Joint Effects of Phthalate Exposure and Serum Paraoxonase (PON1) Activity on Estrogen-dependent Diseases in Taiwanese Women

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ABSTRACT

This study was to examine whether the estrogen-dependent diseases are associated with phthalate exposure and how serum PON1, an anti-oxidant protein, may modulate the disease risk. We conducted a case-control study where Group 1 were patients of either endometriosis or adenomyosis (n = 41), Group 2 were patients of leiomyoma (n = 31) and Group 3 were disease-free controls (n = 70). Patients of Group 1 and 2 had significantly lower serum PON1 activity as compared to controls after adjustment for age and second-hand smoking. No significant differences in phthalate metabolites were found between cases and controls. However, we noticed a joint effect on disease risk between serum PON1 activity and phthalates exposure. For those of low serum PON1 activity and high level of mono-2-ethylhexyl phthalate (MEHP), their risk for leiomyoma was significantly increased (OR = 11.9, $p < 0.05$). A similar joint effect was also found for Group 1 patients. The significant interaction between PON1 and phthalates indicates that oxidative stress may play an important role in the pathophysiology of the diseases.

INTRODUCTION

Estrogen-dependent diseases such as leiomyoma, endometriosis and adenomyosis are common gynecologic diseases that affect millions of women of reproductive age. Although the etiology of the diseases is unclear, a link to endocrine disruptors such as phthalates has been proposed[1-3]. Meanwhile, previous studies have also suggested a role of oxidative stress in the development of the diseases. Decreased activity of serum paraoxonase (PON1), an anti-oxidant enzyme, was found in patients of endometriosis as compared to controls[4,5]. Here we conducted a case-control study to examine the association between phthalate exposure and estrogen-dependent diseases and to understand whether an anti-oxidant enzyme, PON1, may modulate the disease risk.

MATERIALS AND METHODS

Patients of endometriosis, adenomyosis and leiomyoma were recruited from Department of Obstetrics and

Gynecology, Kaohsiung Medical University Hospital, from July 2005 to July 2007. All cases were diagnosed with pathologic confirmation. Controls were from either the same department or health check unit and free from the above three diseases. This protocol was approved by the institutional review board of Kaohsiung Medical University, and informed consent was obtained from all subjects before inclusion. Serum PON1 activity was determined using phenyl acetate as substrate and the rate of phenol generation was monitored at 270 nm. A total of 5 urinary phthalate metabolites including monobutyl phthalate (MBP), mono-2-ethylhexyl phthalate (MEHP), mono-ethyl phthalate (MEP), mono-benzyl phthalate (MBzP) and di-2-ethylhexyl phthalate (DEHP) were analyzed using on-line solid phase extraction coupled with liquid chromatography/electro-spray ionization tandem mass spectrometry (LC-ESI-MS/MS). Concentrations of urinary phthalate metabolites were adjusted by creatinine and presented as μg/g of creatinine.

RESULTS AND DISCUSSION

Table 1 showed the demographic characteristics of participants. Age and second-hand smoking rate were higher in patient groups than in controls and therefore, were included as confounding factors for the following analysis. There was no significant difference in body mass index (BMI) and age of menarche. Patients of both groups had significantly lower serum PON1 activity as compared to controls after adjustment for age and second-hand smoking (Table 1). This finding was consistent with a previous report where serum PON1 activity was found decreased in patients of endometriosis[4].

We measured phthalate metabolites in urine to reflect the individual exposure level to phthalates. A large variation in urinary phthalate levels among individuals was noted; however, there was no significant difference in urinary phthalate metabolites between cases and controls (Table 2). We further analyzed the joint effect between PON1 activity and phthalate exposure on disease risk. Subjects were divided into four sub-groups, with the sub-group of high PON1 activity and low phthalate level as the reference group. For patients of endometriosis/adenomyosis, a clear trend for disease risk was found when PON1 activity was decreasing while MBP, MEP or MBzP level was increasing (Table 3). Subjects who had low PON1 activity and high exposure showed the highest risk for the diseases (OR = 4.26 for MBP; OR = 3.22 for MEP; OR = 3.66 for MBzP). A similar joint effect between PON1 and phthalates was also found for leiomyoma, although the significant trend of risk was only shown in MEHP (Table 4).

The present study showed a significant interaction between serum PON1 activity and phthalates exposure on risk for endometriosis, adenomyosis and leiomyoma. Although the mechanism by which phthalates may contribute to the development of estrogen-dependent diseases is not clear, our finding suggests that PON1, a major anti-oxidant serum protein, is likely to play a modulating role in the pathogenesis.

Table 1. Demographic characteristics of study subjects

	Endometriosis or Adenomyosis (n=41)	Leiomyoma (n=31)	Controls (n=70)
	Mean ± SD	Mean ± SD	Mean ± SD
Age (years) [a]	37.6 ± 8.4 *	41.3 ± 6.6 *	35.2 ± 7.6
BMI (kg/m2) [a]	22.7 ± 3.7	23.6 ± 3.9	21.9 ± 3.4
Age of menarche (years) [a]	13.2 ± 1.4	13.3 ± 1.9	13.2 ± 1.3
Second-hand smoking N (%)[a]	39 (48.2) *	25 (45.5) *	19 (21.4)
PON1 activity [b]	97.4 ± 24.3 *	102.2 ± 29.1 *	117 ± 23.1

[a] Differences were analyzed by ANOVA.

[b] Differences were analyzed by linear regression, adjusted for age and second-hand smoking.

*$P < 0.05$ versus controls.

Table 2. Levels of urinary phthalate metabolites in study subjects

Urinary phthalate metabolites	Endometriosis or Adenomyosis(n=41)	Leiomyoma (n=31)	Controls (n=69)
MBP	77.82(56.44-107.30)	86.50(58.55-127.79)	60.90(48.87-78.89)
MEHP	4.48(3.11-6.44)	8.04(4.23-15.29)	4.03(2.91-5.60)
MEP	68.25(50.72-91.83)	89.56(61.56-130.29)	67.53(53.31-85.55)
MBzP	11.7(8.92-15.36)	15.08(10.94-20.79)	12.33(9.7-15.68)
DEHP	40.03(25.59-62.25)	55.41(29.55-103.90)	38.85(28.37-53.22)

Data were presented as geometric mean (95% CI); unit =μg/g of creatinine.

Differences were analyzed by ANOVA.

Table 3. Joint effect between serum PON1 activity and phthalates exposure on risk for endometriosis and adenomyosis

PON1 Activity	Urinary phthalate metabolites	N	Prevalence (%)	Endometriosis or Adenomyosis OR (95% CI)
	MBP.			
High	Low	20	20.00	reference
	High	25	24.00	0.78 (0.15-3.99)
Low	Low	31	38.71	1.97 (0.48-8.13)
	High	35	54.29	4.26 (1.10-16.45) [a]
	MEP			
High	Low	28	21.43	reference
	High	17	23.53	0.84(0.17-4.11)
Low	Low	27	48.15	2.66(0.76-9.36)
	High	39	46.15	3.22(1.01-10.25) [a]
	MBzP			
High	Low	22	22.73	reference
	High	23	21.74	0.86(0.18 – 4.07)
Low	Low	32	43.75	2.44(0.64 - 9.25)
	High	34	50.00	3.66 (1.02 - 13.10) [a]
	DEHP			
High	Low	22	18.18	reference
	High	23	26.09	1.36(0.26-7.16)
Low	Low	33	45.45	4.69(1.07-20.51) [a]
	High	33	48.48	4.31(1.13-16.47) [a]
	MEHP			
High	Low	21	23.81	reference
	High	24	20.83	0.60(0.12-2.99)
Low	Low	35	42.86	2.84(0.74-10.86)
	High	31	51.61	3.28(0.91-11.86)

PON1 Activity: high (115.04-168.07 U/L), low (70.45-115.04 U/L)

MBP: high (52.11-1202.54), low (11.33-52.11); MEP: high (55.89-1123.40), low (9.88-55.89); MBzP: high (11.02-205.99), low (0.98-11.02); DEHP: high (32.88-833.28), low (2.27-32.88); MEHP: high (3.26-433.66), low (0.44-3.26)

Unit of phthalate metabolites = μg/g of creatinine

Differences were analyzed by multiple logistic regression and adjusted for age and second-hand smoking.

[a] $P < 0.05$

Table 4. Joint effect between serum PON1 activity and phthalates exposure on risk for leiomyoma

PON1 Activity	Urinary phthalate metabolites	N	Prevalence (%)	Leiomyoma OR (95% CI)
	MBP.			
High	High	18	11.11	reference
	High	24	20.83	1.88(0.29-12.18)
Low	Low	28	32.14	3.96(0.69-22.78)
	High	31	48.39	4.75(0.78-28.90)
	MEP			
High	Low	23	4.35	reference
	High	19	31.58	9.67(0.97-96.05)
Low	Low	23	39.13	15.34(1.56-151.26) [a]
	High	36	41.67	10.80(1.18-98.51) [a]
	MBzP			
High	Low	18	5.56	reference
	High	24	25.00	4.12(0.42-41.03)
Low	Low	29	37.93	9.52(0.99-91.94)
	High	30	43.33	8.23(0.89-75.74)
	DEHP			
High	Low	19	5.26	reference
	High	23	26.09	5.15(0.53-49.72)
Low	Low	30	40.00	27.40(1.76-425.92) [a]
	High	29	41.38	10.10(1.04-98.54) [a]
	MEHP			
High	Low	17	5.88	reference
	High	25	24.00	4.88(0.50-47.25)
Low	Low	29	31.03	8.80(0.90-85.99)
	High	30	50.00	11.86(1.28-109.65) [a]

PON1 Activity: high (115.04-168.07 U/L), low (70.45-115.04 U/L)

MBP: high (52.11-1202.54), low (11.33-52.11); MEP: high (55.89-1123.40), low (9.88-55.89); MBzP: high (11.02-205.99), low (0.98-11.02); DEHP: high (32.88-833.28), low (2.27-32.88); MEHP: high (3.26-433.66), low (0.44-3.26)

Unit of phthalate metabolites = μg/g of creatinine

Differences were analyzed by multiple logistic regression and adjusted for age and second-hand smoking.

[a] $P < 0.05$

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