Westernization of dietary habits among Japanese and Koreans may contribute significantly to the increased morbidity and mortality of prostate cancer in those country [3]. One of the reasons why Asians have a low risk of prostate cancer may be the ample intake of soy. Soy foods have been postulated to reduce the risk of a number of chronic diseases, including coronary heart disease, osteoporosis, and cancers [4–6]. The amount of dietary soy intake is also associated with a decreasing risk of prostate cancer [4–6].

Soy isoflavones are nonsteroidal diphenolic compounds that inhibit prostate cancer cell growth in vitro as well as tumor growth in mice [7–12]. The isoflavone aglycones are genistein, daidzen, and glycitein. Genistein, the predominant isoflavone in soy, has been shown to have anti-oxidant activity and to inhibit tumor growth through anti-proliferative and anti-angiogenic mechanisms [13]. In addition to estrogenic activities, genistein can induce apoptosis and inhibit the activation of the anti-apoptotic protection factor, nuclear factor-κB (NF-κB) in prostate cancer cells [14]. Intestinal bacteria convert daidzein into equol. Among Japanese, those who are able to convert daidzein to equol are less prone to develop prostate cancer [15]. Thus the ability to produce equol from daidzein or equol itself is closely related to a lower incidence of prostate cancer. The serum level of active isoflavones is found to be markedly lower in Americans as compared to Japanese and Koreans. The proportion of equol producers is also lower in former than in the latter [16]. Several clinical trials investigating the efficacy of soy isoflavones on prostate-specific antigen (PSA) have been previously undertaken, although the results are not consistent.

Epidemiological studies suggest that natural dietary ingredients used in Asian countries have anti-carcinogenic potential against prostate cancer [17]. Curcumin is a major yellow pigment in turmeric, which is widely used as a spice and coloring agent in several foods such as curry. Extensive studies have revealed that curcumin regulates the expression of inflammatory enzymes, cytokines, adhesion molecules, and cell survival proteins [18]. Curcumin exerts strong anti-oxidant and anti-inflammatory activities by suppressing both constitutive and inducible NF-κB and activator protein-1 activation [19]. In addition, curcumin has chemosensitive and radiosensitive effects by down-regulating the MDM2 oncogene through the PI3K/mTOR/ETS2 pathway [20]. Thus the potential therapeutic role of curcumin in prostate cancer is worthy of further evaluation and clinical trials.

In this study, we examined the effects of isoflavones and curcumin on the expression of PSA and androgen receptor in prostate cancer cells. Furthermore, we conducted a double-blind placebo-controlled clinical trial to evaluate the effects of soy isoflavone and curcumin on serum PSA levels in men who did not have detectable prostate cancer by biopsy.

MATERIALS AND METHODS

Cells and Culture Conditions

The human prostate cancer cell line LNCaP was obtained from the American Type Culture Collection (Rockville, MD). The cells were routinely maintained in RPMI 1640 supplemented with 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. Curcumin (Medi Herb, Inc., Bangalore, India) was dissolved in ethanol at a concentration of 10 mM and stored at −20°C. Isoflavones (NICHIMO Co., Ltd, Tokyo, Japan) were dissolved in DMSO at a concentration of 20 mg/ml and stored at −20°C in the dark.

Measurement of PSA in Conditioned Medium

LNCaP cells were cultured in culture medium for 24 hr and then treated with curcumin, isoflavones, or a combination of the two agents. After a 48 hr treatment, the conditioned media were collected, centrifuged to remove residual cells, and stored at −20°C. For quantitative analysis of the amount of PSA secreted by LNCaP cells, an immunoassay procedure was performed using a commercial PSA assay kit (R&D Systems, Inc., Minneapolis, MN). MTS assay was performed at the same time, and results were used for normalization of PSA levels.

Immunoblotting

Subconfluent LNCaP cells were treated with curcumin, isoflavones, or a combination of the two agents for 24 hr. Cells were washed twice with cold PBS and then lysed in RIPA buffer on ice for 30 min. The cell lysate was centrifuged at 15,000 rpm for 30 min at 4°C, and the supernatant was collected. Protein concentrations were measured by a BCA protein assay kit (Pierce, Inc., Rockford, IL). Protein samples were separated by SDS–PAGE and transferred onto a PVDF membrane (Millipore, Inc., Tokyo, Japan). Immunoblotting was performed using rabbit anti-androgen receptor antibody (Santa Cruz Biotechnology, Inc., CA; 1:2,000), mouse anti-PSA antibody (DakoCytomation, Kyoto, Japan; 1:1,000), or mouse anti-human β-actin antibody (Sigma-Aldrich Co., St. Louis, MO; 1:10,000 dilution) as an internal loading control. Immunoreactive proteins were visualized with ECL detection reagents (GE Healthcare Biosciences, Tokyo, Japan).

Randomized Placebo-Controlled Double-Blind Study for the Effect of Isoflavones and Curcumin on the Serum PSA Levels

One hundred men were recruited who underwent systematic prostate biopsy (14 cores) at the Teikyo University Hospital consecutively because of elevated levels of PSA and were not found to have either cancer or prostatic intraepithelial neoplasia (PIN). Serum levels of PSA were measured by Chemiluminescence Enzyme Immunoassay with a LUMIPULSE kit (FUJIREBIO, Tokyo, Japan). Patients who had allergies against either soy or turmeric; who had already taken supplements containing either isoflavones or curcumin; or who had been treated for cardiovascular disease, liver disease, renal failure, or asthma were excluded from the study. Patients who had a history of any malignancy and who took anti-androgen drugs such as finasteride for their lower urinary tract syndrome were excluded.

Patients were randomized to receive either isoflavones + curcumin (supplement) or placebo in a double-blind study. Participants were asked to take either isoflavones (40 mg) and curcumin (100 mg) or placebo for 6 months. Isoflavones contained 66% daidzen, 24% glycitin, and 10% genistin. Tablets of supplements and placebo were designed and manufactured by Angfa, Inc. (Tokyo, Japan) and SECOM Medical System Co., Ltd (Tokyo, Japan). Each subject gave informed written consent to participate in this study, which was approved by the local ethics committee. In 100 subjects, 42 out of 50 who were assigned to take placebo and 43 out of 50 to take supplements completed the trial. There were no significant adverse effects either in the placebo or supplement groups except one subject on placebo who experienced severe diarrhea during the trial and dropped out subsequently. Fourteen other subjects dropped out of the trial either because of the inability to visit the ambulatory clinic due to the deterioration of other medical conditions. We compared PSA levels at 6 months between the supplement and placebo groups.

Statistical Analysis

Statistical analysis was performed using the SAS statistical software package version 9.13 (SAS Institute, Inc., Cary, NC). *T*-test was used to compare the differences in 6-month PSA levels between the supplement and placebo groups. A *P*-value of <0.05 was considered to be statistically significant. Since PSA levels at the baseline affected ones at 6 months, we performed analysis of covariance (ANCOVA) using baseline PSA levels, which was changed binary variable. We divided subjects into subgroups by a cut-off of 10 ng/ml of baseline PSA level; Subgroup 1: PSA < 10; Subgroup 2: PSA ≥ 10. We compared the change of PSA levels between the baseline and the end of the study. We examined the statistical interactions of the intake of supplement and PSA levels at the baseline by ANCOVA.

RESULTS

Combined Inhibitory Effects of Isoflavones and Curcumin on PSA Production and Expression of Androgen Receptor

To determine the effects of isoflavones, curcumin, and their combination on PSA production, immunoassay was performed on LNCaP cells at various concentrations. Treatment of the cells with 10 μg/ml isoflavones alone caused 40% inhibition of PSA secretion to the supernatant compared to control, whereas treatment of the cells with 20 μM curcumin caused 20% inhibition. A combination treatment with 10 μg/ml isoflavones and 20 μM curcumin caused almost complete inhibition of PSA production in LNCaP cells (Fig. 1). Analysis by immunoblotting was also performed on LNCaP cells before and after treatment of isoflavones and/or curcumin. These results showed that the amounts of PSA production by LNCaP cells were significantly reduced when cells were treated with 25 μM curcumin. A further decrease in PSA level was observed when cells were treated with a combination of 10 μg/ml isoflavones and 25 μM curcumin (Fig. 2). We also examined the effects of these two compounds on the expression of androgen receptors. After 24 hr incubation, the expression of androgen receptor was inhibited by treatment with 10 μg/ml isoflavones or 25 μM curcumin (Fig. 2). The combination of isoflavones and curcumin inhibited the expression of androgen receptors additively.

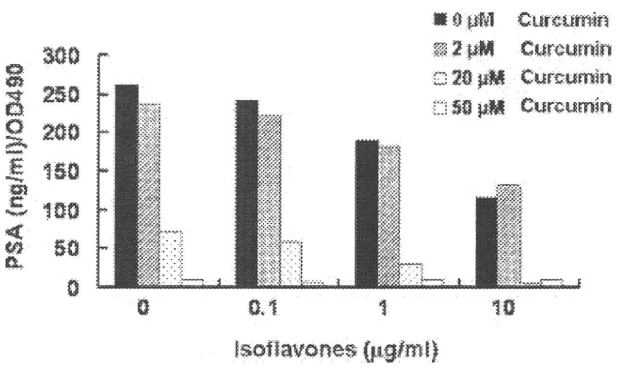

Fig. 1. PSA secretion in LNCaP cells after treatment with curcumin and/or isoflavones. Cells were treated with various doses of curcumin and/or isoflavones for 48 hr.

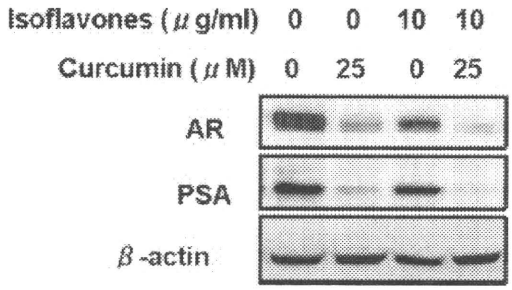

Fig. 2. Androgen receptor and PSA expression in LNCaP cells after treatment with curcumin and/or isoflavones. Immunoblot analysis demonstrated synergistic effects of isoflavones and curcumin after 24 hr. Immunoblots were probed for mouse anti-human β-actin antibody as an internal control.

Effects of Supplements on PSA Levels in Men Without Prostate Cancer

The 85 men who completed the clinical trial were all Japanese. At baseline, the median age of the population was 73 years (range: 50–86). Serum PSA levels at the baseline and at the end of the study were not different between supplement and placebo groups (Table I). The number of subjects in the individual subgroups were: Subgroup 1 (PSA at the baseline <10): Placebo: 32 (37.6%), Supplement: 28 (32.9%); Subgroup 2 (PSA at the baseline ≥ 10): Placebo: 10 (11.8%), Supplement: 15 (17.7%) (Table II). In two subgroups divided by the cut-off of PSA ≥10 ng/ml, serum PSA levels at the baseline and the end of the study between supplement and placebo groups were not different (Table II; Fig. 3). Considering the interaction of initial PSA levels and the intake of supplements, analysis by ANCOVA showed that supplement group had significantly larger decrease in PSA levels in Subgroup 2 (initial PSA ≥ 10 ng/ml) (Table III).

DISCUSSION

Several lines of evidence indicate that oxidative stress may play an important role in carcinogenesis. In the prostate, the aging process shifts this redox balance towards a more oxidative state which may be due in part to a decline of anti-oxidative enzymes like super-oxide dismutase and catalase [21]. Proposed prostate cancer preventive agents like isoflavones, selenium, lycopene, or green tea have well-known anti-oxidant activities. In addition to these agents, curcumin is also a promising candidate [17,22]. Curcumin has been shown to cause apoptosis and cell-cycle arrest with inhibited cell growth, activation of signal transduction, and transforming activities in both androgen-dependent and independent prostate cancer cells [18,22,23]. The present study was undertaken to evaluate the potential efficacy of isoflavones, curcumin, and their combination as prostate cancer preventive agents. The central findings of this study are that a combined treatment of curcumin and isoflavones enhance the inhibition of PSA production and the expression of androgen receptor in LNCaP cells.

PSA is the leading marker for prostate cancer, although it has a low sensitivity, and does not have a clear cut-off level to produce a dichotomous result. In the Prostate Cancer Prevention Trial, even within the 0–4.0 ng/ml interval, the PSA level was a continuously increasing marker of prostate cancer risk including high-grade tumors, with no boundary below which no prostate cancer was found [24,25]. Thus lowering the PSA threshold has been proposed to increase the detection of cancers, although the unavoidable tradeoff is an increased number of subjects who are false positives [26]. Currently there is no consensus for the management of biopsy-negative subjects. Repeated biopsies are recommended based on the fact that a second biopsy can detect cancer in around 30% of subjects [27]. However, patients may not agree with the immediate re-biopsy. Furthermore, subjects who do not have detectable cancer after a second biopsy may be

TABLE I. Patient Characteristics and PSA Changes

	Supplement (n = 42)	Placebo (n = 43)
Age[a]	72.4 (59–86)	71.3 (50–84)
PSA at the baseline[b]	10.5 ± 9.5	8.0 ± 6.7
PSA at 6 months[b]	7.4 ± 4.6	7.1 ± 5.6

[a]Mean (min–max).
[b]Mean ± SD.

TABLE II. Patient Characteristics and PSA Level of Subgroup

Subgroup	Supplement	Age	PSA at the baseline	PSA at 6 months
1: PSA < 10	Placebo (n = 32)	72 (50–82)	5.2 ± 2.2	4.8 ± 2.2
	Supplement (n = 28)	74 (61–86)	6.1 ± 1.9	5.9 ± 2.6
2: PSA ≥ 10	Placebo (n = 10)	74 (71–84)	17.0 ± 8.4	14.2 ± 7.2
	Supplement (n = 15)	73 (59–82)	18.8 ± 12.4	10.2 ± 6.2

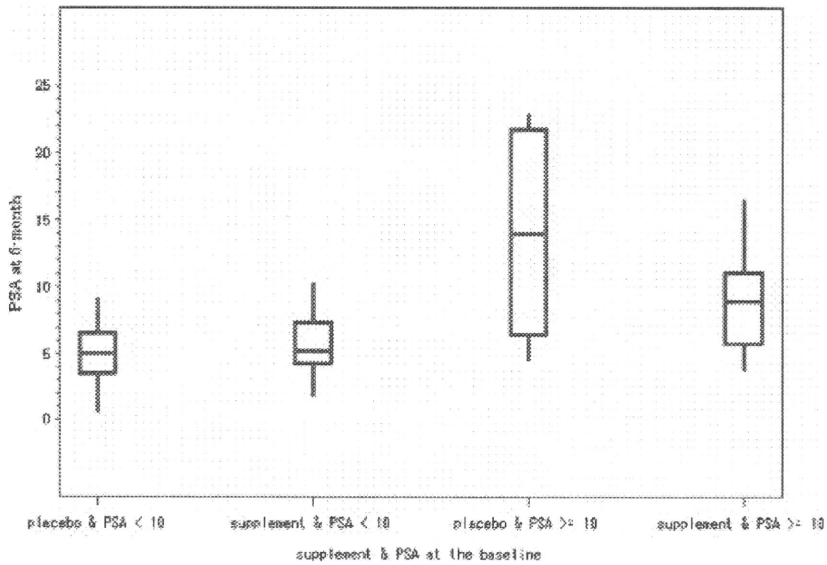

Fig. 3. Serum PSA levels at 6 months between subgroups: Subgroup 1: PSA < 10; Subgroup 2: PSA ≥ 10. PSA levels were compared among subgroups by *t*-test, and ANCOVA was used to compare the differences in PSA levels at 6 months. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

left for observation to see if there will be a further rise of PSA.

Elevated serum levels of PSA reflect not only the existence of cancer but also inflammation in the prostate. Sustained chronic inflammation in the prostate may promote prostate carcinogenesis [28]. In addition, PSA, a serine protease, plays a significant role in prostate tumor growth by regulating various proangiogenic and anti-angiogenic growth factors [29]. Thus intervention to improve the PSA value might have beneficial effects for the prevention of the development of prostate cancer. Several trials to date have examined the effects of isoflavones on serum PSA levels, although the results are not consistent [17,30–32]. The strengths of this study are the randomized, controlled, parallel-arm design; the blinding of investigators and participants; the relatively long (6 months) duration of the intervention; and the larger sample size than in previous studies. In our study, PSA levels significantly decreased in the supplement-treated subgroup compared with that of the placebo group only in those subjects whose baseline PSA levels were over 10 ng/ml ($P = 0.02$). Currently, we do not have a solid explanation for our findings that the effects of soy isoflavones and curcumin were seen only in those subjects whose PSA is over 10. One speculation is that isoflavones and curcumin might improve the asymptomatic inflammation in prostates with high serum PSA levels.

The limitation of this preliminary clinical study was that cohort of patients accrued likely had already been placed on diets rich in soy isoflavones or curcumin and they were not controlled for the content of diets. We did not measure the plasma levels of soy isoflavones and curcumin to see whether their basal levels might affect the observed changes in serum PSA levels, or to show supplementation achieved a biologically relevant increase in serum levels for each of these agents. To further identify the role of derivatives of isoflavones, we need to evaluate the serum levels of equol and daidzen influenced by the intake of soy isoflavones.

TABLE III. ANCOVA with Interaction

Variable	Estimate	SE	95% CI	*P*-value
Supplement 1[a]	1.05	1.05	(−1.03, 3.13)	0.32
Supplement 2[b]	−4.00	1.65	(−7.28, −0.71)	0.02
PSA at the baseline (≥10/<10)	9.36	1.46	(6.45, 12.27)	<0.0001

[a]Supplement use in PSA < 10.
[b]Supplement use in PSA ≥ 10.

CONCLUSIONS

A combined treatment of soy isoflavones and curcumin inhibited the production of PSA and the expression of androgen receptor in cultured prostate cancer cells in vitro. In a randomized, placebo-controlled clinical trial, a combined treatment of soy isoflavones and curcumin decreased serum PSA in those subjects whose baseline PSA was more than 10 ng/ml. Our data show that the effects of isoflavones and curcumin on PSA production in prostate cells, particularly in combination, may have therapeutic advantages in the patients with high PSA level who has negative prostate biopsies.

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Characterization of the anatomical extension pattern of localized prostate cancer arising in the peripheral zone

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Study Type – Diagnostic (non-consecutive series)
Level of Evidence 3b

OBJECTIVES

To characterize the anatomical extension pattern of prostate cancer arising in the peripheral zone (PZ) in radical prostatectomy (RP) specimens and to evaluate its prognostic significance.

PATIENTS AND METHODS

Of 174 consecutive patients undergoing RP, 128 diagnosed as having PZ cancer (PZC) were enrolled. The maximum tumour area (MTA) and maximum tumour volume (MTV) in RP specimens were measured using digital planimetry. A circle with an area equal to the MTA, in which the central point was the intersection of the longest line of the MTA and the line perpendicularly bisecting the first line, was defined as a hypothetical extension area, regardless of anatomical structure. The area within this circle that did not overlap the MTA was defined as ΔTA.

RESULTS

There was a significant correlation between the MTV and ΔTA/MTA, introduced as a variable representing the degree of PZC extension along the anatomical shape of the PZ. The ΔTA/MTA in patients with a MTV of >5 mL was significantly greater than that in those with a MTV of ≤5 mL. Furthermore, ΔTA/MTA was significantly associated with several prognostic indicators, including extracapsular extension, surgical margin status and perineural invasion. Multivariate analysis identified ΔTA/MTA in addition to preoperative serum prostate-specific antigen level, extracapsular extension and surgical margin status as independent predictors of biochemical recurrence after RP.

CONCLUSIONS

PZC tends to extend along the anatomical shape of the PZ during progression, resulting in higher ΔTA/MTA value in advanced PZC than that in early PZC.

KEYWORDS

prostate cancer, radical prostatectomy, peripheral zone, biochemical recurrence

INTRODUCTION

Since McNeal [1] first described three different anatomical zones in the human prostate, several investigators have evaluated the characteristics of prostate cancer according to the zonal origin [2–4] The peripheral zone (PZ) is the predominant location of the origin of prostate cancer, and the proximity of these cancers to the rectal surface can facilitate the diagnosis by palpation and needle biopsy, while prostate cancers arising in the transition zone (TZ) are frequently found incidentally in TURP specimens [5]. Interestingly, despite higher serum PSA levels and tumour volumes, TZ cancer (TZC) more frequently shows favourable pathological findings and better biochemical cure rate after radical prostatectomy (RP) than PZ cancer (PZC) [2–4]. Furthermore, several recent studies have reported that PZC might have more aggressive biological features than TZC [6,7]. Collectively, these findings suggest that it would be suitable to consider PZC and TZC as diseases having different malignant phenotypes.

Despite the intensive efforts investigating differences in the clinicopathological characteristic between PZC and TZC [2–7], there have been few studies systematically analysing the extension pattern of each disease within the prostate gland. In our previous study evaluating the effect of the location of positive biopsy cores on the findings of RP specimens, locally advanced prostate cancers were shown to frequently involve the anterior lateral horn, representing the extreme lateral and anterior PZ that surrounds the TZ, although whether cancer was detected in the ALH or other areas does not seem to affect the biological features [8]. In that study, we also found that tumours detected from the anterior lateral horn had an incidence of TZ involvement similar to that detected from other anatomical sites [8]. These findings suggest that tumours derived from the PZ seem to spread through the PZ and/or across the capsule, but not through the TZ. In the present study therefore, we precisely analysed the RP specimens obtained from patients diagnosed as having disease arising from the PZ, to clarify the extension pattern of PZC and to determine its prognostic significance in patients undergoing RP.

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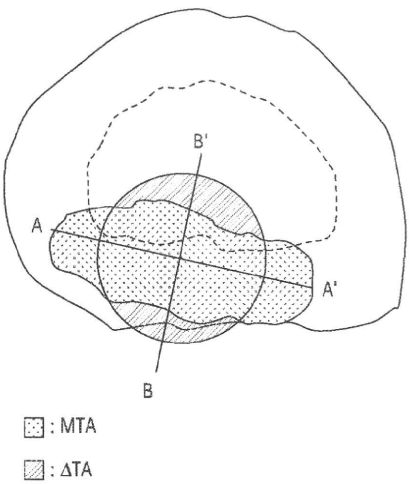
FIG. 1. Schematic presentation of the whole-mount section of a RP specimen. The MTA of the largest single tumour focus was determined using digital planimetry. Line AA' and BB' is the longest line in the MTA and the line perpendicularly bisecting AA', respectively. The circle has the same area as the MTA, and the central point is the intersection of AA' and BB'. The area within this circle that did not overlap the MTA was measured and defined as ΔTA. Solid line, prostate capsule; broken line, boundary of PZ and TZ.



PATIENTS AND METHODS

The study included 174 patients who were diagnosed as having clinically organ-confined prostate cancer according to the staging procedures used at our institution, including a DRE, TRUS, serum PSA assay, pelvic CT, MRI and/or a bone scan. The patients subsequently had RP and bilateral pelvic lymphadenectomy, based on the surgical procedure described by Walsh *et al.* [9], between May 2003 and December 2007m with no neoadjuvant therapy. The median (range) follow-up of the patients was 44.3 (13–67) months. Informed consent for the current study using RP specimens was obtained from each of the patients, and the study design was approved by the Research Ethics Committee of our institution.

RP specimens were prepared by the whole-mount technique, the surface was inked, and the specimen fixed in 10% neutral formalin. After fixation, the apex and base were cut off and serially sectioned along the vertical parasagittal plane. The seminal vesicles were sectioned parallel to their junction with the prostate and submitted entirely for evaluation. The remaining specimen was serially sectioned perpendicularly to the long axis of the gland from the apex to the base at ≈5-mm intervals. All sections were processed with haematoxylin and eosin on slides for microscopic evaluation.

TABLE 1 The characteristics of 128 patients who had RP for PZC

Variable	Median (range) or *n* (%)
Age, years	67.5 (50–78)
Pathological stage	
pT2	91 (71.1)
pT3a	19 (14.8)
pT3b	18 (14.1)
PSA level, ng/mL	9.5 (2.8–6.2)
Gleason score	
≤6	24 (18.7)
3 + 4	44 (34.4)
4 + 3	55 (43.0)
≥8	5 (3.9)
Lymph node metastasis	
Negative	128 (100.0)
Positive	0
SMS	
Negative	97 (75.8)
Positive	31 (24.2)
Lymphatic invasion	
Negative	100 (78.1)
Positive	28 (21.9)
Vascular invasion	
Negative	91 (71.1)
Positive	37 (28.9)
PNI	
Negative	21 (16.4
Positive	107 (83.6)

In this series, the samples were examined pathologically by one pathologist according to the 2002 TNM classification system. Surgical margins were diagnosed as positive if the cancer cells reached the inked margin at any location in the RP specimen.

After RP patients were followed by periodic measurement of serum PSA levels at least every 3 months for the first 2 years, and every 6 months thereafter. Biochemical recurrence was defined as a PSA level persistently >0.2 ng/mL. Irrespective of pathological findings suggesting a poor prognosis, none of the patients received any adjuvant therapy until their serum PSA levels reached ≥0.4 ng/mL.

All tumour areas were marked on whole-mount slides with a water-resistant pen. The maximum tumour area (MTA) of the largest single tumour focus was determined using a digital planimeter (Uchida Yoko, Tokyo, Japan), and the maximum tumour volume (MTV) was calculated as the sum of surface areas for that tumour multiplied by the thickness of the prostate slice as previously described [7]. The MTA and the MTV *in vivo* were corrected for tissue shrinkage during formalin fixation by multiplying by a factor of 1.21 and 1.33, respectively. The boundary between PZ and TZ is marked by a stromal band that is nearly devoid of glandular elements. TZC was considered when >70% of the cancer area was located in the TZ, while the remaining cases were defined as PZC.

To characterize the extension pattern of PZC using an objective method we used a new variable calculated as described below. As shown in Fig. 1, we drew a circle with an area equal to MTA, in which the central point was the intersection of the longest line in the MTA (AA') and a line perpendicularly bisecting AA' (BB'). This was defined as the hypothetical extension pattern regardless of anatomical structure. The area within this circle that did not overlap MTA was measured and defined as ΔTA. Finally, the ΔTA/MTA value was calculated by dividing ΔTA by MTA.

The variables for different groups were compared statistically using the Mann–Whitney *U*-test and chi-square test. Survival curves were compared using the Kaplan–Meier method and analysed by log-rank tests. Cox proportional hazards models were used to assess the hazard ratio with 95% CI, under univariate and multivariate analyses. In all tests $P < 0.05$ was considered to indicate significance.

RESULTS

According to the definition described above, 128 of 174 patients were diagnosed as having PZC and were further analysed to evaluate the extension pattern of PZC within the prostate gland. The clinicopathological characteristics of these 128 patients are summarized in Table 1.

The median (range) values of MTA, MTV, ΔTA and ΔTA/MTA in the 128 patients with PZC were 1.79 (0.15–14.64) cm^2, 1.86

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FIG. 2. Correlation between MTV and ΔTA/MTA in RP specimens from 128 patients with PZC.

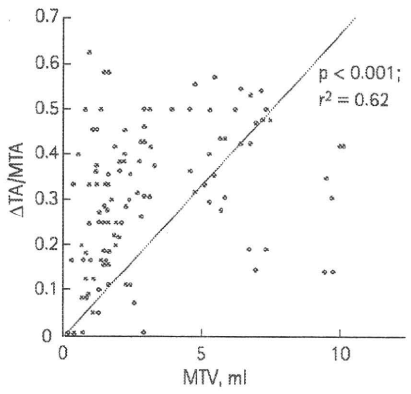


FIG. 3. A comparison of PZ and TZ ΔTA/MTA values according to MTV in RP specimens from 128 patients with PZC.

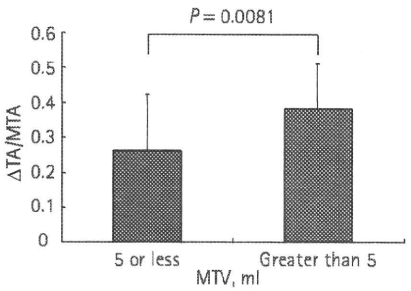


TABLE 2 The association between ΔTA/MTA and clinicopathological factors in 128 patients who had RP for PZC

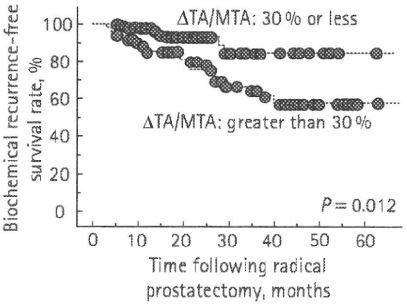
Variables	ΔTA/MTA ≤30	ΔTA/MTA >30	P
N	60	68	
Age, years			
≤65	29	35	0.720
>65	31	33	
PSA level, ng/mL			
≤10	28	36	0.530
>10	32	32	
ECE			
Negative	51	40	0.011
Positive	9	28	
SVI			
Negative	54	56	0.210
Positive	6	12	
Gleason score			
3 + 4, ≤6	33	35	0.690
4 + 3, ≥8	27	33	
SMS			
Negative	51	46	0.022
Positive	9	22	
Lymphatic invasion			
Negative	46	54	0.710
Positive	14	14	
Vascular invasion			
Negative	40	51	0.300
Positive	20	17	
PNI			
Negative	16	5	0.032
Positive	44	63	

TABLE 3 Univariate and multivariate analyses of several variables as predictors of biochemical recurrence after RP

Variable	Hazard ratio (95% CI), P Univariate	Multivariate
Age, years (≤65 vs >65)	1.30 (0.44–1.74), 0.610	1.25 (0.51–1.38), 0.730
PSA level, ng/mL (≤10 vs >10)	3.24 (1.22–3.98), 0.025	3.51 (1.17–4.32), 0.022
ECE (–ve vs +ve)	3.87 (1.47–5.35), 0.043	4.17 (2.11–5.80), 0.032
SVI (–ve vs +ve)	4.17 (1.87–7.10), 0.010	5.22 (1.57–9.46), 0.008
Gleason score (3 + 4, ≤6 vs 4 + 3, ≥8)	1.33 (0.91–2.87), 0.370	1.28 (0.54–2.87), 0.210
SMS (–ve vs +ve)	2.97 (1.17–5.41), 0.009	3.94 (1.18–4.81), 0.010
Lymphatic invasion (–ve vs +ve)	1.28 (0.43–3.02), 0.540	1.47 (0.33–2.45), 0.260
Vascular invasion (–ve vs +ve)	1.54 (0.71–3.64), 0.320	0.89 (0.25–1.32), 0.540
PNI (–ve vs +ve)	1.59 (0.75–2.95), 0.460	1.41 (1.65–3.98), 0.360
ΔTA/MTA (≤30 vs >30)	2.41 (1.28–6.41), 0.031	2.88 (1.22–6.33), 0.042

(0.090–10.11) cm^3, 0.48 (0–3.59) cm^2 and 0.30 (0–0.63), respectively. We initially assessed the relationship between MTV and ΔTA/MTA values in these 128 patients; there was a significant linear relation with MTV values (Fig. 2). Furthermore, when the 128 patients were divided into two groups according to the value of MTV, the ΔTA/MTA value in patients with a MTV of >5 mL was significantly greater than that in those with a MTV of ≤5 mL (Fig. 3). The association between ΔTA/MTA and several clinicopathological variables was then evaluated (Table 2); the ΔTA/MTA was significantly associated with extracapsular extension (ECE), surgical margin status (SMS) and perineural invasion (PNI), but there were no significant relations between ΔTA/MTA and the remaining factors, including age, preoperative serum PSA level, seminal vesicle invasion (SVI), Gleason score, lymphatic invasion and vascular invasion.

FIG. 4. Biochemical recurrence-free survival of 128 patients with PZC who had RP, according to ΔTA/MTA values.



During the observation period of the study, 26 of the 128 patients had a biochemical recurrence after RP. The effect of ΔTA/MTA on biochemical recurrence after RP in the 128 patients with PZC was investigated using univariate and multivariate analyses (Table 3). The univariate analysis identified ΔTA/MTA value in addition to preoperative serum PSA level, ECE, SVI and SMS as significant predictors of the biochemical outcomes. In addition, these five variables, including ΔTA/MTA, were independently associated with biochemical recurrence on multivariate analysis. There was a significant difference in the biochemical recurrence-free survival in the 128 patients according to ΔTA/MTA value (Fig. 4).

DISCUSSION

Prostate cancer arising in the TZ has been shown to have several characteristics differing from those of tumour arising in the PZ [2–5]. Of these, the most important difference is that TZC might have less aggressive features than PZC, although TZC is

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characterized by obviously high serum PSA value and a greater tumour volume than PZC [2–4]. Furthermore, several studies analysing morphological, genetic and biological differences between TZC and PZC were reported recently [6,7]. These findings suggest a potential effect of zonal origin on the phenotype of prostate cancer; accordingly, it would be of interest to assess the extension pattern of prostate cancer in the prostate and its relation to conventional clinicopathological variables.

To date, there have been few studies quantitatively addressing the extension pattern of prostate cancer; hence, in the present study we introduced a new variable, ΔTA/MTA, representing the extension pattern of prostate cancer arising in the PZ, to objectively evaluate its significance. It was initially hypothesized that if prostate cancer arising in the PZ progresses regardless of any anatomical limitations, including the prostate capsule and the boundary between PZ and TZ, these tumours would extend concentrically from the centre of the tumour. Based on this notion, a measurement of the tumour volume that does not overlap either the real tumour shape or the virtual sphere with an identical centre and sharing the same volume as the real tumour, might represent how this tumour progresses along the anatomical shape of the PZ. Furthermore, the measurement of this volume could be reasonably substituted by measuring the tumour areas in the section at MTA; therefore, we introduced ΔTA/MTA value as a variable reflecting the extension pattern of PZC.

In this series of 128 patients diagnosed as having PZC, the ΔTA/MTA value had a linear correlation with the MTV. This outcome strongly suggests that PZC might extend along the anatomical shape of the PZ in the prostate. However, some cases with a large MTV had a comparatively low ΔTA/MTA. This phenomenon could be explained as follows: during progression, PZC eventually invaded the surrounding components and grew spherically irrespective of anatomical limitations, resulting in a tendency to a lower ΔTA/MTA. Furthermore, there is a substantially varied distribution of ΔTA/MTA values in cases with a small MTV, particularly those with a MTV of <1 mL. In these cases, the tumour volume is so small that tumours tend to extend irrespective of the shape of PZ. However, the shapes of MTA are not always circular, despite the lack of anatomical limitation for extending until they grow inot large tumours.

We then assessed the association between ΔTA/MTA and several clinicopathological factors. Of these, ECE, SMS and PNI were identified as significant and closely related to ΔTA/MTA. Collectively, these findings suggest that ΔTA/MTA, which represents the extension pattern of PZC along the anatomical shape of the PZ, could be used as a novel factor reflecting the degree of PZC extension.

We determined whether ΔTA/MTA had an effect on the prognosis of patients with PZC undergoing RP. Multivariate analysis using the Cox proportional hazards model identified ΔTA/MTA in addition to several conventional factors, including preoperative serum PSA level, ECE, SVI and SMS, as independent predictors of biochemical recurrence after RP. There was a significant difference in biochemical recurrence-free survival in the 128 patients according to their ΔTA/MTA value. Considering these findings, the degree of disease extension along the anatomical shape of the PZ quantified by ΔTA/MTA could be used to predict the biochemical outcome after RP.

There are several limitations to the present study. To draw definitive conclusions it would be necessary to undertake prospective studies with more patients and with a longer follow-up. In addition, this study analysed the extension patterns of PZC alone; therefore, the significance of ΔTA/MTA in TZC remains unknown. However, considering the progression patterns of TZC growing spherically in the TZ [10], it might be necessary to introduce other variables representing the extension pattern of TZC. Finally, with respect to the time required for calculating ΔTA/MTA, routine use of this variable for pathological examination could not always be recommended; hence, the introduction of a simpler variable than ΔTA/MTA would be expected.

In conclusion, the present findings suggest that PZC extends along the anatomical shape of the PZ during the process of extension in the prostate gland, and that the extension pattern of PZC can be closely represented by the ΔTA/MTA value. Despite the need for further studies, ΔTA/MTA, which is significantly associated with certain kinds of adverse pathological factors, could be used as an independent indicator of biochemical outcome in patients undergoing RP.

CONFLICT OF INTEREST

None declared.

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Abbreviations: **RP**, radical prostatectomy; **(M)TA**, (maximum) tumour area; **MTV**, maximum tumour volume; **PZ(C)**, peripheral zone (cancer); **TZ(C)**, transitional zone (cancer); **ECE**, extracapsular extension; **SVI**, seminal vesicle invasion; **PNI**, perineural invasion; **SMS**, surgical margin status.

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Clinical Study

Impact of Sacral Surface Therapeutic Electrical Stimulation on Early Recovery of Urinary Continence after Radical Retropubic Prostatectomy: A Pilot Study

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Objectives. To investigate whether sacral surface therapeutic electrical stimulation (SSTES) initiated during the early postoperative period would be effective towards early recovery of postprostatectomy urinary continence. *Methods.* A total of 35 consecutive patients who underwent radical prostatectomy by a single surgeon were enrolled in this study. Twenty early patients began pelvic floor muscle exercise (PME). Fifteen subsequent patients received SSTES postoperatively with no instruction for PME provided. Immediate urinary function just after catheter removal was evaluated with frequency-volume chart and 24-hour pad test. *Results.* There were no differences between the SSTES and PME groups in maximum voided volume capacity (MVV) and urine loss ratio (ULR) on the first day after removal of urethral catheter. However, on day 3 MVV was significantly larger and ULR was also significantly lower in the SSTES group. *Conclusions.* SSTES treatment is feasible and appears to be effective for early recovery of urinary continence after radical prostatectomy.

1. Introduction

Urinary incontinence and sexual dysfunction are representative long-term complications of radical prostatectomy. During several months after radical prostatectomy, urinary incontinence develops in most patients, significantly lowering their quality of life (QOL). One year after the surgery, the incidence of urinary incontinence, the ratio of patients who require pads, and the ratio of those who experience urinary incontinence, even if only slightly, reach 5%–15%, 33%, and 66%, respectively [1, 2]. The main mechanism of postprostatectomy incontinence is considered to be damage of the sphincter muscle caused upon separation of the prostate and urethra. It has been clarified in recent years that preservation of the neurovascular bundle could be involved in the recovery of urinary continence [3], and thus a neurogenic mechanism for the sphincter muscle was indicated. In addition, detrusor overactivity can develop as a consequence of traction of the bladder. It has been reported that urinary incontinence related to detrusor overactivity occurs in 40% and detrusor overactivity incontinence occurs in 13% of patients after radical prostatectomy [4]. To improve the postoperative urinary continence, we should consider not only urinary sphincter muscle damage but also bladder-related factors.

Pelvic floor exercise (PME) has been widely performed after radical prostatectomy with the aim of the prevention and treatment of urinary incontinence. The effectiveness of the PME depends on its instruction method, and recent reports have suggested that the effectiveness would become higher by using the preoperative biofeedback method [5].

Neuromodulation, using electrical or magnetic stimulation, was developed for urgency incontinence as well as stress incontinence [6]. These methods were used to treat postprostatectomy incontinence (PPI) [7–10]. A certain effect was observed in these studies, while these studies were applied only for patients with urinary incontinence. We developed sacral surface therapeutic electrical stimulation

(SSTES) as a therapy for urinary incontinence using neuromodulation [11]. In this therapy, skin surface electrodes are applied on the sacral surface to provide stimulation, making the treatment very easy to perform. It has been shown that SSTES has not only an inhibitory effect on detrusor overactivity but also an efferent stimulant effect to the pudendal nerve [11]. It is thus expected that SSTES initiated in an early postoperative period would be effective for early recovery of postoperative urinary continence.

2. Materials and Methods

The study population consisted of 40 consecutive patients who underwent retropubic radical prostatectomy for newly-diagnosed, clinicallylocalized prostate cancer from November 2004 to November 2006. All of the operations were performed by a single surgeon (Y.A.) and technical modifications were not made except smaller skin incision during the study period. This surgeon experienced more than 500 radical retropubic prostatectomy procedures over 20 years. The patients with prolonged indwelling urethral catheter due to anastomotic leakage (two cases) and who required reinsertion of the urethral catheter because of transient dysuria (one case) were excluded from the study, since the long duration of the indwelling urethral catheter might affect the immediate continence postcatheter removal. The patients who could not complete the frequency volume chart (one case) and withdrew his consent for the use of the SSTES (one case) were also excluded. Thus, a total of 35 patients were enrolled in the study. Among them, 20 early patients (from November 2004 to December 2005) received instruction for the PME and began the exercise one day before the surgery and continued for 1 week or longer. Fifteen subsequent patients (from January 2006 to November 2006) received SSTES, which was started at postoperative day 1 with no PME instruction provided. None of the patients were prescribed anticholinergic drugs during this study. For SSTES, the stimulator was specially designed for this purpose (Nodoka, Lintec Co. Ltd., Tokyo, Japan) (Figure 1), and a pair of speciallydesigned plate electrodes with a contact surface 4-cm × 9-cm (width × height; Electrode type A, Lintec, Tokyo, Japan) (Figure 1) were placed symmetrically on the skin surface over the second through fourth posterior sacral foramens. Pulses of 30-Hz frequency at 200-μs pulse width and maximum output of 80 V were used for 15 minutes twice a day for 1 week. Intensity was controlled by each patient below the pain threshold. The urethral catheter was removed on day 5 or 6 postsurgery. Immediate urinary function just after catheter removal was evaluated with a daily frequency-volume chart and 24-hour pad test. The urine loss ratio [12] was defined as the weight of urine loss in the pad divided by the daily urine volume, that is, micturition volume plus incontinence volume. The maximum voided volume (MVV) was defined as largest voided volume during single micturition from daily frequency-volume chart. The Ethics Committee of the Tohoku University School of Medicine approved this study and informed consent was obtained from all of the patients. Statistical software (JMP Statistical Discovery Software, SAS Institute, Cary, NC, USA) was used for all analyses. Tested groups were compared by unpaired Student's t-test; P-values $< .05$ were considered statistically significant.

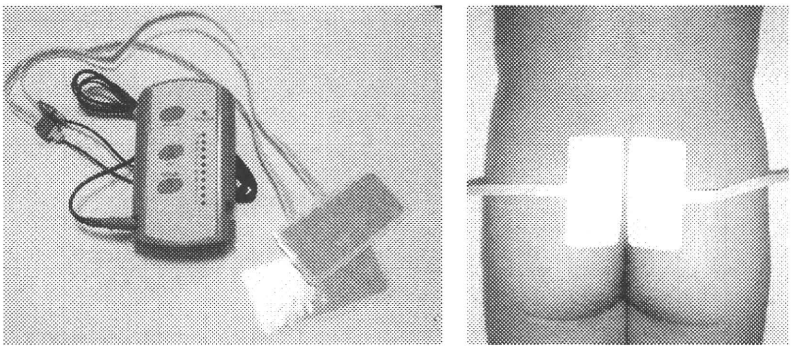
3. Results

Table 1 presents the patient demographics and pathological characteristics. There were no significant differences in any of the baseline clinical or pathologic parameters between the two groups: age, PSA, tumor stage, biopsy Gleason score, or degree of nerve preservation. No differences were observed in any of the postoperative parameters: operative time, estimated blood loss, prostate weight, pathological stage, or positive surgical margin status. During hospitalization, each group received the scheduled PME or SSTES treatment, respectively, under the instruction of the nursing staff. One patient in the SSTES group, who experienced sinus tachycardia and discomfort during electrical stimulation, with a fever of 38 degrees centigrade and expressed a desire to stop electrical stimulation, was excluded from the analysis.

On the first day after removal of the urethral catheter, there were no significant differences between the SSTES and PME groups in maximum voided volume (229.3 ± 79.2 ml (mean ± standard deviation) versus 217.4 ± 99.5 ml, resp.; $P = .35$) or urine loss ratio (13.8 ± 19.9% versus 14.5 ± 23.7%, resp.; $P = .46$). However, the maximum voided volume and urine loss ratio were rapidly improved in the SSTES group (Figures 2 and 3). On the third day after removal of the urethral catheter, the maximum bladder capacity was significantly larger in the SSTES group than in the PME group (315.0 ± 59.9 ml versus 268.1 ± 94.6 ml, resp.; $P < .05$). Urine loss ratio was also significantly lower in the SSTES group (1.18 ± 1.36% versus 10.32 ± 22.7%, $P < .05$). During the study period, there were no significant adverse effects observed, except for the one case described above. No patients showed the symptom of difficulty on urination. No patients complained dysuria or urinary retention during the hospital stay.

4. Discussion

In the literature, urinary incontinence is one cause of lowering the quality of life for patients following radical prostatectomy. Many efforts, including neuromodulation, have been made to achieve early urinary continence. Yokoyama et al. reported that extracorporeal magnetic stimulation improved continence in 60% of patients with PPI [9, 10]. On the other hand, two randomized control studies failed to show an additional effect of neuromodulation for PPI compared with PME alone [7, 8]. In these studies, electrical stimulation was applied with an anal surface electrode and was initiated after urethral catheter removal. Furthermore, in all of the studies noted above, neuromodulatory stimulation was applied in patients with existing urinary incontinence. It is well known that early rehabilitation has an advantage for early and



(a) (b)

FIGURE 1: Portable electrical stimulator (Nodoka, Lintec Co. Ltd., Tokyo, Japan) and a pair of the specially-designed electrodes. A pair of electrodes was placed symmetrically on the skin surface over the second through fourth posterior sacral foramens.

TABLE 1: The demographic and clinical characteristics of the patient population.

	PFE (n = 20)	SS- TES (n = 15)	P*
Age (year)	63.1 ± 6.4	61.4 ± 6.6	.46
PSA	10.7 ± 9.1	9.8 ± 6.6	.72
Clinical stage			
T1	70%	80%	
T2	15%	13%	
T3	15%	7%	.72 c
Biopsy Gleason sum			
6	5%	7%	
7	85%	86%	
8	5%	7%	
9	5%	0%	.84 c
Nerve sparing			
No nerve sparing	10%	13%	
Unilateral	50%	33%	
Bilateral	40%	53%	.62 c
Operation time	221 ± 66	226 ± 71	.85
Blood loss	1170 ± 721	838 ± 360	.11
Prostate weight	59.7 ± 30.1	45.1 ± 12.8	.09
Pathological stage			
pT2	80%	77%	
pT3	20%	23%	.74 c
Positive margin	20%	13%	.60 c

c: Chi-square test.
* Unpaired t-test unless otherwise noted.

satisfactory functional recovery in nonurological fields, such as orthopedics [13], cardiac surgery [14], neurosurgery [15], and spinal cord trauma [16]. PPI occurs due to surgical damage of the urethral sphincter, pelvic floor muscle, and bladder. From this perspective, we generated the idea of initiating electrical stimulation on the first day after radical prostatectomy. As a result of this early electrical rehabilitation, significant effects on early recovery of continence and maximum voided volume were observed. To our knowledge, this is the first report on the possible rehabilitative role of neuromoduration for PPI.

The present study showed the possible utility of SSTES for early recovery of urinary function following radical prostatectomy. We previously shown that postcatheter removal incontinence is significantly related to postoperative urinary function after radical prostatectomy [17]. Therefore, it is expected that minimizing the postcatheter removal incontinence could ultimately affect the postoperative urinary quality of life. In this study, on the first day after catheter removal, there were no differences in maximum voided volume or urine loss ratio between the SSTES and PME groups. On the other hand, on the third day after

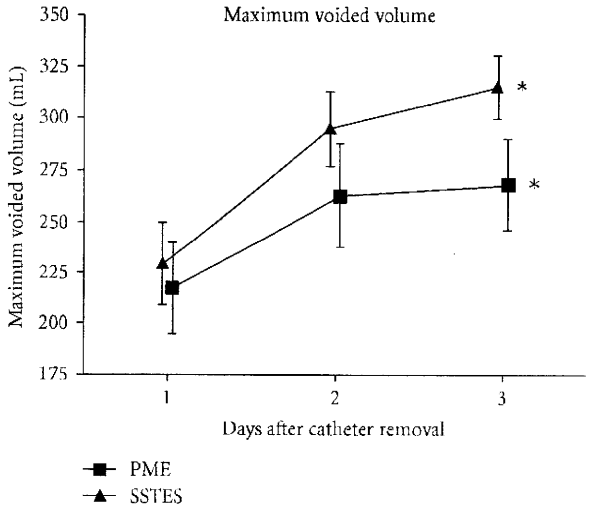


FIGURE 2: Maximum voided volume at day 1, 2, and 3 after removal of the urethral catheter. Error bars represent SEs. $^*P < .05$.

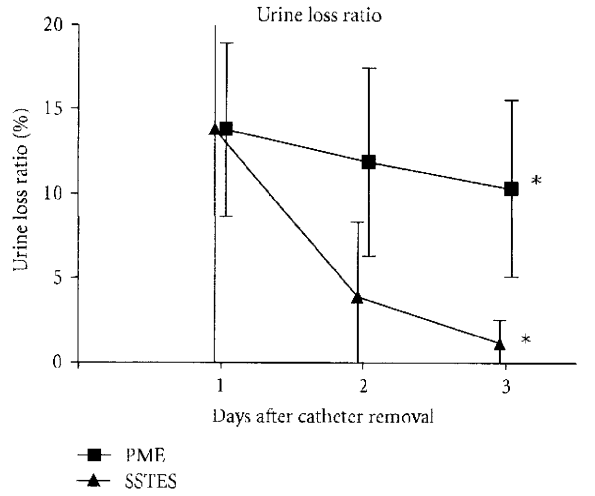


FIGURE 3: Percentage of urine loss ratio at day 1, 2, and 3 after removal of the urethral catheter. The urine loss ratio was defined as the weight of urine loss in the pad divided by the daily urine volume. Error bars represent SEs. $^*P < .05$.

catheter removal, the maximum voided volume and urine loss ratio rapidly improved with SSTES.

It has been reported that SSTES exhibits not only an inhibitory effect on detrusor overactivity but also an efferent stimulant effect via the pudendal nerve [11]. The effect may be partly due to decreasing urgency with electrical stimulation [18]. Pelliccioni and scarpino reported the external anal sphincter response with S3 surface electrical stimulation [19]. Indeed, we macroscopically observed that contraction of the pelvic floor muscle including the urethral sphincter and levator muscle was synchronized with SSTES during open radical prostatectomy (data not shown). The efferent effect on the pudendal nerve, which is equivalent to the effect of pelvic floor muscle exercise, and the afferent inhibitory effect can be expected for injured pelvic floor muscle and detrusor overactivity that develops after radical prostatectomy. Based on the results of this study, SSTES appears to have an early rehabilitative role on postprostatectomy urinary function.

We acknowledge several limitations in this pilot study. First, our study had relatively few patients. Second, although there was no statistical difference, the prostate volume and blood loss were smaller in the SSTES group, which may have influenced the results. Third, the study was not performed in a randomized fashion but as a historical control study. However, all operations were performed by a single, well-experienced surgeon and technical modifications were not made during the study that might minimize the intraoperator's bias, such as a learning effect. Fourth, one of the drawbacks of the neuromodulatory approach is the short carry-over effect. It is unknown whether 1-week of electrical stimulation could affect the recovery of urinary function for 1 month or longer after surgery. Indeed, it was difficult to accurately evaluate urine loss ratio using the 24-hour pad test on an outpatient basis. In the present pilot study, the optimal duration of SSTES remains to be elucidated. Nevertheless, the results show the possible rehabilitative role of SSTES in the early phase of recovery of urinary function following radical prostatectomy

A multi-institutional, randomized controlled study with a large number of subjects is now on going.

5. Conclusion

We investigated the rehabilitative role of SSTES for recovery of urinary function following radical prostatectomy. This treatment is feasible and appears to be effective for early recovery of urinary continence after surgery. A randomized controlled trial with a large study population is warranted to confirm its effectiveness.

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Decline of the Red Blood Cell Count in Patients Receiving Androgen Deprivation Therapy for Localized Prostate Cancer: Impact of ADT on Insulin-like Growth Factor-1 and Erythropoiesis

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OBJECTIVES To elucidate the mechanism of blood hemoglobin loss in patients with prostate cancer during androgen deprivation therapy (ADT), and to examine the activity of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis during ADT, which plays an important role in hematopoiesis.

METHODS A total of 83 patients with localized prostate cancer, who received ADT, were prospectively studied on the basis of their blood samples at the baseline and after ADT for 6 months.

RESULTS Before ADT, the IGF-1 level was correlated with the red blood cell (RBC) count (Spearman's rank correlation coefficient analysis [rs]= 0.315, $P = .011$), hemoglobin (rs = 0.278, $P = .018$), and mean corpuscular volume (rs = 0.266, $P = .020$), but such relationships disappeared after ADT. After ADT, the serum IGF-1 level increased compared with that at the baseline (21 ± 6 vs 18 ± 5 nmol/L, respectively, $P < .001$), but no change was observed in the serum GH level ($P = .691$). There was no difference between erythropoietin and interleukin-6 concentrations before and after ADT ($P = .852$ and $P = .208$, respectively). The hemoglobin concentration and RBC count declined after ADT compared with those before treatment ($P < .001$ for each). Although the mean corpuscular volume declined after ADT ($P = .002$), the mean cell hemoglobin was comparable between before and after ADT ($P = .676$).

CONCLUSIONS Despite the unaffected GH, erythropoietin, and interleukin-6 levels, the serum IGF-1 concentration was elevated by ADT. Even with the increased IGF-1 level, the RBC count and hemoglobin concentration declined after ADT. IGF-1 in the bone marrow erythroid progenitor cells might be functionally inactivated during ADT. UROLOGY 75: 1441–1445, 2010. © 2010 Elsevier Inc.

Treatment involving androgen deprivation has become the therapeutic mainstay for patients with metastatic prostate cancer or nonmetastatic disease to prevent recurrence. Recently, the combined or monotherapeutic use of androgen deprivation therapy (ADT) has also increased to a maximum of 30% for localized prostate cancer patients in the United States.[1,2] By contrast, ADT has been associated with unfavorable events such as hemoglobin loss and changes in body composition.[3-5]

The concentration of blood hemoglobin declines with aging in elderly populations, without demonstrable disorders.[6] Men have a higher hemoglobin concentration than women in all age brackets. In younger to middle-aged populations, the difference between genders is approximately 20 g/L, and this becomes smaller with aging; at 70 years, 14 g/L and at 81 years, 10 g/L. After 81 years, a further decline, which is more predominant in men, is noted.[6]

Insulin-like growth factor-1 (IGF-1) is a peptide involved in the regulation of cell proliferation and differentiation, and exerts multiple effects on glucose, fat, protein, bone, and hematopoiesis.[7] Although several tissues secrete IGF-1, more than 90% of IGF-1 in the serum is synthesized in the liver.[7] The production of IGF-1 in

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Table 1. Patients' characteristics

No. patients	83
Age at diagnosis, mean (range)	68.7 (54-79)
PSA at diagnosis (μg/L), mean (range)	15.8 (5.2-100.9)
Gleason score, mean (range)	7.1 (5-9)

PSA = prostate-specific antigen.

the liver is predominantly dependent on growth hormone (GH) secretion. GH secretion declines with aging, and in the so-called somatopause state, the reduced IGF-1 level accelerates age-related physiological changes.[8,9] It is known that the GH/IGF-1 axis plays a role in the regulation of erythropoiesis.[10,11] Correspondingly, GH administration has been shown to improve physiological disorders, including the decreased hemoglobin concentration, by normalizing the GH/IGF-1 axis.[10,12,13] Thus, GH and IGF-1 show synergistic actions on erythropoiesis.

Accordingly, hemoglobin loss as a result of ADT is suggested to be based on iatrogenic andropause. Although many previous studies have supported this, it is still unclear how androgens regulate erythropoiesis.[3,4,6] By contrast, despite the significance of GH and IGF-1 in hematopoiesis,[12,13] the influence of ADT on the GH/IGF-1 axis in terms of erythropoiesis has not been reported. In the present study, we prospectively studied patients who received ADT for localized or locally advanced prostate cancer to identify responsible factors in hemoglobin loss during ADT, measuring various biochemical parameters and hormones before and after treatment. Also, we focused on the activity of GH/IGF-1 axis during ADT, and found an interesting discordance/dissociation between the GH/IGF-1 axis and erythropoiesis during ADT, which has not been reported but is of potential significance in ADT-associated hemoglobin loss.

MATERIAL AND METHODS

Patients

A total of 83 consecutive patients who were treated with radiotherapy for localized or locally advanced prostate cancer (cT1c–3 N0 M0) at the Department of Urology, Niigata University Hospital, and the Department of Urology, Niigata cancer center Hospital, were enrolled between May 2004 and December 2006. The patients received a subcutaneous injection of a gonadotropin-releasing hormone (GnRH) agonist, goserelin acetate (3.6 mg, every 4 weeks), and peroral nonsteroidal antiandrogen flutamide (375 mg/d, n = 71) or bicalutamide (80 mg/d, n = 12) for 6 months, before radiotherapy. Patients' demographics are shown in Table 1. The study was prospectively designed, and the procedure for this research project was approved by the Ethics Committee of our institution. Informed consent was obtained from all patients.

Blood Sampling and Analytic Measurements

In all patients, blood samples were evaluated at the baseline as well as after ADT for 6 months. All blood samples were obtained between 11 AM and 2 PM. Hormonal examinations including measurement of androgens, erythropoietin, GH, and IGF-1 were performed, in addition to routine blood and serum examinations (red blood cell [RBC] count, hemoglobin, hematocrit, iron, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ- glutamyl transpeptidase, cholinesterase, triglycerides, total cholesterol, high-density lipoprotein, cholesterol and low-density lipoprotein, cholesterol, blood urea nitrogen, and uric acid). Hormonal parameters were quantified by SRL, Tokyo, Japan. Testosterone was determined by electrochemiluminescence immunoassay. Prostate-specific antigen (PSA), dihydrotestosterone, androstenedione, IGF-1, GH, erythropoietin, interleukin-6 (IL-6), adrenocorticotropic hormone, and cortisol were measured by radioimmunoassay. Luteinizing hormone and follicle-stimulating hormone were determined by chemiluminescent immunoassay. Dehydroepiandrosterone sulfate was quantified using the chemiluminescent enzyme immunoassay.

Histopathologic Diagnosis

An independent urological pathologist performed histopathologic diagnoses based on biopsy cores from all 83 patients. Standardized grading was carried out according to the Gleason classification system.

Statistical Analysis

The Wilcoxon signed-rank test was used to compare changes in paired parameters before and after ADT. The test was two-sided, and $P < .05$ was considered significant. Correlations between serum IGF-1 levels and other parameters after ADT and their ratio of before to after ADT were analyzed using Spearman's rank correlation coefficient analysis (rs). All analyses were performed using the SPSS version 11.0 J (SPSS Inc., Chicago, IL) in a windows-based computer.

RESULTS

Adverse Events

Flutamide treatment was discontinued in 28 patients because of adverse effects: at 1 month, 6 patients; at 2 months, 4 patients; at 3 months, 8 patients; at 4 months, 4 patients; and at 5 months, 6 patients. Elevation of the serum transaminase levels and diarrhea were the causes of discontinuation in 24 and 4 patients, respectively. To prevent severe liver dysfunction, the administration of flutamide was immediately discontinued when elevated serum transaminase levels were noted. Transaminase levels recovered within 4 weeks, and diarrhea disappeared immediately after the discontinuation of flutamide. All 28 patients received GnRH agonist monotherapy for the remaining treatment period.

Increased Serum IGF-1 Level After ADT

Analytic measurements of the GH/IGF-1 axis are presented in Table 2. After ADT for 6 months, the serum IGF-1 level increased (21 ± 6 nmol/L) compared with that at the baseline (18 ± 5 nmol/L) ($P < .001$), but no change was observed in the serum GH concentration between before and after ADT (1.5 ± 2.3 vs 0.9 ± 0.9 μg/L, respectively, $P = .691$).

Table 2. Comparison of GH, IGF-1, erythropoietin, and erythropoietic markers between before and after ADT

	Before ADT Mean (SD)	After ADT Mean (SD)	P
GH (μg/L)	1.5 (2.3)	0.9 (0.9)	.691
IGF-1 (nmol/L)	18 (5)	21 (6)	<.001
Erythropoietin (mIU/mL)	28.0 (13.9)	27.8 (17.3)	.852
IL-6 (pg/mL)	2.10 (2.45)	2.06 (3.68)	.208
Hemoglobin (g/L)	146 (15)	131 (12)	<.001
RBC count	451 (44)	408 (53)	<.001
MCV (fL)	95.6 (4.5)	94.7 (4.7)	.002
MCH (pg)	32.3 (2.2)	32.3 (2.0)	.676
MCHC (g/dL)	34.1 (1.1)	33.8 (1.0)	.004
Iron (μg/dL)	94.0 (26.7)	98.9 (30.1)	.234

GH = growth hormone; IGF-1 = insulin-like growth factor-1; ADT = androgen deprivation therapy; IL-6 = interleukin-6; RBC = red blood cell; MCV = mean corpuscular volume; MCH = mean cell hemoglobin; MCHC = mean cell hemoglobin concentration.

Also, we analyzed measurements of the GH/IGF-1 axis separately in the antiandrogen-continued and flutamide-discontinued patients, to eliminate the influence of liver dysfunction on the serum IGF-1 level. In the antiandrogen-continued group, the serum IGF-1 level increased significantly (P <.001) over 6 months. In the flutamide-discontinued group, the serum IGF-1 level also increased, but the difference was not significant (P = .281).

Erythropoietic Parameters

Data on erythropoietic parameters are also shown in Table 2. Although the erythropoietin and iron concentrations were not different between before and after ADT (P = .852 and P = .234, respectively), the hemoglobin concentration declined after ADT (131 ± 12 g/L) compared with that before ADT (146 ± 15 g/L) (P <.001), and the post-treatment RBC count was also decreased compared with that before therapy (408 ± 53 vs 451 ± 44, P <.001). Although the mean corpuscular volume (MCV) declined after ADT (94.7 ± 4.7 fL) in comparison with that before ADT (95.6 ± 4.5 fL) (P = .002), the mean cell hemoglobin (MCH) was equally distributed between before and after ADT (32.3 ± 2.2 and 32.3 ± 2.0 pg, respectively, P = .676). Also, the MCH concentration after ADT (33.8 ± 1.0 g/dL) was lower than that before therapy (34.1 ± 1.1 g/dL) (P = .004). The serum IL-6 level was not different between before and after ADT (2.10 ± 2.45 and 2.06 ± 3.68 pg/mL, respectively, P = .208).

Influence on PSA, Androgens, and Associated Hormones

Table 3 shows the influence of ADT on PSA, androgens, and gonadotropins. The serum levels of PSA, testosterone, dihydrotestosterone, dehydroepiandrosterone sulfate, androstenedione, luteinizing hormone, and follicle-stimulating hormone reduced after ADT (P <.001 in all). The androgen levels were not correlated with any hematopoietic parameters and/or markers either before or after ADT (data not shown).

Table 3. Influence of ADT on PSA and sex hormones

	Before ADT Mean (SD)	After ADT Mean (SD)	P
PSA (μg/L)	15.8 (13.8)	0.1 (0.3)	<.001
Testosterone (nmol/L)	16.0 (5.0)	0.5 (0.3)	<.001
DHT (ng/mL)	0.91 (0.40)	0.04 (0.02)	<.001
Androstenedione (nmol/L)	5.4 (1.7)	2.8 (1.4)	<.001
DHEA-S (μmol/L)	28.9 (19.7)	15.5 (13.4)	<.001
Luteinizing hormone (IU/L)	6.29 (4.65)	0.07 (0.18)	<.001
Follicle-stimulating hormone (IU/L)	12.20 (8.92)	11.12 (8.40)	<.001

DHT = dihydrotestosterone; DHEA-S = dehydroepiandrosterone sulfate; ACTH = adrenocorticotropic hormone.

Table 4. Other hormones and parameters before and after ADT

	Before ADT Mean (SD)	After ADT Mean (SD)	P
ACTH (pmol/L)	33 (15)	30 (14)	.136
Cortisol (nmol/L)	366 (127)	391 (104)	.059
AST (μkat/L)	0.43 (0.16)	0.58 (0.33)	<.001
ALT (μkat/L)	0.40 (0.23)	0.61 (0.44)	<.001
Lactate dehydrogenase (μkat/L)	3.14 (0.48)	3.46 (0.57)	<.001
μ-Glutamyltransferase (μkat/L)	0.68 (0.53)	0.79 (0.63)	<.001
Triglycerides (mmol/L)	1.48 (0.68)	1.63 (0.76)	.092
Cholesterol total (mmol/L)	5.16 (0.89)	5.22 (0.98)	.728
Cholesterol, high-density (mmol/L)	1.45 (0.35)	1.47 (0.34)	.456
Cholesterol, low-density (mmol/L)	2.30 (0.76)	2.99 (0.83)	.675
Urea nitrogen (mmol/L)	5.39 (2.15)	5.82 (2.20)	.002
Uric acid (μmol/L)	314 (73)	260 (76)	<.001

AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Other Parameters

For protein metabolism, blood urea nitrogen increased and uric acid levels decreased (P = .002 and P <.001, respectively) (Table 4). There were no significant differences in the total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride levels between before and after ADT.

Relationships Between the IGF-1 Levels and Other Clinicopathologic Parameters

We did not identify any correlations between the pretreatment serum IGF-1 level and Gleason score (rs = 0.084, P = .485) or pretreatment PSA value (rs = −0.029, P = .810). Before ADT, the IGF-1 level was

correlated only with the RBC count (rs = 0.315, P = .011), hemoglobin (rs = 0.278, P = .018), and MCV (rs = 0.266, P = .020). After ADT, however, there were no correlations between serum IGF-1 and these parameters (P = .329, P = .552, and P = .627, respectively) or other values except for the luteinizing hormone level (rs = 0.364, P = .002).

COMMENT

The elevation of the serum IGF-1 level during ADT has most recently been reported,[14] and in the present study, we noted a dissociation/discordance in the GH/IGF-1 axis relevant to erythropoiesis. Despite the unaffected GH levels, the serum IGF-1 concentration increased after ADT. Indeed, the IGF-1 level was correlated with the RBC count and hemoglobin concentration before ADT (rs = 0.315, P = .011 and rs = 0.278, P = .018, respectively); however, the relationships were disrupted after ADT (P = .329 and P = .552, respectively). It remains unknown whether a distinct feedback loop is present among IGF-1, GH, and erythropoietin or the hemoglobin concentration, and we first discuss the discordance between GH and IGF-1. Most serum IGF-1 binds to IGF-binding proteins 1-6. Tissue IGF-1 bioactivity is determined not only by serum IGF-1, but also by the local expression of IGF-1, IGF-binding proteins, and IGF-1 receptors.[15] However, we could not obtain data on the bone marrow expression of these molecules, but they might explain the paradoxical phenomenon in the GH/IGF-1 axis and erythropoiesis.

Second, our study showed that the MCV was reduced but MCH was not affected by ADT, suggesting that ADT suppresses the proliferation of erythroid cells rather than iron-hemoglobin metabolism. Recent evidence showed that IGF-1 supports the proliferation of erythroid progenitor cells in the bone marrow.[16,17] In the present study, the IGF-1 level was elevated after ADT compared with the baseline, but conversely, the hemoglobin concentration and RBC count declined significantly after ADT. We additionally showed that the increased IGF-1 level under ADT possibly exerts its function in the gonadotroph; the post-treatment serum IGF-1 level was strongly correlated only with the luteinizing hormone level after ADT (rs = 0.364, P = .002). This finding suggests that IGF-1 elevated by ADT is bioactive, because gonadotroph cells express IGF-1 receptors;[18,19] and this is also of interest regarding the development of ADT. In contrast, IGF-1 cannot efficiently execute its function in the bone marrow, suggesting that IGF-1 or its receptor in the bone marrow may be functionally inactivated during ADT.

The recovery of the hemoglobin concentration has been reported to be mediated by an initial increase of erythropoietin secretion to a certain extent.[11,12] By contrast, erythropoietin administration does not have an influence on the GH/IGF-1 axis,[20] suggesting no negative or positive feedback from erythropoietin to IGF-1. Erythropoietin is the strongest factor in erythropoiesis in general or anemic conditions.[6,20] Yet, we did not find any difference in the erythropoietin level between before and after ADT or association between this hormone and IGF-1. Also, IL-6, of which elevation in the serum is relevant to anemia, was not changed by ADT.[21] Thus, the discordance in the GH/IGF-1 axis may be more influential than erythropoietin for hematopoiesis during ADT.

Third, as shown additionally in our study, ADT worked in vivo; it leads to androgen deprivation. It has been suggested that andropause is intrinsically relevant to somatopause.[22,23] Yet, the present study showed that GH was preserved under a condition where the gonadotropins and androgens were deficient. An elevated IGF-1 level could not potentially overcome the influence of the reduced androgens on hematopoiesis, leading to the reduced hemoglobin concentration. It has been reported that the pubertal increase in hemoglobin concentration in boys is related to direct androgen effects rather than IGF-1.[24] However, it is still unknown how androgens are implicated in hematopoiesis. In the present study, the androgen levels were not correlated with hematopoietic parameters either before or after ADT. Erythropoiesis suppression during ADT may have to be explained not only by the androgen-androgen receptor interaction but also by other abnormal metabolic networks such as discordance in the GH/IGF-1 axis. An in vitro study using erythroblast cell lines is currently underway.

Although the definite mechanism for the increased serum IGF-1 level during ADT is unclear, the present study also showed that liver-associated enzymes, such as aspartate amino transferase and alanine aminotransferase, were elevated by ADT (Table 4). These data suggest that the production or release of IGF-1 from the liver might be upregulated during ADT. In addition, we eliminated the influence of liver dysfunction on the serum IGF-1 level by analyzing the IGF-1 levels separately in the antiandrogen-continued and -discontinued groups. In the antiandrogen-continued group, the serum IGF-1 level increased (P <.001) over 6 months. In the flutamide-discontinued group, the serum IGF-1 level also increased; however, the difference was not significant (P = .281). The small number of patients in the flutamide-discontinued group may explain the absence of the statistical significance. It is also conceivable that combined androgen blockade has a greater influence on the serum IGF-1 level than GnRH agonist monotherapy.

CONCLUSIONS

Despite the unaffected GH, erythropoietin, and IL-6 levels, serum IGF-1 increases with ADT. The IGF-1 level was correlated with the RBC count and hemoglobin concentration before treatment, but such relationships were disrupted after ADT. Even with the increased IGF-1 level, the RBC count and hemoglobin concentration declined after ADT. Although the definite mechanism for such dissociation among the GH, IGF-1, and eryth-