients were divided into two groups: familial cases (those with a first-degree relative with prostate cancer); and sporadic cases (those with only the index case of prostate cancer).

In all patients, RRP was performed by an anatomic approach. All RRP specimens were evaluated pathologically (whole-mounted and 3-mm step sectioned from the apex to base) at our institute according to a routine technique. Tumor stage was assigned according to the 1997 modification of the TNM classification for all 146 patients.[10] Tumor grade was described according to the Gleason grading system.[11] On the basis of the histologic grade, patients were separated into two different groups: Gleason score 7 (3 + 4) or less and Gleason score 7 (4 + 3) or greater. On the basis of the pathologic stage, patients were stratified into pT2 and pT3 groups. No patient had pT4 in this study.

Preoperative Serum Prostate-Specific Antigen and CgA Determination

On the day of RRP, before the surgical procedure, but at least 3 weeks after any prostatic manipulation, a blood sample was drawn for the determination of the preoperative serum total prostate-specific antigen (PSA) and CgA levels from all patients. Each sample was homogeneously collected in the early morning after an overnight fast. In each case, serum CgA was measured by radioimmunoassay using a commercial kit (CIS Bio International, Cedex, France). The detection limit of this kit is 1.5 ng/mL. The interassay and intra-assay coefficient of variation of the CgA assay is 5.8% and 3.8%, respectively. The normal range reported by the kit for serum CgA is less than 80 ng/mL. In each case, the same serum sample was also used to determine the total PSA level (Hybritech, San Diego, Calif). All samples were evaluated centrally in the laboratory of our university.

TABLE I. ***Clinical and pathologic characteristics***

Patients (n)	146
Family history of prostate cancer	
Yes	28 (19.2)
No	118 (80.8)
Age (yr)	
Mean ± SD	66.0 ± 3.5
Median	67.0
Range	56.0–72.0
PSA (ng/mL)	
Mean ± SD	11.6 ± 4.5
Median	11.5
Range	4.3–21.5
CgA (ng/mL)	
Mean ± SD	53.5 ± 18.9
Median	51.6
Range	16.7–127.0
pT2 stage	93 (63.7)
pT3 stage	53 (36.3)
Lymph node positive (pN+)	4 (2.7)
Gleason score	
≤7 (3 + 4)	99 (67.8)
≥7 (4 + 3)	47 (32.2)

Key: PSA = prostate-specific antigen; CgA = chromogranin A.
Data presented as number of patients, with percentages in parentheses, unless otherwise noted.

Tissue CgA mRNA Extraction and Reverse Transcriptase-Polymerase Chain Reaction

In the first 20 consecutive prostate cancer cases included in this study, we had the opportunity to analyze CgA mRNA expression on tissue samples obtained from RRP. Prostatic tissue samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis. In each specimen, the diagnosis of prostate adenocarcinoma was histologically confirmed. Each sample weighed about 1 g. Gene expression of CgA was evaluated by semiquantitative reverse transcriptase-polymerase chain reaction, using beta-actin as a control. The method has been previously described.[12,13]

Statistical Analysis

The study objectives called for a design that would detect a statistically significant difference between measures of 25% at $P \leq 0.05$ with a power of 90% (type II or beta error of 0.1). Using standard power analysis methods, a sample size of at least 150 subjects was estimated to be sufficient.

Univariate and multivariate analyses were performed to determine significant differences between the familial and sporadic cases (chi-square test, Fisher's exact test). Spearman correlation coefficients were calculated to measure the association among parameters. Variations in the parameters in the familial and sporadic groups were recorded and Fisher's exact test and the

TABLE II. *Clinical and pathologic characteristics stratified by family history*

Characteristic	Positive Family History	Negative Family History	*P* Value
Patients (n)	28 (19.2)	118 (80.8)	
Age (yr)			
Mean ± SD	61.5 ± 3.0	67.1 ± 2.6	<0.0001*
Median	62.0	67.0	
Range	56.0–67.0	60–72	
PSA (ng/mL)			
Mean ± SD	11.5 ± 4.3	11.6 ± 4.6	0.9625*
Median	11.5	11.6	
Range	4.3–20.6	4.3–21.5	
CgA (ng/mL)			
Mean ± SD	43.3 ± 12.7	55.9 ± 19.4	0.0013*
Median	39.9	54.1	
Range	27.8–78.4	16.7–127.0	
Gleason score			
≤7 (3 + 4)	22 (78.6)	84 (71.2)	0.4891†
≥7 (4 + 3)	6 (21.4)	34 (28.8)	
pT2	18 (64.3)	75 (63.6)	0.5324†
pT3	10 (35.7)	43 (36.4)	
CgA mRNA			
Mean ± SD	0.3843 ± 0.2511	0.7694 ± 0.4767	0.0432*
Median	0.3566	0.6479	
Range	0.1070–0.7171	0.1077–1.8420	

Abbreviations as in Table I.
Data presented as number of patients, with percentages in parentheses, unless otherwise noted.
* *Student's* t *test.*
† *Fisher's exact test.*

unpaired *t* test were performed. Some factors, such as Gleason score (7 [3 + 4] or less versus 7 [4 + 3] or greater) and pT stage (pT2 versus pT3) were dichotomized and transformed into indicator variables. PSA and CgA were used as continuous variables. A 5% level of significance was used for all statistical testing.

RESULTS

Of the 146 patients analyzed, 28 (19.2%) had a positive family history. The distribution of affected relatives was the father for 19 patients (67.9%), at least 1 brother for 7 patients (25.0%), and the father and brother for 2 patients (7.1%; Table I).

No significant association was found between the presence of a family history and the serum PSA level (r [Spearman coefficient] = −0.0037, P = 0.9642), pathologic stage (pT2 versus pT3; r = −0.0085, P = 0.9191), or Gleason score (less than 7 [3 + 4] versus greater than 7 [4 + 3]; r = −0.0674, P = 0.4206) of the tumor (Table II). Only 4 patients had positive lymph nodes (pN+), 2 in the familial and 2 in the sporadic group. A positive family history was significantly associated with age (r = −0.5769, P <0.0001) and serum CgA levels (serum CgA, r = −0.2841, P = 0.0005; tissue CgA mRNA, r = −0.3795, P = 0.0489). Thus, when comparing the two groups, familial and sporadic, no significant differences were found in PSA level (P = 0.9625), Gleason score (P = 0.4891), or pT stage (P = 0.5324; Table II). In contrast, the mean patient age in the familial group was significantly (P <0.0001) lower than that in the sporadic group. Moreover, for the first time, we showed significantly (P = 0.0013) lower mean CgA levels in the familial group compared with the sporadic group (Table II). Considering the normal range reported by the kit for serum CgA, no patients in the familial group and 15 patients (12.7%) in the sporadic group had a CgA level greater than the range.

Multivariate Analysis

We used multivariate analysis to determine whether the serum CgA levels were significantly and independently associated with a family history for prostate cancer compared with serum PSA level, age, Gleason score, and pT stage. The analysis was performed with family history as the dependent variable and age, serum CgA, PSA, Gleason score, and pT stage as independent variables. Only serum CgA and age were significantly and independently associated with a positive family history for prostate cancer (CgA, P = 0.0024, coefficient = 0.00986; age, P = 0.0016, coefficient = 0.00858). The relative risk of familial prostate cancer significantly decreased according to CgA serum levels. Using CgA 20 ng/mL (lowest CgA level in our pop-

ulation) as the reference, for CgA values of 40 to 59 ng/mL, the relative risk was 0.7972 ((95%) confidence interval 0.6163 to 1.031; $P = 0.1217$) and decreased to 0.7073 ((95%) confidence interval 0.5554 to 0.9007; $P = 0.0171$) and 0.5585 ((95%) confidence interval 0.4282 to 0.7210; $P = 0.0188$) for CgA levels of 60 to 79 and 80 to 100 ng/mL, respectively.

CgA mRNA Tissue Expression in Subgroup of 20 Patients

In the first 20 consecutive cases included in the study, we analyzed CgA mRNA expression at the tissue level. In this subgroup, 4 patients (20%) had a familial history of prostate cancer. In all samples examined, expression of CgA mRNA was found. Densitometric analysis of CgA prostate adenocarcinoma products, normalized to that of beta-actin, demonstrated that prostate adenocarcinoma samples obtained from familial patients presented with levels of CgA mRNA significantly ($P = 0.0432$) lower than those from sporadic patients (Table II). Moreover, the mRNA levels of CgA were positively and significantly associated with the serum concentration of CgA ($r = 0.4690$, $P = 0.0390$).

COMMENT

To our knowledge, this is the first reported study that specifically analyzed CgA expression in familial compared with sporadic prostate adenocarcinoma. As in previous studies,[4,9,14–16] in this study, we used a population of patients with newly diagnosed clinically localized prostate adenocarcinoma who were undergoing RRP. As in another analysis,[9] the family history was prospectively evaluated at presentation by the physician, and a positive family history was noted when the patient confirmed the diagnosis of prostate cancer in a first-degree relative. Following previous reports,[9] we decided to limit the positivity for a family history to brothers or the father. Also, for most patients, we did not have access to the pathology report for the relative. In our population, familial aggregation of prostate cancer was observed in 19.2%, within the range reported in published studies.[4] Patients with familial prostate cancer had similar presenting features (PSA level) and pathologic findings as those with sporadic prostate cancer. We focused our attention on the expression of CgA as a marker of NE activity in prostate adenocarcinoma. Different investigators[17–19] have demonstrated a correlation between the number of CgA-positive NE cells and serum CgA levels in patients with prostate adenocarcinoma. Berruti *et al.*[20] showed a progressive increase in the median levels of serum CgA from patients with benign prostatic hyperplasia through those with nonmetastatic and metastatic prostate cancer. In previous studies,[8,12,21,22] we found a significant correlation between serum CgA levels and CgA mRNA expression in prostate adenocarcinoma tissue, as well as in nonmetastatic disease. In the present study, we specifically analyzed whether familial prostate adenocarcinoma presents with different CgA levels compared with sporadic disease. The rationale for this kind of analysis is based on the hypothesis that NE activity influences prostate adenocarcinoma, such that one might expect CgA expression, as an NE marker, to correlate with disease aggressiveness. Therefore, our analysis could also confirm whether familial disease is a different and more aggressive disease compared with sporadic disease. For the first time, in patients with prostate adenocarcinoma, we found a statistically significant ($P = 0.0005$) association between a positive family history and serum CgA levels and significantly ($P = 0.0013$) lower mean and median CgA levels in familial compared with sporadic disease. The CgA levels reported in our study are modest if compared with those observed in pure pathologically confirmed NE tumors. We must remember that NE differentiation of prostate adenocarcinoma consists of the presence of NE cells with a focal distribution in common prostate adenocarcinoma.[23] Therefore, particularly in patients with nonmetastatic prostate cancer, it is not possible to expect the same levels and variations in CgA as those found in pure NE tumors. Moreover, at present, we do not have enough data to define a normal range for CgA levels in the presence of prostate adenocarcinoma. On the basis of the results of a previous multivariate analysis in patients with nonmetastatic prostate cancer selected for RRP, we proposed a cutoff value of 60 ng/mL for serum CgA.[8] For all these reasons, the differences in serum CgA levels observed in the present study remain significant. Also, in most patients, the values were less than the normal reference range for the commercial kit (0% in familial and 12.7% in sporadic). The differences in serum CgA levels reported in our study may also have been influenced by the different age range between the two groups (familial versus sporadic). However, as in previous studies,[8] in our population, a weak and not significant association was found between CgA level and age ($r = 0.1012$, $P = 0.3266$). Moreover, we used multivariate analysis to confirm the independent and significant association of serum CgA levels with family history.

The possible limitations of our study must be acknowledged. As previously published,[12,17] none of our patients presented with a history of NE malignancies or other diseases known to interfere with CgA levels. In a subgroup of our patients, we also analyzed by reverse transcriptase-polymerase chain reaction, CgA mRNA expression on tissue

samples obtained from RRP.[12,13] The confirmation at tissue level of lower CgA expression in patients with familial cancer and the significant correlation between serum and tissue expression of CgA, suggest that, in our population, serum CgA levels were significantly influenced by the NE activity in prostate adenocarcinoma.

CONCLUSIONS

In our experience, age and CgA levels were the only features that correlated significantly with a positive family history of prostate cancer. It should be emphasized that any conclusions regarding this association and its clinical implication can only be drawn from larger, controlled clinical trials. The finding of significantly lower CgA expression in familial compared with sporadic prostate cancer confirms that familial prostate adenocarcinoma is not a more aggressive disease in terms of NE activity.

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