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## Correlation between Steroid Hormone Metabolites and Leiomyomas of Uterus

Sang Wook Bai<sup>1</sup>, Byung Hwa Jung<sup>2</sup>, Bong Chul Chung<sup>2</sup>, Jin Dong Jeon<sup>1</sup>, Hyun Jung Lee<sup>1</sup>,  
 Han Sung Kwon<sup>1</sup>, Kyung Ah Chung<sup>1</sup>, Sei Kwang Kim<sup>1</sup>, Ki Hyun Park<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, Yonsei University College of Medicine

<sup>2</sup>Bioanalysis and Biotransformation Research Center, Korea Institute of Science and  
 Technology, Seoul, Korea

**Objective:** To elucidate 1) whether there are any differences in the urine concentrations of steroid hormone metabolites between patients with leiomyoma and normal controls 2) the correlation between urinary profiles of steroid hormones and leiomyomas of the uterus according to their type, location, volume, and weight.

**Materials of Methods:** The study population consisted of 37 premenopausal patients with uterine leiomyoma and the control group consisted of 25 premenopausal normal volunteer women without uterine leiomyoma. Confirmation of the existence of uterine leiomyoma was done by ultrasonography and histopathological examination after surgery. The volume of the leiomyoma was estimated by trans-abdominal and/or trans-vaginal ultrasonography. The Leiomyomas were divided into 3 types (subserosal, intramural and submucosal). Seventeen patients had subserosal type of leiomyoma, 10 with the intramural type and 10 with the submucosal type. The locations of the leiomyoma were also divided into 3 groups (fundus, body and isthmus). Seventeen patients showed a fundus location, 10 in body, and 10 in isthmus. We compared urinary profiles of the endogenous steroids between patients with leiomyomas and normal controls, and also investigated the relationship between urinary profiles of the endogenous steroids and leiomyomas according to their type, location, volume and weight by using highly sensitive Gas Chromatography-Mass Spectrometry (GC-MS) system.

**Results:** The mean ages of the patients with leiomyomas and the control group were 43.1 ±5.6 and 40.6 ±7.2 years, the weights were 63.4 ±7.3 and 59.4 ±8.1 kg, and their heights were 155.4 ±4.8 and 159.3 ±4.8 cm respectively. Seventeen patients had subserosal, 10 had intramural, and 10 had submucosal leiomyomas. There were 17 patients with leiomyoma located in fundus, 10 in body and 10 in isthmus. 17β-estradiol, 5-AT, 11-keto ET, 11β-hydroxy An, 11β-hydroxy Et, THS, THA, THE, a-cortolone, a-cortol, β-cortol, 11β-OH Et/11β-OH An and E2/E1 were significantly increased in patients with leiomyoma than in the control group. 17β-estradiol was significantly increased in the intramural and the submucosal types than in the subserosal type. There was no significant difference in

: , ) 120-752 134,  
 Tel: (02)02-361-5490, Fax: (02)02-313-8357, e-mail: swbai@yumc.yonsei.ac.kr

the concentrations of urinary steroids according to the locations of leiomyomas. There was no significant relationship between the concentration of urinary steroids and the volume of the leiomyomas. 17 $\beta$ -estradiol significantly decreased as the weight of uterus increased ( $r=-0.322$ ,  $p=0.04$ ).

**Conclusion:** The concentrations of steroid hormone metabolites were generally increased in patients with leiomyoma but were not significantly related to the volume and weight of the leiomyomas. Our study suggests that steroid hormones may be involved in the initiation of leiomyomas but may not be involved in their progression. In addition, the concentrations of steroid hormone metabolites are not related to the leiomyoma type and location.

**Key Words:** Steroid hormone metabolites, Leiomyomas

가 30 , , 20%~30% .<sup>1</sup> 17 , 10 , 10 , estrogen , , , , , 가 17 , 10 , 10 , profile Gas .<sup>2-5</sup> proge- Chromatography-Mas s Spectrometry (GC-MS) antiproge- .  
sterone RU-486 2.  
sterone .  
Androgen estrogen standard Sigma (St. Louis, Mo, USA) . Estrogen profile internal standard d<sub>2</sub>-17 $\beta$ -Estradiol MSD Isotope (Montreal, Canada) . analytical grade 가 . Serdolit AD-2 resin (particle size: 0.1~0.2 mm) Serva (Heidelberg, Germany) . Helix Pomatia  $\beta$ -glucuronidase/arylsulfatase Boeringer Mannheim (Germany) .  $\beta$ -glucuronidase 5.5 U/ml (at 39 ) arylsulfatase 2.6 U/ml (at 38 ) . Silylating reagents MSHFB (N-methyl-N-trimethylsilyheptafluorobutyramide) Machery-Nagel (Duren, Germany) , MSTFA (N-Methyl-N-trimethylsilyl trifluoroacetamide), TMCS (trimethylsilylchloride) TMSIm (N-trimethylsilylimidazole) Sigma (St. Louis, MO, USA) . Ethyl acetate ether "HPLC solvent" grade ether .  
1/6p  $\times$ L1  $\times$ L2  $\times$ L3 (L1, L2, L3: 24 (mid-luteal phase)

10 phy (HP 5972) mass spectrometry (HP 5989B mass  
 20 engine) . Estrogen  
 3. Gas Chromatography - Mass Spectrometry GC column 5% phenylmethyl siloxane (length:  
 (GC - MS) 25 m; inner diameter: 0.2 mm; film thickness: 0.33 µm)  
 cross-link fused-silica capillary  
 Hewlett-Packard GC-MS gas chromatogra- androgen corticoid GC column me-

**Table 1.** General characteristics

	Patients with leiomyoma (n=37)	Control group (n=25)	P
Age (yr)	43.1 ±5.6	40.6 ±7.2	NS
Weight (kg)	63.4 ±7.3	59.4 ±8.1	NS
Height (cm)	155.4 ±4.8	159.3 ±4.8	NS

NS: not significant

**Table 2.** Concentrations (µmole/g creatinine, mean ± SD) and concentration ratios of urinary steroids in normal female subjects (n=25) and patients (n=37) with leiomyoma

Compounds/Concentration ratio	Normal females	Patients with leiomyoma	p value
Estrone (E1)	22.8 ±8.81	22.9 ±10.2	NS
17β-estradiol (E2)	20.8 ±4.46	29.4 ±11.1	<0.05
Androsterone (An)	1.05 ±0.45	1.26 ±1.26	NS
Etiocholanolone (Et)	0.96 ±0.41	1.04 ±1.08	NS
5-Androstene-3β, 16β, 17β-triol (5-AT)	0.26 ±0.10	1.06 ±0.94	<0.01
11-Keto Et	0.32 ±0.18	0.98 ±1.38	<0.05
11β-hydroxy An (11β-OHA n)	0.69 ±0.29	1.25 ±1.11	<0.05
11β-hydroxy Et (11β-OH Et)	0.29 ±0.12	1.22 ±1.87	<0.05
Tetrahydro-11-deoxycortison (THS)	0.27 ±0.10	0.61 ±0.63	<0.05
Tetrahydro-11-dehydrocorticosterone (THA)	0.40 ±0.13	1.72 ±1.55	<0.05
Tetrahydrocortisone (THE)	10.0 ±5.07	3.19 ±4.35	<0.01
5β-Tetrahydrocortisol (THF)	1.25 ±0.75	2.16 ±3.75	NS
α-cortolone	2.16 ±0.79	3.37 ±4.05	<0.05
β-cortolone	2.14 ±1.27	0.94 ±0.71	NS
α-cortol	0.60 ±0.22	1.11 ±1.06	<0.05
β-cortol	0.12 ±0.31	1.64 ±1.92	<0.01
5α-THB	0.55 ±0.14		
5α-THF	0.56 ±0.08		
Et/An	1.12 ±0.34	0.95 ±0.38	NS
11β-OH Et/11β-OH An	0.45 ±0.35	0.84 ±0.76	<0.05
E2/E1	0.89 ±0.78	2.01 ±1.60	<0.01

NS: not significant

thyl siloxane (length; 17 m, inner diameter; 0.2 mm, film thickness: 0.11  $\mu$ m) fused silica capillary

Helium carrier gas split ratio 1:10 GC .180

260 20 가

6 275 2 가

8 300

15 가 10 . Androgen corticoid 180

4 가 300 2

. Injector 300 transfer line

300 200 . Mass spectrometer electron-impact (E1) mode 70 eV

. ion monitoring mode 20

estrogen 21 androgen

. ion dwell time 50 msec .

#### 4. Estrogen, androgen, corticoid

Serdolit AD-2 resin Pasteur pipett 3 cm

(3 ml) internal standard ( $d_2$ -17 $\beta$ -estradiol), (1.5  $\mu$ g for estrogens and methyl testosterone, and 5  $\mu$ g for androgens and corticoids)

column . column 3 ml

free and conjugate 1 ml methanol 3

. 가  $\beta$ -glucuronidase/arylsulfatase (from Helix Pomatia) 55

acetate buffer (0.2 N, pH 5.0) 3

. Cathecol estrogen estrogen 가

1 mg/ml ascorbic acid 가 . 가

potassium carborate 가 pH 9.0

. estrogen ethyl acetate 5 ml

**Table 3.** Concentrations of urinary steroids in patients with leiomyoma according to leiomyoma type

	Leiomyoma type			P
	Subserosal (n=17)	Intramural (n=10)	Submucosal (n=10)	
<b>Androgen metabolites</b>				
Androsterone	1.01 $\pm$ 0.93	1.54 $\pm$ 1.59	0.50 $\pm$ 0.18	NS
Etiocholanolone	1.26 $\pm$ 1.64	1.24 $\pm$ 1.21	0.31 $\pm$ 0.19	NS
5-AT	0.69 $\pm$ 0.52	1.34 $\pm$ 1.17	0.46 $\pm$ 0.33	NS
11-Keto Et	0.68 $\pm$ 0.92	1.29 $\pm$ 1.77	0.34 $\pm$ 0.27	NS
11 $\beta$ -hydroxy An	0.84 $\pm$ 0.60	1.53 $\pm$ 1.37	0.51 $\pm$ 0.36	NS
11 $\beta$ -hydroxy Et	0.92 $\pm$ 0.79	1.44 $\pm$ 2.44	0.27 $\pm$ 0.22	NS
THS	0.46 $\pm$ 0.39	0.77 $\pm$ 0.79	0.22 $\pm$ 0.11	NS
THA	0.82 $\pm$ 0.81	2.63 $\pm$ 2.10	1.19 $\pm$ 0.0	NS
THE	2.12 $\pm$ 2.31	3.79 $\pm$ 5.94	0.77 $\pm$ 0.38	NS
$\beta$ -THF/a-THF	1.59 $\pm$ 1.29	2.56 $\pm$ 4.92		NS
$\beta$ -cortol	1.43 $\pm$ 0.0	1.30 $\pm$ 1.24	0.33 $\pm$ 0.18	NS
a-cortol	0.61 $\pm$ 0.82	2.07 $\pm$ 2.43		NS
<b>Estrogen metabolites</b>				
Estrone (E <sub>1</sub> )	11.80 $\pm$ 10.87	26.05 $\pm$ 22.33	16.77 $\pm$ 0.0	NS
17 $\beta$ -estradiol (E <sub>2</sub> )	16.45 $\pm$ 8.32	31.17 $\pm$ 16.49	28.32 $\pm$ 0.0	0.033
E2/E1	2.16 $\pm$ 1.62	1.79 $\pm$ 1.10	1.69 $\pm$ 0.0	NS

NS: not significant

, androgen corticoid 5 ml ether [E3], 16-epiestriol [16-Epi E3], 16, 17-epiestriol, 16a-hydroxyestrone [16a-OH E1], 17-epiestriol, 6-ketoestriol, 2-methoxyestriol, 6hydroxyestriol, and 16-ketoestradiol [16-Keto E2]) 21 androgen corticoid (androgen [An], etiocholanolone [Et], dehydroepiandrosterone [DHEA], 4-androstenedione [<sup>4</sup>-dione], testosterone [Te], 5-androstenediol [<sup>5</sup>-diol], 11 $\beta$ -hydroxy An [11 $\beta$ -OH An], 11 $\beta$ -hydroxy Et [11 $\beta$ -OH Et], 16a-hydroxy DHEA [16a-OH DHEA], 5-androstene-3a, 16 $\beta$ , 17 $\beta$ -triol [<sup>5</sup>-AT], tetrahydro-11-deoxycortisol [5a-THS], tetrahydrocortisone [THE], 5 $\alpha$ -tetrahydrocortisol [5a-THF], 3 $\beta$ -tetrahydrocortisol [THF], a-cortolone,  $\beta$ -cortolone, cholesterol, a-cortol,  $\beta$ -cortol, 5 $\alpha$ -tetrahydrocorticosterone [5a-THB] and 5 $\beta$ -tetrahydrocorticosterone [THB]) creatinine

P2O5/KOH . estrogen MSHFB/ androgen corticoid 60 30 MSHFB/ TMCS (100:1, volume ratio) 60 10 MSHFB/TMCS/TMSIm (2:2:1, volume ratio) 2  $\mu$ l aliquots auto sampler GC column .

5.

20 estrogen (estrone [E1], 17 $\beta$ -estradiol [E2], 2-hydroxyestrone [2-OH E1], 2-hydroxyestradiol, 2-methoxyestrone [2-Meo E1], 17 $\beta$ -estradiol, 6-dehydroestrone, 6a-hydroxyestradiol, 4-methoxyestradiol, estriol

**Table 4.** Concentrations of urinary steroids in patients with leiomyoma according to leiomyoma location

	Leiomyoma type			P
	Fundus (n=17)	Body (n=10)	Isthmus (n=10)	
<b>Androgen metabolites</b>				
Androsterone	2.05 $\pm$ 2.04	1.24 $\pm$ 1.36	0.3 $\pm$ 0.0	NS
Etiocholanolone	1.79 $\pm$ 2.07	1.08 $\pm$ 1.16	0.16 $\pm$ 0.0	NS
5-AT	0.74 $\pm$ 0.66	1.17 $\pm$ 1.07	0.17 $\pm$ 0.0	NS
11-Keto Et	0.90 $\pm$ 0.84	1.08 $\pm$ 1.61	0.29 $\pm$ 0.0	NS
11 $\beta$ -hydroxy An	0.90 $\pm$ 0.78	1.34 $\pm$ 1.25	0.20 $\pm$ 0.0	NS
11 $\beta$ -hydroxy Et	0.41 $\pm$ 0.45	1.31 $\pm$ 2.15	0.10 $\pm$ 0.0	NS
THS	0.37 $\pm$ 0.36	0.69 $\pm$ 0.72	0.10 $\pm$ 0.0	NS
THA		1.86 $\pm$ 1.79		NS
THE	3.08 $\pm$ 2.69	3.01 $\pm$ 5.14	0.35 $\pm$ 0.0	NS
$\beta$ -THF/a-THF	1.33 $\pm$ 0.84	2.22 $\pm$ 4.35	0.24 $\pm$ 0.0	NS
$\beta$ -cortol	0.77 $\pm$ 0.78	1.35 $\pm$ 1.22	0.20 $\pm$ 0.0	NS
a-cortol	2.00 $\pm$ 0.0	1.74 $\pm$ 2.10		NS
<b>Estrogen metabolites</b>				
Estrone (E <sub>1</sub> )	34.21 $\pm$ 31.03	18.97 $\pm$ 16.53	30.87 $\pm$ 15.33	NS
17 $\beta$ -estradiol (E <sub>2</sub> )	27.62 $\pm$ 16.93	27.28 $\pm$ 15.80	26.25 $\pm$ 14.35	NS
E2/E1	1.13 $\pm$ 0.85	2.07 $\pm$ 1.21	1.57 $\pm$ 0.98	NS

NS: not significant

**Table 5.** Correlation between concentrations of urinary steroids and volume of leiomyoma

	r	p
Androgen metabolites		
Androsterone	0.012	NS
Etiocholanolone	-0.041	NS
5-AT	0.041	NS
11-Keto Et	0.058	NS
11 $\beta$ -hydroxy An	0.081	NS
11 $\beta$ -hydroxy Et	0.023	NS
THS	0.064	NS
THA	-0.093	NS
THE	0.024	NS
$\beta$ -THF/a-THF	0.194	NS
$\beta$ -cortol	0.261	NS
a-cortol	-0.296	NS
Estrogen metabolites		
Estrone (E <sub>1</sub> )	-0.209	NS
17 $\beta$ -estradiol (E <sub>2</sub> )	-0.257	NS
E2/E1	0.078	NS

NS: not significant

**Table 6.** Correlation between concentrations of urinary steroids and weight of uterus

	r	p
Androgen metabolites		
Androsterone	-0.021	NS
Etiocholanolone	-0.053	NS
5-AT	-0.26	NS
11-Keto Et	-0.011	NS
11 $\beta$ -hydroxy An	0.021	NS
11 $\beta$ -hydroxy Et	0.021	NS
THS	0.016	NS
THA	-0.254	NS
THE	0.015	NS
$\beta$ -THF/a-THF	0.266	NS
$\beta$ -cortol	0.055	NS
a-cortol	-0.286	NS
Estrogen metabolites		
Estrone (E <sub>1</sub> )	-0.269	NS
17 $\beta$ -estradiol (E <sub>2</sub> )	-0.322	0.048
E2/E1	0.164	NS

NS: not significant

6.

SPSS (SPSS Inc, Chicago, III)  
ANOVA test Pearson  
p 0.05

43.1  $\pm$  5.6 ,  
40.6  $\pm$  7.2 63.4  $\pm$  7.3 kg, 59.4  $\pm$   
8.1 kg 155.4  $\pm$  4.8 cm, 159.3  $\pm$  4.8 cm  
(Table 1). 17 $\beta$ -estradiol, 5-AT, 11-keto ET, 11 $\beta$ -  
hydroxy An, 11 $\beta$ -hydroxy Et, THS, THA, THE, a-cor-  
tolone, a-cortol,  $\beta$ -cortol, 11 $\beta$ -OH Et/11 $\beta$ -OH An  
E2/E1  
가 (Table 2).

17 $\beta$ -estradiol

가 (Table 3),  
(Table 4, 5).  
17 $\beta$ -estradiol

가 가  
(Table 6).

가 30  
20%~30% .  
가

estrogen  
. GnRH-agonist  
estrogen

estradiol 가<sup>6</sup> Otubu  
 estradiol<sup>7</sup>  
 estrone  
 가 Estriol  
 2-methoxyestrone hydroxylated methoxyla-  
 ted 가  
 E2/E1  
 17β-estradiol estrone  
 17β-estradiol 17β-hydroxy steroid dehydrogenase  
 estrone  
 Yamamoto  
 estradiol estrone<sup>8</sup>  
 Yamamoto<sup>8</sup> estrogen 가  
 11β-OH Et/11β-OH An androgen 5α-re-  
 ductase 11β-OH Et/11β-OH An  
 5α-reductase  
 Reddy Rose  
 가 가 5α-reductase<sup>9</sup>  
 progesterone androgen  
 androgen  
 androgen estro-  
 gen 17β-estradiol  
 가  
 17β-estradiol  
 가  
 가 가

Progesterone androgen andro-  
 gen level progesterone  
 Progesterone  
 estrogen progesterone  
 progesterone<sup>10</sup>  
 Adams estrogen estrogen progesterone  
 progesterone  
 estrogen pro-  
 gesterone local growth factor  
 growth factor<sup>11</sup>  
 androgen estrogen  
 17β-estradiol  
 (regression coefficiency:  
 r)가 (r=-0.322)  
<sup>12</sup>

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