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LEVELS OF INSULIN-LIKE GROWTH FACTOR I (IGF-I) AND IGF BINDING PROTEINS 2 AND 3 IN SERIAL POSTOPERATIVE SERUM SAMPLES AND RISK OF PROSTATE CANCER RECURRENCE

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ABSTRACT

Objectives. To determine changes of insulin-like growth factor I (IGF-I), IGF binding protein-2 (IGFBP-2), and IGFBP-3 levels in serial postoperative serum samples from prostate cancer patients with and without relapse and to evaluate the prognostic value of these molecules in the recurrence of prostate cancer.

Methods. From a group of patients with prostate cancer who had been followed for disease recurrence for almost 5 years after radical prostatectomy, we selected 38 patients (cases) who developed recurrent disease and 40 patients (controls) who were in remission. Of these patients, 70 had 4 and 8 had 3 serial postoperative serum samples collected. The median times for serum collection after surgery were 1.5 years for the first serial samples, 2.6 years for the second, 3.5 years for the third, and 4.5 years for the fourth. Serum levels of IGFBP-2, IGFBP-3, and IGF-I were measured, using commercial immunoassay kits.

Results. The study showed lower serum levels of IGFBP-2 and IGFBP-3 in the cases than in controls ($P < 0.05$) but no difference in IGF-I levels between the two groups ($P = 0.277$). In the sequential samples, IGFBP-2 levels increased over time in the controls ($P = 0.014$) but did not change in the cases ($P = 0.528$). There were no increasing or decreasing trends for IGF-I and IGFBP-3 in either case or control group ($P > 0.05$).

Conclusions. The study suggests that IGFBP-2 may play a role in the progression of prostate cancer, but serum levels of IGFBP-2 as well as IGF-I and IGFBP-3 have no predictive values in the prognosis of prostate cancer. UROLOGY **57:** 471–475, 2001.

Insulin-like growth factors (IGFs), including IGF-I and IGF-II, are mitogenic peptides involved in regulation of cell proliferation, differentiation, and apoptosis.[1] Studies[2,3] have shown that IGFs are potent mitogens for a variety of cancer cells, including prostate cancer. They not only stimulate cancer cell growth, but IGFs also suppress programmed cell death.[4] The mitogenic and anti-apoptotic actions of IGFs are mediated by a specific cell membrane receptor, IGF-I receptor (IGF-IR).[5] The receptor has tyrosine kinase activity, and binding of IGFs to the receptor activates the kinase that triggers signal transduction pathways, involving mitogen-activated protein (MAP) kinase and phosphoinositide 3 (PI3) kinase. The interaction between IGFs and IGF-IR is regulated by a group of specific IGF binding proteins (IGFBPs) that can either inhibit or enhance the actions of IGFs.[6] The dual regulatory effects of IGFBPs on IGFs are determined by their post-translational modification in which IGFBP proteases play an important role.[7] Prostate-specific antigen (PSA) has been found to be one of the IGFBP proteases.

Most members of the IGF family, including IGFs, IGF-IR, as well as some IGFBPs and their proteases, are found in the prostate.[8] In vitro stud-

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ies show that IGFs are strong mitogens for prostate cancer cells and that suppressing the mitogenic stimulation of IGFs can slow down the growth of prostate cancer.[3,9] In humans, epidemiologic studies have found that high plasma levels of IGF-I are associated with increased risk of prostate cancer.[10,11] The effect of IGFs on the progression of prostate cancer has also been evaluated in a number of clinical studies.[12–14] These studies found that serum IGFBP-2 levels were elevated and that IGFBP-3 levels were decreased in patients with prostate cancer compared to those without the cancer, suggesting that the two binding proteins may have clinical implications in the prognosis of prostate cancer. To further examine this possibility, we compared changes of IGF-I, IGFBP-2, and IGFBP-3 levels in sequentially collected postoperative serum samples between prostate cancer patients with and without recurrence.

MATERIAL AND METHODS

Patients

In a clinical study that used prostate-specific antigen (PSA) to monitor prostate cancer recurrence after radical prostatectomy, we recruited 347 patients with prostate cancer consecutively from a prostate clinic at the Toronto Hospital between February 1993 and November 1994.[15] Among these patients, we collected 3 or more serial postoperative serum samples from 148 patients. Of the 148 patients, 51 had biochemical relapse as determined by substantial increases in PSA levels in serial postoperative serum samples. From the group of patients who had at least 3 postoperative serum samples stored in our serum repository, we selected for the study 38 patients (cases) who had developed recurrence and 40 patients (controls) who remained in remission by the end of 1997. The selection was based on the amount of serum specimens available for the study. The median follow-up time was 53 months for the cases and 55 months for the controls.

All patients in the study underwent ultrasound-guided transrectal needle biopsy to establish their histologic diagnosis before surgery; the patients also had a complete evaluation before the surgery, including bone scan, chest x-ray, serum levels of alkaline phosphatase, and physical examination, to exclude the presence of metastatic lesions. During radical prostatectomy, an open pelvic lymphadenectomy was performed to determine the presence of regional nodal metastases before prostatectomy. Pathologic staging was done, based on the complete surgical specimens; parameters recorded in the pathology report include Gleason score, capsular penetration of tumor tissue, nodal status, and tumor involvement in the surgical margins, periprostatic tissues, bladder neck, and seminal vesicle. On the basis of the TNM staging, 8.6% patients had T1a disease, 1.4% T1b, 30% T1c, 48.6% T2a, 8.6% T2b, and 2.8% T3. With regard to histologic grade, 2.6% patients had Gleason score 4 or lower, 10.5% had 5, 18.4% had 6, 46.1% had 7, 18.4% had 8, and 2.9% had Gleason score 9. Because the majority of patients were considered in remission when their postoperative blood samples were collected, information on postoperative treatment was not available.

Serum Samples

Serial serum samples were collected from the patients for PSA testing during their follow-up after radical prostatectomy. Of the 38 cases, 32 had 4 serial samples and 6 had 3 serial samples. On average, the first serial samples were collected 1.4 years after the operation, the second samples 2.6 years, the third samples 3.4 years, and the fourth samples 4.6 years. For the controls, 38 had 4 serial samples and only 2 had 3 serial samples. The average time for serum collection was 1.5 years after the surgery for the first serial samples, 2.6 years for the second ones, 3.8 years for the third ones, and 4.8 years for the last ones.

PSA Test and Biochemical Relapse

Two PSA assays were used in the study to determine serum PSA concentration. The Abbott IMx PSA assay (Abbott Laboratories, Abbott Park, Ill) was used for prostate cancer diagnosis and patient management after surgery in the clinic. As part of a research project reported in a previous study,[15] an ultrasensitive PSA assay with detection limit of 0.001 ng/mL was used to monitor postoperative serum PSA for early detection of prostate cancer recurrence. PSA results measured by this research assay were used to determine relapse of prostate cancer, which was defined as two consecutive increases in serum PSA that resulted in at least the doubling of initial PSA.[15] Our previous study found that the biochemical relapse was significantly associated with those well-established prognostic indicators for prostate cancer recurrence.[15]

Measurements of IGF-I and IGFBPs

Serum concentrations of IGF-I, IGFBP-2, and IGFBP-3 were measured using three commercially available immunoassay kits (DSL Inc., Webster, Tex). The methods for measuring IGF-I and IGFBP-3 were enzyme-linked immunosorbent assays. A radioimmunoassay was used for IGFBP-2. Earlier studies showed that IGF-I and IGFBPs were fairly stable in blood samples and that the results of measurement were reproducible.[10,16] The coefficient of variation for between-run was less than 10% in all three methods. To further reduce the variation in measurement, we analyzed all serial samples from the same patients in one assay plate.

Statistical Analysis

Median levels of IGF-I, IGFBP-2, and IGFBP-3 in each of the four serial serum samples were compared between the two groups of patients, using the Wilcoxon rank-sum test. The Friedman test was used to compare the differences of IGF-I, IGFBP-2, and IGFBP-3 among four serial samples in each patient group. If levels of these variables were significantly different among the four collections, Page's L test was followed to examine the trend. Overall comparisons between the two groups after adjusting for patient and specimen variations were performed, using the generalized linear model (GLM) in the SAS statistical software (SAS Institute Inc, Cary, NC). Other continuous variables were compared, using Student's *t* test or Wilcoxon rank-sum test. For categorical data, the chi-square or Fisher's exact test was used where appropriate.

RESULTS

Table I shows the comparisons of clinical and pathologic features between the cases (patients with relapse) and controls (patients in remission). The mean ages were 62 years for the cases and 63 years for the controls. The median follow-up times were 53 and 55 months, respectively. There were no statistically significant differences in age and follow-up time between the two groups ($P = 0.444$ and 0.372, respectively). The cases had larger tumor volumes (44% versus 21%, $P < 0.001$), higher Gleason scores (7.3 versus 6.2, $P < 0.001$), and higher PSA levels before surgery (9.8 versus 5.7, $P = 0.012$) than the controls. Comparing the days during which the postopera-

TABLE I. *Clinical and pathologic features of patients with prostate cancer*

Variable	Relapse		Remission		*P* Value
	No.	Mean (SD)	No.	Mean (SD)	
Age (y)	38	62 (6.3)	39	63 (5.9)	0.444*
Tumor volume (%)	29	44 (27)	34	21 (21)	<0.001*
Gleason score	38	7.3 (0.9)	38	6.2 (1.4)	<0.001*
	No.	Median	No.	Median	
Follow-up (mo)	31	53	38	55	0.372†
Preoperative PSA (ng/mL)	35	9.8	38	5.7	0.012†
Days after surgery					
1st sample	38	509	40	558	0.678†
2nd sample	38	958	40	957	0.807†
3rd sample	38	1233	40	1395	0.474†
4th sample	32	1679	38	1736	0.194†
Postoperative PSA (ng/L)					
1st sample	38	61.2	40	1.3	<0.001†
2nd sample	38	164.2	40	1.2	<0.001†
3rd sample	38	279.8	40	1.3	<0.001†
4th sample	31	404.9	39	2.0	<0.001†
	No.	% Positive	No.	% Negative	
Surgical margin	31	61.3	38	26.3	0.003‡
Periprostatic tissue	31	80.6	38	44.7	0.002‡
Capsular penetration	31	90.3	38	65.8	0.016‡
Seminal vesicle	31	29.0	38	7.9	0.021‡
Bladder neck	32	15.6	38	2.6	0.086‡
Lymph node	32	0	38	5.3	0.496‡
Pathologic stage					
T1	5	15.6	23	60.5	
T2	26	81.3	14	36.8	
T3	1	3.1	1	2.6	0.008‡

Key: PSA = prostate-specific antigen.
* *Student's* t *test.*
† *Wilcoxon rank-sum test.*
‡ *Chi-square test or Fisher's exact test.*

tive serum samples were collected, we found no significant differences between the two groups at each of the four serial collections.

In comparison to the controls, higher percentages of the cases had positive surgical margin (61.3% versus 26.3%, $P = 0.003$), periprostatic tissue involvement (80.6% versus 44.7%, $P = 0.002$), capsular penetration (90.3% versus 65.8%, $P = 0.016$), and seminal vesicle involvement (29.0% versus 7.9%, $P = 0.021$). However, there were no significant differences in bladder neck and lymph node involvement between the two groups ($P = 0.086$ and 0.496, respectively). With regard to the pathologic stage of the disease, the majority (81.3%) of the cases had a Stage 2 disease, whereas most of the controls (60.5%) had a Stage 1 disease ($P = 0.008$).

Table II shows serum levels of IGF-I, IGFBP-2, and IGFBP-3 in the cases and controls. IGF-I levels were not significantly different between the two groups, neither in the comparison at each serial collection nor in overall comparison. Also, we observed no increasing or decreasing trends for serum IGF-I in the serial samples for either patient group.

For IGFBP-2, serum concentrations in the first and second serial samples were not significantly different between the two groups ($P = 0.159$ and 0.073, respectively), but in the third and fourth serial samples, the differences became statistically significant, higher in the controls than in cases ($P = 0.005$ and 0.041, respectively). Overall, the controls tended to have higher IGFBP-2 than the cases ($P = 0.025$). Also, in the control group, IGFBP-2 levels were increased with time, and the trend was statistically significant ($P = 0.014$). In the cases, however, no significant difference was observed ($P = 0.528$).

With regard to serum levels of IGFBP-3, there were significant differences in the first and last serial samples ($P = 0.044$ and 0.047, respectively), but the differences in the second and third samples

TABLE II. *Levels of IGF-I, IGFBP-2, and IGFBP-3 in patients with prostate cancer*

Variable	Relapse (n = 38)	Remission (n = 40)	*P* Value*
IFG-I (ng/mL)			
1st sample	107.7 (38.9–161.7)	110.2 (46.6–213.1)	0.772
2nd sample	104.8 (51.4–205.5)	112.5 (15.7–205.6)	0.503
3rd sample	105.6 (31.0–214.0)	108.5 (15.5–230.5)	0.976
4th sample†	111.7 (22.8–190.6)	100.5 (39.7–239.4)	0.914
Friedman test:	$P = 0.838$	$P = 0.762$	
Overall comparison between the 2 groups‡: $P = 0.808$			
IGFBP-2 (ng/mL)			
1st sample	447.5 (50.0–895.0)	507.5 (180.5–1807.5)	0.159
2nd sample	401.3 (172.5–1160.0)	557.5 (125.0–1645.0)	0.073
3rd sample	391.3 (115.0–1025.0)	591.3 (127.5–1937.5)	0.005
4th sample†	500.0 (135.0–1007.5)	597.5 (72.5–1942.5)	0.041
Friedman test:	$P = 0.528$	$P = 0.025$	
Page's L test:		$P = 0.014$	
Overall comparison between the 2 groups‡: $P = 0.005$			
IGFBP-3 (ng/mL)			
1st sample	4091 (2526–7121)	4768 (2953–6773)	0.044
2nd sample	4359 (2552–6398)	4506 (3032–6231)	0.330
3rd sample	4165 (1617–7996)	4398 (2351–9874)	0.559
4th sample†	3943 (2186–6429)	4411 (2978–7881)	0.047
Friedman test:	$P = 0.803$	$P = 0.373$	
Overall comparison between the 2 groups‡: $P = 0.038$			

Key: IGF-I = insulin-like growth factor I; IGFBP = insulin-like growth factor binding protein.
Values to Relapse and Remission are median (range).
* *Wilcoxon rank-sum test.*
† *Specimens available for analysis from 32 cases and 38 controls.*
‡ *Generalized linear models after adjusting for patients and specimens.*

were not statistically significant ($P = 0.330$ and 0.559, respectively). Overall, serum levels of IGFBP-3 were significantly higher in the controls than in cases ($P = 0.037$). IGFBP-3 levels did not show any increasing or decreasing trends in these serial samples for either group ($P = 0.803$ for the cases, $P = 0.373$ for the controls).

COMMENT

The study showed no changes, either increase or decrease, in levels of IGF-I and IGFBP-3 over nearly 5 years of follow-up after radical prostatectomy. These findings suggest that IGF-I and IGFBP-3 have no predictive values in prostate cancer prognosis. However, because the progression of prostate cancer is relatively slow, short follow-up time may limit our ability to detect slow and gradual changes of these markers over a long period of time. In the study, we did find that serum levels of IGFBP-2 and IGFBP-3 were higher in patients in remission than in patients with relapse. Moreover, serum levels of IGFBP-2 increased with time in patients who remained in remission, but did not change in patients who had recurrence. This finding apparently did not support the speculation that high IGFBP-2 might be associated with prostate cancer progression.

IGFBP-2 is the most abundant IGFBP in seminal plasma and one of the major IGFBPs in human prostate epithelial cells.[17,18] Two earlier studies[12,13] compared serum levels of IGFBP-2 and IGFBP-3 between patients with prostate cancer and normal controls. Both studies found substantially increased serum levels of IGFBP-2 in cancer patients, suggesting that IGFBP-2 might be involved in the disease. High serum IGFBP-2 was also observed in patients with prostate cancer who had high serum PSA.[19]

Tennant *et al.*[14] reported that IGFBP-2 levels were higher in prostate cancer cells than in normal prostate epithelial cells and that IGFBP-3 was low in cancer cells. High IGFBP-2 and low IGFBP-3 were also seen in several other tissue studies.[20–22] These observations in tumor tissues seemed to be consistent with those in serum samples. However, further examination of the relationship between IGFBP-2 and prostate cancer prognosis revealed inconsistent findings. One study[22] suggested that high IGFBP-2 was associated with high histologic grade of the tumor, whereas others found no correlation between IGFBP-2 and the Gleason score or pathologic stage.[20,21]

In most cell culture experiments, IGFBP-2 was found to be able to inhibit the mitogenic action of IGFs, but, in certain cell lines, this binding protein

was shown to have a weak stimulatory effect on IGF actions.[1,5,6] This dual regulatory effect of IGFBP-2 on IGFs may partially explain the conflicting findings in clinical studies. A cell culture experiment showed that long-term overexpression of IGFBP-2 increased tumorigenic potential for adrenocortical tumor cells, suggesting that IGFBP-2 has an IGF-independent stimulatory effect on tumor growth.[23] Findings of our study do not support the stimulatory effect of IGFBP-2 on tumor growth. Our observation is in agreement with the inhibitory effect of IGFBP-2 on tumor growth.

Laboratory experiments and epidemiologic studies[3,10,11] all have indicated that IGF-I may play a role in the development of prostate cancer. A potential effect of IGF-I on the progression of prostate cancer was also suspected.[24] However, our study found no evidence that IGF-I was involved in the progression of the disease. Correlations between serum PSA and IGFBP-2 and IGFBP-3 were observed in several previous studies,[12,13,19] but our study found no indications that serum PSA was correlated with IGFBP-2 or IGFBP-3.

In summary, the study found no differences in serum levels of IGF-I between patients with prostate cancer who were in remission and those who developed recurrent disease after radical prostatectomy. The study also showed no changes in serum IGF-I and IGFBP-3 over the time of follow-up. Serum levels of IGFBP-2 and IGFBP-3 were higher in the patients who were in remission than in those who developed recurrent disease. Postoperative serum IGFBP-2 levels increased with time in the patients who were in remission but did not change in the patients who had recurrence. Findings of this study suggest that IGFBP-2 may play a role in prostate cancer progression, but the study does not support the notion that IGF-I, IGFBP-2, or IGFBP-3 has predictive values in the prognosis of prostate cancer.

REFERENCES

1. Jones JI, and Clemmons DR: Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev **16:** 3–34, 1995.

2. LeRoith D, Baserga R, Helman L, *et al:* Insulin-like growth factors and cancer. Ann Intern Med **122:** 54–59, 1995.

3. Pietrzkowski Z, Mulholland G, Gomella L, *et al:* Inhibition of growth of prostate cancer cell lines by peptide analogues of insulin-like growth factor 1. Cancer Res **53:** 1102–1106, 1993.

4. Parrizas M, and LeRoith D: Insulin-like growth factor-1 inhibition of apoptosis is associated with increased expression of the bcl-cL gene product. Endocrinology **138:** 1355–1358, 1997.

5. LeRoith D, Werner H, Beitner-Johnson D, *et al:* Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev **16:** 143–163, 1995.

6. Kelley KM, Oh Y, Gargosky SE, *et al:* Insulin-like growth factor-binding proteins (IGFBPs) and their regulatory dynamics. Int J Biochem Cell Biol **28:** 619–637, 1996.

7. Collett-Solberg PF, and Cohen P: The role of the insulin-like growth factor binding proteins and the IGFBP proteases in modulating IGF action. Endocrinol Metab Clin North Am **25:** 591–614, 1996.

8. Kimura G, Kasuva J, Giannini S, *et al:* Insulin-like growth factor (IGF) system components in human prostatic cancer cell-lines: LNCaP, DU145, and PC-3 cells. Int J Urol **3:** 39–46, 1996.

9. Angelloz-Nicoud P, and Binoux M: Autocrine regulation of cell proliferation by the insulin-like growth factor (IGF) and IGF binding protein-3 protease system in a human prostate carcinoma cell line (PC-3). Endocrinology **136:** 5485–5492, 1995.

10. Chan JM, Stampfer MJ, Giovannucci E, *et al:* Plasma insulin-like growth factor-1 and prostate cancer risk: a prospective study. Science **279:** 563–566, 1998.

11. Wolk A, Mantzoros CS, Andersson S-O, *et al:* Insulin-like growth factor 1 and prostate cancer risk: a population-based, case-control study. J Natl Cancer Inst **90:** 911–915, 1998.

12. Cohen P, Peehl DM, Stamey TA, *et al:* Elevated levels of insulin-like growth factor-binding protein-2 in the serum of prostate cancer patients. J Clin Endocrinol Metab **76:** 1031–1035, 1993.

13. Kanety H, Madjar Y, Dagan Y, *et al:* Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: correlation with serum prostate-specific antigen. J Clin Endocrinol Metab **77:** 229–233, 1993.

14. Tennant MK, Thrasher JB, Twomey PA, *et al:* Insulin-like growth factor-binding protein-2 and -3 expression in benign human prostate epithelium, prostate intraepithelial neoplasia, and adenocarcinoma of the prostate. J Clin Endocrinol Metab **81:** 411–420, 1996.

15. Yu H, Diamandis EP, Wong PY, *et al:* Detection of prostate cancer relapse with prostate specific antigen monitoring at levels of 0.001 to 0.1 μg/L. J Urol **157:** 913–918, 1997.

16. Yu H, Mistry J, Nicar MJ, *et al:* Insulin-like growth factors (IGF-I, free IGF-I, and IGF-II) and insulin-like growth factor binding proteins (IGFBP-2, IGFBP-3, IGFBP-6, and ALS) in blood circulation. J Clin Lab Anal **13:** 166–172, 1999.

17. Rosenfeld RG, Pham H, Oh Y, *et al:* Identification of insulin-like growth factor-binding protein-2 (IGFBP-2) and a low molecular weight IGF-BP in human seminal plasma. J Clin Endocrinol Metab **70:** 551–553, 1990.

18. Cohen P, Peehl DM, Lamson G, *et al:* Insulin-like growth factors (IGFs), IGF receptors and IGF binding proteins in primary cultures of prostate epithelial cells. J Clin Endocrinol Metab **73:** 401–407, 1991.

19. Ho PJ, and Baxter RC: Insulin-like growth factor-binding protein-2 in patients with prostate carcinoma and benign prostatic hyperplasia. Clin Endocrinol **46:** 145–154, 1997.

20. Hampel OZ, Kattan MW, Yang G, *et al:* Quantitative immunohistochemical analysis of insulin-like growth factor binding protein-3 in human prostatic adenocarcinoma: a prognostic study. J Urol **159:** 2220–2225, 1998.

21. Thrasher JB, Tennant MK, Twomey PA, *et al:* Immunohistochemical localization of insulin-like growth factor binding proteins 2 and 3 in prostate tissue: clinical correlations. J Urol **155:** 999–1003, 1996.

22. Figueroa JA, Raad SD, Tadlock L, *et al:* Differential expression of insulin-like growth factor binding proteins in high versus low Gleason score prostate cancer. J Urol **159:** 1379–1383, 1998.

23. Hoeflich A, Fettscher O, Lahm H, *et al:* Overexpression of insulin-like growth factor-binding protein-2 results in increased tumorigenic potential in Y-1 adrenocortical tumor cells. Cancer Res **60:** 834–838, 2000.

24. Pollak M, Beamer W, and Zhang JC: Insulin-like growth factors and prostate cancer. Cancer Metas Res **17:** 383–390, 1998–99.