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Effect of radiotherapy for rectal cancer on ovarian androgen production

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Abstract

Background—The impact of radiotherapy (RT) for rectal cancer on ovarian androgen production is unknown. The aim was to examine the effect of RT for rectal cancer on androgen levels in non-oophorectomised women and the association with female sexual desire.

Method—This prospective cohort study included women treated with surgery for rectal cancer with or without RT. Serum testosterone (T), free T, androstenedione and dehydroepiandrosterone sulfate (DHEA-S) were assessed at baseline, after RT and one year postoperatively. Sexual desire was assessed with the Female Sexual Function Index.

Results—Twenty-seven participants had surgery alone (RT-) and 98 had preoperative RT and surgery (RT+). During the first year after surgery, median T and free T decreased from 0.6 (range 0.1–3.6) to 0.5 (0.1–2.3) nmol/L ($p < 0.001$) and 9.1 (range 1.6–45.8) to 7.9 (1.4–22.7) pmol/L

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($p < 0.001$) respectively in the RT+ group and did not change in the RT- group. Longitudinal regression analysis confirmed a decrease in T and free T after RT. The adjusted change in androstenedione and DHEA-S was not significant in any group. The mean change in T (OR 2.74 (95% CI 1.06–7.11, $p = 0.038$), free T (OR 1.08 (95% CI 1.02–1.15), $p = 0.011$) and androstenedione (OR 1.52 (95% CI 1.07–2.16), $p = 0.019$) was related to change in sexual desire.

Conclusion—Radiotherapy decreases androgens predominantly derived from the ovaries, while androgens of mainly adrenal origin remain unchanged. Reduction in ovarian derived androgens maybe associated with reduced sexual desire.

Introduction

Postmenopausal ovaries still secrete androgens. Testosterone (T) levels do not change as a consequence of the menopause, but decline slowly with age (1) (2) (3). In women, serum androgens are derived from the ovaries, the adrenal glands and from conversion in peripheral tissues. Serum T originates by direct synthesis in the ovarian stroma or by conversion of androstenedione in the adrenal. The relative ovarian androstenedione production decreases with age, from 40% in premenopausal to 20% in postmenopausal women.

Dehydroepiandrosterone sulphate (DHEA-S), a steroid with limited androgenic effect, is synthesised mainly in the adrenal glands (2) (3). Radiotherapy (RT) for rectal cancer induces amenorrhea and infertility in premenopausal women, but the effect on ovarian androgen production is not known (4) (5). The impact of RT on ovarian T production in the treatment of gynaecological cancers is limited and conflicting (6) (7) (8). In men, preoperative RT for rectal cancer has a negative impact on serum T levels due to acute testicular failure related to cumulative testicular dose (9). The negative impact on endocrine testicular function might be permanent in selected men resulting in clinical relevant hypogonadism (10). Androgens may play an important role in female sexual function (11). Serum T levels have been reported to be associated with sexual desire and arousal (11) (12) (13). Animal and human data suggest a direct impact on libido through androgen receptors in the central nervous system, as well as an effect on genital physiological response through regulation of smooth muscle and local blood flow (14) (15) (16).

Radiotherapy is a risk factor for sexual dysfunction after treatment for rectal cancer (17) (18) (19). In women treated for rectal cancer, decreased sexual desire is common, particularly after RT (20) (21). A potentially treatable RT-induced decrease in ovarian T production might be a contributing factor. The aim of this study is to examine the effect of RT for rectal cancer on ovarian T production, and to explore the impact on sexual desire in women.

Methods

Patients were recruited from the outpatient clinics of five Swedish rectal cancer surgery centres (Karolinska University Hospital, Ersta Hospital, Örebro University Hospital, Linköping University Hospital and Norrköping Hospital) between June 2008 and August 2013. Follow-up was one year. Patient data, questionnaires and fasting venous blood samples were drawn at baseline and one year after surgery. Participants treated with preoperative RT had an additional blood sample taken after RT the day before surgery. Clinical information was recorded on case report forms by a research nurse. The study was

approved by the Regional Ethical Review Board in Stockholm (2008/247–31/3, 2012/1730–30/3).

Participants

Women aged over 18 years diagnosed with stage I–III rectal cancer planned for abdominal resection were eligible and included after informed consent. Exclusion criteria were previous pelvic RT and inability to provide informed consent due to linguistic or cognitive restrictions. To define a study population with intact ovarian T production analyses in the present study were restricted to participants without a history of oophorectomy or oophorectomy during surgery for rectal cancer

Oncological therapy

Preoperative RT was administrated as either short-course (5 Gy (Gy) x 5) or long-course treatment (2 Gy x 25 or 1.8 Gy x 25 +/- 3 fractions of boost to primary tumour and radiologically malignant lymph nodes). The use and type of preoperative RT given was decided at the multidisciplinary team meeting. The decision to use RT was based on tumour characteristics and cancer stage without knowledge of individual androgen levels. The clinical target volume (CTV) covered the mesorectum and involved regional lymph node stations, with the upper limit at the level of the bifurcation of the common iliac artery or the sacral promontory. No specific considerations were made to minimise the dose to the ovaries, which are normally exposed to the dose of the planning target volume (PTV) The standard chemoradiotherapy regimen was Capecitabine (825 mg/m² twice daily) concomitantly with 50.4 Gy, followed by surgery and selectively eight cycles of adjuvant CAPOX (Capecitabine 1000 mg/m² day 1–14 and Oxaliplatin 130 mg/m² every three weeks). A few participants were treated within the phase III trial RAPIDO (22), wherein participants were randomised to either standard chemoradiotherapy followed by surgery and CAPOX or short-course RT followed by six cycles of neoadjuvant CAPOX and surgery.

Sex hormone levels and sexual desire

The primary outcome was serum T level. Secondary outcomes were calculated free T level, serum levels of androstenedione and DHEA-S, and sexual desire. Serum was separated after centrifugation and stored at –20 degrees Celsius. All analyses were performed at the same time point in the same batch. Technical details of the analyses performed are provided in appendix 1. Testosterone and androstenedione were measured with liquid chromatography –mass spectrometry using the LC-MS/MS Quattro Premier Xevo TQ MS (Waters Corporation, Milford, MA, USA). The total coefficients of variation (CV_{tot}) were 7% at 2.8 nmol/L and 6% at 15 nmol/L for T and 10% at 3 nmol/L for androstenedione. DHEA-S and sexual hormone binding globulin (SHBG) were measured with electrochemiluminiscens assay using the Cobas 8000 instrument from Roche (Roche Diagnostics International Ltd, Rotkreutz, Switzerland). The CV_{tot} for DHEA-S were 11% at 6.0 µmol/L and 9% at 20 µmol/L. The CV_{tot} for SHBG were 9% at 18 and at 36 nmol/L. Serum oestradiol was determined by radioimmunoassay (Estradiol-Ultrasensitive-Coated tube RIA, Cisbio Bioassays, Codolet, France). The CV_{tot} for oestradiol was 16% at 57 pmol/L. Follicle Stimulating Hormone (FSH) and luteinising hormone (LH) were measured with fluorescence immunoassay using Perkin Elmers AutoDELFIA immounassay system

(PerkinElmers, Waltham, MA, USA). The CV_{tot} for FSH were 3% at 7.6 IU/L and 4% at 22 and at 38 IU/L. The CV_{tot} for LH were 4% at 1.6 IU/L, 3% at 20.7 IU/L and 6% at 62 IU/L. Albumin was measured photometrically using the Cobas 8000 instrument from Roche. The CV_{tot} for albumin were 4% at 24 and at 40 g/L. Free T was calculated using a new multistep dynamic model based on T's binding to SHBG (23).

Sexual desire was assessed with the domain score for sexual desire of the Female Sexual Function Index (FSFI) (24). Scores range from 1.2 to 6, with a high score representing high sexual desire. A score of 5 or less is proposed as a cut-off value to identify premenopausal women with low sexual desire (25). Women treated for rectal cancer are usually postmenopausal and older. Therefore sexual desire scores in the cohort were expected to be lower. To compare women with high vs. low sexual desire in the present study, the scores for sexual desire were dichotomised by a cut-off value of 3 points, which reflects the 75th percentile of the sexual desire score at baseline in women included in this study.

Bias

Participation in the study was not restricted by age, sexual activity or current relationship to reduce selection bias risk. Androgen levels and gynaecological status were unknown at inclusion. Blood samples were collected between 8 and 11 am after overnight fasting to avoid information bias due to hormone level circadian oscillation. To further minimise information bias, serum analyses were undertaken in the same batch. To reduce the negative influence of the recently diagnosed cancer on the baseline score of sexual function, patients were asked to answer according to their pre-diagnosis situation.

Statistical Methods

Study data were analysed with Stata version 14 (StataCorp LP, College Station, TX, USA). Groups were compared with Wilcoxon rank-sum, Wilcoxon matched-pairs signed rank and Fisher's exact tests as appropriate, with 2-sided 5% significance levels.

A generalized estimating equation (GEE) approach analysed the change in androgen levels for the exposed and unexposed group over time. This approach gives robust estimates of population averages even with unbalanced data or mis-specified correlation structure. The fitted generalized linear regression models contained linear splines with one knot the day before surgery to account for non-linear trends of change in androgen levels. The specification of the applied GEE models were gaussian distribution of the dependent variable (androgens), exchangeable within-group correlation structure and conventional variance estimator. The reported coefficients represent the main effect on androgen levels (serum T, free T, serum androstenedione and DHEA-S) over the study period.

The following covariates were considered as confounders and/or effect modifiers: Age, body mass index (BMI), American Society of Anaesthesiologists physical status classification (ASA), menopausal status (FSH > 25 IU/l was regarded as postmenopausal), oestradiol level, LH level, type of RT, type of surgery (abdominoperineal excision of rectum (APER) resection vs. low anterior resection with temporary ileostomy), and postoperative chemotherapy. Covariates that changed the unadjusted point estimates by more than 10 per cent and significant interaction terms were included in the final models.

The association between sexual desire and change in androgen levels was also assessed by GEE models without linear splines, specified by binomial distribution of the dependent variable (sexual desire high vs low), exchangeable within-group correlation structure and conventional variance estimator. The effect of change in androgen levels on sexual desire is reported as odds ratio (OR) comparing the odds to experience high versus low sexual desire for a one unit increase of respective androgen level. The final longitudinal regression models were adjusted for confounding and effect modification in analogy to the models with androgen levels as outcome described above.

Results

Participants and descriptive data

Four hundred and four women with rectal cancer were assessed for eligibility, and 142 were included in the study (Fig 1). Seventeen had oophorectomy, and were not analysed in the current cohort study. In total, 125 women were included. Baseline characteristics of the study cohort are presented in Table 1. Ninety-eight women were treated with preoperative RT (RT +). Forty-two patients had short-course RT with immediate surgery, 20 with delayed surgery, and one had short-course RT followed by chemotherapy and delayed surgery. Thirty-five patients had long-course RT with delayed surgery and of these 29 had concomitant chemotherapy.

Patients in the RT + group were younger, more likely to undergo APER and had more severe perioperative events compared with patients in the surgery alone (RT -) group. There were no differences in menopausal status, ASA-classification, BMI, or pathological tumour stage. The final histology of one patient in the unexposed group was an adenoma, even though assessed as adenocarcinoma on histopathological examination of preoperative biopsies. The proportion of patients treated with adjuvant chemotherapy did not differ between the groups.

Twelve women had uni- or bilateral oophorectomy concomitantly with the resection of the primary tumour, and were excluded from analysis one year after surgery. Twenty-seven women were lost to follow-up (figure 1).

Androgen levels

Serum levels of androgens are presented in Table 2 and Fig. 2. Baseline levels of T, free T and androstenedione were similar in the RT+ group compared to the RT- group. Median DHEA-S was higher in RT+ women. In the RT+ group a significant decline during the first year after surgery was registered for T, free T, androstenedione and DHEA-S. In the RT- group the decrease was not significant for T, free T and androstenedione but was in DHEA-S. The cross-sectional comparisons one year after surgery showed no significant differences between RT+ and RT- women regarding T, free T, androstenedione and DHEA-S (table 2).

Adjusted longitudinal regression analysis with linear spline models are displayed in Table 3. The RT- group had no significant changes in androgen levels from baseline to one year postoperatively. In women exposed to preoperative RT there was a decline in T and free T levels from baseline to one year after surgery. The interactions with time were significant for menopausal status in the T model, for oestrogen in the androstenedione model and for

postoperative chemotherapy and age in the DHEA-S model. No interaction was found in the model for free T.

Response rates regarding sexual desire was 120 of 125 (96.0%) at baseline and 86 of 86 (100.0%) one year postoperatively. The score for sexual desire did not differ between groups at baseline ($p=0.322$). During the first year after surgery, the median sexual desire score declined in RT+ women from 2.2 (1.2–6.0) to 1.8 (1.2–5.4), $p=0.016$. In the RT- group the median sexual desire score did not change (1.8 (1.2–4.2) and 1.8 (1.2–4.8) respectively, $p=0.571$). The adjusted longitudinal regression analysis showed an association between sexual desire and mean change in T, free T, androstenedione and DHEAS (table 4).

Discussion

This prospective cohort study showed that RT was associated with a decrease in androgens of ovarian origin (T and free T). No association was observed between RT and androgens produced mainly in the adrenals (androstenedione and DHEA-S). The change in T, free T and androstenedione levels was associated with a decline in sexual desire.

Effect of RT for rectal cancer and reduction in ovarian androgens has not previously been described. The effect of RT on T levels after cervical cancer has been examined in three smaller studies, reporting divergent results (6–8). The ovarian stroma, where T synthesis takes place, may be more RT resistant than the oestrogen producing follicular tissue (8). A possible mechanism for the observed association between RT and T reduction could be slow fibrosis and vascular injury that negatively affects the androgen producing stroma cells. Fibrosis development over time may explain why no significant decline in T and free T was seen shortly after RT.

The reported levels of T at baseline and one year postoperatively in both the RT+ and RT- groups are within the normal range for postmenopausal women and the clinical relevance is not clear. In the cohort, serum levels of T, free T and androstenedione were positively associated with sexual desire. Sexual interest and desire are reported to decline with low T levels in women (12) (13) (26). Reference androgen levels with associations to specific symptoms are not available, and there is no lower limit of endogenous androgens to identify women with sexual dysfunction. Several randomised trials with exogenous testosterone treatment report improved sexual desire, arousal and other aspects of female sexual function in naturally and surgically postmenopausal women with hypoactive sexual desire disorder (27) (28). Sexual dysfunction has been well described after treatment for rectal cancer, with RT as a clear risk factor (17) (19) (21) (29).

Treatment decisions about preoperative RT use were made at multidisciplinary treatment conferences, based on staging and general health without knowledge of androgen levels. The lower age of the RT+ group is not surprising as multimodal treatment for rectal cancer is more frequently used in younger patients with limited comorbidity. Ovarian androgen production does not decline in the same manner as oestrogens at menopause and age did not relevantly change the longitudinal models for T, free T and androstendione in this study. Women with potentially intact ovarian androgen production were assessed in this study to

define a study population at risk. The ovarian androgen levels at baseline were similar despite older participants in the unexposed group. The high frequency of APER in the cohort reflects the rectal cancer population treated at tertiary referral centers with a high proportion of locally advanced and low rectal cancers. In this study 21.6% of patients were lost to follow up, the association between RT and decrease in T is not expected to be different from those included in the study.

The most accurate method to measure serum total T, liquid chromatography tandem mass spectrometry, was used in this study (30). Free T was calculated with a new multi-step dynamic model (23). This model is more accurate than the Vermeulen's model, as the systematic error in calculated free T values in females is significantly reduced.

The longitudinal regression models were adjusted for age, body mass index, ASA, menopausal status, oestradiol level, LH level, type of RT, type of surgery (anterior resection with anastomosis or APER) and postoperative chemotherapy if models containing the covariates above indicated relevant confounding or effect modulation. It is not known whether treatment with Oxaliplatin and Fluorouracil has an impact on ovarian androgen production. Observational studies indicate a moderate transient (<6 months) gonadotoxic effect in premenopausal women treated with Oxaliplatin (5) (31). Fluorouracil is considered to have almost no effect on human reproductive function.

Preoperative RT for rectal cancer was associated with a decrease in T and free T of mainly ovarian origin, which was not the case for androstenedione and DHEA-S, androgens of mainly adrenal origin. These results indicate that RT has a negative impact on ovarian androgen-producing stroma cells. The decrease in T and free T was observed under the first year after surgery for rectal cancer, which raises concerns about a permanent adverse effect of RT. The clinical relevance of the decline in androgens warrants further exploration. Possible adverse effects from decreased T levels include negative effects on body composition, sarcopenia and bone turnover (3). The present study suggests that androgens of ovarian origin were related to sexual desire. A reduced sexual desire is a common complaint in women treated for rectal cancer. This possible link between a decline in androgens and sexual desire could be taken into account during the preoperative information and rehabilitation process as testosterone replacement therapy may be considered in women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The study was registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT01216189).

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Figure 1.
Inclusion and exclusion of patients. RT-, surgery alone group; RT+, preoperative radiotherapy and surgery group.

Figure 2.

Mean values of androgen levels over time in the surgery alone (RT-) and preoperative radiotherapy and surgery (RT+) groups. (Four panels: T, free T, androstenedione, DHEA-S)

Table 1.

Patient characteristics and treatment in the unexposed (RT-) and exposed (RT+) groups.

Variable	RT- (n=27)	RT+ (n=98)	*p-value
Age **	71 (46–90)	61.5 (26–81)	<0.001
BMI **	25.0 (20.8–39.5)	24.8 (18.3–37.4)	0.853
ASA			0.181
I	7	31	
II	11	53	
III	8	14	
Missing	1	0	
Menopausal status before surgery +/- RT ***			0.355
Premenopausal	2	15	
Postmenopausal	25	78	
Missing	0	5	
Severe perioperative adverse event (Clavien grade > 2)	2	27	0.037
Type of surgery			0.005
Anterior resection (Partial or total mesorectal excision)	21	49	
Hartmann's procedure	1	1	
Abdominoperineal excision of rectum	4	46	
Transanal resection	1	1	
No surgery	0	1	
Postoperative chemotherapy ****	6	32	0.348
Pathological stage			0.580
0	1	9	
I	9	22	
II	5	24	
III	11	36	
IV	0	5	
Missing	1	2	

* (Mann-Whitney or Fisher's exact)

** **Median** (range).

*** Definition, premenopausal: FSH<25 E/l, postmenopausal: FSH>25.

**** Within three months from surgery; RT-, surgery alone group; RT+, preoperative radiotherapy and surgery group; BMI, body mass index; ASA, American Society of Anesthesiologists physical status classification.

Table 2.

Serum levels of androgens at baseline, after preoperative radiotherapy (RT) and one year after surgery in the unexposed (RT-) and exposed (RT+) groups

	RT-			RT+			p-value, cross-sectional (rank-sum)
	Serum levels of androgens	n	p-value, longitudinal (sign rank)	Serum levels of androgens	n	p-value, longitudinal (sign rank)	
T (nmol/L)							
Baseline	0.6(0.2-4.8)	27		0.6 (0.1-3.6)	98		0.662
Missing		-			-		
After RT	-	-		0.6 (0.2-3.8)	87	0.944 *	-
Missing					11		
1 year after surgery	0.6 (0.2-1.3)	20	0.720 ***	0.5 (0.1-2.3)	66	0.002 ** <0.001 ***	0.459
Missing		7			32		
Free T (pmol/L)							
Baseline	8.5 (3.3-57.1)	27		9.1 (1.6-45.8)	98		0.824
After RT	-	-		9.3 (2.8-36.2)	87	0.872 *	-
Missing		-			11		
1 year after surgery	8.3 (3.4-21.7)	20	0.823 ***	7.9 (1.4-22.7)	64	0.010 ** <0.001 ***	0.549
Missing		7			34		
Androstenedione (nmol/L)							
Baseline	1.4 (0.3-2.3)	27		1.6 (0.3-7.8)	97		0.078
Missing		-			1		
After RT	-	-		1.6 (0.3-7.0)	87	0.896 *	-
Missing		-			11		
1 year after surgery	1.2 (0.3-3.5)	20	0.667 ***	1.4 (0.3-3.9)	66	0.024 ** 0.014 ***	0.693
Missing		7			32		
DHEA-S (micromol/L)							
Baseline	2.1 (0.2-6.9)	27		2.9 (0.4-9.5)	96		0.033
Missing		-			2		
After RT	-	-		2.7 (0.5-8.8)	87	0.040 *	-

	RT-			RT+			p-value, cross-sectional (rank-sum)
	Serum levels of androgens	n	p-value, longitudinal (sign rank)	Serum levels of androgens	n	p-value, longitudinal (sign rank)	
<u>Missing</u>		-			11		
One year after surgery	1.4 (0.1-7.1)	20	0.002 ***	2.1 (0.4-8.4)	64	<0.001 ** <0.001 ***	0.078
<u>Missing</u>		7			34		

Hormone measures reported as median (range). RT-, surgery alone group; RT+, preoperative radiotherapy and surgery group; T, testosterone; DHEA-S, dehydroepiandrosterone sulfate.

* baseline vs after RT

** after RT vs one year after surgery

*** baseline vs one year after surgery

Table 3.

Longitudinal regression analysis with linear spline models displaying adjusted change over time in androgen levels from baseline to one year postoperatively.

	Main effect over time in serum levels of androgens	95% confidence interval	p value
T (nmol/L) *			
RT-	-0.011	-0.097, 0.075	0.805
RT+	-0.138	-0.232, -0.044	0.004
Free T (pmol/L)			
RT-	-0.529	-1.831, 0.774	0.426
RT+	-1.601	-3.032, -0.170	0.028
Androstenedione (nmol/L) **			
RT-	0.006	-0.186, 0.198	0.950
RT+	-0.055	-1.315, 0.205	0.680
DHEA-S (micromol/L) ***			
RT-	-0.180	-0.436, 0.074	0.165
RT+	-0.119	-0.445, 0.208	0.476

RT-, surgery alone group; RT+, preoperative radiotherapy and surgery group; Adjusted for

* menopausal status,

** S-LH, S-oestradiol (interaction),

*** postoperative chemotherapy (interaction), preoperative RT, age.

Table 4.

Longitudinal regression analysis of the association between mean change in androgen levels and change in sexual desire.

	Odds ratio	95% confidence interval	p value
T [*]	2.74	1.06, 7.11	0.038
Free T ^{**}	1.08	1.02, 1.15	0.011
Androstenedione [*]	1.52	1.07, 2.16	0.019
DHEA-S ^{***}	0.49	0.27, 0.89	0.019

Scores for sexual desire dichotomized on the 75th percentile at baseline. The odds ratio signifies the probability of reporting a sexual desire above the 75th percentile if the androgen level increases with one unit.

^{*} Adjusted for follicle stimulating hormone (FSH),

^{**} adjusted for FSH and Body Mass index (BMI),

^{***} interaction with FSH and adjusted for BMI